Sec16 as an integrator of signaling to the endoplasmic reticulum

Dissertation submitted for the degree of Doctor of Natural Sciences

(Dr. rer. nat.)

Presented by

Kerstin Tillmann

at the

Faculty of Sciences
Department of Biology
University of Konstanz

Date of the oral examination: September 11th, 2015
First referee: Prof. Dr. Daniel Legler
Second referee: PD Dr. Hesso Farhan
Third referee: Prof. Dr. Sebastian Springer

Konstanzer Online-Publikations-System (KOPS)
URL: http://nbn-resolving.de/urn:nbn:de:bsz:352-0-305723

Table of Contents

Summary

Newly synthesized proteins leave the endoplasmic reticulum (ER) at ER exit sites (ERES) in COPII coated vesicles. Among several proteins that regulate ERES, there is consensus that Sec16A plays a key role. Sec16A is a protein of ~250 kDa that localizes to ERES where it regulates ERES number and COPII vesicle formation by acting as a scaffold for COPII components.

The results show that Sec16A is an integrator of growth factor signaling at the level of ERES. Sec16A is regulated by growth factor signaling in two ways: first, short-term growth factor signaling was found to increase the mobility of Sec16A via phosphorylation. Second, long-term growth factor signaling increases Sec16A expression via the Egr transcription factor family. This mode of regulation places Sec16A as the central node in a coherent feed-forward loop.

In addition, mathematical modeling of Sec16A dynamics at ERES in response to signaling provides new insights into the biogenesis of ERES as mediated by Sec16A. Furthermore, recruitment of Sec16A to ERES was found to be COPII-dependent.

Lastly, Sec16A as well as functional ER export is required for cell proliferation, which links Sec16A to hyperproliferative diseases such as cancer.

These findings provide a direct link between mitogenic stimulation, secretion, and proliferation.

Zusammenfassung

Neu synthetisierte Proteine verlassen das endoplasmatische Retikulum (ER) an so genannten „ER exit sites (ERES)“ in COPII-ummantelten Vesikeln. Neben einer Vielzahl an Proteinen, die ERES regulieren, wird das Protein Sec16 allgemein als Schlüsselkomponente in der Regulation von ERES betrachtet. Sec16 hat eine Größe von circa 250 kDa und lokalisiert an ERES, wo es neben der Anzahl an ERES auch die Entstehung von COPII-Vesikeln reguliert, da es für die Komponenten des COPII-Mantels eine stabilisierende, gerüstbildende Funktion übernimmt.

Die Ergebnisse zeigen, dass Sec16A die Signale nach Stimulation mit Wachstumsfaktoren auf dem Level von ERES integriert. Sec16A wird dabei auf zwei Arten durch Wachstumsfaktoren reguliert. Durch kurz andauernde Stimulation mit Wachstumsfaktoren wird die Mobilität von Sec16A durch Phosphorylierung erhöht, während durch lang andauernde Stimulation mit Wachstumsfaktoren die Expression von Sec16A durch Egr Transkriptionsfaktoren gesteigert wird. Diese Art der Regulation setzt Sec16A an die Stelle eines zentralen Knotens in einer kohärenten feed-forward Schleife.

Zusätzlich ermöglicht das mathematische Modellieren der Sec16A-Dynamik an ERES in Reaktion auf Signalierung neue Einblicke in die durch Sec16 mediierte Biogenese von ERES. Des Weiteren sind Hinweise entdeckt worden, dass die Rekrutierung von Sec16A zu ERES COPII-abhängig ist.

Schließlich wird gezeigt, dass sowohl Sec16A, als auch ein funktioneller ER Export essentiell für Zellproliferation sind. Dies stellt Sec16A in den Kontext von hyperproliferativen Krankheiten.

Diese Erkenntnisse stellen einen direkten Zusammenhang zwischen Wachstumsfaktorstimulation, Sekretion und Proliferation her.

Introduction

The Secretory Pathway

1 Structure of the Secretory Pathway

The eukaryotic secretory pathway consists of various different membranous organelles that coordinate protein secretion. This tightly regulated process is essential for cellular function. Transmembrane proteins or proteins that are destined to be secreted from the cell must travel through the secretory pathway not only to be able to reach their destination but also to be post-translationally modified. This is the case for approximately one third of all proteins synthesized in a eukaryotic cell, with an estimated 11% of proteins being soluble secretory proteins and 21% transmembrane proteins [1, 2, 3].

The transport of proteins through the secretory pathway starts at the endoplasmic reticulum (ER). The ER is involved with a variety of cellular functions, including protein synthesis, modification and secretion, lipid synthesis and calcium homeostasis [4, 5]. The ER is the largest membranous organelle in the cell which consists of a single continuous membrane which surrounds the nucleus forming the nuclear envelope, and forms a net-like structure consisting of cisternae and tubules spanning the cytoplasm known as peripheral ER [6]. Based on ultrastructural morphological analyses, it is subdivided into the rough ER, which is covered with ribosomes on its cytosolic surface and has a sheet-like morphology, and the smooth ER which is ribosome-free and has a more tubular structure [7].

Newly synthesized proteins that are destined for the secretory pathway are transferred into the ER lumen or, in the case of transmembrane proteins, into the ER membrane. After secretory proteins are properly folded, they leave the ER at specialized, ribosome-free regions of the rough ER known as transitional ER (tER) or ER exit sites (ERES) [8, 9, 10, 11]. These structures are very stable and long-lived, although a certain degree of mobility has been assigned to them [12]. ERES are organized on cup-shaped structures, the existence of which has been clearly demonstrated in Drosophila cells [13], but has also been seen in animal cells, although only in a third of cases [14]. At ERES, secretory clients are packaged into COPII-coated vesicles [15]. After vesicles have budded from ERES, they deliver their cargo to the ER-Golgi Intermediate Compartment (ERGIC) also known as vesicular-tubular cluster (VTC) [16, 17], which is formed by COPII vesicle fusion. The ERGIC is a stable membrane compartment located between the ER and the Golgi and has been shown to sort cargo for retrograde and anterograde

trafficking via COPI-coated vesicles [17]. Retrograde trafficking from the ERGIC and the Golgi back to the ER ensures that ER resident proteins (ie chaperones, cargo receptors) that are trafficked together with their substrates are recycled back to the ER. Anterograde trafficking transports proteins destined for the Golgi and beyond to the Golgi [16, 17, 18] which fuse with the Golgi membrane. In mammalian cells, the Golgi is located next to the centrosome and forms a ribbon-like stack comprised of cisternae, which are stacks of flattened membrane compartments that are interconnected by tubules. Adjacent to the Golgi stack two reticular membrane networks are found, which, together with the cisternae, form the Golgi complex. Depending on the composition of Golgi enzymes responsible for glycosylation or other post-translational modifications of cargo proteins, the Golgi is subdivided into the cis-Golgi, the trans-Golgi and the Trans Golgi Network (TGN). The *cis*-Golgi faces the ERGIC and the ER and receives vesicles from the ERGIC. The medial-Golgi lies between the cis-Golgi and the TGN, which faces the plasma membrane. At the TGN, proteins are once again sorted and transported to the plasma membrane (PM) or to intracellular compartments via Clathrin-coated vesicles [18, 19, 20].

To ensure that proteins localize to the correct compartments, for example if they are ER-resident proteins or need to be secreted, proteins contain sorting motifs. These are short specific amino acid motifs located in the cytosolic domains of transmembrane proteins that are recognized by cargo adaptors. Soluble proteins require cargo receptors to be sorted into COPII-coated vesicles, whereby the cargo receptors are recognized by cargo adaptors. Cargo adaptors concentrate cargo into vesicles and thereby mediate the transport of proteins to their allocated compartments [3, 21].

Although the general structure of the secretory pathway is universally similar in all mammalian cell types, some differences can be found in specialized cell types. Specialized secretory cells that produce large quantities of proteins that are to be secreted face a large secretory burden as their secretory organelles need to handle this increase. Differentiated B cells or plasma cells that produce and secrete antibodies increase the volume of both ER and Golgi [22, 23, 24, 25].

Neuronal cells on the other hand must be able to transport cargo not only within the cell body but also along their dendrites and axons in order to sustain them. To achieve this, the ER in neurons spans not only the cell body but is found as a highly elaborate network in dendrites. Additionally, small Golgi outposts have been observed in dendrites [26, 27, 28, 29, 30].

The organization of the secretory pathway in mammalian cells is quite different from that in lower organisms. In the budding yeast *Saccharomyces cerevisiae* (*S.cerevisiae*) for example, there are no clearly defined ERES as in most other cell types. Consequently, COPII vesicles are capable of stochastically forming at any region of the ER. Lately, the term ERES was adapted to define clusters of COPII forming vesicles in *S.cerevisiae*, but these are on the ultrastructural level not the same as bona fide ERES in other cell types. [31, 32, 33, 34]. The Golgi also differs markedly between mammalian cells and other cell types. In mammals the Golgi is a single copy organelle that is organized as stacks of flattened cisternae that are laterally anastomosing to form the Golgi ribbon. In S.cerevisiae, the Golgi cisternae do not form stack and are dispersed in the cytoplasm. However, these cisternae can still be classified as *cis*, *medial* or *trans* or TGN based on their protein composition [31, 35, 36, 37, 38]. Another budding yeast that is commonly used as a model organism in trafficking is *Pichia pastoris* (*P.pastoris*). In *P.pastoris*, around two to five distinct ERES are found that are faced by the same number of Golgis, which are composed of stacks of three to four cisternae. These cisternae are not laterally connected as in mammalian cells but are still polarized into *cis*, *medial*, and *trans* cisternae [31, 33, 35, 39, 40]. Similarly to *S.cerevisiae*, the fission yeast *Schizosaccharomyces pombe* (*S.pombe*) does not have clearly defined, easily distinguishable ERES [41], its Golgi however is organized into similar stacks as found in *P.pastoris* [31, 38, 42]. The secretory pathway in plants has traditionally received less attention. However, work in the decade showed that plant cells also have ERES and stacked Golgis that form secretory units dispersed throughout the plant cytosol. A difference to other cell types is that ERES and Golgis are more mobile [43, 44, 45, 46, 47, 48, 49]. Interestingly, neither yeast or plant cells have so far been shown to have an intermediate compartment between ER and Golgi comparable to the mammalian ERGIC [16, 17]. Another popular model organism in cell biology is the fruit fly *Drosophila melanogaster (D.melanogaster*). The secretory pathway in *D.melanogaster* most closely resembles that of *P.pastoris*. *D.melanogaster* lacks an intermediate compartment such as the ERGIC: instead, the tER and Golgi stacks are organized into tER-Golgi units. On average, 20 ERES are found in *D.melanogaster* cells that are closely associated with several Golgi stacks consisting of two to three individual cisternae which also show *cis*- and *trans*-like orientation [13, 50, 51, 52, 53].

As described above, the secretory pathway differs between species and has continuously developed and gained complexity during evolution. In prokaryotes, proteins are directly secreted via the plasma membrane, whereas eukaryotic cells have developed multiple membrane-bound compartments [54]. In many eukaryotic species, such as yeasts and *Drosophila*, the early secretory pathway is organized into secretory units, where ER exit sites at the ER face several Golgis. In contrast, mammalian cells have an additional compartment, the ERGIC, that coordinates anterograde and retrograde protein trafficking [4, 17]. Other species, such as plants and yeasts, contain a vacuole, which plays an important role in protein degradation [31, 55]. Despite these differences in the structure of the secretory pathway, the components of the secretory pathway machinery are largely conserved. However, due to gene duplications, mammalian cells have developed several isoforms of COPII components that allow a more specific regulation of protein trafficking [56].

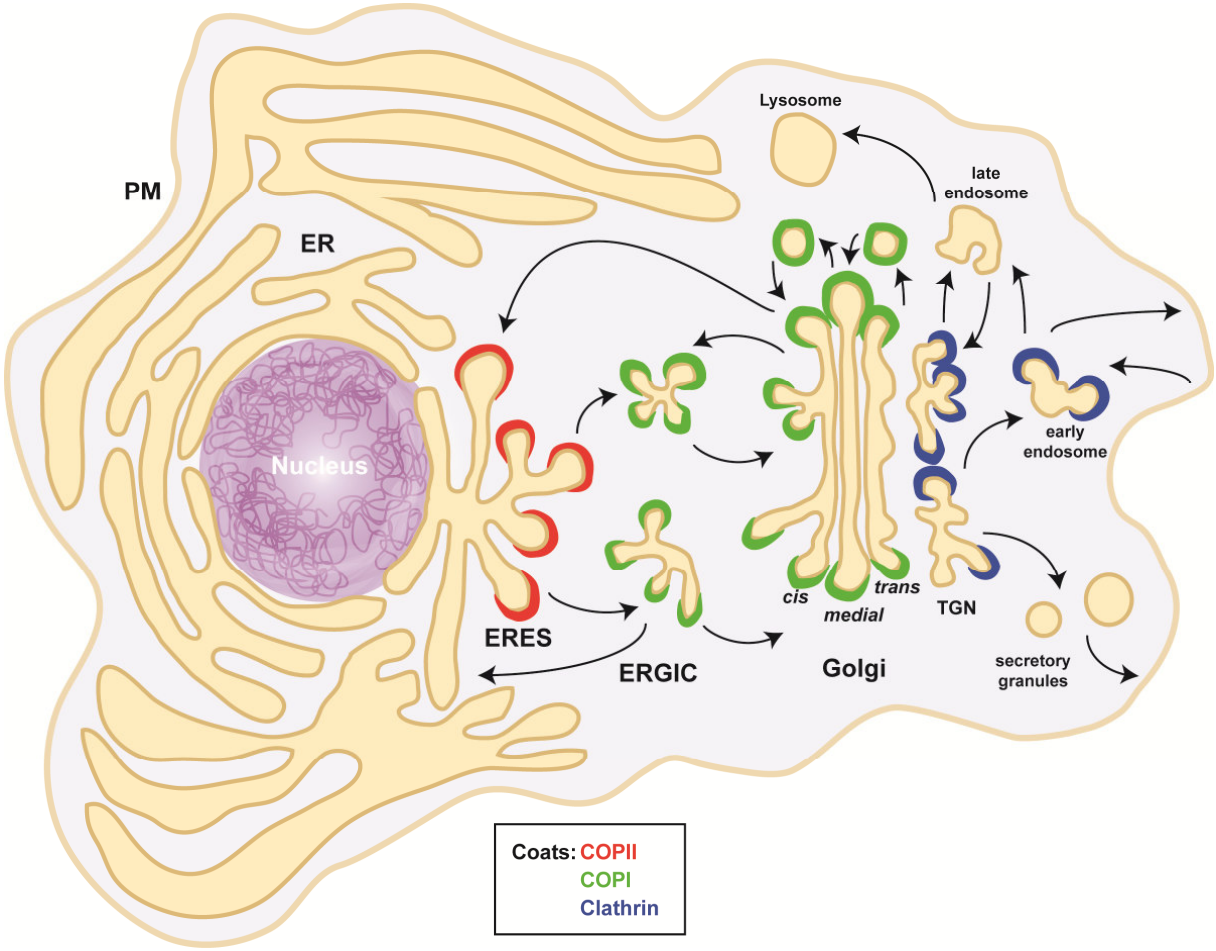


Figure 1: The secretory pathway

Schematic depicting the different compartments of the secretory pathway, whereby transport steps and their direction are indicated by arrows. The vesicle coats are indicated by different colors: COPII (red), COPI (green), and Clathrin (blue). Nascent proteins leave the endoplasmic reticulum (ER) after passing quality control at ER exit sites (ERES) in COPII-coated vesicles. Proteins are transported to the ER-Golgi intermediate compartment (ERGIC), where they are sorted and transported in COPI-coated vesicles back to the ER (retrograde transport) or to the *cis*-Golgi (anterograde transport). Proteins travel through the Golgi from the *cis*-, to the *medial*-, and finally to the *trans*-Golgi and the Trans-Golgi-Network (TGN). At the TGN, proteins are sorted and transported in Clathrin-coated vesicles to the plasma membrane (PM), to early and late endosomes, or to secretory granules.

2 Endoplasmic reticulum

As described above, the ER consists of membranous sheets and tubules, whereby the sheets mostly contain the ribosomes forming the rough ER where protein translation and translocation, post-translational modifications, as well as folding of proteins and quality control takes place [6, 57]. These processes will be discussed briefly below.

2.1 Protein translocation into the ER

Import of proteins into the ER can occur either during protein translation while the emerging protein is bound to the ribosome (co-translationally or ribosome-coupled protein translation), or post-translationally, after the protein has been fully synthesized and is no longer associated with the ribosome (ribosome un-coupled protein translocation). Although both translation modes utilize the same translocation process through the ER membrane via the heterotrimeric Sec61 complex, they require different accessory proteins to assist with the ATP-dependent translocation process [31, 58, 59, 60, 61, 62, 63].

Co-translational translocation is dependent on the signal peptide, a cleavable sequence of 15-30 amino acids with a hydrophobic core flanked by polar and uncharged residues. The signal sequence is recognized by the cytosolic signal recognition particle (SRP) as the precursor polypeptide emerges from the ribosome. Next, SRP binds its SRP receptor on the ER membrane, thereby transporting the nascent protein (bound to the ribosome) to the heterotrimeric Sec61 translocation complex that forms an aqueous pore in the ER membrane. During translocation, the ribosome is tightly bound to the Sec61 pore. Many other proteins assist in and regulate the co-translational translocation process, particularly the ER luminal protein called Binding immunoglobulin protein (BiP). BiP is a member of the heat shock protein 70 (HSP70) family and is a molecular chaperone that binds the nascent protein as it reaches the ER lumen. In addition, BiP gates the Sec61 channel by mediating both sealing and opening of the pore [31, 58, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76].

In post-translational translocation, the fully synthesized polypeptide is kept soluble in the cytosol and transported to the Sec61 translocation complex by cytosolic chaperones, for example members of the Hsp70 and Hsp40 chaperone families. As in co-translational translocation, the Sec61 complex is regulated by the ER luminal BiP, among other proteins [31, 58, 64, 73, 74, 77, 78, 79].

2.2 Protein maturation in the ER lumen

After synthesis of the polypeptide chain and translocation of the nascent protein into the ER, proteins need to be properly folded in order to function. The ER lumen contains a high level of folding enzymes or molecular chaperones. Chaperones are defined as proteins that assist incorrectly or unfolded proteins to reach their native, properly folded state, but are not a part of the final structure of the folded protein. Several categories of folding enzymes are present in the ER lumen, such as the lectins (which include Calnexin and Calreticulin), heat shock family chaperones (such as BiP, GRP94), protein disulfide isomerase (PDI) family, peptidyl-propyl *cis/trans* isomerases (PPIs), and chaperones responsible for special substrates [57, 80].

The most prominent chaperones in the lectin chaperone family are Calnexin and Calreticulin. Calnexin is a type I ER integral membrane protein, whereas Calreticulin is a soluble protein present in the ER lumen. Consequently, Calnexin recognizes proteins that are in proximity of the ER membrane, whilst Calreticulin binds proteins found in the ER lumen [81, 82, 83, 84, 85, 86]. Lectin chaperones require monoglucosylated *N*-linked glycans and unfolded protein regions in order to recognize their protein substrates. Most proteins entering the ER lumen immediately become N-glycosylated, on asparagines within the consensus sequence N-X-S/T by the oligosaccharyl transferase which covalently attaches the core carbohydrate, which consists of two N-acetylglucosamine residues, nine mannose residues, and three terminal glucose residues. As soon as the oligosaccharide is attached to the nascent protein, residues of the oligosaccharide are removed, or trimmed off. Glucosidase I and II remove the two terminal glucose residues, followed by removal of a terminal mannose by ER mannosidases. These trimmings result in monoglucosylated side chains which are recognized by Calnexin and Calreticulin [87, 88, 89, 90, 91, 92, 93, 94, 95, 96]. Removal of the last glucose residue of the oligosaccharide by Glucosidase II results in the release of the nascent protein from either Calnexin or Calreticulin, and prevents re-binding of the protein with the lectin chaperones, as these are unable to recognize unglucosylated proteins. Therefore, the binding of lectin chaperones to their substrates is regulated by the actions of glucosidases and transferases. By binding nascent proteins, lectin chaperones prevent protein aggregation and slow down the folding reaction, thereby increasing the efficiency of the folding. Additionally, they present nascent proteins to other chaperones that assist with protein folding, such as PDIs that are responsible for disulfide bond formation. After release from the lectin chaperones, proteins can either be properly folded, or require additional folding. In this case, the de-glucosylated proteins are recognized as misfolded by UDP-glucose: glycoprotein

glucosyltransferase (GT1) and receive back a glucose residue which once again makes them recognizable for Calnexin or Calreticulin, and they undergo an additional round of folding [57, 82, 87, 92, 94, 97, 98, 99, 100, 101, 102, 103].

The most important member of the heat shock chaperone family is BiP, which is also involved in protein translocation, as described above. Substrates recognized by BiP are mostly non-glycosylated, as BiP recognizes short hydrophobic regions that are usually not accessible on folded proteins. In addition to binding unfolded proteins and preventing their aggregation, BiP also has an ATPase domain. When bound to ATP, BiP is in its low-affinity conformation for unfolded proteins. ATP hydrolysis is induced by other accessory proteins such as DnaJ (Hsp40), which causes BiP to transfer to its high affinity conformation and tightly bind unfolded proteins. Nucleotide exchange factors then exchange ADP for ATP causing BiP to release its substrate. This cycle is repeated numerous times until the protein is folded properly [57, 104, 105, 106, 107, 108, 109, 110, 111, 112]. Another highly abundant glycoprotein chaperone that is only found in vertebrates is GRP94, which is more specialized than BiP and required for the maturation of immunoglobulins and toll-like receptors [57, 113, 114, 115, 116, 117, 118].

An important step in creating the native protein fold is to establish the correct disulfide bonds within the nascent protein, as these are critical for the formation and stability of proteins. This reaction is assisted by a large family of protein disulfide isomerases (PDIs). PDIs not only act as electron donors and acceptors during the formation and rearrangement of disulfide bonds in their target substrates but also function as chaperones [57, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128].

In addition to the described chaperone families there are also highly specialized chaperones present in the ER that show great substrate-specificity. For example, Hsp47 is a chaperone that is required specifically for the maturation of collagen, while the previously mentioned GRP94 is required for assembly for MHCI [113, 114, 116, 117, 129, 130, 131, 132, 133].

2.3 Quality control and ERAD

Various reasons like mutations or shortage in chaperones might result in the accumulation of terminally misfolded proteins. To prevent toxicity due to these unfolded proteins, the ER has evolved strategies to send these proteins to degradation, a process summarized as ER-associated protein degradation (ERAD). However, since

misfolded proteins are located in the ER lumen, they must be transported out of the ER back into the cytoplasm, in a process called „re-translocation“. The ERAD pathway can be divided into four distinct steps, beginning with substrate recognition, dislocation or re-translocation into the cytoplasm, ubiquitination and finally degradation via the proteasome [134, 135].

This sequence of events remains the same, irrespective of which substrate is being targeted. However, the ERAD pathway may be subdivided based on the type of lesion that the ERAD client harbors. The Doa10 complex targets misfolded domains on the cytoplasmic side of the membrane (ERAD-C substrates), whereas proteins with misfolded luminal domains (ERAD-L substrates) or misfolded intramembrane domains (ERAD-M) are processed by the Hrd1 complex. Although most of these studies were performed in yeast, the system is generally believed to act similarly in mammalian cells [136, 137].

After proteins are targeted for degradation by ERAD, they need to be transported across the ER membrane back into the cytoplasm to be degraded by the proteasome. Certain proteins have been suggested to form the translocon for the re-translocation of misfolded proteins, such as the Sec61 complex or Hrd1 [138, 139, 140]. Several proteins assist in the ATP-dependent translocation of misfolded proteins, such as the AAA-ATPase Valosin-containing protein (VCP) also known as p97 [134, 141, 142, 143]. Translocated proteins are targeted for proteasomal degradation by ubiquitylation mediated by ERAD-specific ubiquitin-ligase complexes, whereby substrate specificity is achieved via the E3 ligases [134, 136, 137, 144].

3 ER exit sites

As described above, ER exit sites (ERES) localize to cup-shaped structures on ribosome-free regions of the rough ER. ERES mediate the highly controlled process of protein export from the ER in COPII-coated vesicles [8, 9].

3.1 Cargo selection

Soluble proteins present in the ER lumen can be unselectively enclosed in COPII-coated vesicles and transported in a process known as „bulk flow“, this process is inefficient and only takes place in a few cases where the proteins are further enriched in later compartments. Most secretory cargo is enriched in COPII vesicles by sorting proteins and adaptors[145, 146, 147, 148]. These cargo receptors recognize different short amino acid sequences on secretory proteins and are themselves able to interact with COPII-components via cytosolic domains. Generally, ER export of cargo and cargo receptors requires a combination of export signals and oligomerization of cargo receptors; this might be necessary to ensure that only fully assembled cargos are exported. The best described export signals are di-acidic sequence (DXE) motifs and di-hydrophobic motifs [149].

Several families of transmembrane cargo receptors have been identified so far, these are ERGIC-53 and the ERGIC-53 family, as well as the p24 protein family of which several ER vesicle (Erv) proteins have been studied [3, 146, 150, 151, 152, 153]. The ERGIC-53 family consists of Ca^{2+}-dependent L-type lectins that recognize glycoproteins, of which the ERGIC marker ERGIC-53 has been the most intensively studied; other proteins of this family include ERGL, VIP36 and VIPL. Members of this family localize to different compartments where they most likely act as cargo receptors for either anterograde or retrograde trafficking, and each shows different affinities for differently modified oligosaccharides [3, 151, 154, 155, 156, 157]. Members of the p24 family are present in most eukaryotic cells and are subdivided into four subfamilies (p24α, p24β, p24γ, p24δ). These around 24-kDa proteins shuttle between the compartments of the early secretory pathway and are responsible for the transport of glycosylphosphatidylinositol (GPI)-anchored proteins [158]. Among the best studied p24 family cargo receptors are the Erv41/46 complex and the Emp46/47 complex as well as other individual receptors [159, 160, 161, 162, 163, 164, 165, 166, 167, 168]. However, a systematic overview of cargo protein and cargo receptor pairing is still missing. What is known is that cargo receptors directly interact with components of the COPII coat and that this interaction is required for packaging into COPII-coated vesicles [160, 162, 168, 169].

3.2 COPII

For proteins to leave the ER, they must be packaged into COPII-coated vesicles at ER exit sites. The core machinery and the general assembly process of COPII vesicle formation has been researched in great detail in a variety of model organisms, however, many open questions still exist. For example, it is still unclear how vesicle formation is initiated in detail, and how ERES are formed. The general principle appears to be conserved, and it is responsible for inducing membrane curvature at the ERES, to concentrate cargo into nascent vesicles, and to promote vesicle formation and release. The core machinery that drives COPII vesicle formation consists of five proteins, which are Sar1, Sec23, Sec24, Sec13, and Sec31. These five components have been shown to be sufficient to form vesicles from membranes in vitro [8, 170, 171]. These COPII components are evolutionarily conserved, although gene duplication in metazoan cells resulted in 2 isoforms for Sar1 and Sec23, and four isoforms of Sec24 [8, 56].

Formation of COPII vesicles begins with the transmembrane protein Sec12, which is located at the ER membrane and functions as a guanine nucleotide exchange factor (GEF) for Sar1. Sec12 activates and recruits the GTPase Sar1 to ERES [172, 173, 174]. Sar1 is activated by binding GTP, which results in a conformational change that exposes an N-terminal amphipathic alpha helix which inserts into the ER membrane [175]. Active Sar1 then binds Sec23 and thereby recruits the inner COPII coat consisting of the Sec23/Sec24 heterodimer. Together, Sar1 and Sec23/Sec24 form the so called pre-budding complex, whereby Sec24 is responsible for cargo recruitment into the vesicle and binds cargo and cargo receptors [176, 177, 178]. Sec23 is not involved in cargo binding but functions as the GTPase activating protein (GAP) for Sar1 and therefore stimulates GTP hydrolysis. After the pre-budding complex has formed, the outer coat consisting of Sec13/Sec31 heterodimers is recruited by Sec23 interacting with Sec31 [179, 180, 181]. Sec13/Sec31 forms the outer coat of the nascent vesicle by building a cage-like layer, thereby driving membrane curvature. In addition, Sec31 promotes Sar1 GTP-hydrolysis by increasing the GAP activity of Sec23 by 10-fold [21, 179, 182, 183]. Therefore, once the vesicle is formed and the outer coat is recruited, vesicle scission occurs which is dependent upon GTP hydrolysis mediated by Sar1 [8, 21, 184]. Although the process of COPII vesicle formation appears to be tightly regulated and structured, evidence for regulation is only now occurring. One of the most important proteins involved in the regulation of COPII vesicle formation is the large, 250 kDa protein Sec16A. This protein is believed to regulate COPII vesicle formation by acting as a scaffold protein, as it was

shown to interact with all components of the COPII coat. However, its exact role is still unknown, as well as its recruitment to ERES [185]. The components of the COPII coat, the regulatory protein Sec16, and the ultrastructural formation of the COPII coat will be discussed in detail below.

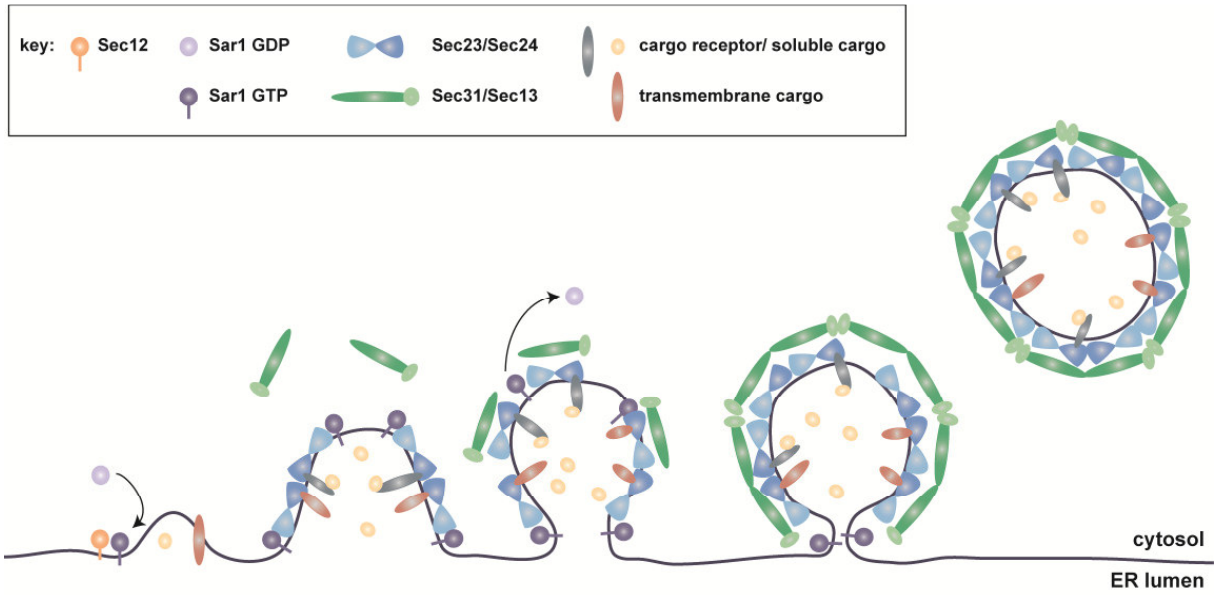


Figure 2: Schematic of COPII vesicle formation

COPII vesicle formation at ERES is initiated by Sec12 (orange), which recruits inactive, GDP-bound Sar1 (light purple) to the membrane and mediates GDP-GTP exchange of Sar1. GTP-bound Sar1 (purple) inserts an amphipathic α-helix into the membrane and recruits the inner COPII coat consisting of Sec23 (light blue) and Sec24 (dark blue). Sec24 recognizes transmembrane cargo (red), or soluble cargo (yellow) via cargo receptors (grey). After formation of the pre-budding complex consisting of Sar1-Sec23/Sec24, the outer COPII coat consisting of Sec13 (light green) and Sec31 (dark green) is recruited which forms a cage-like structure. Binding of the outer coat increases Sar1-GTPase activity and thereby vesicle scission and budding.

a. Sec12/Sar1

As described above, the process of vesicle formation is initiated by the ER-resident type II transmembrane protein Sec12 which recruits Sar1 to the ER membrane and is required for ERES function [172, 174, 175, 186, 187]. In *S.cerevisiae*, Sec12 was found to be localized at the ER membrane by the combined actions of ER-retention and retrieval from the Golgi [188, 189]. Interestingly, not much is known about how and where Sec12 initiates COPII vesicle formation, and how Sec12 is regulated. In contrast to other species where Sec12 is found distributed across the entire ER membrane, Sec12 in *P.pastoris* is found at ribosome-free patches of the rough ER where ERES are formed [190]. Studies in *P.pastoris* led to the hypothesis that localization of *P.pastoris* Sec12 to ERES is dependent upon binding of an unidentified tER-localized partner component. It is likely that in other species, Sec12 requires the interaction with unidentified proteins, although Sec12 in all other species is not concentrated at ERES, and overexpression of *P.pastoris* Sec12 in *S.cerevisiae* resulted in a uniform distribution of Sec12 across the ER membrane. Interestingly, Sec16 has been suggested to be required in the localization of Sec12 to ERES in *P.pastoris* [191]. Increasing Sec12 levels by overexpression of the protein was shown to disrupt the ERES localization of Sec12, but this was rescued by co-expression of Sec16, indicating that stochiometric amounts of both proteins are required for proper Sec12 localization. This rescue required the presence of the C-terminal domain of Sec16. Whether Sec16 fulfills the same function in other species remains to be investigated [190, 191]. Recently, a protein called cutaneous T-cell lymphoma-associated antigen 5 (cTAGE5) was shown to concentrate Sec12 at ERES in mammalian cells [192]. cTAGE5 is a transmembrane protein that colocalizes at ERES with TANGO1 [193]. The transmembrane protein TANGO1 is required ER export of Collagen by recognizing Collagen via its ER-luminal domain and binding Sec23/Sec24 with its cytoplasmic, proline-rich part [194, 195]. In addition, TANGO1 recruits the TRAPP complex component Sedlin. Sedlin directly binds Sar1 and ensures efficient Sar1 GTPase cycling, which was shown to be required for the formation of large vesicles [196]. cTAGE5 was first shown to interact with TANGO1 and Sec23/Sec24 at ERES where it was proposed to function as a co-receptor for TANGO1 to mediate Collagen export [193]. A recent study showed that cTAGE5 concentrates Sec12 at ERES, and that this enrichment of Sec12 was required for the export of Collagen, but not of other proteins [192]. These findings suggest that a complex of several proteins – TANGO1, cTAGE5, and Sedlin – is required for the export of collagen, and that this complex mediates vesicle formation at the level of Sec12 and Sar1.

In addition to Sec12, in *S.cerevisiae*, a Sec12-like protein called Sed4 has been described, although it has not been found in higher species [56, 197]. Sed4 was shown to interact with Sec16 and is believed to assist in the recruitment of COPII. It may inhibit the activity of Sec23, although it was shown to stimulate the GTPase activity of Sar1 in the absence of Bet1 [197, 198, 199, 200].

In a conserved process, Sec12 activates Sar1 by exchanging Sar1-bound GDP for GTP. Sec12 contains a highly conserved K-loop which is required for the interaction with Sar1 and to displace GDP in a process stabilized by potassium [201, 202]. Activation of Sar1 by GTP-binding induces a conformational change in Sar1 and the exposure of an N-terminal amphipathic alpha helix [174, 175, 203]. The helix is inserted superficially into the ER membrane via the interaction of hydrophobic residues in the helix with phospholipid groups. This insertion was shown to induce membrane curvature and to lower membrane rigidity [204, 205]. Membrane association of active Sar1 is required for the recruitment of the inner coat components Sec23/Sec24, whereby Sar1 binds Sec23 [177, 203, 206]. In addition to recruitment of COPII components, Sar1 is also required for concentration of cargo into the vesicle [207, 208]. Furthermore, Sar1 mediates the release of the vesicle from the ER membrane by vesicle scission. During these processes, several rounds of Sar1-GTP hydrolysis may take place [175, 209, 210].

In mammalian cells, two highly similar isoforms of Sar1 have been identified, Sar1A and Sar1B, that might fulfill slightly different functions [211]. Sar1B for example has been implicated in regulation of lipid homeostasis and cholesterol transport, as it is required for export of pre-chylomicron transport vesicles (PCTV) from the ER in enterocytes [212, 213]. Dietary lipids are rapidly converted to triacylglycerols (TAG) at the level of the ER [214]. TAGs are then incorporated into specialized TAG-rich transport vesicles called chylomicrons. These large, 250 nm chylomicrons are exported from the ER in a manner dependent on the formation of PCTVs [215, 216]. Interestingly, Sar1B, but not Sar1A, was found to be required for this process, and it was shown to respond to intracellular dietary lipid levels and enhance assembly of chylomicrons [213, 217, 218, 219]. Sar1B was further shown to be phosphorylated by PKCζ, which was necessary for PCTVs to form [220, 221]. In addition, mutations in Sar1B have been identified that cause Anderson's disease or chylomicron retention disease, which is a rare hereditary hypocholesterolemic syndrome. As the name suggests, patients suffer from low cholesterol levels, as well as chronic diarrhea with steatorrhea and in general a failure to thrive; a similar phenotype is observed in Sar1B-knockout mice [212, 222, 223, 224, 225, 226].

b. Sec23/Sec24

After Sar1 activation, the Sec23/Sec24 heterodimer is recruited, forming the pre-budding complex. Sec23 acts as the GTPase activating protein (GAP) for Sar1 and therefore stimulates Sar1 GTPase activity [173]. Sec23 and Sar1 were shown to have a large interaction interface, and Sar1-binding to Sec23 drives recruitment of the inner COPII coat. In the binding interface between Sar1 and Sec23, the Sec23 arginine-finger plays a major role, as it is inserted into the catalytic pocket of Sar1. This interaction between Sar1 and Sec23 is stabilized by the Sar1-bound GTP [177]. Assembly of the pre-budding complex by binding of Sec23/Sec24 to Sar1 is required for the ERES association of Sec13/Sec31 [171]. Recruitment of Sec31 to the vesicle causes re-orientation of the arginine-finger of Sec23, further increasing Sar1 GTPase activity by 10-fold [179, 183]. Furthermore, Sec23 is targeted by the TRAPP complex, Rab1, and in *S.cerevisiae* by the *cis*-Golgi kinase Hrr25p. Sequential binding of these proteins to Sec23, after it is released form Sar1, is believed to be required for directional transport of the COPII vesicle and to prevent the vesicle from re-fusing with the ER membrane [227].

In mammalian cells, two isoforms of Sec23 have been found, Sec23A and Sec23B. Although not much is known about different functions of these two isoforms, they have been implicated in different diseases and may therefore have specialized functions. For example, missense mutations in Sec23A cause craniolenticulosutural dysplasia (CLSD) due to defective collagen secretion [228, 229, 230, 231]. Mutations of Sec23B however cause congenital dyserythropoietic anemia type II, which is characterized by dysfunctional erythroid differentiation in human patients. In mice however, loss of Sec23B does not cause anemia, but massive defects in secretory tissues causing death soon after birth [232, 233, 234, 235].

The COPII component Sec24 is recruited to ERES together with Sec23, forming the inner coat and stabilizing the pre-budding complex. Sec24 is responsible for cargo sorting and recruitment of cargo in the forming vesicle. For cargo recruitment to take place, Sec24 recognizes specific export signals on cargo proteins or membrane-spanning cargo receptors, but Sec24 itself was also shown to contain cargo-binding sites on its surface. Several isoforms of Sec24 have been described, with Iss1/Sfb2 and Lst1/Sfb3 in yeast, and Sec24A-D in mammals. Within the mammalian isoforms, Sec24A and Sec24B are most similar to one another, as are Sec24C and Sec24D [56, 145, 176, 206, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246]. These isoforms show specificity towards

different cargos, although there is some overlap in the recognition of transport signals and the different isoforms can compensate the loss of one isoform to varying degrees. This broadens the range of potential cargos and enables selective enrichment of cargo into vesicles. In addition, Sec24 isoforms may selectively recognize properly folded proteins due to recognition of folded epitopes instead of mere signal sequences, as reported for Sec22 [236, 240, 241, 247, 248, 249]. Sec24A for example is especially required for the export of cargos containing aromatic/hydrophobic signals, whereas GPi-anchored proteins are selectively recognized by the Sec24C and Sec24D isoforms, and the serotonin transporter (SERT) export was found to be dependent exclusively upon Sec24C, while its close homologue the GABA transporter 1 (GAT1) was shown to be a client for Sec24D [250, 251, 252, 253].

The different functions of the Sec24 isoforms also become evident from the highly different phenotypes of knockout mice. Interestingly, loss of Sec24A and Sec24B, which are more similar to the yeast Sec24 proteins and therefore considered as the evolutionarily older isoforms [56], is not lethal in knockout mice. Sec24A knockout mice show normal survival and development, but with reduced plasma cholesterol levels due to an inability of the mice to secrete PCSK9, a secreted protease which mediates degradation and regulation of the cell surface LDL receptor [254, 255]. Loss of Sec24B function on the other hand causes severe craniorachischisis due to defects in neural tube closure. Closure of the neuronal tube requires the establishment of planar cell polarity. In this process, the vang-like protein 2 (Vangl2) is a key signaling component which exclusively requires Sec24B for its export from the ER. Sec24B mutations have been suggested to contribute to human neuronal tube defects [256, 257, 258]. This difference in phenotype severity between loss of Sec24A and Sec24B can be explained by differences in ER export signal recognition of these isoforms, as investigated by Wendeler et al. [236]. This study compared ER export selectivity of the different Sec24 isoforms and found that while the isoforms are in part functionally redundant, Sec24A is the most important isoform, as double knockdown combinations of the four isoforms containing Sec24A resulted in the strongest inhibition of trafficking. However, the other three Sec24 isoforms were shown to be able to compensate loss of Sec24A due to redundancy in cargo recognition [236]. This corresponds to the relatively mild phenotype of Sec24A knockout mice, as a large part of Sec24A function appears to be compensated by the other isoforms *in vivo*. Therefore, the other Sec24 isoforms may have evolved later and developed specificity for additional cargos, while largely retaining Sec24A function. This is in line with findings in Sec24C and Sec24D knockout mice. Loss of Sec24C causes early embryonic lethality in mice, but tissue-specific

deletion of Sec24C in various cell types did not result in any phenotype, indicating that Sec24C is not required in all tissues [259]. Interestingly, Sec24C was also shown to be involved in the transport of dietary fat across intestinal cells, as Sec24C was found to be specifically required for docking of the pre-chylomicron transport vesicle (PCTV) at the Golgi [260]. As in the case of Sec24C, loss of Sec24D causes early embryonic lethality in mice. However, three patients were identified that were compound heterozygous for two of three identified Sec24D mutations and showed severe defects in bone development, possibly due to defects in collagen secretion. This phenotype is in accordance with the phenotypes described in Sec24D mutant fish and zebrafish [261, 262, 263, 264]. As mentioned above, the fact that loss of Sec24C and Sec24D in knockout mice is lethal may be reconciled with the fact that these isoforms arose later in evolution and possibly evolved highly specialized functions required in complex multicellular organisms, and can therefore not be compensated by other isoforms [56].

Although the primary role of Sec24 is cargo binding, it also plays a role in the regulation of the COPII coat. For example, a mutant version of Sec24 with reduced binding to Sec16 caused defects in COPII vesicle formation, because the ability of Sec16 to inhibit Sar1 GTPase activity was impaired [265]. In addition, the Sec24 isoform Sec24D was shown to be phosphorylated by Akt, and phosphorylated Sec24C and Sec24D show reduced binding to Sec23, indicating a regulatory mechanism targeting Sec24 [266].

c. Sec13/Sec31

After formation of the pre-budding complex, the outer COPII coat consisting of Sec13/Sec31 is recruited. These proteins assembly into units comprised of two Sec13 and two Sec31 proteins, forming a heterotetramer [8, 180, 267, 268]. Sec13 is the smallest component forming the COPII coat with only 33 kDa, and only was found to have two isoforms in the plant *A.thaliana* [56, 269, 270, 271, 272]. Its main function seems to be to provide stability and rigidity to the COPII coat, which was shown to be especially important for large or asymmetrically distributed cargo, and a reduction of cargo burden rescued loss of Sec13 [228, 273, 274, 275]. Interestingly, Sec13 is also found in the nuclear pore complex where it forms a complex with the nuclear pore proteins Nup84 and Nup154C that is similar in structure to the Sec13-Sec31 edge element, as well as other nuclear pore components [276, 277]. Sec13 has not been implicated in human diseases as of yet, however, it was shown to be required for cell survival and collagen transport in the early developmental stage of retina development in zebrafish [278]. In this process, both

the function of Sec13 in the COPII complex and in the nuclear pore complex were shown to be required [279]. In addition, loss of Sec13 in zebrafish also inhibited the development of the digestive system due to induction of UPR-induced apoptosis and growth arrest, as well as epithelial morphogenesis due to defects in secretion of collagen and other extracellular matrix components [280, 281].

The essential protein Sec31 forms the outer COPII coat together with Sec13, and since it is a large protein at 150 kDa, it is the major contributor to the outer COPII coat structure [8, 282]. As most COPII components, Sec31 has two isoforms, Sec31A and Sec31B. While Sec31A resembles yeast Sec31p, human Sec31B shares less than 50% similarity with Sec31A, and it has not been the subject of further research [270, 283, 284, 285]. Upon binding of the Sec13-Sec31A heterotetramer, Sec31A rearranges the interaction between Sec23 and Sar1, and thereby increases Sar1 GTPase activity, which ultimately leads to vesicle scission [177, 179]. Although the COPII coat is structured, it is not rigid but slightly variable and can increase in size to accommodate large cargos such as pro-collagen and chylomicrons. Expansion of vesicle and coat size was shown to be mediated by monoubiquitylation of Sec31A by Cul3, but how this increases the size of the COPII coat is still unclear [286]. In addition, phosphorylation of Sec31 might play a regulatory role. Already upon its discovery, Sec31 was identified as a phosphoprotein, and treatment of samples with alkaline phosphatase inhibited vesicle formation in in vitro budding assays. Later, the casein kinase 2 (CK2) was shown to phosphorylate Sec31A, and thereby Sec31A affinity for Sec23 and turnover of Sec31 at ERES [270, 287].

d. Structure of the COPII coat

A lot of effort has been invested into resolving the assembly of the COPII coat at the ultrastructural level, and recently, the COPII coat was shown not be a rigid assembly of COPII components, but to form a flexible inner and outer coat that can adapt to different sized vesicles and therefore allow export of a variety of different cargos. In general, COPII vesicles can have a size ranging from 60 to 120 nm, but ER export of large cargos such as the 300 nm pro-collagen fibers is also possible [170, 288, 289].

The inner coat consisting of Sec23/Sec24 forms a heterodimer which has a size of approximately 200 kDa. Although both proteins differ strongly in their sequence, their

folding was shown to be similar, and the heterodimer forms a shape that resembles a bow-tie [177, 241]. The complex was shown to be positively charged and curved on the membrane-facing side, which is believed to aid in binding to the negatively charged curved membranes of the nascent vesicles, or to mediate membrane curvature [179, 289].

The outer cage consists of Sec13/Sec31 complexes. Sec31 is larger than Sec13 and contributes mostly to the structure of the coat. A single unit of the coat consist of heterotetramers formed by Sec13 and Sec31, whereby two Sec31 molecules form a rod-like structure by dimerizing tail-to-tail via their α-solenoid structures in their C-terminal region, where they form a flexible hinge (135°-165°) [180, 181, 288, 290]. The β-propeller of Sec13 binds Sec31 at its N-terminal β-propeller. At this Sec13-Sec31 binding area, four Sec13-Sec31 rods attach to form a cage-like latticework that surrounds the vesicle, whereby Sec13 provides stability to the coat [180, 273, 291]. Two vertexes were measured at this interface, a rigid α-vertex at 60°, and a flexible β-vertex which can form between 90-108°, allowing accommodation of differently sized and curved vesicles. Sec13-Sec31 rods were shown to have a length of approximately 30 nm [181, 288, 289, 290]. Sec31 binds Sec23 in the inner coat via a large 300 amino acid long, proline-rich unstructured region in Sec31 that is located near the C-terminus [181, 182].

A recent study investigated the structure of the COPII coat assembled on membranes, and proposed a model that incorporated previous ultrastructural findings in membrane-free environments, as well as the question of how the COPII coat can accommodate differently sized cargos [288]. This model suggests that the inner coat dictates the structure of the outer coat, and that in the case of small cargos, the inner coat does not form a continuous lattice surrounding the vesicles, but instead forms patches where cargo is bound. The area between these patches would allow the transport of slightly larger cargos. The outer coat then associates on top of these inner coat patches in a cage-like manner as described previously, although this cage-like structure was shown to be flexible and not to form a rigid net. The transport of large cargos requires the formation of tubular-shaped vesicles that are surrounded continuously by the inner coat. The outer coat would then be able to bind a higher percentage of inner coat components, and to form a more rigid coat, which was shown to be required for the transport of large cargos such as pro-collagen [196, 288].

How this process is regulated is still unclear, but certain proteins have been identified that are specifically required for the export of large cargos such as collagen and chylomicrons, as discussed above.

3.3 Sec16

An important regulator of COPII vesicle formation is the large, approximately 240 kDa protein Sec16, which localizes to ERES and interacts with all COPII components. This protein was first identified in *S.cerevisiae*, and is absent in some eukaryotic lineages, as is Sec12. A recent genomic and phylogenetic comparative study found the core COPII machinery (Sar1, Sec23, Sec24, Sec13, and Sec31) to be present in all analyzed species, indicating that these components and the mechanism of COPII-vesicle trafficking was already present in the Last Eukaryotic Common Ancestor (LECA). Regulatory proteins, such as Sec12 and importantly Sec16, seem to have evolved later in evolution, as they are missing in certain species. A homology search of 74 eukaryotic genomes revealed that both proteins were absent in eight organisms from five different taxa which are known for their reduced cellular complexity, and appear to have lost Sec12 and Sec16 in the process of undergoing cellular reduction [56]. Sec16 was found to be absent in mostly unicellular parasitic organisms such *as Theileria parva*, *Toxoplasma gondii*, or *Encephalitozoon cuniculi*, but also the multicellular sea anemone *Nematostella vectensis*. Regulatory proteins such as Sec12 and Sec16 may have become essential at later stages in evolution, enabling increased speed and efficiency of COPII vesicle formation, as trafficking became more complex [56]. Sec16 has been identified in all model organisms so far, such as unicellular organisms as *Trypanosoma brucei*, *S.cerevisiae*, *Pichia pastoris*, and other yeasts, as well as multicellular organisms such *Caenorhabditis elegans, Drosophila melanogaster*, and mammals, and even in plants [13, 49, 56, 185, 292, 293, 294, 295, 296, 297, 298, 299, 300]. In mammalian cells, two Sec16 homologues were identified, the larger 231 kDa Sec16A, and a smaller, 117 kDa Sec16B. Sec16A is similar to Sec16 found in other organisms, and it has been more extensively investigated than Sec16B. Although Sec16A and Sec16B were shown to be present in the same complex and may therefore act fulfill similar functions [298], Sec16B was shown to have a distinct role in peroxisome biogenesis. In addition, Sec16B, not Sec16A is found to be mutated in a large number of association studies investigating genetic variants in obesity and related diseases [298, 301, 302, 303, 304, 305, 306, 307, 308].

Three domains of Sec16 have been identified that are conserved, which are responsible for membrane binding and interaction with COPII components. The first domain is at the C-terminus of Sec16 called the C-terminal conserved domain (CTCD) containing the last 158 to 226 residues depending upon the species. This region is present in most organisms, except for *C.elegans*, and it is also absent in mammalian Sec16B [185, 298]. The CTCD is required for the interaction of Sec16A with Sec23 and

Sec12 in mammalian cells and in *P.pastoris*. In *S.cerevisiae*, it was shown to mediate interaction between Sec16p with Sec23 and the Sec12-analogue Sed4 [34, 191, 197, 298, 309]. The second domain is the 400 - 500 amino acids spanning central conserved domain (CCD) located in the middle of the Sec16-sequence. This sequence is highly conserved and found in all Sec16 variants described so far [13, 34, 56, 298, 299, 310]. It is required for interaction with Sec13 in *S.cerevisiae* and mammalian cells, as well as for Sec24-interaction in *S.cerevisiae* [265, 267]. In addition, the CCD is required for oligomerization of Sec16 [34]. The third domain lies 300 residues upstream of the CCD and is called ERES localization domain (ELD) or upstream conserved region (UCR), as it is required for ERES localization of human and Drosophila Sec16 [13, 34, 309]. In addition, this domain was shown to mediate the interaction of Sec16 with Sec23, Sec24 and Sec31 in *S.cerevisiae* and *P.pastoris* [34, 265, 309].

The ELD/UCR and the CCD together were shown to be required for efficient ERES localization of Sec16. Within the ELD, an arginine-rich stretch of 90 amino acids was shown to mediate ERES localization in *Drosophila* that is also found in mammalian Sec16A. The CCD mediates Sec16 oligomerization, which was found to be required for localization of Sec16 to ERES, as monomeric Sec16 was found mostly in the cytosol [267, 309]. In mammalian cells, Sec16 forms complexes of unknown size that contain both Sec16A and Sec16B, and both proteins localize to ERES [298].

The role of Sec16 in COPII vesicle formation and the manner in which it is recruited to ERES is still a matter of debate. Sec16 interacts with all components of the COPII machinery, and it is generally believed to act as a scaffold protein that is required for recruitment and stability of the COPII coat [311]. In addition, Sec16 is required for ERES integrity, as depletion of Sec16 leads to a reduction of ERES number in all species. However, it is unclear whether Sec16 initiates COPII vesicle formation and recruits the COPII components, or if it is recruited by COPII components and stabilizes the coat. In Drosophila S2 cells, Sec16 localization to ERES was shown to be independent of COPII, as depletion of Sec23 did not affect the localization of Sec16. In addition, Sec16 localization was independent of Sar1, as both overexpression of inactive Sar1 and Sar1 depletion did not disrupt Sec16 localization to ERES. Loss of Sec16 on the other hand disrupted Sar1 localization to ERES in *Drosophila*, indicating that Sec16 acts upstream of Sar1 [13]. Similar to these findings in *Drosophila* cells, Sec16 was found to localize to ERES independently of Sec23/Sec24 and Sec13/Sec31 in mammalian cells [14]. In contrast to *Drosophila* cells however, the localization of Sec16 in mammalian cells was found to be dependent upon Sar1 activity [312]. In in vitro experiments, it was further shown that Sec16 requires Sar1 to localize to neutral liposomes or microsomal

membranes [292, 313]. These findings show that Sec16 acts upstream of COPII assembly, and downstream of Sar1 in the case of mammalian cells. In addition, although both Sec16 and COPII localize to ERES, they only rarely co-localize, indicating that Sec16 is not part of the vesicle coat and functions before the vesicle is formed. This supports the role of Sec16 as a scaffold protein that recruits and regulates COPII vesicle formation. Additional support for this model is given by a study that investigated the structure of a complex between a Sec13 and a shortened version of Sec16. Whittle et al. found that Sec16 contains an ancestral coatomer element (ACE1) that is also found in Sec31. The structure of the Sec13-Sec16 tetramer was found to be similar to the structure of the Sec13-Sec31 tetramer, with Sec16 forming a dimer in the middle of the structure and two Sec13 molecules flanking the dimer on each side [180, 267]. Formation of a coat-template by Sec13-Sec16 could assist in the assembly of the COPII coat, whereby Sec13-Sec16 tetramers would ultimately be replaced by Sec13-Sec31 tetramers that form the outer coat. This model is in line with the fact that Sec16 has a regulatory effect on Sar1 GTPase activity[34, 292]. Sec16 was shown to interact with Sec23 and Sar1, and this interaction prevented recruitment of Sec31 in early stages of COPII vesicle formation. Upon recruitment to the COPII coat, Sec31 increases Sec23-mediated Sar1 GTPase activity by 10-fold which ultimately induces vesicle scission. Therefore, in addition to stabilizing the COPII coat, Sec16 prevents premature vesicle scission. In *S.cerevisiae*, this function of Sec16 is supported by the actions of Sec24, as loss of Sec24 function resulted in the production of smaller, prematurely budded vesicles in vitro [34, 265].

Sec16A was also found to interact with proteins that do not belong to the COPII coat machinery. For example, Sec16A was shown to be a target of kinase signaling, as Farhan et al. showed that Sec16A is phosphorylated by ERK2 at position T415. Several lines of evidence suggest a role for mitogenic signaling in ERES regulation via phosphorylation of Sec16, such as a decrease in ERES number observed upon ERK2 depletion and a decrease in the number of ERES and ERGIC-53 dots in cells overexpressing the phosphoablating Sec16A-mutant T415I [314].

Another member of the MAPK family was suggested to regulate Sec16 function in Drosophila. ERK7 is an atypical MAP kinase, whose degradation by the proteasome is inhibited upon serum and amino acid starvation, resulting in higher protein levels of ERK7. Under these conditions, protein secretion was inhibited due to disassembly of ERES mediated by ERK7 [310]. This ERK7-mediated disassembly of ERES was shown

to be due to a relocation of Sec16 and other COPII components away from ERES to newly identified structures called Sec-bodies. These Sec-bodies were induced upon amino acid starvation and are possibly similar to other intracellular stress assemblies, although they were so far shown to only contain COPII components [315]. Interestingly, while ERK2 was shown to phosphorylate Sec16A in mammalian cells, ERK7 was not found to phosphorylate *Drosophila* Sec16 directly. However, Sec16 was shown to be phosphorylated in response to amino acid starvation on a newly identified 'starvation response domain' at the C-terminus [310]. In addition, the dispersion of Sec16 away from ERES observed upon overexpression of wild type ERK7 was abolished in cells overexpressing kinase-dead ERK7 mutants. It is therefore likely that ERK7 phosphorylates Sec16, or mediates a phosphorylation upon amino acid starvation. Interestingly, phosphorylation of Sec16 by ERK2 was not observed in Drosophila cells [310]. Similar to mammalian cells, serum starvation caused a decrease in ERES number in Drosophila cells; however, this response was shown to be mediated by ERK7, not ERK2. The induction of ERES disassembly via Sec16 in order to inhibit secretion upon different types of starvation is therefore a conserved mechanism, whereas the manner in which this is signaled to Sec16 is mediated by different components.

The conserved Trk-fused gene (TFG) was identified as a conserved regulator of COPII-mediated vesicle transport in C.elegans. Depletion of TFG resulted in a decrease of ER-to-Golgi trafficking and a decrease in Sec16B and COPII levels at ERES, which was shown to be mediated in mammalian cells by interaction of TFG with Sec16B. As TFG and Sec16B were shown to co-localize at ERES, it is likely that Sec16A might also be regulated by TFG in the same manner [300]. Interestingly, a later study showed that depletion of TFG did not cause a decrease in Sec16A-positive structures, but significantly decreased the number of Sec31-positive ERES as well as the association or vicinity of Sec16A and ERGIC53 positive structures [316]. As TFG forms hexamers, it was suggested to form polymers at ERES, possibly via interaction with Sec16, and to retain COPII vesicles at their point of formation after vesicle budding to facilitate fusion with the ERGIC, which is in close proximity. In accordance with the role of TFG in the stabilization of the ERES/ERGIC interface, depletion of TFG decreased the tight association usually observed between COPII- or Sec16-positive structures with ERGIC clusters [300, 316].

Sec16A was also shown to be an interaction partner of tumor necrosis factor receptor-associated factor-3 (TRAF3). TRAF3 mediates the induction of interferon (IFN)

production as a reaction to intracellular double-stranded RNA (dsRNA) upon viral infection. TRAF3 was found to be localized to the early secretory pathway under normal conditions, as it was found at ERES, the ERGIC and the cis-Golgi. In addition, TRAF3 was shown to interact with Sec16A and p115. Upon sensing of dsRNA, TRAF3 localizes to Mitochondrial AntiViral Signaling (MAVS), a mitochondria-associated adaptor protein. This triggers the initiation of signaling cascade required in the cellular response to viral infection. The relocalization of TRAF3 to MAVS was shown to be dependent upon Sec16A and p115, as depletion of these proteins led to a dispersal of TRAF3 away from early secretory pathway membranes and inhibited the relocalization of TRAF3 to MAVS upon dsRNA sensing. In addition, depletion of Sec16A and p115 inhibited anti-viral gene expression and IFN production [317].

Finally, the kinase Leucine-rich repeat kinase 2 (Lrrk2) was shown to regulate ER-to-Golgi transport through interaction with Sec16A [318]. Lrrk2 is involved in the regulation of different cellular processes, for example the assembly of microtubules and the actin cytoskeleton, in protein translation, endocytosis and autophagy-mediated protein degradation [319], and it has been associated primarily with Parkinson's disease [320, 321]. In a recent study, Lrrk2 was found to co-localize with Sec16A at ERES, and depletion of Lrrk2 resulted in a dissociation of Sec16A away from ERES, as Sec16A was found to shift to the cytosolic fraction in Lrrk2-depleted cells. In addition, Sec31 formed enlarged ERES or clusters upon loss of Lrrk2, and trafficking of VSVG from the ER to the cell surface was impaired. Although LRRK2 is a kinase, the effect of Lrrk2 on Sec16A was independent of its kinase activity, and Sec16A was not shown to be phosphorylated by Lrrk2. Interestingly, Lrrk2 also contains a GTPase domain that was reported to be similar to that of Sar1. Loss of GTPase function by missense mutation of the GTPase domain was shown to be required for the effect of Lrrk2 on Sec16A and secretion, although the precise mechanism of this regulation has not been elucidated [318]. These findings show that Lrrk2 has a role in upstream regulation of Sec16A, suggests a role for ERES functionality and COPII vesicle trafficking in the pathogenesis of Parkinson's disease [318].

3.4 Regulation of ER Export

Apart from Sec16A, other proteins have been shown to influence ERES structure and ER export by binding different components of the COPII vesicle machinery.

In S.cerevisiae, the protein Nel1 was identified as a paralog of Sec23. Although Nel1 was not found to bind any of the other COPII components and was not incorporated into the COPII coat, it showed strong GTPase GAP activity [322].

In addition to size of cargo, also the amount of cargo at the ER was shown to influence ER export and ERES. Farhan et al. showed that ERES respond differently to acute and chronic increases in cargo load. Acute increase in cargo load at the ER, as induced by BFA treatment, resulted in fusion of ERES creating larger ERES. For this adaptive response the presence of the ERES regulators Sec16A and PI4-kinase IIIα (PI4K- IIIα) is required. In contrast, a chronic increase by overexpression of the anterograde cargo GABA transporter 1 (GAT1) led to an increase in ERES number in a Sec16A-dependent manner. In addition, the UPR was activated [323]. These findings are in line with an earlier study that showed that overexpression of biosynthetic cargo (VSVG) modulated vesicle budding [324].

Further proteins have been found to affect ERES and COPII vesicle formation. For example, phospholipase D (PLD) was shown to be activated by Sar1, and activated PLD was shown to support recruitment of the COPII coat, possibly via changing lipid composition of the membrane at ERES [325].

Another protein that is involved with the regulation of ERES is the mammalian protein p125A or Sec23-interacting protein (Sec23-IP) [326, 327]. p125A is a member of the phosphatic acid-preferring phospholipase A1 enzyme family which was shown *in vitro* to recognize phosphatic acid and phosphatidylinositol phosphates, and to be targeted to PI4P-rich membranes via its C-terminal DDHD-domain, which may also mediate phospholipase activity [328, 329]. Lipid recognition of p125A was found to be supported by the C-terminal SAM domain, which is also required for oligomerization of p125A [326, 327]. In addition, p125A was shown to interact with Sec23 via its N-terminal proline-rich region and with Sec31, thereby linking the inner and outer COPII coat [327, 329, 330]. Consequently, p125A localizes to ERES, but was also found to localize to the ERGIC and cis-Golgi where it co-localizes with p115 and GM130 [329, 331]. Both overexpression and loss of p125A caused a disruption of ERES, indicating that stochiometric amounts of p125A are required for ERES regulation. In a recent study, p125A was proposed to

be required for dispersal of Sec16A away from ERES in late stages of vesicle formation, as temperature-induced traffic blocks at the level of ERES and ERGIC led to a segregation of Sec16-positive and p125A/Sec23/Sec31-positive structures [326]. p125A-mediated spatial segregation of Sec16A away from late-stage COPII-vesicles may be a mechanism how vesicle scission may be regulated, as Sec16A inhibits Sar1-GTPase activity by shielding Sec23 from Sec31 [326]. Surprisingly, under conditions where p125A was either depleted or overexpressed, trafficking of VSVG was still functional, indicating that vesicle scission is possible without p125A mediation. This is in line with the fact that p125A may not be a conserved protein, as it was not found in *P.pastoris* [313, 326, 331]. In addition, despite its role in ERES regulation, p125A is not an essential protein, as the only impairment in p125A-knockout mice is reduced male fertility due to defects in spermiogenesis [332]. In humans, p125A has been associated with Waardenburg-syndrome [333]. As p125A recognizes lipids, these findings support the role of lipid signaling in the regulation of ERES [323, 334].

Sec23 also interacts with the serine/threonine kinases PCTAIRE, which belong to the group of CDK family of protein kinases. Sec23 was shown to interact with PCTAIRE1 and PCTAIRE3, and overexpression of kinase-dead mutant of PCTAIRE1 resulted in a more diffuse staining of Sec24, indicating decreased ERES association of Sec24. In accordance with a disruption of ERES, overexpression of a kinase-dead version of PCTAIRE1 led to decrease in transport of VSVG to the plasma membrane [335].

Furthermore, a Ca2+-binding protein called apoptosis-linked gene 2 (ALG-2) was shown to regulate COPII vesicle formation via binding Sec31. ALG-2 localizes to ERES in a manner dependent on Sec31, and was found to stabilize the interaction between Sec31 and Sec23, which required calcium binding. In addition to ALG-2, another calcium-binding protein called Annexin A11 was also shown to influence Sec31A localization to ERES, as depletion of both proteins decreased the amount of ERES-associated Sec31A. These findings also indicate that luminal calcium might play a role in regulation of COPII vesicle trafficking [336, 337, 338, 339].

The protein Huntingtin, which is mutated in Huntington's disease, was previously shown to be involved in the regulation of various membrane trafficking processes. A recent study showed that Huntingtin is required for efficient ER-to-Golgi trafficking of a fluorescent reporter, but the role of action or a relation to COPII machinery components was not investigated [340].

In addition to regulatory proteins, lipid composition of the membrane was suggested to have a regulatory role in ERES formation [20]. Blumental-Perry et al. showed that

formation of ERES required the presence of the lipid PI4-phosphate in the ER membrane [334], which may be generated by PI4-kinase IIIα (PI4K-IIIα) at the ER [323, 341]. The role of lipids in the regulation of ERES function was supported by Farhan et al., who showed that loss of PI4K-IIIα caused a decrease in ERES number, and that PI4K-IIIα is required for the response of ERES to an acute increase in cargo load [323]. In addition, Shindiapina et al. showed that depletion of phosphatidylinositol inhibited COPII vesicle budding in yeast, whereas ERES remained intact [186]. Phospholipase D (PLD) is another lipid modifying enzyme which was shown to be required for ER export [325]. PLD increases the amount of acidic lipids in the membrane by catalyzing the formation of phosphatic acid [342]. PLD activity was shown to be initiated by Sar1, and this activity was required for recruitment of COPII components and ER export [325]. Diacylglyerol kinase (DGK) is another protein required for the formation of phosphatic acid, as it phosphorylates diacylglycerol [343]. The isoform DGKδ was shown to associate to the ER via its C-terminal SAM domain, which mediates protein oligomerization. Interestingly, overexpression of DGKδ inhibited COPII assembly and ER-to-Golgi trafficking, which was not mediated by the kinase domain of DGKδ, indicating that DGKδ may have an additional scaffolding function [344]. The previously described p125A shares SAM domain with DGK and was shown to regulate ERES, a similar mechanism may be found for DGKδ [326]. Therefore, lipid composition of the ER membrane as well as regulatory functions of lipid-modifying enzymes seem to be an important factor in the regulation of ER export.

Taken together, these individual findings show that ERES respond to regulation by proteins and lipids, and that ER export is a complex and tightly regulated process, which we are only beginning to understand.

4 Post-ERES trafficking in the early secretory pathway

Export of sorted proteins from the ER in COPII-coated vesicles at ERES is only the first step in the secretory pathway. Proteins are further sorted, modified and transported in the early secretory pathway. Post-ERES trafficking between the organelles of the early secretory pathway takes place not in COPII, but in COPI-coated vesicles. This transport machinery, as well as the ERGIC and Golgi as components of the early secretory pathway will be discussed below.

4.1 COPI coat

The process of COPI-mediated vesicle transport is generally similar to COPII-mediated vesicle transport. However, the components have more isoforms than COPII vesicle components. Usage of different isoforms is believed to regulate directionality of COPI-mediated vesicle transport between different organelles. These routes include retrograde trafficking from the ERGIC back to the ER, as well as retrograde trafficking from the *cis*-Golgi back to the ER. Anterograde trafficking from the ERGIC to the *cis*-Golgi also requires COPI function. Additionally, transport of vesicles between the different Golgi compartments as well as within the ERGIC is COPI-dependent. Specificity of transport is acquired via the specialization and localization of different isoforms ; therefore, much more components are involved in COPI-mediated vesicle trafficking compared to COPII [184, 345, 346].

The COPI coat consists of coatomers, which are 600 - 800 kDa large heptameric complexes (α/ β/ β'/ ε/ γ/ δ/ ζ) that are, as opposed to COPII, recruited *en bloc*, as pre-formed complexes from the cytosol [347]. Whilst the COPII coat and the Clathrin coat form coats of regularly formed lattice-like structures with a clearly defined inner and outer coat, this is not the case for the COPI which is more heterogenous. However, two subcomplexes can still be defined. The trimeric or B-complex consists of the α-, β'- and ε-subunits and, since it forms a cage-like complex, structurally resembles the outer COPII coat formed by Sec13/Sec31. Interestingly, this outer coat-like B-subcomplex is responsible for cargo binding, as opposed to the inner coat in COPII [346, 348, 349, 350]. The second subcomplex is the tetrameric or F- subcomplex which consists of the γ-, δ-, ζ- and β-subunits and functions as an adaptor subcomplex.

The procedure of COPI-vesicle formation is comparable to COPII-vesicle formation. As opposed to Sar1A or B in COPII vesicle formation, the GTPase involved in COPI

vesicle formation is Arf1. Arf1 belongs to the family of GTPases which in mammalian cells has 5 to 6 members. Of this group, only Arf1 was shown to be required for COPI vesicle formation, although Arf4 and Arf5 may have a similar function. In contrast to Sar1, Arf1 has an additional function in sorting cargo into COPI vesicles and directly interacts with cargo proteins [351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363]. During initiation of COPI vesicle formation, the Arf1 GTPase is activated by an Arf GEF, which was shown to be Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1), which, despite its name, colocalizes with COPI and both the ERGIC and the Golgi and was shown to activate Arf1, Arf4 and Arf5. As Sec12 does for Sar1, the Arf-GEF activates cytosolic GDP-bound Arf1, which causes a conformational change exposing an amphipathic N-terminal helix that is required for Arf1 association with the membrane. After being recruited to the membrane, Arf1 recruits the COPI coatomer complexes and directly interacts with the β-, β'-, γ-, ε-, and δ-subunits [364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376]. In addition, Arf-GAPs play an important role in the final stages of COPI vesicle formation. They directly interact with the γ- and β'-subunits of the COPI coat and activate Arf GTPase function, thereby inducing vesicle scission and uncoating. Additionally, the Arf-GAPs may have an additional role in cargo recognition as they directly interact with the cytoplasmic tails of cargo proteins, This is in contrast to the COPII machinery, whereby these two functions are distributed among Sec23 and Sec24. Binding of both coat and cargo activates the GAP activity which induces GTP-hydrolysis in Arfs, and dissociation of GDP-bound Arf from the membrane. It is not known whether the Arf-GAP remains associated with the COPI-coat, as it is not a structural component of the coat as Sec23 is in the COPII coat [355, 369, 377, 378, 379, 380, 381].

As mentioned above, the components of the COPI machinery each have multiple isoforms, that by differences in localization and affinity to other COPI-components ensure specificity in vesicle transport. In the case of Arf, there are six mammalian isoforms that are divided into three classes, whereby Class I (Arf1 and Arf3) and Class II (Arf4 and Arf5) are localized to the secretory pathway. It is believed that the different Arfs localize to different locations ; Arf1 appears to be generally distributed whilst Arf4 was found to localize to the ERGIC [355, 368, 382, 383]. Of the COPI subunits, the γ- and the ζ-subunits were found to have two distict isoforms each (γ1 and γ2 ; ζ1 and ζ2), that show differences in subcellular localization. COPI coats containing the γ1- and the ζ2-subunits are enriched at the *cis*-Golgi, whereas the γ2-subunit is enriched at the *trans*-Golgi [345, 384, 385]. The mode of anterograde protein transport to the Golgi and within the Golgi is still debated, as it is unclear whether protein transport takes place in COPI coated vesicles. It was shown however that different components of the COPI coat

machinery are required for efficient anterograde trafficking [363, 379, 386, 387, 388, 389]. Therefore, as opposed to the COPII coat, which is responsible solely for anterograde transport from ERES to the ERGIC and cis-Golgi, COPI-coated vesicles might transport cargo in multiple directions and towards multiple organelles. However, according to the cisternal maturation model described below, protein transport within the Golgi may be independent of vesicular transport. Specificity of COPI-mediated vesicular transport is regulated via use of different GTPases, as the Arf GTPase family involved in COPI vesicle formation contains six different isoforms as opposed to only two Sar1 isoforms in mammalian cells. Additionally, as opposed to COPII-coated vesicles, COPI-coated vesicles are transported along microtubules between the organelles, which might help to ensure directionality of vesicle transport [17, 345, 390, 391, 392, 393, 394, 395].

4.2 ERGIC

The ER-Golgi intermediate compartment (ERGIC), also known as vesicular-tubular clusters (VTC) or sometimes Intermediate Compartment (IC) or tubovesicular complexes (TCs), is an organelle that developed later in evolution as it is only present in mammalian cells, not in yeast or other model organisms. In addition, it was identified much later than other organelles and its function is still not completely resolved. It is clear however that the ERGIC is an independent structure, which is not connected with or a domain of either the ER or the *cis*-Golgi. An older model for ERGIC structure, which is now deemed unlikely, is called the transport complex (TC) model, which is based on older studies using transport of the artificial temperature-sensitive viral GFP-fusion protein Vesicular Stomatitis Virus G (tsO45-VSV-G-GFP) as read-out. In the TC model, the ERGIC clusters are described as transient cargo containers that either fuse with or form the *cis*-Golgi [396, 397, 398]. Identification of ERGIC-53 (also known as LMAN1), a 53 kDa transmembrane type I protein and cargo receptor as marker for the ERGIC compartment led to a new, alternative model that is more consistent with observations from other studies concerning ER-Golgi trafficking, as well as observations from life-cell imaging [390]. Now, the ERGIC is seen as a stable and long-lived compartment where proteins are further sorted for anterograde or retrograde trafficking after leaving the ER. It retains its structure by a continous flux of incoming membrane and material from the ER and an outgoing flux of vesicles that the ERGIC sends to the ER or the Golgi [17, 150, 151, 390, 399]. Additional functions for the ERGIC or IC have been proposed by Saraste et

al, based on observations from studies using an additional ERGIC marker, the GTPase Rab1A. While ERGIC-53 is a transmembrane protein that shuttles between the ER, ERGIC and *cis*-Golgi, Rab1A is recruited to membranes and remains associated with ERGIC-membranes upon BFA treatment, strengthening the view of the ERGIC as a compartment that is independent from the ER or the Golgi. Furthermore, studies using Rab1A indicate a connection between the ERGIC and the endocytic pathway [399, 400, 401, 402].

The ERGIC was suggested to be formed by homotypic fusion of ER-derived COPII-coated vesicles. This process is tightly regulated to ensure directional transport and involves the sequential binding of effectors to Sec23 in order to direct the COPII-vesicles to the ERGIC or *cis*-Golgi. In addition, back-fusion of COPII vesicles may be prevented by ER-resident proteins such as the yeast protein Tip20 [403]. Much research in COPII-vesicle tethering has been performed in *S.cerevisiae* which lacks an intermediate compartment, however, the mammalian homologues of proteins involved in this process which are located at the *cis*-Golgi in *S.cerevisiae* are located at the ERGIC in mammalian cells. COPII vesicle tethering is initiated at ERES by the TRAPPI complex which includes binding of the TRAPPI subunit Bet3 to Sec23 after Sar1 GTP hydrolysis is complete. Next, the small GTPase Rab1 (Ypt1p in *S.cerevisiae*) is activated by the TRAPPI complex and the log, coiled-coil membrane-tethering factor p115 (Uso1p in *S.cerevisiae*) is recruited.

P115/Uso1p also recognizes the yeast kinase Hrr25p. In *S.cerevisiae*, Hrr25p is located at the *cis*-Golgi where it initiates vesicle uncoating and fusion, possibly by displacing TRAPPI and phosphorylating the COPII coat [227, 404, 405, 406, 407, 408]. The mammalian orthologue of Hrr25p was identified to be Casein kinase I delta (CKIδ), which has a conserved and essential role in the circadian clock. CKIδ may act at the ERGIC where it is required for the binding of Arf GAP1 to membranes. Consequently, loss of CKIδ function, either by pharmacological inhibition or overexpression of a dominant negative mutant, was shown to inhibit ER to Golgi trafficking [227, 390, 409, 410, 411, 412]. Vesicle fusion with the acceptor membrane not only delivers membrane material for the ERGIC but also the vesicle cargo which contains a wide variety of different proteins, such as proteins destined for the Golgi and beyond, ERGIC-resident proteins but also ER-resident proteins such as chaperones and cargo receptors that were transported to the ERGIC. These proteins are sorted in the ERGIC and transported back to the ER (retrograde transport) or further on to the Golgi (anterograde transport). In order for protein sorting to take place, cargo proteins destined for the Golgi must dissociate from their cargo receptors which have to be transported back to the ER. This

dissociation has been suggested to be induced by differences in pH and calcium levels. The ER lumen is a neutral environment with a pH of 7.4, whereas the pH in the ERGIC is believed to be more acidic; this change in pH might affect the binding affinities between cargo receptors and their substrates [391, 413, 414, 415]. In addition, while calcium levels were found to be high in both the ER and the Golgi, calcium could not be detected in the ERGIC, indicating lower calcium levels there [415, 416, 417]. The binding efficiency of the cargo receptor ERGIC-53 was shown to be more sensitive to changes in pH levels in an environment with low calcium levels. It is therefore suggested that the combination of low calcium levels and lower pH might induce protein dissociation [17, 399, 413, 414, 415, 416]. A lower pH level in the ERGIC also assists with protein sorting, as the affinity of the KDEL receptors for substrates bearing a KDEL-sequence is increased in low pH environment. The KDEL receptors localize to both the ERGIC and *cis*-Golgi and are responsible for returning ER-resident proteins bearing a KDEL-sequence back to the ER. Binding of KDEL ligands to the KDEL receptor is also required to stimulate retrograde transport of receptor and substrate back to ER in COPI-coated vesicles. Another common ER-retrieval signal is the evolutionary conserved di-lysine motif; ER-resident proteins bearing this di-lysine motif can directly interact with COPI [346, 391, 418, 419, 420, 421, 422, 423, 424].

The selection of cargo for anterograde transport from the ERGIC to the *cis*-Golgi is less well researched; proteins might be packaged into COPI vesicles destined for the Golgi by default and specifically retained or selected only for retrograde transport [147]. However, the identification of an ERGIC-specific hydrophobic export signal in the GABA transporter 1 (GAT1) indicates that export from the ERGIC might also be more tightly regulated via specific export signals [425].

4.3 Golgi

The mammalian Golgi stack consists of several cisternae, with the *cis*-Golgi side facing the ER and ERGIC, and the *trans*-side facing the Trans Golgi network (TGN) and the plasma membrane. Although the cisternae differ from each other, for example in terms of protein content, a unifying principle on Golgi compartmentation does not exist [18, 19, 36, 345, 426].

A recent model aims to incorporate differences in protein content, function, structure and trafficking to divide the Golgi roughly into three functional compartments [426, 427].

Generally, post-translational modification of proteins, such as processing of N-linked oligosaccharides, is a multi-step process involving several different enzymes. One advantage of spatially separated compartments is that potentially competing enzyme reactions can be spatially separated and thereby regulated optimally ; furthermore, separation allows to create optimal environments for different enzymes for example by adjusting pH, ion composition or substrate concentrations. In addition to protein content, Papanikou and Glick as well as Day et al. propose to include the ability of cisternae to receive cargo into the model of Golgi compartmentation. The first stage, called Cisternal Assembly, includes the ERGIC and the *cis*-Golgi, as at this stage the Golgi is assembled and COPII- and COPI-coated vesicles can fuse with the membrane; furthermore, early acting glycosylation enzymes are concentrated in the *cis*-Golgi which are mostly absent in later cisternae. The second stage, called Carbohydrate Synthesis, encompasses the *medial*- and *trans*-Golgi, where the cisternae contain different sets of glycosylation enzymes and glycosylate proteins and lipids, but also synthesize complex polysaccharide. The cisternae in this stage can receive COPI-coated vesicles of a different type than the *cis*-Golgi, and therefore not from the ERGIC. The final stage, or Carrier Formation stage, includes the TGN but also certain cisternae from the *trans*-Golgi, if they are able to package cargo into various types of transport carriers en route to post-Golgi targets. Some processing of secretory proteins can still take place in this third stage, but its main function is seen as packaging of cargo for onward transport. The membranes in this stage are not competent to receive COPI-coated vesicles [394, 426, 427, 428, 429, 430].

Vesicle transport to and within the Golgi is a tightly regulated process. The Golgi receives COPI-coated vesicles from the ERGIC at the *cis*-Golgi, where vesicles fuse with the *cis*-Golgi membrane in a manner that is believed to be mostly similar to vesicle tethering at the ERGIC. The same process of vesicle budding and tethering is used for transport between the different Golgi cisternae which also takes place in COPI-coated vesicles, although different ARFs and associated GEFs and GAPs as well as tethering proteins are used. At the *cis*- and *medial*-Golgi the tethering protein p115 is a likely candidate, as it localizes to these compartments. For fusion of COPI vesicles at the *cis*-Golgi, in addition to p115 the tethers GM130 and GRASP65 are involved, as well as the GTPase Rab1. In intra-Golgi vesicle transport, Rab1 works together with tethers localized exclusively at the Golgi, such as Golgin84, CASP and COG. In anterograde intra-Golgi trafficking, the GTPase Rab2 was found to interact with the tether GRASP55, which is localized at the *medial*-Golgi, as well as with the tethers Golgin45 and GM130 [431].

Although components of the intracellular trafficking machinery have been identified, it is unclear how proteins move through the Golgi, and how cisternae keep their identity. Two models offer an explanation; in the anterograde vesicular transport model, cargo proteins are transported through the Golgi from one cisternae to the next in COPI-coated vesicles, whereas the Golgi-resident enzymes remain in their cisternae. In the cisternal maturation model, which is regarded as more likely, the *cis*-Golgi is formed by fusion of vesicles derived from the ERGIC, and the cisternae mature from *cis*- to *trans*-Golgi by receiving the appropriate Golgi resident enzymes via retrograde transport from the previous cisternae, whereas the cargo proteins remain in the cisternae and travel together with their membrane compartment. This latter model accomodates the fact that large cargos such as pro-collagen do not fit into COPI-coated vesicles, and in the cisternal maturation model, this cargo would not need to be transported [394, 395].

After secretory proteins have received their appropriate post-translational modifations, they are exported from the TGN in Clathrin-coated vesicles to their further destinations [430].

5 References

1. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N*, et al.* Global analysis of protein expression in yeast. *Nature* 2003, **425**(6959)**:** 737-741.
2. Kanapin A, Batalov S, Davis MJ, Gough J, Grimond S, Kawaji H*, et al.* Mouse proteome analysis. *Genome Res* 2003, **13:** 1335-1344.
3. Dancourt J, Barlowe C. Protein sorting receptors in the early secretory pathway. *Annu Rev Biochem* 2010, **79:** 777-802.
4. Bauman O, Walz B. Endoplasmic reticulum of animal cells and its organization into structural and functional domains. *Int Rev Cytol* 2001, **205:** 149-214.
5. Orci L, Ravazzola M, Meda P, Holocomb C, Moore HP, Hicke L*, et al.* Mammalian Sec23p homologue is restricted to the endoplasmic reticulum transitional cytoplasm. *Proc Natl Acad Sci U S A* 1991, **88**(19)**:** 8611-8615.
6. Westrate LM, Lee JE, Prinz WA, Voeltz GK. Form Follows Function: The Importance of Endoplasmic Reticulum Shape. *Annu Rev Biochem* 2015, **84**.
7. Chen S, Novick P, Ferro-Novick S. ER structure and function. *Curr Opin Cell Biol* 2013, **25**(4)**:** 428-433.
8. D'Arcangelo JG, Stahmer KR, Miller EA. Vesicle-mediated export form the ER: COPII coat function and regulation. *Biochim Biophys Acta* 2013, **1833:** 2464-2472.
9. Venditti R, Wilson C, De Matteis MA. Exiting the ER: what we know and what we don't. *Trends Cell Biol* 2014, **24**(1)**:** 9-18.
10. Zeuschner D, Geerts WJ, van Donselaar E, Humbel BM, Slot JW, Koster AJ*, et al.* Immuno-electron tomography of ER exit sites reveals the existence of free COPII-coated transport carriers. *Nat Cell Biol* 2006, **8**(4)**:** 377-383.
11. Bannykh SI, Rowe T, Balch WE. The organization of endoplasmic reticulum export complexes. *J Cell Biol* 1996, **135**(1)**:** 19-35.
12. Stephens DJ. De novo formation, fusion and fission of mammalian COPII-coated endoplasmic reticulum exit sites. *EMBO Rep* 2003, **4**(2)**:** 210-217.
13. Ivan V, de Voer G, Xanthakis D, Spoorendonk KM, Kondylis V, Rabouille C. Drosophila Sec16 mediates the biogenesis of tER sites upstream of Sar1 through an arginine-rich motif. *Mol Biol Cell* 2008, **19**(10)**:** 4352-4365.
14. Hughes H, Budnik A, Schmidt K, Palmer KJ, Mantell J, Noakes C*, et al.* Organisation of human ER-exit sites: requirements for the localisation of Sec16 to transitional ER. *J Cell Sci* 2009, **122**(Pt 16)**:** 2924-2934.
15. Hammond AT, Glick BS. Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. *Mol Biol Cell* 2000, **11**(9)**:** 3013-3030.
16. Hauri HP, Kappeler F, Andersson H, Appenzeller C. ERGIC-53 and traffic in the secretory pathway. *J Cell Sci* 2000, **113:** 587-596.
17. Appenzeller-Herzog C, Hauri HP. The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. *J Cell Sci* 2006, **119:** 2173-2183.
18. Jackson CL. Mechanisms of transport through the Golgi complex. *J Cell Sci* 2009, **122:** 443-452.
19. Bonifacino JS, Glick BS. The mechanisms of vesicle budding and fusion. *Cell* 2004, **116**(2)**:** 153-166.
20. Farhan H, Rabouille C. Signalling to and from the secretory pathway. *J Cell Sci* 2011, **124:** 171-180.
21. Zanetti G, Pahuja KB, Studer S, Shim S, Schekman R. COPII and the regulation of protein sorting in mammals. *Nat Cell Biol* 2012, **14**(1)**:** 20-28.
22. Wiest DL, Burkhardt JK, Hester S, Hortsch M, Meyer DI, Argon Y. Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately. *J Cell Biol* 1990, **110**(5)**:** 1501-1511.
23. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, Zhao H*, et al.* XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* 2004, **21**(1)**:** 81-93.
24. Brewer JW, Hendershot LM. Building an antibody factory: a job for the unfolded protein response. *Nat Immunol* 2005, **6**(1)**:** 23-29.
25. Shohat M, Janossy G, Dourmashkin RR. Development of rough endoplasmic reticulum in mouse splenic lymphocytes stimulated by mitogens. *Eur J Immunol* 1973, **3**(11)**:** 680-687.

26. Cui-Wang T, Hanus C, Helton T, Bourne J, Watson D, Harris KM*, et al.* Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. *Cell* 2012, **148:** 309-321.
27. Hanus C, Ehlers MD. Secretory outposts for the local processing of membrane cargo in neuronal dendrites. *Traffic* 2008, **9**(9)**:** 1437-1445.
28. Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J Neurosci* 2002, **22**(6)**:** 2215-2224.
29. Spacek J, Harris KM. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J Neurosci* 1997, **17**(1)**:** 190-203.
30. Horton AC, Ehlers MD. Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. *J Neurosci* 2003, **23**(15)**:** 6188-6199.
31. Delic M, Valli M, Graf AB, Pfeffer M, Mattanovich D, Gasser B. The secretory pathway: exploring yeast diversity. *FEMS Mi robiol Rev* 2013, **37**(6)**:** 872-914.
32. Castillon GA, Watanabe R, Taylor M, Schwabe TM, Riezman H. Concentration of GPI-anchored proteins upon ER exit in yeast. *Traffic* 2009, **10**(2)**:** 186-200.
33. Rossanese OW, Soderholm J, Bevis BJ, Sears IB, O'Connor J, Williamson EK*, et al.* Golgi structure correlates with transitional endoplasmic reticulum organization in Pichia pastoris and Saccharomyces cerevisiae. *J Cell Biol* 1999, **145**(1)**:** 69-81.
34. Yorimitsu T, Sato K. Insights into structural and regulatory roles of Sec16 in COPII vesicle formation at ER exit sites. *Mol Biol Cell* 2012, **23**(15)**:** 2930-2942.
35. Suda Y, Nakano A. The Yeast Golgi Apparatus. *Traffic* 2012, **13**(4)**:** 505-510.
36. Lowe M. Structural organization of the Golgi apparatus. *Curr Opin Cell Biol* 2011, **23**(1)**:** 85-93.
37. Shorter J, Warren G. Golgi architecture and inheritance. *Annu Rev Cell Dev Biol* 2002, **18:** 379-420.
38. Rambourg A, Clermont Y, Ovtracht L, Képès F. Three-dimensional structure of tubular networks, presumably Golgi in nature, in various yeast strains: a comparative study. *Anat Rec* 1995, **243**(3)**:** 283-293.
39. Mogelsvang S, Gomez-Ospina N, Soderholm J, Glick BS, Staehelin LA. Tomographic evidence for continuous turnover of Golgi cisternae in Pichia pastoris. *Mol Biol Cell* 2003, **14**(6)**:** 2277-2291.
40. Gould SJ, McCollum D, Spong AP, Heyman JA, Subramani S. Development of the yeast Pichia pastoris as a model organism for a genetic and molecular analysis of peroxisome assembly. *Yeast* 1992, **8**(8)**:** 613-628.
41. Vjestica A, Tang XZ, Oliferenko S. The actomyosin ring recruits early secretory compartments to the division site in fission yeast. *Mol Biol Cell* 2008, **19**(3)**:** 1125-1138.
42. Preuss D, Mulholland J, Franzusoff A, Segev N, Botstein D. Characterization of the Saccharomyces Golgi complex through the cell cycle by immunoelectron microscopy. *Mol Biol Cell* 1992, **3**(7)**:** 789-803.
43. daSilva LL, Snapp EL, Denecke J, Lipponcott-Schwartz J, Hawes C, Brandizzi F. Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. *Plant Cell* 2004, **16**(7)**:** 1753-1771.
44. Hanton SL, Bartolotti LE, Renna L, Stefano G, Brandizzi F. Crossing the divide--transport between the endoplasmic reticulum and Golgi apparatus in plants. *Traffic* 2005, **6**(267-77).
45. Nebenführ A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, Meehl JB*, et al.* Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. *Plant Physiol* 1999, **121**(4)**:** 1127-1142.
46. Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C. Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *Plant J* 1998, **15**(3)**:** 441-447.
47. Paris N, Stanley CM, Jones RL, Rogers JC. Plant cells contain two functionally distinct vacuolar compartments. *Cell* 1996, **85**(4)**:** 563-572.
48. Xiang L, Etxeberria E, Van den Ende W. Vacuolar protein sorting mechanisms in plants. *FEBS J* 2013, **280**(4)**:** 979-993.
49. Takagi J, Renna L, Takahashi H, Koumoto Y, Tamura KS, G., Fukao Y*, et al.* MAIGO5 functions in protein export from Golgi-associated endoplasmic reticulum exit sites in Arabidopsis. *Plant Cell* 2013, **25**(11)**:** 4658-4675.

50. Kondylis V, Rabouille C. A novel role for dp115 in the organization of tER sites in Drosophila. *J Cell Biol* 2003, **162**(2)**:** 185-198.
51. Kondylis V, Rabouille C. The Golgi apparatus: lessons from Drosophila. *FEBS Lett* 2009, **283**(23)**:** 3827-3838.
52. Kondylis V, van Nispen tot Pannerden HE, Herpers B, Friggi-Grelin F, Rabouille C. The golgi comprises a paired stack that is separated at G2 by modulation of the actin cytoskeleton through Abi and Scar/WAVE. *Dev Cell* 2007, **12**(6)**:** 901-915.
53. Kondylis V, Tang Y, Fuchs F, Boutros M, Rabouille C. Identification of ER proteins involved in the functional organisation of the early secretory pathway in Drosophila cells by a targeted RNAi screen. *PLoS One* 2011, **6**(2)**:** e17173.
54. Becker B, Melkonian M. The secretory pathway of protists: spatial and functional organization and evolution. *Microbiological reviews* 1996, **60**(4)**:** 697-721.
55. Drakakaki G, Dandekar A. Protein secretion: How many secretory routes does a plant cell have? *Plant Sci* 2013, **203:** 74-78.
56. Schlacht A, Dacks JB. Unexpected ancient paralogues and an evolutionary model for the COPII coat complex. *Genome Biol Evol* 2015.
57. Hebert DN, Molinari M. In and Out of the ER: Protein Folding, Quality Control, Degradation, and Related Human Diseases. *Physiol Rev* 2007, **87**(4)**:** 1377-1408.
58. Zimmermann R, Eyrisch S, Ahmad M, Helms V. Protein translocation across the ER membrane. *Biochim Biophys Acta* 2011, **1808**(3)**:** 912-924.
59. von Heijne G. Patterns of amino acids near signal-sequence cleavage sites. *Eur J Biochem* 1983, **133**(1)**:** 17-21.
60. von Heijne G. Analysis of the distribution of charged residues in the N-terminal region of signal sequences: implications for protein export in prokaryotic and eukaryotic cells. *EMBO J* 1984, **3**(10)**:** 2315-2318.
61. Von Heijne G. Signal sequences. The limits of variation. *J Mol Biol* 1985, **184**(1)**:** 99-105.
62. Von Heijne G. Towards a comparative anatomy of N-terminal topogenic protein sequences. *J Mol Biol* 1986, **189**(1)**:** 239-242.
63. Martoglio B, Dobberstein B. Signal sequences: more than just greasy peptides. *Trends Cell Biol* 1998, **8**(10)**:** 410-415.
64. Dudek J, Pfeffer S, Lee PH, Jung M, Cavalié A, Helms V*, et al.* Protein Transport into the Human Endoplasmic Reticulum. *J Mol Biol* 2015, **427:** 1159-1175.
65. Görlich D, Rapoport TA. Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell* 1993, **75**(4)**:** 615-630.
66. Haigh NG, Johnson AE. A new role for BiP: closing the aqueous translocon pore during protein integration into the ER membrane. *J Cell Biol* 2002, **156**(2)**:** 261-270.
67. Matlack KE, Misselwitz B, Plath K, Rapoport TA. BiP acts as a molecular ratchet during posttranslational transport of prepro-alpha factor across the ER membrane. *Cell* 1999, **97**(5)**:** 553-564.
68. Lyman SK, Schekman R. Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of Saccharomyces cerevisiae. *J Cell Biol* 1995, **131**(5)**:** 1163-1171.
69. Alder NN, Shen Y, Brodsky JL, Hendershot LM, Johnson AE. The molecular mechanisms underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum. *J Cell Biol* 2005, **168**(3)**:** 389-399.
70. Hamman BD, Hendershot LM, Johnson AE. BiP maintains the permeability barrier of the ER membrane by sealing the lumenal end of the translocon pore before and early in translocation. *Cell* 1998, **92**(6)**:** 747-758.
71. Görlich D, Prehn S, Hartmann E, Kalies KU, Rapoport TA. A mammalian homolog of SEC61p and CYp is associated with ribosomes and nascent polypeptides during translocation. *Cell* 1992, **71**(3)**:** 489-503.
72. Tyedmers J, Lerner M, Wiedmann M, Volkmer J, Zimmermann R. Polypeptide-binding proteins mediate completion of co-translational protein translocation into the mammalian endoplasmic reticulum. *EMBO Rep* 2003, **4**(5)**:** 505-510.
73. Deshaies RJ, Koch BD, Werner-Washburne M, Craig EA, Schekman R. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature* 1988, **332**(6167)**:** 800-805.

74. Brodsky JL, Goeckeler J, Schekman R. BiP and Sec63p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum. *Proc Natl Acad Sci U S A* 1995, **92:** 9643-9646.
75. Neuhof A, Rolls MM, Jungnickel B, Kalies KU, Rapoport TA. Binding of signal recognition particle gives ribosome/nascent chain complexes a competitive advantage in endoplasmic reticulum membrane interaction. *Mol Biol Cell* 1998, **9**(1)**:** 103-115.
76. Halic M, Blau M, Becker T, Mielke T, Pool MR, Wild K*, et al.* Following the signal sequence from ribosomal tunnel exit to signal recognition particle. *Nature* 2006, **444**(7118)**:** 507-511.
77. Ast T, Cohen G, Schuldiner M. A Network of Cytosolic Factors Targets SRP-Independent Proteins to the Endoplasmic Reticulum. *Cell* 2013, **152**(5)**:** 1134-1145.
78. Chirico WJ, Waters MG, Blobel G. 70K heat shock related proteins stimulate protein translocation into microsomes. *Nature* 1988, **332:** 805-810.
79. Grove DE, Fan CY, Ren HY, Cyr DM. The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508. *Mol Biol Cell* 2011, **22**(3)**:** 301-314.
80. Halperin L, Jung J, Michalak M. The many functions of the endoplasmic reticulum chaperones and folding enzymes. *IUBMB Life* 2014, **66**(5)**:** 318-326.
81. Schrag JD, Bergeron JJ, Li Y, Borisova S, Hahn M, Thomas DY*, et al.* The Structure of calnexin, an ER chaperone involved in quality control of protein folding. *Mol Cell* 2001, **8**(3)**:** 633-644.
82. Ou WJ, Cameron PH, Thomas DY, Bergeron JJ. Association of folding intermediates of glycoproteins with calnexin during protein maturation. *Nature* 1993, **364**(6440)**:** 771-776.
83. Otteken A, Moss B. Calreticulin interacts with newly synthesized human immunodeficiency virus type 1 envelope glycoprotein, suggesting a chaperone function similar to that of calnexin. *J Biol Chem* 1996, **271**(1)**:** 97-103.
84. Peterson JR, Ora A, Van PN, Helenius A. Transient, lectin-like association of calreticulin with folding intermediates of cellular and viral glycoproteins. *Mol Biol Cell* 1995, **6**(9)**:** 1173-1184.
85. Kapoor M, Srinivas H, Kandiah E, Gemma E, Ellgaard L, Oscarson S*, et al.* Interactions of substrate with calreticulin, an endoplasmic reticulum chaperone. *J Biol Chem* 2003, **278**(8)**:** 6194-6200.
86. Michalak M, Groenendyk J, Szabo E, Gold LI, Opas M. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *Biochem J* 2009, **417**(3)**:** 651-666.
87. Caramelo JJ, Parodi AJ. Getting in and out from calnexin/calreticulin cycles. *J Biol Chem* 2008, **283**(16)**:** 10221-10225.
88. Aebi M, Bernasconi R, Clerc S, Molinari M. N-glycan structures: recognition and processing in the ER. *Trends Biochem Sci* 2010, **35**(2)**:** 74-82.
89. Kozlov G, Pocanschi CL, Rosenauer A, Bastos-Aristizabal S, Gorelik A, Williams DB*, et al.* Structural basis of carbohydrate recognition by calreticulin. *J Biol Chem* 2010, **285**(49)**:** 38612-38620.
90. Hammond C, Braakman I, Helenius A. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. *Proc Natl Acad Sci U S A* 1994, **91**(3)**:** 913-917.
91. Swanton E, High S, Woodman P. Role of calnexin in the glycan-independent quality control of proteolipid protein. *The EMBO journal* 2003, **22**(12)**:** 2948-2958.
92. Hebert DN, Foellmer B, Helenius A. Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. *Cell* 1995, **81**(3)**:** 425-433.
93. Danilczyk UG, Williams DB. The lectin chaperone calnexin utilizes polypeptide-based interactions to associate with many of its substrates in vivo. *J Biol Chem* 2001, **276**(27)**:** 25532-25540.
94. Williams DB. Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. *J Cell Sci* 2006, **119**(Pt 4)**:** 615-623.
95. Ware FE, Vassilakos A, Peterson PA, Jackson MR, Lehrman MA, Williams DB. The molecular chaperone calnexin binds Glc1Man9GlcNAc2 oligosaccharide as an initial step in recognizing unfolded glycoproteins. *J Biol Chem* 1995, **270**(9)**:** 4697-4704.

96. Avezov E, Frenkel Z, Ehrlich M, Herscovics A, Lederkremer GZ. Endoplasmic reticulum (ER) mannosidase I is compartmentalized and required for N-glycan trimming to Man5-6GlcNAc2 in glycoprotein ER-associated degradation. *Mol Biol Cell* 2008, **19**(1)**:** 216-225.
97. Ihara Y, Cohen-Doyle MF, Saito Y, Williams DB. Calnexin discriminates between protein conformational states and functions as a molecular chaperone in vitro. *Mol Cell* 1999, **4**(3)**:** 331-341.
98. Stronge VS, Saito Y, Ihara Y, Williams DB. Relationship between calnexin and BiP in suppressing aggregation and promoting refolding of protein and glycoprotein substrates. *J Biol Chem* 2001, **276**(43)**:** 39779-39787.
99. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem* 2004, **73:** 1019-1049.
100. Guerin M, Parodi AJ. The UDP-glucose:glycoprotein glucosyltransferase is organized in at least two tightly bound domains from yeast to mammals. *J Biol Chem* 2003, **278**(23)**:** 20540-20546.
101. Ritter C, Helenius A. Recognition of local glycoprotein misfolding by the ER folding sensor UDP-glucose:glycoprotein glucosyltransferase. *Nature structural biology* 2000, **7**(4)**:** 278-280.
102. Ritter C, Quirin K, Kowarik M, Helenius A. Minor folding defects trigger local modification of glycoproteins by the ER folding sensor GT. *The EMBO journal* 2005, **24**(9)**:** 1730-1738.
103. Sousa M, Parodi AJ. The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. *The EMBO journal* 1995, **14**(17)**:** 4196-4203.
104. Blond-Elguindi S, Cwirla SE, Dower WJ, Lipshutz RJ, Sprang SR, Sambrook JF*, et al.* Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. *Cell* 1993, **75**(4)**:** 717-728.
105. Chevalier M, Rhee H, Elguindi EC, Blond SY. Interaction of murine BiP/GRP78 with the DnaJ homologue MTJ1. *J Biol Chem* 2000, **275**(26)**:** 19620-19627.
106. Chung KT, Shen Y, Hendershot LM. BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. *J Biol Chem* 2002, **277**(49)**:** 47557-47563.
107. Shen Y, Meunier L, Hendershot LM. Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. *J Biol Chem* 2002, **277**(18)**:** 15947-15956.
108. Kabani M, Kelley SS, Morrow MW, Montgomery DL, Sivendran R, Rose MD*, et al.* Dependence of endoplasmic reticulum-associated degradation on the peptide binding domain and concentration of BiP. *Mol Biol Cell* 2003, **14**(8)**:** 3437-3448.
109. Sorgjerd K, Ghafouri B, Jonsson BH, Kelly JW, Blond SY, Hammarstrom P. Retention of misfolded mutant transthyretin by the chaperone BiP/GRP78 mitigates amyloidogenesis. *J Mol Biol* 2006, **356**(2)**:** 469-482.
110. Shen Y, Hendershot LM. ERdj3, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates. *Mol Biol Cell* 2005, **16**(1)**:** 40-50.
111. Awad W, Estrada I, Shen Y, Hendershot LM. BiP mutants that are unable to interact with endoplasmic reticulum DnaJ proteins provide insights into interdomain interactions in BiP. *Proc Natl Acad Sci U S A* 2008, **105**(4)**:** 1164-1169.
112. Chien V, Aitken JF, Zhang S, Buchanan CM, Hickey A, Brittain T*, et al.* The chaperone proteins HSP70, HSP40/DnaJ and GRP78/BiP suppress misfolding and formation of beta-sheet-containing aggregates by human amylin: a potential role for defective chaperone biology in Type 2 diabetes. *Biochem J* 2010, **432**(1)**:** 113-121.
113. Melnick J, Dul JL, Argon Y. Sequential interaction of the chaperones BiP and GRP94 with immunoglobulin chains in the endoplasmic reticulum. *Nature* 1994, **370**(6488)**:** 373-375.
114. Melnick J, Aviel S, Argon Y. The endoplasmic reticulum stress protein GRP94, in addition to BiP, associates with unassembled immunoglobulin chains. *J Biol Chem* 1992, **267**(30)**:** 21303-21306.
115. Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. *Nat Cell Biol* 2001, **3**(10)**:** 891-896.

116. Linderoth NA, Simon MN, Rodionova NA, Cadene M, Laws WR, Chait BT*, et al.* Biophysical analysis of the endoplasmic reticulum-resident chaperone/heat shock protein gp96/GRP94 and its complex with peptide antigen. *Biochemistry* 2001, **40**(5)**:** 1483-1495.
117. Biswas C, Sriram U, Ciric B, Ostrovsky O, Gallucci S, Argon Y. The N-terminal fragment of GRP94 is sufficient for peptide presentation via professional antigen-presenting cells. *International immunology* 2006, **18**(7)**:** 1147-1157.
118. Wu S, Hong F, Gewirth D, Guo B, Liu B, Li Z. The molecular chaperone gp96/GRP94 interacts with Toll-like receptors and integrins via its C-terminal hydrophobic domain. *J Biol Chem* 2012, **287**(9)**:** 6735-6742.
119. Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. *Cold Spring Harb Perspect Biol* 2013, **5**(5)**:** a013201.
120. Ellgaard L, Ruddock LW. The human protein disulphide isomerase family: substrate interactions and functional properties. *EMBO Rep* 2005, **6**(1)**:** 28-32.
121. Schwaller M, Wilkinson B, Gilbert HF. Reduction-reoxidation cycles contribute to catalysis of disulfide isomerization by protein-disulfide isomerase. *J Biol Chem* 2003, **278**(9)**:** 7154-7159.
122. Darby NJ, Penka E, Vincentelli R. The multi-domain structure of protein disulfide isomerase is essential for high catalytic efficiency. *J Mol Biol* 1998, **276**(1)**:** 239-247.
123. Quan H, Fan G, Wang CC. Independence of the chaperone activity of protein disulfide isomerase from its thioredoxin-like active site. *J Biol Chem* 1995, **270**(29)**:** 17078-17080.
124. Cai H, Wang CC, Tsou CL. Chaperone-like activity of protein disulfide isomerase in the refolding of a protein with no disulfide bonds. *J Biol Chem* 1994, **269**(40)**:** 24550-24552.
125. Puig A, Lyles MM, Noiva R, Gilbert HF. The role of the thiol/disulfide centers and peptide binding site in the chaperone and anti-chaperone activities of protein disulfide isomerase. *J Biol Chem* 1994, **269**(29)**:** 19128-19135.
126. Appenzeller-Herzog C, Ellgaard L. The human PDI family: versatility packed into a single fold. *Biochim Biophys Acta* 2008, **1783**(4)**:** 535-548.
127. Horibe T, Gomi M, Iguchi D, Ito H, Kitamura Y, Masuoka T*, et al.* Different contributions of the three CXXC motifs of human protein-disulfide isomerase-related protein to isomerase activity and oxidative refolding. *J Biol Chem* 2004, **279**(6)**:** 4604-4611.
128. Lappi AK, Lensink MF, Alanen HI, Salo KE, Lobell M, Juffer AH*, et al.* A conserved arginine plays a role in the catalytic cycle of the protein disulphide isomerases. *J Mol Biol* 2004, **335**(1)**:** 283-295.
129. Nagai N, Hosokawa M, Itohara S, Adachi E, Matsushita T, Hosokawa N*, et al.* Embryonic lethality of molecular chaperone hsp47 knockout mice is associated with defects in collagen biosynthesis. *J Cell Biol* 2000, **150**(6)**:** 1499-1506.
130. Nagata K. Hsp47: a collagen-specific molecular chaperone. *Trends Biochem Sci* 1996, **21**(1)**:** 22-26.
131. Matsuoka Y, Kubota H, Adachi E, Nagai N, Marutani T, Hosokawa N*, et al.* Insufficient folding of type IV collagen and formation of abnormal basement membrane-like structure in embryoid bodies derived from Hsp47-null embryonic stem cells. *Mol Biol Cell* 2004, **15**(10)**:** 4467-4475.
132. Kubota H, Nagata K. Roles of collagen fibers and its specific molecular chaperone: analysis using HSP47-knockout mice. *Uchu Seibutsu Kagaku* 2004, **18**(3)**:** 118-119.
133. Hu G, Gura T, Sabsay B, Sauk J, Dixit SN, Veis A. Endoplasmic reticulum protein Hsp47 binds specifically to the N-terminal globular domain of the amino-propeptide of the procollagen I alpha 1 (I)-chain. *J Cell Biochem* 1995, **59**(3)**:** 350-367.
134. Olzmann JA, Kopito RR, Christianson JC. The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harb Perspect Biol* 2013, **5**.
135. Ruggiano A, Foresti O, Carvalho P. Quality control: ER-associated degradation: protein quality control and beyond. *J Cell Biol* 2014, **204**(6)**:** 869-879.
136. Carvalho P, Goder V, Rapoport TA. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell* 2006, **126**(2)**:** 361-373.
137. Christianson JC, Olzmann JA, Shaler TA, Sowa ME, Bennett EJ, Richter CM*, et al.* Defining human ERAD networks through an integrative mapping strategy. *Nat Cell Biol* 2012, **14**(1)**:** 93-105.

138. Plemper RK, Bohmler S, Bordallo J, Sommer T, Wolf DH. Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. *Nature* 1997, **388**(6645)**:** 891-895.
139. Scott DC, Schekman R. Role of Sec61p in the ER-associated degradation of short-lived transmembrane proteins. *J Cell Biol* 2008, **181**(7)**:** 1095-1105.
140. Carvalho P, Stanley AM, Rapoport TA. Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p. *Cell* 2010, **143**(4)**:** 579-591.
141. Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 2001, **414**(6864)**:** 652-656.
142. Wang Q, Li L, Ye Y. Inhibition of p97-dependent protein degradation by Eeyarestatin I. *J Biol Chem* 2008, **283**(12)**:** 7445-7454.
143. Rabinovich E, Kerem A, Frohlich KU, Diamant N, Bar-Nun S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol Cell Biol* 2002, **22**(2)**:** 626-634.
144. Kim H, Bhattacharya A, Qi L. Endoplasmic reticulum quality control in cancer: Friend or foe. *Semin Cancer Biol* 2015.
145. Malkus P, Jiang F, Schekman R. Concentrative sorting of secretory cargo proteins into COPII-coated vesicles. *J Cell Biol* 2002, **159:** 915-921.
146. Sato K, Nakano A. Mechanisms of COPII vesicle formation and protein sorting. *FEBS Lett* 2007, **581:** 2076-2082.
147. Martínez-Menárguez JA, Geuze HJ, Slot JW, Klumperman J. Vesicular tubular clusters between the ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. *Cell* 1999, **98:** 81-90.
148. Wieland FT, Gleason ML, Serafini TA, Rothman JE. The rate of bulk flow from the endoplasmic reticulum to the cell surface. *Cell* 1987, **50:** 289-300.
149. Barlowe C. Signals for COPII-dependent export from the ER: what's the ticket out? *Trends Cell Biol* 2003, **13:** 295-300.
150. Schweizer A, Fransen JA, Bächi T, Ginsel L, Hauri HP. Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. *J Cell Biol* 1988, **107:** 1643-1653.
151. Appenzeller C, Andersson H, Kappeler F, Hauri HP. The lectin ERGIC-53 is a cargo transport receptor for glycoproteins. *Nat Cell Biol* 1999, **1:** 330-334.
152. Stamnes MA, Craighead MW, Hoe MH, Lampen N, Geromanos S, Tempst P*, et al.* An integral membrane component of coatomer-coated transport vesicles defines a family of proteins involved in budding. *Proc Natl Acad Sci U S A* 1995, **92:** 8011-8015.
153. Schimmöller F, Singer-Krüger B, Schröder S, Krüger U, Barlowe C, Riezman H. The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. *EMBO J* 1995, **14:** 1329-1339.
154. Nufer O, Mitrovic S, Hauri HP. Profile-based data base scanning for animal L-type lectins and characterization of VIPL, a novel VIP36-like endoplasmic reticulum protein. *J Biol Chem* 2003, **278:** 15886-15896.
155. Kamiya Y, Kamiya D, Yamamoto K, Nyfeler B, Hauri HP, Kato K. Molecular basis of sugar recognition by the human L-type lectins ERGIC-53, VIPL, and VIP36. *J Biol Chem* 2008, **283:** 1857-1861.
156. Hara-Kuge S, Ohkura T, Ideo H, Shimada O, Atsumi S, Yamashita K. Involvement of VIP36 in intracellular transport and secretion of glycoproteins in polarized Madin-Darby canine kidney (MDCK) cells. *J Biol Chem* 2002, **277:** 16332-16339.
157. Neve EP, Svensson K, Fuxe J, Pettersson RF. VIPL, a VIP36-like membrane protein with a putative function in the export of glycoproteins from the endoplasmic reticulum. *Exp Cell Res* 2003, **288:** 70-83.
158. Strating JR, Martens GJ. The p24 family and selective transport processes at the ER-Golgi interface. *Biol Cell* 2009, **101:** 495-509.
159. Dominguez M, Dejgaard K, Füllekrug J, Dahan S, Fazel A, Paccaud JP*, et al.* gp25L/emp24/p24 protein family members of the cis-Golgi network bind both COP I and II coatomer. *J Cell Biol* 1998, **140:** 751-765.
160. Herzig Y, Sharpe HJ, Elbaz Y, Munro S, Schuldiner M. A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14. *PLoS One* 2012, **10:** e1001329.
161. Otte S, Barlowe C. The Erv41p-Erv46p complex: multiple export signals are required in trans for COPII-dependent transport from the ER. *EMBO J* 2002, **21:** 6095-6104.

162. Otte S, Barlowe C. Sorting signals can direct receptor-mediated export of soluble proteins into COPII vesicles. *Nat Cell Biol* 2004, **6:** 1189-1194.
163. Belden WJ, Barlowe C. Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient endoplasmic reticulum to Golgi transport. *J Biol Chem* 1996, **271:** 26939-26946.
164. Belden WJ, Barlowe C. Distinct roles for the cytoplasmic tail sequences of Emp24p and Erv25p in transport between the endoplasmic reticulum and Golgi complex. *J Biol Chem* 2001, **276:** 43040-43048.
165. Nickel W, Sohn K, Bünning C, Wieland FT. p23, a major COPI-vesicle membrane protein, constitutively cycles through the early secretory pathway. *Proc Natl Acad Sci U S A* 1997, **94:** 11393-11398.
166. Füllekrug J, Suganuma T, Tang BL, Hong W, Storrie B, Nilsson T. Localization and recycling of gp27 (hp24γ3): complex formation with other p24 family members. *Mol Biol Cell* 1999, **10:** 1939-1955.
167. Marzioch M, Henthorn DC, Herrmann JM, Wilson R, Thomas DY, Bergeron JJ*, et al.* Erp1p and Erp2p, partners for Emp24p and Erv25p in a yeast p24 complex. *Mol Biol Cell* 1999, **10:** 1923-1938.
168. Takida S, Maeda Y, Kinoshita T. Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane. *Biochem J* 2008, **409:** 555-562.
169. Barlowe C. Membrane Trafficking: ER Export Encounters Dualism. *Curr Biol* 2015, **25:** 151-153.
170. Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N*, et al.* COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell* 1994, **77**(6)**:** 895-907.
171. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R*, et al.* COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. *Cell* 1998, **93**(2)**:** 263-275.
172. Barlowe C, Schekman R. SEC12 encodes a guanine-nucleotide-exchange factor essential for transport vesicle budding from the ER. *Nature* 1993, **365**(6444)**:** 347-349.
173. Yoshihisa T, Barlowe C, Schekman R. Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. *Science* 1993, **259**(5100)**:** 1466-1468.
174. Weissman JT, Plutner H, Balch WE. The mammalian guanine nucleotide exchange factor mSec12 is essential for activation of the Sar1 GTPase directing endoplasmic reticulum export. *Traffic* 2001, **2**(7)**:** 465-475.
175. Lee MC, Orci L, Hamamoto S, Futai E, Ravazzola M, Schekman R. Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell* 2005, **122**(4)**:** 605-617.
176. Miller E, Antonny B, Hamamoto S, Schekman R. Cargo selection into COPII vesicles is driven by the Sec24p subunit. *The EMBO journal* 2002, **21**(22)**:** 6105-6113.
177. Bi X, Corpina RA, Goldberg J. Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat. *Nature* 2002, **419**(6904)**:** 271-277.
178. Miller EA, Beilharz TH, Malkus PN, Lee MC, Hamamoto S, Orci L*, et al.* Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* 2003, **114**(4)**:** 497-509.
179. Bi X, Mancias JD, Goldberg J. Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31. *Dev Cell* 2007, **13**(5)**:** 635-645.
180. Stagg SM, Gurkan C, Fowler DM, LaPointe P, Foss TR, Potter CS*, et al.* Structure of the Sec13/31 COPII coat cage. *Nature* 2006, **439**(7073)**:** 234-238.
181. Bhattacharya N, J OD, Stagg SM. The structure of the Sec13/31 COPII cage bound to Sec23. *J Mol Biol* 2012, **420**(4-5)**:** 324-334.
182. Fath S, Mancias JD, Bi X, Goldberg J. Structure and organization of coat proteins in the COPII cage. *Cell* 2007, **129**(7)**:** 1325-1336.
183. Antonny B, Madden D, Hamamoto S, Orci L, Schekman R. Dynamics of the COPII coat with GTP and stable analogues. *Nat Cell Biol* 2001, **3**(6)**:** 531-537.
184. Faini M, Beck R, Wieland FT, Briggs JA. Vesicle coats: structure, function, and general principles of assembly. *Trends Cell Biol* 2013, **23:** 279-288.
185. Sprangers J, Rabouille C. SEC16 in COPII coat dynamics at ER exit sites. *Biochem Soc Trans* 2015, **43:** 97-103.

186. Shindiapina P, Barlowe C. Requirements for transitional endoplasmic reticulum site structure and function in Saccharomyces cerevisiae. *Mol Biol Cell* 2010, **21**(9)**:** 1530-1545.
187. Nakano A, Brada D, Schekman R. A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast. *J Cell Biol* 1988, **107**(3)**:** 851-863.
188. Sato K, Nishikawa S, Nakano A. Membrane protein retrieval from the Golgi apparatus to the endoplasmic reticulum (ER): characterization of the RER1 gene product as a component involved in ER localization of Sec12p. *Mol Biol Cell* 1995, **6**(11)**:** 1459-1477.
189. Sato M, Sato K, Nakano A. Endoplasmic reticulum localization of Sec12p is achieved by two mechanisms: Rer1p-dependent retrieval that requires the transmembrane domain and Rer1p-independent retention that involves the cytoplasmic domain. *J Cell Biol* 1996, **134:** 279-293.
190. Soderholm J, Bhattacharyya D, Strongin D, Markovitz V, Connerly PL, Reineke CA*, et al.* The transitional ER localization mechanism of Pichia pastoris Sec12. *Dev Cell* 2004, **6**(5)**:** 64959.
191. Montegna EA, Bhave M, Liu Y, Bhattacharyya D, Glick BS. Sec12 binds to Sec16 at transitional ER sites. *PLoS One* 2012, **7**(2)**:** e31156.
192. Saito K, Yamashiro K, Shimazu N, Tanabe T, Kontani K, Katada T. Concentration of Sec12 at ER exit sites via interaction with cTAGE5 is required for collagen export. *J Cell Biol* 2014, **206**(6)**:** 751-762.
193. Saito K, Yamashiro K, Ichikawa Y, Erlmann P, Kontani K, Malhotra V*, et al.* cTAGE5 mediates collagen secretion through interaction with TANGO1 at endoplasmic reticulum exit sites. *Mol Biol Cell* 2011, **22**(13)**:** 2301-2308.
194. Saito K, Chen M, Bard F, Chen S, Zhou H, Woodley D*, et al.* TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. *Cell* 2009, **136**(5)**:** 891-902.
195. Bard F, Casano L, Mallabiabarrena A, Wallace E, Saito K, Kitayama H*, et al.* Functional genomics reveals genes involved in protein secretion and Golgi organization. *Nature* 2006, **439**(7076)**:** 604-607.
196. Venditti R, Scanu T, Santoro M, Di Tullio G, Spaar A, Gaibisso R*, et al.* Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science* 2012, **337**(6102)**:** 1668-1672.
197. Gimeno RE, Espenshade P, Kaiser CA. SED4 encodes a yeast endoplasmic reticulum protein that binds Sec16p and participates in vesicle formation. *J Cell Biol* 1995, **131**(2)**:** 325-338.
198. Kodera C, Yorimitsu T, Nakano A, Sato K. Sed4p stimulates Sar1p GTP hydrolysis and promotes limited coat disassembly. *Traffic* 2011, **12**(5)**:** 591-599.
199. Saito-Nakano Y, Nakano A. Sed4p functions as a positive regulator of Sar1p probably through inhibition of the GTPase activation by Sec23p. *Genes Cells* 2000, **5**(12)**:** 1039-1048.
200. Hardwick KG, Boothroyd JC, Rudner AD, Pelham HR. Genes that allow yeast cells to grow in the absence of the HDEL receptor. *The EMBO journal* 1992, **11**(11)**:** 4187-4195.
201. McMahon C, Studer SM, Clendinen C, Dann GP, Jeffrey PD, Hughson FM. The structure of Sec12 implicates potassium ion coordination in Sar1 activation. *J Biol Chem* 2012, **287:** 43599-43606.
202. Long KR, Yamamoto Y, Baker AL, Watkins SC, Coyne CB, Conway JF*, et al.* Sar1 assembly regulates membrane constriction and ER export. *J Cell Biol* 2010, **190**(1)**:** 115-128.
203. Huang M, Weissman JT, Beraud-Dufour S, Luan P, Wang C, Chen W*, et al.* Crystal structure of Sar1-GDP at 1.7 A resolution and the role of the NH2 terminus in ER export. *J Cell Biol* 2001, **155**(6)**:** 937-948.
204. Settles EI, Loftus AF, McKeown AN, Parthasarathy R. The vesicle trafficking protein Sar1 lowers lipid membrane rigidity. *Biophysical journal* 2010, **99**(5)**:** 1539-1545.
205. Loftus AF, Hsieh VL, Parthasarathy R. Modulation of membrane rigidity by the human vesicle trafficking proteins Sar1A and Sar1B. *Biochem Biophys Res Commun* 2012, **426**(4)**:** 585-589.
206. Sato K, Nakano A. Dissection of COPII subunit-cargo assembly and disassembly kinetics during Sar1p-GTP hydrolysis. *Nature structural & molecular biology* 2005, **12**(2)**:** 167-174.

207. Tabata KV, Sato K, Ide T, Nishizaka T, Nakano A, Noji H. Visualization of cargo concentration by COPII minimal machinery in a planar lipid membrane. *The EMBO journal* 2009, **28**(21)**:** 3279-3289.
208. Kuehn MJ, Herrmann JM, Schekman R. COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. *Nature* 1998, **391**(6663)**:** 187-190.
209. Hariri H, Bhattacharya N, Johnson K, Noble AJ, Stagg SM. Insights into the mechanisms of membrane curvature and vesicle scission by the small GTPase Sar1 in the early secretory pathway. *J Mol Biol* 2014, **426**(22)**:** 3811-3826.
210. Bielli A, Haney CJ, Gabreski G, Watkins SC, Bannykh SI, Aridor M. Regulation of Sar1 NH2 terminus by GTP binding and hydrolysis promotes membrane deformation to control COPII vesicle fission. *J Cell Biol* 2005, **171**(6)**:** 919-924.
211. Cutrona MB, Beznoussenko GV, Fusella A, Martella O, Moral P, Mironov AA. Silencing of mammalian Sar1 isoforms reveals COPII-independent protein sorting and transport. *Traffic* 2013, **14:** 691-708.
212. Shoulders CC, Stephens DJ, Jones B. The intracellular transport of chylomicrons requires the small GTPase, Sar1b. *Current opinion in lipidology* 2004, **15**(2)**:** 191-197.
213. Siddiqi SA, Gorelick FS, Mahan JT, Mansbach CM, 2nd. COPII proteins are required for Golgi fusion but not for endoplasmic reticulum budding of the pre-chylomicron transport vesicle. *J Cell Sci* 2003, **116**(Pt 2)**:** 415-427.
214. Mansbach CM, 2nd, Nevin P. Intracellular movement of triacylglycerols in the intestine. *Journal of lipid research* 1998, **39**(5)**:** 963-968.
215. Cartwright IJ, Higgins JA. Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes. *J Biol Chem* 2001, **276**(51)**:** 48048-48057.
216. Neeli I, Siddiqi SA, Siddiqi S, Mahan J, Lagakos WS, Binas B*, et al.* Liver fatty acid-binding protein initiates budding of pre-chylomicron transport vesicles from intestinal endoplasmic reticulum. *J Biol Chem* 2007, **282**(25)**:** 17974-17984.
217. Sane A, Seidman E, Spahis S, Lamantia V, Garofalo C, Montoudis A*, et al.* New Insights in Intestinal Sar1B GTPase Regulation and Role in Cholesterol Homeostasis. *J Cell Biochem* 2015.
218. Levy E, Harmel E, Laville M, Sanchez R, Emonnot L, Sinnett D*, et al.* Expression of Sar1b enhances chylomicron assembly and key components of the coat protein complex II system driving vesicle budding. *Arterioscler Thromb Vasc Biol* 2011, **31:** 2692-2699.
219. Fryer LG, Jones B, Duncan EJ, Hutchison CE, Ozkan T, Williams PA*, et al.* Expression of Sar1b enhances chylomicron assembly and key components of the coat protein complex II system driving vesicle budding. *J Biol Chem* 2014, **289:** 4244-4261.
220. Siddiqi S, Mansbach CM, 2nd. Phosphorylation of Sar1b protein releases liver fatty acid-binding protein from multiprotein complex in intestinal cytosol enabling it to bind to endoplasmic reticulum (ER) and bud the pre-chylomicron transport vesicle. *J Biol Chem* 2012, **287**(13)**:** 10178-10188.
221. Siddiqi SA, Mansbach CM, 2nd. PKC zeta-mediated phosphorylation controls budding of the pre-chylomicron transport vesicle. *J Cell Sci* 2008, **121**(Pt 14)**:** 2327-2338.
222. Magnolo L, Najah M, Fancello T, Di Leo E, Pinotti E, Brini I*, et al.* Novel mutations in SAR1B and MTTP genes in Tunisian children with chylomicron retention disease and abetalipoproteinemia. *Gene* 2013, **512**(1)**:** 28-34.
223. Okada T, Miyashita M, Fukuhara J, Sugitani M, Ueno T, Samson-Bouma ME*, et al.* Anderson's disease/chylomicron retention disease in a Japanese patient with uniparental disomy 7 and a normal SAR1B gene protein coding sequence. *Orphanet journal of rare diseases* 2011, **6:** 78.
224. Levic DS, Minkel JR, Wang WD, Rybski WM, Melville DB, Knapik EW. Animal model of Sar1b deficiency presents lipid absorption deficits similar to Anderson disease. *Journal of molecular medicine (Berlin, Germany)* 2015, **93**(2)**:** 165-176.
225. Charcosset M, Sassolas A, Peretti N, Roy CC, Deslandres C, Sinnett D*, et al.* Anderson or chylomicron retention disease: molecular impact of five mutations in the SAR1B gene on the structure and the functionality of Sar1b protein. *Molecular genetics and metabolism* 2008, **93**(1)**:** 74-84.
226. Jones B, Jones EL, Bonney SA, Patel HN, Mensenkamp AR, Eichenbaum-Voline S*, et al.* Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders. *Nat Genet* 2003, **34**(1)**:** 29-31.

227. Lord C, Bhandari D, Menon S, Ghassemian M, Nycz D, Hay J*, et al.* Sequential interactions with Sec23 control the direction of vesicle traffic. *Nature* 2011, **473:** 181-186.
228. Townley AK, Feng Y, Schmidt K, Carter DA, Porter R, Verkade P*, et al.* Efficient coupling of Sec23-Sec24 to Sec13-Sec31 drives COPII-dependent collagen secretion and is essential for normal craniofacial development. *J Cell Sci* 2008, **121:** 3025-3034.
229. Boyadjiev SA, Kim SD, Hata A, Haldeman-Englert C, Zackai EH, Naydenov C*, et al.* Cranio-lenticulo-sutural dysplasia associated with defects in collagen secretion. *Clinical genetics* 2011, **80**(2)**:** 169-176.
230. Boyadjiev SA, Fromme JC, Ben J, Chong SS, Nauta C, Hur DJ*, et al.* Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. *Nat Genet* 2006, **38**(10)**:** 1192-1197.
231. Lang MR, Lapierre LA, Frotscher M, Goldenring JR, Knapik EW. Secretory COPII coat component Sec23a is essential for craniofacial chondrocyte maturation. *Nat Genet* 2006, **38**(10)**:** 1198-1203.
232. Schwarz K, Iolascon A, Verissimo F, Trede NS, Horsley W, Chen W*, et al.* Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II. *Nat Genet* 2009, **41**(8)**:** 936-940.
233. Tao J, Zhu M, Wang H, Afelik S, Vasievich MP, Chen XW*, et al.* SEC23B is required for the maintenance of murine professional secretory tissues. *Proc Natl Acad Sci U S A* 2012, **109**(29)**:** E2001-2009.
234. Russo R, Langella C, Esposito MR, Gambale A, Vitiello F, Vallefuoco F*, et al.* Hypomorphic mutations of SEC23B gene account for mild phenotypes of congenital dyserythropoietic anemia type II. *Blood cells, molecules & diseases* 2013, **51**(1)**:** 17-21.
235. Khoriaty R, Vasievich MP, Jones M, Everett L, Chase J, Tao J*, et al.* Absence of a red blood cell phenotype in mice with hematopoietic deficiency of SEC23B. *Mol Cell Biol* 2014, **34**(19)**:** 3721-3734.
236. Wendeler MW, Paccaud JP, Hauri HP. Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum. *EMBO Rep* 2007, **8**(3)**:** 258-264.
237. Pagano A, Letourneur F, Garcia-Estefania D, Carpentier JL, Orci L, Paccaud JP. Sec24 proteins and sorting at the endoplasmic reticulum. *J Biol Chem* 1999, **274**(12)**:** 7833-7840.
238. Tang BL, Kausalya J, Low DY, Lock ML, Hong W. A family of mammalian proteins homologous to yeast Sec24p. *Biochem Biophys Res Commun* 1999, **258**(3)**:** 679-684.
239. Mossessova E, Bickford LC, Goldberg J. SNARE Selectivity of the COPII Coat. *Cell* 2003, **114:** 483-495.
240. Campbell JL, Schekman R. Selective packaging of cargo molecules into endoplasmic reticulum-derived COPII vesicles. *Proc Natl Acad Sci U S A* 1997, **94**(3)**:** 837-842.
241. Mancias JD, Goldberg J. Structural basis of cargo membrane protein discrimination by the human COPII coat machinery. *EMBO J* 2008, **27**(21)**:** 2918-2928.
242. Roberg KJ, Crotwell M, Espenshade P, Gimeno R, Kaiser CA. LST1 is a SEC24 homologue used for selective export of the plasma membrane ATPase from the endoplasmic reticulum. *J Cell Biol* 1999, **145**(4)**:** 659-672.
243. Shimoni Y, Kurihara T, Ravazzola M, Amherdt M, Orci L, Schekman R. Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in Saccharomyces cerevisiae. *J Cell Biol* 2000, **151**(5)**:** 973-984.
244. Kurihara T, Hamamoto S, Gimeno RE, Kaiser CA, Schekman R, Yoshihisa T. Sec24p and Iss1p function interchangeably in transport vesicle formation from the endoplasmic reticulum in Saccharomyces cerevisiae. *Mol Biol Cell* 2000, **11**(3)**:** 983-998.
245. Belden WJ, Barlowe C. Role of Erv29p in collecting soluble secretory proteins into ER-derived transport vesicles. *Science* 2001, **294**(5546)**:** 1528-1531.
246. Otte S, Barlowe C. Sorting signals can direct receptor-mediated export of soluble proteins into COPII vesicles. *Nat Cell Biol* 2004, **6**(12)**:** 1189-1194.
247. Mancias JD, Goldberg J. The transport signal on Sec22 for packaging into COPII-coated vesicles is a conformational epitope. *Mol Cell* 2007, **26**(3)**:** 403-414.
248. Otsu W, Kurooka T, Otsuka Y, Sato K, Inaba M. A new class of endoplasmic reticulum export signal PhiXPhiXPhi for transmembrane proteins and its selective interaction with Sec24C. *J Biol Chem* 2013, **288**(25)**:** 18521-18532.

249. Manzano-Lopez J, Perez-Linero AM, Aguilera-Romero A, Martin ME, Okano T, Silva DV*, et al.* COPII coat composition is actively regulated by luminal cargo maturation. *Curr Biol* 2015, **25:** 152-162.
250. Sucic S, Koban F, El-Kasaby A, Kudlacek O, Stockner T, Sitte HH*, et al.* Switching the clientele: a lysine residing in the C terminus of the serotonin transporter specifies its preference for the coat protein complex II component SEC24C. *J Biol Chem* 2013, **288**(8)**:** 5330-5341.
251. Montgomery TR, Steinkellner T, Sucic S, Koban F, Schuchner S, Ogris E*, et al.* Axonal targeting of the serotonin transporter in cultured rat dorsal raphe neurons is specified by SEC24C-dependent export from the endoplasmic reticulum. *J Neurosci* 2014, **34**(18)**:** 6344-6351.
252. Sucic S, El-Kasaby A, Kudlacek O, Sarker S, Sitte HH, Marin P*, et al.* The serotonin transporter is an exclusive client of the coat protein complex II (COPII) component SEC24C. *J Biol Chem* 2011, **286**(18)**:** 16482-16490.
253. Bonnon C, Wendeler MW, Paccaud JP, Hauri HP. Selective export of human GPI-anchored proteins from the endoplasmic reticulum. *J Cell Sci* 2010, **123**(Pt 10)**:** 1705-1715.
254. Chen XW, Wang H, Bajaj K, Zhang P, Meng ZX, Ma D*, et al.* SEC24A deficiency lowers plasma cholesterol through reduced PCSK9 secretion. *eLife* 2013, **2:** e00444.
255. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *Journal of lipid research* 2009, **50 Suppl:** S172-177.
256. Wansleeben C, Feitsma H, Montcouquiol M, Kroon C, Cuppen E, Meijlink F. Planar cell polarity defects and defective Vangl2 trafficking in mutants for the COPII gene Sec24b. *Development* 2010, **137**(7)**:** 1067-1073.
257. Merte J, Jensen D, Wright K, Sarsfield S, Wang Y, Schekman R*, et al.* Sec24b selectively sorts Vangl2 to regulate planar cell polarity during neural tube closure. *Nat Cell Biol* 2010, **12**(1)**:** 41-46; sup pp 41-48.
258. Yang XY, Zhou XY, Wang QQ, Li H, Chen Y, Lei YP*, et al.* Mutations in the COPII vesicle component gene SEC24B are associated with human neural tube defects. *Human mutation* 2013, **34**(8)**:** 1094-1101.
259. Adams EJ, Chen XW, O'Shea KS, Ginsburg D. Mammalian COPII coat component SEC24C is required for embryonic development in mice. *J Biol Chem* 2014, **289**(30)**:** 20858-20870.
260. Siddiqi S, Saleem U, Abumrad NA, Davidson NO, Storch J, Siddiqi SA*, et al.* A novel multiprotein complex is required to generate the prechylomicron transport vesicle from intestinal ER. *Journal of lipid research* 2010, **51**(7)**:** 1918-1928.
261. Garbes L, Kim K, Riess A, Hoyer-Kuhn H, Beleggia F, Bevot A*, et al.* Mutations in SEC24D, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta. *American journal of human genetics* 2015, **96**(3)**:** 432-439.
262. Sarmah S, Barrallo-Gimeno A, Melville DB, Topczewski J, Solnica-Krezel L, Knapik EW. Sec24D-dependent transport of extracellular matrix proteins is required for zebrafish skeletal morphogenesis. *PLoS One* 2010, **5**(4)**:** e10367.
263. Baines AC, Adams EJ, Zhang B, Ginsburg D. Disruption of the Sec24d gene results in early embryonic lethality in the mouse. *PLoS One* 2013, **8**(4)**:** e61114.
264. Ohisa S, Inohaya K, Takano Y, Kudo A. sec24d encoding a component of COPII is essential for vertebra formation, revealed by the analysis of the medaka mutant, vbi. *Dev Biol* 2010, **342**(1)**:** 85-95.
265. Kung LF, Pagant S, Futai E, D'Arcangelo JG, Buchanan R, Dittmar JC*, et al.* Sec24p and Sec16p cooperate to regulate the GTP cycle of the COPII coat. *The EMBO journal* 2012, **31**(4)**:** 1014-1027.
266. Sharpe LJ, Luu W, Brown AJ. Akt phosphorylates Sec24: new clues into the regulation of ER-to-Golgi trafficking. *Traffic* 2011, **12**(1)**:** 19-27.
267. Whittle JR, Schwartz TU. Structure of the Sec13-Sec16 edge element, a template for assembly of the COPII vesicle coat. *J Cell Biol* 2010, **190**(3)**:** 347-361.
268. Salama NR, Yeung T, Schekman RW. The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. *The EMBO journal* 1993, **12**(11)**:** 4073-4082.
269. Hino T, Tanaka Y, Kawamukai M, Nishimura K, Mano S, Nakagawa T. Two Sec13p homologs, AtSec13A and AtSec13B, redundantly contribute to the formation of COPII

transport vesicles in Arabidopsis thaliana. *Bioscience, biotechnology, and biochemistry* 2011, **75**(9)**:** 1848-1852.
270. Salama NR, Chuang JS, Schekman RW. Sec31 encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum. *Mol Biol Cell* 1997, **8**(2)**:** 205-217.
271. Shaywitz DA, Orci L, Ravazzola M, Swaroop A, Kaiser CA. Human SEC13Rp functions in yeast and is located on transport vesicles budding from the endoplasmic reticulum. *J Cell Biol* 1995, **128**(5)**:** 769-777.
272. Swaroop A, Yang-Feng TL, Liu W, Gieser L, Barrow LL, Chen KC*, et al.* Molecular characterization of a novel human gene, SEC13R, related to the yeast secretory pathway gene SEC13, and mapping to a conserved linkage group on human chromosome 3p24-p25 and mouse chromosome 6. *Hum Mol Genet* 1994, **3**(8)**:** 1281-1286.
273. Copic A, Latham CF, Horlbeck MA, D'Arcangelo JG, Miller EA. ER cargo properties specify a requirement for COPII coat rigidity mediated by Sec13p. *Science* 2012, **335**(6074)**:** 1359-1362.
274. Stagg SM, LaPointe P, Razvi A, Gurkan C, Potter CS, Carragher B*, et al.* Structural basis for cargo regulation of COPII coat assembly. *Cell* 2008, **134**(3)**:** 474-484.
275. Pryer NK, Salama NR, Schekman R, Kaiser CA. Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. *J Cell Biol* 1993, **120**(4)**:** 865-875.
276. Enninga J, Levay A, Fontoura BM. Sec13 shuttles between the nucleus and the cytoplasm and stably interacts with Nup96 at the nuclear pore complex. *Mol Cell Biol* 2003, **23**(20)**:** 7271-7284.
277. Siniossoglou S, Wimmer C, Rieger M, Doye V, Tekotte H, Weise C*, et al.* A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores. *Cell* 1996, **84**(2)**:** 265-275.
278. Schmidt K, Cavodeassi F, Feng Y, Stephens DJ. Early stages of retinal development depend on Sec13 function. *Biology open* 2013, **2**(3)**:** 256-266.
279. Niu X, Hong J, Zheng X, Melville DB, Knapik EW, Meng A*, et al.* The nuclear pore complex function of Sec13 protein is required for cell survival during retinal development. *J Biol Chem* 2014, **289**(17)**:** 11971-11985.
280. Niu X, Gao C, Jan Lo L, Luo Y, Meng C, Hong J*, et al.* Sec13 safeguards the integrity of the endoplasmic reticulum and organogenesis of the digestive system in zebrafish. *Dev Biol* 2012, **367**(2)**:** 197-207.
281. Townley AK, Schmidt K, Hodgson L, Stephens DJ. Epithelial organization and cyst lumen expansion require efficient Sec13-Sec31-driven secretion. *J Cell Sci* 2012, **125**(Pt 3)**:** 673-684.
282. Belden WJ, Barlowe C. Purification of functional Sec13p-Sec31p complex, a subunit of COPII coat. *Methods in enzymology* 2001, **329:** 438-443.
283. Stankewich MC, Stabach PR, Morrow JS. Human Sec31B: a family of new mammalian orthologues of yeast Sec31p that associate with the COPII coat. *J Cell Sci* 2006, **119**(Pt 5)**:** 958-969.
284. Shugrue CA, Kolen ER, Peters H, Czernik A, Kaiser C, Matovcik L*, et al.* Identification of the putative mammalian orthologue of Sec31P, a component of the COPII coat. *J Cell Sci* 1999, **112 (Pt 24):** 4547-4556.
285. Tang BL, Zhang T, Low DY, Wong ET, Horstmann H, Hong W. Mammalian homologues of yeast sec31p. An ubiquitously expressed form is localized to endoplasmic reticulum (ER) exit sites and is essential for ER-Golgi transport. *J Biol Chem* 2000, **275**(18)**:** 13597-13604.
286. Jin L, Pahuja KB, Wickliffe KE, Gorur A, Baumgartel C, Schekman R*, et al.* Ubiquitin-dependent regulation of COPII coat size and function. *Nature* 2012, **482**(7386)**:** 495-500.
287. Koreishi M, Yu S, Oda M, Honjo Y, Satoh A. CK2 phosphorylates Sec31 and regulates ER-To-Golgi trafficking. *PLoS One* 2013, **8**(1)**:** e54382.
288. Zanetti G, Prinz S, Daum S, Meister A, Schekman R, Bacia K*, et al.* The structure of the COPII transport-vesicle coat assembled on membranes. *eLife* 2013, **2:** e00951.
289. Lederkremer GZ, Cheng Y, Petre BM, Vogan E, Springer S, Schekman R*, et al.* Structure of the Sec23p/24p and Sec13p/31p complexes of COPII. *Proc Natl Acad Sci U S A* 2001, **98**(19)**:** 10704-10709.

290. Noble AJ, Zhang Q, O'Donnell J, Hariri H, Bhattacharya N, Marshall AG*, et al.* A pseudoatomic model of the COPII cage obtained from cryo-electron microscopy and mass spectrometry. *Nature structural & molecular biology* 2013, **20**(2)**:** 167-173.
291. Matsuoka K, Schekman R, Orci L, Heuser JE. Surface structure of the COPII-coated vesicle. *Proc Natl Acad Sci U S A* 2001, **98**(24)**:** 13705-13709.
292. Supek F, Madden DT, Hamamoto S, Orci L, Schekman R. Sec16p potentiates the action of COPII proteins to bud transport vesicles. *J Cell Biol* 2002, **158**(6)**:** 1029-1038.
293. Novick P, Field C, Schekman R. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* 1980, **21**(1)**:** 205-215.
294. Kaiser CA, Schekman R. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell* 1990, **61**(4)**:** 723-733.
295. Espenshade P, Gimeno RE, Holzmacher E, Teung P, Kaiser CA. Yeast SEC16 gene encodes a multidomain vesicle coat protein that interacts with Sec23p. *J Cell Biol* 1995, **131**(2)**:** 311-324.
296. Neumann N, Lundin D, Poole AM. Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor. *PLoS One* 2010, **5**(10)**:** e13241.
297. Sealey-Cardona M, Schmidt K, Demmel L, Hirschmugl T, Gesell T, Dong G*, et al.* Sec16 determines the size and functioning of the Golgi in the protist parasite, Trypanosoma brucei. *Traffic* 2014, **15**(6)**:** 613-629.
298. Bhattacharya D, Glick BS. Two Mammalian Sec16 Homologues Have Nonredundant Functions in Endoplasmic Reticulum (ER) Export and Transitional ER Organization. *Mol Biol Cell* 2007, **18:** 839-849.
299. Connerly PL, Esaki M, Montegna EA, Strongin DE, Levi S, Soderholm J*, et al.* Sec16 is a Determinant of Transitional ER Organization. *Curr Biol* 2005, **15**(16)**:** 1439-1447.
300. Witte K, Schuh AL, Hegermann J, Sarkeshik A, Mayers JR, Schwarze K*, et al.* TFG-1 function in protein secretion and oncogenesis. *Nat Cell Biol* 2011, **13**(5)**:** 550-558.
301. Budnik A, Heesom KJ, Stephens DJ. Characterization of human Sec16B: indications of specialized, non-redundant functions. *Sci Rep* 2011, **77**.
302. Tani K, Tagaya M, Yonekawa S, Baba T. Dual function of Sec16B: Endoplasmic reticulum-derived protein secretion and peroxisome biogenesis in mammalian cells. *Cellular logistics* 2011, **1**(4)**:** 164-167.
303. Yonekawa S, Furuno A, Baba T, Fujiki Y, Ogasawara Y, Yamamoto A*, et al.* Sec16B is involved in the endoplasmic reticulum export of the peroxisomal membrane biogenesis factor peroxin 16 (Pex16) in mammalian cells. *Proc Natl Acad Sci U S A* 2011, **108**(31)**:** 12746-12751.
304. Lv D, Zhang DD, Wang H, Zhang Y, Liang L, Fu JF*, et al.* Genetic variations in SEC16B, MC4R, MAP2K5 and KCTD15 were associated with childhood obesity and interacted with dietary behaviors in Chinese school-age population. *Gene* 2015, **560**(2)**:** 149-155.
305. Ng MC, Tam CH, So WY, Ho JS, Chan AW, Lee HM*, et al.* Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese. *The Journal of clinical endocrinology and metabolism* 2010, **95**(5)**:** 2418-2425.
306. Albuquerque D, Nobrega C, Rodriguez-Lopez R, Manco L. Association study of common polymorphisms in MSRA, TFAP2B, MC4R, NRXN3, PPARGC1A, TMEM18, SEC16B, HOXB5 and OLFM4 genes with obesity-related traits among Portuguese children. *Journal of human genetics* 2014, **59**(6)**:** 307-313.
307. Hotta K, Nakamura M, Nakamura T, Matsuo T, Nakata Y, Kamohara S*, et al.* Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. *Journal of human genetics* 2009, **54**(12)**:** 727-731.
308. Ahmad S, Rukh G, Varga TV, Ali A, Kurbasic A, Shungin D*, et al.* Gene x physical activity interactions in obesity: combined analysis of 111,421 individuals of European ancestry. *PLoS genetics* 2013, **9**(7)**:** e1003607.
309. Bharucha N, Liu Y, Papanikou E, McMahon C, Esaki M, Jeffrey PD*, et al.* Sec16 influences transitional ER sites by regulating rather than organizing COPII. *Mol Biol Cell* 2013, **24:** 3406-3419.

310. Zacharogianni M, Kondylis V, Tang Y, Farhan H, Xanthakis D, Fuchs F*, et al.* ERK7 is a negative regulator of protein secretion in response to amino-acid starvation by modulating Sec16 membrane association. *The EMBO journal* 2011, **30**(18)**:** 3684-3700.
311. Gimeno RE, Espenshade P, Kaiser CA. COPII coat subunit interactions: Sec24p and Sec23p bind to adjacent regions of Sec16p. *Mol Biol Cell* 1996, **7**(11)**:** 1815-1823.
312. Watson P, Townley AK, Koka P, Palmer KJ, Stephens DJ. Sec16 defines endoplasmic reticulum exit sites and is required for secretory cargo export in mammalian cells. *Traffic* 2006, **7**(12)**:** 1678-1687.
313. Iinuma T, Shiga A, Nakamoto K, O'Brien MB, Aridor M, Arimitsu N*, et al.* Mammalian Sec16/p250 plays a role in membrane traffic from the endoplasmic reticulum. *J Biol Chem* 2007, **282**(24)**:** 17632-17639.
314. Farhan H, Wendeler MW, Mitrovic S, Fava E, Silberberg Y, Sharan R*, et al.* MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *J Cell Biol* 2010, **189**(6)**:** 997-1011.
315. Zacharogianni M, Gomez AA, Veenendaal T, Smout J, Rabouille C. A stress assembly that confers cell viability by preserving ERES components during amino-acid starvation. *eLife* 2014, **3**.
316. Johnson A, Bhattacharya N, Hanna M, Pennington JG, Schuh AL, Wang L*, et al.* TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization. *EMBO J* 2015.
317. van Zuylen WJ, Doyon P, Clement JF, Khan KA, D'Ambrosio LM, Do F*, et al.* Proteomic profiling of the TRAF3 interactome network reveals a new role for the ER-to-Golgi transport compartments in innate immunity. *PLoS pathogens* 2012, **8**(7)**:** e1002747.
318. Cho HJ, Yu J, Xie C, Rudrabhatla P, Chen X, Wu J*, et al.* Leucine-rich repeat kinase 2 regulates Sec16A at ER exit sites to allow ER-Golgi export. *EMBO J* 2014, **33:** 2314-2331.
319. Wallings R, Manzoni C, Bandopadhyay R. Cellular processes associated with LRRK2 function and dysfunction. *FEBS J* 2015.
320. Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A*, et al.* LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J* 2007, **405**(2)**:** 307-317.
321. Taymans JM, Baekelandt V. Phosphatases of alpha-synuclein, LRRK2, and tau: important players in the phosphorylation-dependent pathology of Parkinsonism. *Frontiers in genetics* 2014, **5:** 382.
322. Kodera C, Yorimitsu T, Sato K. Sec23 homolog Nel1 is a novel GTPase-activating protein for Sar1 but does not function as a subunit of the coat protein complex II (COPII) coat. *J Biol Chem* 2014, **289**(31)**:** 21423-21432.
323. Farhan H, Weiss M, Tani K, Kaufman RJ, Hauri HP. Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load. *EMBO J* 2008, **27**(15)**:** 2043-2054.
324. Aridor M, Bannykh SI, Rowe T, Balch WE. Cargo can modulate COPII vesicle formation from the endoplasmic reticulum. *J Biol Chem* 1999, **274**(7)**:** 4389-4399.
325. Pathre P, Shome K, Blumental-Perry A, Bielli A, Haney CJ, Alber S*, et al.* Activation of phospholipase D by the small GTPase Sar1p is required to support COPII assembly and ER export. *The EMBO journal* 2003, **22**(16)**:** 4059-4069.
326. Klinkenberg D, Long KR, Shome K, Watkins SC, Aridor M. A cascade of ER exit site assembly that is regulated by p125A and lipid signals. *J Cell Sci* 2014, **127**(Pt 8)**:** 1765-1778.
327. Tani K, Mizoguchi T, Iwamatsu A, Hatsuzawa K, Tagaya M. p125 is a novel mammalian Sec23p-interacting protein with structural similarity to phospholipid-modifying proteins. *J Biol Chem* 1999, **274**(29)**:** 20505-20512.
328. Inoue H, Baba T, Sato S, Ohtsuki R, Takemori A, Watanabe T*, et al.* Roles of SAM and DDHD domains in mammalian intracellular phospholipase A1 KIAA0725p. *Biochim Biophys Acta* 2012, **1823**(4)**:** 930-939.
329. Mizoguchi T, Nakajima K, Hatsuzawa K, Nagahama M, Hauri HP, Tagaya M*, et al.* Determination of functional regions of p125, a novel mammalian Sec23p-interacting protein. *Biochem Biophys Res Commun* 2000, **279**(1)**:** 144-149.
330. Ong YS, Tang BL, Loo LS, Hong W. p125A exists as part of the mammalian Sec13/Sec31 COPII subcomplex to facilitate ER-Golgi transport. *J Cell Biol* 2010, **190**(3)**:** 331-345.

331. Shimoi W, Ezawa I, Nakamoto K, Uesaki S, Gabreski G, Aridor M*, et al.* p125 is localized in endoplasmic reticulum exit sites and involved in their organization. *J Biol Chem* 2005, **280**(11)**:** 10141-10148.
332. Arimitsu N, Kogure T, Baba T, Nakao K, Hamamoto H, Sekimizu K*, et al.* p125/Sec23-interacting protein (Sec23ip) is required for spermiogenesis. *FEBS Lett* 2011, **585**(14)**:** 2171-2176.
333. McGary KL, Park TJ, Woods JO, Cha HJ, Wallingford JB, Marcotte EM. Systematic discovery of nonobvious human disease models through orthologous phenotypes. *Proc Natl Acad Sci U S A* 2010, **107**(14)**:** 6544-6549.
334. Blumental-Perry A, Haney CJ, Weixel KM, Watkins SC, Weisz OA, Aridor M. Phosphatidylinositol 4-phosphate formation at ER exit sites regulates ER export. *Dev Cell* 2006, **11**(5)**:** 671-682.
335. Palmer KJ, Konkel JE, Stephens DJ. PCTAIRE protein kinases interact directly with the COPII complex and modulate secretory cargo transport. *J Cell Sci* 2005, **118**(Pt 17)**:** 3839-3847.
336. Yamasaki A, Tani K, Yamamoto A, Kitamura N, Komada M. The Ca2+-binding protein ALG-2 is recruited to endoplasmic reticulum exit sites by Sec31A and stabilizes the localization of Sec31A. *Mol Biol Cell* 2006, **17**(11)**:** 4876-4887.
337. la Cour JM, Schindler AJ, Berchtold MW, Schekman R. ALG-2 attenuates COPII budding in vitro and stabilizes the Sec23/Sec31A complex. *PLoS One* 2013, **8**(9)**:** e75309.
338. Shibata H, Kanadome T, Sugiura H, Yokoyama T, Yamamuro M, Moss SE*, et al.* A new role for annexin A11 in the early secretory pathway via stabilizing Sec31A protein at the endoplasmic reticulum exit sites (ERES). *J Biol Chem* 2015, **290**(8)**:** 4981-4993.
339. Helm JR, Bentley M, Thorsen KD, Wang T, Foltz L, Oorschot V*, et al.* Apoptosis-linked gene-2 (ALG-2)/Sec31 interactions regulate endoplasmic reticulum (ER)-to-Golgi transport: a potential effector pathway for luminal calcium. *J Biol Chem* 2014, **289**(34)**:** 23609-23628.
340. Brandtstaetter H, Kruppa AJ, Buss F. Huntingtin is required for ER-to-Golgi transport and for secretory vesicle fusion at the plasma membrane. *Dis Model Mech* 2014, **7**(12)**:** 1335-1340.
341. Bianco A, Reghellin V, Donnici L, Fenu S, Alvarez R, Baruffa C*, et al.* Metabolism of phosphatidylinositol 4-kinase IIIalpha-dependent PI4P Is subverted by HCV and is targeted by a 4-anilino quinazoline with antiviral activity. *PLoS pathogens* 2012, **8**(3)**:** e1002576.
342. Bi K, Roth MG, Ktistakis NT. Phosphatidic acid formation by phospholipase D is required for transport from the endoplasmic reticulum to the Golgi complex. *Curr Biol* 1997, **7**(5)**:** 301-307.
343. Topham MK, Prescott SM. Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions. *J Biol Chem* 1999, **274**(17)**:** 11447-11450.
344. Nagaya H, Wada I, Jia YJ, Kanoh H. Diacylglycerol kinase delta suppresses ER-to-Golgi traffic via its SAM and PH domains. *Mol Biol Cell* 2002, **13**(1)**:** 302-316.
345. Szul T, Sztul E. COPII and COPI traffic at the ER-Golgi interface. *Physiology (Bethesda)* 2011, **26**(5)**:** 348-364.
346. Jackson LP. Structure and mechanism of COPI vesicle biogenesis. *Curr Opin Cell Biol* 2014, **29:** 67-73.
347. Hara-Kuge S, Kuge O, Orci L, Amherdt M, Ravazzola M, Wieland FT*, et al.* En bloc incorporation of coatomer subunits during the assembly of COP-coated vesicles. *J Cell Biol* 1994, **124:** 883-892.
348. Cosson P, Letourneur F. Coatomer interaction with di-lysine endoplasmic reticulum retention motifs. *Science* 1994, **263:** 1629-1631.
349. Schröder-Köhne S, Letourneur F, Riezman H. Alpha-COP can discriminate between distinct, functional di-lysine signals in vitro and regulates access into retrograde transport. *J Cell Sci* 1998, **111:** 3459-3470.
350. Eugster A, Frigerio G, Dale M, Duden R. The alpha- and beta'-COP WD40 domains mediate cargo-selective interactions with distinct di-lysine motifs. *Mol Biol Cell* 2004, **15:** 1011-1023.
351. Ren X, Farias GG, Canagarajah BJ, Bonifacino JS, Hurley JH. Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1. *Cell* 2013, **152**(4)**:** 755-767.

352. Pepperkok R, Whitney JA, Gomez M, Kreis TE. COPI vesicles accumulating in the presence of a GTP restricted arf1 mutant are depleted of anterograde and retrograde cargo. *J Cell Sci* 2000, **113 (Pt 1):** 135-144.
353. Zhu Y, Traub LM, Kornfeld S. ADP-ribosylation factor 1 transiently activates high-affinity adaptor protein complex AP-1 binding sites on Golgi membranes. *Mol Biol Cell* 1998, **9**(6)**:** 1323-1337.
354. Crottet P, Meyer DM, Rohrer J, Spiess M. ARF1.GTP, tyrosine-based signals, and phosphatidylinositol 4,5-bisphosphate constitute a minimal machinery to recruit the AP-1 clathrin adaptor to membranes. *Mol Biol Cell* 2002, **13**(10)**:** 3672-3682.
355. Nie Z, Randazzo PA. Arf GAPs and membrane traffic. *J Cell Sci* 2006, **119**(Pt 7)**:** 1203-1211.
356. Nickel W, Malsam J, Gorgas K, Ravazzola M, Jenne N, Helms JB*, et al.* Uptake by COPI-coated vesicles of both anterograde and retrograde cargo is inhibited by GTPgammaS in vitro. *J Cell Sci* 1998, **111 (Pt 20):** 3081-3090.
357. Balch WE, Kahn RA, Schwaninger R. ADP-ribosylation factor is required for vesicular trafficking between the endoplasmic reticulum and the cis-Golgi compartment. *J Biol Chem* 1992, **267**(18)**:** 13053-13061.
358. Kahn RA, Randazzo P, Serafini T, Weiss O, Rulka C, Clark J*, et al.* The amino terminus of ADP-ribosylation factor (ARF) is a critical determinant of ARF activities and is a potent and specific inhibitor of protein transport. *J Biol Chem* 1992, **267**(18)**:** 13039-13046.
359. Lanoix J, Ouwendijk J, Lin CC, Stark A, Love HD, Ostermann J*, et al.* GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. *The EMBO journal* 1999, **18**(18)**:** 4935-4948.
360. Serafini T, Orci L, Amherdt M, Brunner M, Kahn RA, Rothman JE. ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. *Cell* 1991, **67**(2)**:** 239-253.
361. Donaldson JG, Cassel D, Kahn RA, Klausner RD. ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein beta-COP to Golgi membranes. *Proc Natl Acad Sci U S A* 1992, **89**(14)**:** 6408-6412.
362. Liang JO, Sung TC, Morris AJ, Frohman MA, Kornfeld S. Different domains of mammalian ADP-ribosylation factor 1 mediate interaction with selected target proteins. *J Biol Chem* 1997, **272**(52)**:** 33001-33008.
363. Zhang CJ, Rosenwald AG, Willingham MC, Skuntz S, Clark J, Kahn RA. Expression of a dominant allele of human ARF1 inhibits membrane traffic in vivo. *J Cell Biol* 1994, **124**(3)**:** 289-300.
364. Eugster A, Frigerio G, Dale M, Duden R. COP I domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP. *EMBO J* 2000, **19:** 3905-3917.
365. Palmer DJ, Helms JB, Beckers CJ, Orci L, Rothman JE. Binding of coatomer to Golgi membranes requires ADP-ribosylation factor. *J Biol Chem* 1993, **268**(16)**:** 12083-12089.
366. Teal SB, Hsu VW, Peters PJ, Klausner RD, Donaldson JG. An activating mutation in ARF1 stabilizes coatomer binding to Golgi membranes. *J Biol Chem* 1994, **269**(5)**:** 3135-3138.
367. Sun Z, Anderl F, Frohlich K, Zhao L, Hanke S, Brugger B*, et al.* Multiple and stepwise interactions between coatomer and ADP-ribosylation factor-1 (Arf1)-GTP. *Traffic* 2007, **8**(5)**:** 582-593.
368. Randazzo PA, Nie Z, Miura K, Hsu VW. Molecular aspects of the cellular activities of ADP-ribosylation factors. *Science's STKE : signal transduction knowledge environment* 2000, **2000**(59)**:** re1.
369. Reinhard C, Schweikert M, Wieland FT, Nickel W. Functional reconstitution of COPI coat assembly and disassembly using chemically defined components. *Proc Natl Acad Sci U S A* 2003, **100**(14)**:** 8253-8257.
370. Spang A, Matsuoka K, Hamamoto S, Schekman R, Orci L. Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes. *Proc Natl Acad Sci U S A* 1998, **95**(19)**:** 11199-11204.
371. Jackson CL. GEF-effector interactions. *Cellular logistics* 2014, **4**(2)**:** e943616.
372. Quilty D, Gray F, Summerfeldt N, Cassel D, Melancon P. Arf activation at the Golgi is modulated by feed-forward stimulation of the exchange factor GBF1. *J Cell Sci* 2014, **127**(Pt 2)**:** 354-364.

373. Zhao X, Claude A, Chun J, Shields DJ, Presley JF, Melancon P. GBF1, a cis-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic. *J Cell Sci* 2006, **119**(Pt 18)**:** 3743-3753.
374. Szul T, Garcia-Mata R, Brandon E, Shestopal S, Alvarez C, Sztul E. Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor GBF1. *Traffic* 2005, **6**(5)**:** 374-385.
375. Kawamoto K, Yoshida Y, Tamaki H, Torii S, Shinotsuka C, Yamashina S*, et al.* GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the cis-Golgi and involved in membrane association of the COPI coat. *Traffic* 2002, **3**(7)**:** 483-495.
376. Niu TK, Pfeifer AC, Lippincott-Schwartz J, Jackson CL. Dynamics of GBF1, a Brefeldin A-sensitive Arf1 exchange factor at the Golgi. *Mol Biol Cell* 2005, **16**(3)**:** 1213-1222.
377. Bai M, Pang X, Lou J, Zhou Q, Zhang K, Ma J*, et al.* Mechanistic insights into regulated cargo binding by ACAP1 protein. *J Biol Chem* 2012, **287**(34)**:** 28675-28685.
378. Yang JS, Lee SY, Gao M, Bourgoin S, Randazzo PA, Premont RT*, et al.* ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. *J Cell Biol* 2002, **159**(1)**:** 69-78.
379. Kartberg F, Asp L, Dejgaard SY, Smedh M, Fernandez-Rodriguez J, Nilsson T*, et al.* ARFGAP2 and ARFGAP3 are essential for COPI coat assembly on the Golgi membrane of living cells. *J Biol Chem* 2010, **285**(47)**:** 36709-36720.
380. Lee SY, Yang JS, Hong W, Premont RT, Hsu VW. ARFGAP1 plays a central role in coupling COPI cargo sorting with vesicle formation. *J Cell Biol* 2005, **168**(2)**:** 281-290.
381. Nie Z, Boehm M, Boja ES, Vass WC, Bonifacino JS, Fales HM*, et al.* Specific regulation of the adaptor protein complex AP-3 by the Arf GAP AGAP1. *Dev Cell* 2003, **5**(3)**:** 513-521.
382. Chun J, Shapovalova Z, Dejgaard SY, Presley JF, Melançon P. Characterization of class I and II ADP-ribosylation factors (Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1. *Mol Biol Cell* 2008, **19:** 3488-3500.
383. Chun J, Shapovalova Z, Dejgaard SY, Presley JF, Melancon P. Characterization of class I and II ADP-ribosylation factors (Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1. *Mol Biol Cell* 2008, **19**(8)**:** 3488-3500.
384. Moelleken J, Malsam J, Betts MJ, Movafeghi A, Reckmann I, Meissner I*, et al.* Differential localization of coatomer complex isoforms within the Golgi apparatus. *Proc Natl Acad Sci U S A* 2007, **104:** 4425-4430.
385. Blagitko N, Schulz U, Schinzel AA, Ropers HH, Kalscheuer VM. gamma2-COP, a novel imprinted gene on chromosome 7q32, defines a new imprinting cluster in the human genome. *Hum Mol Genet* 1999, **8:** 2387-2396.
386. Dascher C, Balch WE. Dominant inhibitory mutants of ARF1 block endoplasmic reticulum to Golgi transport and trigger disassembly of the Golgi apparatus. *J Biol Chem* 1994, **269**(2)**:** 1437-1448.
387. Reiterer V, Maier S, Sitte HH, Kriz A, Ruegg MA, Hauri HP*, et al.* Sec24- and ARFGAP1-dependent trafficking of GABA transporter-1 is a prerequisite for correct axonal targeting. *J Neurosci* 2008, **28**(47)**:** 12453-12464.
388. Stephens DJ, Pepperkok R. Imaging of procollagen transport reveals COPI-dependent cargo sorting during ER-to-Golgi transport in mammalian cells. *J Cell Sci* 2002, **115**(Pt 6)**:** 1149-1160.
389. Shima DT, Scales SJ, Kreis TE, Pepperkok R. Segregation of COPI-rich and anterograde-cargo-rich domains in endoplasmic-reticulum-to-Golgi transport complexes. *Curr Biol* 1999, **9**(15)**:** 821-824.
390. Ben-Tekaya H, Miura K, Pepperkok R, Hauri HP. Live imaging of bidirectional traffic from the ERGIC. *J Cell Sci* 2005, **118:** 357-367.
391. Wilson DW, Lewis MJ, Pelham HR. pH-dependent binding of KDEL to its receptor in vitro. *J Biol Chem* 1993, **268:** 7465-7468.
392. Lippincott-Schwartz J, Cole NB, Marotta A, Conrad PA, Bloom GS. Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. *J Cell Biol* 1995, **128:** 293-306.
393. Roghi C, Allan VJ. Dynamic association of cytoplasmic dynein heavy chain 1a with the Golgi apparatus and intermediate compartment. *J Cell Sci* 1999, **112:** 4673-4685.

394. Glick BS, Elston T, Oster G. A cisternal maturation mechanism can explain the asymmetry of the Golgi stack. *FEBS Lett* 1997, **414:** 177-181.
395. Beznoussenko GV, Parashuraman S, Rizzo R, Polishchuk R, Martella O, Di Giandomenico D*, et al.* Transport of soluble proteins through the Golgi occurs by diffusion via continuities across cisternae. *eLife* 2014, **3**.
396. Lippincott-Schwartz J, Roberts TH, Hirschberg K. Secretory protein trafficking and organelle dynamics in living cells. *Annu Rev Cell Dev Biol* 2000, **16:** 557-589.
397. Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J. ER-to-Golgi transport visualized in living cells. *Nature* 1997, **389**(6646)**:** 81-85.
398. Scales SJ, Pepperkok R, Kreis TE. Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell* 1997, **90**(6)**:** 1137-1148.
399. Zhang YC, Zhou Y, Yang CZ, Xiong DS. A review of ERGIC-53: its structure, functions, regulation and relations with diseases. *Histol Histopathol* 2009, **24:** 1193-1204.
400. Saraste J, Dale HA, Bazzocco S, Marie M. Emerging new roles of the pre-Golgi intermediate compartment in biosynthetic-secretory trafficking. *FEBS Lett* 2009, **583**(23)**:** 3804-3810.
401. Marie M, Dale HA, Sannerud R, Saraste J. The function of the intermediate compartment in pre-Golgi trafficking involves its stable connection with the centrosome. *Mol Biol Cell* 2009, **20:** 4458-4470.
402. Sannerud R, Marie M, Nizak C, Dale HA, Pernet-Gallay K, Perez F*, et al.* Rab1 defines a novel pathway connecting the pre-Golgi intermediate compartment with the cell periphery. *Mol Biol Cell* 2006, **17:** 1514-1526.
403. Kamena F, Spang A. Tip20p prohibits back-fusion of COPII vesicles with the endoplasmic reticulum. *Science* 2004, **304:** 286-289.
404. Yu S, Satoh A, Pypaert M, Mullen K, Hay JC, Ferro-Novick S. mBet3p is required for homotypic COPII vesicle tethering in mammalian cells. *J Cell Biol* 2006, **174:** 359-368.
405. Cai H, Yu S, Menon S, Cai Y, Lazarova D, Fu C*, et al.* TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature* 2007.
406. Cai Y, Chin HF, Lazarova D, Menon S, Fu C, Cai H*, et al.* The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell* 2008, **133:** 1202-1213.
407. Allan BB, Moyer BD, Balch WE. Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science* 2000, **289:** 444-448.
408. Alvarez C, Fujita H, Hubbard A, Sztul E. ER to Golgi transport: Requirement for p115 at a pre-Golgi VTC stage. *J Cell Biol* 1999, **147:** 1205-1222.
409. Milne DM, Looby P, Meek DW. Catalytic activity of protein kinase CK1 delta (casein kinase 1delta) is essential for its normal subcellular localization. *Exp Cell Res* 2001, **263:** 43-54.
410. Yu S, Roth MG. Casein kinase I regulates membrane binding by ARF GAP1. *Mol Biol Cell* 2002, **13**(8)**:** 2559-2570.
411. Lee H, Chen R, Lee Y, Yoo S, Lee C. Essential roles of CKIdelta and CKIepsilon in the mammalian circadian clock. *Proc Natl Acad Sci U S A* 2009, **106**(50)**:** 21359-21364.
412. Fan JY, Preuss F, Muskus MJ, Bjes ES, Price JL. Drosophila and vertebrate casein kinase Idelta exhibits evolutionary conservation of circadian function. *Genetics* 2009, **181**(1)**:** 139-152.
413. Appenzeller-Herzog C, Roche AC, Nufer O, Hauri HP. pH-induced conversion of the transport lectin ERGIC-53 triggers glycoprotein release. *J Biol Chem* 2004, **279:** 12943-12950.
414. Paroutis P, Touret N, Grinstein S. The pH of the secretory pathway: measurement, determinants, and regulation. *Physiology (Bethesda)* 2004, **19:** 207-215.
415. Alvarez J, Montero M. Measuring [Ca2+] in the endoplasmic reticulum with aequorin. *Cell calcium* 2002, **32**(5-6)**:** 251-260.
416. Kawasaki N, Ichikawa Y, Matsuo I, Totani K, Matsumoto N, Ito Y*, et al.* The sugar-binding ability of ERGIC-53 is enhanced by its interaction with MCFD2. *Blood* 2008, **111**(4)**:** 1972-1979.
417. Demaurex N, Frieden M. Measurements of the free luminal ER Ca(2+) concentration with targeted "cameleon" fluorescent proteins. *Cell calcium* 2003, **34**(2)**:** 109-119.
418. Jackson MR, Nilsson T, Peterson PA. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J* 1990, **9:** 3153-3162.

419. Jackson LP, Lewis M, Kent HM, Edeling MA, Evans PR, Duden R*, et al.* Molecular basis for recognition of dilysine trafficking motifs by COPI. *Dev Cell* 2012, **23:** 1255-1262.
420. Ma W, Goldberg J. Rules for the recognition of dilysine retrieval motifs by coatomer. *EMBO J* 2013, **32:** 926-937.
421. Capitani M, Sallese M. The KDEL receptor: new functions for an old protein. *FEBS Lett* 2009, **583:** 3863-3871.
422. Pelham HR. Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment. *EMBO J* 1988, **7:** 913-918.
423. Munro S, Pelham HR. A C-terminal signal prevents secretion of luminal ER proteins. *Cell* 1987, **48:** 899-907.
424. Tang BL, Wong SH, Qi XL, Low SH, Hong W. Molecular cloning, characterization, subcellular localization and dynamics of p23, the mammalian KDEL receptor. *J Cell Biol* 1993, **120:** 325-338.
425. Farhan H, Reiterer V, Kriz A, Hauri HP, Pavelka M, Sitte HH*, et al.* Signal-dependent export of GABA transporter 1 from the ER-Golgi intermediate compartment is specified by a C-terminal motif. *J Cell Sci* 2008, **121:** 753-761.
426. Day KJ, Staehelin LA, Glick BS. A three-stage model of Golgi structure and function. *Histochem Cell Biol* 2013, **140:** 239-249.
427. Papanikou E, Glick BS. Golgi compartmentation and identity. *Curr Opin Cell Biol* 2014, **29:** 74-81.
428. Stanley P. Golgi glycosylation. *Cold Spring Harb Perspect Biol* 2011, **3:** a005199.
429. Dick G, Akslen-Hoel LK, Grøndahl F, Kjos I, Prydz K. Proteoglycan synthesis and Golgi organization in polarized epithelial cells. *J Histochem Cytochem* 2012, **60:** 926-935.
430. Anitei M, Hoflack B. Exit from the trans-Golgi network: from molecules to mechanisms. *Curr Opin Cell Biol* 2011, **23:** 443-451.
431. Short B, Preisinger C, Körner R, Kopajtich R, Byron O, Barr FA. A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic. *J Cell Biol* 2001, **155:** 877-883.

Response of the early secretory pathway to the environment

The secretory pathway not only transports secretory proteins to the extracellular environment, it is also involved in maintaining cellular homeostasis and in general in supporting cellular function. However, cellular requirements change, for example during cell growth or differentiation, or in response to changes in the environment, which induces cell stress and requires survival mechanisms. The secretory pathway must adapt to these changes, and it must therefore be able to sense these changes and receive signals from the environment.

1 Response of the early secretory pathway to signaling

It is now commonly accepted that the secretory pathway is the recipient of kinase signaling in order to be able to react to changes in the environment and adapt its function accordingly. Below, the process of signal transduction, in particular MAPK signaling, will be briefly introduced, followed by what is currently known about signaling to the early secretory pathway.

1.1 The MAPK signaling pathways

The translation of extracellular stimuli into cellular responses is a highly conserved mechanism. Membrane-spanning cell surface receptors bind extracellular molecules or ligands. Upon ligand binding, receptors initiate intracellular signaling cascades with the help of adaptor proteins. Different kinds of receptors exist that recognize different ligands and make use of different intracellular transduction pathways, such as for example integrins which bind the extracellular matrix, cytokine receptors, G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTK) [1, 2]. The most common signal transduction mechanism is via the addition of phosphates to specific amino acid residues (mostly serine, threonine, and tyrosine) on target proteins, in a process called phosphorylation. This conserved process is mediated by proteins called kinases, which recognize specific sequence patterns in their target proteins [1, 3]. Possibly the best studied of intracellular signaling cascades are the mitogen-activated protein kinase (MAPK) signaling cascades. Four different MAPK cascades have been described

depending on which MAPK components are involved, and they each fulfill different physiological activities (see Figure 3) [4, 5].

The extracellular signal-regulated kinase 1 and 2 (ERKs) are involved in proliferation and differentiation, whereas the two cascades around c-Jun N-terminal kinase (JNK; also known as stress-activated protein kinase 1 (SAPK1)) and p38 MAPK (also known as SAPK2-4 or p38 α-δ) are involved in the cellular stress response and apoptosis. The fourth group consists mainly of ERK5, also known as Big MAPK (BMK), and other identified MAP kinases that do not fit in another group, such as ERK7 and 8. ERK5 appears to be involved in both mitogenic and stress signaling, whereas activation of ERK7 and 8 remains elusive [2, 4, 5, 6, 7].

The general principle of activation within these MAPK cascades is the same, although different proteins are used. The MAPK cascades can be activated in various ways, these could be through small GTP-binding proteins, adaptor proteins, or via a mediator kinase (MAP4K) that activates the MAPK kinase kinase (MAP3K). The activated MAP3K then signals to the MAPK kinase (MAPKK) which in turn activates the MAP kinase (MAPK), which finally activates the MAPK-activated protein kinase (MAPKAPK). Within these cascades, MAP3K, MAPKK and MAPK are seen as core components, whereas MAP4K and MAPKAPK are involved in specific cases. Furthermore, although MAP4K, MAP3K and MAPKK mainly function in signal transmission to the next level within the cascade, both MAPK and MAPKAPK can each phosphorylate many different substrates which regulate different cellular outcomes [2, 4, 5, 8].

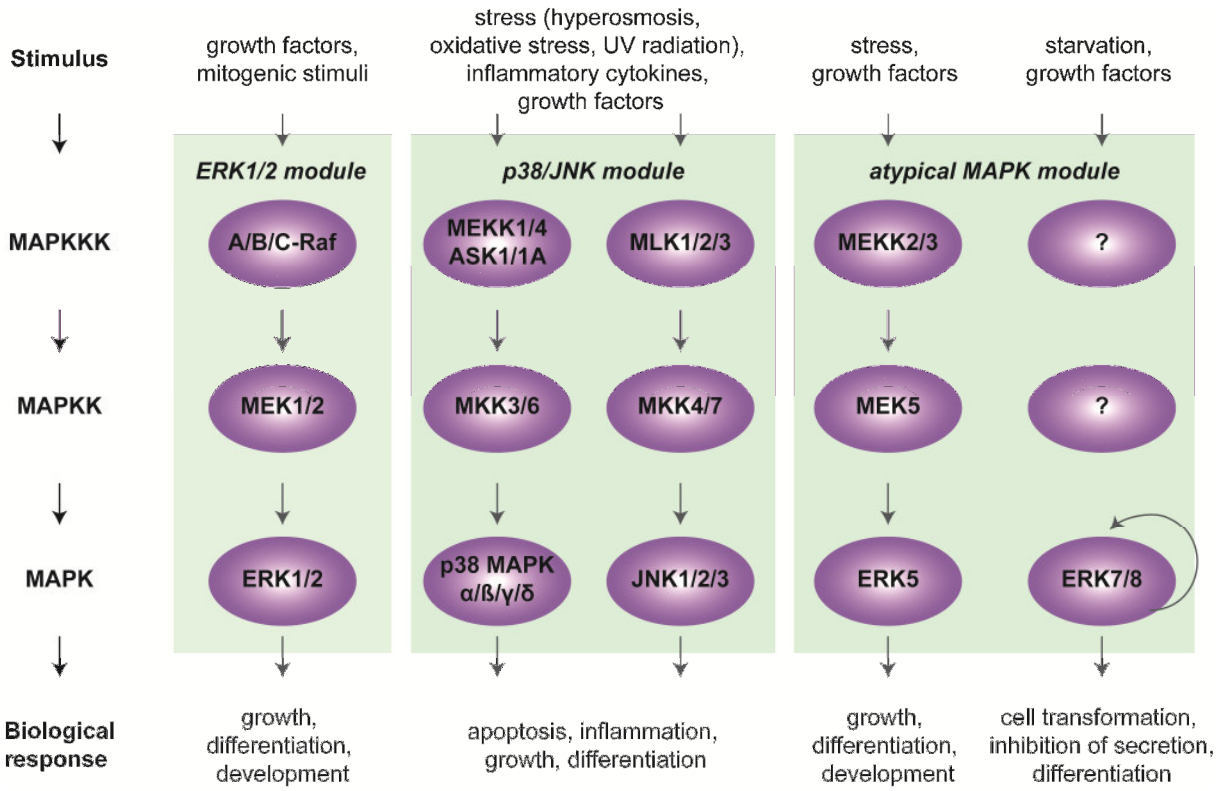


Figure 3: Schematic representation of the different MAPK cascades, indicating the activating stimuli, the kinases involved (purple), and the biological outcome

1.2 Growth factor signaling via the ERK1/2 cascade

The ERK1/2 cascade is the best investigated of the MAPK cascades, and it is mostly involved in the induction and regulation of cell growth, proliferation, differentiation and motility. Dysregulation of this cascade has been found to be a major contribution to various pathologies, such as neurodegenerative disease, developmental diseases, diabetes, and most importantly in different types of cancer [8, 9, 10, 11].

Activation of the ERK1/2 cascade usually takes place in response to extracellular stimuli, due to binding of different growth factors, hormones or neurotransmitters to receptors that activate the cascade [12]. One of these stimuli can be signaling induced by binding of epidermal growth factor (EGF) to the EGF receptor (EGFR). The EGFR is a single spanning transmembrane protein; additionally, it has an extracellular ligand-binding domain and an intracytoplasmic tyrosine kinase domain, as it belongs to a family of receptor tyrosine kinases. Binding of the ligand to EGFR induces a conformational change in the receptor, which causes the receptor to dimerize with another EGFR. Upon dimerization, the receptors autophosphorylate several tyrosine

residues within their C-terminal tail. These phosphorylated tyrosine residues provide docking sites for specific cytosolic proteins that contain Scr homology 2 (SH2) and phosphotyrosine-binding domains. Next, the adaptor protein Grb2 associates to EGFR with the help of the adaptor molecule Shc, and the exchange factor SOS is recruited. An exchange factor protein catalyzes the exchange of GDP to GTP, thereby activating a G-protein; in this case, SOS recruits GDP-bound Ras and activates it (GTP-Ras). Ras then activates a MAP3K, which could be the protein kinases Raf-1, B-Raf or A-Raf. It is known that the Raf kinases are recruited to the membrane in order to be activated, however, the mechanism by which this activation takes place is still not clear. Next, the MAP3K activates the MAP2K, which is MEK1 or 2 (MEK1/2) via phosphorylation. Activated MEK1/2 then phosphorylates ERK1/2 at the MAPK level, which are seen as executors of the biological response [13, 14]. ERK1 and ERK2 share high sequence homology, therefore they fulfil many overlapping functions. ERK1, also known as p44, has a size of 44 kDa, whereas ERK2, also known as p42, is slightly smaller with 42 kDa. Both ERK1 and ERK2 phosphorylate serine or threonine residues that are located next to proline residues [15, 16, 17, 18]. ERK1/2 have been shown to be able to phosphorylate more than 200 different substrates localized in different cellular compartments, such as the cytoplasm, the nucleus, and the cytosolic face of various organelles [19, 20]. In the nucleus, ERK1/2 phosphorylate transcription factors such as c-Fos, c-Jun or Elk1. The transcription factor Elk1 is one of the main regulators of immediate early genes; these are genes whose transcription factors must be activated within minutes after the extracellular stimulus to provide a fast response. Elk1 forms a ternary complex with serum response factor (SRF), a transcription factor that has been shown to transcriptionally regulate several growth factor-induced genes, such as the transcription factor Early growth response-1 and -3 (Egr1 and 3) [21, 22, 23, 24]. The Egr1 transcription factor is part of a family of transcription factors that will be described below.

1.3 The non-ERK1/2 cascades

The non-ERK1/2 signaling cascades are also known as stress signaling cascades, as they are activated by both growth factor signaling and different stress stimuli.

The p38 MAPK cascade shows some crosstalk and overlap in substrates with the ERK1/2 and the JNK cascade, and is implicated in a variety of cellular processes, such as response to stress, apoptosis, cellular senescence, cell cycle checkpoint regulation,

cell survival, and in the regulation of immunological effects [8, 11, 25, 26]. It is activated by stress-related stimuli including UV light, heat, osmotic shock, or in response to stimulation with inflammatory cytokines such as Tumor Necrosis Factor-α and β (TNF-α and β) and Interleukin-1 (IL1), or to growth factors such as Colony Stimulating Factor-1 (CSF-1) [25, 27, 28, 29]. Receptor activation or environmental cues lead to activation of upstream adaptor proteins and ultimately to activation of the p38 MAPK cascade. This activation is mediated by MAP3K kinases such as ASK1 or MEKK4, or by low molecular weight GTP-binding proteins of the Rho family such as the small GTPases Cdc42 and Rac1. In this context, p21-activated kinases (PAKs) such as PAK1, PAK2 and PAK3 have been shown to be activated by Cdc42 and Rac [8, 27, 30]. After activation of the cascade at the MAP3K level, activation of the following kinases takes place via phosphorylation, similar to the ERK1/2 cascade. The kinases at the MAPKK level are mostly Mitogen-activated protein kinase kinase 3 (MKK3) and MKK6, although MKK4 is also involved [26, 31, 32]. These kinases then activate the MAPKs in the cascade, which are the four isoforms (α, β, γ, δ) of p38, although some alternative splice variants also exist. The four p38 isoforms have a molecular weight of 38 kDa; two isoforms, p38α and p38β are expressed ubiquitously, whereas expression of p38γ and p38δ is dependent upon cell type. p38γ is expressed most abundantly in skeletal muscle, whereas p38δ is found in the pituitary and adrenal gland. Additionally, p38γ and p38δ are key components in the innate immune response. The p38 kinases contain a threonine-glycine-tyrosine dual phosphorylation motif, which is phosphorylated upon activation [32, 33, 34, 35, 36]. The activated p38s can then phosphorylate their substrates, which can be either MAPKAPK components, such as MAPKAPK2 and 3, MNK and MSK, which are also activated by the ERK1/2 cascade, or MAPKAPK5 or regulatory molecules such as PLA2 and heat shock proteins. Additionally, phosphorylated p38 can translocate to the nucleus and phosphorylate and thereby activate a variety of transcription factors, such as activating transcription factor 1,2 and 6 (ATF-1/2/6), growth arrest and DNA damage inducible gene 153 (CHOP), p53, myocyte enhance factor 2C (MEF2C), cAMP response element binding protein (CREB), and Elk1 [27, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48].

Dysregulation of the p38 MAPK has been implicated in pathologies involving the immune system, such as inflammation-related diseases or autoimmune diseases, and in cardiovascular disease. Additionally, it might play a role in neurodegenerative diseases, diabetes, and cancer [49, 50, 51, 52]. The p38 MAPK cascade has opposing roles in cancer, as it induces apoptosis and inhibits transformation and invasion. However, it

was also shown to induce cancer progression. These opposing effects are likely due to different functions of the various p38 isoforms [8, 11, 53, 54, 55].

The c-Jun N-terminal kinase (JNK) cascade was named after the transcription factor c-Jun, which is a target of phosphorylation of this cascade. In general, the JNK cascade is stimulated by stresses such as UV radiation and oxidative DNA damage, but also mitogens [12, 25, 56, 57]. Dysregulation of the JNK cascade play a role in several diseases, especially in neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and amyotrophic lateral sclerosis (ALS). The JNK cascade has also been implicated in other diseases such as diabetes, chronic inflammatory diseases, as well as in several types of cancer [8, 9, 50, 58, 59].

The JNK cascade shares many components with the previously described p38 cascade, and both cascades are often activated simultaneously as they also respond to similar stimuli. Activation at the MAP3K level can take place either via small GTPases such as Cdc42 or Rac1, via adaptor molecules such as Tumor necrosis factor receptor-associated factors (TRAFs), or via MAP4Ks. Kinases at the MAP3K level are largely shared with the p38 cascade, such as Apoptosis signal-regulating kinase 1 (ASK1), TGFβ-activated kinase (TAK1), Mixed-lineage kinases 3 (MLK3) or the MEK kinases 1-3 (MEKK1/2/3). However, some MAP3Ks that are specific for the JNK cascade exist, these are ASK2, Leucine-zipper bearing kinase 1 (LZK1), MLK1 and Leucine-zipper and sterile-alpha motif kinase (ZAK). Signal transmission to the MAP3Ks involves interaction with specific scaffold proteins under specific conditions, such as the JNK-interacting proteins (JIPs) [60, 61, 62]. Upon activation, the MAP3Ks activate and phosphorylate Threonine and Serine residues in the activation loop of kinases at the MAPKK level, which are MKK4 and MKK7. These then phosphorylate the kinases at the MAPK level at their Tyrosine and Threonine residues in the activation loop [62]. In the JNK cascade, three basic MAP kinases exist, which are JNK1, JNK2 and JNK3. While JNK1 and JNK2 are expressed ubiquitously, JNK3 is exclusively expressed in the brain, heart and testis. Each kinase further undergoes differential splicing which results in multiple isoforms of all three JNKs. Additionally, each JNK has a short form (46 kDa) and a long form (54 kDa). The resulting large number of different JNK forms are involved in different cellular processes and thereby convey specificity of signaling, as they prefer different substrate proteins [8, 57, 58, 61, 63, 64, 65]. A large variety of substrates for the JNK cascade have been identified, that are located in the cytoplasm and the nucleus. Following activation, JNKs translocate to the

nucleus where they phosphorylate transcription factors such as c-Jun, ATF and Elk [57, 61].

The ERK5 cascade is named after ERK5, as this is the only MAPK currently assigned to this cascade. ERK5 is a large protein with a size of 110 kDa, and is therefore also known as Big MAPK (BMK1) [66, 67]. This cascade is activated in response to cellular stress such as oxidative stress and hyperosmolarity, but activation by mitogenic stimulation is equally common, and it has been implicated in cancer, although its involvement in physiological processes is still largely unknown [68, 69]. Activation of the ERK5 cascade appears to involve binding adaptor proteins such as Lad, and certain kinases that act as MAP4Ks, including WNK1. At the MAP3K level MEKK2 and 3 have been identified, although other kinases might be involved [70, 71, 72, 73, 74]. At the MAPKK level, MEK5 is the only protein involved, which phosphorylates ERK5. Similarly to ERK1/2, ERK5 is phosphorylated on the threonine and tyrosine residues within a sequence similar to ERK1/2, but no cross-reactivity between the ERK1/2 cascade and ERK5 has been reported [66, 70, 75]. Upon activation, ERK5 is able to phosphorylate several transcription factors, including c-Myc, MEF2 family members, and c-Fos [8, 76, 77, 78, 79, 80].

In addition to ERK5, other MAPK have been identified that are referred to as atypical MAPKs; these are ERK3/4, Nemo-like kinase (NLK), and ERK7. ERK3/4 and NLK are classified as atypical MAP kinases because they lack the characteristic Thr-Glu-Tyr phosphorylation site in their activation loop, whereas ERK7 is not a substrate of the MAPKK family [7].

The atypical kinase ERK7/8 (also known as MAPK15) was first identified as human ERK8, due to its lower than expected similarity to the previously identified rat ERK7. Therefore, this kinase is referred to as both ERK7 and ERK8, although in human cells it is still mostly referred to as ERK8 [7, 81, 82]. Similar to other classical MAP kinases, ERK7/8 contains the typical Thr-Glu-Tyr phosphorylation site in its activation loop, as well as a kinase domain that is 45% identical to the ERK1 kinase domain [83]. In contrast to other MAP kinases, ERK7/8 has a C-terminal extension of over 200 residues which was shown to be required for its autophosphorylation. In line with its autophosphorylating function, ERK7/8 has a high constitutive activity in serum starved cells, which cannot be suppressed by MAPK inhibitors. The activity of ERK7/8 may be mediated by de-phosphorylation and protein turnover, as ERK7/8 was shown to be ubiquitylated and rapidly degraded by the proteasome in proliferating cells [81, 84, 85, 86].

Despite its constitutive activity, ERK7/8 can be further activated in response to serum, DNA damage and by activity of human oncogenes [82, 86, 87]. The physiological function of ERK7/8 is still unclear, but overexpressed ERK7/8 was shown to localize to the nucleus, and ERK7/8 was implicated in the regulation of several nuclear processes. For example, ERK8 was found to be highly expressed in human colorectal cancer cells and promoted transformation by directly phosphorylating the transcription factor c-Jun [88]. ERK8 was also shown to mediate telomerase activity, as loss of ERK8 also decreased telomerase activity [89]. Another study found a role for ERK8 in protecting genomic integrity by directly interacting with chromatin and stabilizing Proliferating cell nuclear antigen (PCNA), as loss of PCNA leads to increased DNA damage [90]. Furthermore, a general role for ERK7/8 in regulation of nuclear receptors was suggested, as ERK7/8 has been shown to be a co-repressor for the estrogen-related-receptor α (ERRα) by inducing its relocation to the cytoplasm, thereby inhibiting its transcriptional activity [91]. ERK8 was also shown to downregulate transcription of the Glucocorticoid receptor α (GRα) by directly interacting with hydrogen peroxide inducible clone-5 (Hic-5), also known as androgen receptor activator 55 (ARA55), which is a co-activator of several nuclear receptors [92]. Recently, ERK7/8 was shown to mediate different processes within the secretory pathway. ERK8 was identified as negative regulator of ER O-glycosylation, and loss of ERK8 caused hyperactivation of ER O-glycosylation and increased cell motility. ER O-glycosylation is increased in cancer cells and promotes cell motility. Consequently, ERK8 was found to be downregulated in human breast and lung carcinomas [93]. ERK8 activity was also shown to be required for the induction of autophagy in basal conditions and upon amino acid starvation, by directly interacting with ATG8-like proteins and by indirectly decreasing inhibitory LC3 phosphorylation [94]. In *Drosophila* cells, ERK7 was found to be upregulated in insulin-producing median neurosecretory cells (IPCs) in larvae upon limiting dietary conditions. Upregulation of ERK7 was shown to inhibit secretion of insulin-like peptides (dILPs) from IPCs to inhibit tissue growth upon nutrient starvation [95]. In addition, ERK7 was shown to inhibit ER export by inducing disassembly of ERES in response to amino acid starvation. ERK7 was shown to target ERES via Sec16, as amino acid starvation stabilizes ERK7 protein levels, which caused a modification in the C-terminal region of Sec16 [96].

1.4 Regulation of MAPK cascades

As pointed out earlier, protein kinases are key regulators of cellular homeostasis. Thus, kinase signaling cascades are subject to sophisticated regulatory mechanisms that help fine-tune and adjust the strength and duration of signaling. These regulatory mechanisms include feed-back loops as well as de-activation of kinase signaling by phosphatases. In addition, signaling may be contained locally by scaffolding and compartmentalization [5].

An important factor in regulation of signaling is the strength and duration of a signal, and therefore, deactivation of kinases by phosphatases is an important process. Dephosphorylation of kinases by removal of phosphates is mediated by MAPK phosphatases (MKPs). As for kinases, different groups of phosphatases exist that differ upon their specificity for certain residues. Phosphatases that only dephosphorylate tyrosine residues or only serine/threonine residues exist, as well as dual specificity phosphatases (DUSPs) that can remove phosphogroups from either residues [4, 97, 98, 99]. In addition, some MKPs are able to regulate several MAPKs from different cascades, such as DUSP1/MKP-1 that dephosphorylates ERK, JNK and p38 in the nucleus, whereas other phosphatases show specificity, as the cytoplasmic phosphatase DUSP6/MKP-3 that exclusively dephosphorylates ERK, and DUSP10/MKP-5 and DUSP16/MKP-7 that prefer JNK and p38 over ERK [100, 101, 102, 103, 104, 105].

MAPK cascades function via sequential activation of the involved kinases, however, there is evidence for the involvement of scaffold proteins in these signaling cascades that may mediate the interaction between two kinases or even organize signaling complexes. Scaffold complexes regulate signaling by locally increasing kinase concentration and thereby allowing for localized activation of substrates. In addition, binding of scaffold proteins to kinases prevents activation of kinases by irrelevant stimuli by shielding the kinase from other proteins [5, 106]. Several scaffold proteins have been identified that act at different points within their cascade. Some of the best understood scaffold proteins are Kinase suppressor of Ras 1&2 (KSR1&2) that regulate the ERK1/2 cascade. KSR is a highly conserved protein that interacts with a variety of signaling molecules. KSR1 is described as both a pseudokinase and a scaffold protein, and its function is believed to bring together ERK, MEK and activated Raf-1 at the plasma membrane, to provide a docking platform for signaling molecules and to facilitate the sequential phosphorylation steps within the cascade [107, 108, 109]. In addition to signaling molecules within the cascade, KRS has been shown to interact

with other proteins that regulate its function and localization, thereby adding another layer to MAPK signaling regulation [106, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120]. Another protein with ERK scaffolding properties is MEK-partner 1 (MP1), which selectively promotes signaling from MEK1 to ERK1, but cannot bind MEK2 or ERK2. MP1 also functions in compartmentalization as it localizes ERK1 to endosomes [121, 122, 123, 124]. Another group of newly emerging scaffolding proteins are the *β*-arrestins that have been shown to be involved in the regulation of G-protein-coupled receptor (GPCR) signaling as well as in GPCR signal transduction and MAPK scaffolding. This dual function is believed to facilitate the activation of the MAPK cascade following GPCR activation. In the ERK1/2 cascades, the *β*-arrestins-1 and 2 have been shown to be involved [125, 126, 127, 128, 129].

For the stress-activated protein kinase cascades, several scaffolding proteins have been identified. The most investigated scaffolding proteins are the group of JNK interacting proteins (JIPs), which interact with JNK and p38. Association of JIP proteins with JNK have been shown to increase JNK activation; this activation is in turn regulated by a large variety of proteins interacting with JIP [60, 106, 130, 131, 132, 133, 134, 135]. Arrestins have also been shown to facilitate activation of the JNK cascade by GPCR signaling. For example, *β*-arrestin-2 was shown to act as a scaffold for JNK3 [106, 136, 137, 138, 139]. Recently, also the ERK5 cascade was shown to require *β*-arrestin-mediated activation following GPCR activation [140].

Relocalization of kinases and restriction of components to certain locations in the cell are important means of regulation of signaling. This process is best understood in the ERK1/2 cascade and is mediated by scaffolding and anchoring proteins. For example, MEK1/2 and ERK1/2 are bound to cytoplasmic anchors in their inactive state and are released upon phosphorylation. Although ERK1/2 preferentially localizes to the nucleus after release from its cytoplasmic anchor, it can localize to other subcellular compartments with the help of specific directing proteins [2, 141, 142, 143, 144, 145]. For example, the scaffold protein MP1 ensures ERK1 localization to endosomes by forming a scaffolding complex with its partner protein p14 and the kinases MEK1 and ERK1. The MP1-p14 scaffolding complex also interact with ERK2, but the biological relevance of this interaction is still unclear [121, 122, 146, 147, 148]. Sef1 is required for the Golgi localization of ERK1/2, and VDAC1 directs ERK1/2 to mitochondria [149, 150, 151, 152].

1.5 The Egr transcription factor family

The family of Early Growth Response (Egr) transcription factors is a group of four transcription factors now named Egr1-4 that share a highly conserved DNA-binding domain consisting of three zinc-finger motifs. This domain recognizes a 9 base pair DNA segment, whereby each zinc finger recognizes three nucleotides [153, 154, 155, 156, 157, 158, 159, 160, 161]. Apart from this conserved domain, Egrs differ in their structure to varying degrees. Egr2 and Egr3 are the most closely related, followed by Egr1 and Egr4 which are more distantly related [162]. Egr3 is the only member of the Egr transcription factor family for whom the presence of several isoforms has been reported [163, 164].

In general, these transcription factors are rapidly induced mainly in response to growth factor stimulation, but other stress-related stimuli have also been shown to induce Egr transcription factors. The expression of the Egr transcription factors appears to be tightly regulated, as stimuli inducing Egrs also induce the nuclear co-repressor NGFI-A binding protein-2 (NAB2) that suppresses transcriptional activity of Egr1, Egr2 and Egr3, whereby it has been shown that they also induce their suppressor NAB2 [154, 165, 166]. Regulation of Egr4 differs from that of the other members of the Egr family, as it does not contain a NAB2-binding site. Instead, Egr4 appears to have autoregulatory properties, as it binds a region in its own promoter and represses its own transcription [167].

The Egr transcription factors seem to fulfill different functions in different processes. In general, they are thought to be involved in processes such as cell survival, proliferation, differentiation, and apoptosis [154].

Much research has focused on the role of Egrs in neurons with regard to neuronal differentiation, memory and learning [162]. Egr1, Egr2 and Egr3 are rapidly upregulated in neuronal cells after stimulation of the cells by induction of long-term potentials (LTPs) [162, 168, 169, 170, 171]. Furthermore, much research has been performed in Egr-deficient mice, which are viable, except for mice lacking Egr2. Mice lacking Egr1 and Egr4 show problems with fertility, whereas mice lacking Egr3 have severe motor abnormalities due to lack of muscle spindles [153, 172, 173, 174, 175, 176, 177]. Mice lacking Egr1 show deficiencies in maintenance of late LTPs and interestingly, they are unable to form long term memories as tested in a variety of behavioral tasks. However, no impairment in short-term memory formation was found [178, 179]. This is in contrast to Egr3-deficient mice that show deficits in short-term memory formation and as a consequence, also in long-term memory formation. These defects are in addition to the previously mentioned motor abnormalities and abnormal reaction and adaptation to stress and in social interactions

[162, 180, 181]. The involvement in neurological processes of the Egr transcription factors is reminiscent with human association studies that indicate an involvement of Egr1 with Alzheimer's disease and Egr3 in Schizophrenia in several populations, as well as in Bipolar Disorder and even psychosis [182, 183, 184, 185, 186, 187, 188, 189, 190, 191]. Additionally, Egr2 and Egr3 are involved in immunity as they contribute to the regulation of proliferation and differentiation of B and T cells and also of dendritic cells [192, 193, 194, 195].

Since Egr1 and Egr3 are involved in proliferation, they have also been found to play a role in cancer. However, for Egr1, opposing roles have reported in different types of cancer. In prostate cancer cells, Egr1 is overexpressed and has been shown to be required for tumor progression [22, 196, 197, 198]. In a transgenic mouse model, loss of Egr1 delayed tumor progression from neoplasia to invasive carcinoma. Generally, Egr1 is overexpressed in prostate cancer and promotes tumor progression, possibly by controlling proteins involved in cell cycle regulation such as Cyclin D2. Egr1 also induces other proteins important for tumor progression such as insulin-like growth factor-II, transforming growth factor-β1 (TGF- β1), and platelet-derived growth factor-A. In addition, Egr1 promotes translocation of the androgen receptor to the nucleus [154, 198, 199, 200, 201, 202, 203, 204, 205]. Furthermore, the Egr1 repressor Nab2 is downregulated in both human and mouse prostate tumors [206]. In contrast, in several other types of cancer including breast cancer, Egr1 was lost [207, 208, 209, 210, 211]. Interestingly, in breast cancer, Egr1 was found to induce expression of the tumor suppressor BRCA1 [212]. In general, in some cancers, Egr1 might act as a tumor suppressor and inhibit tumor progression, while in other cancer types, Egr1 is overexpressed, promotes tumor progression and was even shown to enhance drug resistance of the tumor [210, 213, 214, 215, 216, 217, 218]. Of note, apoptosis induction by several chemotherapeutic agents was shown to be mediated by Egr1 [219, 220, 221, 222]. More research is required to integrate these opposing findings into a coherent model of Egr1 function.

Research on the involvement of Egr3 in cancer has been less extensive so far, but it was found to play a role in prostate cancer and breast cancer. Egr3 was found to be highly overexpressed in non-relapsing prostate cancer, but showed lower expression in relapsing prostate cancer. In addition, expression patterns of inflammatory genes that are known to be involved in prostate cancer correlated with Egr3 expression levels, indicating a regulatory role for Egr3. Upregulation of inflammatory cytokines and growth factors, in particular of Interleukin-6 (IL6) and IL8 was found to be Egr3 dependent in prostate cancer by a later study, and this was suggested to be an important part of prostate cancer progression [223, 224]. In breast cancer, Egr3 was shown to be induced by estrogen-mediated signaling, and increased expression of Egr3 in patient samples was

associated with an increased risk of recurrence of the cancer as well as an adverse clinical outcome. These correlations are in line with the finding that overexpression of Egr3 increased cancer cell migration and invasion properties [225, 226]. In contrast, in gastric cancer, Egr3 was found to be expressed at lower levels compared with matched non-tumour tissues, and decreased Egr3 expression levels correlated with poor prognosis [227].

2 Integration of extracellular signaling to the early secretory pathway

Previously, the secretory pathway was seen as a steady homeostatic membrane system. Many recent studies however show that compartments of the secretory pathway are specifically targeted by kinase signaling, and that the secretory pathway does not just consist of steady membranous compartments, but that these are adaptable to different cellular needs in both structure and function [228, 229]. The secretory pathway undergoes dramatic changes during cell division, a process which is also regulated in part by kinase signaling; signaling during mitosis will not be discussed below. The main signaling events discussed in the following chapters are illustrated in Figure 4.

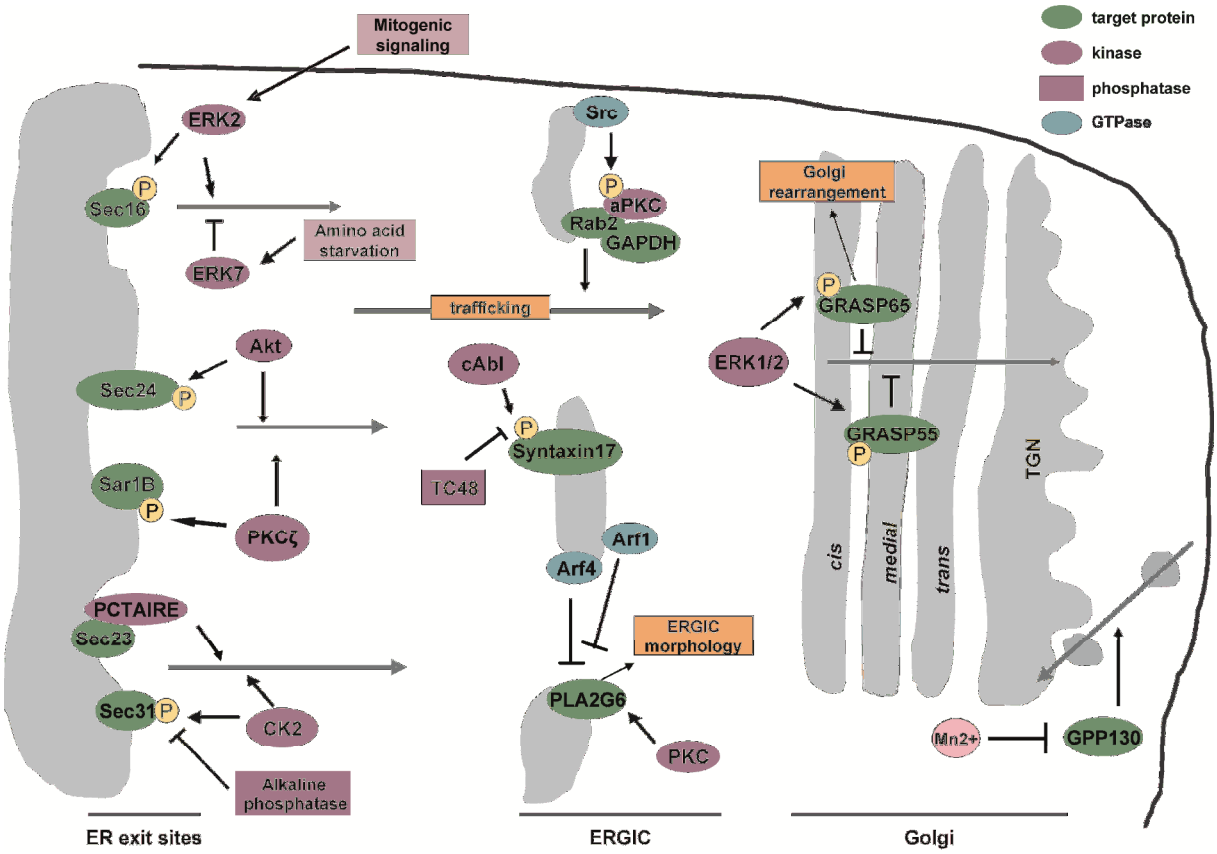


Figure 4: Schematic illustration of key signaling events occurring at the ER-Golgi interface

2.1 ERES

The regulation of ERES by kinase signaling has probably been the most extensively studied, and the first insights came from the observations of two studies using the isoquinolinesulfonamide H89, which is a serine/threonine kinase inhibitor. Lee et al. showed that H89 treatment blocked ER export, but not retrograde trafficking from the ERGIC or the Golgi. Additionally, Sec13 recruitment to ERES was disturbed by H89 treatment, but not the localization of βCOP to the Golgi [230]. These findings are in line with the observations of Aridor et al., who also saw inhibition of ER export by H89 treatment due to loss of Sar1 recruitment by H89 treatment, followed by an inhibition of Sec23/24 recruitment to membranes, which explains the defect of Sec13 recruitment found by Lee et al [230, 231]. Recent studies revisited this problem, and Nakagawa et al. found ER-coupled β-tubulin to be a downstream effector of the still elusive H89-sensitive kinase [232]. Using microsome binding assays and recombinant Sar1, they were able to verify an inhibition of Sar1 recruitment to microsomes in the presence of H89, and an inverse relationship of phosphorylated β-tubulin and Sar1 at the microsome depending upon H89 concentration. These results may indicate a role for β-tubulin phosphorylation at the ER by H89-sensitive kinase in the recruitment of Sar1 to ERES [232, 233]. In another study, inhibition of Sar1 recruitment to ER membranes was found to be mediated by the ER-resident Gi2-protein using the Gi protein activator Mastoparan 7. Mastoparan 7 treatment suppressed Sar1 translocation onto microsomes presumably via the actions of ER-resident Gi2-protein, as the effect of Mastoparan 7 was abolished by co-treatment with the G-protein inhibitor pertussis toxin [234]. These studies indicate that two distinct mechanisms exist, targeted by different signaling cascades, that suppress and support ER export via modulation of Sar1 recruitment to the ER membrane [232, 233, 234]. Furthermore, the phosphatase inhibitor okadaic acid was shown to inhibit ER export, indicating a role for phosphatases as well as kinases in the regulation of ER export [235].

Recent studies have focused more on phosphorylation of components of the ER export machinery. Sar1b was found to be phosphorylated by the atypical protein kinase C ζ (PKCζ) in intestinal cells, which stimulated budding of pre-chylomicron transport vesicles from the ER. Recently, PKCζ was found to be activated by dietary phosphatidylcholine, and that PKCζ activation correlated with intracellular phosphatidylcholine concentrations. In this way, dietary phosphatidylcholine directly increases its export from the ER by stimulating this rate-limiting step [236, 237, 238]. The COPII-coat component Sec23 also appears to be a target of signaling. The kinase PCTAIRE-1, which belongs to a group of PCTAIRE kinases that are part of the cyclin-

dependent kinase (CDK) family, was shown to interact directly with Sec23. Sec23 was not shown to be phosphorylated by PCTAIRE, indicating that PCTAIRE might act as a scaffold protein [239]. However, kinase activity of PCTAIRE is still required for secretory pathway function, as loss of PCTAIRE as well as overexpression of a kinase-dead mutant resulted in a decrease in ERES number as well as a disturbance in ERGIC structure and Golgi fragmentation. In addition, overexpression of the kinase-dead mutant decreased ER-to-Golgi trafficking, as measured using trafficking of temperature-sensitive-VSVG, while overexpression of an active form further increased trafficking [239]. More is known about phosphorylation of Sec24. Sec24C is phosphorylated upon entry of the cell into mitosis, which has been suggested to somehow contribute to the disassembly of ERES during mitosis; this process will be discussed later [240]. Another study found that the protein kinase Akt phosphorylates Sec24C and Sec24D on serine/threonine residues, thereby increasing their binding to Sec23 [241]. This was proposed as a general pathway of Akt-dependent stimulation of ER-to-Golgi trafficking, as previous studies have shown increased trafficking in response to stimuli mediated by the Pi3K/Akt pathway. Trafficking of sterol regulatory element binding protein-2 (SREBP-2) was shown to be increased by Akt, insulin stimulation of rat brown adipose cells selectively increased trafficking of MHC-I, and ER-to-Golgi trafficking of the lipid ceramide was affected by inhibition of the PI3K/Akt pathway [242, 243, 244]. Sec31 is another COPII component that has been shown to be phosphorylated. The first indications came from a yeast in vitro study that showed that pre-treatment of Sec31 with alkaline phosphatase strongly inhibited the formation of COPII vesicles in an in vitro budding assay [245]. A later study in mammalian cells identified the constitutively active Casein Kinase II (CK2) as a serine/threonine kinase responsible for Sec31 phosphorylation. Loss or inhibition of CK2 reduced membrane trafficking, as secretion of secretory alkaline phosphatase into the supernatant was reduced, as well as ER-to-Golgi trafficking of temperature-sensitive VSVG. Additionally, Koreishi et al. found that phosphorylation of Sec31 decreased its affinity for Sec23, thereby stimulating ER export, while a non-phosphorylatable Sec31 mutant bound Sec23 more strongly and remained at ERES for longer [246]. CK2 is involved in processes such as cell cycle, cell survival and transcriptional regulation, however, which stimulus initiates CK2-dependent Sec31 phosphorylation was not reported and therefore all examples highlighted above do not indicate which “signal” is responsible for targeting a certain kinase to the COPII machinery.

Accessory proteins that assist in COPII-vesicle formation but are not part of the vesicle coat may also be phosphorylated. For example, Sec16A has been shown to be

phosphorylated by ERK2, and loss of ERK2 was shown to decrease ERES number [247]. In line with these findings, overexpression of oncogenic Ras, which increases ERK2 activity, also caused an increase in ERES number [247]. Additionally, a functional consequence of ERK2-dependent Sec16A phosphorylation was found, as phosphorylation increased Sec16A mobility following EGF stimulation in FRAP assays [247]. Sec16 has also been suggested to be phosphorylated by ERK7 in response to amino acid starvation in Drosophila S2 cells, which will be discussed in more detail below. Although direct phosphorylation of Sec16 was not shown, amino acid starvation led to a disassembly of ERES mediated by ERK7 [96].

2.2 ERGIC

The ERGIC may also be a target of signaling. One example is the ERGIC-localized kinase CK1δ, which is a homologue of the yeast Hrr25p which localizes to the yeast cis-Golgi, and possibly regulates COPII-vesicle fusion. CK1δ was shown to phosphorylate the GTPase Arf1 and thereby mediate the recruitment of Arf1 to membranes. Arf1 is required for the recruitment of COPI components and for the formation of COPI vesicles. Consequently, loss of CK1δ function disrupted Arf1 localization to membranes, and inhibition with the CK1δ-specific inhibitor IC261 led to a block of temperature sensitive VSV-G trafficking at the pre-Golgi level, indicating that CK1δ has a regulatory function in post-ER membrane trafficking [248, 249, 250, 251]. In addition, a signaling complex recruited by the small GTPase Rab2 was shown to be important for trafficking. Rab2 is a small GTPase that was proposed to be involved in the recruitment of coatomer to the ERGIC and is essential for anterograde and retrograde vesicle trafficking from the ERGIC [252, 253, 254]. Early studies have shown that Rab2 requires the atypical protein kinase C (aPKC) for the recruitment of β-COP to the membrane, and that only the aPKCλ and aPKCι isoforms were found to be required for Rab2-dependent β-COP recruitment in in vitro membrane association and vesicle budding assays [253, 255]. Rab2 was also shown to recruit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to the ERGIC, which is phosphorylated at the ERGIC by aPKCλ/ι [255, 256, 257]. Since GAPDH is able to interact with α-tubulin, it was suggested that Rab2 controls microtubules (MT) and MT motor proteins at the ERGIC, via GAPDH, in a manner which was shown to be dependent upon aPKCλ/ι activity [258, 259]. With regard to vesicle trafficking at the ERGIC, GAPDH also plays an important role, as inhibition of GAPDH by antibody injection blocked ER-to-Golgi transport [256].

Furthermore, Src kinase, which is a member of the non-receptor tyrosine kinase family, was shown to regulate Rab2 activity at the ERGIC. Phosphorylation of aPKC by Src was shown to be required for the interaction between aPKC and Rab2, and in turn, Rab2 was also required for Src recruitment to the ERGIC [255, 257, 260, 261]. Additionally, Src was shown to also phosphorylate GAPDH, and this phosphorylation was required for Rab2-mediated effects, as overexpression of a non-phosphorylatable GAPDH mutant blocked ER-to-Golgi trafficking, but not GAPDH association to the ERGIC [262].

Syntaxin17 is another ERGIC-associated protein that is targeted by signaling. Syntaxin17 was identified as a SNARE protein that is necessary for secretion and might act as a receptor protein, but its function is still unclear and it has been implicated in autophagosome regulation [263]. It localizes mostly to the ER and ERGIC membranes and is required to maintain ERGIC and Golgi architecture [264]. It was recently shown to be phosphorylated by the tyrosine kinase c-Abl in response to serum stimulation, and possibly due to growth factor receptor stimulation, as phosphorylation of Syntaxin17 was reduced if cells were pre-treated with EGFR inhibitors prior to serum stimulation. This phosphorylation was further shown to affect the association of Syntaxin17 with β-COP, but phosphorylation status of Syntaxin17 was found to not influence β-COP distribution in the cell [265]. Syntaxin17 was also shown to be a target of the TC48 tyrosine phosphatase that has been previously implicated to be a regulator of the early secretory pathway. The T-cell protein tyrosine phosphatase (TCPTP) has two splice variants, TC45 which localizes exclusively to the nucleus acting as a nuclear pore protein, and TC48. TC48 localizes mainly the ER, but shuttles through the ERGIC and *cis*-Golgi before being retrieved by retrograde transport to the ER via interaction with p24 family members. TC48 was suggested to serve a regulatory function in the components of the secretory pathway, possibly by regulation of vesicular trafficking [266, 267].

The characteristic discontinuous structure of the ERGIC was shown to be regulated by the group VI phospholipase A2 (PLA2G6) [268], which is a calcium-independent PLA2 form. Ben-Tekaya et al. showed that PLA2G6 localizes to the ERGIC and that it has an essential role in maintaining ERGIC structure. Loss of both of the GTPases Arf1 and Arf4 led to a hyperactivation of the PLA2G6-A isoform, which led to tubulation of the ERGIC and connection of the normally separated ERGIC clusters [268]. A dysfunctional ERGIC due to PLA2G6 misregulation very likely contributes to apoptotic phenotype of cells with misregulated PLA2G6 activity. Furthermore, PLA2G6 was linked to inflammatory signaling in a type 1 diabetes autoimmunity model, as proinflammatory cytokines induced PLA2G6 and ultimately apoptosis of β-cells [269]. Pharmacological

inhibition of PLA2G6 in female mice reduced incidence of diabetes development due to preservation of β-cells [270]. Additionally, PLA2G6 was also shown to be a downstream target of PKC signaling in immune cells [271, 272]. Taken together, a variety of stimuli target PLA2G6 and thereby the ERGIC indicating that the ERGIC is regulated by external stimuli to a greater extent than it is recognized so far.

2.3 Golgi

The Golgi is the organelle that has been the most extensively studied with regard to signaling, as the Golgi disassembles during mitosis and re-assembles afterwards in a process that is regulated by kinases and phosphatases [273]. The Golgi matrix proteins GRASP65 and GRASP55 are involved in Golgi architecture, and phosphorylation and subsequent de-phosphorylation of GRASP55 and GRASP65 were shown to mediate Golgi disassembly and re-assembly before and after mitosis [274, 275, 276, 277, 278, 279, 280]. Additionally, targeting GRASP55/65 by signaling might regulate the velocity of trafficking through the Golgi, as GRASP65 and GRASP55 have been suggested to negatively regulate protein trafficking. A delay in trafficking might be necessary to ensure proper glycosylation of proteins, as adverse effects on protein glycosylation were observed due to accelerated Golgi-to-cell-surface trafficking in GRASP55/56 knockdown [281, 282]. ERK1/2 MAPK signaling at the Golgi is also tightly regulated, and an important negative regulator of ERK1/2 signaling at the Golgi was identified to be Bcl-2 inhibitor of transcription (Bit1) [283]. Although this protein was first shown to localize to mitochondria, it was also found to be present in the ER and Golgi, where it negatively regulates ERK activity. Bit1 at the Golgi is required for stress resistance, and was also shown to be involved in anoikis, which is a form of apoptosis found in epithelial cells. In mitochondria, protein kinase D was found to positively regulate Bit1 [283, 284, 285, 286]. Interestingly, ion signaling also appears to have an effect on localization of Golgi proteins. Increased intracellular Manganese (Mn) concentration causes a relocalization of the cycling cis-Golgi glycoprotein Golgi phosphoprotein of 130 kDa (GPP130) first to multivesicular bodies and then to lysosomes where it is degraded. [287, 288, 289]. Apart from Manganese, Calcium also appears to play a role in Golgi function and trafficking, as loss of the secretory pathway Ca(2+)-ATPase isoform 1 (SPCA1), which is a Golgi-localized Ca2+-pump, disturbed Golgi structure and inhibited trafficking of proteins through the Golgi [290, 291].

2.4 Evidence from screens for kinase/phosphatase regulation of the early secretory pathway

A large amount of evidence for signaling to the early secretory pathway comes from several different screens that were performed with the aim to uncover regulators of structure and function of the secretory pathway. Many proteins, not only kinases and phosphatases, were uncovered that appear to regulate the organelles of the early secretory pathway. These screens will be discussed in relation to signaling to the early secretory pathway briefly below. The screens show very little overlap with regard to hits that were identified, but this can be explained by different species used (Drosophila versus mammalian cells), different set-ups due to different contexts that were researched, and consequently very different read-outs, as discussed in a recent review [292].

The first RNAi screen to identify regulators of secretion in metazoans was performed in Drosophila S2 cells by Bard et al. Secretion of soluble horseradish peroxide (HRP) into the supernatant was used as a read-out, and potential hits were further tested by overexpression and colocalization with the Golgi using overexpressed GFP-ManII. A major criticism towards this approach was that secreted HRP was not normalized to the amount of living cells. Still, many known regulators of secretion were identified in this screen, as well as many new potential regulators, one of which, a protein termed Transport and Golgi organization 1 (TANGO1) has been extensively researched and found to be localized to ERES where it is involved in cargo export [293]. Another screen in Drosophila S2 cells by Wendler et al. did correct for cell viability and used Luciferase secretion as a read-out. Interestingly, the overlap between these two screens was very low. A third screen in Drosophila S2 cells focused on the identification of proteins that regulate the morphology of the early secretory pathway, or tER-Golgi units in Drosophila cells, rather than effect on anterograde trafficking. Furthermore, this screen focused on proteins that are predicted to be associated to the ER. This screen found a variety of new regulators of the early secretory pathway. Interestingly, hits that upon depletion led to an increase in number of tER-Golgi units also increased cell size in general. Cell size is an important factor in the regulation of cell division, and this finding therefore provides a link between the secretory pathway and cell division in Drosophila cells [294].

This important link between proliferation and the secretory pathway was also established in an earlier screen using mammalian cells [247]. This RNAi screen by Farhan et al. investigated changes in morphology of the early secretory pathway in

mammalian HeLa cells using ERGIC-53 as a marker, as this protein cycles between the different compartments of the early secretory pathway and it was therefore possible to stain ERES, the ERGIC, as well as the cis-Golgi. This screen targeted the entire human kinome and phosphatome, and identified 122 kinases and phosphatases that regulate the early secretory pathway. Interestingly, a role for the ERK1/2 signaling cascade in regulation of ERES was found [247]. A later RNAi screen also in the mammalian HeLa cells by the Pepperkok group targeted the entire human genome and used trafficking of the model secretory cargo protein temperature sensitive VSVG (VSVG-ts045) as a read-out [295]. This screen identified a total of 554 hits that influence secretion, and the two screens showed an overlap of 39%. This large number of regulators of the early secretory pathway supports the emerging view that the secretory pathway is not a homeostatic membranous system but highly involved in a large number of cellular processes, and therefore tightly regulated by internal and external stimuli. Another RNAi screen focused exclusively on the identification of kinases and phosphatases that influence Golgi morphology using markers for the *cis*, the *medial* and the *trans*-Golgi. This approach revealed 159 hits that were clustered into sub-networks, one of them being the ERK1/2 signaling cascade that was also found to be involved in regulation of the early secretory pathway by the Hauri group [247, 296].

3 Response of the endomembrane system to nutrients and nutrient starvation

Adaptation to nutrient availability is an essential factor in cell survival, and mechanisms to monitor nutrient availability and to adapt to lack of nutrients are largely conserved. Upon decreased nutrient availability, such as lack of glucose or amino acids, cells must decrease their energy expenditure and protein synthesis. As mentioned previously, the secretory pathway handles a large portion of nascent proteins; therefore, upon nutrient starvation, this cargo load is decreased. In addition, the secretory pathway is a large, multi-organelle structure and is involved in many cellular processes. Its maintenance and function requires energy and the synthesis of proteins and lipids. Therefore, the secretory pathway must adapt to changes in nutrient availability by decreasing its secretory processes and energy expenditure. However, the response of the secretory pathway to nutrient starvation and signaling starvation has not been researched in great detail.

The most extensively studied mechanism in response to nutrient starvation is macroautophagy, hereafter referred to autophagy. This process involves the bulk degradation process of proteins and organelles that are engulfed in the cytoplasm by the phagophore, which is a double-membraned cup-shaped structure that eventually closes, forming the autophagosome. These large vesicular structures then fuse with lysosomes, and fusion forms auto(phago)lysosomes and the contents of the autophagosome are degraded by lysosomal enzymes [297, 298]. This enables the cell to degrade components not required during nutrient starvation, and to liberate amino acids. The most important pathway for induction of autophagy is the highly conserved mammalian or mechanistic target of rapamycin (mTOR) pathway. mTOR is present in two protein complexes, called mTOR complex 1 (mTORC1) and 2 (mTORC2), whereby the mTORC1 complex is responsible for induction of autophagy. mTORC1 is activated in the presence of nutrients and inhibits autophagy under basal conditions [299]. Decreased glucose/ATP or amino acid levels lead to an inhibition of mTORC1, thereby the phosphorylation-dependent inhibition of the serine/threonine kinases Unc-51-like Kinases 1 and 2 (ULK1/2) by mTORC1 is lost. Activation of ULK complex (ATG1 in yeast) is believed to be upstream of the recruitment of ATG proteins and initiates the formation of phagophores by recruiting two ubiquitination-like reaction complexes to the phagophore membranes. The first step in phagophore formation is the membrane association of the E3-ligase-like Atg5-Atg12-Atg16L complex. This is followed by the

conjugation of the ubiquitin-like Atg8 family, which is divided into the three subfamilies LC3 (which consist of LC3A, -B, B2 and, -C), GABARAP and GATE-16 [297, 298, 300].

In contrast to yeast, where only one phagophore is present which originates from the phagophore assembly site (PAS) on the endoplasmic reticulum, mammalian cells form several phagophores. Contrary to yeast, the origination site of mammalian phagophores and the source of membranes during the elongation phase remain elusive, and many organelles of the secretory pathway have been shown to be involved [300, 301].

The ER for example has been suggested to supply membrane material, as formation of the autophagosome requires phosphatidylinositol 3-phosphate (PI(3)P), and a PI(3)-P-binding protein called DFCP1, that normally localizes to the ER, was shown to translocate to punctate compartments upon amino acid starvation where it partially co-localized with the autophagosome markers LC3 and Atg5 [302]. In addition, overexpressed ULK1 was found to frequently associate with the ER and to localize in close proximity to the ER reticular structures [303]. Other studies using electron tomography showed direct interconnections between the ER and developing phagophores [304, 305, 306, 307]. The developing phagophore was also shown to form contacts with mitochondria, which were previously implicated to form contacts with phagophores [307, 308]. Interestingly, a recent study showed that autophagosomes form at ER-mitochondria contact sites, since the autophagosome marker Atg14 localized to ER-mitochondria contact sites during starvation, and that the ER-resident SNARE protein Syntaxin17 was responsible for the recruitment of Atg14 [309]. Another ER-associated compartment, ER exit sites, has emerged as a key compartment for autophagosome formation, as suggested by several lines of evidence. In mammalian cells, autophagosomes were shown to form in close proximity to the ER at sites termed omegasomes [302, 310]. Using the omegasome-marker DFCP1, Ge et al. showed that pharmacological inhibition of COPII vesicle transport by H89 treatment caused a decrease in omegasome number [311]. In addition, inhibition of ER export by overexpression of dominant-negative or constitutively active Sar1-mutants was shown to decrease the number of DFCP1-positive omegasomes and ATG14-positive early autophagosomes, indicating that functional ER export is required for autophagosome initiation [311]. This is further supported by recent studies in yeast showing that phagophores form in close proximity to ERES [312, 313]. Suzuki et al. showed that the isolation membrane, which elongates to form the closed autophagosome, forms in

close proximity to ERES [313]. Graef et al. verified these findings in both yeast and mammalian cells. In addition, Graef et al. showed that the edge of the expanding isolation membrane localized next to several COPII components in over 90% of cases [312]. In addition, COPII components were suggested to directly interact with components of the autophagosome machinery as shown by mass spectrometry [312]. In a recent review, the findings regarding the role of ERES in autophagosome formation were summarized in two different models [314]. In the first model, ERES deliver membrane to the growing phagophore by COPII-mediated vesicle transport. In the second model, ERES act as scaffolds where autophagosome formation takes place. Although both models are possible considering the current data, the scaffolding model is supported by additional ultrastructural studies showing that omegasomes are connected to the ER through thin tubular structures [315, 316]. If the phagophore and ER remained connected during elongation of the isolation membrane, ERES were suggested to act as a scaffold enabling this connection. [314]. This connection might be further stabilized by the TRAPPIII complex which binds to Sec23 and mediates vesicle fusion. Tan et al. showed that the TRAPPIII complex is recruited to the phagophore assembly site (PAS). However, in support of the vesicular transport model, TRAPPIII might direct COPII vesicles to the phagophore, as COPII vesicles were shown to accumulate at the PAS when autophagy was inhibited [317, 318]. Further studies are likely to provide a clearer picture. However, although ERES are a strong candidate as membrane source for autophagosome formation, other compartments have been implicated.

Biochemical studies have shown that the ERGIC is an important membrane source for the phagophore that provides small LC3-lipidation active vesicles. This process was shown to be dependent upon the activity PI3K as well as on functional COPII-vesicle transport from the ER to the ERGIC [311, 319, 320]. The findings in mammalian cells that indicate an important role for the early secretory pathway and COPII-vesicle trafficking in the formation of the autophagosome are in line with earlier findings in *S.cerevisiae* that also show that functional COPII-vesicle transport is required in this process [321, 322].

Another compartment that provides membranes to the forming phagosome is the Golgi as well as the endosomes. Although this has been researched in more detail in yeast, Atg9-associated vesicle trafficking from the Golgi and endosomes to autophagosomes also seems to take place in mammalian cells. As mentioned above, the transmembrane protein Atg9 is localized to the Golgi, the trans-Golgi network and late endosomes, but translocates to autophagosomes during amino acid starvation where it co-localizes with LC3. This process was shown to be negatively regulated by p38 [323,

[324, 325, 326]. As a last compartment, the plasma membrane may also provide membrane material to the autophagosome [327, 328, 329].

Given the extensive involvement of the secretory pathway in autophagosome formation, it is likely that compartments of secretory pathway are able to receive nutritional signaling input and to react to a lack of nutrients. Interestingly, certain proteins that are secreted by eukaryotic cells upon starvation make use of components of the secretory pathway for non-conventional secretion. In *S.cerevisiae*, starvation-induced secretion of the Acyl-CoA binding protein 1 (Acb1) makes use of a membrane-bound compartment that requires the yeast orthologue of the mammalian Golgi-associated GRASP55 and GRASP65 proteins, Grh1. This compartment forms near ER exit sites upon starvation due to relocalization of the Golgi-resident protein Grh1 to ERES, and was called compartment for unconventional protein secretion, or CUPS. These CUPS were further shown to be induced upon glucose, but not nitrogen starvation, and they are independent of COPII- and COPI-vesicle transport, and contain COPII and Golgi tethering proteins, but no Golgi enzymes. Once starvation ceases, CUPS are absorbed into the ER [330, 331, 332]. This process was also described for starvation-dependent secretion of Acb1 in *P.pastoris* and in *Dictyostelium discoideum*, therefore a similar mechanism might exist in mammalian cells, as Interleukin 1β (IL-1β) is secreted independently of the ER-Golgi route, the same is true for cytoplasmic protein fibroblast growth factor 2 (FGF2) [332, 333, 334, 335, 336, 337, 338]. Furthermore, it was shown in Drosophila S2 cells that ERES react to nutrient starvation via ERK7 [96]. ERK7 is an atypical MAPK that might play an important role in the cellular starvation response, as it was shown to stimulate autophagy by binding to LC3. Regulation of ERK7 still remains unclear, but it was shown to be regulated by the ubiquitin-proteasome pathway, and to be highly active during starvation, possibly due to the ability of ERK7 to autophosphorylate [81, 84, 85, 94]. Both serum starvation and amino-acid starvation were shown to lead ERES disassembly and a dispersion and aggregation of Sec16 and Sec23. This was shown to be dependent upon ERK7 activity, and overexpression of ERK7 also caused ERES disassembly and aggregation of Sec16 [96]. These Sec16-containing aggregates that develop in response to amino-acid starvation were shown to contain other COPII components and have lipid-droplet like qualities, but they were different from ERES and other stress-induced aggregates. These aggregates were named Sec-bodies and seem to be Drosophila-specific, as this phenomenon was not found in mammalian cells [339].

Taken together, evidence is accumulating that the secretory pathway plays an active and important role in the adaptation of the cell to nutrient starvation to ensure survival that goes further than simply decreasing energy expenditure, but instead includes induction and support of autophagy and other adaptive responses.

4 References

1. Downward J. The ins and outs of signalling. *Nature* 2001, **411**(6839)**:** 759-762.
2. Wortzel I, Seger R. The ERK Cascade: Distinct Functions within Various Subcellular Organelles. *Genes Cancer* 2011, **2:** 195-209.
3. Hunter T. The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease. *Philos Trans R Soc Lond B Biol Sci* 1998, **353:** 583-605.
4. Rubinfeld H, Seger R. The ERK cascade: a prototype of MAPK signaling. *Mol Biotechnol* 2005, **31:** 151-174.
5. Imajo M, Tsuchiya Y, Nishida E. Regulatory Mechanisms and Functions of MAP Kinase Signaling Pathways. *IUBMB Life* 2006, **58:** 312-317.
6. Sturgill TW, Ray LB. Muscle proteins related to microtubule associated protein-2 are substrates for an insulin-stimulatable kinase. *Biochem Biophys Res Commun* 1986, **134:** 565-571.
7. Coulombe P, Meloche S. Atypical mitogen-activated protein kinases: structure, regulation and functions. *Biochim Biophys Acta* 2007, **1773:** 1376-1387.
8. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim Biophys Acta* 2011, **1813:** 1619-1633.
9. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta* 2010, **1802:** 396-405.
10. Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* 2009, **19:** 230-236.
11. Lavoie JN, L'Allemain G, Brunet A, Müller R, Pouysségur J. Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway. *J Biol Chem* 1996, **271:** 20608-20616.
12. Assefa Z, Garmyn M, Bouillon R, Merlevede W, Vandenheede JR, Agostinis P. Differential stimulation of ERK and JNK activities by ultraviolet B irradiation and epidermal growth factor in human keratinocytes. *J Invest Dermatol* 1997, **108:** 886-891.
13. Chadee DN, Kyriakis JM. MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation. *Nat Cell Biol* 2004, **6**(8)**:** 770-776.
14. Seger R, Ahn NG, Posada J, Munar ES, Jensen AM, Cooper JA*, et al.* Purification and characterization of mitogen-activated protein kinase activator(s) from epidermal growth factor-stimulated A431 cells. *J Biol Chem* 1992, **267**(20)**:** 14373-14381.
15. Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD*, et al.* ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 1991, **65**(4)**:** 663-675.
16. Robbins DJ, Cobb MH. Extracellular signal-regulated kinases 2 autophosphorylates on a subset of peptides phosphorylated in intact cells in response to insulin and nerve growth factor: analysis by peptide mapping. *Mol Biol Cell* 1992, **3**(3)**:** 299-308.
17. Yung Y, Yao Z, Hanoch T, Seger R. ERK1b, a 46-kDa ERK isoform that is differentially regulated by MEK. *J Biol Chem* 2000, **275**(21)**:** 15799-15808.
18. Aebersold DM, Shaul YD, Yung Y, Yarom N, Yao Z, Hanoch T*, et al.* Extracellular signal-regulated kinase 1c (ERK1c), a novel 42-kilodalton ERK, demonstrates unique modes of regulation, localization, and function. *Mol Cell Biol* 2004, **24**(22)**:** 10000-10015.
19. Carlson SM, Chouinard CR, Labadorf A, Lam CJ, Schmelzle K, Fraenkel E*, et al.* Large-scale discovery of ERK2 substrates identifies ERK-mediated transcriptional regulation by ETV3. *Science signaling* 2011, **4**(196)**:** rs11.
20. Casar B, Pinto A, Crespo P. Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes. *Mol Cell* 2008, **31**(5)**:** 708-721.
21. Herndon CA, Ankenbruck N, Fromm L. The Erk MAP kinase pathway is activated at muscle spindles and is required for induction of the muscle spindle-specific gene Egr3 by neuregulin1. *J Neurosci Res* 2014, **92:** 174-184.
22. Gregg J, Fraizer G. Transcriptional Regulation of EGR1 by EGF and the ERK Signaling Pathway in Prostate Cancer Cells. *Genes Cancer* 2011, **2**(9)**:** 900-909.
23. Tsai JC, Liu L, Zhang J, Spokes KC, Topper JN, Aird WC. Epidermal growth factor induces Egr-1 promoter activity in hepatocytes in vitro and in vivo. *American journal of physiology Gastrointestinal and liver physiology* 2001, **281**(5)**:** G1271-1278.

24. Tsai JC, Liu L, Guan J, Aird WC. The Egr-1 gene is induced by epidermal growth factor in ECV304 cells and primary endothelial cells. *American journal of physiology Cell physiology* 2000, **279**(5)**:** C1414-1424.
25. Mendelson KG, Contois LR, Tevosian SG, Davis RJ, Paulson KE. Independent regulation of JNK/p38 mitogen-activated protein kinases by metabolic oxidative stress in the liver. *Proc Natl Acad Sci U S A* 1996, **93:** 12908-12913.
26. Derijard B, Raingeaud J, Barrett T, Wu IH, Han J, Ulevitch RJ*, et al.* Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 1995, **267**(5198)**:** 682-685.
27. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 2005(11-8).
28. Wang L, Ma R, Flavell RA, Choi ME. Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for activation of p38alpha and p38delta MAPK isoforms by TGF-beta 1 in murine mesangial cells. *J Biol Chem* 2002, **277**(49)**:** 47257-47262.
29. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ*, et al.* Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 1995, **270**(13)**:** 7420-7426.
30. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T*, et al.* Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 1997, **275**(5296)**:** 90-94.
31. Han J, Lee JD, Jiang Y, Li Z, Feng L, Ulevitch RJ. Characterization of the structure and function of a novel MAP kinase kinase (MKK6). *J Biol Chem* 1996, **271**(6)**:** 2886-2891.
32. Wang XS, Diener K, Manthey CL, Wang S, Rosenzweig B, Bray J*, et al.* Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. *J Biol Chem* 1997, **272**(38)**:** 23668-23674.
33. Goedert M, Cuenda A, Craxton M, Jakes R, Cohen P. Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. *The EMBO journal* 1997, **16**(12)**:** 3563-3571.
34. Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L*, et al.* Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. *J Biol Chem* 1997, **272**(48)**:** 30122-30128.
35. Mertens S, Craxton M, Goedert M. SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases. *FEBS Lett* 1996, **383**(3)**:** 273-276.
36. Li Z, Jiang Y, Ulevitch RJ, Han J. The primary structure of p38 gamma: a new member of p38 group of MAP kinases. *Biochem Biophys Res Commun* 1996, **228**(2)**:** 334-340.
37. Gong X, Ming X, Deng P, Jiang Y. Mechanisms regulating the nuclear translocation of p38 MAP kinase. *J Cell Biochem* 2010, **110**(6)**:** 1420-1429.
38. Zer C, Sachs G, Shin JM. Identification of genomic targets downstream of p38 mitogen-activated protein kinase pathway mediating tumor necrosis factor-alpha signaling. *Physiological genomics* 2007, **31**(2)**:** 343-351.
39. Iordanov M, Bender K, Ade T, Schmid W, Sachsenmaier C, Engel K*, et al.* CREB is activated by UVC through a p38/HOG-1-dependent protein kinase. *The EMBO journal* 1997, **16**(5)**:** 1009-1022.
40. Swart JM, Bergeron DM, Chiles TC. Identification of a membrane Ig-induced p38 mitogen-activated protein kinase module that regulates cAMP response element binding protein phosphorylation and transcriptional activation in CH31 B cell lymphomas. *Journal of immunology (Baltimore, Md : 1950)* 2000, **164**(5)**:** 2311-2319.
41. Deak M, Clifton AD, Lucocq LM, Alessi DR. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *The EMBO journal* 1998, **17**(15)**:** 4426-4441.
42. Wang XZ, Ron D. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP Kinase. *Science* 1996, **272**(5266)**:** 1347-1349.
43. Han J, Jiang Y, Li Z, Kravchenko VV, Ulevitch RJ. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* 1997, **386**(6622)**:** 296-299.
44. Yang SH, Galanis A, Sharrocks AD. Targeting of p38 mitogen-activated protein kinases to MEF2 transcription factors. *Mol Cell Biol* 1999, **19**(6)**:** 4028-4038.
45. She QB, Chen N, Dong Z. ERKs and p38 kinase phosphorylate p53 protein at serine 15 in response to UV radiation. *J Biol Chem* 2000, **275**(27)**:** 20444-20449.

46. Huang C, Ma WY, Maxiner A, Sun Y, Dong Z. p38 kinase mediates UV-induced phosphorylation of p53 protein at serine 389. *J Biol Chem* 1999, **274**(18)**:** 12229-12235.
47. Luo S, Lee AS. Requirement of the p38 mitogen-activated protein kinase signalling pathway for the induction of the 78 kDa glucose-regulated protein/immunoglobulin heavy-chain binding protein by azetidine stress: activating transcription factor 6 as a target for stress-induced phosphorylation. *Biochem J* 2002, **366**(Pt 3)**:** 787-795.
48. Thuerauf DJ, Arnold ND, Zechner D, Hanford DS, DeMartin KM, McDonough PM*, et al.* p38 Mitogen-activated protein kinase mediates the transcriptional induction of the atrial natriuretic factor gene through a serum response element. A potential role for the transcription factor ATF6. *J Biol Chem* 1998, **273**(32)**:** 20636-20643.
49. Tang X, Deng L, Xiong H, Li G, Lin J, Liu J*, et al.* Expression profile of mitrogen-activated protein kinase (MAPK) signaling genes in the skeletal muscle & liver of rat with type 2 diabetes: Role in disease pathology. *Indian J Med Res* 2014, **140:** 744-755.
50. Cheng TH, Shih NL, Chen CH, Lin H, Liu JC, Chao HH*, et al.* Role of mitogen-activated protein kinase pathway in reactive oxygen species-mediated endothelin-1-induced beta-myosin heavy chain gene expression and cardiomyocyte hypertrophy. *J Biomed Sci* 2005, **12:** 123-133.
51. Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL*, et al.* Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. *J Clin Invest* 2003, **112:** 197-208.
52. Cho HJ, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EBr*, et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 2001, **292:** 1728-1731.
53. Kennedy NJ, Cellurale C, Davis RJ. A Radical Role for p38 MAPK in Tumor Initiation. *Cancer Cell* 2007, **11:** 101-103.
54. Junttila MR, Ala-Aho R, Jokilehto T, Peltonen J, Kallajoki M, Grenman R*, et al.* p38alpha and p38delta mitogen-activated protein kinase isoforms regulate invasion and growth of head and neck squamous carcinoma cells. *Oncogene* 2007, **26**(36)**:** 5267-5279.
55. Chen G, Hitomi M, Han J, Stacey DW. The p38 pathway provides negative feedback for Ras proliferative signaling. *J Biol Chem* 2000, **275**(50)**:** 38973-38980.
56. Dèrijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T*, et al.* JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 1994, **76:** 1025-1037.
57. Sluss HK, Barrett T, Dèrijard B, Davis RJ. Signal transduction by tumor necrosis factor mediated by JNK protein kinases. *Mol Cell Biol* 1994, **14:** 8376-8384.
58. Johnson GL, Nakamura K. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. *Biochim Biophys Acta* 2007, **1773:** 1341-1348.
59. Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M. Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. *Nature* 1991, **354:** 494-496.
60. Whitmarsh AJ. The JIP family of MAPK scaffold proteins. *Biochem Soc Trans* 2006, **34:** 828-832.
61. Bogoyevitch MA, Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev* 2006, **70**(4)**:** 1061-1095.
62. Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell* 2000, **103:** 239-252.
63. Martin JH, Mohit AA, Miller CA. Developmental expression in the mouse nervous system of the p493F12 SAP kinase. *Brain Res Mol Brain Res* 1996, **35**(1-2)**:** 47-57.
64. Mohit AA, Martin JH, Miller CA. p493F12 kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. *Neuron* 1995, **14**(1)**:** 67-78.
65. Gupta S, Barrett T, Whitmarsh AJ, Cavanagh J, Sluss HK, Derijard B*, et al.* Selective interaction of JNK protein kinase isoforms with transcription factors. *The EMBO journal* 1996, **15**(11)**:** 2760-2770.
66. Zhou G, Bao ZQ, Dixon JE. Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 1995, **270**(21)**:** 12665-12669.
67. Lee JD, Ulevitch RJ, Han J. Primary structure of BMK1: a new mammalian map kinase. *Biochem Biophys Res Commun* 1995, **213**(2)**:** 715-724.

68. Simoes AE, Pereira DM, Gomes SE, Brito H, Carvalho T, French A*, et al.* Aberrant MEK5/ERK5 signalling contributes to human colon cancer progression via NF-kappaB activation. *Cell death & disease* 2015, **6:** e1718.
69. Kato Y, Tapping RI, Huang S, Watson MH, Ulevitch RJ, Lee JD. Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* 1998, **395**(6703)**:** 713-716.
70. Nishimoto S, Nishida E. MAPK signalling: ERK5 versus ERK1/2. *EMBO Rep* 2006, **7**(8)**:** 782-786.
71. Takeda AN, Oberoi-Khanuja TK, Glatz G, Schulenburg K, Scholz RP, Carpy A*, et al.* Ubiquitin-dependent regulation of MEKK2/3-MEK5-ERK5 signaling module by XIAP and cIAP1. *The EMBO journal* 2014, **33**(16)**:** 1784-1801.
72. Nakamura K, Johnson GL. PB1 domains of MEKK2 and MEKK3 interact with the MEK5 PB1 domain for activation of the ERK5 pathway. *J Biol Chem* 2003, **278**(39)**:** 36989-36992.
73. Chao TH, Hayashi M, Tapping RI, Kato Y, Lee JD. MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway. *J Biol Chem* 1999, **274**(51)**:** 36035-36038.
74. Sun W, Kesavan K, Schaefer BC, Garrington TP, Ware M, Johnson NL*, et al.* MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway. *J Biol Chem* 2001, **276**(7)**:** 5093-5100.
75. Honda T, Obara Y, Yamauchi A, Couvillon AD, Mason JJ, Ishii K*, et al.* Phosphorylation of ERK5 on Thr732 is associated with ERK5 nuclear localization and ERK5-dependent transcription. *PLoS One* 2015, **10**(2)**:** e0117914.
76. Buschbeck M, Ullrich A. The unique C-terminal tail of the mitogen-activated protein kinase ERK5 regulates its activation and nuclear shuttling. *J Biol Chem* 2005, **280**(4)**:** 2659-2667.
77. Wang X, Pesakhov S, Harrison JS, Danilenko M, Studzinski GP. ERK5 pathway regulates transcription factors important for monocytic differentiation of human myeloid leukemia cells. *Journal of cellular physiology* 2014, **229**(7)**:** 856-867.
78. Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, Lee JD. BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *The EMBO journal* 1997, **16**(23)**:** 7054-7066.
79. Kasler HG, Victoria J, Duramad O, Winoto A. ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain. *Mol Cell Biol* 2000, **20**(22)**:** 8382-8389.
80. Terasawa K, Okazaki K, Nishida E. Regulation of c-Fos and Fra-1 by the MEK5-ERK5 pathway. *Genes Cells* 2003, **8**(3)**:** 263-273.
81. Abe MK, Kuo WL, Hershenson MB, Rosner MR. Extracellular signal-regulated kinase 7 (ERK7), a novel ERK with a C-terminal domain that regulates its activity, its cellular localization, and cell growth. *Mol Cell Biol* 1999, **19**(2)**:** 1301-1312.
82. Abe MK, Saelzler MP, Espinosa R, 3rd, Kahle KT, Hershenson MB, Le Beau MM*, et al.* ERK8, a new member of the mitogen-activated protein kinase family. *J Biol Chem* 2002, **277**(19)**:** 16733-16743.
83. Strambi A, Mori M, Rossi M, Colecchia D, Manetti F, Carlomagno F*, et al.* Structure prediction and validation of the ERK8 kinase domain. *PLoS One* 2013, **8**(1)**:** e52011.
84. Kuo WL, Duke CJ, Abe MK, Kaplan EL, Gomes S, Rosner MR. ERK7 expression and kinase activity is regulated by the ubiquitin-proteosome pathway. *J Biol Chem* 2004, **279**(22)**:** 23073-23081.
85. Abe MK, Kahle KT, Saelzler MP, Orth K, Dixon JE, Rosner MR. ERK7 is an autoactivated member of the MAPK family. *J Biol Chem* 2001, **276**(24)**:** 21272-21279.
86. Klevernic IV, Stafford MJ, Morrice N, Peggie M, Morton S, Cohen P. Characterization of the reversible phosphorylation and activation of ERK8. *Biochem J* 2006, **394**(Pt 1)**:** 365-373.
87. Iavarone C, Acunzo M, Carlomagno F, Catania A, Melillo RM, Carlomagno SM*, et al.* Activation of the Erk8 mitogen-activated protein (MAP) kinase by RET/PTC3, a constitutively active form of the RET proto-oncogene. *J Biol Chem* 2006, **281**(15)**:** 10567-10576.
88. Xu YM, Zhu F, Cho YY, Carper A, Peng C, Zheng D*, et al.* Extracellular signal-regulated kinase 8-mediated c-Jun phosphorylation increases tumorigenesis of human colon cancer. *Cancer Res* 2010, **70**(8)**:** 3218-3227.

89. Cerone MA, Burgess DJ, Naceur-Lombardelli C, Lord CJ, Ashworth A. High-throughput RNAi screening reveals novel regulators of telomerase. *Cancer Res* 2011, **71**(9)**:** 3328-3340.
90. Groehler AL, Lannigan DA. A chromatin-bound kinase, ERK8, protects genomic integrity by inhibiting HDM2-mediated degradation of the DNA clamp PCNA. *J Cell Biol* 2010, **190**(4)**:** 575-586.
91. Rossi M, Colecchia D, Iavarone C, Strambi A, Piccioni F, Verrotti di Pianella A*, et al.* Extracellular signal-regulated kinase 8 (ERK8) controls estrogen-related receptor alpha (ERRalpha) cellular localization and inhibits its transcriptional activity. *J Biol Chem* 2011, **286**(10)**:** 8507-8522.
92. Saelzler MP, Spackman CC, Liu Y, Martinez LC, Harris JP, Abe MK. ERK8 down-regulates transactivation of the glucocorticoid receptor through Hic-5. *J Biol Chem* 2006, **281**(24)**:** 16821-16832.
93. Chia J, Tham KM, Gill DJ, Bard-Chapeau EA, Bard FA. ERK8 is a negative regulator of O-GalNAc glycosylation and cell migration. *eLife* 2014, **3:** e01828.
94. Colecchia D, Strambi A, Sanzone S, Iavarone C, Rossi M, Dall'Armi C*, et al.* MAPK15/ERK8 stimulates autophagy by interacting with LC3 and GABARAP proteins. *Autophagy* 2012, **8**(12)**:** 1724-1740.
95. Hasygar K, Hietakangas V. p53- and ERK7-dependent ribosome surveillance response regulates Drosophila insulin-like peptide secretion. *PLoS genetics* 2014, **10**(11)**:** e1004764.
96. Zacharogianni M, Kondylis V, Tang Y, Farhan H, Xanthakis D, Fuchs F*, et al.* ERK7 is a negative regulator of protein secretion in response to amino-acid starvation by modulating Sec16 membrane association. *The EMBO journal* 2011, **30**(18)**:** 3684-3700.
97. Camps M, Nichols A, Arkinstall S. Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J* 2000, **14:** 6-16.
98. Alessi DR, Gomez N, Moorhead G, Lewis T, Keyse SM, Cohen P. Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. *Curr Biol* 1995, **5:** 283-295.
99. Franklin CC, Kraft AS. Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J Biol Chem* 1997, **272:** 16917-16923.
100. Owens DM, Keyse SM. Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* 2007, **26:** 3203-3213.
101. Groom LA, Sneddon AA, Alessi DR, Dowd S, Keyse SM. Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase. *EMBO J* 1996, **15:** 3621-3632.
102. Muda M, Theodosious A, Rodrigues N, Boschert U, Camps M, Gillieron C*, et al.* The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J Biol Chem* 1996, **271:** 27205-27208.
103. Slack DN, Seternes OM, Gabrielsen M, Keyse SM. Distinct binding determinants for ERK2/p38alpha and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J Biol Chem* 2001, **276:** 16491-16500.
104. Tanoue T, Moriguchi T, Nishida E. Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5. *J Biol Chem* 1999, **274:** 19949-19956.
105. Tanoue T, Yamamoto T, Maeda R, Nishida E. A Novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 alpha and beta MAPKs. *J Biol Chem* 2001, **276:** 26629-26639.
106. Morrison DK, Davis RJ. Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu Rev Cell Dev Biol* 2003, **19:** 91-118.
107. Yu W, Fantl WJ, Harrowe G, Williams LT. Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK. *Curr Biol* 1998, **8**(1)**:** 56-64.
108. Roy F, Laberge G, Douziech M, Ferland-McCollough D, Therrien M. KSR is a scaffold required for activation of the ERK/MAPK module. *Genes Dev* 2002, **16**(4)**:** 427-438.
109. Stewart S, Sundaram M, Zhang Y, Lee J, Han M, Guan KL. Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization. *Mol Cell Biol* 1999, **19**(8)**:** 5523-5534.
110. Kornfeld K, Hom DB, Horvitz HR. The ksr-1 gene encodes a novel protein kinase involved in Ras-mediated signaling in C. elegans. *Cell* 1995, **83:** 903-913.

111. Sundaram M, Han M. The C. elegans ksr-1gene encodes a novel Raf-related kinase involvedin Ras-mediated signal transduction. *Cell* 1995, **83:** 889-901.
112. Therrien M, Chang HC, Solomon NM, Karim FD, Wassarman DA, Rubin GM. KSR, a novel protein kinase required for RAS signal transduction. *Cell* 1995, **83:** 879-888.
113. Therrien M, Michaud NR, Rubin GM, Morrison DK. KSR modulates signal propagation within the MAPK cascade. *Genes Dev* 1996, **10:** 2684-2695.
114. Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K. Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev* 1999, **13:** 163-175.
115. Cacace AM, Michaud NR, Therrien M, Mathes K, Copeland TD, Rubin GM*, et al.* Identification of constitutive and ras-inducible phosphorylation sites of KSR: implications for 14-3-3 binding, mitogen-activated protein kinase binding, and KSR overexpression. *Mol Cell Biol* 1999, **19:** 229-240.
116. Müller J, Cacace AM, Lyons WE, McGill CB, Morrison DK. Identification of B-KSR1, a novel brain-specific isoform of KSR1 that functions in neuronal signaling. *Mol Cell Biol* 20, **20:** 5529-5539.
117. Ohmachi M, Rocheleau CE, Church D, Lambie E, Schedl T, Sundaram MV. C. elegans ksr-1 and ksr-2 have both unique and redundant functions and are required for MPK-1 ERK phosphorylation. *Curr Biol* 2002, **12:** 427-433.
118. Zhang H, Koo CY, Stebbing J, Giamas G. The dual function of KSR1: a pseudokinase and beyond. *Biochem Soc Trans* 2013, **41:** 1078-1082.
119. Müller J, Ory S, Copeland TD, Piwnica-Worms H, Morrison DK. C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. *Mol Cell* 2001, **8:** 983-993.
120. Xing H, Kornfeld K, Muslin AJ. The protein kinase KSR interacts with 14-3-3 protein and Raf. *Curr Biol* 1997, **7:** 294-300.
121. Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, Weber MJ. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. *Science* 1998, **281:** 1668-1671.
122. Teis D, Wunderlich W, Huber LA. Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. *Dev Cell* 2002, **3:** 803-814.
123. Sharma C, Vomastek T, Tarcsafalvi A, Catling AD, Schaeffer HJ, Eblen ST*, et al.* MEK partner 1 (MP1): regulation of oligomerization in MAP kinase signaling. *J Cell Biochem* 2005, **94:** 708-719.
124. Pullikuth A, McKinnon E, Schaeffer HJ, Catling AD. The MEK1 scaffolding protein MP1 regulates cell spreading by integrating PAK1 and Rho signals. *Mol Cell Biol* 2005, **25:** 5119-5133.
125. Tohgo A, Choy EW, Gesty-Palmer D, Pierce KL, Laporte S, Oakley RH*, et al.* The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Biol Chem* 2003, **278:** 6258-6267.
126. Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S*, et al.* beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J Biol Chem* 2006, **281:** 1261-1273.
127. Tohgo A, Pierce KL, Choy EW, Lefkowitz RJ, Luttrell LM. beta-Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. *J Biol Chem* 2002, **277:** 9429-9436.
128. Wei H, Ahn S, Barnes WG, Lefkowitz RJ. Stable interaction between beta-arrestin 2 and angiotensin type 1A receptor is required for beta-arrestin 2-mediated activation of extracellular signal-regulated kinases 1 and 2. *J Biol Chem* 2004, **279:** 48255-48261.
129. DeFea KA, Zalevsky J, Thoma MS, Déry O, Mullins RD, Bunnett NW. beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J Cell Biol* 2000, **148:** 1267-1281.
130. Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern Jr*, et al.* A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 1997, **277:** 693-696.
131. Negri S, Oberson A, Steinmann M, Sauser C, Nicod P, Waeber G*, et al.* cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. *Genomics* 2000, **64:** 324-330.

132. Whitmarsh AJ, Cavanagh J, Tournier C, Yasuda J, Davis RJ. A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* 1998, **281:** 1671-1674.
133. Yasuda J, Whitmarsh AJ, Cavanagh J, Sharma M, Davis RJ. The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol Cell Biol* 1999, **19:** 7245-7254.
134. Willoughby EA, Perkins GR, Collins MK, Whitmarsh AJ. The JIP-1 scaffold protein targets MKP-7 to dephosphorylate JNK. *J Biol Chem* 2003, **278:** 10731-10736.
135. Kim AH, Yano H, Cho HJ, Meyer DI, Monks B, Margolis B*, et al.* Akt1 regulates a JNK scaffold during excitotoxic apoptosis. *Neuron* 2002, **35:** 697-709.
136. Miller WE, Lefkowitz RJ. Expanding roles for beta-arrestins as scaffolds and adapters in GPCR signaling and trafficking. *Curr Opin Cell Biol* 2001, **13:** 139-145.
137. McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT*, et al.* Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* 2000, **290:** 1574-1577.
138. Zhan X, Kaoud TS, Kook S, Dalby KN, Gurevich VV. JNK3 enzyme binding to arrestin-3 differentially affects the recruitment of upstream mitogen-activated protein (MAP) kinase kinases. *J Biol Chem* 2013, **288:** 28535-28547.
139. Kook S, Zhan X, Kaoud TS, Dalby KN, Gurevich VV, Gurevich EV. Arrestin-3 binds c-Jun N-terminal kinase 1 (JNK1) and JNK2 and facilitates the activation of these ubiquitous JNK isoforms in cells via scaffolding. *J Biol Chem* 2013, **288:** 37332-37342.
140. Sánchez-Fernández G, Cabezudo S, García-Hoz C, Tobin AB, Mayor FJ, Ribas C. ERK5 activation by Gq-coupled muscarinic receptors is independent of receptor internalization and β-arrestin recruitment. *PLoS One* 2013, **8:** e84174.
141. Yao Z, Seger R. The ERK signaling cascade--views from different subcellular compartments. *Biofactors* 2009, **35:** 407-416.
142. Rubinfeld H, Hanoch T, Seger R. Identification of a cytoplasmic-retention sequence in ERK2. *J Biol Chem* 1999, **274:** 30349-30352.
143. Shibayama S, Shibata-Seita R, Miura K, Kirino Y, Takishima K. Identification of a C-terminal region that is required for the nuclear translocation of ERK2 by passive diffusion. *J Biol Chem* 2002, **277:** 37777-37782.
144. Wolf I, Rubinfeld H, Yoon S, Marmor G, Hanoch T, Seger R. Involvement of the activation loop of ERK in the detachment from cytosolic anchoring. *J Biol Chem* 2001, **276:** 24490-24497.
145. Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E*, et al.* Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* 1998, **93**(4)**:** 605-615.
146. Wunderlich W, Fialka I, Teis D, Alpi A, Pfeiffer A, Parton RG*, et al.* A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mp1 on a late endosomal/lysosomal compartment. *J Cell Biol* 2001, **152:** 765-776.
147. Lunin VV, Munger C, Wagner J, Ye Z, Cygler M, Sacher M. The structure of the MAPK scaffold, MP1, bound to its partner, p14. A complex with a critical role in endosomal map kinase signaling. *J Biol Chem* 2004, **279**(22)**:** 23422-23430.
148. Brahma A, Dalby KN. Regulation of protein phosphorylation within the MKK1-ERK2 complex by MP1 and the MP1*P14 heterodimer. *Archives of biochemistry and biophysics* 2007, **460**(1)**:** 85-91.
149. Torii S, Kusakabe M, Yamamoto T, Maekawa M, Nishida E. Sef is a spatial regulator for Ras/MAP kinase signaling. *Dev Cell* 2004, **7:** 33-44.
150. Galli S, Jahn O, Hitt R, Hesse D, Opitz L, Plessmann U*, et al.* A new paradigm for MAPK: structural interactions of hERK1 with mitochondria in HeLa cells. *PLoS One* 2009, **4:** e7541.
151. Poderoso C, Converso DP, Maloberti P, Duarte A, Neuman I, Galli S*, et al.* A mitochondrial kinase complex is essential to mediate an ERK1/2-dependent phosphorylation of a key regulatory protein in steroid biosynthesis. *PLoS One* 2008, **3**(1)**:** e1443.
152. Duarte A, Castillo AF, Podesta EJ, Poderoso C. Mitochondrial fusion and ERK activity regulate steroidogenic acute regulatory protein localization in mitochondria. *PLoS One* 2014, **9**(6)**:** e100387.
153. O'Donovan KJ, Tourellotte WG, Millbrandt J, Baraban JM. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci* 1999, **22:** 167-173.

154. Pagel JI, Deindl E. Early growth response 1--a transcription factor in the crossfire of signal transduction cascades. *Indian J Biochem Biophys* 2011, **48:** 226-235.
155. Chavrier P, Lemaire P, Revelant O, Bravo R, Charnay P. Characterization of a mouse multigene family that encodes zinc finger structures. *Mol Cell Biol* 1988, **8:** 1319-1326.
156. Christy B, Nathans D. DNA binding site of the growth factor-inducible protein Zif268. *Proc Natl Acad Sci U S A* 1989, **86:** 8737-8741.
157. Nardelli J, Gibson TJ, Vesque C, Charnay P. Base sequence discrimination by zinc-finger DNA-binding domains. *Nature* 1991, **349:** 175-178.
158. Sukhatme VP, Cao XM, Chang LC, Tsai-Morris CH, Stamenkovich D, Ferreira PC*, et al.* A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. *Cell* 1988, **53:** 37-43.
159. Joseph LJ, Le Beau MM, Jamieson GAJ, Acharya S, Shows TB, Rowley JD*, et al.* Molecular cloning, sequencing, and mapping of EGR2, a human early growth response gene encoding a protein with "zinc-binding finger" structure. *Proc Natl Acad Sci U S A* 1988, **85:** 7164-7168.
160. Kharbanda S, Nakamura T, Stone R, Hass R, Bernstein S, Datta R*, et al.* Expression of the early growth response 1 and 2 zinc finger genes during induction of monocytic differentiation. *J Clin Invest* 1991, **88:** 571-577.
161. Patwardhan S, Gashler A, Siegel MG, Chang LC, Joseph LJ, Shows TB*, et al.* EGR3, a novel member of the Egr family of genes encoding immediate-early transcription factors. *Oncogene* 1991, **6:** 917-928.
162. Poirier R, Cheval H, Mailhes C, Garel S, Charnay P, Davis S*, et al.* Distinct functions of egr gene family members in cognitive processes. *Front Neurosci* 2008, **2:** 47-55.
163. O'Donovan KJ, Levkovitz Y, Ahn D, Baraban JM. Functional comparison of Egr3 transcription factor isoforms: identification of an activation domain in the N-terminal segment absent from Egr3beta, a major isoform expressed in brain. *J Neurochem* 2000, **75:** 1352-1357.
164. O'Donovan KJ, Baraban JM. Major Egr3 isoforms are generated via alternate translation start sites and differ in their abilities to activate transcription. *Mol Cell Biol* 1999, **19:** 4711-4718.
165. Kumbrink J, Gerlinger M, Johnson JP. Egr-1 induces the expression of its corepressor nab2 by activation of the nab2 promoter thereby establishing a negative feedback loop. *J Biol Chem* 2005, **280:** 42785-42793.
166. Kumbrink J, Kirsch KH, Johnson JP. EGR1, EGR2, and EGR3 activate the expression of their coregulator NAB2 establishing a negative feedback loop in cells of neuroectodermal and epithelial origin. *J Cell Biochem* 2010, **111:** 207-217.
167. Zipfel PF, Decker EL, Holst C, Sherka C. The human zinc finger protein EGR-4 acts as autoregulatory transcriptional repressor. *Biochim Biophys Acta* 1997, **1354:** 134-144.
168. Cole AJ, Saffen DW, Baraban JM, Worley PF. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* 1989, **340:** 474-476.
169. Williams J, Dragunow M, Lawlor P, Mason S, Abraham WC, Leah J*, et al.* Krox20 may play a key role in the stabilization of long-term potentiation. *Brain Res Mol Brain Res* 1995, **28:** 87-93.
170. Worley PF, Bhat RV, Baraban JM, Erickson CA, McNaughton BL, Barnes CA. Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. *J Neurosci* 1993, **13:** 4776-4786.
171. Yamagata K, Kaufmann WE, Lanahan A, Papapavlou M, Barnes CA, Andreasson KI*, et al.* Egr3/Pilot, a zinc finger transcription factor, is rapidly regulated by activity in brain neurons and colocalizes with Egr1/zif268. *Learn Mem* 1994, **1:** 140-152.
172. Tourtellotte WG, Milbrandt J. Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3. *Nat Genet* 1998, **20:** 87-91.
173. Tourtellotte WG, Keller-Peck C, Milbrandt J, Kucera J. The transcription factor Egr3 modulates sensory axon-myotube interactions during muscle spindle morphogenesis. *Dev Biol* 2001, **232:** 388-399.
174. Swiatek PJ, Gridley T. Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. *Genes Dev* 1993, **7:** 2071-2084.

175. Topilko P, Schneider-Maunoury S, Levi G, Trembleau A, Gourdji D, Driancourt MA*, et al.* Multiple pituitary and ovarian defects in Krox-24 (NGFIA/Egr-1) targeted mice. *Mol Endocrinol* 1998, **12:** 107-122.
176. Lee SL, Sadovsky Y, Swirnoff AH, Polish JA, Goda P, Gavrilina G*, et al.* Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). *Science* 1996, **273:** 1219-1221.
177. Tourtellotte WG, Nagarajan R, Auyeung A, Mueller C, Milbrandt J. Infertility associated with incomplete spermatogenic arrest and oligozoospermia in Egr4-deficient mice. *Development* 1999, **126:** 5061-5071.
178. Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S*, et al.* A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat Neurosci* 2001, **4:** 289-296.
179. Penke Z, Morice E, Veyrac A, Gros A, Chagneau C, LeBlanc P*, et al.* Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Philos Trans R Soc Lond B Biol Sci* 2013, **369:** 20130159.
180. Li L, Yun SH, Keblesh J, Trommer BL, Xiong H, Radulovic J*, et al.* Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. *Mol Cell Neurosci* 2007, **35:** 76-88.
181. Gallitano-Mendel A, Izumi Y, Tokuda K, Zorumski CF, Howell MP, Muglia LJ*, et al.* The immediate early gene early growth response gene 3 mediates adaptation to stress and novelty. *Neuroscience* 2007, **148:** 633-643.
182. Kim SH, Song JY, Joo EJ, Lee KY, Ahn YM, Kim YS. EGR3 as a potential susceptibility gene for schizophrenia in Korea. *Am J Med Genet B Neuropsychiatr Genet* 2010, **153B:** 1355-1360.
183. Gallitano AL, Tillman R, Dinu V, Geller B. Family-based association study of early growth response gene 3 with child bipolar I disorder. *J Affect Disord* 2012, **138:** 387-396.
184. Zhang R, Lu S, Meng L, Min Z, Tian J, Valenzuela RK*, et al.* Genetic evidence for the association between the early growth response 3 (EGR3) gene and schizophrenia. *PLoS One* 2012, **7:** e30237.
185. Maple AM, Zhao X, Elizalde DI, McBride AK, Gallitano AL. Htr2a Expression Responds Rapidly to Environmental Stimuli in an Egr3-Dependent Manner. *ACS Chem Neurosci* 2015.
186. Hendrickx A, Pierrot N, Tasiaux B, Schakman O, Kienlen-Campard P, De Smet C*, et al.* Epigenetic regulations of immediate early genes expression involved in memory formation by the amyloid precursor protein of Alzheimer disease. *PLoS One* 2014, **9:** e99467.
187. Koldamova R, Schug J, Lefterova M, Cronican AA, Fitz NF, Davenport FA*, et al.* Genome-wide approaches reveal EGR1-controlled regulatory networks associated with neurodegeneration. *Neuobiol Dis* 2014, **63:** 107-114.
188. Gómez Ravetti M, Rosso OA, Berretta R, Moscato P. Uncovering molecular biomarkers that correlate cognitive decline with the changes of hippocampus' gene expression profiles in Alzheimer's disease. *PLoS One* 2010, **5:** e10153.
189. MacGibbon GA, Lawlor PA, Walton M, Sirimanne E, Faull RL, Synek B*, et al.* Expression of Fos, Jun, and Krox family proteins in Alzheimer's disease. *Exp Neurol* 1997, **147:** 316-332.
190. Kurian SM, Le-Niculescu H, Patel SD, Bertram D, Davis J, Dike C*, et al.* Identification of blood biomarkers for psychosis using convergent functional genomics. *Molecular psychiatry* 2011, **16**(1)**:** 37-58.
191. Yamada K, Gerber DJ, Iwayama Y, Ohnishi T, Ohba H, Toyota T*, et al.* Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. *Proc Natl Acad Sci U S A* 2007, **104**(8)**:** 2815-2820.
192. Li S, Miao T, Sebastian M, Bhullar P, Ghaffari E, Liu M*, et al.* The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells. *Immunity* 2012, **37**(4)**:** 685-696.
193. Miah MA, Byeon SE, Ahmed MS, Yoon CH, Ha SJ, Bae YS. Egr2 induced during DC development acts as an intrinsic negative regulator of DC immunogenicity. *Eur J Immunol* 2013, **43**(9)**:** 2484-2496.

194. Du N, Kwon H, Li P, West EE, Oh J, Liao W*, et al.* EGR2 is critical for peripheral naive T-cell differentiation and the T-cell response to influenza. *Proc Natl Acad Sci U S A* 2014, **111**(46)**:** 16484-16489.
195. Safford M, Collins S, Lutz MA, Allen A, Huang CT, Kowalski J*, et al.* Egr-2 and Egr-3 are negative regulators of T cell activation. *Nat Immunol* 2005, **6:** 472-480.
196. Eid MA, Kumar MV, Iczkowski KA, Bostwick DG, Tindall DJ. Expression of early growth response genes in human prostate cancer. *Cancer Res* 1998, **58**(11)**:** 2461-2468.
197. Thigpen AE, Cala KM, Guileyardo JM, Molberg KH, McConnell JD, Russell DW. Increased expression of early growth response-1 messenger ribonucleic acid in prostatic adenocarcinoma. *The Journal of urology* 1996, **155**(3)**:** 975-981.
198. Yang SZ, Eltoum IA, Abdulkadir SA. Enhanced EGR1 activity promotes the growth of prostate cancer cells in an androgen-depleted environment. *J Cell Biochem* 2006, **97**(6)**:** 1292-1299.
199. Yang SZ, Abdulkadir SA. Early growth response gene 1 modulates androgen receptor signaling in prostate carcinoma cells. *J Biol Chem* 2003, **278**(41)**:** 39906-39911.
200. Abdulkadir SA, Qu Z, Garabedian E, Song SK, Peters TJ, Svaren J*, et al.* Impaired prostate tumorigenesis in Egr1-deficient mice. *Nature medicine* 2001, **7**(1)**:** 101-107.
201. Svaren J, Ehrig T, Abdulkadir SA, Ehrengruber MU, Watson MA, Milbrandt J. EGR1 target genes in prostate carcinoma cells identified by microarray analysis. *J Biol Chem* 2000, **275**(49)**:** 38524-38531.
202. Baron V, Duss S, Rhim J, Mercola D. Antisense to the early growth response-1 gene (Egr-1) inhibits prostate tumor development in TRAMP mice. *Annals of the New York Academy of Sciences* 2003, **1002:** 197-216.
203. Virolle T, Krones-Herzig A, Baron V, De Gregorio G, Adamson ED, Mercola D. Egr1 promotes growth and survival of prostate cancer cells. Identification of novel Egr1 target genes. *J Biol Chem* 2003, **278**(14)**:** 11802-11810.
204. Ma J, Ren Z, Ma Y, Xu L, Zhao Y, Zheng C*, et al.* Targeted knockdown of EGR-1 inhibits IL-8 production and IL-8-mediated invasion of prostate cancer cells through suppressing EGR-1/NF-kappaB synergy. *J Biol Chem* 2009, **284:** 34600-34606.
205. Parra E, Ferreira J, Ortega A. Overexpression of EGR-1 modulates the activity of NF-kappaB and AP-1 in prostate carcinoma PC-3 and LNCaP cell lines. *International journal of oncology* 2011, **39**(2)**:** 345-352.
206. Abdulkadir SA, Carbone JM, Naughton CK, Humphrey PA, Catalona WJ, Milbrandt J. Frequent and early loss of the EGR1 corepressor NAB2 in human prostate carcinoma. *Human pathology* 2001, **32**(9)**:** 935-939.
207. Chen L, Wang S, Zhou Y, Wu X, Entin I, Epstein J*, et al.* Identification of early growth response protein 1 (EGR-1) as a novel target for JUN-induced apoptosis in multiple myeloma. *Blood* 2010, **115:** 61-70.
208. Huang RP, Fan Y, de Belle I, Niemeyer C, Gottardis MM, Mercola D*, et al.* Decreased Egr-1 expression in human, mouse and rat mammary cells and tissues correlates with tumor formation. *International journal of cancer Journal international du cancer* 1997, **72**(1)**:** 102-109.
209. Joslin JM, Fernald AA, Tennant TR, Davis EM, Kogan SC, Anastasi J*, et al.* Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. *Blood* 2007, **110**(2)**:** 719-726.
210. Calogero A, Arcella A, De Gregorio G, Porcellini A, Mercola D, Liu C*, et al.* The early growth response gene EGR-1 behaves as a suppressor gene that is down-regulated independent of ARF/Mdm2 but not p53 alterations in fresh human gliomas. *Clin Cancer Res* 2001, **7**(9)**:** 2788-2796.
211. Levin WJ, Press MF, Gaynor RB, Sukhatme VP, Boone TC, Reissmann PT*, et al.* Expression patterns of immediate early transcription factors in human non-small cell lung cancer. The Lung Cancer Study Group. *Oncogene* 1995, **11**(7)**:** 1261-1269.
212. Shin SY, Kim CG, Lee YH. Egr-1 regulates the transcription of the BRCA1 gene by etoposide. *BMB Rep* 2013, **46:** 92-96.
213. Sun T, Tian H, Feng YG, Zhu YQ, Zhang WQ. Egr-1 promotes cell proliferation and invasion by increasing β-catenin expression in gastric cancer. *Dig Dis Sci* 2013, **58:** 423-430.
214. Peng WX, Pan FY, Liu XJ, Ning S, Xu N, Meng FL*, et al.* Hypoxia stabilizes microtubule networks and decreases tumor cell chemosensitivity to anticancer drugs through Egr-1. *Anat Rec* 2010, **293:** 414-420.

215. Mahaligham D, Natoni A, Keane M, Samali A, Szegezdi E. Early growth response-1 is a regulator of DR5-induced apoptosis in colon cancer cells. *Br J Cancer* 2010, **102:** 754-764.
216. Egerod FL, Bartels A, Fristrup N, Borre M, Ørntoft TF, Oleskiewicz MB*, et al.* High frequency of tumor cells with nuclear Egr-1 protein expression in human bladder cancer is associated with disease progression. *BMC Cancer* 2009, **30**(9)**:** 385.
217. Tao W, Shi JF, Zhang Q, Xue B, Sun YJ, Li CJ. Egr-1 enhances drug resistance of breast cancer by modulating MDR1 expression in a GGPPS-independent manner. *Biomed Pharmacother* 2013, **67:** 197-202.
218. Myung DS, Park YL, Kim N, Chung CY, Park HC, Kim JS*, et al.* Expression of early growth response-1 in colorectal cancer and its relation to tumor cell proliferation and apoptosis. *Oncol Rep* 2014, **31:** 788-794.
219. Woo SM, Min KJ, Kim S, Park JW, Kim DE, Chun KS*, et al.* Silibinin induces apoptosis of HT29 colon carcinoma cells through early growth response-1 (EGR-1)-mediated non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) up-regulation. *Chem Biol Interact* 2014, **211:** 36-43.
220. Shin SY, Kim JH, Lee JH, Lim Y, Lee YH. 2'-Hydroxyflavanone induces apoptosis through Egr-1 involving expression of Bax, p21, and NAG-1 in colon cancer cells. *Mol Nutr Food Res* 2012, **56:** 761-774.
221. Lim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB*, et al.* NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. *Apoptosis : an international journal on programmed cell death* 2007, **12**(2)**:** 411-421.
222. Kwon O, Soung NK, Thimmegowda NR, Jeong SJ, Jang JH, Moon DO*, et al.* Patulin induces colorectal cancer cells apoptosis through EGR-1 dependent ATF3 up-regulation. *Cell Signal* 2012, **24:** 943-950.
223. Pio R, Jia Z, Baron VT, Mercola D. Early growth response 3 (Egr3) is highly over-expressed in non-relapsing prostate cancer but not in relapsing prostate cancer. *PLoS One* 2013, **8**(1)**:** e54096.
224. Baron VT, Pio R, Jia Z, Mercola D. Early Growth Response 3 regulates genes of inflammation and directly activates IL6 and IL8 expression in prostate cancer. *Br J Cancer* 2015, **112:** 755-764.
225. Inoue A, Omoto Y, Yamaguchi Y, Kiyama R, Hayashi SI. Transcription factor EGR3 is involved in the estrogen-signaling pathway in breast cancer cells. *J Mol Endocrinol* 2004, **32:** 649-661.
226. Suzuki T, Inoue A, Miki Y, Moriya T, Akahira J, Ishida T*, et al.* Early growth responsive gene 3 in human breast carcinoma: a regulator of estrogen-meditated invasion and a potent prognostic factor. *Endocrine-related cancer* 2007, **14**(2)**:** 279-292.
227. Liao F, Ji MY, Shen L, Qiu S, Guo XF, Dong WG. Decreased EGR3 expression is related to poor prognosis in patients with gastric cancer. *J Mol Histol* 2013, **44:** 463-468.
228. Farhan H, Rabouille C. Signalling to and from the secretory pathway. *J Cell Sci* 2011, **124:** 171-180.
229. Cancino J, Luini A. Signaling circuits on the Golgi complex. *Traffic* 2013, **14**(2)**:** 121-134.
230. Lee TH, Linstedt AD. Potential role for protein kinases in regulation of bidirectional endoplasmic reticulum-to-Golgi transport revealed by protein kinase inhibitor H89. *Mol Biol Cell* 2000, **11**(8)**:** 2577-2590.
231. Aridor M, Balch WE. Kinase Signaling Initiates Coat Complex II (COPII) Recruitment and Export from the Mammalian Endoplasmic Reticulum. *J Biol Chem* 2000, **275**(46)**:** 35673-35676.
232. Nakagawa H, Miyazaki S, Abe T, Umadome H, Tanaka K, Nishimura K*, et al.* H89 sensitive kinase regulates the translocation of Sar1 onto the ER membrane through phosphorylation of ER-coupled beta-tubulin. *The international journal of biochemistry & cell biology* 2011, **43**(3)**:** 423-430.
233. Nakagawa H, Ishizaki M, Miyazaki S, Abe T, Nishimura K, Komori M*, et al.* Sar1 translocation onto the ER-membrane for vesicle budding has different pathways for promotion and suppression of ER-to-Golgi transport mediated through H89-sensitive kinase and ER-resident G protein. *Molecular and cellular biochemistry* 2012, **366**(1-2)**:** 175-182.

234. Nakagawa H, Umadome H, Miyazaki S, Tanaka K, Nishimura K, Komori M*, et al.* ER-resident Gi2 protein controls sar1 translocation onto the ER during budding of transport vesicles. *J Cell Biochem* 2011, **112**(9)**:** 2250-2256.
235. Pryde JG, Farmaki T, Lucocq JM. Okadaic acid induces selective arrest of protein transport in the rough endoplasmic reticulum and prevents export into COPII-coated structures. *Mol Cell Biol* 1998, **18**(2)**:** 1125-1135.
236. Siddiqi S, Mansbach CM, 2nd. Dietary and biliary phosphatidylcholine activates PKCzeta in rat intestine. *Journal of lipid research* 2015, **56**(4)**:** 859-870.
237. Siddiqi SA, Mansbach CM, 2nd. PKC zeta-mediated phosphorylation controls budding of the pre-chylomicron transport vesicle. *J Cell Sci* 2008, **121**(Pt 14)**:** 2327-2338.
238. Siddiqi S, Mansbach CM, 2nd. Phosphorylation of Sar1b protein releases liver fatty acid-binding protein from multiprotein complex in intestinal cytosol enabling it to bind to endoplasmic reticulum (ER) and bud the pre-chylomicron transport vesicle. *J Biol Chem* 2012, **287**(13)**:** 10178-10188.
239. Palmer KJ, Konkel JE, Stephens DJ. PCTAIRE protein kinases interact directly with the COPII complex and modulate secretory cargo transport. *J Cell Sci* 2005, **118**(Pt 17)**:** 3839-3847.
240. Dudognon P, Maeder-Garavaglia C, Carpentier JL, Paccaud JP. Regulation of a COPII component by cytosolic O-glycosylation during mitosis. *FEBS Lett* 2004, **561**(1-3)**:** 44-50.
241. Sharpe LJ, Luu W, Brown AJ. Akt phosphorylates Sec24: new clues into the regulation of ER-to-Golgi trafficking. *Traffic* 2011, **12**(1)**:** 19-27.
242. Du X, Kristiana I, Wong J, Brown AJ. Involvement of Akt in ER-to-Golgi transport of SCAP/SREBP: a link between a key cell proliferative pathway and membrane synthesis. *Mol Biol Cell* 2006, **17**(6)**:** 2735-2745.
243. Malide D, Yewdell JW, Bennink JR, Cushman SW. The export of major histocompatibility complex class I molecules from the endoplasmic reticulum of rat brown adipose cells is acutely stimulated by insulin. *Mol Biol Cell* 2001, **12**(1)**:** 101-114.
244. Giussani P, Brioschi L, Bassi R, Riboni L, Viani P. Phosphatidylinositol 3-kinase/AKT pathway regulates the endoplasmic reticulum to golgi traffic of ceramide in glioma cells: a link between lipid signaling pathways involved in the control of cell survival. *J Biol Chem* 2009, **284**(8)**:** 5088-5096.
245. Salama NR, Chuang JS, Schekman RW. Sec31 encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum. *Mol Biol Cell* 1997, **8**(2)**:** 205-217.
246. Koreishi M, Yu S, Oda M, Honjo Y, Satoh A. CK2 phosphorylates Sec31 and regulates ER-To-Golgi trafficking. *PLoS One* 2013, **8**(1)**:** e54382.
247. Farhan H, Wendeler MW, Mitrovic S, Fava E, Silberberg Y, Sharan R*, et al.* MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *J Cell Biol* 2010, **189**(6)**:** 997-1011.
248. Yu S, Roth MG. Casein kinase I regulates membrane binding by ARF GAP1. *Mol Biol Cell* 2002, **13**(8)**:** 2559-2570.
249. Lord C, Bhandari D, Menon S, Ghassemian M, Nycz D, Hay J*, et al.* Sequential interactions with Sec23 control the direction of vesicle traffic. *Nature* 2011, **473:** 181-186.
250. Reiterer V, Maier S, Sitte HH, Kriz A, Ruegg MA, Hauri HP*, et al.* Sec24- and ARFGAP1-dependent trafficking of GABA transporter-1 is a prerequisite for correct axonal targeting. *J Neurosci* 2008, **28**(47)**:** 12453-12464.
251. Dascher C, Balch WE. Dominant inhibitory mutants of ARF1 block endoplasmic reticulum to Golgi transport and trigger disassembly of the Golgi apparatus. *J Biol Chem* 1994, **269**(2)**:** 1437-1448.
252. Tisdale EJ, Balch WE. Rab2 is essential for the maturation of pre-Golgi intermediates. *J Biol Chem* 1996, **271**(46)**:** 29372-29379.
253. Tisdale EJ, Jackson MR. Rab2 protein enhances coatomer recruitment to pre-Golgi intermediates. *J Biol Chem* 1998, **273**(27)**:** 17269-17277.
254. Tisdale EJ. A Rab2 mutant with impaired GTPase activity stimulates vesicle formation from pre-Golgi intermediates. *Mol Biol Cell* 1999, **10**(6)**:** 1837-1849.
255. Tisdale EJ. Rab2 requires PKC iota/lambda to recruit beta-COP for vesicle formation. *Traffic* 2000, **1**(9)**:** 702-712.

256. Tisdale EJ. Glyceraldehyde-3-phosphate dehydrogenase is required for vesicular transport in the early secretory pathway. *J Biol Chem* 2001, **276**(4)**:** 2480-2486.
257. Tisdale EJ. Rab2 interacts directly with atypical protein kinase C (aPKC) iota/lambda and inhibits aPKCiota/lambda-dependent glyceraldehyde-3-phosphate dehydrogenase phosphorylation. *J Biol Chem* 2003, **278**(52)**:** 52524-52530.
258. Tisdale EJ. Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase Ciota /lambda and plays a role in microtubule dynamics in the early secretory pathway. *J Biol Chem* 2002, **277**(5)**:** 3334-3341.
259. Tisdale EJ, Azizi F, Artalejo CR. Rab2 utilizes glyceraldehyde-3-phosphate dehydrogenase and protein kinase C{iota} to associate with microtubules and to recruit dynein. *J Biol Chem* 2009, **284**(9)**:** 5876-5884.
260. Tisdale EJ, Artalejo CR. Src-dependent aprotein kinase C iota/lambda (aPKCiota/lambda) tyrosine phosphorylation is required for aPKCiota/lambda association with Rab2 and glyceraldehyde-3-phosphate dehydrogenase on pre-golgi intermediates. *J Biol Chem* 2006, **281**(13)**:** 8436-8442.
261. Wooten MW, Vandenplas ML, Seibenhener ML, Geetha T, Diaz-Meco MT. Nerve growth factor stimulates multisite tyrosine phosphorylation and activation of the atypical protein kinase C's via a src kinase pathway. *Mol Cell Biol* 2001, **21**(24)**:** 8414-8427.
262. Tisdale EJ, Artalejo CR. A GAPDH mutant defective in Src-dependent tyrosine phosphorylation impedes Rab2-mediated events. *Traffic* 2007, **8**(6)**:** 733-741.
263. Itakura E, Kishi-Itakura C, Mizushima N. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. *Cell* 2012, **151**(6)**:** 1256-1269.
264. Muppirala M, Gupta V, Swarup G. Syntaxin 17 cycles between the ER and ERGIC and is required to maintain the architecture of ERGIC and Golgi. *Biol Cell* 2011, **103**(7)**:** 333-350.
265. Muppirala M, Gupta V, Swarup G. Tyrosine phosphorylation of a SNARE protein, syntaxin 17: implications for membrane trafficking in the early secretory pathway. *Biochim Biophys Acta* 2012, **1823**(12)**:** 2109-2119.
266. Muppirala M, Gupta V, Swarup G. Emerging role of tyrosine phosphatase, TCPTP, in the organelles of the early secretory pathway. *Biochim Biophys Acta* 2013, **1833**(5)**:** 1125-1132.
267. Gupta V, Swarup G. Evidence for a role of transmembrane protein p25 in localization of protein tyrosine phosphatase TC48 to the ER. *J Cell Sci* 2006, **119**(Pt 9)**:** 1703-1714.
268. Ben-Tekaya H, Kahn RA, Hauri HP. ADP ribosylation factors 1 and 4 and group VIA phospholipase A(2) regulate morphology and intraorganellar traffic in the endoplasmic reticulum-Golgi intermediate compartment. *Mol Biol Cell* 2010, **21**(23)**:** 4130-4140.
269. Lei X, Bone RN, Ali T, Zhang S, Bohrer A, Tse HM*, et al.* Evidence of contribution of iPLA2beta-mediated events during islet beta-cell apoptosis due to proinflammatory cytokines suggests a role for iPLA2beta in T1D development. *Endocrinology* 2014, **155**(9)**:** 3352-3364.
270. Bone RN, Gai Y, Magrioti V, Kokotou MG, Ali T, Lei X*, et al.* Inhibition of Ca2+-independent phospholipase A2beta (iPLA2beta) ameliorates islet infiltration and incidence of diabetes in NOD mice. *Diabetes* 2015, **64**(2)**:** 541-554.
271. Akiba S, Ohno S, Chiba M, Kume K, Hayama M, Sato T. Protein kinase Calpha-dependent increase in Ca2+-independent phospholipase A2 in membranes and arachidonic acid liberation in zymosan-stimulated macrophage-like P388D1 cells. *Biochemical pharmacology* 2002, **63**(11)**:** 1969-1977.
272. Akiba S, Mizunaga S, Kume K, Hayama M, Sato T. Involvement of group VI Ca2+-independent phospholipase A2 in protein kinase C-dependent arachidonic acid liberation in zymosan-stimulated macrophage-like P388D1 cells. *J Biol Chem* 1999, **274**(28)**:** 19906-19912.
273. Colanzi A, Sutterlin C. Signaling at the Golgi during mitosis. *Methods in cell biology* 2013, **118:** 383-400.
274. Feinstein TN, Linstedt AD. GRASP55 regulates Golgi ribbon formation. *Mol Biol Cell* 2008, **19**(7)**:** 2696-2707.
275. Jesch SA, Lewis TS, Ahn NG, Linstedt AD. Mitotic phosphorylation of Golgi reassembly stacking protein 55 by mitogen-activated protein kinase ERK2. *Mol Biol Cell* 2001, **12**(6)**:** 1811-1817.

276. Bisel B, Wang Y, Wei JH, Xiang Y, Tang D, Miron-Mendoza M*, et al.* ERK regulates Golgi and centrosome orientation towards the leading edge through GRASP65. *J Cell Biol* 2008, **182**(5)**:** 837-843.
277. Wei JH, Seemann J. Remodeling of the Golgi structure by ERK signaling. *Communicative & integrative biology* 2009, **2**(1)**:** 35-36.
278. Yoshimura S, Yoshioka K, Barr FA, Lowe M, Nakayama K, Ohkuma S*, et al.* Convergence of cell cycle regulation and growth factor signals on GRASP65. *J Biol Chem* 2005, **280**(24)**:** 23048-23056.
279. Tang D, Yuan H, Vielemeyer O, Perez F, Wang Y. Sequential phosphorylation of GRASP65 during mitotic Golgi disassembly. *Biology open* 2012, **1**(12)**:** 1204-1214.
280. Xiang Y, Wang Y. GRASP55 and GRASP65 play complementary and essential roles in Golgi cisternal stacking. *J Cell Biol* 2010, **188**(2)**:** 237-251.
281. Xiang Y, Zhang X, Nix DB, Katoh T, Aoki K, Tiemeyer M*, et al.* Regulation of protein glycosylation and sorting by the Golgi matrix proteins GRASP55/65. *Nature communications* 2013, **4:** 1659.
282. Tillmann KD, Millarte V, Farhan H. Regulation of traffic and organelle architecture of the ER-Golgi interface by signal transduction. *Histochem Cell Biol* 2013, **140**(3)**:** 297-306.
283. Yi P, Nguyen DT, Higa-Nishiyama A, Auguste P, Bouchecareilh M, Dominguez M*, et al.* MAPK scaffolding by BIT1 in the Golgi complex modulates stress resistance. *J Cell Sci* 2010, **123**(Pt 7)**:** 1060-1072.
284. Biliran H, Jan Y, Chen R, Pasquale EB, Ruoslahti E. Protein kinase D is a positive regulator of Bit1 apoptotic function. *J Biol Chem* 2008, **283**(42)**:** 28029-28037.
285. Jenning S, Pham T, Ireland SK, Ruoslahti E, Biliran H. Bit1 in anoikis resistance and tumor metastasis. *Cancer letters* 2013, **333**(2)**:** 147-151.
286. Kairouz-Wahbe R, Biliran H, Luo X, Khor I, Wankell M, Besch-Williford C*, et al.* Anoikis effector Bit1 negatively regulates Erk activity. *Proc Natl Acad Sci U S A* 2008, **105**(5)**:** 1528-1532.
287. Mukhopadhyay S, Bachert C, Smith DR, Linstedt AD. Manganese-induced trafficking and turnover of the cis-Golgi glycoprotein GPP130. *Mol Biol Cell* 2010, **21**(7)**:** 1282-1292.
288. Mukhopadhyay S, Linstedt AD. Manganese blocks intracellular trafficking of Shiga toxin and protects against Shiga toxicosis. *Science* 2012, **335**(6066)**:** 332-335.
289. Mukhopadhyay S, Redler B, Linstedt AD. Shiga toxin-binding site for host cell receptor GPP130 reveals unexpected divergence in toxin-trafficking mechanisms. *Mol Biol Cell* 2013, **24**(15)**:** 2311-2318.
290. Micaroni M, Perinetti G, Berrie CP, Mironov AA. The SPCA1 Ca2+ pump and intracellular membrane trafficking. *Traffic* 2010, **11**(10)**:** 1315-1333.
291. Sepulveda MR, Vanoevelen J, Raeymaekers L, Mata AM, Wuytack F. Silencing the SPCA1 (secretory pathway Ca2+-ATPase isoform 1) impairs Ca2+ homeostasis in the Golgi and disturbs neural polarity. *J Neurosci* 2009, **29**(39)**:** 12174-12182.
292. Farhan H. Systems biology of the secretory pathway: What have we learned so far? *Biol Cell* 2015.
293. Bard F, Casano L, Mallabiabarrena A, Wallace E, Saito K, Kitayama H*, et al.* Functional genomics reveals genes involved in protein secretion and Golgi organization. *Nature* 2006, **439**(7076)**:** 604-607.
294. Kondylis V, Tang Y, Fuchs F, Boutros M, Rabouille C. Identification of ER proteins involved in the functional organisation of the early secretory pathway in Drosophila cells by a targeted RNAi screen. *PLoS One* 2011, **6**(2)**:** e17173.
295. Simpson JC, Joggerst B, Laketa V, Verissimo F, Cetin C, Erfle H*, et al.* Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway. *Nat Cell Biol* 2012, **14**(7)**:** 764-774.
296. Chia J, Goh G, Racine V, Ng S, Kumar P, Bard F. RNAi screening reveals a large signaling network controlling the Golgi apparatus in human cells. *Molecular systems biology* 2012, **8:** 629.
297. Rubinsztein DC, Shpilka T, Elazar Z. Mechanisms of autophagosome biogenesis. *Curr Biol* 2012, **22**(1)**:** R29-34.
298. Weidberg H, Shvets E, Elazar Z. Biogenesis and cargo selectivity of autophagosomes. *Annu Rev Biochem* 2011, **80:** 125-156.
299. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012, **149**(2)**:** 274-293.

300. Russell RC, Yuan HX, Guan KL. Autophagy regulation by nutrient signaling. *Cell Res* 2014, **24**(1)**:** 42-57.
301. Carlsson SR, Simonsen A. Membrane dynamics in autophagosome biogenesis. *J Cell Sci* 2015, **128**(2)**:** 193-205.
302. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A*, et al.* Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* 2008, **182**(4)**:** 685-701.
303. Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* 2010, **6**(6)**:** 764-776.
304. Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat Cell Biol* 2009, **11**(12)**:** 1433-1437.
305. Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. Electron tomography reveals the endoplasmic reticulum as a membrane source for autophagosome formation. *Autophagy* 2010, **6**(2)**:** 301-303.
306. Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. *Autophagy* 2009, **5**(8)**:** 1180-1185.
307. Biazik J, Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. Ultrastructural relationship of the phagophore with surrounding organelles. *Autophagy* 2015, **25**.
308. Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK*, et al.* Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 2010, **141**(4)**:** 656-667.
309. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N*, et al.* Autophagosomes form at ER-mitochondria contact sites. *Nature* 2013, **495**(7441)**:** 389-393.
310. Nanao T, Koike M, Yamaguchi J, Sasaki M, Uchiyama Y. Cellular localization and tissue distribution of endogenous DFCP1 protein. *Biomedical research (Tokyo, Japan)* 2015, **36**(2)**:** 121-133.
311. Ge L, Melville D, Zhang M, Schekman R. The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. *eLife* 2013, **2:** e00947.
312. Graef M, Friedman JR, Graham C, Babu M, Nunnari J. ER exit sites are physical and functional core autophagosome biogenesis components. *Mol Biol Cell* 2013, **24**(18)**:** 2918-2931.
313. Suzuki K, Akioka M, Kondo-Kakuta C, Yamamoto H, Ohsumi Y. Fine mapping of autophagy-related proteins during autophagosome formation in Saccharomyces cerevisiae. *J Cell Sci* 2013, **126**(Pt 11)**:** 2534-2544.
314. Sanchez-Wandelmer J, Ktistakis NT, Reggiori F. ERES: sites for autophagosome biogenesis and maturation? *J Cell Sci* 2015, **128**(2)**:** 185-192.
315. Uemura T, Yamamoto M, Kametaka A, Sou YS, Yabashi A, Yamada A*, et al.* A cluster of thin tubular structures mediates transformation of the endoplasmic reticulum to autophagic isolation membrane. *Mol Cell Biol* 2014, **34**(9)**:** 1695-1706.
316. Duke EM, Razi M, Weston A, Guttmann P, Werner S, Henzler K*, et al.* Imaging endosomes and autophagosomes in whole mammalian cells using correlative cryo-fluorescence and cryo-soft X-ray microscopy (cryo-CLXM). *Ultramicroscopy* 2014, **143:** 77-87.
317. Tan D, Cai Y, Wang J, Zhang J, Menon S, Chou HT*, et al.* The EM structure of the TRAPPIII complex leads to the identification of a requirement for COPII vesicles on the macroautophagy pathway. *Proc Natl Acad Sci U S A* 2013, **110**(48)**:** 19432-19437.
318. Wang J, Tan D, Cai Y, Reinisch KM, Walz T, Ferro-Novick S. A requirement for ER-derived COPII vesicles in phagophore initiation. *Autophagy* 2014, **10**(4)**:** 708-709.
319. Ge L, Zhang M, Schekman R. Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER-Golgi intermediate compartment. *eLife* 2014, **3:** e04135.
320. Ge L, Schekman R. The ER-Golgi intermediate compartment feeds the phagophore membrane. *Autophagy* 2014, **10**(1)**:** 170-172.
321. Ishihara N, Hamasaki M, Yokota S, Suzuki K, Kamada Y, Kihara A*, et al.* Autophagosome requires specific early Sec proteins for its formation and NSF/SNARE for vacuolar fusion. *Mol Biol Cell* 2001, **12**(11)**:** 3690-3702.

322. Hamasaki M, Noda T, Ohsumi Y. The early secretory pathway contributes to autophagy in yeast. *Cell structure and function* 2003, **28**(1)**:** 49-54.
323. Young AR, Chan EY, Hu XW, Kochl R, Crawshaw SG, High S*, et al.* Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J Cell Sci* 2006, **119**(Pt 18)**:** 3888-3900.
324. Ohashi Y, Munro S. Membrane delivery to the yeast autophagosome from the Golgi-endosomal system. *Mol Biol Cell* 2010, **21**(22)**:** 3998-4008.
325. Yamamoto H, Kakuta S, Watanabe TM, Kitamura A, Sekito T, Kondo-Kakuta C*, et al.* Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J Cell Biol* 2012, **198**(2)**:** 219-233.
326. Webber JL, Tooze SA. Coordinated regulation of autophagy by p38alpha MAPK through mAtg9 and p38IP. *The EMBO journal* 2010, **29**(1)**:** 27-40.
327. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat Cell Biol* 2010, **12**(8)**:** 747-757.
328. Ravikumar B, Moreau K, Rubinsztein DC. Plasma membrane helps autophagosomes grow. *Autophagy* 2010, **6**(8)**:** 1184-1186.
329. Puri C, Renna M, Bento CF, Moreau K, Rubinsztein DC. ATG16L1 meets ATG9 in recycling endosomes: additional roles for the plasma membrane and endocytosis in autophagosome biogenesis. *Autophagy* 2014, **10**(1)**:** 182-184.
330. Bruns C, McCaffery JM, Curwin AJ, Duran JM, Malhotra V. Biogenesis of a novel compartment for autophagosome-mediated unconventional protein secretion. *J Cell Biol* 2011, **195**(6)**:** 979-992.
331. Cruz-Garcia D, Curwin AJ, Popoff JF, Bruns C, Duran JM, Malhotra V. Remodeling of secretory compartments creates CUPS during nutrient starvation. *J Cell Biol* 2014, **207**(6)**:** 695-703.
332. Malhotra V. Unconventional protein secretion: an evolving mechanism. *EMBO J* 2013, **32:** 1660-1664.
333. Manjithaya R, Anjard C, Loomis WF, Subramani S. Unconventional secretion of Pichia pastoris Acb1 is dependent on GRASP protein, peroxisomal functions, and autophagosome formation. *J Cell Biol* 2010, **188**(4)**:** 537-546.
334. Duran JM, Anjard C, Stefan C, Loomis WF, Malhotra V. Unconventional secretion of Acb1 is mediated by autophagosomes. *J Cell Biol* 2010, **188**(4)**:** 527-536.
335. Kinseth MA, Anjard C, Fuller D, Guizzunti G, Loomis WF, Malhotra V. The Golgi-associated protein GRASP is required for unconventional protein secretion during development. *Cell* 2007, **130**(3)**:** 524-534.
336. Rubartelli A, Cozzolino F, Talio M, Sitia R. A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. *The EMBO journal* 1990, **9**(5)**:** 1503-1510.
337. Dupont N, Jiang S, Pilli M, Ornatowski W, Bhattacharya D, Deretic V. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. *The EMBO journal* 2011, **30**(23)**:** 4701-4711.
338. Steringer JP, Bleicken S, Andreas H, Zacherl S, Laussmann M, Temmerman K*, et al.* Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2)-dependent oligomerization of fibroblast growth factor 2 (FGF2) triggers the formation of a lipidic membrane pore implicated in unconventional secretion. *J Biol Chem* 2012, **287**(33)**:** 27659-27669.
339. Zacharogianni M, Gomez AA, Veenendaal T, Smout J, Rabouille C. A stress assembly that confers cell viability by preserving ERES components during amino-acid starvation. *eLife* 2014, **3**.

The secretory pathway in cell growth and cancer

Cell growth and proliferation are important processes to ensure viability and optimal functionality of multicellular organisms, but dysregulation can cause a variety of diseases. As a central part of cellular function, the secretory pathway plays an important role in these processes, which will be discussed in this chapter.

1 Signaling pathways controlling proliferation and cell growth

Two major signaling pathways exist that control key cellular processes such as cell survival, differentiation, proliferation, and metabolism; these are the Ras-ERK pathway and the PI3K-mTOR pathway. The Ras-ERK pathway is the most important pathway in the regulation of proliferation, and was introduced previously.

The PI3K-mTOR pathway is a key signaling pathway that controls cell size and growth and is named after two main components, PI3K (phosphatidylinositol 3-kinase (PI3K)) and mammalian/mechanistic target of rapamycin (mTOR). The atypical serine/threonine kinase mTOR belongs to the family of the phosphoinositide 3-kinase (PI3K)-related kinases and is present in two distinct complexes called mTOR complex 1 or 2 (mTORC1 and 2) [1]. The complexes consist of 6 or 7 protein components. mTOR as the kinase is shared between the complexes, as well as five more proteins that are shared. These are the two scaffold proteins Tti and Tel2 that form a complex, mammalian lethal with sec-13 protein 8 (mLST8) whose function is unknown, and the mTOR inhibitor DEP domain containing mTOR-interacting protein (deptor). Other regulatory and scaffolding proteins are complex-specific. The mTORC1 complex additionally contains regulatory-associated protein of mammalian target of rapamycin (raptor) and proline-rich Akt substrate 40 kDa (PRAS40), whereas mTORC2 contains rapamycin-insensitive companion of mTOR (rictor), mammalian stress-activated map kinase-interacting protein 1 (mSin1) as well as protein observed with rictor 1 and 2 (protor1/2) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The mTOR pathway is activated by a variety of external and internal stimuli, such as growth factors, cellular stress, amino acid levels or energy status. Growth factor signaling to RTKs leads to their activation and to intracellular recruitment of the docking proteins insulin receptor substrate (IRS) or

GRB2-associated binder (GAB), which leads to the activation of the lipid kinase PI3K. PI3K then generates phosphatidyl inositol 3,4,5 tri-phosphate (PIP3), which directly or indirectly leads to an activation of several kinases including mTOR in the mTORC2 complex, and protein kinase B (Akt) which leads to an activation of mTORC1. Apart from activating mTORC1, Akt has a large variety of targets and stimulates processes such as cell survival, proliferation, and metabolism. The activated mTORC1 complex also controls a variety of cellular functions related to energy and nutrient homeostasis in the cell, such as inhibition of autophagy and lysosome biogenesis, and stimulation of protein synthesis, lipid synthesis and energy metabolism, ultimately via control of transcription. For example, mTORC1 controls protein synthesis by directly phosphorylating and thereby activating S6 kinase 1 (S6K1), and inhibiting the translation regulators eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), which promotes protein synthesis [1, 17, 18]. The function of mTORC2 complex is less well researched, but it was shown that activated mTORC2 is involved in organization of the cytoskeleton and promotes cell survival and metabolism by directly activating Akt and serum/glucocorticoid regulated kinase (SGK1), which inhibit the transcription factor Forkhead box protein O (FoxO), that, when active, positively regulates the translation of genes involved in induction of apoptosis and of cell cycle inhibitors while negatively regulating the translation of cell cycle activators [1].

These two key signaling pathways can promote proliferation on their own, but evidence for a large amount of cross-talk between these two pathways and other signaling pathways has been found. For example, both Akt and ERK phosphorylate and thereby inhibit the heterodimer of tuberous sclerosis 1 and 2 (TSC1/2), which is an upstream inhibitor of mTORC1 [19, 20, 21, 22, 23, 24, 25]. In addition, many of the downstream targets of the Ras-ERK pathway and the PI3K-mTOR pathway are shared between the two pathways, such as the transcription factors FOXO and c-Myc [18, 26, 27, 28, 29, 30].

One of the hallmarks of cancer is uncontrolled cell division or hyperproliferation. In the majority of cases, this is due to activating mutations in proto-oncogenes or loss-of-function mutations in tumor suppressors. These mutations cause constitutive activation of the Ras-ERK or PI3K-mTOR pathway and therefore continuous proliferation signaling [1, 17, 31, 32]. The Ras-ERK or MAPK pathway has been widely researched in relation to cancer. It has been found to be mutated in a large variety of different cancer types, and mutations are found at all levels of pathway regulation. Common mutations found in various different cancer types are overexpression or mutations of the EGF

receptor, which causes increased activation of the MAPK signaling pathway due to aberrant receptor activation. The Ras proteins are often found to have an activating mutation leaving the Ras protein in its GTP-bound state, which causes Ras to continuously stimulate the MAPK pathway. Mutations are also found in the Raf proteins, which are direct effectors of Ras. Their activating mutations show a similar increased ERK activity. Raf mutations are more varied among different tumors. Since the outcome of oncogenic mutations in this pathway generally is an increased ERK1/2 activity, the Raf-MEK-ERK cascade is an attractive target for anti-cancer therapy. Many drugs have been developed in recent years that target different components of the ERK cascade in several cancer including colonic cancer, lung cancer and melanoma [31, 33, 34, 35].

As mentioned before, the PI3K-Akt-mTOR pathway is also often hyperactived in cancer cells due to cross-talk with the Ras-ERK pathway. In addition, key components of the pathway such as PI3K and Akt are oncogenes which are often found to be mutated in different cancers [1, 36, 37, 38]. Furthermore, inhibitory regulators within the pathway may gain loss-of-function mutations. This is the case for PTEN, which restricts the production of PIP3, as well as the TSC1/2 proteins which normally inhibit mTORC1 activation and are themselves inhibited by Akt and ERK among others. In addition, loss of the tumor suppressor p53, which is a very common event in cancer cells, further promotes activation of the mTORC1 complex [1, 17, 39].

2 The role of the secretory pathway in proliferation

Proliferation requires de novo protein synthesis, therefore, growth factor signaling leads to an increase in protein synthesis. This is mediated via activation of S6K1 and inhibition of 4E-BP1 by the Ras-ERK pathway and by the mTOR pathway. Additionally, mTOR activity also induces ribosome biogenesis. The increase in synthesized proteins consequently also leads to an increased protein cargo load in the secretory pathway, to which the secretory pathway adapts. These mechanisms will be discussed in more detail in the next chapter.

For cell division to take place, the secretory pathway undergoes a variety of adaptations, as it partly disassembles during mitosis. Interestingly, during mitosis, protein synthesis is downregulated to 25-30% compared to interphase levels, which is accompanied by a reduction in general protein trafficking through the secretory pathway [40, 41, 42]. Several studies in mammalian cells have shown that during mitosis, different cargos are retained in the ER. For example, studies found that the membrane-spanning VSV-G was retained in the ER during mitosis. The same was true for a truncated version of VSV-G lacking its transmembrane domain, which acts similar to a soluble cargo protein [40, 43, 44, 45]. Endogenous transmembrane cargos such as ERGIC-53 and CD8 were also found to accumulate in the ER during mitosis [42, 46]. Other studies investigated secretion levels of soluble cargos during mitosis and found that secretion of the human growth hormone was decreased to a 10-fold lower level during mitosis by CHO cells, and histamine secretion during mitosis by rat mast cells was also profoundly decreased [40, 45, 47]. The observed decrease in general protein trafficking during mitosis is accompanied by major structural re-arrangements of the early secretory pathway. This enables inheritance of secretory pathway components during mitosis [40, 48]. In animal cells, as opposed to *S.cerevisiae*, the nuclear envelope is broken down during the early stages of mitosis, which is accompanied by a sheet-to-tubule reorganization of the ER, whereas the nature of the ER reorganization is controversial due to differences in technical approaches [40, 49, 50, 51, 52].

The block of ER export during mitosis has been suggested to be caused by ERES disassembly. ERES number has been shown to be decreased during mitosis, and the localization of COPII components was reported to be shifted towards the cytosol [42, 53, 54, 55]. Interestingly, in the case of Sec24, this decreased membrane association has been suggested to be due to phosphorylation and loss of O-glycosylation of Sec24 which was found during mitosis but not interphase [56]. In contrast to the components of the COPII coat, Sec16 was shown to remain associated with ERES during mitosis. The

presence of Sec16 at ERES was proposed to aid a rapid re-assembly of ERES and thereby in the restoration of the secretory pathway upon completion of mitosis [55, 57]. Disassembly of ERES has been proposed to be regulated by the mitotic cyclin-dependent kinase 1 (CDK1), which is a serine/threonine kinase. Addition of mitotic CDK1-complex or mitotic cytosol to permeabilized cells was shown to cause ERES disassembly. However, it has to be stressed that the protein Yip1A was used to visualize ERES, which is not commonly used as ERES marker. Yip1A cycles between ER and Golgi and is rather a marker for active bidirectional ER-Golgi transport [58, 59]. Thus, ER retention of Yip1A is indicative of a block in ER export, but not per se an indication for a disassembly of ERES.

Another important adaptation during mitosis is the disassembly of the Golgi, which enables Golgi inheritance during cell division [48, 60]. Early studies led to a model in which the Golgi fragments into small vesicles and tubules upon mitosis, resulting in punctate Golgi clusters and a Golgi haze formed by small vesicles [61, 62, 63]. A second model called the ER recycling model was based on the fact that during mitosis, ER-to-Golgi trafficking is blocked. This COPII trafficking block, in combination with unhindered COPI vesicle-mediated recycling of proteins, would cause an accumulation of Golgi proteins at the ER, similar to cells treated with brefeldin A (BFA) [63, 64, 65, 66]. In contrast to this second model, more recent studies showed a separation between markers of the ER and Golgi remnants during mitosis, indicating that the Golgi disassembles but does not fuse with the ER [63, 67, 68, 69]. The first model is further supported by studies unraveling the molecular mechanism underlying mitotic Golgi disassembly. The first step in Golgi disassembly is the disconnection of the Golgi ribbon which yields several disconnected Golgi stacks. The stacks are further converted into ‘Golgi blobs’, which are described as tubular-reticular membranes. These are then broken down further by vesiculation, resulting in a Golgi haze [60, 70]. Mitotic CDK1 was shown to phosphorylate the Golgi tethering factor GM130, disrupting the interaction of GM130, p115, and Giantin at the Golgi membrane. This causes a disruption of COPI vesicle tethering at the Golgi and therefore a block in COPI vesicle trafficking at the Golgi, ultimately causing Golgi disruption [71, 72, 73, 74, 75, 76]. In addition, several kinases were shown to mediate Golgi disassembly by targeting the Golgi tethering proteins GRASP55 and GRASP65. These kinases are CDK1, ERK1c, and Plk1, although other kinases are likely to also be involved. GRASP55/65 contain several phosphorylation sites, and GRASP55/65 phosphorylation was shown to impair the homo-oligomerization capabilities of GRASP55/65, as well as their interaction with Golgins [77, 78, 79, 80, 81, 82, 83].

During cell division, both daughter cells inherit Golgi fragments in a process called partitioning, which is followed by tightly regulated Golgi re-assembly during telophase [84].

3 The role of the ER-to-Golgi trafficking machinery in cancer

As described in the previous chapter, the secretory pathway plays an important role in proliferating cells, and is therefore also important in highly proliferative cancer cells.

Increased de novo lipid synthesis is a hallmark of cancer cells, which is required to meet the increased demand for membrane material in highly proliferating cells [85, 86, 87]. This process is mediated by the mTORC1 signaling pathway, which promotes *de novo* lipid synthesis via the activation and upregulation of the sterol regulatory element-binding protein 1/2 (SREBP1/2). The SREBP1/2 transcription factors initiate the expression of genes involved in fatty acid and cholesterol synthesis. The requirement of this process for cell growth was demonstrated in both mammalian and *Drosophila* cells, and loss of SREBP1 in *Drosophila* even caused decreased organ and body size [1, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97].

The role of lipid synthesis in cancer cells is increasingly recognized, as increased activity and expression of lipogenic enzymes have been found in various cancer types. In cancer cells, a large part of newly synthesized fatty acids was shown to be processed into phospholipids and incorporated into membrane lipids, which was required for cell growth. For plasma membrane expansion to take place, membrane material must be delivered to the plasma membrane, which takes place via the secretory pathway [98, 99, 100, 101, 102].

Importantly, cancer cells have been found to have an increased metabolic demand and increased protein synthesis levels. Cells have adaptive mechanisms which allow them to handle an increased cargo load; these will discussed below.

3.1 The ER stress response

Protein folding and quality control of nascent proteins in the ER lumen is an important and tightly monitored process, which ensures that only properly functional proteins are released into the cell or extracellular space. The importance of this process and the toxicity of misfolded proteins becomes clear by looking at a variety of diseases caused by aggregation of misfolded proteins. Examples for neurodegenerative diseases caused by release of misfolded proteins are Alzheimer's disease, Parkinson's disease, and Huntington's disease; but non-neurological diseases such as diabetes type 2, atherosclerosis and others have been implicated as well. The aim of activating the ER stress response is at first to increase the folding capacity of the ER to enable the folding and chaperone machinery to handle the increased amount of unfolded proteins. Only if this is not sufficient does the Unfolded Protein Response lead to an activation of apoptosis [103, 104, 105, 106].

The ER stress response is mediated by three different pathways named after the key components which act as sensor and activation protein of their respective pathway. These are the two kinases PERK and IRE1 and the transcription factor ATF6. In unstressed cells, these proteins are bound to the luminal chaperone BiP in the ER, which keeps these sensors in their inactive state. An increase in unfolded proteins in the ER lumen leads to binding of these unfolded proteins to BiP, which therefore releases the ER stress sensors which consequently become active [103, 105, 106, 107, 108, 109, 110].

An immediate response to ER stress is the transient attenuation of mRNA translation. This is mediated by the double stranded RNA-activated protein kinase-like ER kinase (PERK) arm of the UPR. PERK is a transmembrane serine/threonine kinase localized at the ER, which is kept inactive by binding to the chaperone BiP. Upon accumulation of unfolded proteins PERK is released from BiP, and activates its kinase function by dimerization and autophosphorylation. PERK then phosphorylates the eukaryotic translation initiation factor 2 on the alpha subunit (eIF2α), thereby inhibiting the function of the guanine nucleotide exchange factor eIF2B, which ultimately leads to a general attenuation of mRNA translation. However, phosphorylation of eIF2α activates transcription of the activating transcription factor 4 (ATF4). ATF4 then induces transcription of approximately one third of all UPR-dependent genes that are required to handle the increased demand on the ER [103, 105, 106, 107, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119].

The second arm of the UPR is controlled by inositol requiring kinase 1 (IRE1), which in mammalian cells has two isoforms that are largely redundant in function; these isoforms are IRE1α, which is expressed in most tissues, and IRE1β, which is found in intestinal epithelial cells. During non-stress conditions, IRE1 is kept in its inactive monomeric state by binding to the luminal chaperone BiP. BiP preferentially binds misfolded proteins, therefore accumulation of misfolded proteins leads to a release of IRE1. Upon release from BiP, IRE1 forms homodimers and autophosphorylates which activates its site-specific endoribonuclease (RNase) activity. In mammalian cells, active IRE1 initiates the removal of 26 base pairs from the mRNA of the transcription factor X-box binding protein 1 (XBP1). This alternative splicing causes a frameshift in the mRNA which results in a larger version of XBP1, called spliced XBP1, or XBP1-s. This then functions as a transcriptional activator of a variety of genes relevant to UPR, but also induces chaperones and components of the ERAD machinery [103, 104, 106, 108, 109, 120, 121, 122, 123, 124, 125, 126, 127, 128].

The third arm of the UPR is mediated by the transcription factor activating transcription factor 6 (ATF6). ATF6 is synthesized as a transmembrane protein that is localized to the ER membrane. ATF6, like other UPR-sensors, is bound to BiP during non-stress conditions, and released upon induction of ER stress. Released ATF6 travels through the secretory pathway to the Golgi apparatus where it is sequentially cleaved by two Golgi-resident proteases, releasing the cytosolic fragment of ATF6 that translocates to the nucleus where it acts as a transcriptional modulator [103, 106, 129, 130, 131, 132, 133, 134, 135, 136].

Taken together, activation of the three arms of the UPR increases the expression of proteins such as chaperones and folding enzymes, components of the ERAD and ER export machinery, and modulators of cellular metabolism. This initially enables the ER to handle the increased amount of misfolded proteins. However, the UPR also induces pro-apoptotic genes, and if chronic ER stress cannot be resolved, ER stress-induced apoptosis is initiated via several mechanisms. A part of UPR induced genes are pro-apoptotic genes, and an important ER stress-induced apoptotic pathway is mediated via C/EBP homologous protein (CHOP) which is also known as growth arrest and DNA damage 153 (GADD153). CHOP is a transcription factor which is induced via the PERK and ATF6 pathways and activates the transcription of genes that promote apoptosis. CHOP-dependent upregulation of growth arrest and DNA damage-inducible protein 34 (GADD34) enhances dephosphorylation of eIF2α. This leads to an upregulation of protein synthesis and therefore pushes the chronic UPR further towards apoptotic signaling. Furthermore, CHOP induces expression of the cell surface death

receptor 5 (DR5) and inhibits transcription of anti-apoptotic genes such as B cell lymphoma 2 (Bcl-2) [103, 106, 137, 138, 139, 140, 141, 142, 143, 144].

Another pathway how ER stress signals to the apoptotic machinery is via IRE1. Upon activation, IRE1 recruits a complex of TNF-receptor-associated factor 2 (TRAF2) and the apoptosis-signal-regulating kinase 1 (ASK1). This complex activates the stress MAP kinases p38 and JNK. JNK directly affects the cell death machinery by phosphorylating members of the Bcl2 protein family. Phosphorylation of ER-localized Bcl2 by JNK suppresses its anti-apoptotic activity, whereas phosphorylation of the pro-apoptotic Bcl2 family member Bcl2-interacting mediator of cell death (Bim, also known as BH3) initiates the apoptotic cascade at the ER and the mitochondrial membranes. In unstressed cells, Bim, which has at least three isoforms, is bound to the dynein motor complex which keeps it inactive. JNK-induced phosphorylation releases Bim, which translocates to the ER and mitochondrial membranes inducing oligomerization and activation of the membrane-associated pro-apoptotic proteins (Bax) and (Bak), whose ER-membrane recruitment is enhanced by the ER-localized pro-apoptotic protein Bcl2-interacting killer (BIK). Activation of Bax and Bak is believed to allow Ca^{2+}-flux from the ER and the mitochondria into the cytoplasm. The increase of cytoplasmic Ca^{2+}-levels leads to the activation of mitochondrial- dependent and – independent caspase cascades and ultimately apoptosis. In addition, translocation of Bim to the ER membrane also activates Caspase-12 at the ER membrane which induces apoptosis by activating Caspase-9 which then activates Caspase-3 [103, 106, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166].

The UPR also has an effect on ER export by targeting COPII vesicle formation. However, only few studies exist that show the relationship between the UPR and ER export. COPII components have been shown to be upregulated in response to ER stress [167, 168, 169]. This was shown to be mediated by the leucine-zipper type transcription factor homologous to ATG6/CREB (Hac1), which is induced directly by ER stress and via UPR-induced differential splicing similar to ATG6 [168, 170]. An increase in COPII components could increase ER export, which may serve to relieve the burden on the ER [169]. Indeed, early studies in *S.cerevisiae* showed that COPII components were required for normal growth upon induction of ER stress [169, 171]. In addition, components required for ER-to-Golgi trafficking have been shown to influence components of the UPR. For example, ATF6 transport to the Golgi is mediated by COPII vesicle formation [172], and the *S.cerevisiae* Golgi GTPase Ypt1 stabilizes unspliced Hac1 mRNA under basal conditions [173, 174].

3.2 The response of the early secretory pathway to increased cargo load

In addition to the UPR which takes places at the ER, other organelles in the early secretory pathway must adapt to an increase in cargo load. For example, ER exit sites respond to changes in cargo load at the ER. Studies have shown that an acute increase in cargo load at the ER leads to fusion of ERES and to an increased COPII assembly at ERES [167, 175]. In contrast, prolonged increase in cargo load results in biogenesis of new ERES and an increase in their number [167]. Interestingly, cargo load was shown to influence COPII turnover at ERES. Fluorescence Recovery After Photobleaching (FRAP) assays showed that a decrease in cargo load at the ER decreased the turnover rate of COPII components at individual ERES [176].

Cargo load is also tightly monitored at the level of the Golgi. A proportion of cargo that arrives at the Golgi consists of ER chaperones that contain a KDEL-retrieval sequence which ensures their transport back to ER. This KDEL sequence is recognized by the Golgi-resident KDEL receptor, which upon binding was shown to activate G proteins, and therefore appears to also act as a sensor of incoming traffic at the Golgi. One of the targets that becomes activated is the kinase Src, which stimulates retrograde protein transport from the Golgi back to ER. Src also stimulates trafficking of proteins through the Golgi, which ensures the maintenance of protein flow through the secretory pathway and may thereby relieve the secretory burden at the Golgi [177, 178, 179, 180, 181].

As described above, increased secretory load affects the secretory pathway both via an increased secretory burden and via the UPR. These responses are especially important in the context of cancer, as cancer cells use these mechanisms to adapt to their increased protein synthesis levels, making them essential for cancer cell survival. Therefore, targeting the UPR and ER export are attractive targets for chemotherapy [182, 183].

4 References

1. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012, **149**(2)**:** 274-293.
2. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A*, et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 2004, **6**(11)**:** 1122-1128.
3. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H*, et al.* mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002, **110**(2)**:** 163-175.
4. Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H*, et al.* GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol Cell* 2003, **11**(4)**:** 895-904.
5. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM*, et al.* DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* 2009, **137**(5)**:** 873-886.
6. Kaizuka T, Hara T, Oshiro N, Kikkawa U, Yonezawa K, Takehana K*, et al.* Tti1 and Tel2 are critical factors in mammalian target of rapamycin complex assembly. *J Biol Chem* 2010, **285**(26)**:** 20109-20116.
7. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S*, et al.* Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 2002, **110**(2)**:** 177-189.
8. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E*, et al.* PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* 2007, **25**(6)**:** 903-915.
9. Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, Jeno P*, et al.* PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS One* 2007, **2**(11)**:** e1217.
10. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* 2007, **9**(3)**:** 316-323.
11. Wang L, Harris TE, Roth RA, Lawrence JC, Jr. PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. *J Biol Chem* 2007, **282**(27)**:** 20036-20044.
12. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H*, et al.* Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 2004, **14**(14)**:** 1296-1302.
13. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA*, et al.* mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol* 2006, **16**(18)**:** 1865-1870.
14. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY*, et al.* SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 2006, **127**(1)**:** 125-137.
15. Pearce LR, Huang X, Boudeau J, Pawlowski R, Wullschleger S, Deak M*, et al.* Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J* 2007, **405**(3)**:** 513-522.
16. Pearce LR, Sommer EM, Sakamoto K, Wullschleger S, Alessi DR. Protor-1 is required for efficient mTORC2-mediated activation of SGK1 in the kidney. *Biochem J* 2011, **436**(1)**:** 169-179.
17. Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. *Molecular bioSystems* 2015.
18. Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci* 2011, **36**(6)**:** 320-328.
19. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 2003, **17**(15)**:** 1829-1834.
20. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 2002, **4**(9)**:** 648-657.
21. Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc Natl Acad Sci U S A* 2002, **99**(21)**:** 13571-13576.

22. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* 2005, **121**(2)**:** 179-193.
23. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002, **10**(1)**:** 151-162.
24. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. *Nat Cell Biol* 2002, **4**(9)**:** 658-665.
25. Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc Natl Acad Sci U S A* 2004, **101**(37)**:** 13489-13494.
26. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* 2000, **14**(19)**:** 2501-2514.
27. Zhu J, Blenis J, Yuan J. Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. *Proc Natl Acad Sci U S A* 2008, **105**(18)**:** 6584-6589.
28. Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X*, et al.* ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat Cell Biol* 2008, **10**(2)**:** 138-148.
29. Rena G, Guo S, Cichy SC, Unterman TG, Cohen P. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J Biol Chem* 1999, **274**(24)**:** 17179-17183.
30. Tang ED, Nunez G, Barr FG, Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. *J Biol Chem* 1999, **274**(24)**:** 16741-16746.
31. Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. *Oncogene* 2007, **26**(22)**:** 3279-3290.
32. Burotto M, Chiou VL, Lee JM, Kohn EC. The MAPK pathway across different malignancies: a new perspective. *Cancer* 2014, **120:** 3446-3456.
33. Rubinfeld H, Seger R. The ERK cascade: a prototype of MAPK signaling. *Mol Biotechnol* 2005, **31:** 151-174.
34. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nature reviews Cancer* 2003, **3**(1)**:** 11-22.
35. Santarpia L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. *Expert opinion on therapeutic targets* 2012, **16**(1)**:** 103-119.
36. Miller TW, Rexer BN, Garrett JT, Arteaga CL. Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer. *Breast cancer research : BCR* 2011, **13**(6)**:** 224.
37. Kim MS, Jeong EG, Yoo NJ, Lee SH. Mutational analysis of oncogenic AKT E17K mutation in common solid cancers and acute leukaemias. *Br J Cancer* 2008, **98**(9)**:** 1533-1535.
38. Soung YH, Lee JW, Nam SW, Lee JY, Yoo NJ, Lee SH. Mutational analysis of AKT1, AKT2 and AKT3 genes in common human carcinomas. *Oncology* 2006, **70**(4)**:** 285-289.
39. Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci U S A* 2005, **102**(23)**:** 8204-8209.
40. Yeong FM. Multi-step down-regulation of the secretory pathway in mitosis: a fresh perspective on protein trafficking. *BioEssays : news and reviews in molecular, cellular and developmental biology* 2013, **35**(5)**:** 462-471.
41. Le Breton M, Cormier P, Belle R, Mulner-Lorillon O, Morales J. Translational control during mitosis. *Biochimie* 2005, **87**(9-10)**:** 805-811.
42. Farmaki T, Ponnambalam S, Prescott AR, Clausen H, Tang BL, Hong W*, et al.* Forward and retrograde trafficking in mitotic animal cells. ER-Golgi transport arrest restricts protein export from the ER into COPII-coated structures. *J Cell Sci* 1999, **112 (Pt 5):** 589-600.
43. Warren G, Featherstone C, Griffiths G, Burke B. Newly synthesized G protein of vesicular stomatitis virus is not transported to the cell surface during mitosis. *J Cell Biol* 1983, **97**(5 Pt 1)**:** 1623-1628.
44. Featherstone C, Griffiths G, Warren G. Newly synthesized G protein of vesicular stomatitis virus is not transported to the Golgi complex in mitotic cells. *J Cell Biol* 1985, **101**(6)**:** 2036-2046.

45. Kreiner T, Moore HP. Membrane traffic between secretory compartments is differentially affected during mitosis. *Cell regulation* 1990, **1**(5)**:** 415-424.
46. Hauri HP, Kappeler F, Andersson H, Appenzeller C. ERGIC-53 and traffic in the secretory pathway. *J Cell Sci* 2000, **113:** 587-596.
47. Hesketh TR, Beaven MA, Rogers J, Burke B, Warren GB. Stimulated release of histamine by a rat mast cell line is inhibited during mitosis. *J Cell Biol* 1984, **98**(6)**:** 2250-2254.
48. Barr FA. Inheritance of the endoplasmic reticulum and Golgi apparatus. *Curr Opin Cell Biol* 2002, **14**(4)**:** 496-499.
49. Guttinger S, Laurell E, Kutay U. Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nat Rev Mol Cell Biol* 2009, **10**(3)**:** 178-191.
50. Lu L, Ladinsky MS, Kirchhausen T. Cisternal organization of the endoplasmic reticulum during mitosis. *Mol Biol Cell* 2009, **20**(15)**:** 3471-3480.
51. Puhka M, Vihinen H, Joensuu M, Jokitalo E. Endoplasmic reticulum remains continuous and undergoes sheet-to-tubule transformation during cell division in mammalian cells. *J Cell Biol* 2007, **179**(5)**:** 895-909.
52. Puhka M, Joensuu M, Vihinen H, Belevich I, Jokitalo E. Progressive sheet-to-tubule transformation is a general mechanism for endoplasmic reticulum partitioning in dividing mammalian cells. *Mol Biol Cell* 2012, **23**(13)**:** 2424-2432.
53. Hammond AT, Glick BS. Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. *Mol Biol Cell* 2000, **11**(9)**:** 3013-3030.
54. Prescott AR, Farmaki T, Thomson C, James J, Paccaud JP, Tang BL*, et al.* Evidence for prebudding arrest of ER export in animal cell mitosis and its role in generating Golgi partitioning intermediates. *Traffic* 2001, **2**(5)**:** 321-335.
55. Stephens DJ. De novo formation, fusion and fission of mammalian COPII-coated endoplasmic reticulum exit sites. *EMBO Rep* 2003, **4**(2)**:** 210-217.
56. Dudognon P, Maeder-Garavaglia C, Carpentier JL, Paccaud JP. Regulation of a COPII component by cytosolic O-glycosylation during mitosis. *FEBS Lett* 2004, **561**(1-3)**:** 44-50.
57. Hughes H, Stephens DJ. Sec16A defines the site for vesicle budding from the endoplasmic reticulum on exit from mitosis. *J Cell Sci* 2010, **123**(Pt 23)**:** 4032-4038.
58. Dykstra KM, Pokusa JE, Suhan J, Lee TH. Yip1A structures the mammalian endoplasmic reticulum. *Mol Biol Cell* 2010, **21**(9)**:** 1556-1568.
59. Kano F, Tanaka AR, Yamauchi S, Kondo H, Murata M. Cdc2 kinase-dependent disassembly of endoplasmic reticulum (ER) exit sites inhibits ER-to-Golgi vesicular transport during mitosis. *Mol Biol Cell* 2004, **15**(9)**:** 4289-4298.
60. Colanzi A, Corda D. Mitosis controls the Golgi and the Golgi controls mitosis. *Curr Opin Cell Biol* 2007, **19**(4)**:** 386-393.
61. Lucocq JM, Pryde JG, Berger EG, Warren G. A mitotic form of the Golgi apparatus in HeLa cells. *J Cell Biol* 1987, **104**(4)**:** 865-874.
62. Shima DT, Haldar K, Pepperkok R, Watson R, Warren G. Partitioning of the Golgi apparatus during mitosis in living HeLa cells. *J Cell Biol* 1997, **137**(6)**:** 1211-1228.
63. Barr FA. Golgi inheritance: shaken but not stirred. *J Cell Biol* 2004, **164**(7)**:** 955-958.
64. Zaal KJ, Smith CL, Polishchuk RS, Altan N, Cole NB, Ellenberg J*, et al.* Golgi membranes are absorbed into and reemerge from the ER during mitosis. *Cell* 1999, **99**(6)**:** 589-601.
65. Thyberg J, Moskalewski S. Reorganization of the Golgi complex in association with mitosis: redistribution of mannosidase II to the endoplasmic reticulum and effects of brefeldin A. *Journal of submicroscopic cytology and pathology* 1992, **24**(4)**:** 495-508.
66. Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD. Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* 1989, **56**(5)**:** 801-813.
67. Jesch SA, Linstedt AD. The Golgi and endoplasmic reticulum remain independent during mitosis in HeLa cells. *Mol Biol Cell* 1998, **9**(3)**:** 623-635.
68. Pecot MY, Malhotra V. Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells. *Cell* 2004, **116**(1)**:** 99-107.
69. Axelsson MA, Warren G. Rapid, endoplasmic reticulum-independent diffusion of the mitotic Golgi haze. *Mol Biol Cell* 2004, **15**(4)**:** 1843-1852.
70. Colanzi A, Sutterlin C. Signaling at the Golgi during mitosis. *Methods in cell biology* 2013, **118:** 383-400.

71. Misteli T, Warren G. COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system. *J Cell Biol* 1994, **125**(2)**:** 269-282.
72. Nakamura N, Lowe M, Levine TP, Rabouille C, Warren G. The vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner. *Cell* 1997, **89**(3)**:** 445-455.
73. Lowe M, Rabouille C, Nakamura N, Watson R, Jackman M, Jamsa E*, et al.* Cdc2 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis. *Cell* 1998, **94**(6)**:** 783-793.
74. Levine TP, Rabouille C, Kieckbusch RH, Warren G. Binding of the vesicle docking protein p115 to Golgi membranes is inhibited under mitotic conditions. *J Biol Chem* 1996, **271**(29)**:** 17304-17311.
75. Radulescu AE, Mukherjee S, Shields D. The Golgi protein p115 associates with gamma-tubulin and plays a role in Golgi structure and mitosis progression. *J Biol Chem* 2011, **286**(24)**:** 21915-21926.
76. Puthenveedu MA, Linstedt AD. Evidence that Golgi structure depends on a p115 activity that is independent of the vesicle tether components giantin and GM130. *J Cell Biol* 2001, **155**(2)**:** 227-238.
77. Lin CY, Madsen ML, Yarm FR, Jang YJ, Liu X, Erikson RL. Peripheral Golgi protein GRASP65 is a target of mitotic polo-like kinase (Plk) and Cdc2. *Proc Natl Acad Sci U S A* 2000, **97**(23)**:** 12589-12594.
78. Wang Y, Seemann J, Pypaert M, Shorter J, Warren G. A direct role for GRASP65 as a mitotically regulated Golgi stacking factor. *The EMBO journal* 2003, **22**(13)**:** 3279-3290.
79. Tang D, Mar K, Warren G, Wang Y. Molecular mechanism of mitotic Golgi disassembly and reassembly revealed by a defined reconstitution assay. *J Biol Chem* 2008, **283**(10)**:** 6085-6094.
80. Xiang Y, Wang Y. GRASP55 and GRASP65 play complementary and essential roles in Golgi cisternal stacking. *J Cell Biol* 2010, **188**(2)**:** 237-251.
81. Acharya U, Mallabiabarrena A, Acharya JK, Malhotra V. Signaling via mitogen-activated protein kinase kinase (MEK1) is required for Golgi fragmentation during mitosis. *Cell* 1998, **92**(2)**:** 183-192.
82. Sütterlin C, Lin CY, Feng Y, Ferris DK, Erikson RL, Malhotra V. Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis. *Proc Natl Acad Sci U S A* 2001, **98**(16)**:** 9128-9132.
83. Shaul YD, Seger R. ERK1c regulates Golgi fragmentation during mitosis. *J Cell Biol* 2006, **172**(6)**:** 885-897.
84. Wang Y, Seemann J. Golgi biogenesis. *Cold Spring Harb Perspect Biol* 2011, **3**(10)**:** a005330.
85. Daniels VW, Smans K, Royaux I, Chypre M, Swinnen JV, Zaidi N. Cancer cells differentially activate and thrive on de novo lipid synthesis pathways in a low-lipid environment. *PLoS One* 2014, **9**(9)**:** e106913.
86. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nature reviews Cancer* 2007, **7**(10)**:** 763-777.
87. Zhang F, Du G. Dysregulated lipid metabolism in cancer. *World journal of biological chemistry* 2012, **3**(8)**:** 167-174.
88. Laplante M, Sabatini DM. An emerging role of mTOR in lipid biosynthesis. *Curr Biol* 2009, **19**(22)**:** R1046-1052.
89. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL*, et al.* Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* 2010, **39**(2)**:** 171-183.
90. Li S, Ogawa W, Emi A, Hayashi K, Senga Y, Nomura K*, et al.* Role of S6K1 in regulation of SREBP1c expression in the liver. *Biochem Biophys Res Commun* 2011, **412**(2)**:** 197-202.
91. Takashima M, Ogawa W, Emi A, Kasuga M. Regulation of SREBP1c expression by mTOR signaling in hepatocytes. *The Kobe journal of medical sciences* 2009, **55**(2)**:** E45-52.
92. Wang BT, Ducker GS, Barczak AJ, Barbeau R, Erle DJ, Shokat KM. The mammalian target of rapamycin regulates cholesterol biosynthetic gene expression and exhibits a rapamycin-resistant transcriptional profile. *Proc Natl Acad Sci U S A* 2011, **108**(37)**:** 15201-15206.

93. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S*, et al.* SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell metabolism* 2008, **8**(3)**:** 224-236.
94. Porstmann T, Santos CR, Lewis C, Griffiths B, Schulze A. A new player in the orchestra of cell growth: SREBP activity is regulated by mTORC1 and contributes to the regulation of cell and organ size. *Biochem Soc Trans* 2009, **37**(Pt 1)**:** 278-283.
95. Porstmann T, Griffiths B, Chung YL, Delpuech O, Griffiths JR, Downward J*, et al.* PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. *Oncogene* 2005, **24**(43)**:** 6465-6481.
96. Goldstein JL, Rawson RB, Brown MS. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. *Archives of biochemistry and biophysics* 2002, **397**(2)**:** 139-148.
97. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997, **89**(3)**:** 331-340.
98. Milgraum LZ, Witters LA, Pasternack GR, Kuhajda FP. Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. *Clin Cancer Res* 1997, **3**(11)**:** 2115-2120.
99. Kuhajda FP. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition (Burbank, Los Angeles County, Calif)* 2000, **16**(3)**:** 202-208.
100. Chajes V, Cambot M, Moreau K, Lenoir GM, Joulin V. Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival. *Cancer Res* 2006, **66**(10)**:** 5287-5294.
101. Yoon S, Lee MY, Park SW, Moon JS, Koh YK, Ahn YH*, et al.* Up-regulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells. *J Biol Chem* 2007, **282**(36)**:** 26122-26131.
102. Brusselmans K, De Schrijver E, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the acetyl-CoA-carboxylase-alpha gene induces growth inhibition and apoptosis of prostate cancer cells. *Cancer Res* 2005, **65**(15)**:** 6719-6725.
103. Malhotra V, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. *Semin Cell Dev Biol* 2007, **18:** 716-731.
104. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E*, et al.* Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004, **306**(5695)**:** 457-461.
105. Kaufman RJ. Orchestrating the unfolded protein response in health and disease. *J Clin Invest* 2002, **110**(10)**:** 1389-1398.
106. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 2006, **7**(9)**:** 880-885.
107. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* 2000, **5**(5)**:** 897-904.
108. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001, **107**(7)**:** 881-891.
109. Sidrauski C, Walter P. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* 1997, **90**(6)**:** 1031-1039.
110. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol* 2000, **2**(6)**:** 326-332.
111. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999, **397**(6716)**:** 271-274.
112. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P*, et al.* Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell* 2001, **7**(6)**:** 1165-1176.
113. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M*, et al.* An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 2003, **11**(3)**:** 619-633.
114. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M*, et al.* Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 2000, **6**(5)**:** 1099-1108.

115. Jiang HY, Wek RC. Phosphorylation of the alpha-subunit of the eukaryotic initiation factor-2 (eIF2alpha) reduces protein synthesis and enhances apoptosis in response to proteasome inhibition. *J Biol Chem* 2005, **280**(14)**:** 14189-14202.
116. Shi Y, Vattem KM, Sood R, An J, Liang J, Stramm L*, et al.* Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. *Mol Cell Biol* 1998, **18**(12)**:** 7499-7509.
117. Sood R, Porter AC, Ma K, Quilliam LA, Wek RC. Pancreatic eukaryotic initiation factor-2alpha kinase (PEK) homologues in humans, Drosophila melanogaster and Caenorhabditis elegans that mediate translational control in response to endoplasmic reticulum stress. *Biochem J* 2000, **346 Pt 2:** 281-293.
118. Liu CY, Schroder M, Kaufman RJ. Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum. *J Biol Chem* 2000, **275**(32)**:** 24881-24885.
119. Rutkowski DT, Kaufman RJ. All roads lead to ATF4. *Dev Cell* 2003, **4**(4)**:** 442-444.
120. Cox JS, Walter P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* 1996, **87**(3)**:** 391-404.
121. Tirasophon W, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev* 1998, **12**(12)**:** 1812-1824.
122. Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *The EMBO journal* 1998, **17**(19)**:** 5708-5717.
123. Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 2000, **101**(3)**:** 249-258.
124. Niwa M, Sidrauski C, Kaufman RJ, Walter P. A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. *Cell* 1999, **99**(7)**:** 691-702.
125. Yoshida H, Haze K, Yanagi H, Yura T, Mori K. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J Biol Chem* 1998, **273**(50)**:** 33741-33749.
126. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP*, et al.* IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 2002, **415**(6867)**:** 92-96.
127. Back SH, Schroder M, Lee K, Zhang K, Kaufman RJ. ER stress signaling by regulated splicing: IRE1/HAC1/XBP1. *Methods (San Diego, Calif)* 2005, **35**(4)**:** 395-416.
128. Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T*, et al.* IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* 2002, **16**(4)**:** 452-466.
129. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 1999, **10**(11)**:** 3787-3799.
130. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M*, et al.* Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response. *Mol Cell Biol* 2001, **21**(4)**:** 1239-1248.
131. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M*, et al.* ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol Cell Biol* 2000, **20**(18)**:** 6755-6767.
132. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R*, et al.* ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 2000, **6**(6)**:** 1355-1364.
133. Okada T, Yoshida H, Akazawa R, Negishi M, Mori K. Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. *Biochem J* 2002, **366**(Pt 2)**:** 585-594.

134. Adachi Y, Yamamoto K, Okada T, Yoshida H, Harada A, Mori K. ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. *Cell structure and function* 2008, **33**(1)**:** 75-89.
135. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H*, et al.* Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev Cell* 2007, **13**(3)**:** 365-376.
136. Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J*, et al.* ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. *Dev Cell* 2007, **13**(3)**:** 351-364.
137. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R*, et al.* CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* 2004, **18**(24)**:** 3066-3077.
138. Kojima E, Takeuchi A, Haneda M, Yagi A, Hasegawa T, Yamaki K*, et al.* The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: elucidation by GADD34-deficient mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2003, **17**(11)**:** 1573-1575.
139. Novoa I, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J Cell Biol* 2001, **153**(5)**:** 1011-1022.
140. Ma Y, Brewer JW, Diehl JA, Hendershot LM. Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J Mol Biol* 2002, **318**(5)**:** 1351-1365.
141. Ron D, Habener JF. CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes Dev* 1992, **6**(3)**:** 439-453.
142. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H*, et al.* CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev* 1998, **12**(7)**:** 982-995.
143. Yamaguchi H, Wang HG. CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells. *J Biol Chem* 2004, **279**(44)**:** 45495-45502.
144. McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol Cell Biol* 2001, **21**(4)**:** 1249-1259.
145. Morishima N, Nakanishi K, Tsuchiya K, Shibata T, Seiwa E. Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signaling for activation of caspase-12 during ER stress-induced apoptosis. *J Biol Chem* 2004, **279**(48)**:** 50375-50381.
146. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T*, et al.* BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science* 2003, **300**(5616)**:** 135-139.
147. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J*, et al.* Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J Cell Biol* 2003, **162**(1)**:** 59-69.
148. Klee M, Pallauf K, Alcala S, Fleischer A, Pimentel-Muinos FX. Mitochondrial apoptosis induced by BH3-only molecules in the exclusive presence of endoplasmic reticular Bak. *The EMBO journal* 2009, **28**(12)**:** 1757-1768.
149. Mathai JP, Germain M, Shore GC. BH3-only BIK regulates BAX,BAK-dependent release of Ca2+ from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death. *J Biol Chem* 2005, **280**(25)**:** 23829-23836.
150. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. *Genes Dev* 2001, **15**(12)**:** 1481-1486.
151. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T*, et al.* BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol Cell* 2001, **8**(3)**:** 705-711.
152. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP*, et al.* Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000, **287**(5453)**:** 664-666.

153. Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B*, et al.* Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. *Science* 2006, **312**(5773)**:** 572-576.
154. Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K*, et al.* ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev* 2002, **16**(11)**:** 1345-1355.
155. Mauro C, Crescenzi E, De Mattia R, Pacifico F, Mellone S, Salzano S*, et al.* Central role of the scaffold protein tumor necrosis factor receptor-associated factor 2 in regulating endoplasmic reticulum stress-induced apoptosis. *J Biol Chem* 2006, **281**(5)**:** 2631-2638.
156. Zhang C, Kawauchi J, Adachi MT, Hashimoto Y, Oshiro S, Aso T*, et al.* Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine. *Biochem Biophys Res Commun* 2001, **289**(3)**:** 718-724.
157. Cheung HH, Lynn Kelly N, Liston P, Korneluk RG. Involvement of caspase-2 and caspase-9 in endoplasmic reticulum stress-induced apoptosis: a role for the IAPs. *Exp Cell Res* 2006, **312**(12)**:** 2347-2357.
158. Dahmer MK. Caspases-2, -3, and -7 are involved in thapsigargin-induced apoptosis of SH-SY5Y neuroblastoma cells. *J Neurosci Res* 2005, **80**(4)**:** 576-583.
159. Tan Y, Dourdin N, Wu C, De Veyra T, Elce JS, Greer PA. Ubiquitous calpains promote caspase-12 and JNK activation during endoplasmic reticulum stress-induced apoptosis. *J Biol Chem* 2006, **281**(23)**:** 16016-16024.
160. Morishima N, Nakanishi K, Takenouchi H, Shibata T, Yasuhiko Y. An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J Biol Chem* 2002, **277**(37)**:** 34287-34294.
161. Nishitoh H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M*, et al.* ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol Cell* 1998, **2**(3)**:** 389-395.
162. Boya P, Cohen I, Zamzami N, Vieira HL, Kroemer G. Endoplasmic reticulum stress-induced cell death requires mitochondrial membrane permeabilization. *Cell death and differentiation* 2002, **9**(4)**:** 465-467.
163. Deniaud A, Sharaf el dein O, Maillier E, Poncet D, Kroemer G, Lemaire C*, et al.* Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene* 2008, **27**(3)**:** 285-299.
164. Hacki J, Egger L, Monney L, Conus S, Rosse T, Fellay I*, et al.* Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2. *Oncogene* 2000, **19**(19)**:** 2286-2295.
165. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ*, et al.* Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 2001, **292**(5517)**:** 727-730.
166. Lei K, Davis RJ. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci U S A* 2003, **100**(5)**:** 2432-2437.
167. Farhan H, Weiss M, Tani K, Kaufman RJ, Hauri HP. Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load. *EMBO J* 2008, **27**(15)**:** 2043-2054.
168. Vembar SS, Brodsky JL. One step at a time: endoplasmic reticulum-associated degradation. *Nat Rev Mol Cell Biol* 2008, **9**(12)**:** 944-957.
169. Higashio H, Kohno K. A genetic link between the unfolded protein response and vesicle formation from the endoplasmic reticulum. *Biochem Biophys Res Commun* 2002, **296**(3)**:** 568-574.
170. Ogawa N, Mori K. Autoregulation of the HAC1 gene is required for sustained activation of the yeast unfolded protein response. *Genes Cells* 2004, **9**(2)**:** 95-104.
171. Sato M, Sato K, Nakano A. Evidence for the intimate relationship between vesicle budding from the ER and the unfolded protein response. *Biochem Biophys Res Commun* 2002, **296**(3)**:** 560-567.
172. Schindler AJ, Schekman R. In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles. *Proc Natl Acad Sci U S A* 2009, **106**(42)**:** 17775-17780.
173. Tsvetanova NG, Riordan DP, Brown PO. The yeast Rab GTPase Ypt1 modulates unfolded protein response dynamics by regulating the stability of HAC1 RNA. *PLoS genetics* 2012, **8**(7)**:** e1002862.

174. Tsvetanova NG. The secretory pathway in control of endoplasmic reticulum homeostasis. *Small GTPases* 2013, **4**(1)**:** 28-33.
175. Guo Y, Linstedt AD. COPII-Golgi protein interactions regulate COPII coat assembly and Golgi size. *J Cell Biol* 2006, **174**(1)**:** 53-63.
176. Forster R, Weiss M, Zimmermann T, Reynaud EG, Verissimo F, Stephens DJ*, et al.* Secretory cargo regulates the turnover of COPII subunits at single ER exit sites. *Curr Biol* 2006, **16**(2)**:** 173-179.
177. Giannotta M, Ruggiero C, Grossi M, Cancino J, Capitani M, Pulvirenti T*, et al.* The KDEL receptor couples to Galphaq/11 to activate Src kinases and regulate transport through the Golgi. *The EMBO journal* 2012, **31**(13)**:** 2869-2881.
178. Luini A, Mavelli G, Jung J, Cancino J. Control systems and coordination protocols of the secretory pathway. *F1000prime reports* 2014, **6:** 88.
179. Bard F, Mazelin L, Pechoux-Longin C, Malhotra V, Jurdic P. Src regulates Golgi structure and KDEL receptor-dependent retrograde transport to the endoplasmic reticulum. *J Biol Chem* 2003, **278**(47)**:** 46601-46606.
180. Cancino J, Capalbo A, Di Campli A, Giannotta M, Rizzo R, Jung JE*, et al.* Control systems of membrane transport at the interface between the endoplasmic reticulum and the Golgi. *Dev Cell* 2014, **30**(3)**:** 280-294.
181. Pulvirenti T, Giannotta M, Capestrano M, Capitani M, Pisanu A, Polishchuk RS*, et al.* A traffic-activated Golgi-based signalling circuit coordinates the secretory pathway. *Nat Cell Biol* 2008, **10**(8)**:** 912-922.
182. Clarke HJ, Chambers JE, Liniker E, Marciniak SJ. Endoplasmic reticulum stress in malignancy. *Cancer Cell* 2014, **25**(5)**:** 563-573.
183. Rutkowski DT, Kaufman RJ. That which does not kill me makes me stronger: adapting to chronic ER stress. *Trends Biochem Sci* 2007, **32**(10)**:** 469-476.

Aim of the thesis

Newly synthesized proteins leave the endoplasmic reticulum (ER) at ER exit sites (ERES) in COPII coated vesicles. Among several proteins that regulate ERES, there is consensus that Sec16A plays a key role. Sec16A is a large protein of ~ 250 kDa that localizes to ERES, regulates their number, and interacts with several components of the COPII coat.

Previously, it was shown that Sec16 is phosphorylated by ERK2 after mitogenic stimulation, which was shown to increase ERES number. Thus, it was proposed that Sec16 acts as an integrator of mitogenic signaling to ERES. The aim of this thesis was to understand the role of Sec16 in the response of the secretory pathway to mitogenic signaling. Therefore, we will investigate the impact of the presence and absence of growth factor signaling on Sec16A dynamics and expression levels. In addition, we will aim to unravel the impact of phosphorylation on Sec16A at the molecular level; this will involve a combination of biochemical and microscopic techniques. Together, these results will be incorporated in a mathematical model describing Sec16A-dependent ERES biogenesis. Furthermore, we will investigate whether Sec16A (and therefore ER export) has an impact on cell growth and proliferation, which would highlight the importance of Sec16A as an integrator of signaling and places Sec16A at the center of potential targets to modulate ER export as a therapeutic strategy against cancer.

Materials and Methods

1 Cell culture and transfection

HeLa cells were cultured in DMEM supplemented with 10% FBS and antibiotics (Penicillin-streptomycin). Cells were trypsinized every 72-96 h and media were changed every other day. Transfection of cDNA was performed using FuGene6 (Promega) following the manufacturer's instructions. For knockdown experiments, cells were plated into 6-well plates and transfected with 5 nM siRNA (Qiagen) using HiPerFect (Qiagen) according to the manufacturer's instructions.

2 Immunofluorescence staining

HeLa cells were fixed by incubating in 3% paraformaldehyde, pH 7.4, for 20 min at room temperature, permeabilized for 5 min with PBS supplemented with 0.2% Triton X-100, and 3% bovine serum albumin (BSA). After washing, cells were incubated with the first antibody (anti-Sec16 and anti-Sec31A diluted at 1:1000 in PBS containing 3% BSA) for 60 min at room temperature. After washing, cells were incubated with the proper secondary antibody (diluted 1:200 in PBS containing 3% BSA) for 60 min at room temperature. Cells were washed and mounted in polyvinylalcohol.

3 ERES quantification

ERES were quantified in cells immunostained for Sec16 or Sec31 or in cells expressing GFP-Sec16. Images were acquired using a LeicaSP5 confocal microscope, with a 63x/1.4NA oil immersion objective at 3 fold digital magnification. ERES were quantified using ImageJ by applying uniform thresholding to the images to exclude non-specific structures. Structures smaller than 2 pixels in size were excluded from analysis as these typically represented noise originating from image pixelization. In the case of GFP-Sec16 expressing cells, total fluorescence intensity of cells was measured, and ERES were only quantified from cells displaying comparable fluorescence intensity (within twofold intensity range), thereby excluding possible effects due to the grade of GFP-Sec16 overexpression.

4 In vitro recruitment assay

HeLa cells were plated on glass cover slips in 2 cm cell culture dishes. After 24 h cells were washed in Buffer-1 (25 mM HEPES, pH 7.2, 125 mM KOAc, 2.5 mM MgOAc,

5 mM EGTA) at room temperature and permeabilized for 6 min at room temperature in Buffer-2 (Buffer-1 supplemented with 1 mM DTT and 30 µg/mL digitonin). Cells were washed six times in Buffer-3 on ice (Buffer-1 with 1 mM DTT). Cytosol was prepared from HeLa cells by mechanical shearing using a 25G needle in Buffer-4 (25 mM HEPES, pH 7.2, 75 mM KOAc, 5 mM MgOAc, 5 mM EGTA, 1 mM DTT, 1.8 mM $CaCl_2$, 10 µM ATP, proteinase inhibitor) on ice. Homogenate was centrifuged at 1000xg to remove nuclei and the post-nuclear supernatant was centrifuged at 20'000x g for 15 min at 4°C. The supernatant was collected, aliquoted and stored at -80°C. For recruitment experiments, cytosol was added at a concentration of 2.5 mg/mL and semi-intact cells were incubated for 30 min at room temperature followed by fixation.

5 Retention Using Selective Hooks (RUSH) assay

HeLa cells were plated on glass cover slips in 2 cm cell culture dishes and transfected with siRNA according to standard procedure. 24 h before the experiment, plasmids encoding RUSH-constructs [1] were transfected according to standard protocol. For ER-to-Golgi trafficking, Biotin was added for indicated time points. For Golgi-to-plasma membrane trafficking, cell culture medium was supplemented with 20 mM HEPES at the same time as Biotin addition, and cells were subjected to 20°C for 1 h before being returned to 37°C. After indicated time points, cells were fixed in 3% paraformaldehyde and immunostained for Giantin, followed by confocal microscopy.

6 Fluorescence Recovery After Photobleaching (FRAP)

FRAP was performed with a Leica SP5 confocal laser scanning microscope using a 63x/1.4NA oil immersion objective at 5 fold digital magnification. All experiments were performed at 37°C. Glass cover slips were transferred to a Ludin chamber (Life Imaging Services GmbH) and covered with imaging medium (DMEM supplemented with 20 mM Hepes, pH 7.4). After acquisition of a pre-bleach image, the ERES was bleached at 100% laser intensity for 750 ms. After bleaching, images were acquired for the indicated time at one image per second. Images were analyzed using ImageJ. The mobile fraction was calculated via $q=(F_\infty - F_0)/(F_i - F_0)$, where F_∞ is fluorescence in the bleached region after recovery, F_i is the fluorescence in the bleached region before bleaching, and F_0 is the fluorescence in the bleached region directly after bleaching.

7 Fluorescence Correlation Spectroscopy (FCS)

FCS measurements were performed on a Leica SP5 SMD system equipped with a custom-made climate chamber for 37°C incubation. Samples were illuminated at 488nm via a water immersion objective (HCX PL APO 63x1.2W CORR), fluorescence detection used a bandpass filter (500-530nm); pinhole was set to one Airy unit. FCS data were fitted using the fitting function for two non-interacting populations with normal diffusion:

$$C(\tau) = \frac{fA}{\left(1+\tau/\tau_D^{(1)}\right)} + \frac{(1-f)A}{\left(1+\tau/\tau_D^{(2)}\right)}$$

The first term with amplitude *f* and diffusion time $\tau_D^{(1)} = r_0^2/(4D_c)$ describes a fast diffusing species, e.g. cytosolic Sec16-GFP, while the second term with amplitude $(1-f)$ and diffusion time $\tau_D^{(2)} = r_0^2/(4D_m)$ describes a slow diffusing species, e.g. membrane-bound Sec16-GFP. Both diffusion times are determined by the radius of the confocal volume, $r_0 \approx 220\text{nm}$, and the respective diffusion constants, D_c and D_m. For simplicity, we have neglected a factor $\sqrt{\left(1+\tau/(S^2\tau_D^{(2)})\right)}$ in the denominator of the first summand. This factor captures diffusion along the optical axis, yet due to the unavoidable elongation of the confocal volume (described by $S^2 \approx 25$) it had little influence on the fit parameters reported here. The prefactor *A* is proportional to the inverse number of GFP-tagged Sec16 molecules in the focus (here: typically 20-100) and it also encodes GFP's photophysics on time scales of ~10µs. Since all diffusion times were well beyond 300µs, the photophysics' contribution to *A* was negligible for the fitting process. Autocorrelation curves were collected for 60 seconds in regions of the peripheral ER, *i.e.* away from the nuclear rim, for several loci in a variety of cells and treatments. FCS curves were fitted individually, and the mean of the obtained diffusion coefficients (24 curves for untreated cells; 16 for starved and mitogen-treated cells) are reported in the main text.

Theoretical predictions for diffusion constants of Sec16 in cytosol and on membranes were derived as follows. We assumed Sec16 to be globular with a mass about 10fold larger than GFP and a hydrodynamic radius ~2.2fold larger than GFP, i.e. R=3.3nm. Based on the Einstein-Stokes equation $D = k_B T/(6\pi\eta R)$ the diffusion constant is D_c=17µm^2/s. We used a cytosolic viscosity η 4fold larger than that of water [2]; k_BT is

thermal energy. For Sec16's diffusion on membranes, we employed the Saffman-Delbruck relation for peripheral membrane proteins [3], from which one infers a diffusion constant of Sec16 in the gross range of D_m=1μm^2/s.

8 Modeling

ERES formation was modelled as a Flory-Huggins unmixing scenario in which a clustering protein pool (here: Sec16) demixes from the surrounding lipid solvent into larger patches. This approach is a simplified version of a previously reported computational model [4]. Here, an emerging ERES will grow by recruiting free proteins from a basin of attraction with radius λ until the lifetime of the protein on the membrane (i.e. its inverse dissociation rate, $1/\Gamma$) becomes equal to the time needed to reach the ERES by diffusion, $\tau=\pi\lambda^2/(4D_0)$; D_0 denotes the proteins' diffusion coefficient. ERES therefore keep a well-defined distance, 2λ. By construction, the entire ER membrane area, L^2, is equal to the sum of all ERES basins, i.e. $L^2=\pi\lambda^2 N_{ERES}$. With $\pi\lambda^2/(4D_0)=1/\Gamma$, the number of ERES is hence given by $N_{ERES}= \Gamma L^2/D_0$. Since almost all membrane-bound clustering proteins are by definition in the ERES, the protein pool's area fraction is given by $\phi = N_{ERES}\pi R_{ERES}^2 / L^2$. From this, the radius of ERES can be determined as $R_{ERES} = \sqrt{4\phi D_0/(\pi k_{off})}$.

9 Regulatory sequence analysis

We used the PRIMA algorithm [5] to scan the 500bp region upstream to the transcription start site for enriched binding sites. Enrichment was calculated with respect to similar 500bp regions upstream to the transcription start site for the entire genome. This analysis revealed 184 enriched matrices corresponding to 180 transcription factors (TFs). We further scored each enriched TF according to its shortest distance in a protein-protein interaction network from either EGFR or IGF1R and ranked the TFs accordingly. Sequences and annotations for the regions, based on Human Genome build 19, were downloaded from EMBL on 30/8/12. Transcription factor binding sites, represented as position weigh matrices were downloaded from TRANSFAC [6] database release 11.1. A protein-protein interaction network was taken from ANAT [7].

10 Cell lysis and Western blotting

HeLa cells were plated into 2 cm cell culture dishes and lysed after treatment in 100 µl Lysis Buffer (50 mM Tris-HCL pH 7.4, 10 mM EDTA, 100 mM NaCl, 0.1 % SDS, 1 % NP-40, protease inhibitor) on ice for 20 min, followed by centrifugation at max. speed for 10 min at 4°C. Samples were diluted 1:5 in 5XSDS sample buffer (225 mM Tris-HCL pH 6.8, 5 %SDS, 50 % Glycerol, 0.05 % Bromophenolblue, 4 % β-Mercaptoethanol) and boiled for 5 min at 95°C. Samples were loaded onto 1 mm Tris-HCL gels, followed by standard SDS-PAGE procedure and semi-dry transfer. For Sec16 detection, gels containing 6.5% polyacrylamide were used.

11 Co-immunoprecipitation

HeLa cells were plated into 10 cm cell culture dishes. After 24 h, cells were transfected with a GFP-tagged version Sec16. After further 24 h, cells were lysed in buffer (50mM Tris-HCL, pH 7.5, 50 mM NaCl, 0.5% NP40 protease inhibitors). The cell lysate was loaded onto pre-equilibrated GFP-trap-A beads and the lysate was incubated over night at 4°C. After washing, elution of bound material was performed by boiling in sample buffer.

12 Subcellular fractionation assay

HeLa cells were plated into 10 cm cell culture and gently lysed by osmotic pressure. Cells were washed in PBS, followed by ice-cold hyptonic buffer (20 mM HEPES pH 7.4, 15 mM KCl, 250 mM Sucrose) for 1 min, and a 1 min wash in ice-cold hypertonic buffer (20 mM HEPES pH 7.4, 300 mM KCl, 250 mM Sucrose). Cells were taken up in ice-cold hypotonic buffer containing protease inhibitor. Nuclei were removed by centrifugation at 800 g, followed by centrifugation at 2000 g for clean-up. Cytosolic and membrane fractions were separated by centrifugation of the supernatant at max.speed.

13 References

1. Boncompain G, Divoux S, Gareil N, de Forges H, Lescure A, Latreche L*, et al.* Synchronization of secretory protein traffic in populations of cells. *Nature methods* 2012, **9**(5)**:** 493-498.
2. Elsner M, Hashimoto H, Simpson JC, Cassel D, Nilsson T, Weiss M. Spatiotemporal dynamics of the COPI vesicle machinery. *EMBO Rep* 2003, **4**(10)**:** 1000-1004.
3. Morozova D, Guigas G, Weiss M. Dynamic structure formation of peripheral membrane proteins. *PLoS computational biology* 2011, **7**(6)**:** e1002067.
4. Heinzer S, Worz S, Kalla C, Rohr K, Weiss M. A model for the self-organization of exit sites in the endoplasmic reticulum. *J Cell Sci* 2008, **121**(Pt 1)**:** 55-64.
5. Elkon R, Linhart C, Sharan R, Shamir R, Shiloh Y. Genome-Wide In Silico Identification of Transcriptional Regulators Controlling the Cell Cycle in Human Cells. *Genome Research* 2003, **13**(5)**:** 773-780.
6. Matys V, Kel-Margoulis OV, Fricke E, Liebich I, Land S, Barre-Dirrie A*, et al.* TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res* 2006, **34**(Database issue)**:** D108-110.
7. Yosef N, Zalckvar E, Rubinstein AD, Homilius M, Atias N, Vardi L*, et al.* ANAT: a tool for constructing and analyzing functional protein networks. *Science signaling* 2011, **4**(196)**:** pl1.

Results

Regulation of Sec16A at the transcriptional and posttranslational level links proliferation and secretion

Tillmann KD, Reiterer V, Baschieri F, Hoffmann J, Millarte V, Hauser MA, Mazza A, Atias N, Legler DF, Sharan R, Weiss M, Farhan H. **J Cell Sci**. 2015 Feb 15;128(4):670-82. Epub 2014 Dec 19.

1 Sec16A integrates growth factor signaling at the level of ERES

It was reported that growth factor (GF) signaling leads to phosphorylation of Sec16A, which changes the number of ER exit sites (ERES) [1]. To determine the role of Sec16A in integrating GF signaling, we first determined the effect of GF depletion on ERES. Therefore, we serum-starved cells for 6 h which resulted in a robust reduction of ERES as quantified by immunostaining of Sec31, indicating that GF signaling indeed has a role in the regulation of the early secretory pathway by maintaining a certain ERES number. Importantly, knockdown of Sec16A inhibited this response, showing that the reduction in ERES number by GF starvation causally depends on Sec16A (Fig.5A). However, this observation raises the question whether any condition that reduces ERES number renders them unresponsive to changes in GF levels. Therefore, we performed knockdown experiments with four kinases (NME5, NME6, NME7, and PiP5K1C), which have previously been identified to reduce ERES number in a kinome screen [1]. Knockdown cells displayed on average ~ 25% reduction in ERES number, but did not affect Sec16A protein levels (Fig.5B+C), indicating that the reduction of ERES number occurs via a mechanism independent of Sec16A protein levels. Serum starvation in addition to knockdown of any of these four kinases led to a further decrease in ERES number when compared to steady state conditions, therefore loss of these kinases did not affect the ability of ERES to respond to loss of GF signaling. We also performed knockdown of Sar1A, the GTPase that initiates the COPII assembly cascade, as well as of the COPII components Sec23A and Sec23B, in the presence and absence of GFs (Fig.5D). Depletion of Sar1A reduces the number of ERES and renders them insensitive to the amount of growth factors, similarly to what we observed in Sec16A depleted cells.

Therefore, the ability of ERES to respond to growth factors requires the presence of Sec16A and COPII. Unfortunately, in the case of Sec23 depletion, no definitive conclusion can be drawn due to large differences found between experiments (Fig.5D+E).

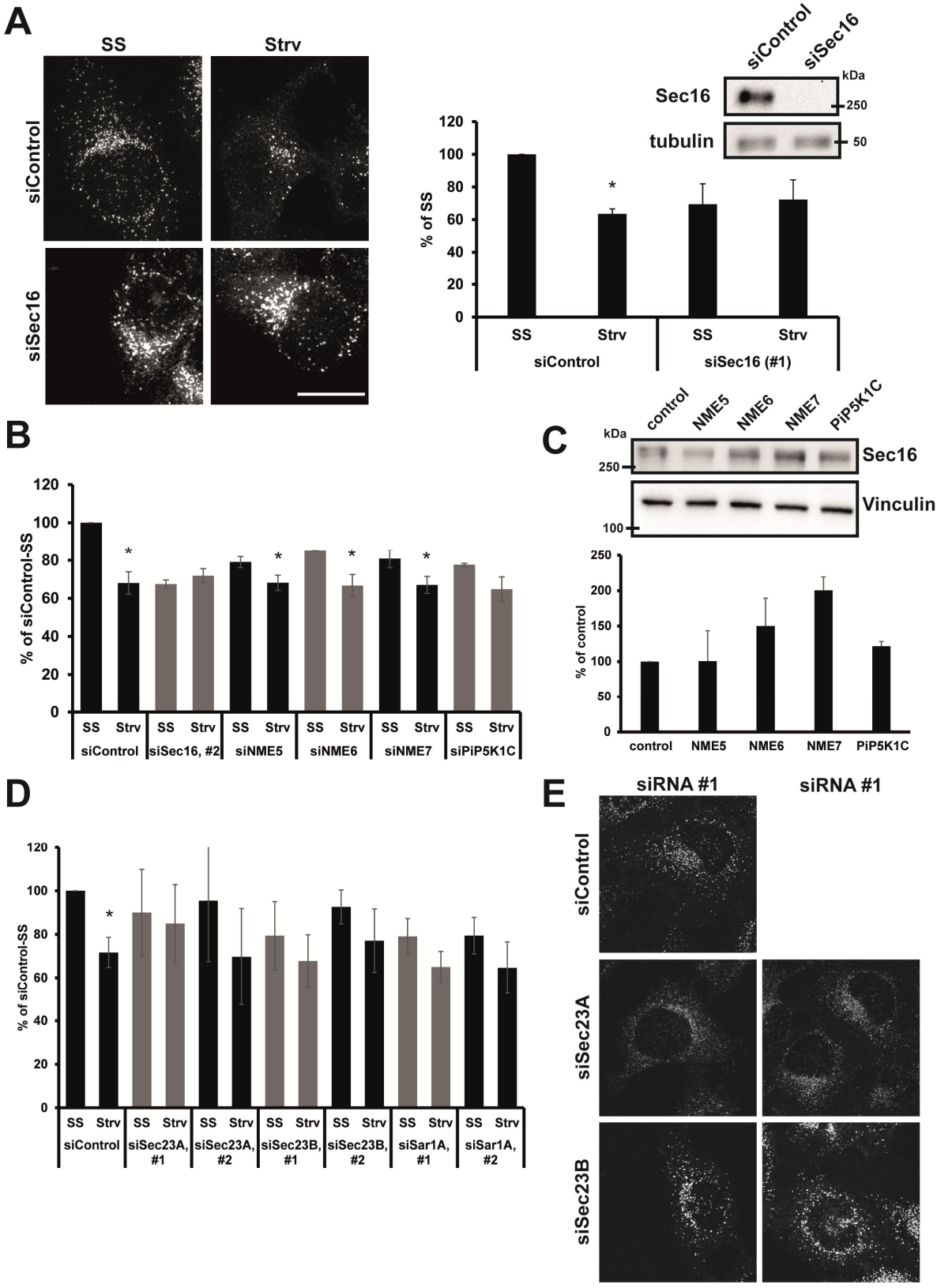


Figure 5: *Effect of growth factor signaling status in ERES number is independent of NME5, NME5, NME7 or PiP5K1C but not Sec16A orSar1A.* **A,** HeLa cells were transfected with control siRNA (control) or siRNA to Sec16 (siSec16). After 72 h, cells were left in steady state (SS), or were serum starved for 6 h (Strv) followed by fixation, Sec31 immunostaining, and confocal microscopy. Right panel shows an immunoblot

demonstrating efficiency of Sec16 knockdown and a graphic representation of the number of ERES per cell presented as percent of control in steady state. This value amounts to 156.4 ± 32 ERES. Results are means ± SD from three independent experiments with more than 50 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.01$) as determined by paired two-tailed Student's t-test. Scale bar: 10 µm. **B,** HeLa cells were transfected with control siRNA (control) or siRNA to NME5, NME6, NME7, PiP5K1C or Sec16 (siRNA clone #2) and treated as in A. Panel shows graphic representation of the number of ERES per cell. Results are presented as percent of control in steady state to account for inter-assay variance. This value amounts to 228.56 ± 12.3 ERES. Results are means ± SD from three independent experiments where at least 30 cells were evaluated per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **C,** HeLa cells were transfected with non-targeting siRNA (control) or with siRNA against NME5, NME6, NME7 or PiP5K1C. After 72h, cells were lysed and subjected to SDS-PAGE followed by immunoblotting against the indicated proteins. The top panel shows a representative experiment and the bottom panel shows an evaluation of three independent experiments depicting levels of Sec16 normalized to Vinculin. Values are ±SD and are represented as percent of control. **D,** HeLa cells were transfected with control siRNA (control) or siRNA to Sec23A, Sec23B or Sar1A and treated as in A. Panel shows graphic representation of the number of ERES per cell. Results are presented as percent of control in steady state to account for inter-assay variance. This value amounts to 309.01 ± 35.6 ERES. Results are means ± SD from three independent experiments where at least 30 cells were evaluated per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **E,** HeLa cells were transfected with control siRNA (control) or siRNA to Sec23A or Sec23B. After 72h, cells were fixed, immunostained for Sec31 and imaged by confocal microscopy. Scale bar: 10 µm.

2 Absence of growth factor signaling decreases Sec16A synthesis

We hypothesized that the absence of GFs might alter ERES by changing the levels of Sec16A and therefore performed a time-course experiment, which revealed that serum starvation leads to a reduction of Sec16A levels with a halftime of about 2-3 h (Fig.6A). In contrast, Sec31 levels remained largely unchanged. We next probed the possibility that GFs regulate Sec16A synthesis. If true, then treatment with GFs after serum-starvation ought to increase Sec16A levels on a time scale of a few hours. After serum-starvation for four hours, cells were treated with serum for different periods of time. Indeed, we found that serum treatment increased Sec16A levels after approximately 3-4 hours of stimulation (Fig.6B), but not after 0.5-2 hours of treatment, indicating that a slow process such as protein synthesis, might be responsible for the serum-dependent increase in Sec16A protein levels. Interestingly, Sec31 protein levels did not change in response to serum treatment. To test whether this effect was dependent upon *de novo* protein synthesis, steady-state cells were stimulated with serum for four hours in the presence or absence of cycloheximide (CHX), which blocks protein translation (Fig.6C). The serum-dependent increase in Sec16A protein levels was absent in cells treated with CHX, indicating that Sec16A levels are controlled by GF signaling.

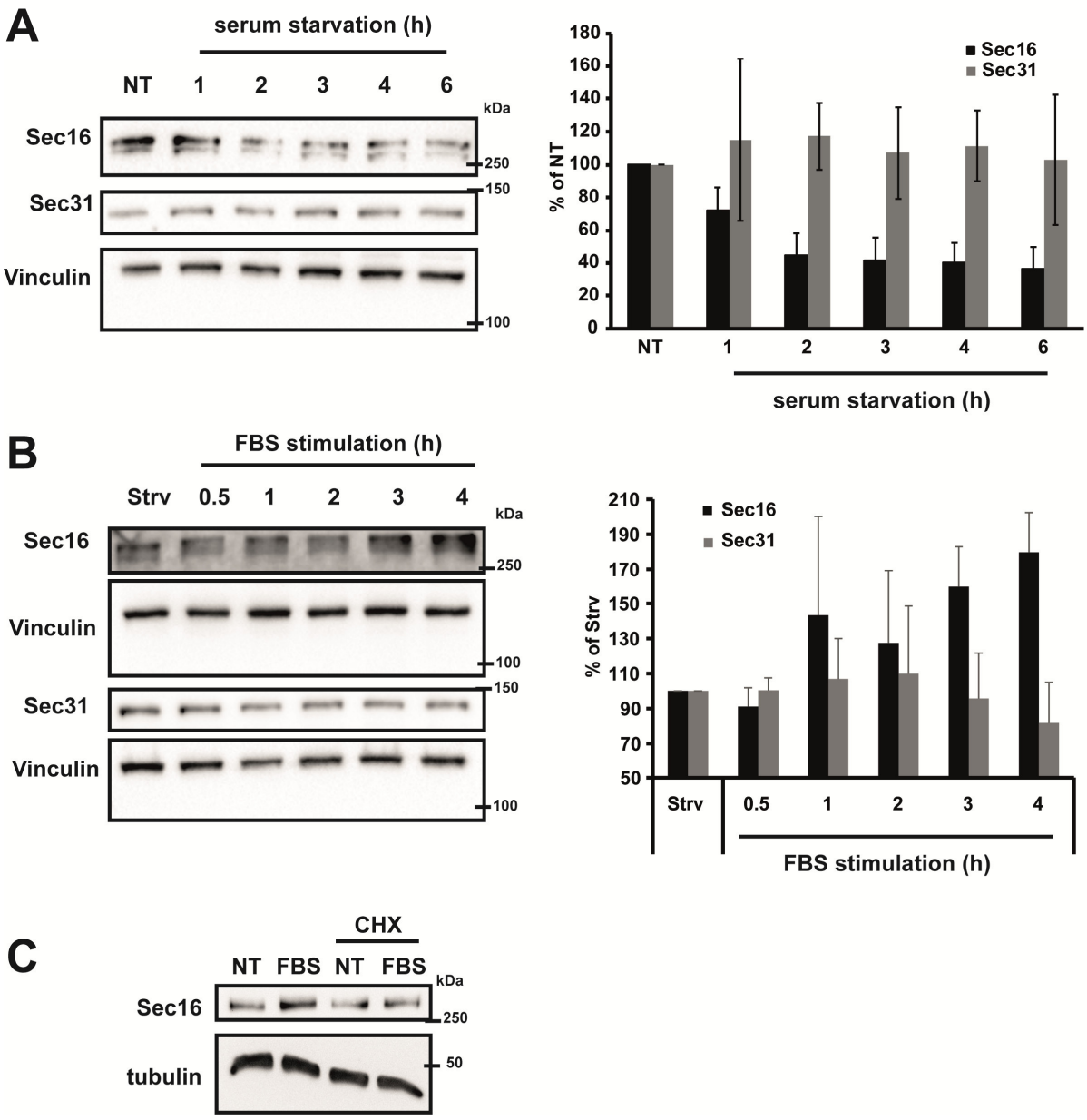


Figure 6: *GF levels regulate Sec16 expression levels.* **A,** HeLa cells were either left untreated (NT) or serum-starved for the indicated time points. Cell lysates were immunoblotted against the indicated proteins. Left panel shows a representative experiment and the right panel shows an evaluation of three independent experiments of Sec16 (black bars) and Sec31 (grey bars) levels. Values are ±SD and are represented as percent of NT. **B,** HeLa cells were serum-starved for 4 h (Strv) or treated with 10% FBS as indicated. Cell lysates were immunoblotted against the indicated proteins. The left panel shows a representative experiment and the right panel shows an evaluation of three independent experiments depicting expression of Sec16 (black bars) and Sec31 (grey bars). Values are ±SD and presented as % of the serum-starved condition. **C,** HeLa cells were either left untreated (NT), or stimulated with 10% FBS for 4h. CHX indicates cycloheximide treatment for 30 min prior to FBS stimulation. Cell lysates were immunoblotted against the indicated proteins.

The results shown above imply that Sec16A is a short-lived protein. Indeed, chasing Sec16A levels after blocking translation by cycloheximide treatment revealed that Sec16A has a half-life of approximately 2-3 h (Fig.7A). Serum starvation likewise resulted in a decrease of Sec16A levels (Fig.7B). The decrease of Sec16A levels can be explained either by an increase of protein degradation, by a reduction in the rate of synthesis, or by a combination of both. First, we determined whether Sec16A is in principle degraded by the proteasome, which was the case since treatment with MG132 increased Sec16A levels and prevented its decay in cycloheximide treated cells. If an increased degradation is the main cause for the reduction of Sec16A levels under serum starvation, then we might expect that the decay kinetics under serum-starvation are higher than under cycloheximide treatment. This was not the case (Fig. 7B). Therefore, we conclude that, while degradation of Sec16A is in principle mediated via the proteasome, the decay in Sec16A levels under serum-starvation is not caused by an increase in the rate of proteasomal degradation. Thus, we are tempted to speculate that GF signaling regulates Sec16A synthesis. However, we first wanted to exclude another possibility. Serum starvation could for instance lead to the formation of stress granules, where Sec16A mRNA is trapped and prevented from translation. We therefore tested whether serum starvation leads to the formation of stress granules by serum starving cells and evaluating stress granule formation by staining for the stress granule marker DDX6 (Fig.7C) [2]. However, while induction of stress granules by a combination of heat shock and Saponin treatment caused an increase in DDX6-positive punctae, serum starvation did not, indicating that serum starvation does not induce stress granule formation. It is therefore unlikely that Sec16A mRNA is trapped in stress granules in response to serum starvation. Recently, amino-acid starvation was shown to lead to a relocalization of Sec16A and COPII components away from ERES and to specialized, starvation-induced Sec bodies in Drosophila S2 cells [3]. We therefore tested whether a similar phenomenon might take place upon serum starvation in mammalian cells. However, GFP-Sec16A and Sec31 did not co-localize with the stress granule marker DDX6 in steady state or after serum starvation, and also not after induction of stress granule formation by 2 hours heat-shock at 43°C and 1 hour treatment with 600mM Sorbitol, which was shown to increase SG number (Fig.7C+D).

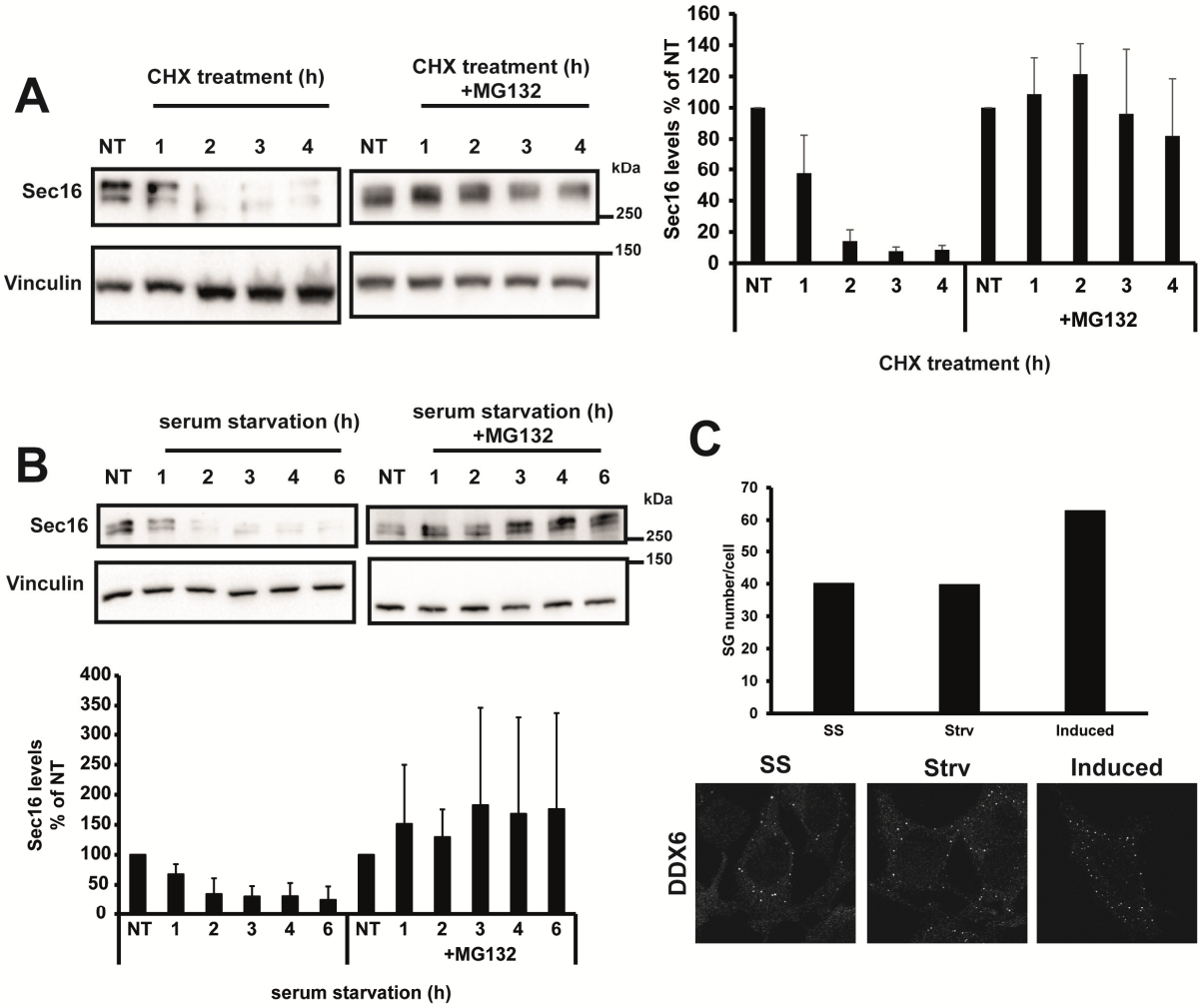


Figure 7: *Sec16 is a short-lived protein which is rescued by proteasomal inhibition.* **A,** HeLa cells were either left untreated (NT), or treated with cycloheximide (CHX) as indicated. Alternatively cells were pre-treated with MG132 for 30 min prior to cycloheximide addition (+MG132). Cells were lysed and immunoblotted against the indicated proteins. The left panel shows a representative experiment and the

lower right shows an evaluation of three independent experiments. Values are ±SD and presented as percent of NT. **B,** HeLa cells were either left untreated (NT), or serum-starved for the indicated time points. Alternatively cells were pre-treated with MG132 for 30 min prior to serum-starvation (+MG132). Cells were lysed and lysates were subjected to SDS-PAGE and immunoblotting against the indicated proteins. The upper panel shows a representative experiment and the lower panel shows an evaluation of three independent experiments depicting expression of Sec16. Values are ±SD and are represented as percent of NT. **C,** HeLa cells were grown on glass coverslips and were left in steady state (SS), serum starved for 6 h (Strv) or subjected to heat shock at 43°C for 2 h in combination with 1 h treatment with 600 mM Sorbitol to induce stress granules (Induced). Cells were fixed, followed by DDX6 immunostaining and confocal microscopy. Top panel shows a graphic representation of the number of stress granules per cell presented as percent of control in steady state. This value amounts to 40.1 stress granules/cell. Results are mean of one experiments with 30 cells per condition. **D,** HeLa cells expressing wild –type GFP-Sec16A were treated as in C, fixed and immunostained against Sec31 and DDX6, followed by confocal microscopy. Panel shows representative images from three independent experiments.

3 Sec16A expression might be controlled by Egr1+3 transcription factors

As mentioned above, a possible explanation for the decrease in Sec16A protein levels is that Sec16A expression might be tightly regulated and halted upon loss of GF signaling, which is in line with the finding that Sec16A has a short half-life. In addition, Sec16A levels increase on a short time-scale in response to GF stimulation. We therefore hypothesized that growth factors sensitive transcription factors control Sec16A levels. To test whether this is true, we first needed to identify which transcription factors are possible candidates. Together with the group of Rhoded Sharan (University of Tel-Aviv, Israel), we bioinformatically analyzed a 500 bp region upstream of the transcription start site of the Sec16A gene using the PRIMA algorithm [4] to identify enrichment of transcription factor binding sites in this region compared to similar 500 bp regions upstream of the transcription start site of the entire genome. This revealed over 90 candidates which were ranked based on two criteria (see Table 1). First, the number of potential binding sites in the putative Sec16A promoter region was taken into account. Secondly, the transcription factors were ranked by the sum of their distance (in a protein-protein interaction network) to the two growth factor receptors EGFR and IGRF, as we were mainly interested in identifying transcription factors involved in the rapid increase in Sec16A levels upon GF stimulation. This approach revealed the Egr transcription factor family as the most likely candidates, which belong to the group of immediate early genes (Fig.8A). Individual knockdown of Egr1 or Egr3 did not reduce Sec16A expression (Fig.8D), but co-knockdown of Egr1 and Egr3 resulted in a clear reduction of Sec16A levels (Fig.8B+C). Next, we determined whether the induction of Sec16A levels by mitogen treatment is dependent on Egr1/3. We therefore treated cells with serum for 4 h as previously, which resulted in a robust induction of Sec16A levels. However, this response was completely ablated in Egr1 or Egr3 single knockdown cells (Fig.8D). Since Egr transcription factors are downstream targets of the ERK1/2 MAPK cascade, we tested whether ERK2, which was previously shown to phosphorylate Sec16A, also had an effect on Sec16A protein levels. Indeed, depletion of ERK2 reduced Sec16A protein levels (Fig.8E).

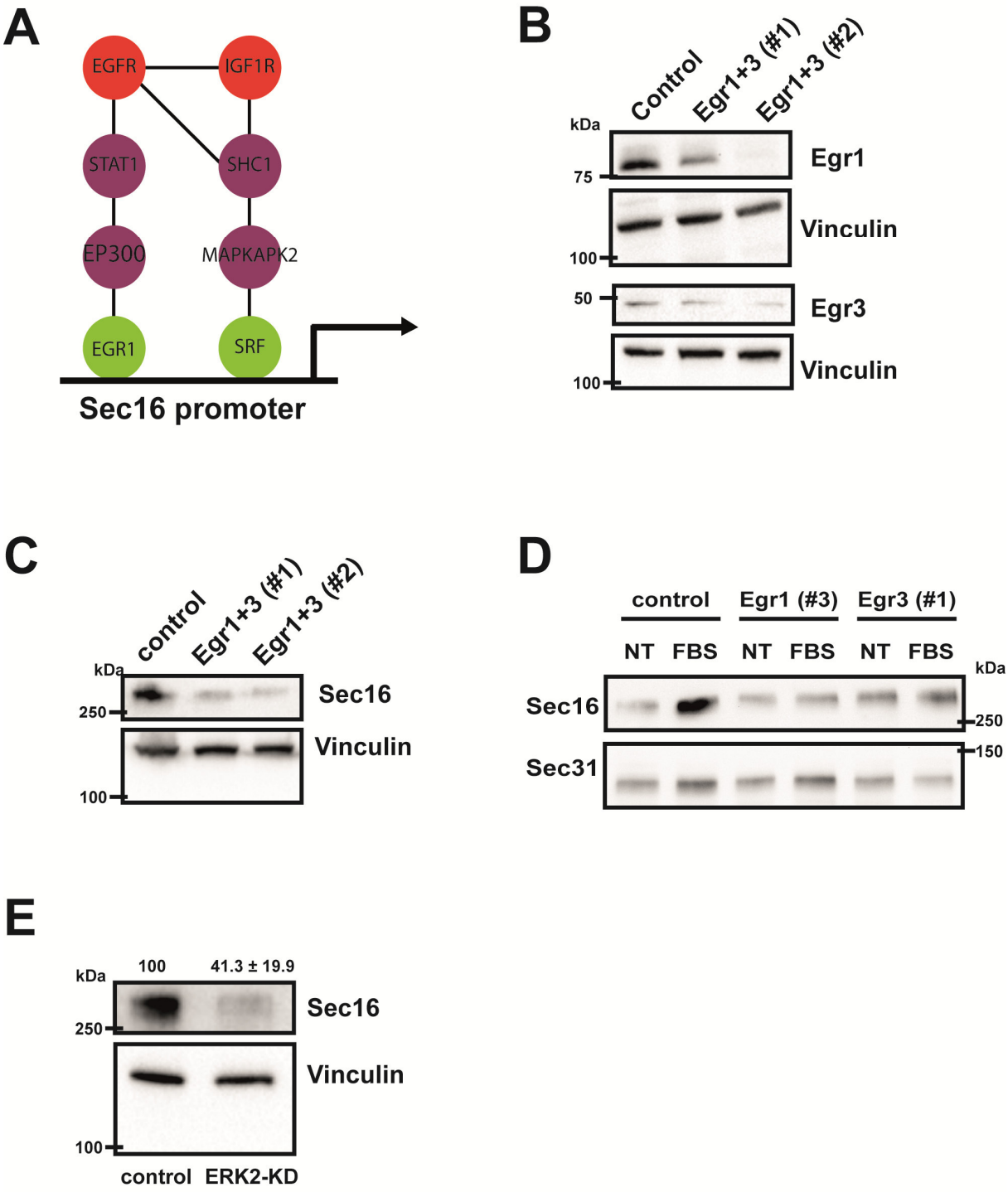


Figure 8: *Egr family transcription factors regulate Sec16 expression.* **A,** Schematic representation of the result of our bioinformatic analysis as described in the text. Green nodes are TFs predicted to bind to the Sec16 promoter. Red nodes are the two growth factor receptors to which the transcription factors were linked to via intermediate proteins (purple nodes). Edges are experimentally documented physical interactions. **B&C,** HeLa cells were transfected with non-targeting siRNA (control) or with two different combinations of siRNA against Egr1 and Egr3. After 72 h, cells were lysed and immunoblotted against the indicated proteins. **D,** HeLa cells were transfected with non-targeting siRNA (control) or with siRNA against Egr1 or Egr3. After 72 h, cells were either harvested directly (NT) or treated with 10% FBS (FBS) for 4 h prior to lysis and immunoblotting as indicated. HeLa cells were transfected with non-targeting siRNA

(control) or with siRNA against ERK2 (ERK2-KD). **E,** Cells were lysed after 72 h and immunoblotted against the indicated proteins.

Table 1: Hits of transcription factors that were predicted to bind to the putative Sec16A promoter region ranked based on the number of potential binding sites and by the sum of their distances (in a protein-protein interaction network) to the growth factor receptors EGFR and IGFR

Score	Gene
13	MAZ
9	EGR2, EGR1, EGR3
7	EGR4
6	PATZ1
5	NR2F2, NR2F1, CREB1, E2F1
4	E2F3, E2F4, TFDP1
3	HIF1A, TFAP2A, E4F1, SMAD3, CREM, TFAP2C, SP3
2	ATF3, TFAP2B, HIC1, TCF3, RXRA, PPARA
1	HOXA9, MEIS1, PPARG, ETS1, ETS2, ERF, ERG, FLI1, ELF2, ELK4, ETV7, MYC, MAX, USF1, GATA1, MYOD1, NR1I3, NR1I2, VDR, PAX6, TCF4, TAL1, ATF2

As we showed that the Egr1/3 transcription factors control the expression of the secretory pathway component Sec16A, we next tested the effect of Egr1+3 depletion on the early secretory pathway. In accordance with their ability to regulate Sec16A levels, co-depletion of Egr1+3 resulted in a reduction in the number of ERES (Fig.9A) which was comparable to the reduction in ERES number observed in Sec16A knockdown cells. Next, we wanted to test the effect of Egr1+3 depletion on ER-to-Golgi trafficking. To do this, we used the recently described Retention Using Selective Hooks (RUSH) system [5], which is illustrated in Fig.9B. The RUSH system relies on the retention of a specific secretory cargo of choice in a donor compartment by using a streptavidin-based retention: the secretory cargo protein is tagged with GFP for visualization, as well as with streptavidin binding protein (SBP). The SBP part of the cargo or reporter protein binds to the streptavidin part in the hook, which is co-expressed and also contains a sequence that targets it to a specific secretory compartment, in this case the ER. Therefore, the hook, which is retained in the ER, binds the reporter via the interaction between streptavidin and SBP, keeping the reporter in the ER. Upon addition of biotin, the interaction is disrupted, as biotin has a stronger affinity for streptavidin than SBP, thereby releasing the reporter from the hook. The reporter is then free to travel through the secretory pathway and reaches its destined secretory compartment, which can be the Golgi, the plasma membrane or it can be secreted. We used cells stably expressing GFP-tagged MannosidaseII (ManII-RUSH) which is retained in the ER and travels to the Golgi upon addition of biotin. Knockdown of Egr1+3 led to a marked delay in the arrival of ManII-RUSH from the ER at the Golgi (Fig.9C), and the effect was comparable to cells depleted of Sec16A (Fig.9D). The Egr1+3 transcription factors may in theory control the expression of other components of the secretory pathway and therefore affect other trafficking routes apart from ER-to-Golgi trafficking. To exclude this possibility, we tested the effect of Egr1+3 depletion on post-Golgi trafficking by concentrating the GFP-tagged VSVG-RUSH construct at the Golgi using a 20°C temperature block after biotin addition, before returning the cells to 37°C and chasing the reporter for the indicated time points. No effect of Egr1+3 knockdown on Golgi to plasma membrane trafficking was observed (Fig.9E), thus excluding pleiotropic effects on the secretory pathway.

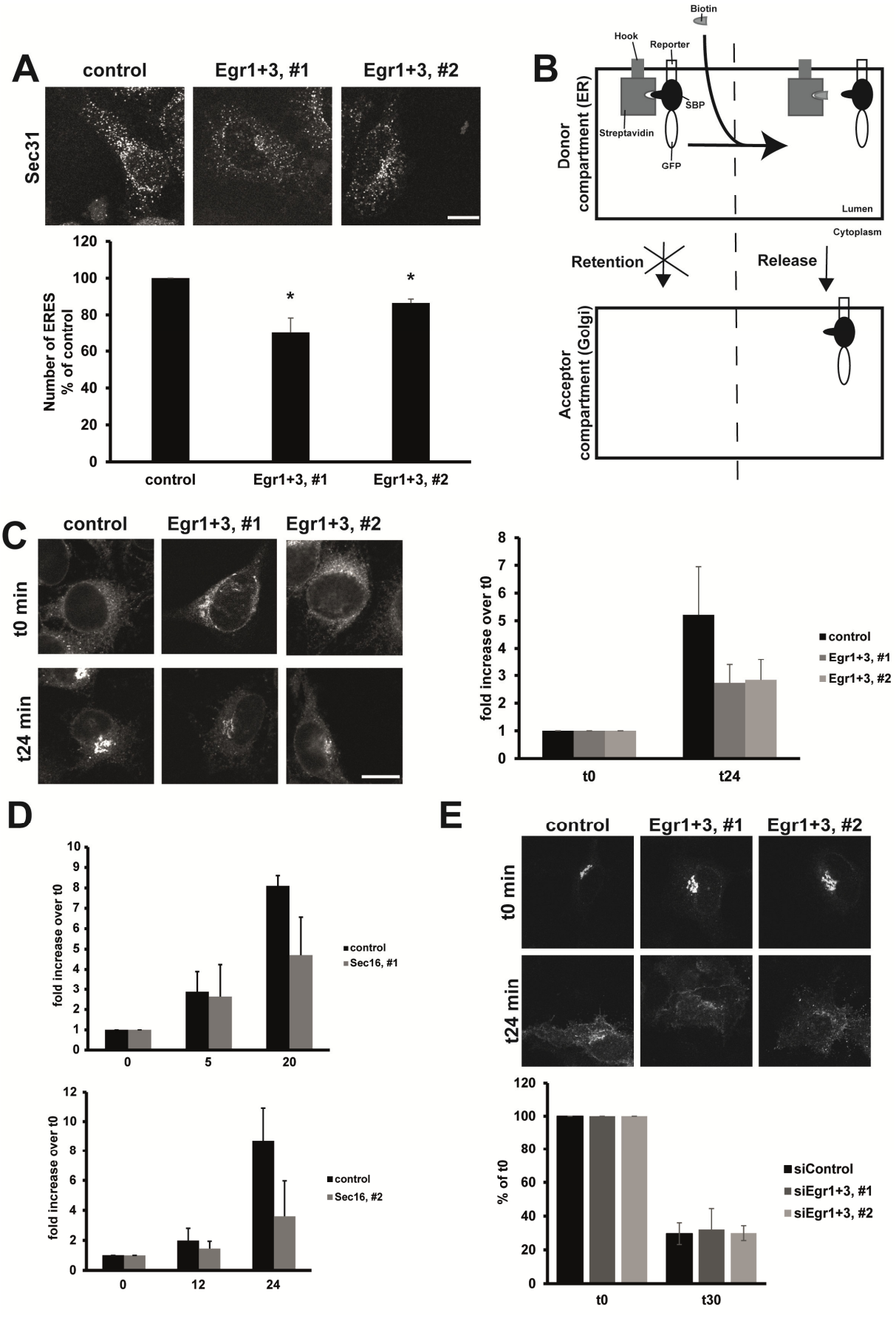

A
control
Egr1+3, #1
Egr1+3, #2
Sec31
Number of ERES
% of control
120
100
80
60
40
20
0
control
Egr1+3, #1
Egr1+3, #2
B
Biotin
Hook
Reporter
SBP
Streptavidin
GFP
Donor compartment (ER)
Lumen
Cytoplasm
Retention
Release
Acceptor compartment (Golgi)
C
control
Egr1+3, #1
Egr1+3, #2
t0 min
t24 min
fold increase over t0
control
Egr1+3, #1
Egr1+3, #2
t0
t24
D
fold increase over t0
control
Sec16, #1
0
5
20
fold increase over t0
control
Sec16, #2
0
12
24
E
control
Egr1+3, #1
Egr1+3, #2
t0 min
t24 min
% of t0
siControl
siEgr1+3, #1
siEgr1+3, #2
t0
t30

Figure 9: *Decrease in ER-to-Golgi trafficking in Egr1+3- and Sec16-depleted cells.* **A,** HeLa cells were transfected with control siRNA (control) or siRNA to Egr1 and Egr3 (Egr1+3). After 72 h, cells were fixed, stained for Sec31 and imaged using confocal microscopy. ERES were counted using ImageJ. Results are presented as percent of control and this value amounts to 244.25 ± 13.62 ERES. Results are means ± SD from three independent experiments where at least 50 cells were evaluated per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) **B,** Schematic illustrating the principle of the RUSH-system. **C,** HeLa cells stably expressing GFP-tagged MannosidaseII RUSH-construct (ManII-RUSH) were transfected with control siRNA (control) or siRNA to Egr1 and Egr3 (Egr1+3). After 72 h, ManII-GFP was released by adding Biotin and cells were fixed at the indicated subsequent time points. Lower panel shows a bar graph of fluorescence intensity at Golgi area, normalized to ER fluorescence, presented as fold increase over t0. This value amounts to 9.52 ± 3.6 AU in control cells and 9.27 ± 3 AU or 12.32 ± 2.6 AU in clones #1 and #2 in Egr1+3 knockdown cells, respectively. Results are means ± SD from three independent experiments with at least 50 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **D,** HeLa cells expressing GFP-tagged MannosidaseII RUSH-construct (ManII-RUSH) were transfected with control siRNA (control) or siRNA to Sec16 (Sec16). After 48 h, ManII-GFP was released by adding Biotin and cells were fixed at the indicated subsequent time points. Bar graphs represent fluorescence intensity at Golgi area, normalized to ER fluorescence, presented as fold increase over t0. This value amounts to 8.4 ± 0.6 AU in control cells and 7.3 ± 2.1 AU in Sec16 knockdown cells in the upper panel and 8.64 ± 2.8 AU in control cells and 9.98 ± 1.9 AU in Sec16 knockdown cells in the lower panel. Results are means ± SD from three independent experiments with at least 50 cells per experiment. E, HeLa cells transiently expressing the GFP-tagged VSVG RUSH-construct were transfected with control siRNA (control) or siRNA to Egr1+3 (siEgr1+3). After 72 h, GFP-VSVG was released at 20°C for 1 h. Subsequently, cells were either fixed directly, or were placed back to 37°C to release VSVG from the Golgi followed by fixation after 30 minutes. Cells were immunostained for Giantin to label the Golgi and the rate of fluorescence decay from the Golgi was measured to estimate exit of secretory cargo from this organelle. Upper panel shows representative images from three independent experiments. Lower panel shows a bar graph of fluorescence intensity at Golgi area, normalized to ER fluorescence, presented as percent of t0. Results are means ± SD from three independent experiments with at least 30 cells per experiment.

Cargo load has previously been shown to affect ERES number, and therefore we determined whether the observed reduction of ERES in Egr1+3 cells is due to a change in Sec16A levels or due to alterations in the synthesis of secretory proteins [6, 7]. To test this, we used the hepatic cell line HepG2, which is a stronger secretory cell than HeLa cells. Similar to our findings in HeLa cells, Egr1+3 knockdown in HepG2 cells decreased the number of ERES (Fig.10A). However, silencing Egr1+3 did not affect the levels of alpha1 antitrypsin (AAT1) (Fig.10B), a major secretory protein in HepG2 cells [8, 9]. A similar result was obtained with albumin, the most prevalent cargo in hepatocytes (Fig.10C). Therefore, it is unlikely that Egr1+3 knockdown reduces secretory cargo load.

Altogether, these results indicate that Sec16A expression might be controlled by Egr1+3 transcription factors and further experiments are required to validate whether this is the case.

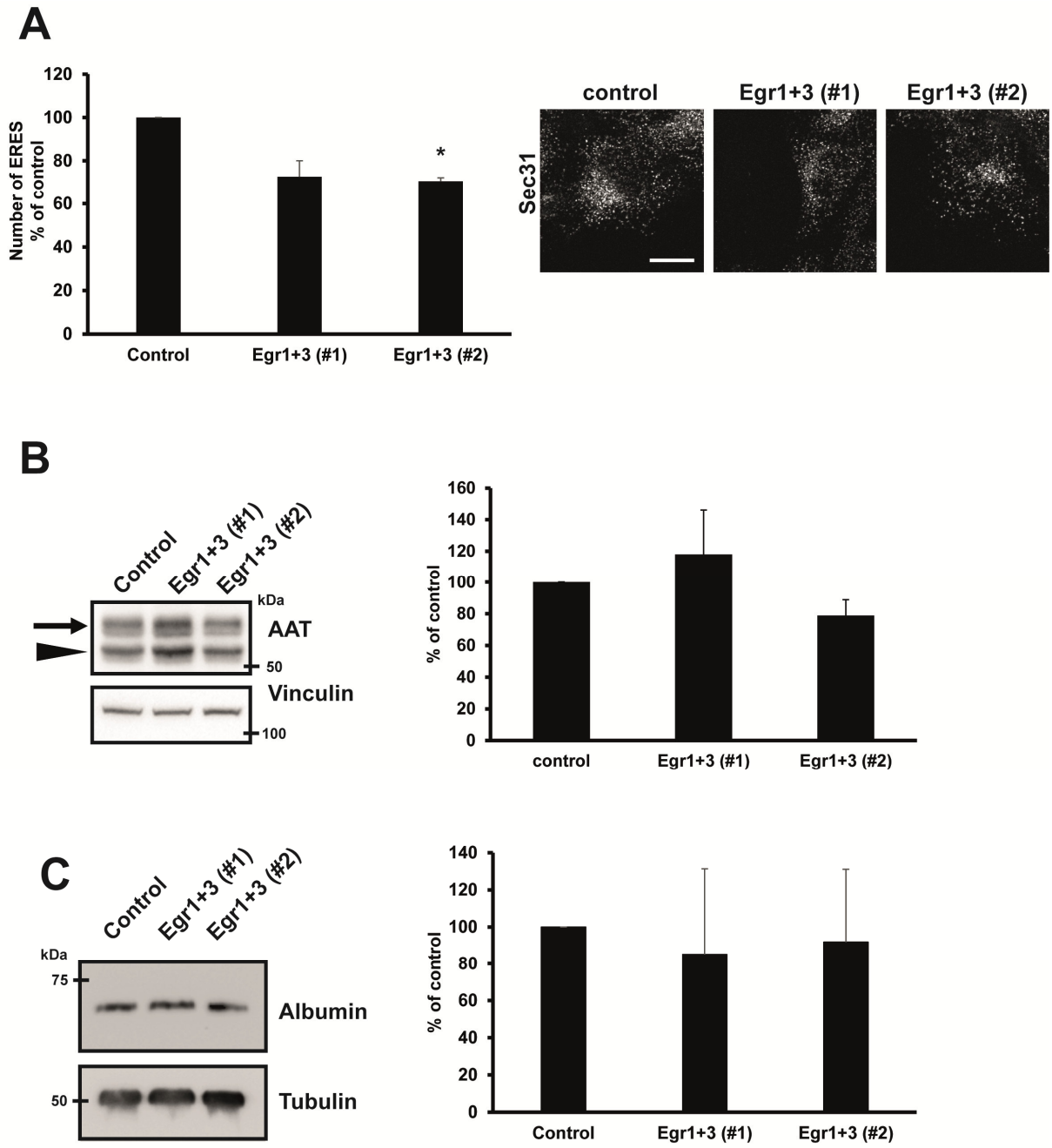


Figure 10: *Decrease of ERES number after loss of Egr1+3 is independent of cargo load in HepG2 cells.* **A,** HepG2 cells were transfected with control siRNA (control) or siRNA to Egr1 and Egr3 (Egr1+3). After 72 h, cells were fixed and stained for Sec31, and images were acquired by confocal microscopy. Left panel shows graphic representation of the number of ERES per cell. Results are presented as percent of control to account for inter-assay variance. This value amounts to 314 ± 51 ERES. The results are means ± SD from three independent experiments where at least 50 cells were evaluated per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **B&C,** HepG2 cells were transfected with non-targeting siRNA (control) or with siRNA against Egr1 and Egr3. After 72 h, cells were lysed and immunoblotted against a1-antitrypsin (AAT) in panel B or against Albumin in panel C. The left parts show representative experiments and the right parts show evaluation of three independent experiments. Values are ±SD and are represented as percent of control.

4 Sec16A as part of a coherent feed-forward loop (CFFL)

Our results so far revealed that GF signaling regulates Sec16A transcription, resulting in an increase of Sec16A levels on a time-scale of 2-3 h after GF stimulation. In addition, GF signaling pathways are expected to induce translation, thereby increasing secretory cargo load. This scenario is reminiscent of a coherent feed-forward loop [10, 11] (CFFL; Fig.11A). In a CFFL, an input node (GFs) triggers a central node (Sec16A) that subsequently triggers an output node (secretion or ERES number). In addition, the input node also triggers the output node, but with slower kinetics than the aforementioned links. CFFLs are typically found as part of persistence detectors, which ensure that transient stimuli that are able to trigger the central node do not affect the output node to any appreciable extent. Only a prolonged (i.e. a persistent) stimulus is able to trigger the output node. A CFFL necessitates the presence of a fast connection between input and central node (i.e. between GFs and Sec16A). The connection ought to be considerably faster than between input and output node (i.e. between GFs and secretion). To investigate the fast connection between input and central node, we next tested whether and how a brief GF treatment affects Sec16A and ERES organization.

5 Growth factor treatment increases ERES number and alters Sec16A dynamics

To test whether short term GF signaling has an effect on ERES, we serum-starved cells which results in a decrease in ERES number (Fig.5A), and then briefly treated the cells with fetal bovine serum (FBS) for 10 to 15 min. On this short timescale, we observed a rapid increase in the number of ERES. The response of ERES to GF treatment was absent in Sec16A depleted cells (Fig.11B). This response is unlikely to be mediated by induction of Sec16A levels, because a 30 min FBS treatment did not induce Sec16A levels to any appreciable extent (Fig.6B). Furthermore, we tested whether the fast response of ERES was also dependent on ERK2 (Fig.11C). Indeed, depletion of ERK2 ablated the response of ERES to GF stimulation similar to Sec16A depleted cells, which is in line with previous findings showing that ERK2 phosphorylates Sec16A [1].

To gain more detailed insights into the mechanism that underlies the observed increase of ERES number, we adapted a previously reported simulation approach for the self-assembly of ERES [12]. In agreement with experimental observations, this *in silico* simulation approach revealed a quasi-crystalline arrangement of ERES with a fairly uniform ERES size, and a comparatively low density of Sec16A or COPII proteins on ER membranes between ERES. The steady state of ERES formation therefore can be described by an unmixing scenario of the Flory-Huggins type [13]: Analogous to a domain formation of small amphipathic polymers in water, Sec16A and/or COPII show a dynamic segregation into patchy domains on ER membranes (=ERES). However, in contrast to a standard Flory-Huggins scenario, Sec16A and/or COPII have finite residence times on ER membranes, *i.e.* they dissociate on average with rate Γ from ER membranes. Extending the Flory-Huggins scenario to include this aspect (see *Materials and Methods* for details), allowed us to analytically predict the steady-state number and radius of ERES (N_{ERES} and R_{ERES}, respectively) as a function of the protein density, ϕ, and the proteins' dissociation rate and diffusion coefficient (Γ and D, respectively): $N_{ERES} = \Gamma L^2/D$ and $R_{ERES} = \sqrt{4\phi D/(\pi\Gamma)}$ (L^2 = area of the ER membrane). The model predicts that increasing the average dissociation constant of Sec16, Γ, increases the number of Sec16A-positive ERES irrespective of the available protein amount, ϕ. However, this should come at the expense of ERES size since the ERES radius depends inversely on the dissociation rate Γ. In other words, Sec16A molecules must be able to leave ERES faster on average to be able to nucleate new, but smaller ERES at remote locations (Fig11D). We tested this hypothesis experimentally by stimulating serum-starved cells with FBS for 15 min, followed by Sec31A immunostaining. Indeed, a brief FBS stimulation leads to a reduction of the average size of ERES (Fig.11E). We also performed live imaging of YFP-Sec31A and found that a brief stimulation with FBS increased ERES number and reduced their size (Fig.11F), which agrees with the findings in fixed cells (Fig.11E+F). In addition to a decrease in ERES size, the model predicts an increased 'mobilization' of Sec16A, *i.e.* a larger dissociation rate Γ, upon mitogen treatment.

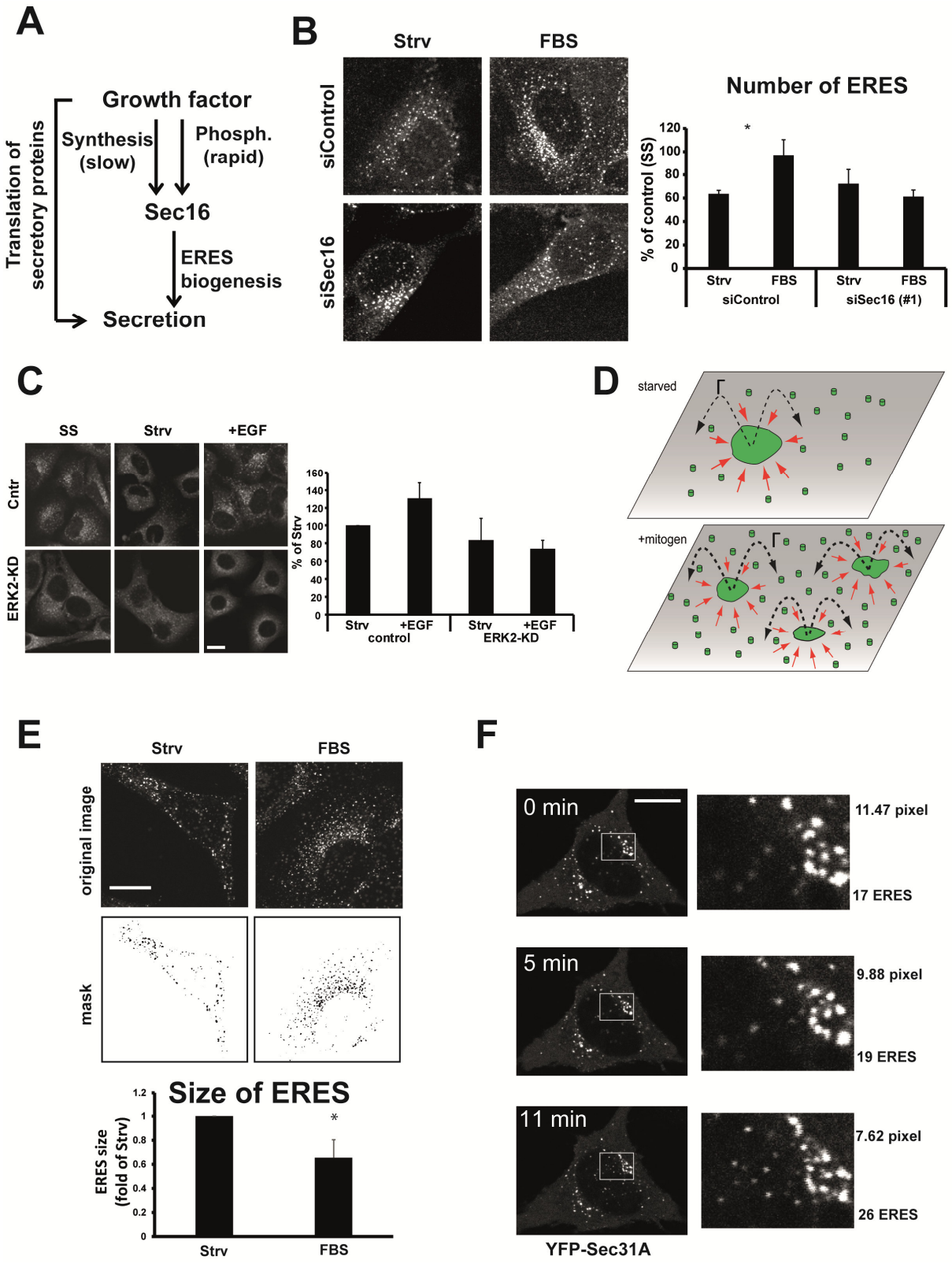


Figure 11: *Fast response of Sec16 to growth factors.* **A,** Schematic representation of the proposed coherent feed-forward loop. **B,** HeLa cells were transfected with control siRNA (siControl) or siRNA to Sec16 (siSec16). After 72 h, cells were serum starved for 6 h (Strv) and fixed, or followed by 10% FBS treatment for 15 min (FBS) and fixation. Cells were stained for Sec31, and imaged by confocal microscopy. Right panel shows graphic representation of the number of ERES per cell. Results are

presented as % of control (Strv) to account for inter-assay variance. Results are means ± SD from three independent experiments where at least 50 cells were evaluated per experiment. **C,** HeLa cells were transfected with control siRNA (control) or siRNA to ERK2 (ERK2). After 72 hours, cells were serum starved for 6 h (strv), or serum starved for 6 h and stimulated with 50 ng/mL EGF for 15 min (EGF). After treatment, cells were fixed and stained for Sec31, and images were acquired by confocal microscopy. Right panel shows graphic representation of the number of ERES per cell. Results are presented as percent of control (Strv) to account for inter-assay variance. This value amounts to 97.9 ± 30.1 ERES. Scale bar in this image is 10µm. **D,** Schematic representation of the results of our mathematical modeling. In starved cells, Sec16 rarely leave the ERES (thin black dashed arrows). Those that escaped the ERES will re-bind to and diffuse on ER membranes from where they get captured by existing ERES (red arrows). In mitogen-treated cells, dissociation is enhanced (thick black dashed arrows), and rebinding Sec16 molecules can form local assemblies on ER membranes that attract even more Sec16, thereby growing new ERES. E**,** HeLa cells were serum starved for 4 h (Strv) and stimulated with 10% FBS for 15 min (FBS) followed by fixation, immunostaining, and confocal microscopy. Upper panel shows representative images of ERES as well as masks of counted particles as appearing for analysis. Lower panel shows graphic representation of ERES size presented as fold change of Strv. This value amounts to 96.9 ± 19.2 pixels/ERES (~9.8x9.8 pixels, or 462x462 nm for each ERES). Results are means ± SD from three independent experiments with at least 10 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by paired Student's t-test. **F,** HeLa cells expressing YFP-Sec31A were serum starved for 4 h. Live imaging was started upon addition of 10% FBS and an image was acquired every 60 seconds. Stills are shown of indicated time points. The right most parts are magnified areas and number next to them are the average ERES size (in pixels) and their number within the displayed region.

We next tested this prediction by fluorescence recovery after photobleaching (FRAP) of single ERES in HeLa cells expressing GFP-Sec16A (Fig.12A). We evaluated FRAP curves of individual ERES by assuming a simple binding reaction, i.e. using a single-exponential recovery. Since ERES are small structures, Sec16A association/dissociation events necessarily display a stochastic nature, i.e. a considerable variation of fitting parameters between individual FRAP curves is anticipated. Therefore, averaging FRAP curves to obtain a smoothed 'master' curve may introduce serious artefacts like stretched-exponential or even power-law recoveries. We therefore fitted curves individually and averaged the obtained fitting parameters. Averaged typical time scale of fluorescence recovery did not vary systematically between starved and mitogen-treated cells with recovery half-time of about 10s was observed. Yet, we observed differences in the extent of the recovery: As compared to their pre-bleach fluorescence (set to unity), the average extent of fluorescence recovery of ERES was larger and the mobile fraction higher when cells had been stimulated with GFs (Fig. 12A), which is in line with our previous observations [1].

If only a single pool of Sec16A-GFP was present on ERES, one would expect either a full recovery (q=1) or no recovery at all (q =0) on the time scale of the FRAP experiment. Observing a recovery with a maximum value of q between zero and unity therefore requires the existence of a fast Sec16A pool that carries the observed recovery (relative amount q), and a slow Sec16A pool that does not contribute to the fluorescence recovery on the time scale of the FRAP experiment (relative amount 1-q). Consequently, the arithmetically averaged dissociation rate of the entire Sec16A population is $\Gamma = q\Gamma_{fast} + (1-q)\Gamma_{slow}$. Observing an increase in q after GF stimulation therefore points towards a faster turnover kinetics of Sec16A on average (Γ is increased), while the half time of the recovery is only determined by Γ_{fast} (since the experiment takes much less time than $1/\Gamma_{slow}$). Yet, an increase in the average dissociation rate Γ is exactly the prediction of the above model, and we therefore conclude that indeed a mobilization of Sec16A after mitogen stimulation is a factor that can drive formation of new ERES.

An increased dissociation rate of Sec16A upon GF stimulation suggests that upon GF stimulation, less Sec16A should be present on membranes and more in the cytosol, as Sec16A is mobilized away from membranes. The cytosolic Sec16A then rebinds the ER membrane to form new ERES in different locations (Fig.12B). Indeed, using a very gentle lysis and fractionation protocol to not introduce artefacts due to mechanical

stress, we found that during serum starvation, Sec16A is found mostly at membranes, but disperses partly to the cytosol upon brief FBS treatment (Fig.12C). As ERK2 phosphorylates Sec16A at T415, we hypothesized that membrane association of Sec16A might be influenced by its phosphorylation status. We performed fractionation assays as before using phosphomimetic (T415E) and phosphoablating (T415I) GFP-tagged Sec16A mutants (Fig.12D). Interestingly, the phosphomimetic mutant of GFP-Sec16A was found to have a higher membrane/cytosol ratio, indicating that phosphorylation-induced Sec16A turnover at ERES is also required for membrane association in general. The phosphoablating Sec16A mutant had a lower membrane/cytosol ratio, but was present in both fractions, therefore the regulation of Sec16A association to ERES is more complexly regulated than by phosphorylation and de-phosphorylation alone.

Number and size of ERES also depend on the diffusion constant of Sec16A on ER membranes, D. Thus, if our above rationale is to hold true, D must not vary strongly between starved and mitogen-treated cells. To probe this, we have used fluorescence correlation spectroscopy (FCS) that allowed us to determine the diffusion constants of GFP-Sec16A in cytoplasm and on ER membranes (representative FCS curves in Fig.12E). As a result, we found that the diffusion constant of GFP-Sec16A in cytoplasm agreed well with theoretical predictions for a soluble protein of ~280 kDa irrespective of the treatment (D≈16 $\mu m^2/s$). In contrast, the membrane-bound pool of Sec16A showed a minor, but significant reduction in the diffusion constant under starvation conditions (D≈0.43 $\mu m^2/s$) as compared to mitogen-treated cells (D≈0.65 $\mu m^2/s$) and completely untreated cells (D≈0.62 $\mu m^2/s$). Most likely, the slightly slower diffusion of ER-bound Sec16A in starved cells is due to more pronounced interactions with other ER-bound proteins. Indeed, most likely a change in D and in Γ may be linked: Γ is the weighted average of two dissociation constants, is $\Gamma = q\Gamma_{fast} + (1-q)\Gamma_{slow}$, i.e. increasing Γ most likely means that the fast dissociation gains more weight (q increases). Given that Γ_{slow} is associated with a pool of Sec16A that is stuck in ERES (immobile on the time scale of our experiments), this “mobilization” increases the amount of Sec16A molecules on ER regions between ERES. Increasing the pool of these free Sec16A molecules possibly leads to a more pronounced interaction with other ER-resident components, e.g. resulting in a transient formation of oligomeric structures, which consequently reduces the average diffusion of Sec16A copies on ER patches between ERES. Nevertheless, Sec16A is regulated on the time scale of minutes consistent with being

part of a CFFL. We next aimed at understanding how mobilization of Sec16A generates more ERES.

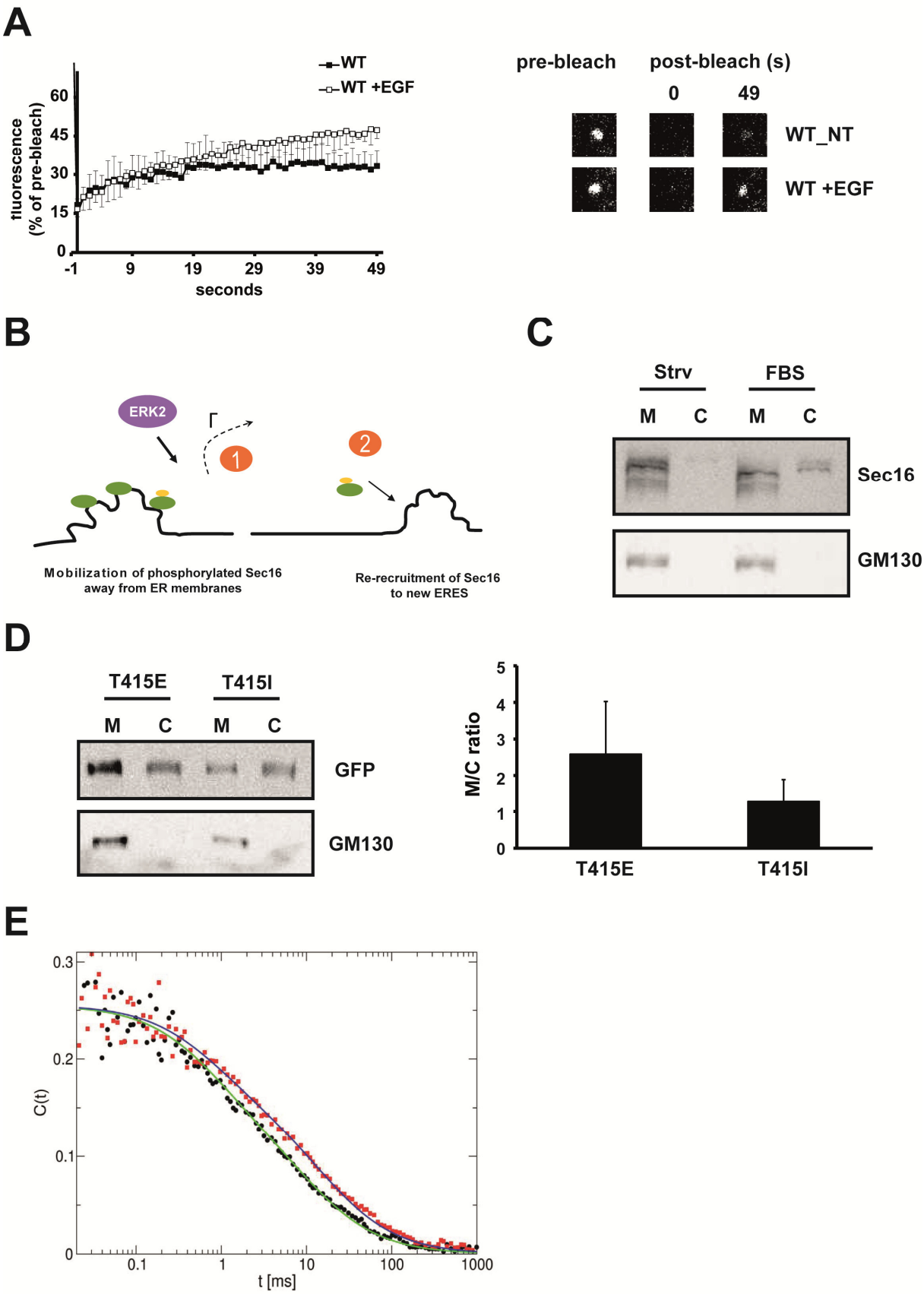


Figure 12: *Analysis of Sec16 dynamics.* **A,** HeLa cells expressing wild type GFP-Sec16. Cells were serum starved for 6 h. FRAP curves were recorded from the same coverslip before (black symbols) and after stimulation with 50 ng/ml EGF (+EGF; white symbols). FRAP curves are shown as mean of three

independent experiments where at least 5 curves were recorded in each experiment. Fluorescence values were normalized to the pre-bleach intensity. Images in the right panel depict representative examples of bleached ERES before (pre-bleach) and after bleaching at the indicated time points. **B,** Schematic illustrating two-step process of Sec16A mobilization and re-binding in the biogenesis of ERES. **C,** HeLa cells were serum starved for 6 h (Strv) and then stimulated with 10% FBS for 15 min (FBS) before lysis by osmotic shock, followed by separation of membrane (M) and cytosolic (C) fraction by centrifugation. Samples were subjected to SDS-PAGE and immunoblotting against the indicated proteins. Panel shows a representative experiment of three independent experiments. **D,** HeLa cells transiently expressing phosphomimetic (T415E) or phosphoablating (T415I) GFP-Sec16A mutant were lysed by osmotic shock, followed by separation of membrane (M) and cytosolic (C) fraction by centrifugation. Samples were subjected to SDS-PAGE and immunoblotting against the indicated proteins. Left panel shows a representative experiment of three independent experiments. Right panel shows an evaluation of three independent experiments. Values are ±SD and are represented as membrane to cytosol ratio (M/C ratio). **E,** Fluorescence Correlation Spectroscopy (FCS) of Sec16A. The decay of the fluorescence autocorrelation, C(t), for GFP-Sec16A in starved cells (red symbols) and serum-treated cells (black symbols) both include a fast fraction that represents cytosolic Sec16A molecules. The slower, ER-bound fraction of Sec16A molecules, however, is significantly different. Starved cells show a shift of the correlation decay towards a larger time scale, i.e. membrane-bound Sec16A diffuses slower. Green and blue lines are best fits according to the fitting function stated in *Materials and Methods.*

6 Interaction with COPII modulates the turnover of Sec16A on ERES

We hypothesized that an interaction with COPII is responsible for the observed existence of a fast and a slow Sec16A pool and that thereby COPII plays a role in the ability of Sec16A to generate new ERES. If true, then decreasing the binding of COPII to ERES ought to affect the amount of fluorescence recovery, q, in FRAP experiments. As a first step, we treated cells with cycloheximide to reduce the protein load in the ER and concomitant the association of COPII with ERES [6, 14]. While GF treatment in control cells led to an increase in Sec16A fluorescence recovery, the extent of recovery and the mobile fraction in cycloheximide treated cells was the same for starved and mitogen-treated cells (Fig.12A+13A). To obtain further insights into the role of COPII, we performed FRAP experiments with a truncation mutant of Sec16A that lacks the last C-terminal 431 amino acids which we called Sec16-Maddin. This mutant still contains the previously described ERK2 phosphorylation site Thr415 but it lacks the domain that was described to bind Sec23A and Sec12 [1, 15, 16]. This mutant still localizes to ERES (Fig.13B), but in fractionation assays shows a lower membrane association than the wild type (Fig.13C). Using co-immunoprecipitation we confirmed that Sec16-Maddin fails to interact with Sec23A, but is still able to interact with Sec31 (Fig.13D) as the Sec31-binding region has been previously mapped to the central region of Sec16A [17]. In FRAP experiments, treatment with GFs did not increase but rather decreased the extent of recovery and mobile fraction of Sec16-Maddin (Fig.13E&H). To further support the notion on the role of COPII, we depleted Sar1A and found EGF treatment did not change GFP-Sec16 mobility (Fig.13F&H). Of note, depletion of Sar1A also affected the ability of Sec16A to form new ERES in response to growth factor treatment (Fig.13I). As depletion of ERES regulators might in general disrupt the ability of ERES to respond to GF stimulation, we tested whether depletion of kinases unrelated to the Sec16A-COPII pathway are similarly unresponsive to GF stimulation. We found that depletion of the kinases NME6, NME7, and PiP5K1C lowered the number of ERES (Fig.5B), but did not affect the ability of ERES to respond to growth factor treatment, as ERES number still increased after brief (15 min) GF stimulation (Fig.13I). In addition, depletion of NME6 also did not inhibit the increase in GFP-Sec16 mobility after EGF treatment (Fig.13G&H). Thus, the COPII-Sec16A interplay is important for generation of new ERES after growth factor treatment and we next sought to test this conjecture.

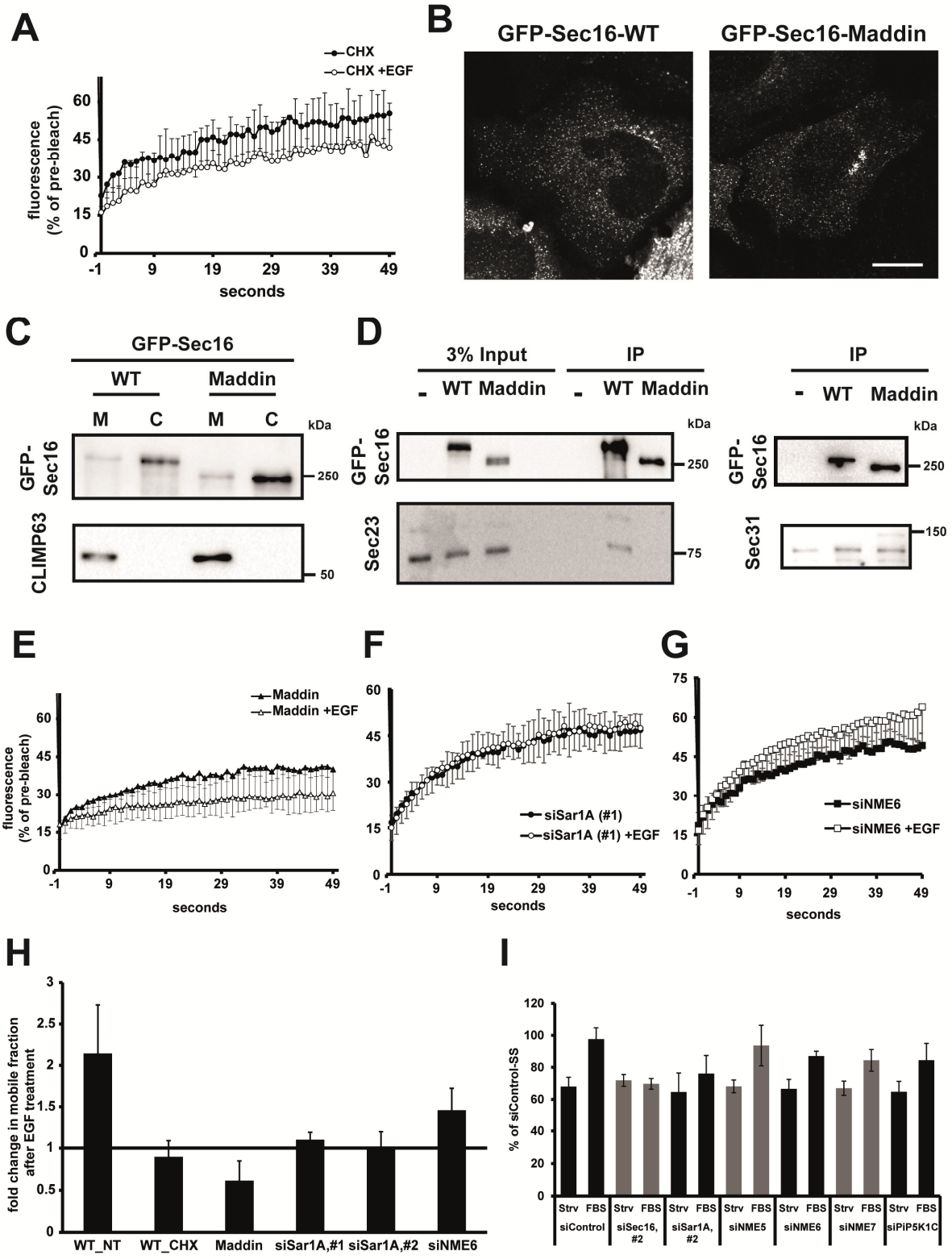


Figure 13: *Role of COPII in Sec16A dynamics at ERES.* **A,** HeLa cells expressing wild type GFP-Sec16A were serum starved for 6 h. Additionally, cells were pre-treated with 50 µg/mL cycloheximide for 2 h (CHX) before starting FRAP measurements. FRAP curves were recorded from the same coverslip before (black symbols) and after stimulation with 50 ng/ml EGF (+EGF; white symbols). FRAP curves are shown as mean of three independent experiments where at least 5 curves were recorded in each experiment. Fluorescence values were normalized to the pre-bleach intensity. **B,** HeLa cells expressing GFP-tagged

wild type Sec16 (left) or Sec16-Maddin (right). **C,** HeLa cells expressing wild-type GFP-Sec16 (WT) or GFP-Sec16-Maddin (Maddin) were lysed by osmotic shock, followed by separation of membrane (M) and cytosolic (C) fraction by centrifugation. Samples were subjected to SDS-PAGE and immunoblotting against the indicated proteins. Panel shows a representative experiment of three independent experiments. **D,** HeLa cells expressing wild-type GFP-Sec16 (WT), GFP-Sec16-Maddin (Maddin) or GFP (-) were lysed and Sec16 was immunoprecipitated using GFP-trap beads. The immunoprecipitate was eluted from beads and Sec16 was detected by immunoblotting. The same membrane was probed for the levels of Sec23A (Sec23) or Sec31. **E,** HeLa cells expressing GFP-Sec16A-Maddin were serum starved for 6 h. FRAP curves were recorded from the same coverslip before (black symbols) and after stimulation with 50 ng/ml EGF (+EGF; white symbols). FRAP curves are shown as mean of three independent experiments where at least 5 curves were recorded in each experiment. Fluorescence values were normalized to the pre-bleach intensity. **F&G,** Similar experimental setting as in panel E except that cells expressing GFP-Sec16-WT were used and cells were transfected with siRNA to Sar1A (F) or NME6 (G) 72 h prior to FRAP measurement of wild type GFP-Sec16. **H,** Bar graph depicts the relative increase in mobile fractions, q, after treatment with EGF determined as indicated in “Materials and Methods”. WT_NT and WT_CHX indicate the condition of wild type GFP-Sec16 expressing cells treated with solvent and cycloheximide, respectively. Values are means ±SD from three independent experiments. **I,** HeLa cells were transfected with control siRNA (control) or siRNA to Sec16A (siRNA clone #2), Sar1A (siRNA clone #2), NME5, NME6, NME7 or PiP5K1C. After 72 h, cells were left in steady state (depicted in Fig.R1), serum starved for 6 h (strv), or serum starved for 6 h and stimulated with 10% FBS for 15 min (FBS). After treatment, cells were fixed and stained for Sec31, and images were acquired by confocal microscopy. Panel shows graphic representation of the number of ERES per cell. Results are presented as percent of control in steady state to account for inter-assay variance. This value amounts to 228.56 ± 12.3 ERES. Results are means ± SD from three independent experiments where at least 30 cells were evaluated per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey’s post hoc test.

7 Interaction with COPII is required for Sec16A to generate more ERES

To test the role of the Sec16A-COPII interplay in the generation of more ERES after mitogen treatment, we determined the ERES number in cells expressing either wild type GFP-Sec16A or Sec23-binding deficient GFP-Sec16-Maddin. We only compared cells within a twofold range of fluorescence intensities to avoid inclusion of cells with a too high variability of exogenous Sec16A overexpression. Cells were fixed before and after treatment with serum for 15 minutes followed by confocal imaging. If the ability of Sec16A to interact with COPII is critical to generate more ERES, then Sec16-Maddin should fail to respond to GF treatment. As observed with endogenous Sec16A, GF treatment increased the number of ERES labeled with wild-type GFP-Sec16A (Fig.14A). As a control, we tested whether the response requires Sec16A phosphorylation of Thr415 and found this to be the case (Fig.14A), as ERES labelled with the phosphoablating GFP-Sec16A-T415I mutant did not increase in number as response to FBS treatment. ERES number also did not increase after mitogen stimulation in cells with GFP-Sec16-Maddin (Fig.14A). Hence, an interaction with COPII (or at least the Sec16-Sec23 interaction) seems to be relevant for the increase in the number of ERES upon mitogen treatment.

Finally, we tested the role of COPII using an in vitro recruitment assay (Fig.14B). Semi-intact cells were extensively washed to strip off the majority of Sec16A and COPII from their endomembranes, followed by incubation with fresh cytosol which led to recruitment of COPII and Sec16A and formation of new ERES (Fig.14B+C). We compared control cells, which were untreated, with cells pre-treated with cycloheximide. Reducing the load of secretory cargo leads to significantly less de novo formation of Sec16A- and COPII-positive ERES (Fig.14C-E). Altogether, this data underscores the necessity for Sec16A mobilization for ERES generation, and an active role of COPII, which cooperates with Sec16A in the formation of new ERES after mitogen treatment.

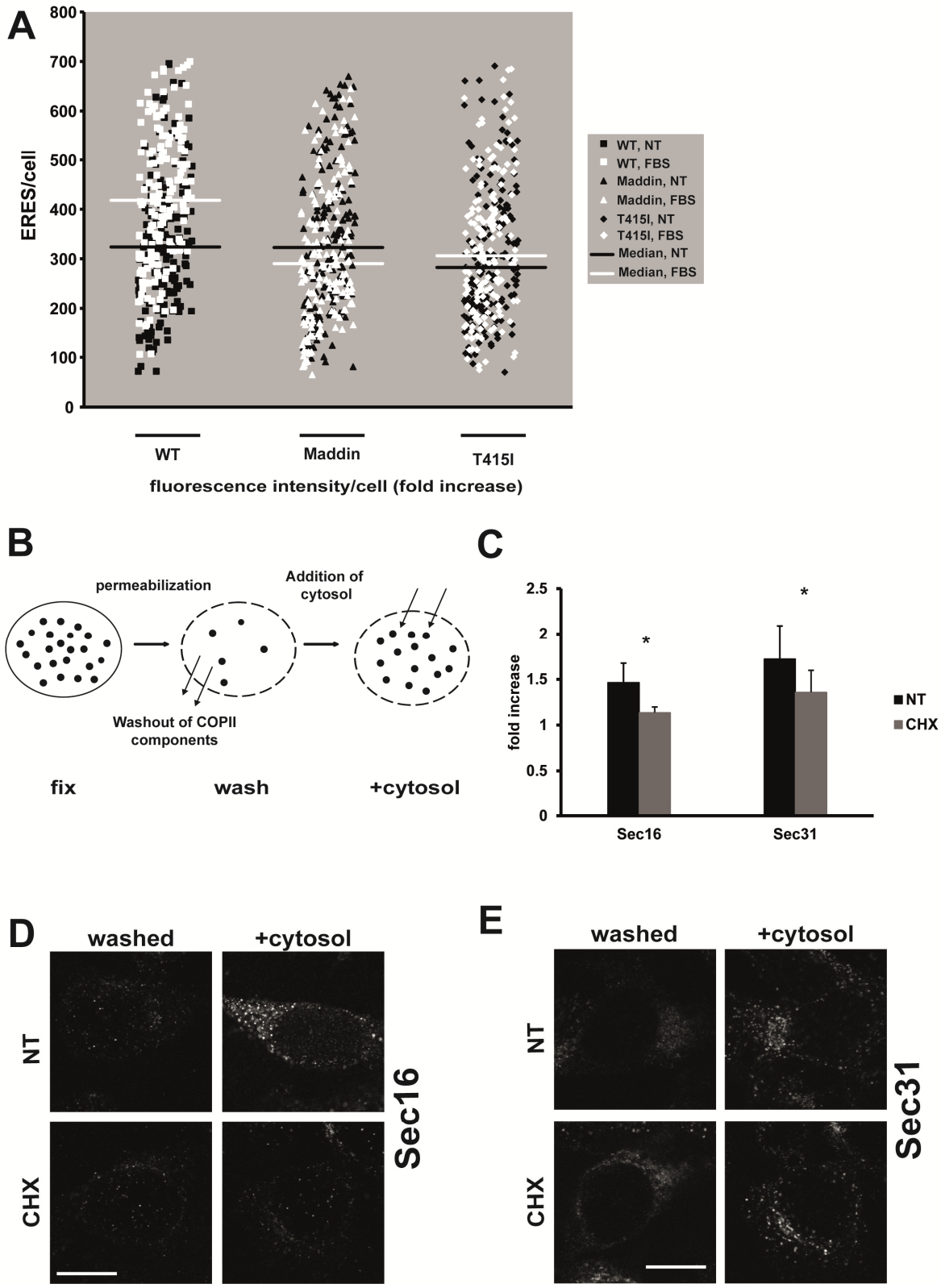
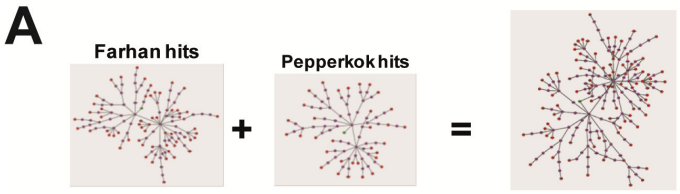


Figure 14: *Role of COPII and Sec16A in biogenesis of ERES.* **A,** HeLa cells on glass cover slips were transfected with plasmid encoding wild type GFP-Sec16 (WT), or GFP-Sec16-Maddin (Maddin), or GFP-Sec16-T415I (T415I). After 24 h, cells were either fixed directly (NT) or treated with 10% FBS for 15 min (+FBS) followed by fixation. The number of Sec16-positive ERES was counted using ImageJ. Lines indicate the median number of ERES/cell from non-treated cells (black lines) or FBS-treated cells (white

lines). **B,** Schematic illustrating the different steps in the recruitment assay. **C-E,** HeLa cells were plated on glass cover slips. After 24 h, cells were either not treated or treated with cycloheximide (CHX) for two hours prior to the experiment. Cells were permeabilized and washed extensively to deplete COPII and Sec16. These cells were either fixed directly after washing (washed) or treated with cytosol harvested from HeLa cells (+cytosol) for 30 min at room temperature followed by fixation. Sec16 was detected using immunofluorescence. The number of Sec16-positive ERES was counted using ImageJ and the bar graph in the right panel shows the relative increase in Sec16-positive ERES in the condition "+cytosol" normalized to the condition "washed". Values are means ± SD from four (Sec16) or three (Sec31) independent experiments. Asterisks indicate statistically significant differences (*, $P < 0.05$) determined by paired Student's t-test.

8 Cell proliferation is dependent on Sec16A

Our results so far clearly showed that Sec16A integrates mitogenic signaling into the secretory pathway on the level of ERES. Yet it is so far unclear whether Sec16A is relevant for the biologic outcome of mitogenic signaling, such a proliferation. In previous work, 64 kinases and phosphatases were identified that, when depleted, reduce the number of ERES [1]. More recently, others described 47 proteins that regulate ERES number [18]. We hypothesized that, if ER export is linked to the regulation of cell proliferation, then a network linking these hits (111) to Sec16A ought to be enriched in cellular processes related to cell proliferation. We therefore constructed an anchored network of these 111 hits with Sec16A as an anchor, using a previously described algorithm [19]. This anchored network was analyzed for enrichment of biological processes (GO term annotation). Indeed, this approach revealed several proliferation related processes that were enriched at a p-value of smaller than 0.001 (Fig.15A). Therefore, our computational analysis suggests a link between ER export and cell proliferation. To test this experimentally, we performed a Sec16A knockdown and measured the increase in cell number for two days. We found that depletion of Sec16A inhibited cell proliferation (Fig.15B), and this effect was not attributed to an increase apoptosis as assessed by determining caspase-3 cleavage (Fig.15C).



List of enriched biological processes related to cell proliferation

GO-ID	Description	corr p-val	Genes
42127	regulation of cell proliferation	3.90E-09	PRKCZ CAV1 CLU NFKBIA FOXO1 TNFRSF5 RPS6KB1 PTEN CTNNB1 CUL2 KRAS GAB2 CTGF PRKCA EGFR AR VHL SMAD4 TP53 RAF1 RB1 KDR CAPN1 MAPK1 SSTR2 NOTCH1 HIF1A HDAC1 NUP62 NME1 JUN VEGFA PDGFRA MDM2 HGS NGFR SST
8284	positive regulation of cell proliferation	3.70E-06	PRKCA EGFR PRKCZ CLU TNFRSF5 RPS6KB1 CTNNB1 KDR CAPN1 MAPK1 NOTCH1 HIF1A KRAS HDAC1 GAB2 CTGF NME1 JUN VEGFA PDGFRA MDM2 NGFR
8285	negative regulation of cell proliferation	1.39E-04	PRKCA AR CAV1 VHL SMAD4 TP53 RAF1 RB1 PTEN CTNNB1 CUL2 SSTR2 NUP62 NME1 JUN HGS SST
50678	regulation of epithelial cell proliferation	1.80E-04	EGFR AR NOTCH1 NME1 VEGFA PTEN KDR CTNNB1
82	G1/S transition of mitotic cell cycle	7.33E-05	EGFR CUL2 PIM1 RPS6KB1 RB1 RHOU CDC25A
45786	negative regulation of cell cycle	9.30E-04	KHDRBS1 EGFR CUL2 HDAC1 MAPK12 TP53 DGKZ NGFR RB1 CDK5
51726	regulation of cell cycle	1.37E-07	KHDRBS1 EGFR ROCK2 PIM1 TP53 RPS6KB1 RB1 CDK7 CDK5 PTEN CDC25A SIRT2 CSNK2A2 CUL2 FBXW7 APP HDAC1 MAPK12 CTGF JUN BUB1B MDM2 DGKZ NGFR

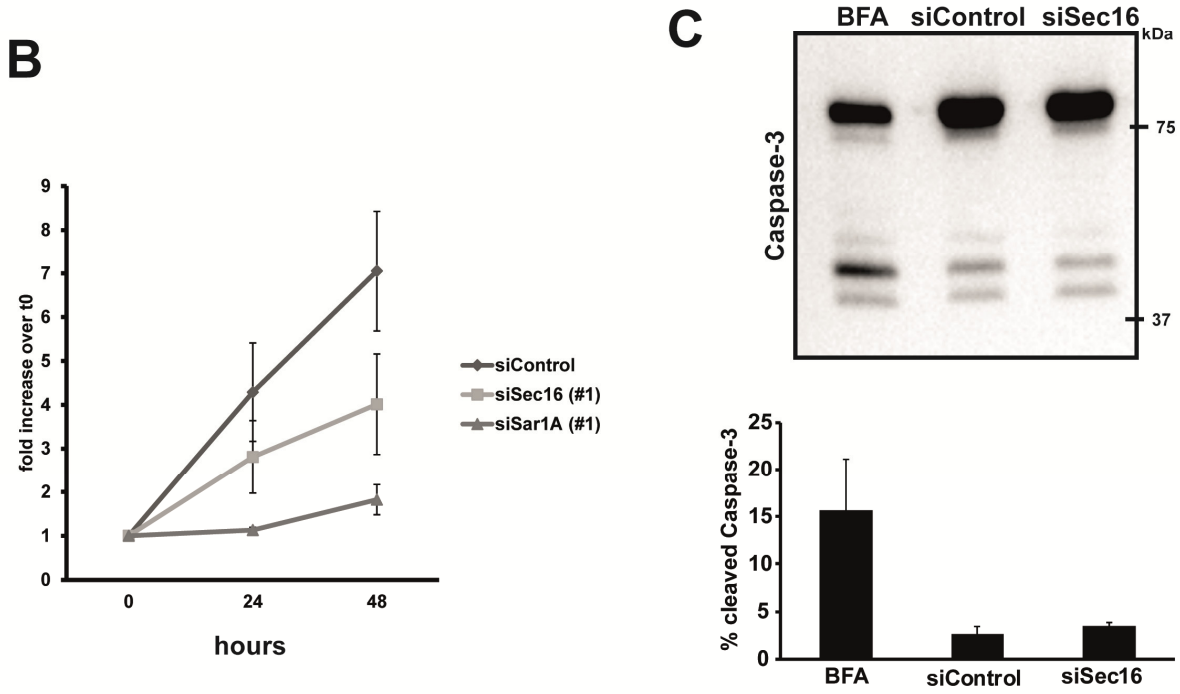


Figure 15: *Sec16A represents a link between the secretory pathway and cell proliferation.* **A,** Upper panel: Hits from previous screens (Farhan et al.,2010; Simpson et al.,2012) (Farhan hits and Pepperkok hits) were also anchored to Sec16 and the networks were merged (right network). Lower panel: All nodes from the merged network were analyzed for enrichment of cellular processes (GO-term annotations) using the BiNGO plugin in Cytoscape. Cellular processes that are significantly enriched and that are linked to cell growth and proliferation are displayed. **B,** HeLa cells were transfected with control siRNA (siControl) or siRNA to Sec16 (siSec16) or Sar1A (siSar1A). After 24 h or 48 h, cells were detached and counted using a counting chamber. Results are presented as fold increase of number of cells plated at time point 0 (t0). **C,** HeLa cells were transfected with control siRNA (siControl) or siRNA to Sec16 (siSec16), or treated with 5 µg/ml BFA overnight. Lysates were immunoblotted against Caspase-3. Upper panel shows a representative experiment and the lower panel an evaluation of three independent experiments depicting the percentage of cleaved versus total Caspase-3.

The Ras-ERK1/2 pathway is a well known regulator of proliferation and it is also known to induce Egr family members, which we experimentally verify (Fig.16A). In line with our finding that Egr members control Sec16A expression, Ras overexpression also induced a 3 fold increase in Sec16A levels (Fig.16A). We tested whether Sec16A is involved in the Egr-dependent control of proliferation. Silencing Egr1+3 resulted in an inhibition of cell proliferation, which was overcome by overexpressing Sec16A (Fig.16C) that is driven by a CMV promoter (and is therefore not Egr-dependent). Overexpressing Sec16A significantly induced proliferation (Fig.16B and C), indicating that Sec16A is necessary and sufficient for proliferation. We also overexpressed the Sec16A-T415I mutant as well as Sec16-Maddin and determined the effect of Sec16A phosphorylation and COPII-binding ability on proliferation. HeLa cells overexpressing these mutants proliferated stronger than GFP-expressing cells, but significantly less than cells expressing wild type Sec16A. Sec16A forms oligomers and Sec16A mutants are expected to oligomerize with endogenous wild type Sec16A. We propose that this is the reason why these mutants themselves induce proliferation, although significantly less than overexpressing wild type Sec16A. We could not test the overexpression of Sec16A variants in cells depleted of endogenous Sec16A because Sec16A silenced cells did not tolerate the overexpression of any plasmid. In general, an inhibition of ER export is expected to inhibit proliferation. To experimentally verify this assumption, we inhibited ER export by depletion of Sar1A and found that it reduces proliferation (Fig.16C). Next, we tested whether the previously observed effect of Sec16A overexpression on proliferation is due to a general increase in ER export. We therefore overexpressed wild type Sar1A. However, overexpression of Sar1A had no detectable effect on proliferation (Fig.16C), indicating that Sec16A is required and sufficient for cells to proliferate.

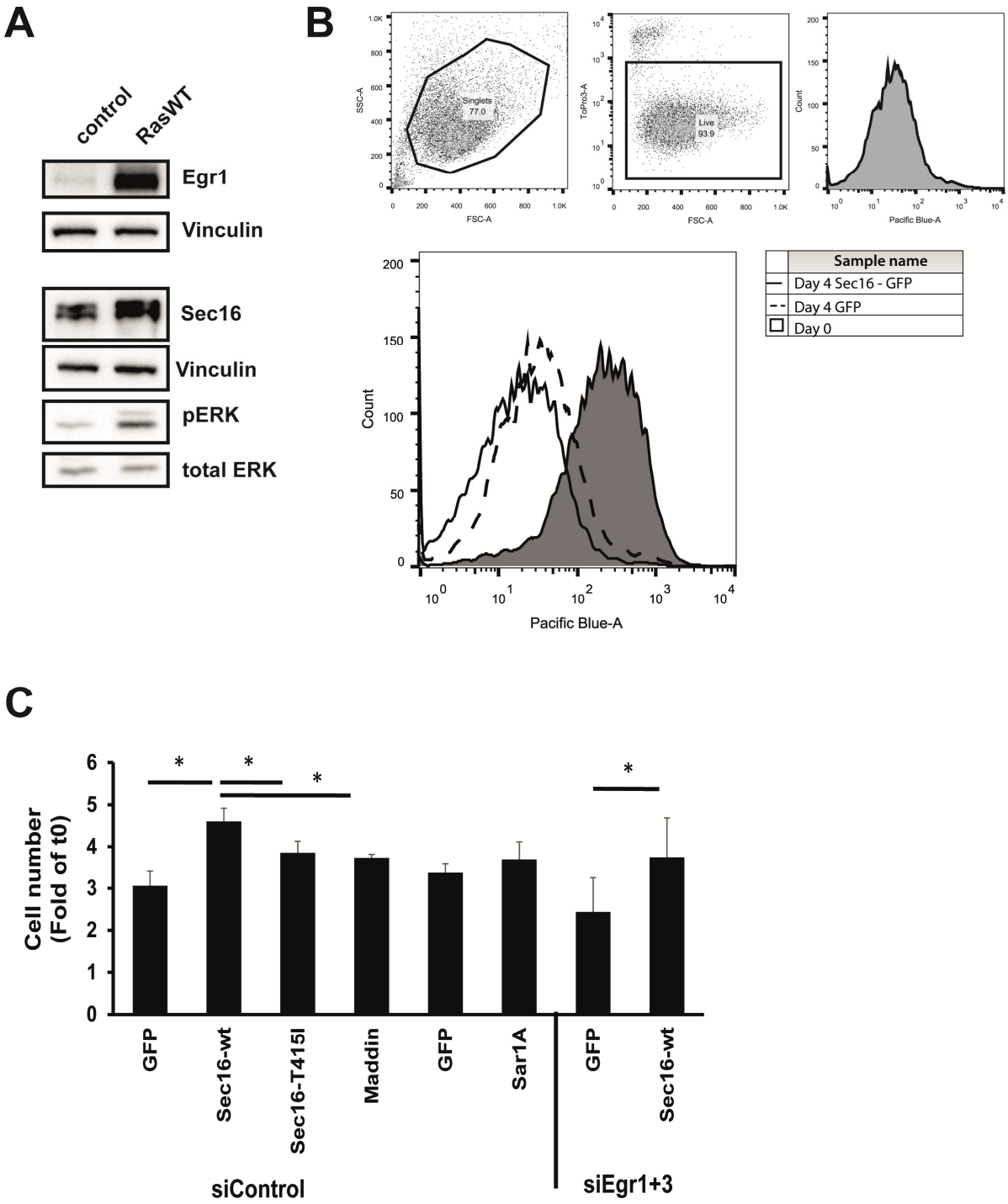


Figure 16: *Sec16A activity is required for cell proliferation.* **A,** HeLa cells were transfected with empty vector (control) or wild type Ras (Ras-WT). 24 hours after transfection, cells were lysed and immunoblotted against indicated proteins. Panel shows representative blot of three independent experiments. **B,** HeLa cells were plated and on the next day, transfected with plasmid encoding GFP, or GFP-tagged Sec16A. After 24 h, cells were loaded with fluorescent dye. The upper row shows the gating strategy to only measure fluorescence in vital cells. The lower row shows FACS curves of cells directly

after loading with dye (grey curve), or 48 h after dye loading from GFP-expressing cells (dotted line) or from cells overexpressing GFP-Sec16A (solid line). Note that cells overexpressing GFP-Sec16A shift strongest to the left, indicating a higher degree of dilution of dye and thus of proliferation. **C,** HeLa cells were transfected with control siRNA (siControl) or siRNA to or Egr1+3 (siEgr1+3). After 24 h, cells were transfected with plasmid encoding GFP (GFP), wild type GFP-Sec16 (Sec16WT), Sec16-Maddin (Maddin), the phosphorylation-deficient Sec16-T415I and Sar1A. 48 hours after transfection, cells were detached and counted using a counting chamber. Results are presented as fold increase of number of cells plated at time point 0 (t0). Asterisks in this figure indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test.

9 Summary

Altogether, the notion that ER export and cell proliferation are linked is supported by our findings and we propose Sec16A as the molecular integrator of these two processes. As summarized in our working model (Fig.17), we have shown that Sec16A is regulated by mitogenic signaling in two ways, first via regulation of Sec16A phosphorylation and secondly via control of Sec16A protein levels via the Egr transcription factor family. Consistent with its role as a signal integrator, Sec16A reversibly influences ERES and ER export in response to GF signaling status.

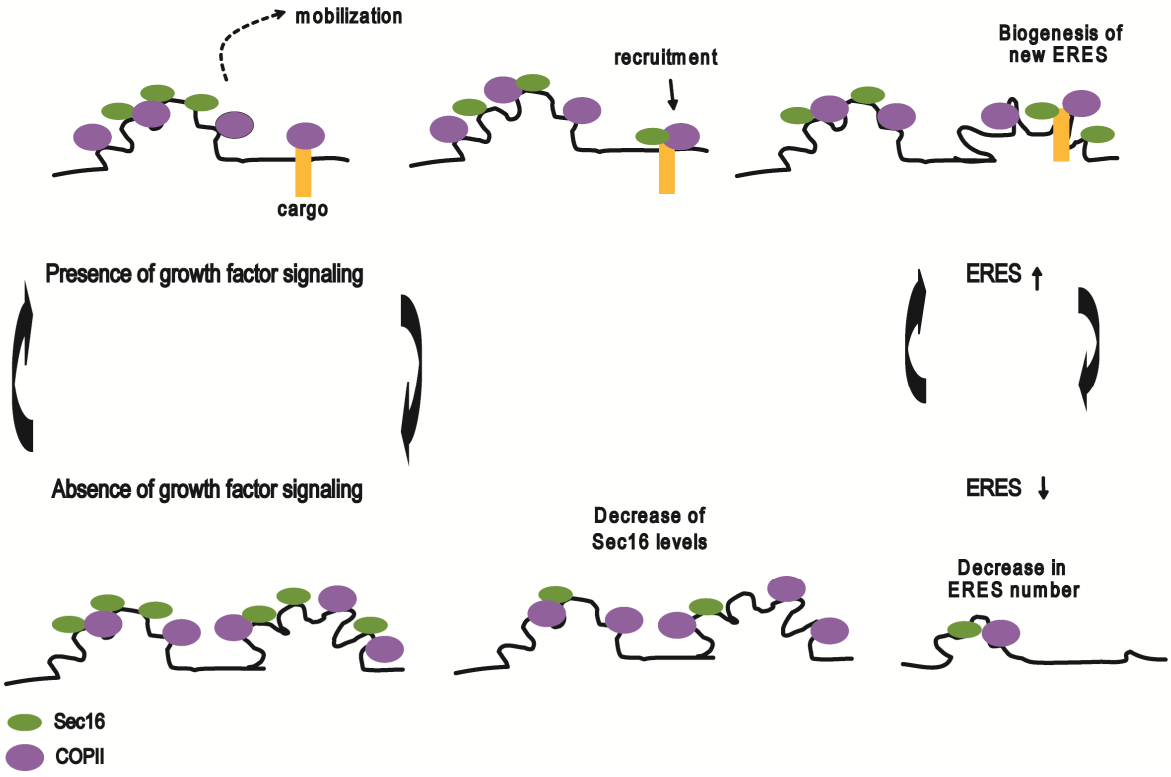


Figure 17: *Working model illustrating Sec16A-mediated ERES regulation in presence and absence of growth factor signaling*

10 References

1. Farhan H, Wendeler MW, Mitrovic S, Fava E, Silberberg Y, Sharan R*, et al.* MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *J Cell Biol* 2010, **189**(6)**:** 997-1011.
2. Nonhoff U, Ralser M, Welzel F, Piccini I, Balzereit D, Yaspo ML*, et al.* Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. *Mol Biol Cell* 2007, **18**(4)**:** 1385-1396.
3. Zacharogianni M, Gomez AA, Veenendaal T, Smout J, Rabouille C. A stress assembly that confers cell viability by preserving ERES components during amino-acid starvation. *eLife* 2014, **3**.
4. Elkon R, Linhart C, Sharan R, Shamir R, Shiloh Y. Genome-wide in silico identification of transcriptional regulators controlling the cell cycle in human cells. *Genome Res* 2003, **13**(5)**:** 773-780.
5. Boncompain G, Divoux S, Gareil N, de Forges H, Lescure A, Latreche L*, et al.* Synchronization of secretory protein traffic in populations of cells. *Nature methods* 2012, **9**(5)**:** 493-498.
6. Farhan H, Weiss M, Tani K, Kaufman RJ, Hauri HP. Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load. *EMBO J* 2008, **27**(15)**:** 2043-2054.
7. Guo Y, Linstedt AD. COPII-Golgi protein interactions regulate COPII coat assembly and Golgi size. *J Cell Biol* 2006, **174**(1)**:** 53-63.
8. Reiterer V, Nyfeler B, Hauri HP. Role of the lectin VIP36 in post-ER quality control of human alpha1-antitrypsin. *Traffic* 2010, **11**(8)**:** 1044-1055.
9. Nyfeler B, Reiterer V, Wendeler MW, Stefan E, Zhang B, Michnick SW*, et al.* Identification of ERGIC-53 as an intracellular transport receptor of alpha1-antitrypsin. *J Cell Biol* 2008, **180**(4)**:** 705-712.
10. Lim WA, Lee CM, Tang C. Design principles of regulatory networks: searching for the molecular algorithms of the cell. *Mol Cell* 2013, **49**(2)**:** 202-212.
11. Shen-Orr SS, Milo R, Mangan S, Alon U. Network motifs in the transcriptional regulation network of Escherichia coli. *Nat Genet* 2002, **31**(1)**:** 64-68.
12. Heinzer S, Worz S, Kalla C, Rohr K, Weiss M. A model for the self-organization of exit sites in the endoplasmic reticulum. *J Cell Sci* 2008, **121**(Pt 1)**:** 55-64.
13. Gedde UW. *Polymer Physics*. Kluwer Academic Publishers: Dordrecht, 1995.
14. Forster R, Weiss M, Zimmermann T, Reynaud EG, Verissimo F, Stephens DJ*, et al.* Secretory cargo regulates the turnover of COPII subunits at single ER exit sites. *Curr Biol* 2006, **16**(2)**:** 173-179.
15. Bhattacharya D, Glick BS. Two Mammalian Sec16 Homologues Have Nonredundant Functions in Endoplasmic Reticulum (ER) Export and Transitional ER Organization. *Mol Biol Cell* 2007, **18:** 839-849.
16. Montegna EA, Bhave M, Liu Y, Bhattacharyya D, Glick BS. Sec12 binds to Sec16 at transitional ER sites. *PLoS One* 2012, **7**(2)**:** e31156.
17. Shaywitz DA, Espenshade PJ, Gimeno RE, Kaiser CA. COPII subunit interactions in the assembly of the vesicle coat. *J Biol Chem* 1997, **272**(41)**:** 25413-25416.
18. Simpson JC, Joggerst B, Laketa V, Verissimo F, Cetin C, Erfle H*, et al.* Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway. *Nat Cell Biol* 2012, **14**(7)**:** 764-774.
19. Yosef N, Zalckvar E, Rubinstein AD, Homilius M, Atias N, Vardi L*, et al.* ANAT: a tool for constructing and analyzing functional protein networks. *Science signaling* 2011, **4**(196)**:** pl1.

Characterization of the role of TECPR2 in the early secretory pathway

In collaboration for a project of the group of Christian Behrends (University of Frankfurt, Germany), I performed a variety of experiments to characterize the effect of TECPR2-depletion on the early secretory pathway. These results are part of a manuscript that has been submitted for publication and I will explain my contribution towards this work below.

1 Background information

The protein tectonin beta-propeller repeat containing 2 (TECPR2) was identified in a proteomic analysis of the autophagy network to have a role in autophagy [1]. The protein is mostly uncharacterized, but due to its large size of more than 1400 amino acid residues it was suggested to function as a molecular linker. TECPR2 contains three WD (tryptophan-aspartic acid dipeptide) domains in its N-terminal region and six TECPR domains in the C-terminal region; both of these domains have been shown to be required for protein-protein interaction [2]. Additionally, TECPR2 was found to be mutated in an autosomal-recessive form of hereditary spastic paraparesis, where a frameshift mutation leads to a shortened protein product which is rapidly degraded. Patients suffer from a variety of severe symptoms, such as developmental abnormalities like a short stature and intellectual disability, but also axonal degeneration of the corticospinal or pyramidal motor and sensory tracks, which causes progressive spasticity, hyperreflexia and hypotonia. Axonal degeneration also causes the severe intellectual disability, gastroesophageal-reflux disease and central-apnea due to loss of muscle control [2, 3]. As mentioned above, TECPR2 levels are decreased in these patients, as well as protein levels of the lipidated form of the autophagy regulator LC3B. These findings support a role for TECPR2 in autophagy, and for autophagy in HSP. However, the mechanism how TECPR2 regulates LC3B is unknown. Microtubule-associated light chain 3 (LC3) is the mammalian homologue of yeast Atg8 and fulfills a similar function in autophagosome formation. LC3 has three isoforms (LC3A, B, and C) that are expressed differentially in rat tissue. Some isoform-specific functions have been reported so far, but in general, the LC3 isoforms are believed to act redundantly[4, 5]. LC3 is a cytosolic protein (LC3-I), but becomes lipidated

with phosphatidylethanolamine (PE) in response to autophagic signaling via an ubiquitin-like conjugation system. The lipidated form of LC3 (LC3-II) associated to the membrane of the phagophore and is required for autophagosome formation [6]. The initiation point of the phagophore as well as its membrane source is still unknown, but different components of the secretory pathway, especially ERES, have been shown to be involved (see Introduction) [7].

Our collaboration partners were interested in further characterizing the role of TECPR2 in autophagy and performed an interactome analysis of TECPR2, which revealed that TECPR2 is present in three networks, the HOPS complex, the BLOC-1 complex, as well as Sec24D. We were interested in the role of TECPR2 in the early secretory pathway, due to its interaction with the COPII-component Sec24D, and the role ERES might play in autophagosome formation.

2 Results

Since TECPR2 was shown to interact with Sec24D, which is a COPII component at ERES, we first tested whether TECPR2 depletion has an effect on ERES number. We used two different ERES markers, Sec31 and Sec16, and found that loss of TECPR2 dramatically decreases ERES number by more than 35% (Fig.18A+B). Additionally, we tested the effect of TECPR2 depletion on the ERGIC and found that the number of ERGIC53-positive dots was significantly decreased (Fig.18C). We next tested whether loss of TECPR2 decreases the protein levels of certain COPII components, which might explain the decrease in ERES number. However, TECPR2 depletion did not decrease protein levels of Sec13, Sec31 or Sec16 (Fig.18D). Therefore, the effect of TECPR2 on ERES must be due to its interaction with Sec24D. However, we found that TECPR2 does not colocalize with the ERES marker Sec31. In fact, the localization of both proteins is best described as mutually exclusive (Fig.18E).

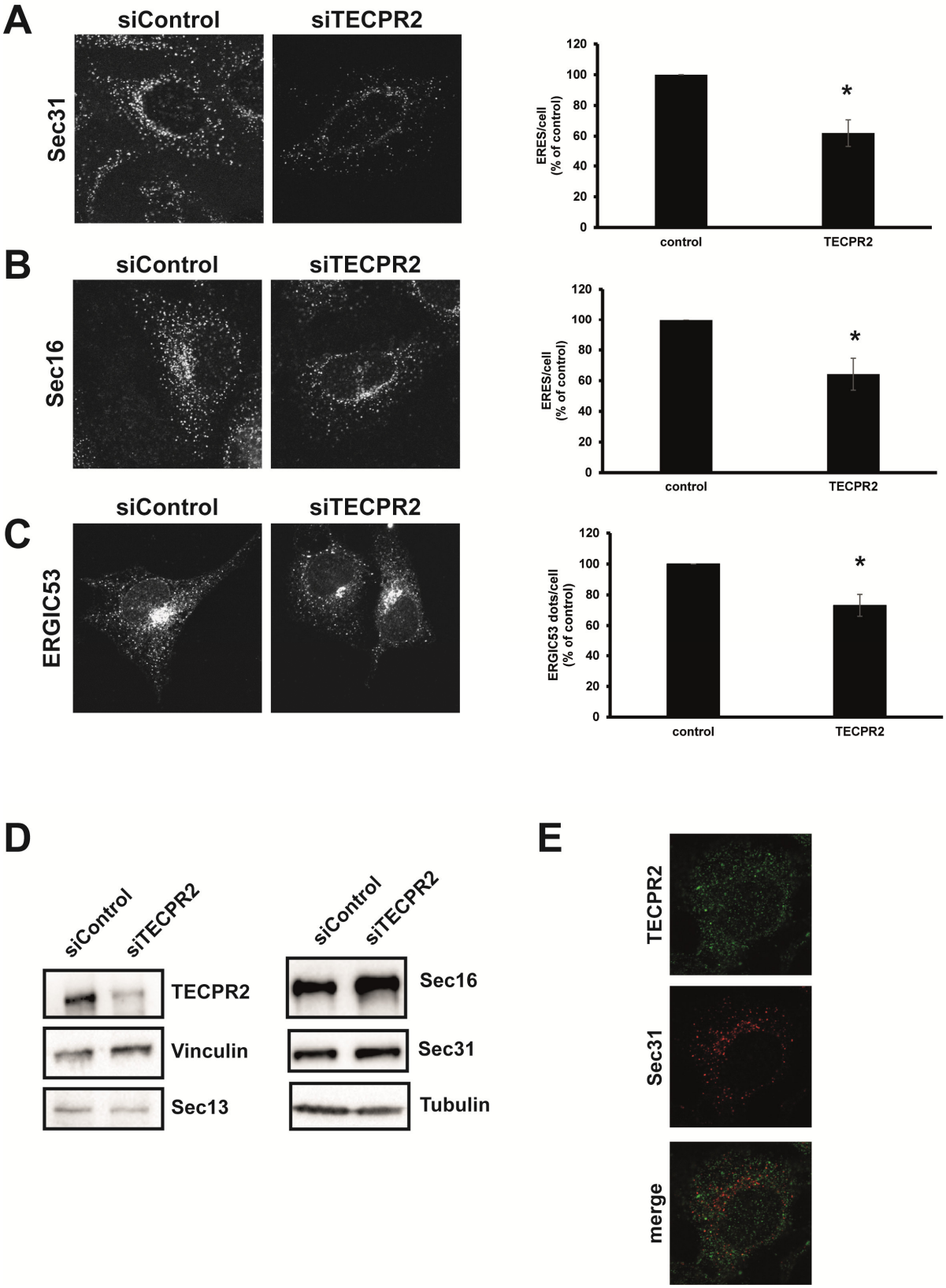


Figure 18: *Effect TECPR2 depletion on ERES and COPII components.* **A-C,** HeLa cells were transfected with control siRNA (siControl) or siRNA to TECPR2 (siTECPR2). After 96 h, cells were fixed and immunostained for the indicated proteins, followed by confocal microscopy and ERES quantification. Left panels show representative images from three independent experiments. Right panels show graphic representation of the number of ERES per cell presented as percent of control. This value amounts to

238.94 ± 48.5 ERES in the case of Sec31, to 204.69 ± 68.1 ERES in the case of Sec16, and to 149.6 ± 30.3 ERES in the case of ERGIC53. Results are means ± SD from three independent experiments with more than 50 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by paired two-tailed Student's t-test. Scale bar: 10 µm. **D,** HeLa cells were transfected with non-targeting siRNA (siControl) or with siRNA against TECPR2 (siTECPR2). After 96 h, cells were lysed and subjected to SDS-PAGE followed by immunoblotting against the indicated proteins. Panels show representative blots from three independent experiments. **E,** HeLa cells were grown on coverslips, followed by fixation, immunostaining against the indicated proteins, and imaging by confocal microscopy.

As TECPR2 interacts with Sec24D, we next tested the effect of TECPR2 depletion on Sec24D dynamics using FRAP assays (Fig.19A). We found that loss of TECPR2 significantly decreases the mobile fraction as well as the half time of recovery of YFP-Sec24D at ERES, indicating that TECPR2 has a role in ERES association of Sec24D. TECPR2-dependent recruitment of Sec24D to ERES might explain the decrease in ERES number upon TECPR2 depletion, as Sec24 is an essential structural component of COPII vesicles and ERES.

A decrease in ERES number should have an effect on ER export. Therefore, we investigated ER-to-Golgi trafficking using the RUSH assay. Since Sec24D interacts with TECPR2, and Sec24D is required especially for the transport of glycosylphosphatidylinositol (GPI)-anchored proteins [8, 9], we used the GFP-GPI-RUSH construct in the assay [10]. As expected, loss of TECPR2 strongly inhibited ER-to-Golgi trafficking of GFP-GPI-RUSH (Fig.19B).

HSP-patients show developmental abnormalities such as short stature, which are often found to be caused by dysfunctional Collagen secretion, as Collagen I is the main extracellular matrix component involved in bone formation [11]. Additionally, Sec24D has been shown to be required for the secretion of extracellular matrix proteins in zebrafish [12, 13]. Given the impaired growth of patients harboring a TECPR2 mutation, as well as the impaired Sec24D recruitment to ERES in TECPR2 depleted cells, we hypothesized that TECPR2 may influence Collagen secretion. We therefore tested the effect of TECPR2 depletion on secretion of the GFP-CollagenX-RUSH, and found that ER-to-Golgi trafficking of Collagen is significantly decreased (Fig.19C). Interestingly, in a transcriptome analysis in TECPR2 depleted cells, our collaborators found the expression of the Collagen IV group to be decreased, which is found in basement membranes [14]. Downregulation of large cargos such as certain Collagen subtypes may be an adaptive response of cells to handle high ER stress levels due to impaired ER export.

Taken together, these findings show that TECPR2 is involved in the organization of ERES by recruiting Sec24D to ERES, and that loss of TECPR2 inhibits ER export of small and large cargos. In addition to Sec24D, TECPR2 was found to bind to components of two protein complexes (HOPS and BLOC-1) as well as the autophagic machinery, we tested whether the role of TECPR2 in those complexes is independent of its role in ERES function. We depleted proteins essential for the function of these complexes and determined ERES number (Fig.19D). We did not observe a decrease in

ERES number, indicating that TECPR2 function in these complexes is independent from its function in ERES regulation.

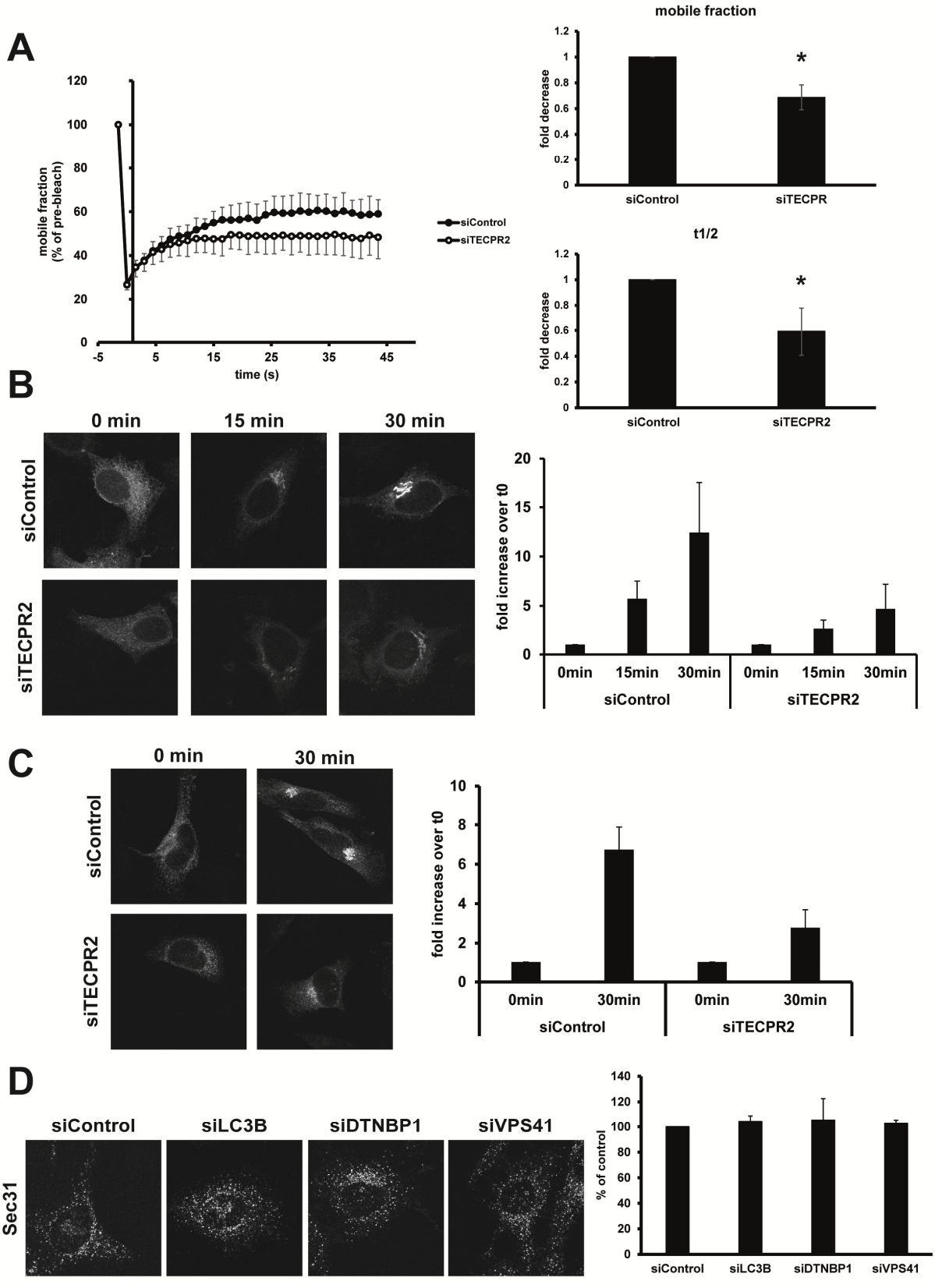


Figure 19: *Effect of TECPR2 depletion on ERES dynamics and ER-to-Golgi trafficking.* **A,** HeLa cells were transfected with control siRNA (siControl) or siRNA to TECPR2 (siTECPR2) for 96 h. 24 h before the start of the experiment, cells were transfected with plasmid encoding YFP-Sec24D. FRAP curves are shown as mean of three independent experiments where at least 5 curves were recorded in each experiment. Fluorescence values were normalized to the pre-bleach intensity. Left panel shows averaged FRAP curves from three independent experiments. Right top panel shows bar graph depicting mobile fraction, right bottom panel shows bar graph depicting half time of recovery. **B,** HeLa cells were transfected with control siRNA (siControl) or siRNA to TECPR2 (siTECPR2) for 96 h. 24 h before the start of the experiment, cells were transfected with plasmid encoding GFP-tagged RUSH-GPi. GFP-GPi was released from the ER by adding Biotin, and cells were fixed at the indicated subsequent time points. Left panel shows representative images from three independent experiments. Right panel shows a bar graph of fluorescence intensity at Golgi area, normalized to ER fluorescence, presented as fold increase over t0. This value amounts to 5.00 ± 3.0 AU in control cells and 10.36 ± 12.1 AU in siTECPR2 cells. Results are means ± SD from three independent experiments with at least 30 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **C,** HeLa cells were transfected with control siRNA (siControl) or siRNA to TECPR2 (siTECPR2) for 96 h. 24 h before the start of the experiment, cells were transfected with plasmid encoding GFP-tagged RUSH-Collagen. GFP-Collagen was released from the ER by adding Biotin, and cells were fixed at the indicated subsequent time points. Left panel shows representative images from three independent experiments. Right panel shows a bar graph of fluorescence intensity at Golgi area, normalized to ER fluorescence, presented as fold increase over t0. This value amounts to 5.67 ± 1.5 AU in control cells and 8.52 ± 3.3 AU in siTECPR2 cells. Results are means ± SD from three independent experiments with at least 30 cells per experiment. Asterisks indicate statistically significant differences (*, $P < 0.05$) as determined by ANOVA with Tukey's post hoc test. **D,** HeLa cells were transfected with control siRNA (siControl) or siRNA to LC3B (siLC3B), to DNTBP1 (siDNTBP1), or VPS41 (siVPS41). After 96 h, cells were fixed, followed by Sec31 immunostaining, confocal microscopy, and ERES quantification. Left panel shows representative images of three independent experiments. Right panel shows a graphic representation of the number of ERES per cell presented as percent of control. This value amounts to 226.71 ± 16.5 ERES. Results are means ± SD from three independent experiments with more than 50 cells per experiment.

3 Summary

To summarize these results, we showed that TECPR2 depletion decreases ERES number as well as the number of ERGIC dots, although TECPR2 itself does not localize to ERES. However, TECPR2 is required for Sec24D recruitment to ERES, as revealed by FRAP assays. In line with these findings, trafficking of Sec24D-specific cargos was found to be massively inhibited by TECPR2 depletion.

4 References

1. Behrends C, Sowa ME, Gygi SP, Harper JW. Network organization of the human autophagy system. *Nature* 2010, **466**(7302)**:** 68-76.
2. Oz-Levi D, Ben-Zeev B, Ruzzo EK, Hitomi Y, Gelman A, Pelak K*, et al.* Mutation in TECPR2 reveals a role for autophagy in hereditary spastic paraparesis. *American journal of human genetics* 2012, **91**(6)**:** 1065-1072.
3. Oz-Levi D, Gelman A, Elazar Z, Lancet D. TECPR2: a new autophagy link for neurodegeneration. *Autophagy* 2013, **9**(5)**:** 801-802.
4. Wild P, McEwan DG, Dikic I. The LC3 interactome at a glance. *J Cell Sci* 2014, **127**(Pt 1)**:** 3-9.
5. Wu J, Dang Y, Su W, Liu C, Ma H, Shan Y*, et al.* Molecular cloning and characterization of rat LC3A and LC3B--two novel markers of autophagosome. *Biochem Biophys Res Commun* 2006, **339**(1)**:** 437-442.
6. Rubinsztein DC, Shpilka T, Elazar Z. Mechanisms of autophagosome biogenesis. *Curr Biol* 2012, **22**(1)**:** R29-34.
7. Sanchez-Wandelmer J, Ktistakis NT, Reggiori F. ERES: sites for autophagosome biogenesis and maturation? *J Cell Sci* 2015, **128**(2)**:** 185-192.
8. Bonnon C, Wendeler MW, Paccaud JP, Hauri HP. Selective export of human GPI-anchored proteins from the endoplasmic reticulum. *J Cell Sci* 2010, **123**(Pt 10)**:** 1705-1715.
9. Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M*, et al.* Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. *J Cell Biol* 2011, **194**(1)**:** 61-75.
10. Boncompain G, Divoux S, Gareil N, de Forges H, Lescure A, Latreche L*, et al.* Synchronization of secretory protein traffic in populations of cells. *Nature methods* 2012, **9**(5)**:** 493-498.
11. Alford AI, Kozloff KM, Hankenson KD. Extracellular matrix networks in bone remodeling. *The international journal of biochemistry & cell biology* 2015.
12. Sarmah S, Barrallo-Gimeno A, Melville DB, Topczewski J, Solnica-Krezel L, Knapik EW. Sec24D-dependent transport of extracellular matrix proteins is required for zebrafish skeletal morphogenesis. *PLoS One* 2010, **5**(4)**:** e10367.
13. Melville DB, Montero-Balaguer M, Levic DS, Bradley K, Smith JR, Hatzopoulos AK*, et al.* The feelgood mutation in zebrafish dysregulates COPII-dependent secretion of select extracellular matrix proteins in skeletal morphogenesis. *Dis Model Mech* 2011, **4**(6)**:** 763-776.
14. Poschl E, Schlotzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 2004, **131**(7)**:** 1619-1628.

Discussion

In recent years, the view of the secretory pathway as a homeostatic membrane system that is solely required for transport of secretory proteins has begun to change, due to numerous findings indicating that the secretory pathway is targeted by intracellular signaling cascades and is also a place of signal initiation [1]. Several large scale siRNA screens have identified numerous proteins, among them many kinases and phosphatases, which mediate the structure and function of the secretory pathway and are not classical core components of the secretory pathway machinery. However, a direct stepwise connection between an extracellular stimulus, a signal cascade, a target protein within the secretory pathway and a change in structure or function of the secretory pathway has not been described. Our results are the first to do so, as we show that mitogenic signaling controls Sec16A phosphorylation as well as protein levels, which in turn mediates ERES number and ER export. We further showed that Sec16A and functional ER export were required for the outcome of mitogenic signaling, which is cell proliferation. Below, these findings will be discussed in context of the current literature.

1 Sec16A as an integrator of signaling and nutritional stimuli

In previous studies, a variety of proteins were identified that influence secretion or the structure of the early secretory pathway, as discussed in detail in the introduction [2, 3, 4]. However, since most of these proteins were identified in large scale siRNA screens, the mechanism behind their influence on the secretory pathway remains unclear. In addition, in the case of kinases and phosphatases, the stimuli leading to their regulation of the secretory pathway are largely unknown. In their kinome/phosphatome screen, Farhan et al. identified an enrichment of the MAPK signaling network among their hits, which led to the identification of ERK2 as a kinase responsible for the phosphorylation of Sec16A [2]. In this thesis, we provide for the first time a mechanism of how an external stimulus (growth factor/EGF signaling) signals to a component of the secretory pathway (Sec16A) via an intracellular signaling cascade (ERK2), and how this influences the secretory pathway (increase in ERES number and size).

Another screen also identified Sec16 as a target of kinase signaling in Drosophila S2 cells [4]. This siRNA screen identified ERK7 as a regulator of Sec16 and ERES. ERK7 is an atypical kinase that is constitutively active and might be autoactivated.

Overexpression of ERK7 caused a disassembly of ERES and the dissociation of Sec16 and COPII components away from ERES. As ERK7 is a stress kinase, Zacharogianni et al. tested whether amino acid starvation, which activates stress signaling cascades, has an effect on ERES organization. Indeed, amino acid starvation caused a rapid dispersion of Sec16 and Sec23 away from ERES in an ERK7-dependent manner. Dissociated Sec16 and Sec23 were not degraded or dispersed in the cytosol but formed aggregates. These aggregates were identified in a later study as Sec bodies, which are specialized, stress granule-like bodies that reversibly form in response to amino acid starvation in Drosophila cells and contain Sec16 and COPII components [5]. ERK7-mediated disassembly of ERES was independent of mTORC1 signaling, which is surprising given that mTORC1 signaling is a key mediator of the cellular response to stress and nutrient deprivation. The response of ERES to amino acid starvation was also found in mammalian cells, where amino acid starvation led to a 20-30 % decrease in ERES number, whereas Sec body formation was not found in mammalian cells. Conversely, serum starvation leads to a decrease in ERES number in mammalian cells as mediated by ERK1/2 signaling, due to a decrease in Sec16A protein levels. In Drosophila cell however, ERK1/2 signaling is not involved in the response of Sec16 and ERES to serum starvation, as pharmacological inhibition of ERK1/2 activity did not cause Sec16 dispersal away from ERES. Furthermore, in Drosophila cells, Sec16 is not degraded but forms aggregates in response to serum starvation, as discussed above.

Despite these differences, the outcome of serum starvation is similar in both species, which is a decrease in ERES number as well as decreased protein secretion. However, these differences in regulation show that although the secretory pathway appears to be highly regulated by external stimuli, the mechanism of regulation might differ dramatically between species. Possible reasons for these differences may be that the secretory pathway has become more complex during evolution as more functions have evolved. For example, while Drosophila only has an innate immune system, mammals have evolved to have an adaptive immune system [6]. The adaptive immune system consists of specific immune cells that place a unique demand on their secretory pathway to enable antigen presentation or antibody production, which the mammalian secretory pathway must adapt to. Furthermore, lower eukaryotic organisms such as *Drosophila* or *P.pastoris* make do with few secretory units, whereas mammalian cells contain hundreds of ERES that are not directly associated with the Golgi [7, 8]. It is tempting to speculate that a more than 10-fold increase in ERES number leads to an increase in secretory capacity. However, an uncoupling of secretory units requires

additional mechanisms to direct vesicular transport. Indeed, mammalian cells contain an ERGIC which is responsible for sorting of cargo derived from the ER [9]. In addition, gene duplications have led to the presence of several isoforms of COPII components in mammalian cells, which may allow for the regulation of a broader range of processes [10]. Due to a variety of differences between the secretory pathway of different species, it is likely that although core machinery components and mechanisms are conserved, regulation and fine tuning of specific processes differ.

2 Translational control of Sec16A by Egr transcription factors mediates ER export

In addition, we show a tight transcriptional regulation of a component of the secretory pathway in response to external stimuli, as transcriptional regulation of components of the secretory pathway was only reported in the context of the Unfolded Protein Response (UPR) or upon cellular differentiation [11]. Our finding is surprising as the secretory pathway is generally seen as part of the general housekeeping machinery of the cell, and its components would therefore be expected to be long-lived. However, we found that Sec16A, as opposed to other COPII components, is a short-lived protein with a half-life of only 2-3 hours. Given the central role of Sec16A in the regulation of ERES and ER export, these findings support the role of Sec16A as an integrator of signaling.

We found that expression of Sec16A in response to growth factor signaling is controlled by two members of the Egr transcription factor family, Egr1 and Egr3. These transcription factors belong to the group of immediate early genes, which are induced rapidly in response to growth factor stimulation. Egr1 and Egr3 are involved in proliferation, and were therefore also found to play a role in cancer [12, 13, 14, 15, 16, 17]. As these transcription factors control Sec16A expression, we investigated the role of Sec16A in proliferation, which will be discussed below.

In addition to proliferation, Egr1 and Egr3 have been investigated in relation to neuronal differentiation, and mice lacking either of these transcription factors show deficits in memory formation, among other impairments [18, 19, 20]. As these transcription factors mediate Sec16A expression, it is possible that functional ER export and Sec16A play a role in differentiation. In highly specialized secretory cells, such as B

cells or β cells, the ER and Golgi are increased in size as compared to non-secretory cells, as the secretory pathway has to handle a larger secretory burden. This is associated with an upregulation of secretory pathway components via induction of ER stress [21, 22]. Recently, eight members of the bZip transcription factor family were identified to regulate this process. Interestingly, Sec16 as well as the COPII coat machinery components were found to be regulated by these transcription factors [11]. This shows that the secretory pathway adapts to novel demands in differentiated cells. Another example is the differentiation of neurons. Neuronal cells have a large surface area, and during differentiation, plasma membrane must be added by secretory organelles to allow the outgrowth of axons and dendrites. In addition, the structure of the secretory pathway in differentiated neurons is markedly different from other cells, as the ER extends hundreds of microns into dendrites. Furthermore, neurons contain Golgi outposts at dendritic branch points in addition to their normal Golgi, as well as ERES in vicinity of these Golgi outposts [23, 24, 25, 26]. In Drosophila cells, dendrite outgrowth was shown to involve the upregulation of COPII components, as the Cut homeodomain transcription factor was able to upregulate Sec31 expression. This was mediated by the transcription factor CrebA, and overexpression of CrebA in combination with overexpression of Sec31 or Sec23 further enhanced dendriting branching and growth as compared to overexpression if CrebA alone. Consequently, loss of Sar1, Sec31 and other COPII components decreased dendritic complexity, indicating that COPII is required for efficient dendrite formation and branching [27]. Although this paper did not investigate the role of Sec16A in this process, it is likely to be also involved. Another paper investigated the role of ER export in axonal development. Aridor et al. found that in early stages of axonal growth, Sar1 was concentrated at the developing axon, and that loss of Sar1 reduced axonal growth [24]. These data show that ER export is required for cellular differentiation, and that this is mediated by translational control of COPII components.

3 Novel insights into regulation of ERES biogenesis by mathematical modeling

Sec16A was previously shown to be phosphorylated by ERK2, which gave rise to the hypothesis that mitogenic signaling influences Sec16A, as ERK2 is activated in response to mitogenic signaling [2]. Indeed, EGF stimulation and Sec16A

phosphorylation status were shown to influence Sec16A mobility as shown by FRAP assays. In this thesis, it was demonstrated that Sec16A integrates growth factor signaling at the level of ERES, and that Sec16A is the central node in a coherent feed-forward loop. Therefore, Sec16A was shown to be regulated by phosphorylation on a short time scale in response to growth factor signaling. Long-term growth factor signaling was shown to increase Sec16A protein levels by induction of the immediate early gene transcription factor family Egr. These findings support a mathematical model of Sec16A dynamics in ERES biogenesis and dynamics in response to growth factor signaling status. This model predicts an increase of Sec16A mobility or turnover at ERES in response to mitogenic signaling, by phosphorylation of Sec16A. Increased mobility or an increased dissociation rate of Sec16A was proposed to be sufficient for the nucleation of more ERES. In a previous model however, the biogenesis of more and smaller ERES was predicted to not be sufficient to handle an increased secretory flux, but that an increase of ERES size was necessary [28]. In line with this prediction, Farhan et al. showed that an acute increase in cargo load led to fusion of ERES in a Sec16A-dependent manner, which resulted in larger ERES. A chronic increase in cargo load however caused an increase in ERES number [29]. The model predicted an increase in ERES number on a short time scale of 15 min, which was also observed experimentally. In this context, the increase in ERES number is at the cost of ERES size, which would not increase secretory flux. Therefore, ERES size must also increase, and an increase in Sec16A protein levels on the timescale of a few hours was observed, which was due to de novo protein synthesis. An increase in Sec16A protein levels increases the size of previously formed ERES on the timescale of a few hours, which is the same time frame as de novo protein synthesis in response to mitogenic signaling should take. Therefore, by the time the secretory burden on ERES increases in response to mitogenic stimulation, ERES have grown first in number and then in size to accommodate the increased secretory flux.

The model further predicted that complex formation of Sec16A might play a role in its membrane association. As determined by Fluorescence Correlation Spectroscopy (FCS), comparing movement of Sec16A particles on the membrane versus movement of Sec16A particles in the cytosol indicated that Sec16A particles of membranes have to have a size that is larger than Sec16A particles that are present in the cytosol. Sec16A was shown to form heteromeric complexes of unknown size in previous studies via its conserved central domain (CCD). A possible scenario would be that Sec16A is present in large complexes at ERES. Phosphorylation of Sec16A might cause a dissociation of Sec16A from the complex and therefore mediate its release, as

a monomer, into the cytosol. This mechanism would also provide an explanation to the question of how phosphorylation increases Sec16A mobility. However, this raises the question of whether the phosphorylation status of Sec16A is generally required for its localization to ERES, or how Sec16A re-binding to ERES is mediated. The data showed that a phosphoablating Sec16A mutant (T415I) still localizes to ERES, although it does no longer respond to growth factor signaling. In addition, previous studies showed that this mutant shows a decreased fluorescence recovery in FRAP experiments, but it localizes to ERES and it does recover, albeit at a decreased rate as compared to the wildtype protein [2]. Therefore, phosphorylation status alone does not mediate membrane association of Sec16A.

4 Role of Sec16A in ERES and COPII-coat dynamics

As discussed above, Sec16A mediates biogenesis of novel ERES after mitogenic stimulation, but the role of Sec16A in COPII vesicle formation is still unclear. In addition, it is unclear how and at which point during COPII vesicle formation Sec16 is recruited to ERES. Recently, two models for the role of Sec16 were discussed in a review [30]. The first model describes a role for Sec16 as a scaffold for COPII vesicle formation, whereas in the second model, Sec16 acts downstream of the assembly of the COPII coat and mediates COPII vesicle release [31].

In the first model, Sec16 localizes independently to ERES and functions as a scaffold that recruits COPII components to ERES, regulating COPII vesicle coat formation. This model is compatible with findings in Drosophila which show that Sec16 localizes to ERES independently of Sar1 [7, 32]. In addition, COPII components were shown to not be required for Sec16 localization to ERES in Drosophila [32, 33]. In contrast, in vitro experiments using human Sec16 showed that Sec16 does require Sar1 for efficient localization of neutral liposomes [31]. In P.pastoris, COPII at ERES was shown to be required for Sec16 localization to ERES [34]. Therefore, the scaffold model agrees with findings in Drosophila, but not with findings in mammalian or yeast cells.

In the second model, Sec16 localizes to ERES in a manner dependent on COPII and acts downstream of Sar1, regulating Sar1 GTPase activity and thereby COPII vesicle release. This model is consistent with Sar1- and COPII- dependent ERES localization

of Sec16 in P.pastoris. In addition, several studies showed that Sec16 modulates Sar1 GTPase activity by delaying the recruitment of Sec31. As Sec31 enhances Sar1 GTPase activity via Sec23, Sec16 prevents premature vesicle budding by keeping Sec31 away from Sec23 during early stages of vesicle formation [31, 35, 36]. In yeast, this Sec16 function was mediated by interaction with Sec24 [37].

It is likely that Sec16 fulfills both functions in vivo, although in some species, either the scaffolding or the GTPase regulating function might be the predominant mode of action, and Sec16 recruitment to ERES might be different in some species. The data suggest that in mammalian cells, Sec16 initiates *de novo* ERES biogenesis upon growth factor stimulation by dissociating from ERES and re-binding the membrane forming novel ERES. This would suggest that Sec16A defines the location of novel ERES and initiates ERES formation, which implicates a Sar1-independent recruitment of Sec16A to the membrane. However, we also found that Sec16A requires the presence of COPII at ERES for efficient ERES localization, and for its ability to generate novel ERES upon growth factor stimulation. These findings are in agreement with earlier studies which found that although the ERES localization domain (ELD) of Sec16A is required for its ERES localization, it is not sufficient. Sec16A also requires the presence of the central conserved domain (CCD), which mediates binding to Sec24 in Drosophila [30, 37]. It is therefore likely that upon mitogenic stimulation, Sec16A localizes to pre-ERES structures that may contain Sar1 and Sec24, which allows Sec16A to bind. Although it is unknown how these pre-ERES form, it is likely that they require Sec16A for stabilization, and therefore Sec16A-binding defines which pre-ERES structures further develop into ERES. This scenario is compatible with a role of Sec16A in the stabilization of the pre-budding complex which consists of Sar1 and Sec23-Sec24 [38].

In addition to findings regarding the regulation of Sec16 via signaling, Sec16A in mammalian cells is likely to have a regulatory function on COPII vesicle formation. This is in line with studies showing that cargo load at the ER influences ERES behavior [29]. Additionally, in yeast, cargo receptors were suggested to actively recruit COPII to ERES dependent upon the level of mature cargo at the ER [39, 40]. Although Sec16A requires presence of COPII at ERES, it may still act as a scaffold protein that stabilizes COPII coat vesicle formation at early stages. As suggested by structural analysis of the Sec13-Sec16 tetramer, Sec16A might interact with Sec24 and Sec12, thereby keeping Sec12 in the vicinity of Sar1, regulating Sar1 GTPase activity. In addition, Sec16, possibly bound to Sec13, would stabilize the pre-budding complex consisting of Sar1 and Sec23/Sec24. As Sec16 and Sec13 were shown to form a structure very similar to

the Sec13-Sc31 tetramer, Sec16 might form a pre-coat together with Sec13. This hypothesis is consistent with the role of Sec16 of shielding Sec23 from Sec31 during early stages of vesicle formation. The Sec16-Sec13 tetramer would then gradually be replaced by the outer coat consisting of Sec13/Sec31 [41]. Therefore, Sec16 might be present at early stages of COPII vesicle formation, whereas Sec31 is present at late stage and budded COPII vesicles, which would explain the low level of co-localization of Sec16 and Sec31 that is generally seen. However, some studies suggest that Sec16 might be incorporated into the COPII coat in mammalian, *Drosophila*, and *S.cerevisiae*, but not in *P.pastoris* [7, 30, 33, 34, 42]. Therefore, Sec16 might only be present at certain places of the COPII vesicle, for example in the vicinity of Sar1 at the vesicle bud, where it could fulfill all of its ascribed functions. However, a recent study by Klinkenberg et al. suggested that p125A spatially segregates Sec16A from COPII at late stages of vesicle formation, thereby forcing Sec16A to make room for the outer coat [43]. These findings provide an additional regulatory mechanism that mediates the transition from nascent to budding vesicle, and also indicates that Sec16A is very likely not a part of the COPII coat [43].

5 Does Sec16A favor vesicular or tubular ER export?

Although it is generally accepted that the COPII coat machinery is required for ER export, the question of whether ER export is mediated by vesicles or tubules is still much debated [44]. Since the organization of the early secretory pathway differs markedly between different species, a direct comparison of findings can be difficult. In general, ERES and the recipient of ER export, either the ERGIC or cis-Golgi, are in close proximity. In plant cells, P.pastoris and Drosophila, cells contain several Golgis that are in close association with ERES. These clusters are referred to as tER-Golgi units or secretory units [7, 45, 46]. In mammalian cells, only one Golgi exists, as well as several hundred ERES. In addition, mammalian cells contain an ERGIC, which receives COPII vesicles from ERES [9]. However, ERGIC clusters are found in close proximity to ERES, and the ERES/ERGIC interface was recently shown to be stabilized by the protein TFG via interaction with Sec16A [47, 48]. S.cerevisiae does not contain an ERGIC, and ERES are not as clearly defined as in other species [49]. Interestingly, the question of whether ER export takes place in vesicles or via tubules has not been

resolved, as current imaging techniques have not been able to provide sufficient evidence in favor of or against the existence of COPII vesicles [44]. Tubules spanning the distance between ERES and ERGIC/*cis*-Golgi have been documented more frequently in EM imaging than COPII vesicles, which argues in favor of transport mediated by tubules, not vesicles. In addition, live cell experiments have provided evidence for direct tubular contact between ER and Golgi, as laser manipulation of the Golgi showed that a moving Golgi often drags along tubules connected to the ER [50]. In addition, protein dynamics in FRAP assays of a Golgi resident protein indicated direct tubular connections to the ER [46]. Furthermore, COPII-vesicle critics claim that the average size of COPII vesicles formed in vivo has a size of approximately 70 nm, which is too small to accommodate large cargos such as pro-collagen that are 300 nm in size. However, the COPII coat has been shown to theoretically be flexible enough to accommodate differently sized and curved vesicles [51, 52]. In addition, recent studies have identified mechanisms modulated by specific proteins such as Sedlin, which might allow for pro-collagen packing into vesicles by delaying Sar1 GTP hydrolysis and vesicle scission [44, 52, 53, 54].

Several arguments are in favor of the existence of COPII vesicles. First, Zeuschner et al. found COPII vesicles in mammalian cells by immune-electron tomography after two different fixation techniques [55]. Furthermore, the COPII coat and associated machinery is similar to that of the COPI and Clathrin coat, which do form coated vesicles [56, 57, 58]. Furthermore, many budding assays have shown that in principle, COPII vesicles do bud from the membrane, and the presence of smaller-than-usual COPII vesicles in these assays is seen as a defect in vesicle formation [57, 59, 60]. Constitutively active Sar1 (Sar1-H79G) causes formation of long, rigid tubules that do not bud off the membrane, as in this mutant, GTP hydrolysis is inhibited. In cells overexpressing another Sar1 mutant that forms long but flexible tubules on membranes, export of pro-collagen, but not VSVG, was inhibited. This finding is remarkable, as especially a large cargo such as pro-collagen has been suggested to require tubules, not vesicles, for ER export [61].

Another argument in favor of vesicles is the nature of a secretory pathway that is composed of distinct membranous compartments, which allows the secretory pathway to provide different environments within different compartments. In addition to major differences in enzyme composition, the compartments differ in their pH level. Slightly different pH levels were shown to modulate binding and release of cargo proteins from cargo receptors; this was reported in most species. Direct connections between the ER and ERGIC/*cis*-Golgi via tubules could allow the flow of proteins and ions through these tubules, thereby disturbing the composition of both organelles [44, 62, 63].

In general, the function of Sec16 can be reconciled with either the vesicular or tubular model. Theoretically, since Sec16 has been suggested to stabilize the COPII coat via its scaffolding function, it could stabilize a coat surrounding vesicles of tubules. In addition, the COPII coat was shown by ultrastructural analysis to be able to surround a tubular structure, as this is required for the export of pro-collagen.

6 Role of ER export in proliferation and cancer

Sec16A integrates growth factor signaling at the level of ERES, and we showed that Sec16A also mediates the outcome of growth factor signaling, which is proliferation. In addition to Sec16A, loss of Sar1 also inhibited proliferation, indicating that proliferation requires functional ER export. The concept that cell growth requires a functional secretory machinery is intuitive, as cells must be able to grow in size to be able to proliferate [4, 64]. Expansion of the plasma membrane requires membrane material to be delivered by the secretory pathway. In addition to our work, several other studies have provided evidence for a close link between secretion and proliferation. In their kinase/phosphatase screen, Farhan et al. identified several members of the ERK1/2 MAPK signaling pathway as a network that influences the secretory pathway [2]. The same is true for a whole genome screen by Simpson et al [3]. The ERK1/2 MAPK signaling pathway is one of the key pathways that modulate cell proliferation, and misregulation of this pathway is found in most types of cancer. Given the tight regulation of the secretory pathway by the ERK1/2 MAPK signaling pathway, and the fact that the secretory pathway plays a major role in proliferation, targeting the secretory pathway is an attractive strategy in developing novel therapeutic approaches against cancer. In addition, Sec16A has been found to be upregulated by twofold in colonic cancer samples in a proteome screen comparing healthy versus cancer tissue [65]. Searching the cancer genomics portal cBioPortal (*www.cbioportal.org*) revealed that depending on the cancer subtype and study, Sec16A was altered, either amplified or mutated, in up to 8% of cases, such as in stomach adenocarcinoma, uterine carcinomas, and melanoma. Interestingly, Sec16B was found to be amplified in up to 11 % of cases in large scale studies mapping the genome in liver hepatocellular carcinoma, breast invasive carcinoma, and lung adenocarcinoma. Furthermore, members of the Egr transcription factor family, which were identified to control expression of Sec16A in response to growth factor signaling, are found to be involved

in a variety of different cancers [12, 13, 15, 16, 66, 67, 68]. At cBioPortal, Egr1 was found to be mutated in 18% of cases in a study of 90 pancreatic adenocarcinoma cases, and amplified in 16% of 415 kidney renal clear cell carcinoma cases. Interestingly, the most common alteration for Egr3 documented by the cBioProtal website is a loss of Egr3. Egr3 was found to be deleted in nearly 16% of cases in two studies investigating 258 and 333 cases of prostate adenocarcinoma, respectively. As described previously, the role of Egr3 in cancer has not been extensively studied, and Egr3 was found to be both overexpressed and decreased in different cancer types [69, 70, 71].

Generally, tumor cells show increased metabolic rate and protein synthesis, which places a large demand on the early secretory pathway. In many cancers, this increased secretory burden leads to an induction of the ER stress response and an increase in COPII vesicle budding from the ER, to increase ER export levels [29, 72, 73, 74, 75]. The ER stress response is an adaptive process that helps the cells to handle an increased cargo load at the ER in initial stages of increased protein synthesis. If the secretory burden further increases or induction of the UPR is not sufficient to resolve the secretory burden, UPR-induced apoptosis is initiated. This makes ER export and the UPR an attractive target for anti-cancer therapy, as tumors show higher ER stress levels compared to healthy cells, and a pharmacologically induced increase in ER stress might push tumor cells towards UPR-induced apoptosis. This approach and the role of the UPR in cancer has gained much interest in the last decade [73, 74, 75, 76, 77]. At this point, several compounds targeting the UPR have been described that show promising effects as anti-tumor drugs, or that might function to sensitize tumor cells to chemotherapeutic agents [78, 79]. Targeting the COPII machinery and Sec16 to inhibit ER export provides additional, cytoplasmic targets for anti-cancer therapy. Advances in ultrastructural analysis of the COPII coat and its components may make the design of small molecules possible that could disrupt the coat at critical connective sites. However, targeting COPII vesicle formation directly is likely to be very toxic, therefore a modulation of ER export would be more useful. As mentioned previously, signaling pathways that among others target the secretory machinery are overactive in cancer. For example, several studies have shown that COPII components are targeted by kinase signaling and other post-translational modifications. Targeting ER export indirectly by pharmacologically modulating hyperactive signaling pathways may therefore be a useful therapeutic strategy [2, 80].

7 References

1. Farhan H, Rabouille C. Signalling to and from the secretory pathway. *J Cell Sci* 2011, **124:** 171-180.
2. Farhan H, Wendeler MW, Mitrovic S, Fava E, Silberberg Y, Sharan R*, et al.* MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *J Cell Biol* 2010, **189**(6)**:** 997-1011.
3. Simpson JC, Joggerst B, Laketa V, Verissimo F, Cetin C, Erfle H*, et al.* Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway. *Nat Cell Biol* 2012, **14**(7)**:** 764-774.
4. Zacharogianni M, Kondylis V, Tang Y, Farhan H, Xanthakis D, Fuchs F*, et al.* ERK7 is a negative regulator of protein secretion in response to amino-acid starvation by modulating Sec16 membrane association. *The EMBO journal* 2011, **30**(18)**:** 3684-3700.
5. Zacharogianni M, Gomez AA, Veenendaal T, Smout J, Rabouille C. A stress assembly that confers cell viability by preserving ERES components during amino-acid starvation. *eLife* 2014, **3**.
6. Agaisse H. An adaptive immune response in Drosophila? *Cell host & microbe* 2007, **1**(2)**:** 91-93.
7. Ivan V, de Voer G, Xanthakis D, Spoorendonk KM, Kondylis V, Rabouille C. Drosophila Sec16 mediates the biogenesis of tER sites upstream of Sar1 through an arginine-rich motif. *Mol Biol Cell* 2008, **19**(10)**:** 4352-4365.
8. Soderholm J, Bhattacharyya D, Strongin D, Markovitz V, Connerly PL, Reineke CA*, et al.* The transitional ER localization mechanism of Pichia pastoris Sec12. *Dev Cell* 2004, **6**(5)**:** 64959.
9. Appenzeller-Herzog C, Hauri HP. The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. *J Cell Sci* 2006, **119:** 2173-2183.
10. Schlacht A, Dacks JB. Unexpected ancient paralogues and an evolutionary model for the COPII coat complex. *Genome Biol Evol* 2015.
11. Fox RM, Andrew DJ. Transcriptional regulation of secretory capacity by bZip transcription factors. *Frontiers in biology* 2015, **10**(1)**:** 28-51.
12. Gregg J, Fraizer G. Transcriptional Regulation of EGR1 by EGF and the ERK Signaling Pathway in Prostate Cancer Cells. *Genes Cancer* 2011, **2**(9)**:** 900-909.
13. Eid MA, Kumar MV, Iczkowski KA, Bostwick DG, Tindall DJ. Expression of early growth response genes in human prostate cancer. *Cancer Res* 1998, **58**(11)**:** 2461-2468.
14. Tsai JC, Liu L, Guan J, Aird WC. The Egr-1 gene is induced by epidermal growth factor in ECV304 cells and primary endothelial cells. *American journal of physiology Cell physiology* 2000, **279**(5)**:** C1414-1424.
15. Shin SY, Kim CG, Lee YH. Egr-1 regulates the transcription of the BRCA1 gene by etoposide. *BMB Rep* 2013, **46:** 92-96.
16. Pio R, Jia Z, Baron VT, Mercola D. Early growth response 3 (Egr3) is highly over-expressed in non-relapsing prostate cancer but not in relapsing prostate cancer. *PLoS One* 2013, **8**(1)**:** e54096.
17. Pagel JI, Deindl E. Early growth response 1--a transcription factor in the crossfire of signal transduction cascades. *Indian J Biochem Biophys* 2011, **48:** 226-235.
18. Li L, Yun SH, Keblesh J, Trommer BL, Xiong H, Radulovic J*, et al.* Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. *Mol Cell Neurosci* 2007, **35:** 76-88.
19. O'Donovan KJ, Tourellotte WG, Millbrandt J, Baraban JM. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci* 1999, **22:** 167-173.
20. Penke Z, Morice E, Veyrac A, Gros A, Chagneau C, LeBlanc P*, et al.* Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Philos Trans R Soc Lond B Biol Sci* 2013, **369:** 20130159.
21. Wiest DL, Burkhardt JK, Hester S, Hortsch M, Meyer DI, Argon Y. Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately. *J Cell Biol* 1990, **110**(5)**:** 1501-1511.
22. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, Zhao H*, et al.* XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* 2004, **21**(1)**:** 81-93.

23. Aridor M, Guzik AK, Bielli A, Fish KN. Endoplasmic reticulum export site formation and function in dendrites. *J Neurosci* 2004, **24**(15)**:** 3770-3776.
24. Aridor M, Fish KN. Selective targeting of ER exit sites supports axon development. *Traffic* 2009, **10**(11)**:** 1669-1684.
25. Hanus C, Kochen L, Tom Dieck S, Racine V, Sibarita JB, Schuman EM*, et al.* Synaptic control of secretory trafficking in dendrites. *Cell Rep* 2014, **7**(6)**:** 1771-1778.
26. Cui-Wang T, Hanus C, Helton T, Bourne J, Watson D, Harris KM*, et al.* Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. *Cell* 2012, **148:** 309-321.
27. Iyer SC, Ramachandran Iyer EP, Meduri R, Rubaharan M, Kuntimaddi A, Karamsetty M*, et al.* Cut, via CrebA, transcriptionally regulates the COPII secretory pathway to direct dendrite development in Drosophila. *J Cell Sci* 2013, **126**(Pt 20)**:** 4732-4745.
28. Heinzer S, Worz S, Kalla C, Rohr K, Weiss M. A model for the self-organization of exit sites in the endoplasmic reticulum. *J Cell Sci* 2008, **121**(Pt 1)**:** 55-64.
29. Farhan H, Weiss M, Tani K, Kaufman RJ, Hauri HP. Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load. *EMBO J* 2008, **27**(15)**:** 2043-2054.
30. Sprangers J, Rabouille C. SEC16 in COPII coat dynamics at ER exit sites. *Biochem Soc Trans* 2015, **43:** 97-103.
31. Supek F, Madden DT, Hamamoto S, Orci L, Schekman R. Sec16p potentiates the action of COPII proteins to bud transport vesicles. *J Cell Biol* 2002, **158**(6)**:** 1029-1038.
32. Watson P, Townley AK, Koka P, Palmer KJ, Stephens DJ. Sec16 defines endoplasmic reticulum exit sites and is required for secretory cargo export in mammalian cells. *Traffic* 2006, **7**(12)**:** 1678-1687.
33. Hughes H, Budnik A, Schmidt K, Palmer KJ, Mantell J, Noakes C*, et al.* Organisation of human ER-exit sites: requirements for the localisation of Sec16 to transitional ER. *J Cell Sci* 2009, **122**(Pt 16)**:** 2924-2934.
34. Bharucha N, Liu Y, Papanikou E, McMahon C, Esaki M, Jeffrey PD*, et al.* Sec16 influences transitional ER sites by regulating rather than organizing COPII. *Mol Biol Cell* 2013, **24:** 3406-3419.
35. Yoshihisa T, Barlowe C, Schekman R. Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. *Science* 1993, **259**(5100)**:** 1466-1468.
36. Antonny B, Madden D, Hamamoto S, Orci L, Schekman R. Dynamics of the COPII coat with GTP and stable analogues. *Nat Cell Biol* 2001, **3**(6)**:** 531-537.
37. Kung LF, Pagant S, Futai E, D'Arcangelo JG, Buchanan R, Dittmar JC*, et al.* Sec24p and Sec16p cooperate to regulate the GTP cycle of the COPII coat. *The EMBO journal* 2012, **31**(4)**:** 1014-1027.
38. Gimeno RE, Espenshade P, Kaiser CA. COPII coat subunit interactions: Sec24p and Sec23p bind to adjacent regions of Sec16p. *Mol Biol Cell* 1996, **7**(11)**:** 1815-1823.
39. Manzano-Lopez J, Perez-Linero AM, Aguilera-Romero A, Martin ME, Okano T, Silva DV*, et al.* COPII coat composition is actively regulated by luminal cargo maturation. *Curr Biol* 2015, **25:** 152-162.
40. Forster R, Weiss M, Zimmermann T, Reynaud EG, Verissimo F, Stephens DJ*, et al.* Secretory cargo regulates the turnover of COPII subunits at single ER exit sites. *Curr Biol* 2006, **16**(2)**:** 173-179.
41. Whittle JR, Schwartz TU. Structure of the Sec13-Sec16 edge element, a template for assembly of the COPII vesicle coat. *J Cell Biol* 2010, **190**(3)**:** 347-361.
42. Espenshade P, Gimeno RE, Holzmacher E, Teung P, Kaiser CA. Yeast SEC16 gene encodes a multidomain vesicle coat protein that interacts with Sec23p. *J Cell Biol* 1995, **131**(2)**:** 311-324.
43. Klinkenberg D, Long KR, Shome K, Watkins SC, Aridor M. A cascade of ER exit site assembly that is regulated by p125A and lipid signals. *J Cell Sci* 2014, **127**(Pt 8)**:** 1765-1778.
44. Robinson DG, Brandizzi F, Hawes C, Nakano A. Vesicles versus Tubes: is ER-Golgi Transport in Plants Fundamentally Different to other Eukaryotes ? *Plant Physiol* 2015.
45. Montegna EA, Bhave M, Liu Y, Bhattacharyya D, Glick BS. Sec12 binds to Sec16 at transitional ER sites. *PLoS One* 2012, **7**(2)**:** e31156.
46. daSilva LL, Snapp EL, Denecke J, Lipponcott-Schwartz J, Hawes C, Brandizzi F. Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. *Plant Cell* 2004, **16**(7)**:** 1753-1771.

47. Johnson A, Bhattacharya N, Hanna M, Pennington JG, Schuh AL, Wang L*, et al.* TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization. *EMBO J* 2015.
48. Witte K, Schuh AL, Hegermann J, Sarkeshik A, Mayers JR, Schwarze K*, et al.* TFG-1 function in protein secretion and oncogenesis. *Nat Cell Biol* 2011, **13**(5)**:** 550-558.
49. Delic M, Valli M, Graf AB, Pfeffer M, Mattanovich D, Gasser B. The secretory pathway: exploring yeast diversity. *FEMS Mi robiol Rev* 2013, **37**(6)**:** 872-914.
50. Sparkes IA, Ketelaar T, de Ruijter NC, Hawes C. Grab a Golgi: laser trapping of Golgi bodies reveals in vivo interactions with the endoplasmic reticulum. *Traffic* 2009, **10**(5)**:** 567-571.
51. Stagg SM, Gurkan C, Fowler DM, LaPointe P, Foss TR, Potter CS*, et al.* Structure of the Sec13/31 COPII coat cage. *Nature* 2006, **439**(7073)**:** 234-238.
52. Zanetti G, Prinz S, Daum S, Meister A, Schekman R, Bacia K*, et al.* The structure of the COPII transport-vesicle coat assembled on membranes. *eLife* 2013, **2:** e00951.
53. Venditti R, Scanu T, Santoro M, Di Tullio G, Spaar A, Gaibisso R*, et al.* Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science* 2012, **337**(6102)**:** 1668-1672.
54. Venditti R, Wilson C, De Matteis MA. Exiting the ER: what we know and what we don't. *Trends Cell Biol* 2014, **24**(1)**:** 9-18.
55. Zeuschner D, Geerts WJ, van Donselaar E, Humbel BM, Slot JW, Koster AJ*, et al.* Immuno-electron tomography of ER exit sites reveals the existence of free COPII-coated transport carriers. *Nat Cell Biol* 2006, **8**(4)**:** 377-383.
56. Faini M, Beck R, Wieland FT, Briggs JA. Vesicle coats: structure, function, and general principles of assembly. *Trends Cell Biol* 2013, **23:** 279-288.
57. Hughson FM. Copy coats: COPI mimics clathrin and COPII. *Cell* 2010, **142**(1)**:** 19-21.
58. Lee C, Goldberg J. Structure of coatomer cage proteins and the relationship among COPI, COPII, and clathrin vesicle coats. *Cell* 2010, **142**(1)**:** 123-132.
59. Futai E, Hammamoto S, Orci L, Schekman R. GTP/GDP exchange by Sec12p enables COPII vesicle bud formation on synthetic liposomes. *EMBO J* 2004, **23**(21)**:** 4146-4155.
60. la Cour JM, Schindler AJ, Berchtold MW, Schekman R. ALG-2 attenuates COPII budding in vitro and stabilizes the Sec23/Sec31A complex. *PLoS One* 2013, **8**(9)**:** e75309.
61. Long KR, Yamamoto Y, Baker AL, Watkins SC, Coyne CB, Conway JF*, et al.* Sar1 assembly regulates membrane constriction and ER export. *J Cell Biol* 2010, **190**(1)**:** 115-128.
62. Wilson DW, Lewis MJ, Pelham HR. pH-dependent binding of KDEL to its receptor in vitro. *J Biol Chem* 1993, **268:** 7465-7468.
63. Paroutis P, Touret N, Grinstein S. The pH of the secretory pathway: measurement, determinants, and regulation. *Physiology (Bethesda)* 2004, **19:** 207-215.
64. Kondylis V, Tang Y, Fuchs F, Boutros M, Rabouille C. Identification of ER proteins involved in the functional organisation of the early secretory pathway in Drosophila cells by a targeted RNAi screen. *PLoS One* 2011, **6**(2)**:** e17173.
65. Wisniewski JR, Ostasiewicz P, Dus K, Zielinska DF, Gnad F, Mann M. Extensive quantitative remodeling of the proteome between normal colon tissue and adenocarcinoma. *Molecular systems biology* 2012, **8:** 611.
66. Thigpen AE, Cala KM, Guileyardo JM, Molberg KH, McConnell JD, Russell DW. Increased expression of early growth response-1 messenger ribonucleic acid in prostatic adenocarcinoma. *The Journal of urology* 1996, **155**(3)**:** 975-981.
67. Yang SZ, Abdulkadir SA. Early growth response gene 1 modulates androgen receptor signaling in prostate carcinoma cells. *J Biol Chem* 2003, **278**(41)**:** 39906-39911.
68. Baron VT, Pio R, Jia Z, Mercola D. Early Growth Response 3 regulates genes of inflammation and directly activates IL6 and IL8 expression in prostate cancer. *Br J Cancer* 2015, **112:** 755-764.
69. Inoue A, Omoto Y, Yamaguchi Y, Kiyama R, Hayashi SI. Transcription factor EGR3 is involved in the estrogen-signaling pathway in breast cancer cells. *J Mol Endocrinol* 2004, **32:** 649-661.
70. Suzuki T, Inoue A, Miki Y, Moriya T, Akahira J, Ishida T*, et al.* Early growth responsive gene 3 in human breast carcinoma: a regulator of estrogen-meditated invasion and a potent prognostic factor. *Endocrine-related cancer* 2007, **14**(2)**:** 279-292.

71. Liao F, Ji MY, Shen L, Qiu S, Guo XF, Dong WG. Decreased EGR3 expression is related to poor prognosis in patients with gastric cancer. *J Mol Histol* 2013, **44:** 463-468.
72. Higashio H, Kohno K. A genetic link between the unfolded protein response and vesicle formation from the endoplasmic reticulum. *Biochem Biophys Res Commun* 2002, **296**(3)**:** 568-574.
73. Clarke HJ, Chambers JE, Liniker E, Marciniak SJ. Endoplasmic reticulum stress in malignancy. *Cancer Cell* 2014, **25**(5)**:** 563-573.
74. Rutkowski DT, Kaufman RJ. That which does not kill me makes me stronger: adapting to chronic ER stress. *Trends Biochem Sci* 2007, **32**(10)**:** 469-476.
75. Dejeans N, Barroso K, Fernandez-Zapico ME, Samali A, Chevet E. Novel roles of the unfolded protein response in the control of tumor development and aggressiveness. *Semin Cancer Biol* 2015.
76. Hetz C, Chevet E. Theme Series - UPR in cancer. *Semin Cancer Biol* 2015.
77. Maurel M, McGrath EP, Mnich K, Healy S, Chevet E, Samali A. Controlling the unfolded protein response-mediated life and death decisions in cancer. *Semin Cancer Biol* 2015.
78. Huang H, Liu H, Liu C, Fan L, Zhang X, Gao A*, et al.* Disruption of the unfolded protein response (UPR) by lead compound selectively suppresses cancer cell growth. *Cancer letters* 2015, **360**(2)**:** 257-268.
79. Ri M, Tashiro E, Oikawa D, Shinjo S, Tokuda M, Yokouchi Y*, et al.* Identification of Toyocamycin, an agent cytotoxic for multiple myeloma cells, as a potent inhibitor of ER stress-induced XBP1 mRNA splicing. *Blood cancer journal* 2012, **2**(7)**:** e79.
80. Koreishi M, Yu S, Oda M, Honjo Y, Satoh A. CK2 phosphorylates Sec31 and regulates ER-To-Golgi trafficking. *PLoS One* 2013, **8**(1)**:** e54382.

Comprehensive Reference List

Abdulkadir, S. A., J. M. Carbone, C. K. Naughton, P. A. Humphrey, W. J. Catalona and J. Milbrandt (2001). "Frequent and early loss of the EGR1 corepressor NAB2 in human prostate carcinoma." Hum Pathol 32(9): 935-939.

Abdulkadir, S. A., Z. Qu, E. Garabedian, S. K. Song, T. J. Peters, J. Svaren, J. M. Carbone, C. K. Naughton, W. J. Catalona, J. J. Ackerman, J. I. Gordon, P. A. Humphrey and J. Milbrandt (2001). "Impaired prostate tumorigenesis in Egr1-deficient mice." Nat Med 7(1): 101-107.

Abe, M. K., K. T. Kahle, M. P. Saelzler, K. Orth, J. E. Dixon and M. R. Rosner (2001). "ERK7 is an autoactivated member of the MAPK family." J Biol Chem 276(24): 21272-21279.

Abe, M. K., W. L. Kuo, M. B. Hershenson and M. R. Rosner (1999). "Extracellular signal-regulated kinase 7 (ERK7), a novel ERK with a C-terminal domain that regulates its activity, its cellular localization, and cell growth." Mol Cell Biol 19(2): 1301-1312.

Abe, M. K., M. P. Saelzler, R. Espinosa, 3rd, K. T. Kahle, M. B. Hershenson, M. M. Le Beau and M. R. Rosner (2002). "ERK8, a new member of the mitogen-activated protein kinase family." J Biol Chem 277(19): 16733-16743.

Acharya, U., A. Mallabiabarrena, J. K. Acharya and V. Malhotra (1998). "Signaling via mitogen-activated protein kinase kinase (MEK1) is required for Golgi fragmentation during mitosis." Cell 92(2): 183-192.

Adachi, Y., K. Yamamoto, T. Okada, H. Yoshida, A. Harada and K. Mori (2008). "ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum." Cell Struct Funct 33(1): 75-89.

Adams, E. J., X. W. Chen, K. S. O'Shea and D. Ginsburg (2014). "Mammalian COPII coat component SEC24C is required for embryonic development in mice." J Biol Chem 289(30): 20858-20870.

Aebersold, D. M., Y. D. Shaul, Y. Yung, N. Yarom, Z. Yao, T. Hanoch and R. Seger (2004). "Extracellular signal-regulated kinase 1c (ERK1c), a novel 42-kilodalton ERK, demonstrates unique modes of regulation, localization, and function." Mol Cell Biol 24(22): 10000-10015.

Aebi, M., R. Bernasconi, S. Clerc and M. Molinari (2010). "N-glycan structures: recognition and processing in the ER." Trends Biochem Sci 35(2): 74-82.

Agaisse, H. (2007). "An adaptive immune response in Drosophila?" Cell Host Microbe 1(2): 91-93.

Ahmad, S., G. Rukh, T. V. Varga, A. Ali, A. Kurbasic, D. Shungin, U. Ericson, R. W. Koivula, A. Y. Chu, L. M. Rose, A. Ganna, Q. Qi, A. Stancakova, C. H. Sandholt, C. E. Elks, G. Curhan, M. K. Jensen, R. M. Tamimi, K. H. Allin, T. Jorgensen, S. Brage, C. Langenberg, M. Aadahl, N. Grarup, A. Linneberg, G. Pare, P. K. Magnusson, N. L. Pedersen, M. Boehnke, A. Hamsten, K. L. Mohlke, L. T. Pasquale, O. Pedersen, R. A. Scott, P. M. Ridker, E. Ingelsson, M. Laakso, T. Hansen, L. Qi, N. J. Wareham, D. I. Chasman, G. Hallmans, F. B. Hu, F. Renstrom, M. Orho-Melander and P. W. Franks (2013). "Gene x physical activity interactions in obesity: combined analysis of 111,421 individuals of European ancestry." PLoS Genet 9(7): e1003607.

Akiba, S., S. Mizunaga, K. Kume, M. Hayama and T. Sato (1999). "Involvement of group VI Ca2+-independent phospholipase A2 in protein kinase C-dependent arachidonic acid liberation in zymosan-stimulated macrophage-like P388D1 cells." J Biol Chem 274(28): 19906-19912.

Akiba, S., S. Ohno, M. Chiba, K. Kume, M. Hayama and T. Sato (2002). "Protein kinase Calpha-dependent increase in Ca2+-independent phospholipase A2 in membranes and arachidonic acid liberation in zymosan-stimulated macrophage-like P388D1 cells." Biochem Pharmacol 63(11): 1969-1977.

Albuquerque, D., C. Nobrega, R. Rodriguez-Lopez and L. Manco (2014). "Association study of common polymorphisms in MSRA, TFAP2B, MC4R, NRXN3, PPARGC1A, TMEM18, SEC16B, HOXB5 and OLFM4 genes with obesity-related traits among Portuguese children." J Hum Genet 59(6): 307-313.

Alder, N. N., Y. Shen, J. L. Brodsky, L. M. Hendershot and A. E. Johnson (2005). "The molecular mechanisms underlying BiP-mediated gating of the Sec61 translocon of the endoplasmic reticulum." J Cell Biol 168(3): 389-399.

Alessi, D. R., N. Gomez, G. Moorhead, T. Lewis, S. M. Keyse and P. Cohen (1995). "Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines." Curr Biol 5: 283-295.

Alford, A. I., K. M. Kozloff and K. D. Hankenson (2015). "Extracellular matrix networks in bone remodeling." Int J Biochem Cell Biol.

Allan, B. B., B. D. Moyer and W. E. Balch (2000). "Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion." Science 289: 444-448.

Alvarez, C., H. Fujita, A. Hubbard and E. Sztul (1999). "ER to Golgi transport: Requirement for p115 at a pre-Golgi VTC stage." J Cell Biol 147: 1205-1222.

Alvarez, J. and M. Montero (2002). "Measuring [Ca2+] in the endoplasmic reticulum with aequorin." Cell Calcium 32(5-6): 251-260.

Anitei, M. and B. Hoflack (2011). "Exit from the trans-Golgi network: from molecules to mechanisms." Curr Opin Cell Biol 23: 443-451.

Antonny, B., D. Madden, S. Hamamoto, L. Orci and R. Schekman (2001). "Dynamics of the COPII coat with GTP and stable analogues." Nat Cell Biol 3(6): 531-537.

Appenzeller-Herzog, C. and L. Ellgaard (2008). "The human PDI family: versatility packed into a single fold." Biochim Biophys Acta 1783(4): 535-548.

Appenzeller-Herzog, C. and H. P. Hauri (2006). "The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function." J Cell Sci 119: 2173-2183.

Appenzeller-Herzog, C., A. C. Roche, O. Nufer and H. P. Hauri (2004). "pH-induced conversion of the transport lectin ERGIC-53 triggers glycoprotein release." J Biol Chem 279: 12943-12950.

Appenzeller, C., H. Andersson, F. Kappeler and H. P. Hauri (1999). "The lectin ERGIC-53 is a cargo transport receptor for glycoproteins." Nat Cell Biol 1: 330-334.

Aridor, M. and W. E. Balch (2000). "Kinase Signaling Initiates Coat Complex II (COPII) Recruitment and Export from the Mammalian Endoplasmic Reticulum." J Biol Chem 275(46): 35673-35676.

Aridor, M., S. I. Bannykh, T. Rowe and W. E. Balch (1999). "Cargo can modulate COPII vesicle formation from the endoplasmic reticulum." J Biol Chem 274(7): 4389-4399.

Aridor, M. and K. N. Fish (2009). "Selective targeting of ER exit sites supports axon development." Traffic 10(11): 1669-1684.

Aridor, M., A. K. Guzik, A. Bielli and K. N. Fish (2004). "Endoplasmic reticulum export site formation and function in dendrites." J Neurosci 24(15): 3770-3776.

Arimitsu, N., T. Kogure, T. Baba, K. Nakao, H. Hamamoto, K. Sekimizu, A. Yamamoto, H. Nakanishi, R. Taguchi, M. Tagaya

and K. Tani (2011). "p125/Sec23-interacting protein (Sec23ip) is required for spermiogenesis." FEBS Lett 585(14): 2171-2176.

Assefa, Z., M. Garmyn, R. Bouillon, W. Merlevede, J. R. Vandenheede and P. Agostinis (1997). "Differential stimulation of ERK and JNK activities by ultraviolet B irradiation and epidermal growth factor in human keratinocytes." J Invest Dermatol 108: 886-891.

Ast, T., G. Cohen and M. Schuldiner (2013). "A Network of Cytosolic Factors Targets SRP-Independent Proteins to the Endoplasmic Reticulum." Cell 152(5): 1134-1145.

Avezov, E., Z. Frenkel, M. Ehrlich, A. Herscovics and G. Z. Lederkremer (2008). "Endoplasmic reticulum (ER) mannosidase I is compartmentalized and required for N-glycan trimming to Man5-6GlcNAc2 in glycoprotein ER-associated degradation." Mol Biol Cell 19(1): 216-225.

Awad, W., I. Estrada, Y. Shen and L. M. Hendershot (2008). "BiP mutants that are unable to interact with endoplasmic reticulum DnaJ proteins provide insights into interdomain interactions in BiP." Proc Natl Acad Sci U S A 105(4): 1164-1169.

Axe, E. L., S. A. Walker, M. Manifava, P. Chandra, H. L. Roderick, A. Habermann, G. Griffiths and N. T. Ktistakis (2008). "Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum." J Cell Biol 182(4): 685-701.

Axelsson, M. A. and G. Warren (2004). "Rapid, endoplasmic reticulum-independent diffusion of the mitotic Golgi haze." Mol Biol Cell 15(4): 1843-1852.

Back, S. H., M. Schroder, K. Lee, K. Zhang and R. J. Kaufman (2005). "ER stress signaling by regulated splicing: IRE1/HAC1/XBP1." Methods 35(4): 395-416.

Bai, M., X. Pang, J. Lou, Q. Zhou, K. Zhang, J. Ma, J. Li, F. Sun and V. W. Hsu (2012). "Mechanistic insights into regulated cargo binding by ACAP1 protein." J Biol Chem 287(34): 28675-28685.

Baines, A. C., E. J. Adams, B. Zhang and D. Ginsburg (2013). "Disruption of the Sec24d gene results in early embryonic lethality in the mouse." PLoS One 8(4): e61114.

Balch, W. E., R. A. Kahn and R. Schwaninger (1992). "ADP-ribosylation factor is required for vesicular trafficking between the endoplasmic reticulum and the cis-Golgi compartment." J Biol Chem 267(18): 13053-13061.

Bannykh, S. I., T. Rowe and W. E. Balch (1996). "The organization of endoplasmic reticulum export complexes." J Cell Biol 135(1): 19-35.

Bard, F., L. Casano, A. Mallabiabarrena, E. Wallace, K. Saito, H. Kitayama, G. Guizzunti, Y. Hu, F. Wendler, R. Dasgupta, N. Perrimon and V. Malhotra (2006). "Functional genomics reveals genes involved in protein secretion and Golgi organization." Nature 439(7076): 604-607.

Bard, F., L. Mazelin, C. Pechoux-Longin, V. Malhotra and P. Jurdic (2003). "Src regulates Golgi structure and KDEL receptor-dependent retrograde transport to the endoplasmic reticulum." J Biol Chem 278(47): 46601-46606.

Barlowe, C. (2003). "Signals for COPII-dependent export from the ER: what's the ticket out?" Trends Cell Biol 13: 295-300.

Barlowe, C. (2015). "Membrane Trafficking: ER Export Encounters Dualism." Curr Biol 25: 151-153.

Barlowe, C., L. Orci, T. Yeung, M. Hosobuchi, S. Hamamoto, N. Salama, M. F. Rexach, M. Ravazzola, M. Amherdt and R. Schekman (1994). "COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum." Cell 77(6): 895-907.

Barlowe, C. and R. Schekman (1993). "SEC12 encodes a guanine-nucleotide-exchange factor essential for transport vesicle budding from the ER." Nature 365(6444): 347-349.

Baron, V., S. Duss, J. Rhim and D. Mercola (2003). "Antisense to the early growth response-1 gene (Egr-1) inhibits prostate tumor development in TRAMP mice." Ann N Y Acad Sci 1002: 197-216.

Baron, V. T., R. Pio, Z. Jia and D. Mercola (2015). "Early Growth Response 3 regulates genes of inflammation and directly activates IL6 and IL8 expression in prostate cancer." Br J Cancer 112: 755-764.

Barr, F. A. (2002). "Inheritance of the endoplasmic reticulum and Golgi apparatus." Curr Opin Cell Biol 14(4): 496-499.

Barr, F. A. (2004). "Golgi inheritance: shaken but not stirred." J Cell Biol 164(7): 955-958.

Bauman, O. and B. Walz (2001). "Endoplasmic reticulum of animal cells and its organization into structural and functional domains." Int Rev Cytol 205: 149-214.

Becker, B. and M. Melkonian (1996). "The secretory pathway of protists: spatial and functional organization and evolution." Microbiol Rev 60(4): 697-721.

Behrends, C., M. E. Sowa, S. P. Gygi and J. W. Harper (2010). "Network organization of the human autophagy system." Nature 466(7302): 68-76.

Belden, W. J. and C. Barlowe (1996). "Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient endoplasmic reticulum to Golgi transport." J Biol Chem 271: 26939-26946.

Belden, W. J. and C. Barlowe (2001). "Distinct roles for the cytoplasmic tail sequences of Emp24p and Erv25p in transport between the endoplasmic reticulum and Golgi complex." J Biol Chem 276: 43040-43048.

Belden, W. J. and C. Barlowe (2001). "Purification of functional Sec13p-Sec31p complex, a subunit of COPII coat." Methods Enzymol 329: 438-443.

Belden, W. J. and C. Barlowe (2001). "Role of Erv29p in collecting soluble secretory proteins into ER-derived transport vesicles." Science 294(5546): 1528-1531.

Ben-Tekaya, H., R. A. Kahn and H. P. Hauri (2010). "ADP ribosylation factors 1 and 4 and group VIA phospholipase A(2) regulate morphology and intraorganellar traffic in the endoplasmic reticulum-Golgi intermediate compartment." Mol Biol Cell 21(23): 4130-4140.

Ben-Tekaya, H., K. Miura, R. Pepperkok and H. P. Hauri (2005). "Live imaging of bidirectional traffic from the ERGIC." J Cell Sci 118: 357-367.

Bertolotti, A., Y. Zhang, L. M. Hendershot, H. P. Harding and D. Ron (2000). "Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response." Nat Cell Biol 2(6): 326-332.

Beznoussenko, G. V., S. Parashuraman, R. Rizzo, R. Polishchuk, O. Martella, D. Di Giandomenico, A. Fusella, A. Spaar, M. Sallese, M. G. Capestrano, M. Pavelka, M. R. Vos, Y. G. Rikers, V. Helms, A. A. Mironov and A. Luini (2014). "Transport of soluble proteins through the Golgi occurs by diffusion via continuities across cisternae." Elife 3.

Bharucha, N., Y. Liu, E. Papanikou, C. McMahon, M. Esaki, P. D. Jeffrey, F. M. Hughson and B. S. Glick (2013). "Sec16 influences transitional ER sites by regulating rather than organizing COPII." Mol Biol Cell 24: 3406-3419.

Bhattacharya, D. and B. S. Glick (2007). "Two Mammalian Sec16 Homologues Have Nonredundant

Functions in Endoplasmic Reticulum (ER) Export and

Transitional ER Organization." Mol Biol Cell 18: 839-849.

Bhattacharya, N., O. D. J and S. M. Stagg (2012). "The structure of the Sec13/31 COPII cage bound to Sec23." J Mol Biol 420(4-5): 324-334.

Bi, K., M. G. Roth and N. T. Ktistakis (1997). "Phosphatidic acid formation by phospholipase D is required for transport from the endoplasmic reticulum to the Golgi complex." Curr Biol 7(5): 301-307.

Bi, X., R. A. Corpina and J. Goldberg (2002). "Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat." Nature 419(6904): 271-277.

Bi, X., J. D. Mancias and J. Goldberg (2007). "Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31." Dev Cell 13(5): 635-645.

Bianco, A., V. Reghellin, L. Donnici, S. Fenu, R. Alvarez, C. Baruffa, F. Peri, M. Pagani, S. Abrignani, P. Neddermann and R. De Francesco (2012). "Metabolism of phosphatidylinositol 4-kinase IIIalpha-dependent PI4P Is subverted by HCV and is targeted by a 4-anilino quinazoline with antiviral activity." PLoS Pathog 8(3): e1002576.

Biazik, J., P. Ylä-Anttila, H. Vihinen, E. Jokitalo and E. L. Eskelinen (2015). "Ultrastructural relationship of the phagophore with surrounding organelles." Autophagy 25.

Bielli, A., C. J. Haney, G. Gabreski, S. C. Watkins, S. I. Bannykh and M. Aridor (2005). "Regulation of Sar1 NH2 terminus by GTP binding and hydrolysis promotes membrane deformation to control COPII vesicle fission." J Cell Biol 171(6): 919-924.

Biliran, H., Y. Jan, R. Chen, E. B. Pasquale and E. Ruoslahti (2008). "Protein kinase D is a positive regulator of Bit1 apoptotic function." J Biol Chem 283(42): 28029-28037.

Bisel, B., Y. Wang, J. H. Wei, Y. Xiang, D. Tang, M. Miron-Mendoza, S. Yoshimura, N. Nakamura and J. Seemann (2008). "ERK regulates Golgi and centrosome orientation towards the leading edge through GRASP65." J Cell Biol 182(5): 837-843.

Biswas, C., U. Sriram, B. Ciric, O. Ostrovsky, S. Gallucci and Y. Argon (2006). "The N-terminal fragment of GRP94 is sufficient for peptide presentation via professional antigen-presenting cells." Int Immunol 18(7): 1147-1157.

Blagitko, N., U. Schulz, A. A. Schinzel, H. H. Ropers and V. M. Kalscheuer (1999). "gamma2-COP, a novel imprinted gene on chromosome 7q32, defines a new imprinting cluster in the human genome." Hum Mol Genet 8: 2387-2396.

Blond-Elguindi, S., S. E. Cwirla, W. J. Dower, R. J. Lipshutz, S. R. Sprang, J. F. Sambrook and M. J. Gething (1993). "Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP." Cell 75(4): 717-728.

Blumental-Perry, A., C. J. Haney, K. M. Weixel, S. C. Watkins, O. A. Weisz and M. Aridor (2006). "Phosphatidylinositol 4-phosphate formation at ER exit sites regulates ER export." Dev Cell 11(5): 671-682.

Boevink, P., K. Oparka, S. Santa Cruz, B. Martin, A. Betteridge and C. Hawes (1998). "Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network." Plant J 15(3): 441-447.

Bogoyevitch, M. A. and B. Kobe (2006). "Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases." Microbiol Mol Biol Rev 70(4): 1061-1095.

Boncompain, G., S. Divoux, N. Gareil, H. de Forges, A. Lescure, L. Latreche, V. Mercanti, F. Jollivet, G. Raposo and F. Perez (2012). "Synchronization of secretory protein traffic in populations of cells." Nat Methods 9(5): 493-498.

Bone, R. N., Y. Gai, V. Magrioti, M. G. Kokotou, T. Ali, X. Lei, H. M. Tse, G. Kokotos and S. Ramanadham (2015). "Inhibition of Ca2+-independent phospholipase A2beta (iPLA2beta) ameliorates islet infiltration and incidence of diabetes in NOD mice." Diabetes 64(2): 541-554.

Bonifacino, J. S. and B. S. Glick (2004). "The mechanisms of vesicle budding and fusion." Cell 116(2): 153-166.

Bonnon, C., M. W. Wendeler, J. P. Paccaud and H. P. Hauri (2010). "Selective export of human GPI-anchored proteins from the endoplasmic reticulum." J Cell Sci 123(Pt 10): 1705-1715.

Boulton, T. G., S. H. Nye, D. J. Robbins, N. Y. Ip, E. Radziejewska, S. D. Morgenbesser, R. A. DePinho, N. Panayotatos, M. H. Cobb and G. D. Yancopoulos (1991). "ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF." Cell 65(4): 663-675.

Boya, P., I. Cohen, N. Zamzami, H. L. Vieira and G. Kroemer (2002). "Endoplasmic reticulum stress-induced cell death requires mitochondrial membrane permeabilization." Cell Death Differ 9(4): 465-467.

Boyadjiev, S. A., J. C. Fromme, J. Ben, S. S. Chong, C. Nauta, D. J. Hur, G. Zhang, S. Hamamoto, R. Schekman, M. Ravazzola, L. Orci and W. Eyaid (2006). "Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking." Nat Genet 38(10): 1192-1197.

Boyadjiev, S. A., S. D. Kim, A. Hata, C. Haldeman-Englert, E. H. Zackai, C. Naydenov, S. Hamamoto, R. W. Schekman and J. Kim (2011). "Cranio-lenticulo-sutural dysplasia associated with defects in collagen secretion." Clin Genet 80(2): 169-176.

Braakman, I. and D. N. Hebert (2013). "Protein folding in the endoplasmic reticulum." Cold Spring Harb Perspect Biol 5(5): a013201.

Brahma, A. and K. N. Dalby (2007). "Regulation of protein phosphorylation within the MKK1-ERK2 complex by MP1 and the MP1*P14 heterodimer." Arch Biochem Biophys 460(1): 85-91.

Brandtstaetter, H., A. J. Kruppa and F. Buss (2014). "Huntingtin is required for ER-to-Golgi transport and for secretory vesicle fusion at the plasma membrane." Dis Model Mech 7(12): 1335-1340.

Brewer, J. W. and L. M. Hendershot (2005). "Building an antibody factory: a job for the unfolded protein response." Nat Immunol 6(1): 23-29.

Brodsky, J. L., J. Goeckeler and R. Schekman (1995). "BiP and Sec63p are required for both co- and posttranslational protein translocation into the yeast endoplasmic reticulum." Proc Natl Acad Sci U S A 92: 9643-9646.

Brown, M. S. and J. L. Goldstein (1997). "The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor." Cell 89(3): 331-340.

Bruns, C., J. M. McCaffery, A. J. Curwin, J. M. Duran and V. Malhotra (2011). "Biogenesis of a novel compartment for autophagosome-mediated unconventional protein secretion." J Cell Biol 195(6): 979-992.

Brusselmans, K., E. De Schrijver, G. Verhoeven and J. V. Swinnen (2005). "RNA interference-mediated silencing of the acetyl-CoA-carboxylase-alpha gene induces growth inhibition and apoptosis of prostate cancer cells." Cancer Res 65(15): 6719-6725.

Budnik, A., K. J. Heesom and D. J. Stephens (2011). "Characterization of human Sec16B: indications of specialized, non-redundant functions." Sci Rep 77.

Burotto, M., V. L. Chiou, J. M. Lee and E. C. Kohn (2014). "The MAPK pathway across different malignancies: a new perspective." Cancer 120: 3446-3456.

Buschbeck, M. and A. Ullrich (2005). "The unique C-terminal tail of the mitogen-activated protein kinase ERK5 regulates its activation and nuclear shuttling." J Biol Chem 280(4): 2659-2667.

Cacace, A. M., N. R. Michaud, M. Therrien, K. Mathes, T. D. Copeland, G. M. Rubin and D. K. Morrison (1999). "Identification of constitutive and ras-inducible phosphorylation sites of KSR: implications for 14-3-3 binding, mitogen-activated protein kinase binding, and KSR overexpression." Mol Cell Biol 19: 229-240.

Cai, H., C. C. Wang and C. L. Tsou (1994). "Chaperone-like activity of protein disulfide isomerase in the refolding of a protein with no disulfide bonds." J Biol Chem 269(40): 24550-24552.

Cai, H., S. Yu, S. Menon, Y. Cai, D. Lazarova, C. Fu, K. Reinisch, J. C. Hay and S. Ferro-Novick (2007). "TRAPPI tethers COPII vesicles by binding the coat subunit Sec23." Nature.

Cai, Y., H. F. Chin, D. Lazarova, S. Menon, C. Fu, H. Cai, A. Sclafani, D. W. Rodgers, E. M. De La Cruz, S. Ferro-Novick and K. M. Reinisch (2008). "The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes." Cell 133: 1202-1213.

Calfon, M., H. Zeng, F. Urano, J. H. Till, S. R. Hubbard, H. P. Harding, S. G. Clark and D. Ron (2002). "IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA." Nature 415(6867): 92-96.

Calogero, A., A. Arcella, G. De Gregorio, A. Porcellini, D. Mercola, C. Liu, V. Lombari, M. Zani, G. Giannini, F. M. Gagliardi, R. Caruso, A. Gulino, L. Frati and G. Ragona (2001). "The early growth response gene EGR-1 behaves as a suppressor gene that is down-regulated independent of ARF/Mdm2 but not p53 alterations in fresh human gliomas." Clin Cancer Res 7(9): 2788-2796.

Campbell, J. L. and R. Schekman (1997). "Selective packaging of cargo molecules into endoplasmic reticulum-derived COPII vesicles." Proc Natl Acad Sci U S A 94(3): 837-842.

Camps, M., A. Nichols and S. Arkinstall (2000). "Dual specificity phosphatases: a gene family for control of MAP kinase function." FASEB J. 14: 6-16.

Cancino, J., A. Capalbo, A. Di Campli, M. Giannotta, R. Rizzo, J. E. Jung, R. Di Martino, M. Persico, P. Heinklein, M. Sallese and A. Luini (2014). "Control systems of membrane transport at the interface between the endoplasmic reticulum and the Golgi." Dev Cell 30(3): 280-294.

Cancino, J. and A. Luini (2013). "Signaling circuits on the Golgi complex." Traffic 14(2): 121-134.

Capitani, M. and M. Sallese (2009). "The KDEL receptor: new functions for an old protein." FEBS Lett 583: 3863-3871.

Caramelo, J. J. and A. J. Parodi (2008). "Getting in and out from calnexin/calreticulin cycles." J Biol Chem 283(16): 10221-10225.

Carlson, S. M., C. R. Chouinard, A. Labadorf, C. J. Lam, K. Schmelzle, E. Fraenkel and F. M. White (2011). "Large-scale discovery of ERK2 substrates identifies ERK-mediated transcriptional regulation by ETV3." Sci Signal 4(196): rs11.

Carlsson, S. R. and A. Simonsen (2015). "Membrane dynamics in autophagosome biogenesis." J Cell Sci 128(2): 193-205.

Cartwright, I. J. and J. A. Higgins (2001). "Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes." J Biol Chem 276(51): 48048-48057.

Carvalho, P., V. Goder and T. A. Rapoport (2006). "Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins." Cell 126(2): 361-373.

Carvalho, P., A. M. Stanley and T. A. Rapoport (2010). "Retrotranslocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p." Cell 143(4): 579-591.

Casar, B., A. Pinto and P. Crespo (2008). "Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes." Mol Cell 31(5): 708-721.

Castillon, G. A., R. Watanabe, M. Taylor, T. M. Schwabe and H. Riezman (2009). "Concentration of GPI-anchored proteins upon ER exit in yeast." Traffic 10(2): 186-200.

Cerone, M. A., D. J. Burgess, C. Naceur-Lombardelli, C. J. Lord and A. Ashworth (2011). "High-throughput RNAi screening reveals novel regulators of telomerase." Cancer Res 71(9): 3328-3340.

Chadee, D. N. and J. M. Kyriakis (2004). "MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation." Nat Cell Biol 6(8): 770-776.

Chajes, V., M. Cambot, K. Moreau, G. M. Lenoir and V. Joulin (2006). "Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival." Cancer Res 66(10): 5287-5294.

Chao, T. H., M. Hayashi, R. I. Tapping, Y. Kato and J. D. Lee (1999). "MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway." J Biol Chem 274(51): 36035-36038.

Charcosset, M., A. Sassolas, N. Peretti, C. C. Roy, C. Deslandres, D. Sinnett, E. Levy and A. Lachaux (2008). "Anderson or chylomicron retention disease: molecular impact of five mutations in the SAR1B gene on the structure and the functionality of Sar1b protein." Mol Genet Metab 93(1): 74-84.

Chavrier, P., P. Lemaire, O. Revelant, R. Bravo and P. Charnay (1988). "Characterization of a mouse multigene family that encodes zinc finger structures." Mol Cell Biol 8: 1319-1326.

Chen, G., M. Hitomi, J. Han and D. W. Stacey (2000). "The p38 pathway provides negative feedback for Ras proliferative signaling." J Biol Chem 275(50): 38973-38980.

Chen, L., S. Wang, Y. Zhou, X. Wu, I. Entin, J. Epstein, S. Yaccoby, W. Xiong, B. Barlogie, J. D. J. Shaughnessy and F. Zhan (2010). "Identification of early growth response protein 1 (EGR-1) as a novel target for JUN-induced apoptosis in multiple myeloma." Blood 115: 61-70.

Chen, S., P. Novick and S. Ferro-Novick (2013). "ER structure and function." Curr Opin Cell Biol 25(4): 428-433.

Chen, X. W., H. Wang, K. Bajaj, P. Zhang, Z. X. Meng, D. Ma, Y. Bai, H. H. Liu, E. Adams, A. Baines, G. Yu, M. A. Sartor, B. Zhang, Z. Yi, J. Lin, S. G. Young, R. Schekman and D. Ginsburg (2013). "SEC24A deficiency lowers plasma cholesterol through reduced PCSK9 secretion." Elife 2: e00444.

Cheng, E. H., M. C. Wei, S. Weiler, R. A. Flavell, T. W. Mak, T. Lindsten and S. J. Korsmeyer (2001). "BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis." Mol Cell 8(3): 705-711.

Cheng, T. H., N. L. Shih, C. H. Chen, H. Lin, J. C. Liu, H. H. Chao, J. Y. Liou, Y. L. Chen, H. W. Tsai, Y. S. Chen, C. F. Cheng and J. J. Chen (2005). "Role of mitogen-activated protein kinase

pathway in reactive oxygen species-mediated endothelin-1-induced beta-myosin heavy chain gene expression and cardiomyocyte hypertrophy." J Biomed Sci 12: 123-133.

Cheung, H. H., N. Lynn Kelly, P. Liston and R. G. Korneluk (2006). "Involvement of caspase-2 and caspase-9 in endoplasmic reticulum stress-induced apoptosis: a role for the IAPs." Exp Cell Res 312(12): 2347-2357.

Chevalier, M., H. Rhee, E. C. Elguindi and S. Y. Blond (2000). "Interaction of murine BiP/GRP78 with the DnaJ homologue MTJ1." J Biol Chem 275(26): 19620-19627.

Chia, J., G. Goh, V. Racine, S. Ng, P. Kumar and F. Bard (2012). "RNAi screening reveals a large signaling network controlling the Golgi apparatus in human cells." Mol Syst Biol 8: 629.

Chia, J., K. M. Tham, D. J. Gill, E. A. Bard-Chapeau and F. A. Bard (2014). "ERK8 is a negative regulator of O-GalNAc glycosylation and cell migration." Elife 3: e01828.

Chien, V., J. F. Aitken, S. Zhang, C. M. Buchanan, A. Hickey, T. Brittain, G. J. Cooper and K. M. Loomes (2010). "The chaperone proteins HSP70, HSP40/DnaJ and GRP78/BiP suppress misfolding and formation of beta-sheet-containing aggregates by human amylin: a potential role for defective chaperone biology in Type 2 diabetes." Biochem J 432(1): 113-121.

Chirico, W. J., M. G. Waters and G. Blobel (1988). "70K heat shock related proteins stimulate protein translocation into microsomes." Nature 332: 805-810.

Cho, H. J., J. Mu, J. K. Kim, J. L. Thorvaldsen, Q. Chu, E. B. r. Crenshaw, K. H. Kaestner, M. S. Bartolomei, G. I. Shulman and M. J. Birnbaum (2001). "Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta)." Science 292: 1728-1731.

Cho, H. J., J. Yu, C. Xie, P. Rudrabhatla, X. Chen, J. Wu, L. Parisiadou, G. Liu, L. Sun, B. Ma, J. Ding, Z. Liu and H. Cai (2014). "Leucine-rich repeat kinase 2 regulates Sec16A at ER exit sites to allow ER-Golgi export." EMBO J. 33: 2314-2331.

Christianson, J. C., J. A. Olzmann, T. A. Shaler, M. E. Sowa, E. J. Bennett, C. M. Richter, R. E. Tyler, E. J. Greenblatt, J. W. Harper and R. R. Kopito (2012). "Defining human ERAD networks through an integrative mapping strategy." Nat Cell Biol 14(1): 93-105.

Christy, B. and D. Nathans (1989). "DNA binding site of the growth factor-inducible protein Zif268." Proc Natl Acad Sci U S A 86: 8737-8741.

Chun, J., Z. Shapovalova, S. Y. Dejgaard, J. F. Presley and P. Melancon (2008). "Characterization of class I and II ADP-ribosylation factors (Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1." Mol Biol Cell 19(8): 3488-3500.

Chun, J., Z. Shapovalova, S. Y. Dejgaard, J. F. Presley and P. Melançon (2008). "Characterization of class I and II ADP-ribosylation factors (Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1." Mol Biol Cell 19: 3488-3500.

Chung, K. T., Y. Shen and L. M. Hendershot (2002). "BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP." J Biol Chem 277(49): 47557-47563.

Clarke, H. J., J. E. Chambers, E. Liniker and S. J. Marciniak (2014). "Endoplasmic reticulum stress in malignancy." Cancer Cell 25(5): 563-573.

Colanzi, A. and D. Corda (2007). "Mitosis controls the Golgi and the Golgi controls mitosis." Curr Opin Cell Biol 19(4): 386-393.

Colanzi, A. and C. Sutterlin (2013). "Signaling at the Golgi during mitosis." Methods Cell Biol 118: 383-400.

Cole, A. J., D. W. Saffen, J. M. Baraban and P. F. Worley (1989). "Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation." Nature 340: 474-476.

Colecchia, D., A. Strambi, S. Sanzone, C. Iavarone, M. Rossi, C. Dall'Armi, F. Piccioni, A. Verrotti di Pianella and M. Chiariello (2012). "MAPK15/ERK8 stimulates autophagy by interacting with LC3 and GABARAP proteins." Autophagy 8(12): 1724-1740.

Connerly, P. L., M. Esaki, E. A. Montegna, D. E. Strongin, S. Levi, J. Soderholm and B. S. Glick (2005). "Sec16 is a Determinant of Transitional ER Organization." Curr Biol 15(16): 1439-1447.

Cooney, J. R., J. L. Hurlburt, D. K. Selig, K. M. Harris and J. C. Fiala (2002). "Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane." J Neurosci 22(6): 2215-2224.

Copic, A., C. F. Latham, M. A. Horlbeck, J. G. D'Arcangelo and E. A. Miller (2012). "ER cargo properties specify a requirement for COPII coat rigidity mediated by Sec13p." Science 335(6074): 1359-1362.

Cosson, P. and F. Letourneur (1994). "Coatomer interaction with di-lysine endoplasmic reticulum retention motifs." Science 263: 1629-1631.

Coulombe, P. and S. Meloche (2007). "Atypical mitogen-activated protein kinases: structure, regulation and functions." Biochim Biophys Acta 1773: 1376-1387.

Cox, J. S. and P. Walter (1996). "A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response." Cell 87(3): 391-404.

Crottet, P., D. M. Meyer, J. Rohrer and M. Spiess (2002). "ARF1.GTP, tyrosine-based signals, and phosphatidylinositol 4,5-bisphosphate constitute a minimal machinery to recruit the AP-1 clathrin adaptor to membranes." Mol Biol Cell 13(10): 3672-3682.

Cruz-Garcia, D., A. J. Curwin, J. F. Popoff, C. Bruns, J. M. Duran and V. Malhotra (2014). "Remodeling of secretory compartments creates CUPS during nutrient starvation." J Cell Biol 207(6): 695-703.

Cui-Wang, T., C. Hanus, T. Helton, J. Bourne, D. Watson, K. M. Harris and M. D. Ehlers (2012). "Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites." Cell 148: 309-321.

Cutrona, M. B., G. V. Beznoussenko, A. Fusella, O. Martella, P. Moral and A. A. Mironov (2013). "Silencing of mammalian Sar1 isoforms reveals COPII-independent protein sorting and transport." Traffic 14: 691-708.

D'Arcangelo, J. G., K. R. Stahmer and E. A. Miller (2013). "Vesicle-mediated export form the ER: COPII coat function and regulation." Biochim Biophys Acta 1833: 2464-2472.

Dahmer, M. K. (2005). "Caspases-2, -3, and -7 are involved in thapsigargin-induced apoptosis of SH-SY5Y neuroblastoma cells." J Neurosci Res 80(4): 576-583.

Dancourt, J. and C. Barlowe (2010). "Protein sorting receptors in the early secretory pathway." Annu Rev Biochem 79: 777-802.

Daniels, V. W., K. Smans, I. Royaux, M. Chypre, J. V. Swinnen and N. Zaidi (2014). "Cancer cells differentially activate and thrive on de novo lipid synthesis pathways in a low-lipid environment." PLoS One 9(9): e106913.

Danilczyk, U. G. and D. B. Williams (2001). "The lectin chaperone calnexin utilizes polypeptide-based interactions to associate with many of its substrates in vivo." J Biol Chem 276(27): 25532-25540.

Darby, N. J., E. Penka and R. Vincentelli (1998). "The multi-domain structure of protein disulfide isomerase is essential for high catalytic efficiency." J Mol Biol 276(1): 239-247.

Dascher, C. and W. E. Balch (1994). "Dominant inhibitory mutants of ARF1 block endoplasmic reticulum to Golgi transport and trigger disassembly of the Golgi apparatus." J Biol Chem 269(2): 1437-1448.

daSilva, L. L., E. L. Snapp, J. Denecke, J. Lipponcott-Schwartz, C. Hawes and F. Brandizzi (2004). "Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells." Plant Cell 16(7): 1753-1771.

Davis, R. J. (2000). "Signal transduction by the JNK group of MAP kinases." Cell 103: 239-252.

Day, K. J., L. A. Staehelin and B. S. Glick (2013). "A three-stage model of Golgi structure and function." Histochem Cell Biol 140: 239-249.

Deak, M., A. D. Clifton, L. M. Lucocq and D. R. Alessi (1998). "Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB." EMBO J 17(15): 4426-4441.

DeFea, K. A., J. Zalevsky, M. S. Thoma, O. Déry, R. D. Mullins and N. W. Bunnett (2000). "beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2." J Cell Biol 148: 1267-1281.

Dejeans, N., K. Barroso, M. E. Fernandez-Zapico, A. Samali and E. Chevet (2015). "Novel roles of the unfolded protein response in the control of tumor development and aggressiveness." Semin Cancer Biol.

Delic, M., M. Valli, A. B. Graf, M. Pfeffer, D. Mattanovich and B. Gasser (2013). "The secretory pathway: exploring yeast diversity." FEMS Mi robiol Rev 37(6): 872-914.

Demaurex, N. and M. Frieden (2003). "Measurements of the free luminal ER Ca(2+) concentration with targeted "cameleon" fluorescent proteins." Cell Calcium 34(2): 109-119.

Deniaud, A., O. Sharaf el dein, E. Maillier, D. Poncet, G. Kroemer, C. Lemaire and C. Brenner (2008). "Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis." Oncogene 27(3): 285-299.

Dèrijard, B., M. Hibi, I. H. Wu, T. Barrett, B. Su, T. Deng, M. Karin and R. J. Davis (1994). "JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain." Cell 76: 1025-1037.

Derijard, B., J. Raingeaud, T. Barrett, I. H. Wu, J. Han, R. J. Ulevitch and R. J. Davis (1995). "Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms." Science 267(5198): 682-685.

Deshaies, R. J., B. D. Koch, M. Werner-Washburne, E. A. Craig and R. Schekman (1988). "A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides." Nature 332(6167): 800-805.

Dhillon, A. S., S. Hagan, O. Rath and W. Kolch (2007). "MAP kinase signalling pathways in cancer." Oncogene 26(22): 3279-3290.

Dick, G., L. K. Akslen-Hoel, F. Grøndahl, I. Kjos and K. Prydz (2012). "Proteoglycan synthesis and Golgi organization in polarized epithelial cells." J Histochem Cytochem 60: 926-935.

Dickens, M., J. S. Rogers, J. Cavanagh, A. Raitano, Z. Xia, J. r. Halpern, M. E. Greenberg, C. L. Sawyers and R. J. Davis (1997). "A cytoplasmic inhibitor of the JNK signal transduction pathway." Science 277: 693-696.

Dominguez, M., K. Dejgaard, J. Füllekrug, S. Dahan, A. Fazel, J. P. Paccaud, D. Y. Thomas, J. J. Bergeron and T. Nilsson (1998). "gp25L/emp24/p24 protein family members of the cis-Golgi network bind both COP I and II coatomer." J Cell Biol 140: 751-765.

Donaldson, J. G., D. Cassel, R. A. Kahn and R. D. Klausner (1992). "ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein beta-COP to Golgi membranes." Proc Natl Acad Sci U S A 89(14): 6408-6412.

Downward, J. (2001). "The ins and outs of signalling." Nature 411(6839): 759-762.

Downward, J. (2003). "Targeting RAS signalling pathways in cancer therapy." Nat Rev Cancer 3(1): 11-22.

Drakakaki, G. and A. Dandekar (2013). "Protein secretion: How many secretory routes does a plant cell have?" Plant Sci 203: 74-78.

Du, N., H. Kwon, P. Li, E. E. West, J. Oh, W. Liao, Z. Yu, M. Ren and W. J. Leonard (2014). "EGR2 is critical for peripheral naive T-cell differentiation and the T-cell response to influenza." Proc Natl Acad Sci U S A 111(46): 16484-16489.

Du, X., I. Kristiana, J. Wong and A. J. Brown (2006). "Involvement of Akt in ER-to-Golgi transport of SCAP/SREBP: a link between a key cell proliferative pathway and membrane synthesis." Mol Biol Cell 17(6): 2735-2745.

Duarte, A., A. F. Castillo, E. J. Podesta and C. Poderoso (2014). "Mitochondrial fusion and ERK activity regulate steroidogenic acute regulatory protein localization in mitochondria." PLoS One 9(6): e100387.

Dudek, J., S. Pfeffer, P. H. Lee, M. Jung, A. Cavalié, V. Helms, F. Förster and R. Zimmermann (2015). "Protein Transport into the Human Endoplasmic Reticulum." J Mol Biol 427: 1159-1175.

Dudognon, P., C. Maeder-Garavaglia, J. L. Carpentier and J. P. Paccaud (2004). "Regulation of a COPII component by cytosolic O-glycosylation during mitosis." FEBS Lett 561(1-3): 44-50.

Duke, E. M., M. Razi, A. Weston, P. Guttmann, S. Werner, K. Henzler, G. Schneider, S. A. Tooze and L. M. Collinson (2014). "Imaging endosomes and autophagosomes in whole mammalian cells using correlative cryo-fluorescence and cryo-soft X-ray microscopy (cryo-CLXM)." Ultramicroscopy 143: 77-87.

Dupont, N., S. Jiang, M. Pilli, W. Ornatowski, D. Bhattacharya and V. Deretic (2011). "Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta." EMBO J 30(23): 4701-4711.

Duran, J. M., C. Anjard, C. Stefan, W. F. Loomis and V. Malhotra (2010). "Unconventional secretion of Acb1 is mediated by autophagosomes." J Cell Biol 188(4): 527-536.

Düvel, K., J. L. Yecies, S. Menon, P. Raman, A. I. Lipovsky, A. L. Souza, E. Triantafellow, Q. Ma, R. Gorski, S. Cleaver, M. G. Vander Heiden, J. P. MacKeigan, P. M. Finan, C. B. Clish, L. O. Murphy and B. D. Manning (2010). "Activation of a metabolic gene regulatory network downstream of mTOR complex 1." Mol Cell 39(2): 171-183.

Dykstra, K. M., J. E. Pokusa, J. Suhan and T. H. Lee (2010). "Yip1A structures the mammalian endoplasmic reticulum." Mol Biol Cell 21(9): 1556-1568.

Egerod, F. L., A. Bartels, N. Fristrup, M. Borre, T. F. Ørntoft, M. B. Oleskiewicz, N. Brünner and L. Dyrskjøt (2009). "High frequency of tumor cells with nuclear Egr-1 protein expression in human bladder cancer is associated with disease progression." BMC Cancer 30(9): 385.

Eid, M. A., M. V. Kumar, K. A. Iczkowski, D. G. Bostwick and D. J. Tindall (1998). "Expression of early growth response genes in human prostate cancer." Cancer Res 58(11): 2461-2468.

Elkon, R., C. Linhart, R. Sharan, R. Shamir and Y. Shiloh (2003). "Genome-wide in silico identification of transcriptional regulators controlling the cell cycle in human cells." Genome Res 13(5): 773-780.

Ellgaard, L. and L. W. Ruddock (2005). "The human protein disulphide isomerase family: substrate interactions and functional properties." EMBO Rep 6(1): 28-32.

Elsner, M., H. Hashimoto, J. C. Simpson, D. Cassel, T. Nilsson and M. Weiss (2003). "Spatiotemporal dynamics of the COPI vesicle machinery." EMBO Rep 4(10): 1000-1004.

Enninga, J., A. Levay and B. M. Fontoura (2003). "Sec13 shuttles between the nucleus and the cytoplasm and stably interacts with Nup96 at the nuclear pore complex." Mol Cell Biol 23(20): 7271-7284.

Ersahin, T., N. Tuncbag and R. Cetin-Atalay (2015). "The PI3K/AKT/mTOR interactive pathway." Mol Biosyst.

Espenshade, P., R. E. Gimeno, E. Holzmacher, P. Teung and C. A. Kaiser (1995). "Yeast SEC16 gene encodes a multidomain vesicle coat protein that interacts with Sec23p." J Cell Biol 131(2): 311-324.

Eugster, A., G. Frigerio, M. Dale and R. Duden (2000). "COP I domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP." EMBO J. 19: 3905-3917.

Eugster, A., G. Frigerio, M. Dale and R. Duden (2004). "The alpha- and beta'-COP WD40 domains mediate cargo-selective interactions with distinct di-lysine motifs." Mol Biol Cell 15: 1011-1023.

Faini, M., R. Beck, F. T. Wieland and J. A. Briggs (2013). "Vesicle coats: structure, function, and general principles of assembly." Trends Cell Biol 23: 279-288.

Fan, J. Y., F. Preuss, M. J. Muskus, E. S. Bjes and J. L. Price (2009). "Drosophila and vertebrate casein kinase Idelta exhibits evolutionary conservation of circadian function." Genetics 181(1): 139-152.

Farhan, H. (2015). "Systems biology of the secretory pathway: What have we learned so far?" Biol Cell.

Farhan, H. and C. Rabouille (2011). "Signalling to and from the secretory pathway." J Cell Sci 124: 171-180.

Farhan, H., V. Reiterer, A. Kriz, H. P. Hauri, M. Pavelka, H. H. Sitte and M. Freissmuth (2008). "Signal-dependent export of GABA transporter 1 from the ER-Golgi intermediate compartment is specified by a C-terminal motif." J Cell Sci 121: 753-761.

Farhan, H., M. Weiss, K. Tani, R. J. Kaufman and H. P. Hauri (2008). "Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load." EMBO J. 27(15): 2043-2054.

Farhan, H., M. W. Wendeler, S. Mitrovic, E. Fava, Y. Silberberg, R. Sharan, M. Zerial and H. P. Hauri (2010). "MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening." J Cell Biol 189(6): 997-1011.

Farmaki, T., S. Ponnambalam, A. R. Prescott, H. Clausen, B. L. Tang, W. Hong and J. M. Lucocq (1999). "Forward and retrograde trafficking in mitotic animal cells. ER-Golgi transport arrest restricts protein export from the ER into COPII-coated structures." J Cell Sci 112 (Pt 5): 589-600.

Fath, S., J. D. Mancias, X. Bi and J. Goldberg (2007). "Structure and organization of coat proteins in the COPII cage." Cell 129(7): 1325-1336.

Featherstone, C., G. Griffiths and G. Warren (1985). "Newly synthesized G protein of vesicular stomatitis virus is not transported to the Golgi complex in mitotic cells." J Cell Biol 101(6): 2036-2046.

Feinstein, T. N. and A. D. Linstedt (2008). "GRASP55 regulates Golgi ribbon formation." Mol Biol Cell 19(7): 2696-2707.

Feng, Z., H. Zhang, A. J. Levine and S. Jin (2005). "The coordinate regulation of the p53 and mTOR pathways in cells." Proc Natl Acad Sci U S A 102(23): 8204-8209.

Forster, R., M. Weiss, T. Zimmermann, E. G. Reynaud, F. Verissimo, D. J. Stephens and R. Pepperkok (2006). "Secretory cargo regulates the turnover of COPII subunits at single ER exit sites." Curr Biol 16(2): 173-179.

Fox, R. M. and D. J. Andrew (2015). "Transcriptional regulation of secretory capacity by bZip transcription factors." Front Biol (Beijing) 10(1): 28-51.

Franklin, C. C. and A. S. Kraft (1997). "Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells." J Biol Chem 272: 16917-16923.

Frias, M. A., C. C. Thoreen, J. D. Jaffe, W. Schroder, T. Sculley, S. A. Carr and D. M. Sabatini (2006). "mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s." Curr Biol 16(18): 1865-1870.

Fryer, L. G., B. Jones, E. J. Duncan, C. E. Hutchison, T. Ozkan, P. A. Williams, O. Alder, M. Nieuwdorp, A. K. Townley, A. R. Mensenkamp, D. J. Stephens, G. M. Dallinga-Thie and C. C. Shoulders (2014). "Expression of Sar1b enhances chylomicron assembly and key components of the coat protein complex II system driving vesicle budding." J Biol Chem 289: 4244-4261.

Fujita, M., R. Watanabe, N. Jaensch, M. Romanova-Michaelides, T. Satoh, M. Kato, H. Riezman, Y. Yamaguchi, Y. Maeda and T. Kinoshita (2011). "Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI." J Cell Biol 194(1): 61-75.

Füllekrug, J., T. Suganuma, B. L. Tang, W. Hong, B. Storrie and T. Nilsson (1999). "Localization and recycling of gp27 (hp24γ3): complex formation with other p24 family members." Mol Biol Cell 10: 1939-1955.

Futai, E., S. Hammamoto, L. Orci and R. Schekman (2004). "GTP/GDP exchange by Sec12p enables COPII vesicle bud formation on synthetic liposomes." EMBO J. 23(21): 4146-4155.

Galli, S., O. Jahn, R. Hitt, D. Hesse, L. Opitz, U. Plessmann, H. Urlaub, J. J. Poderoso, E. A. Jares-Erijman and T. M. Jovin (2009). "A new paradigm for MAPK: structural interactions of hERK1 with mitochondria in HeLa cells." PLoS One 4: e7541.

Gallitano-Mendel, A., Y. Izumi, K. Tokuda, C. F. Zorumski, M. P. Howell, L. J. Muglia, D. F. Wozniak and J. Milbrandt (2007). "The immediate early gene early growth response gene 3 mediates adaptation to stress and novelty." Neuroscience 148: 633-643.

Gallitano, A. L., R. Tillman, V. Dinu and B. Geller (2012). "Family-based association study of early growth response gene 3 with child bipolar I disorder." J Affect Disord 138: 387-396.

Garbes, L., K. Kim, A. Riess, H. Hoyer-Kuhn, F. Beleggia, A. Bevot, M. J. Kim, Y. H. Huh, H. S. Kweon, R. Savarirayan, D. Amor, P. M. Kakadia, T. Lindig, K. O. Kagan, J. Becker, S. A. Boyadjiev, B. Wollnik, O. Semler, S. K. Bohlander, J. Kim and C. Netzer (2015). "Mutations in SEC24D, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta." Am J Hum Genet 96(3): 432-439.

Garofalo, R. S., S. J. Orena, K. Rafidi, A. J. Torchia, J. L. Stock, A. L. Hildebrandt, T. Coskran, S. C. Black, D. J. Brees, J. R. Wicks, J. D. McNeish and K. G. Coleman (2003). "Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta." J Clin Invest 112: 197-208.

Ge, L., D. Melville, M. Zhang and R. Schekman (2013). "The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis." Elife 2: e00947.

Ge, L. and R. Schekman (2014). "The ER-Golgi intermediate compartment feeds the phagophore membrane." Autophagy 10(1): 170-172.

Ge, L., M. Zhang and R. Schekman (2014). "Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER-Golgi intermediate compartment." Elife 3: e04135.

Gedde, U. W. (1995). Polymer Physics. Dordrecht, Kluwer Academic Publishers.

Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Belle, N. Dephoure, E. K. O'Shea and J. S. Weissman (2003). "Global analysis of protein expression in yeast." Nature 425(6959): 737-741.

Giannotta, M., C. Ruggiero, M. Grossi, J. Cancino, M. Capitani, T. Pulvirenti, G. M. Consoli, C. Geraci, F. Fanelli, A. Luini and M. Sallese (2012). "The KDEL receptor couples to Galphaq/11 to activate Src kinases and regulate transport through the Golgi." EMBO J 31(13): 2869-2881.

Gimeno, R. E., P. Espenshade and C. A. Kaiser (1995). "SED4 encodes a yeast endoplasmic reticulum protein that binds Sec16p and participates in vesicle formation." J Cell Biol 131(2): 325-338.

Gimeno, R. E., P. Espenshade and C. A. Kaiser (1996). "COPII coat subunit interactions: Sec24p and Sec23p bind to adjacent regions of Sec16p." Mol Biol Cell 7(11): 1815-1823.

Giussani, P., L. Brioschi, R. Bassi, L. Riboni and P. Viani (2009). "Phosphatidylinositol 3-kinase/AKT pathway regulates the endoplasmic reticulum to golgi traffic of ceramide in glioma cells: a link between lipid signaling pathways involved in the control of cell survival." J Biol Chem 284(8): 5088-5096.

Glick, B. S., T. Elston and G. Oster (1997). "A cisternal maturation mechanism can explain the asymmetry of the Golgi stack." FEBS Lett 414: 177-181.

Goedert, M., A. Cuenda, M. Craxton, R. Jakes and P. Cohen (1997). "Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases." EMBO J 16(12): 3563-3571.

Goldstein, J. L., R. B. Rawson and M. S. Brown (2002). "Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis." Arch Biochem Biophys 397(2): 139-148.

Gómez Ravetti, M., O. A. Rosso, R. Berretta and P. Moscato (2010). "Uncovering molecular biomarkers that correlate cognitive decline with the changes of hippocampus' gene expression profiles in Alzheimer's disease." PLoS One 5: e10153.

Gong, X., X. Ming, P. Deng and Y. Jiang (2010). "Mechanisms regulating the nuclear translocation of p38 MAP kinase." J Cell Biochem 110(6): 1420-1429.

Görlich, D., S. Prehn, E. Hartmann, K. U. Kalies and T. A. Rapoport (1992). "A mammalian homolog of SEC61p and CYp is associated with ribosomes and nascent polypeptides during translocation." Cell 71(3): 489-503.

Görlich, D. and T. A. Rapoport (1993). "Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane." Cell 75(4): 615-630.

Gould, S. J., D. McCollum, A. P. Spong, J. A. Heyman and S. Subramani (1992). "Development of the yeast Pichia pastoris as a model organism for a genetic and molecular analysis of peroxisome assembly." Yeast 8(8): 613-628.

Graef, M., J. R. Friedman, C. Graham, M. Babu and J. Nunnari (2013). "ER exit sites are physical and functional core autophagosome biogenesis components." Mol Biol Cell 24(18): 2918-2931.

Gregg, J. and G. Fraizer (2011). "Transcriptional Regulation of EGR1 by EGF and the ERK Signaling Pathway in Prostate Cancer Cells." Genes Cancer 2(9): 900-909.

Groehler, A. L. and D. A. Lannigan (2010). "A chromatin-bound kinase, ERK8, protects genomic integrity by inhibiting HDM2-mediated degradation of the DNA clamp PCNA." J Cell Biol 190(4): 575-586.

Groom, L. A., A. A. Sneddon, D. R. Alessi, S. Dowd and S. M. Keyse (1996). "Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase." EMBO J. 15: 3621-3632.

Grove, D. E., C. Y. Fan, H. Y. Ren and D. M. Cyr (2011). "The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508." Mol Biol Cell 22(3): 301-314.

Guerin, M. and A. J. Parodi (2003). "The UDP-glucose:glycoprotein glucosyltransferase is organized in at least two tightly bound domains from yeast to mammals." J Biol Chem 278(23): 20540-20546.

Guo, Y. and A. D. Linstedt (2006). "COPII-Golgi protein interactions regulate COPII coat assembly and Golgi size." J Cell Biol 174(1): 53-63.

Gupta, S., T. Barrett, A. J. Whitmarsh, J. Cavanagh, H. K. Sluss, B. Derijard and R. J. Davis (1996). "Selective interaction of JNK protein kinase isoforms with transcription factors." EMBO J 15(11): 2760-2770.

Gupta, V. and G. Swarup (2006). "Evidence for a role of transmembrane protein p25 in localization of protein tyrosine phosphatase TC48 to the ER." J Cell Sci 119(Pt 9): 1703-1714.

Guttinger, S., E. Laurell and U. Kutay (2009). "Orchestrating nuclear envelope disassembly and reassembly during mitosis." Nat Rev Mol Cell Biol 10(3): 178-191.

Hacki, J., L. Egger, L. Monney, S. Conus, T. Rosse, I. Fellay and C. Borner (2000). "Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2." Oncogene 19(19): 2286-2295.

Haigh, N. G. and A. E. Johnson (2002). "A new role for BiP: closing the aqueous translocon pore during protein integration into the ER membrane." J Cell Biol 156(2): 261-270.

Hailey, D. W., A. S. Rambold, P. Satpute-Krishnan, K. Mitra, R. Sougrat, P. K. Kim and J. Lippincott-Schwartz (2010). "Mitochondria supply membranes for autophagosome biogenesis during starvation." Cell 141(4): 656-667.

Halic, M., M. Blau, T. Becker, T. Mielke, M. R. Pool, K. Wild, I. Sinning and R. Beckmann (2006). "Following the signal sequence from ribosomal tunnel exit to signal recognition particle." Nature 444(7118): 507-511.

Halperin, L., J. Jung and M. Michalak (2014). "The many functions of the endoplasmic reticulum chaperones and folding enzymes." IUBMB Life 66(5): 318-326.

Hamasaki, M., N. Furuta, A. Matsuda, A. Nezu, A. Yamamoto, N. Fujita, H. Oomori, T. Noda, T. Haraguchi, Y. Hiraoka, A. Amano and T. Yoshimori (2013). "Autophagosomes form at ER-mitochondria contact sites." Nature 495(7441): 389-393.

Hamasaki, M., T. Noda and Y. Ohsumi (2003). "The early secretory pathway contributes to autophagy in yeast." Cell Struct Funct 28(1): 49-54.

Hamman, B. D., L. M. Hendershot and A. E. Johnson (1998). "BiP maintains the permeability barrier of the ER membrane by sealing the lumenal end of the translocon pore before and early in translocation." Cell 92(6): 747-758.

Hammond, A. T. and B. S. Glick (2000). "Dynamics of transitional endoplasmic reticulum sites in vertebrate cells." Mol Biol Cell 11(9): 3013-3030.

Hammond, C., I. Braakman and A. Helenius (1994). "Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control." Proc Natl Acad Sci U S A 91(3): 913-917.

Han, J., Y. Jiang, Z. Li, V. V. Kravchenko and R. J. Ulevitch (1997). "Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation." Nature 386(6622): 296-299.

Han, J., J. D. Lee, Y. Jiang, Z. Li, L. Feng and R. J. Ulevitch (1996). "Characterization of the structure and function of a novel MAP kinase kinase (MKK6)." J Biol Chem 271(6): 2886-2891.

Hanton, S. L., L. E. Bartolotti, L. Renna, G. Stefano and F. Brandizzi (2005). "Crossing the divide--transport between the endoplasmic reticulum and Golgi apparatus in plants." Traffic 6(267-77).

Hanus, C. and M. D. Ehlers (2008). "Secretory outposts for the local processing of membrane cargo in neuronal dendrites." Traffic 9(9): 1437-1445.

Hanus, C., L. Kochen, S. Tom Dieck, V. Racine, J. B. Sibarita, E. M. Schuman and M. D. Ehlers (2014). "Synaptic control of secretory trafficking in dendrites." Cell Rep 7(6): 1771-1778.

Hara-Kuge, S., O. Kuge, L. Orci, M. Amherdt, M. Ravazzola, F. T. Wieland and J. E. Rothman (1994). "En bloc incorporation of coatomer subunits during the assembly of COP-coated vesicles." J Cell Biol 124: 883-892.

Hara-Kuge, S., T. Ohkura, H. Ideo, O. Shimada, S. Atsumi and K. Yamashita (2002). "Involvement of VIP36 in intracellular transport and secretion of glycoproteins in polarized Madin-Darby canine kidney (MDCK) cells." J Biol Chem 277: 16332-16339.

Hara, K., Y. Maruki, X. Long, K. Yoshino, N. Oshiro, S. Hidayat, C. Tokunaga, J. Avruch and K. Yonezawa (2002). "Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action." Cell 110(2): 177-189.

Harding, H. P., I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira and D. Ron (2000). "Regulated translation initiation controls stress-induced gene expression in mammalian cells." Mol Cell 6(5): 1099-1108.

Harding, H. P., Y. Zhang, A. Bertolotti, H. Zeng and D. Ron (2000). "Perk is essential for translational regulation and cell survival during the unfolded protein response." Mol Cell 5(5): 897-904.

Harding, H. P., Y. Zhang and D. Ron (1999). "Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase." Nature 397(6716): 271-274.

Harding, H. P., Y. Zhang, H. Zeng, I. Novoa, P. D. Lu, M. Calfon, N. Sadri, C. Yun, B. Popko, R. Paules, D. F. Stojdl, J. C. Bell, T. Hettmann, J. M. Leiden and D. Ron (2003). "An integrated stress response regulates amino acid metabolism and resistance to oxidative stress." Mol Cell 11(3): 619-633.

Hardwick, K. G., J. C. Boothroyd, A. D. Rudner and H. R. Pelham (1992). "Genes that allow yeast cells to grow in the absence of the HDEL receptor." EMBO J 11(11): 4187-4195.

Hariri, H., N. Bhattacharya, K. Johnson, A. J. Noble and S. M. Stagg (2014). "Insights into the mechanisms of membrane curvature and vesicle scission by the small GTPase Sar1 in the early secretory pathway." J Mol Biol 426(22): 3811-3826.

Hasygar, K. and V. Hietakangas (2014). "p53- and ERK7-dependent ribosome surveillance response regulates Drosophila insulin-like peptide secretion." PLoS Genet 10(11): e1004764.

Hauri, H. P., F. Kappeler, H. Andersson and C. Appenzeller (2000). "ERGIC-53 and traffic in the secretory pathway." J Cell Sci 113: 587-596.

Hayashi-Nishino, M., N. Fujita, T. Noda, A. Yamaguchi, T. Yoshimori and A. Yamamoto (2009). "A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation." Nat Cell Biol 11(12): 1433-1437.

Hayashi-Nishino, M., N. Fujita, T. Noda, A. Yamaguchi, T. Yoshimori and A. Yamamoto (2010). "Electron tomography reveals the endoplasmic reticulum as a membrane source for autophagosome formation." Autophagy 6(2): 301-303.

Haze, K., H. Yoshida, H. Yanagi, T. Yura and K. Mori (1999). "Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress." Mol Biol Cell 10(11): 3787-3799.

Hebert, D. N., B. Foellmer and A. Helenius (1995). "Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum." Cell 81(3): 425-433.

Hebert, D. N. and M. Molinari (2007). "In and Out of the ER: Protein Folding, Quality Control, Degradation, and Related Human Diseases." Physiol Rev 87(4): 1377-1408.

Heinzer, S., S. Worz, C. Kalla, K. Rohr and M. Weiss (2008). "A model for the self-organization of exit sites in the endoplasmic reticulum." J Cell Sci 121(Pt 1): 55-64.

Helenius, A. and M. Aebi (2004). "Roles of N-linked glycans in the endoplasmic reticulum." Annu Rev Biochem 73: 1019-1049.

Helm, J. R., M. Bentley, K. D. Thorsen, T. Wang, L. Foltz, V. Oorschot, J. Klumperman and J. C. Hay (2014). "Apoptosis-linked gene-2 (ALG-2)/Sec31 interactions regulate endoplasmic reticulum (ER)-to-Golgi transport: a potential effector pathway for luminal calcium." J Biol Chem 289(34): 23609-23628.

Hendrickx, A., N. Pierrot, B. Tasiaux, O. Schakman, P. Kienlen-Campard, C. De Smet and J. N. Octave (2014). "Epigenetic regulations of immediate early genes expression involved in memory formation by the amyloid precursor protein of Alzheimer disease." PLoS One 9: e99467.

Herndon, C. A., N. Ankenbruck and L. Fromm (2014). "The Erk MAP kinase pathway is activated at muscle spindles and is required for induction of the muscle spindle-specific gene Egr3 by neuregulin1." J Neurosci Res 92: 174-184.

Herzig, Y., H. J. Sharpe, Y. Elbaz, S. Munro and M. Schuldiner (2012). "A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14." PLoS One 10: e1001329.

Hesketh, T. R., M. A. Beaven, J. Rogers, B. Burke and G. B. Warren (1984). "Stimulated release of histamine by a rat mast cell line is inhibited during mitosis." J Cell Biol 98(6): 2250-2254.

Hetz, C., P. Bernasconi, J. Fisher, A. H. Lee, M. C. Bassik, B. Antonsson, G. S. Brandt, N. N. Iwakoshi, A. Schinzel, L. H. Glimcher and S. J. Korsmeyer (2006). "Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha." Science 312(5773): 572-576.

Hetz, C. and E. Chevet (2015). "Theme Series - UPR in cancer." Semin Cancer Biol.

Higashio, H. and K. Kohno (2002). "A genetic link between the unfolded protein response and vesicle formation from the endoplasmic reticulum." Biochem Biophys Res Commun 296(3): 568-574.

Hino, T., Y. Tanaka, M. Kawamukai, K. Nishimura, S. Mano and T. Nakagawa (2011). "Two Sec13p homologs, AtSec13A and AtSec13B, redundantly contribute to the formation of COPII transport vesicles in Arabidopsis thaliana." Biosci Biotechnol Biochem 75(9): 1848-1852.

Honda, T., Y. Obara, A. Yamauchi, A. D. Couvillon, J. J. Mason, K. Ishii and N. Nakahata (2015). "Phosphorylation of ERK5 on Thr732 is associated with ERK5 nuclear localization and ERK5-dependent transcription." PLoS One 10(2): e0117914.

Horibe, T., M. Gomi, D. Iguchi, H. Ito, Y. Kitamura, T. Masuoka, I. Tsujimoto, T. Kimura and M. Kikuchi (2004). "Different contributions of the three CXXC motifs of human protein-disulfide isomerase-related protein to isomerase activity and oxidative refolding." J Biol Chem 279(6): 4604-4611.

Horton, A. C. and M. D. Ehlers (2003). "Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging." J Neurosci 23(15): 6188-6199.

Horton, J. D., J. C. Cohen and H. H. Hobbs (2009). "PCSK9: a convertase that coordinates LDL catabolism." J Lipid Res 50 Suppl: S172-177.

Hotta, K., M. Nakamura, T. Nakamura, T. Matsuo, Y. Nakata, S. Kamohara, N. Miyatake, K. Kotani, R. Komatsu, N. Itoh, I. Mineo, J. Wada, H. Masuzaki, M. Yoneda, A. Nakajima, T. Funahashi, S. Miyazaki, K. Tokunaga, M. Kawamoto, T. Ueno, K. Hamaguchi, K. Tanaka, K. Yamada, T. Hanafusa, S. Oikawa, H. Yoshimatsu, K. Nakao, T. Sakata, Y. Matsuzawa, N. Kamatani and Y. Nakamura (2009). "Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population." J Hum Genet 54(12): 727-731.

Hu, G., T. Gura, B. Sabsay, J. Sauk, S. N. Dixit and A. Veis (1995). "Endoplasmic reticulum protein Hsp47 binds specifically to the N-terminal globular domain of the amino-propeptide of the procollagen I alpha 1 (I)-chain." J Cell Biochem 59(3): 350-367.

Huang, C., W. Y. Ma, A. Maxiner, Y. Sun and Z. Dong (1999). "p38 kinase mediates UV-induced phosphorylation of p53 protein at serine 389." J Biol Chem 274(18): 12229-12235.

Huang, H., H. Liu, C. Liu, L. Fan, X. Zhang, A. Gao, X. Hu, K. Zhang, X. Cao, K. Jiang, Y. Zhou, J. Hou, F. Nan and J. Li (2015). "Disruption of the unfolded protein response (UPR) by lead compound selectively suppresses cancer cell growth." Cancer Lett 360(2): 257-268.

Huang, M., J. T. Weissman, S. Beraud-Dufour, P. Luan, C. Wang, W. Chen, M. Aridor, I. A. Wilson and W. E. Balch (2001). "Crystal structure of Sar1-GDP at 1.7 A resolution and the role of the NH2 terminus in ER export." J Cell Biol 155(6): 937-948.

Huang, R. P., Y. Fan, I. de Belle, C. Niemeyer, M. M. Gottardis, D. Mercola and E. D. Adamson (1997). "Decreased Egr-1 expression in human, mouse and rat mammary cells and tissues correlates with tumor formation." Int J Cancer 72(1): 102-109.

Hughes, H., A. Budnik, K. Schmidt, K. J. Palmer, J. Mantell, C. Noakes, A. Johnson, D. A. Carter, P. Verkade, P. Watson and D. J. Stephens (2009). "Organisation of human ER-exit sites: requirements for the localisation of Sec16 to transitional ER." J Cell Sci 122(Pt 16): 2924-2934.

Hughes, H. and D. J. Stephens (2010). "Sec16A defines the site for vesicle budding from the endoplasmic reticulum on exit from mitosis." J Cell Sci 123(Pt 23): 4032-4038.

Hughson, F. M. (2010). "Copy coats: COPI mimics clathrin and COPII." Cell 142(1): 19-21.

Hunter, T. (1998). "The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease." Philos Trans R Soc Lond B Biol Sci 353: 583-605.

Iavarone, C., M. Acunzo, F. Carlomagno, A. Catania, R. M. Melillo, S. M. Carlomagno, M. Santoro and M. Chiariello (2006). "Activation of the Erk8 mitogen-activated protein (MAP) kinase by RET/PTC3, a constitutively active form of the RET proto-oncogene." J Biol Chem 281(15): 10567-10576.

Ichijo, H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono and Y. Gotoh (1997). "Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways." Science 275(5296): 90-94.

Ihara, Y., M. F. Cohen-Doyle, Y. Saito and D. B. Williams (1999). "Calnexin discriminates between protein conformational states and functions as a molecular chaperone in vitro." Mol Cell 4(3): 331-341.

Iinuma, T., A. Shiga, K. Nakamoto, M. B. O'Brien, M. Aridor, N. Arimitsu, M. Tagaya and K. Tani (2007). "Mammalian Sec16/p250 plays a role in membrane traffic from the endoplasmic reticulum." J Biol Chem 282(24): 17632-17639.

Imajo, M., Y. Tsuchiya and E. Nishida (2006). "Regulatory Mechanisms and Functions of MAP Kinase Signaling Pathways." IUBMB Life 58: 312-317.

Inoki, K., Y. Li, T. Xu and K. L. Guan (2003). "Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling." Genes Dev 17(15): 1829-1834.

Inoki, K., Y. Li, T. Zhu, J. Wu and K. L. Guan (2002). "TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling." Nat Cell Biol 4(9): 648-657.

Inoue, A., Y. Omoto, Y. Yamaguchi, R. Kiyama and S. I. Hayashi (2004). "Transcription factor EGR3 is involved in the estrogen-signaling pathway in breast cancer cells." J Mol Endocrinol 32: 649-661.

Inoue, H., T. Baba, S. Sato, R. Ohtsuki, A. Takemori, T. Watanabe, M. Tagaya and K. Tani (2012). "Roles of SAM and DDHD domains in mammalian intracellular phospholipase A1 KIAA0725p." Biochim Biophys Acta 1823(4): 930-939.

Iordanov, M., K. Bender, T. Ade, W. Schmid, C. Sachsenmaier, K. Engel, M. Gaestel, H. J. Rahmsdorf and P. Herrlich (1997). "CREB is activated by UVC through a p38/HOG-1-dependent protein kinase." EMBO J 16(5): 1009-1022.

Ishihara, N., M. Hamasaki, S. Yokota, K. Suzuki, Y. Kamada, A. Kihara, T. Yoshimori, T. Noda and Y. Ohsumi (2001). "Autophagosome requires specific early Sec proteins for its

formation and NSF/SNARE for vacuolar fusion." Mol Biol Cell 12(11): 3690-3702.

Itakura, E., C. Kishi-Itakura and N. Mizushima (2012). "The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes." Cell 151(6): 1256-1269.

Itakura, E. and N. Mizushima (2010). "Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins." Autophagy 6(6): 764-776.

Ivan, V., G. de Voer, D. Xanthakis, K. M. Spoorendonk, V. Kondylis and C. Rabouille (2008). "Drosophila Sec16 mediates the biogenesis of tER sites upstream of Sar1 through an arginine-rich motif." Mol Biol Cell 19(10): 4352-4365.

Iyer, S. C., E. P. Ramachandran Iyer, R. Meduri, M. Rubaharan, A. Kuntimaddi, M. Karamsetty and D. N. Cox (2013). "Cut, via CrebA, transcriptionally regulates the COPII secretory pathway to direct dendrite development in Drosophila." J Cell Sci 126(Pt 20): 4732-4745.

Jacinto, E., V. Facchinetti, D. Liu, N. Soto, S. Wei, S. Y. Jung, Q. Huang, J. Qin and B. Su (2006). "SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity." Cell 127(1): 125-137.

Jacinto, E., R. Loewith, A. Schmidt, S. Lin, M. A. Ruegg, A. Hall and M. N. Hall (2004). "Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive." Nat Cell Biol 6(11): 1122-1128.

Jackson, C. L. (2009). "Mechanisms of transport through the Golgi complex." J Cell Sci 122: 443-452.

Jackson, C. L. (2014). "GEF-effector interactions." Cell Logist 4(2): e943616.

Jackson, L. P. (2014). "Structure and mechanism of COPI vesicle biogenesis." Curr Opin Cell Biol 29: 67-73.

Jackson, L. P., M. Lewis, H. M. Kent, M. A. Edeling, P. R. Evans, R. Duden and D. J. Owen (2012). "Molecular basis for recognition of dilysine trafficking motifs by COPI." Dev Cell 23: 1255-1262.

Jackson, M. R., T. Nilsson and P. A. Peterson (1990). "Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum." EMBO J. 9: 3153-3162.

Jacobs, D., D. Glossip, H. Xing, A. J. Muslin and K. Kornfeld (1999). "Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase." Genes Dev 13: 163-175.

Jaleel, M., R. J. Nichols, M. Deak, D. G. Campbell, F. Gillardon, A. Knebel and D. R. Alessi (2007). "LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity." Biochem J 405(2): 307-317.

Jenning, S., T. Pham, S. K. Ireland, E. Ruoslahti and H. Biliran (2013). "Bit1 in anoikis resistance and tumor metastasis." Cancer Lett 333(2): 147-151.

Jesch, S. A., T. S. Lewis, N. G. Ahn and A. D. Linstedt (2001). "Mitotic phosphorylation of Golgi reassembly stacking protein 55 by mitogen-activated protein kinase ERK2." Mol Biol Cell 12(6): 1811-1817.

Jesch, S. A. and A. D. Linstedt (1998). "The Golgi and endoplasmic reticulum remain independent during mitosis in HeLa cells." Mol Biol Cell 9(3): 623-635.

Jiang, H. Y. and R. C. Wek (2005). "Phosphorylation of the alpha-subunit of the eukaryotic initiation factor-2 (eIF2alpha) reduces protein synthesis and enhances apoptosis in response to proteasome inhibition." J Biol Chem 280(14): 14189-14202.

Jiang, Y., H. Gram, M. Zhao, L. New, J. Gu, L. Feng, F. Di Padova, R. J. Ulevitch and J. Han (1997). "Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta." J Biol Chem 272(48): 30122-30128.

Jin, L., K. B. Pahuja, K. E. Wickliffe, A. Gorur, C. Baumgartel, R. Schekman and M. Rape (2012). "Ubiquitin-dependent regulation of COPII coat size and function." Nature 482(7386): 495-500.

Johnson, A., N. Bhattacharya, M. Hanna, J. G. Pennington, A. L. Schuh, L. Wang, M. S. Otegui, S. M. Stagg and A. Audhya (2015). "TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization." EMBO J.

Johnson, G. L. and K. Nakamura (2007). "The c-jun kinase/stress-activated pathway: regulation, function and role in human disease." Biochim Biophys Acta 1773: 1341-1348.

Jones, B., E. L. Jones, S. A. Bonney, H. N. Patel, A. R. Mensenkamp, S. Eichenbaum-Voline, M. Rudling, U. Myrdal, G. Annesi, S. Naik, N. Meadows, A. Quattrone, S. A. Islam, R. P. Naoumova, B. Angelin, R. Infante, E. Levy, C. C. Roy, P. S. Freemont, J. Scott and C. C. Shoulders (2003). "Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders." Nat Genet 34(1): 29-31.

Jones, M. W., M. L. Errington, P. J. French, A. Fine, T. V. Bliss, S. Garel, P. Charnay, B. Bozon, S. Laroche and S. Davis (2001). "A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories." Nat Neurosci 4: 289-296.

Joseph, L. J., M. M. Le Beau, G. A. J. Jamieson, S. Acharya, T. B. Shows, J. D. Rowley and V. P. Sukhatme (1988). "Molecular cloning, sequencing, and mapping of EGR2, a human early growth response gene encoding a protein with "zinc-binding finger" structure." Proc Natl Acad Sci U S A 85: 7164-7168.

Joslin, J. M., A. A. Fernald, T. R. Tennant, E. M. Davis, S. C. Kogan, J. Anastasi, J. D. Crispino and M. M. Le Beau (2007). "Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders." Blood 110(2): 719-726.

Junttila, M. R., R. Ala-Aho, T. Jokilehto, J. Peltonen, M. Kallajoki, R. Grenman, P. Jaakkola, J. Westermarck and V. M. Kahari (2007). "p38alpha and p38delta mitogen-activated protein kinase isoforms regulate invasion and growth of head and neck squamous carcinoma cells." Oncogene 26(36): 5267-5279.

Kabani, M., S. S. Kelley, M. W. Morrow, D. L. Montgomery, R. Sivendran, M. D. Rose, L. M. Gierasch and J. L. Brodsky (2003). "Dependence of endoplasmic reticulum-associated degradation on the peptide binding domain and concentration of BiP." Mol Biol Cell 14(8): 3437-3448.

Kahn, R. A., P. Randazzo, T. Serafini, O. Weiss, C. Rulka, J. Clark, M. Amherdt, P. Roller, L. Orci and J. E. Rothman (1992). "The amino terminus of ADP-ribosylation factor (ARF) is a critical determinant of ARF activities and is a potent and specific inhibitor of protein transport." J Biol Chem 267(18): 13039-13046.

Kairouz-Wahbe, R., H. Biliran, X. Luo, I. Khor, M. Wankell, C. Besch-Williford, J. Pascual, R. Oshima and E. Ruoslahti (2008). "Anoikis effector Bit1 negatively regulates Erk activity." Proc Natl Acad Sci U S A 105(5): 1528-1532.

Kaiser, C. A. and R. Schekman (1990). "Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway." Cell 61(4): 723-733.

Kaizuka, T., T. Hara, N. Oshiro, U. Kikkawa, K. Yonezawa, K. Takehana, S. Iemura, T. Natsume and N. Mizushima (2010). "Tti1 and Tel2 are critical factors in mammalian target of

rapamycin complex assembly." J Biol Chem 285(26): 20109-20116.

Kamena, F. and A. Spang (2004). "Tip20p prohibits back-fusion of COPII vesicles with the endoplasmic reticulum." Science 304: 286-289.

Kamiya, Y., D. Kamiya, K. Yamamoto, B. Nyfeler, H. P. Hauri and K. Kato (2008). "Molecular basis of sugar recognition by the human L-type lectins ERGIC-53, VIPL, and VIP36." J Biol Chem 283: 1857-1861.

Kanapin, A., S. Batalov, M. J. Davis, J. Gough, S. Grimond, H. Kawaji, M. Magrane, H. Matsuda, C. Schönbach, R. D. Teasdale, Z. Yuan, R. G. Group and G. Members (2003). "Mouse proteome analysis." Genome Res 13: 1335-1344.

Kano, F., A. R. Tanaka, S. Yamauchi, H. Kondo and M. Murata (2004). "Cdc2 kinase-dependent disassembly of endoplasmic reticulum (ER) exit sites inhibits ER-to-Golgi vesicular transport during mitosis." Mol Biol Cell 15(9): 4289-4298.

Kapoor, M., H. Srinivas, E. Kandiah, E. Gemma, L. Ellgaard, S. Oscarson, A. Helenius and A. Surolia (2003). "Interactions of substrate with calreticulin, an endoplasmic reticulum chaperone." J Biol Chem 278(8): 6194-6200.

Kartberg, F., L. Asp, S. Y. Dejgaard, M. Smedh, J. Fernandez-Rodriguez, T. Nilsson and J. F. Presley (2010). "ARFGAP2 and ARFGAP3 are essential for COPI coat assembly on the Golgi membrane of living cells." J Biol Chem 285(47): 36709-36720.

Kasler, H. G., J. Victoria, O. Duramad and A. Winoto (2000). "ERK5 is a novel type of mitogen-activated protein kinase containing a transcriptional activation domain." Mol Cell Biol 20(22): 8382-8389.

Kato, Y., V. V. Kravchenko, R. I. Tapping, J. Han, R. J. Ulevitch and J. D. Lee (1997). "BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C." EMBO J 16(23): 7054-7066.

Kato, Y., R. I. Tapping, S. Huang, M. H. Watson, R. J. Ulevitch and J. D. Lee (1998). "Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor." Nature 395(6703): 713-716.

Kaufman, R. J. (2002). "Orchestrating the unfolded protein response in health and disease." J Clin Invest 110(10): 1389-1398.

Kawamoto, K., Y. Yoshida, H. Tamaki, S. Torii, C. Shinotsuka, S. Yamashina and K. Nakayama (2002). "GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the cis-Golgi and involved in membrane association of the COPI coat." Traffic 3(7): 483-495.

Kawasaki, N., Y. Ichikawa, I. Matsuo, K. Totani, N. Matsumoto, Y. Ito and K. Yamamoto (2008). "The sugar-binding ability of ERGIC-53 is enhanced by its interaction with MCFD2." Blood 111(4): 1972-1979.

Kennedy, N. J., C. Cellurale and R. J. Davis (2007). "A Radical Role for p38 MAPK in Tumor Initiation." Cancer Cell 11: 101-103.

Kharbanda, S., T. Nakamura, R. Stone, R. Hass, S. Bernstein, R. Datta, V. P. Sukhatme and D. Kufe (1991). "Expression of the early growth response 1 and 2 zinc finger genes during induction of monocytic differentiation." J Clin Invest 88: 571-577.

Khokhlatchev, A. V., B. Canagarajah, J. Wilsbacher, M. Robinson, M. Atkinson, E. Goldsmith and M. H. Cobb (1998). "Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation." Cell 93(4): 605-615.

Khoriaty, R., M. P. Vasievich, M. Jones, L. Everett, J. Chase, J. Tao, D. Siemieniak, B. Zhang, I. Maillard and D. Ginsburg (2014). "Absence of a red blood cell phenotype in mice with hematopoietic deficiency of SEC23B." Mol Cell Biol 34(19): 3721-3734.

Kim, A. H., H. Yano, H. J. Cho, D. I. Meyer, B. Monks, B. Margolis, M. J. Birnbaum and M. V. Chao (2002). "Akt1 regulates a JNK scaffold during excitotoxic apoptosis." Neuron 35: 697-709.

Kim, D. H., D. D. Sarbassov, S. M. Ali, J. E. King, R. R. Latek, H. Erdjument-Bromage, P. Tempst and D. M. Sabatini (2002). "mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery." Cell 110(2): 163-175.

Kim, D. H., D. D. Sarbassov, S. M. Ali, R. R. Latek, K. V. Guntur, H. Erdjument-Bromage, P. Tempst and D. M. Sabatini (2003). "GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR." Mol Cell 11(4): 895-904.

Kim, E. K. and E. J. Choi (2010). "Pathological roles of MAPK signaling pathways in human diseases." Biochim Biophys Acta 1802: 396-405.

Kim, H., A. Bhattacharya and L. Qi (2015). "Endoplasmic reticulum quality control in cancer: Friend or foe." Semin Cancer Biol.

Kim, M. S., E. G. Jeong, N. J. Yoo and S. H. Lee (2008). "Mutational analysis of oncogenic AKT E17K mutation in common solid cancers and acute leukaemias." Br J Cancer 98(9): 1533-1535.

Kim, S. H., J. Y. Song, E. J. Joo, K. Y. Lee, Y. M. Ahn and Y. S. Kim (2010). "EGR3 as a potential susceptibility gene for schizophrenia in Korea." Am J Med Genet B Neuropsychiatr Genet 153B: 1355-1360.

Kinseth, M. A., C. Anjard, D. Fuller, G. Guizzunti, W. F. Loomis and V. Malhotra (2007). "The Golgi-associated protein GRASP is required for unconventional protein secretion during development." Cell 130(3): 524-534.

Klee, M., K. Pallauf, S. Alcala, A. Fleischer and F. X. Pimentel-Muinos (2009). "Mitochondrial apoptosis induced by BH3-only molecules in the exclusive presence of endoplasmic reticular Bak." EMBO J 28(12): 1757-1768.

Klevernic, I. V., M. J. Stafford, N. Morrice, M. Peggie, S. Morton and P. Cohen (2006). "Characterization of the reversible phosphorylation and activation of ERK8." Biochem J 394(Pt 1): 365-373.

Klinkenberg, D., K. R. Long, K. Shome, S. C. Watkins and M. Aridor (2014). "A cascade of ER exit site assembly that is regulated by p125A and lipid signals." J Cell Sci 127(Pt 8): 1765-1778.

Kodera, C., T. Yorimitsu, A. Nakano and K. Sato (2011). "Sed4p stimulates Sar1p GTP hydrolysis and promotes limited coat disassembly." Traffic 12(5): 591-599.

Kodera, C., T. Yorimitsu and K. Sato (2014). "Sec23 homolog Nel1 is a novel GTPase-activating protein for Sar1 but does not function as a subunit of the coat protein complex II (COPII) coat." J Biol Chem 289(31): 21423-21432.

Kojima, E., A. Takeuchi, M. Haneda, A. Yagi, T. Hasegawa, K. Yamaki, K. Takeda, S. Akira, K. Shimokata and K. Isobe (2003). "The function of GADD34 is a recovery from a shutoff of protein synthesis induced by ER stress: elucidation by GADD34-deficient mice." FASEB J 17(11): 1573-1575.

Koldamova, R., J. Schug, M. Lefterova, A. A. Cronican, N. F. Fitz, F. A. Davenport, A. Carter, E. L. Castranio and I. Lefterov (2014). "Genome-wide approaches reveal EGR1-controlled

regulatory networks associated with neurodegeneration." Neuobiol Dis 63: 107-114.

Kondylis, V. and C. Rabouille (2003). "A novel role for dp115 in the organization of tER sites in Drosophila." J Cell Biol 162(2): 185-198.

Kondylis, V. and C. Rabouille (2009). "The Golgi apparatus: lessons from Drosophila." FEBS Lett 283(23): 3827-3838.

Kondylis, V., Y. Tang, F. Fuchs, M. Boutros and C. Rabouille (2011). "Identification of ER proteins involved in the functional organisation of the early secretory pathway in Drosophila cells by a targeted RNAi screen." PLoS One 6(2): e17173.

Kondylis, V., H. E. van Nispen tot Pannerden, B. Herpers, F. Friggi-Grelin and C. Rabouille (2007). "The golgi comprises a paired stack that is separated at G2 by modulation of the actin cytoskeleton through Abi and Scar/WAVE." Dev Cell 12(6): 901-915.

Kook, S., X. Zhan, T. S. Kaoud, K. N. Dalby, V. V. Gurevich and E. V. Gurevich (2013). "Arrestin-3 binds c-Jun N-terminal kinase 1 (JNK1) and JNK2 and facilitates the activation of these ubiquitous JNK isoforms in cells via scaffolding." J Biol Chem 288: 37332-37342.

Koreishi, M., S. Yu, M. Oda, Y. Honjo and A. Satoh (2013). "CK2 phosphorylates Sec31 and regulates ER-To-Golgi trafficking." PLoS One 8(1): e54382.

Kornfeld, K., D. B. Hom and H. R. Horvitz (1995). "The ksr-1 gene encodes a novel protein kinase involved in Ras-mediated signaling in C. elegans." Cell 83: 903-913.

Kozlov, G., C. L. Pocanschi, A. Rosenauer, S. Bastos-Aristizabal, A. Gorelik, D. B. Williams and K. Gehring (2010). "Structural basis of carbohydrate recognition by calreticulin." J Biol Chem 285(49): 38612-38620.

Kreiner, T. and H. P. Moore (1990). "Membrane traffic between secretory compartments is differentially affected during mitosis." Cell Regul 1(5): 415-424.

Kubota, H. and K. Nagata (2004). "Roles of collagen fibers and its specific molecular chaperone: analysis using HSP47-knockout mice." Biol Sci Space 18(3): 118-119.

Kuehn, M. J., J. M. Herrmann and R. Schekman (1998). "COPII-cargo interactions direct protein sorting into ER-derived transport vesicles." Nature 391(6663): 187-190.

Kuhajda, F. P. (2000). "Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology." Nutrition 16(3): 202-208.

Kumbrink, J., M. Gerlinger and J. P. Johnson (2005). "Egr-1 induces the expression of its corepressor nab2 by activation of the nab2 promoter thereby establishing a negative feedback loop." J Biol Chem 280: 42785-42793.

Kumbrink, J., K. H. Kirsch and J. P. Johnson (2010). "EGR1, EGR2, and EGR3 activate the expression of their coregulator NAB2 establishing a negative feedback loop in cells of neuroectodermal and epithelial origin." J Cell Biochem 111: 207-217.

Kung, L. F., S. Pagant, E. Futai, J. G. D'Arcangelo, R. Buchanan, J. C. Dittmar, R. J. Reid, R. Rothstein, S. Hamamoto, E. L. Snapp, R. Schekman and E. A. Miller (2012). "Sec24p and Sec16p cooperate to regulate the GTP cycle of the COPII coat." EMBO J 31(4): 1014-1027.

Kuo, W. L., C. J. Duke, M. K. Abe, E. L. Kaplan, S. Gomes and M. R. Rosner (2004). "ERK7 expression and kinase activity is regulated by the ubiquitin-proteosome pathway." J Biol Chem 279(22): 23073-23081.

Kurian, S. M., H. Le-Niculescu, S. D. Patel, D. Bertram, J. Davis, C. Dike, N. Yehyawi, P. Lysaker, J. Dustin, M. Caligiuri, J. Lohr, D. K. Lahiri, J. I. Nurnberger, Jr., S. V. Faraone, M. A. Geyer, M. T. Tsuang, N. J. Schork, D. R. Salomon and A. B. Niculescu (2011). "Identification of blood biomarkers for psychosis using convergent functional genomics." Mol Psychiatry 16(1): 37-58.

Kurihara, T., S. Hamamoto, R. E. Gimeno, C. A. Kaiser, R. Schekman and T. Yoshihisa (2000). "Sec24p and Iss1p function interchangeably in transport vesicle formation from the endoplasmic reticulum in Saccharomyces cerevisiae." Mol Biol Cell 11(3): 983-998.

Kwon, O., N. K. Soung, N. R. Thimmegowda, S. J. Jeong, J. H. Jang, D. O. Moon, J. K. Chung, K. S. Lee, Y. T. Kwon, R. L. Erikson, J. S. Ahn and B. Y. Kim (2012). "Patulin induces colorectal cancer cells apoptosis through EGR-1 dependent ATF3 up-regulation." Cell Signal 24: 943-950.

la Cour, J. M., A. J. Schindler, M. W. Berchtold and R. Schekman (2013). "ALG-2 attenuates COPII budding in vitro and stabilizes the Sec23/Sec31A complex." PLoS One 8(9): e75309.

Lang, M. R., L. A. Lapierre, M. Frotscher, J. R. Goldenring and E. W. Knapik (2006). "Secretory COPII coat component Sec23a is essential for craniofacial chondrocyte maturation." Nat Genet 38(10): 1198-1203.

Lanoix, J., J. Ouwendijk, C. C. Lin, A. Stark, H. D. Love, J. Ostermann and T. Nilsson (1999). "GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles." EMBO J 18(18): 4935-4948.

Laplante, M. and D. M. Sabatini (2009). "An emerging role of mTOR in lipid biosynthesis." Curr Biol 19(22): R1046-1052.

Laplante, M. and D. M. Sabatini (2012). "mTOR signaling in growth control and disease." Cell 149(2): 274-293.

Lappi, A. K., M. F. Lensink, H. I. Alanen, K. E. Salo, M. Lobell, A. H. Juffer and L. W. Ruddock (2004). "A conserved arginine plays a role in the catalytic cycle of the protein disulphide isomerases." J Mol Biol 335(1): 283-295.

Lavoie, J. N., G. L'Allemain, A. Brunet, R. Müller and J. Pouysségur (1996). "Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway." J Biol Chem 271: 20608-20616.

Le Breton, M., P. Cormier, R. Belle, O. Mulner-Lorillon and J. Morales (2005). "Translational control during mitosis." Biochimie 87(9-10): 805-811.

Lederkremer, G. Z., Y. Cheng, B. M. Petre, E. Vogan, S. Springer, R. Schekman, T. Walz and T. Kirchhausen (2001). "Structure of the Sec23p/24p and Sec13p/31p complexes of COPII." Proc Natl Acad Sci U S A 98(19): 10704-10709.

Lee, C. and J. Goldberg (2010). "Structure of coatomer cage proteins and the relationship among COPI, COPII, and clathrin vesicle coats." Cell 142(1): 123-132.

Lee, H., R. Chen, Y. Lee, S. Yoo and C. Lee (2009). "Essential roles of CKIdelta and CKIepsilon in the mammalian circadian clock." Proc Natl Acad Sci U S A 106(50): 21359-21364.

Lee, J. D., R. J. Ulevitch and J. Han (1995). "Primary structure of BMK1: a new mammalian map kinase." Biochem Biophys Res Commun 213(2): 715-724.

Lee, K., W. Tirasophon, X. Shen, M. Michalak, R. Prywes, T. Okada, H. Yoshida, K. Mori and R. J. Kaufman (2002). "IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response." Genes Dev 16(4): 452-466.

Lee, M. C., L. Orci, S. Hamamoto, E. Futai, M. Ravazzola and R. Schekman (2005). "Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle." Cell 122(4): 605-617.

Lee, S. L., Y. Sadovsky, A. H. Swirnoff, J. A. Polish, P. Goda, G. Gavrilina and J. Milbrandt (1996). "Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1)." Science 273: 1219-1221.

Lee, S. Y., J. S. Yang, W. Hong, R. T. Premont and V. W. Hsu (2005). "ARFGAP1 plays a central role in coupling COPI cargo sorting with vesicle formation." J Cell Biol 168(2): 281-290.

Lee, T. H. and A. D. Linstedt (2000). "Potential role for protein kinases in regulation of bidirectional endoplasmic reticulum-to-Golgi transport revealed by protein kinase inhibitor H89." Mol Biol Cell 11(8): 2577-2590.

Lei, K. and R. J. Davis (2003). "JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis." Proc Natl Acad Sci U S A 100(5): 2432-2437.

Lei, X., R. N. Bone, T. Ali, S. Zhang, A. Bohrer, H. M. Tse, K. R. Bidasee and S. Ramanadham (2014). "Evidence of contribution of iPLA2beta-mediated events during islet beta-cell apoptosis due to proinflammatory cytokines suggests a role for iPLA2beta in T1D development." Endocrinology 155(9): 3352-3364.

Levic, D. S., J. R. Minkel, W. D. Wang, W. M. Rybski, D. B. Melville and E. W. Knapik (2015). "Animal model of Sar1b deficiency presents lipid absorption deficits similar to Anderson disease." J Mol Med (Berl) 93(2): 165-176.

Levin, W. J., M. F. Press, R. B. Gaynor, V. P. Sukhatme, T. C. Boone, P. T. Reissmann, R. A. Figlin, E. C. Holmes, L. M. Souza and D. J. Slamon (1995). "Expression patterns of immediate early transcription factors in human non-small cell lung cancer. The Lung Cancer Study Group." Oncogene 11(7): 1261-1269.

Levine, T. P., C. Rabouille, R. H. Kieckbusch and G. Warren (1996). "Binding of the vesicle docking protein p115 to Golgi membranes is inhibited under mitotic conditions." J Biol Chem 271(29): 17304-17311.

Levy, E., E. Harmel, M. Laville, R. Sanchez, L. Emonnot, D. Sinnett, E. Ziv, E. Delvin, P. Couture, V. Marcil and A. T. Sane (2011). "Expression of Sar1b enhances chylomicron assembly and key components of the coat protein complex II system driving vesicle budding." Arterioscler Thromb Vasc Biol 31: 2692-2699.

Li, L., S. H. Yun, J. Keblesh, B. L. Trommer, H. Xiong, J. Radulovic and W. G. Tourellotte (2007). "Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory." Mol Cell Neurosci 35: 76-88.

Li, S., T. Miao, M. Sebastian, P. Bhullar, E. Ghaffari, M. Liu, A. L. Symonds and P. Wang (2012). "The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells." Immunity 37(4): 685-696.

Li, S., W. Ogawa, A. Emi, K. Hayashi, Y. Senga, K. Nomura, K. Hara, D. Yu and M. Kasuga (2011). "Role of S6K1 in regulation of SREBP1c expression in the liver." Biochem Biophys Res Commun 412(2): 197-202.

Li, Z., Y. Jiang, R. J. Ulevitch and J. Han (1996). "The primary structure of p38 gamma: a new member of p38 group of MAP kinases." Biochem Biophys Res Commun 228(2): 334-340.

Liang, J. O., T. C. Sung, A. J. Morris, M. A. Frohman and S. Kornfeld (1997). "Different domains of mammalian ADP-ribosylation factor 1 mediate interaction with selected target proteins." J Biol Chem 272(52): 33001-33008.

Liao, F., M. Y. Ji, L. Shen, S. Qiu, X. F. Guo and W. G. Dong (2013). "Decreased EGR3 expression is related to poor prognosis in patients with gastric cancer." J Mol Histol 44: 463-468.

Lim, J. H., J. W. Park, D. S. Min, J. S. Chang, Y. H. Lee, Y. B. Park, K. S. Choi and T. K. Kwon (2007). "NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells." Apoptosis 12(2): 411-421.

Lim, W. A., C. M. Lee and C. Tang (2013). "Design principles of regulatory networks: searching for the molecular algorithms of the cell." Mol Cell 49(2): 202-212.

Lin, C. Y., M. L. Madsen, F. R. Yarm, Y. J. Jang, X. Liu and R. L. Erikson (2000). "Peripheral Golgi protein GRASP65 is a target of mitotic polo-like kinase (Plk) and Cdc2." Proc Natl Acad Sci U S A 97(23): 12589-12594.

Linderoth, N. A., M. N. Simon, N. A. Rodionova, M. Cadene, W. R. Laws, B. T. Chait and S. Sastry (2001). "Biophysical analysis of the endoplasmic reticulum-resident chaperone/heat shock protein gp96/GRP94 and its complex with peptide antigen." Biochemistry 40(5): 1483-1495.

Lippincott-Schwartz, J., N. B. Cole, A. Marotta, P. A. Conrad and G. S. Bloom (1995). "Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic." J Cell Biol 128: 293-306.

Lippincott-Schwartz, J., T. H. Roberts and K. Hirschberg (2000). "Secretory protein trafficking and organelle dynamics in living cells." Annu Rev Cell Dev Biol 16: 557-589.

Lippincott-Schwartz, J., L. C. Yuan, J. S. Bonifacino and R. D. Klausner (1989). "Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER." Cell 56(5): 801-813.

Liu, C. Y., M. Schroder and R. J. Kaufman (2000). "Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum." J Biol Chem 275(32): 24881-24885.

Loftus, A. F., V. L. Hsieh and R. Parthasarathy (2012). "Modulation of membrane rigidity by the human vesicle trafficking proteins Sar1A and Sar1B." Biochem Biophys Res Commun 426(4): 585-589.

Long, K. R., Y. Yamamoto, A. L. Baker, S. C. Watkins, C. B. Coyne, J. F. Conway and M. Aridor (2010). "Sar1 assembly regulates membrane constriction and ER export." J Cell Biol 190(1): 115-128.

Lord, C., D. Bhandari, S. Menon, M. Ghassemian, D. Nycz, J. Hay, P. Ghosh and S. Ferro-Novick (2011). "Sequential interactions with Sec23 control the direction of vesicle traffic." Nature 473: 181-186.

Lowe, M. (2011). "Structural organization of the Golgi apparatus." Curr Opin Cell Biol 23(1): 85-93.

Lowe, M., C. Rabouille, N. Nakamura, R. Watson, M. Jackman, E. Jamsa, D. Rahman, D. J. Pappin and G. Warren (1998). "Cdc2 kinase directly phosphorylates the cis-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis." Cell 94(6): 783-793.

Lu, L., M. S. Ladinsky and T. Kirchhausen (2009). "Cisternal organization of the endoplasmic reticulum during mitosis." Mol Biol Cell 20(15): 3471-3480.

Lucocq, J. M., J. G. Pryde, E. G. Berger and G. Warren (1987). "A mitotic form of the Golgi apparatus in HeLa cells." J Cell Biol 104(4): 865-874.

Luini, A., G. Mavelli, J. Jung and J. Cancino (2014). "Control systems and coordination protocols of the secretory pathway." F1000Prime Rep 6: 88.

Lunin, V. V., C. Munger, J. Wagner, Z. Ye, M. Cygler and M. Sacher (2004). "The structure of the MAPK scaffold, MP1, bound to its partner, p14. A complex with a critical role in

endosomal map kinase signaling." J Biol Chem 279(22): 23422-23430.

Luo, S. and A. S. Lee (2002). "Requirement of the p38 mitogen-activated protein kinase signalling pathway for the induction of the 78 kDa glucose-regulated protein/immunoglobulin heavy-chain binding protein by azetidine stress: activating transcription factor 6 as a target for stress-induced phosphorylation." Biochem J 366(Pt 3): 787-795.

Lv, D., D. D. Zhang, H. Wang, Y. Zhang, L. Liang, J. F. Fu, F. Xiong, G. L. Liu, C. X. Gong, F. H. Luo, S. K. Chen, Z. L. Li and Y. M. Zhu (2015). "Genetic variations in SEC16B, MC4R, MAP2K5 and KCTD15 were associated with childhood obesity and interacted with dietary behaviors in Chinese school-age population." Gene 560(2): 149-155.

Lyman, S. K. and R. Schekman (1995). "Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of Saccharomyces cerevisiae." J Cell Biol 131(5): 1163-1171.

Ma, J., Z. Ren, Y. Ma, L. Xu, Y. Zhao, C. Zheng, Y. Fang, T. Xue, B. Sun and W. Xiao (2009). "Targeted knockdown of EGR-1 inhibits IL-8 production and IL-8-mediated invasion of prostate cancer cells through suppressing EGR-1/NF-kappaB synergy." J Biol Chem 284: 34600-34606.

Ma, L., Z. Chen, H. Erdjument-Bromage, P. Tempst and P. P. Pandolfi (2005). "Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis." Cell 121(2): 179-193.

Ma, W. and J. Goldberg (2013). "Rules for the recognition of dilysine retrieval motifs by coatomer." EMBO J. 32: 926-937.

Ma, Y., J. W. Brewer, J. A. Diehl and L. M. Hendershot (2002). "Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response." J Mol Biol 318(5): 1351-1365.

MacGibbon, G. A., P. A. Lawlor, M. Walton, E. Sirimanne, R. L. Faull, B. Synek, E. Mee, B. Connor and M. Dragunow (1997). "Expression of Fos, Jun, and Krox family proteins in Alzheimer's disease." Exp Neurol 147: 316-332.

Magnolo, L., M. Najah, T. Fancello, E. Di Leo, E. Pinotti, I. Brini, N. M. Gueddiche, S. Calandra, N. M. Slimene and P. Tarugi (2013). "Novel mutations in SAR1B and MTTP genes in Tunisian children with chylomicron retention disease and abetalipoproteinemia." Gene 512(1): 28-34.

Mahaligham, D., A. Natoni, M. Keane, A. Samali and E. Szegezdi (2010). "Early growth response-1 is a regulator of DR5-induced apoptosis in colon cancer cells." Br J Cancer 102: 754-764.

Malhotra, V. (2013). "Unconventional protein secretion: an evolving mechanism." EMBO J. 32: 1660-1664.

Malhotra, V. and R. J. Kaufman (2007). "The endoplasmic reticulum and the unfolded protein response." Semin Cell Dev Biol 18: 716-731.

Malide, D., J. W. Yewdell, J. R. Bennink and S. W. Cushman (2001). "The export of major histocompatibility complex class I molecules from the endoplasmic reticulum of rat brown adipose cells is acutely stimulated by insulin." Mol Biol Cell 12(1): 101-114.

Malkus, P., F. Jiang and R. Schekman (2002). "Concentrative sorting of secretory cargo proteins into COPII-coated vesicles." J Cell Biol 159: 915-921.

Mancias, J. D. and J. Goldberg (2007). "The transport signal on Sec22 for packaging into COPII-coated vesicles is a conformational epitope." Mol Cell 26(3): 403-414.

Mancias, J. D. and J. Goldberg (2008). "Structural basis of cargo membrane protein discrimination by the human COPII coat machinery." EMBO J. 27(21): 2918-2928.

Manjithaya, R., C. Anjard, W. F. Loomis and S. Subramani (2010). "Unconventional secretion of Pichia pastoris Acb1 is dependent on GRASP protein, peroxisomal functions, and autophagosome formation." J Cell Biol 188(4): 537-546.

Manning, B. D., A. R. Tee, M. N. Logsdon, J. Blenis and L. C. Cantley (2002). "Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway." Mol Cell 10(1): 151-162.

Mansbach, C. M., 2nd and P. Nevin (1998). "Intracellular movement of triacylglycerols in the intestine." J Lipid Res 39(5): 963-968.

Manzano-Lopez, J., A. M. Perez-Linero, A. Aguilera-Romero, M. E. Martin, T. Okano, D. V. Silva, P. H. Seeberger, H. Riezman, K. Funato, V. Goder, R. E. Wellinger and M. Muñiz (2015). "COPII coat composition is actively regulated by luminal cargo maturation." Curr Biol 25: 152-162.

Maple, A. M., X. Zhao, D. I. Elizalde, A. K. McBride and A. L. Gallitano (2015). "Htr2a Expression Responds Rapidly to Environmental Stimuli in an Egr3-Dependent Manner." ACS Chem Neurosci.

Marciniak, S. J., C. Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H. P. Harding and D. Ron (2004). "CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum." Genes Dev 18(24): 3066-3077.

Marie, M., H. A. Dale, R. Sannerud and J. Saraste (2009). "The function of the intermediate compartment in pre-Golgi trafficking involves its stable connection with the centrosome." Mol Biol Cell 20: 4458-4470.

Martin, J. H., A. A. Mohit and C. A. Miller (1996). "Developmental expression in the mouse nervous system of the p493F12 SAP kinase." Brain Res Mol Brain Res 35(1-2): 47-57.

Martínez-Menárguez, J. A., H. J. Geuze, J. W. Slot and J. Klumperman (1999). "Vesicular tubular clusters between the ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles." Cell 98: 81-90.

Martoglio, B. and B. Dobberstein (1998). "Signal sequences: more than just greasy peptides." Trends Cell Biol 8(10): 410-415.

Marzioch, M., D. C. Henthorn, J. M. Herrmann, R. Wilson, D. Y. Thomas, J. J. Bergeron, R. C. Solari and A. Rowley (1999). "Erp1p and Erp2p, partners for Emp24p and Erv25p in a yeast p24 complex." Mol Biol Cell 10: 1923-1938.

Mathai, J. P., M. Germain and G. C. Shore (2005). "BH3-only BIK regulates BAX,BAK-dependent release of Ca2+ from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death." J Biol Chem 280(25): 23829-23836.

Matlack, K. E., B. Misselwitz, K. Plath and T. A. Rapoport (1999). "BiP acts as a molecular ratchet during posttranslational transport of prepro-alpha factor across the ER membrane." Cell 97(5): 553-564.

Matsuoka, K., L. Orci, M. Amherdt, S. Y. Bednarek, S. Hamamoto, R. Schekman and T. Yeung (1998). "COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes." Cell 93(2): 263-275.

Matsuoka, K., R. Schekman, L. Orci and J. E. Heuser (2001). "Surface structure of the COPII-coated vesicle." Proc Natl Acad Sci U S A 98(24): 13705-13709.

Matsuoka, Y., H. Kubota, E. Adachi, N. Nagai, T. Marutani, N. Hosokawa and K. Nagata (2004). "Insufficient folding of type IV collagen and formation of abnormal basement membrane-like structure in embryoid bodies derived from Hsp47-null embryonic stem cells." Mol Biol Cell 15(10): 4467-4475.

Maurel, M., E. P. McGrath, K. Mnich, S. Healy, E. Chevet and A. Samali (2015). "Controlling the unfolded protein response-mediated life and death decisions in cancer." Semin Cancer Biol.

Mauro, C., E. Crescenzi, R. De Mattia, F. Pacifico, S. Mellone, S. Salzano, C. de Luca, L. D'Adamio, G. Palumbo, S. Formisano, P. Vito and A. Leonardi (2006). "Central role of the scaffold protein tumor necrosis factor receptor-associated factor 2 in regulating endoplasmic reticulum stress-induced apoptosis." J Biol Chem 281(5): 2631-2638.

McCullough, K. D., J. L. Martindale, L. O. Klotz, T. Y. Aw and N. J. Holbrook (2001). "Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state." Mol Cell Biol 21(4): 1249-1259.

McDonald, P. H., C. W. Chow, W. E. Miller, S. A. Laporte, M. E. Field, F. T. Lin, R. J. Davis and R. J. Lefkowitz (2000). "Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3." Science 290: 1574-1577.

McGary, K. L., T. J. Park, J. O. Woods, H. J. Cha, J. B. Wallingford and E. M. Marcotte (2010). "Systematic discovery of nonobvious human disease models through orthologous phenotypes." Proc Natl Acad Sci U S A 107(14): 6544-6549.

McMahon, C., S. M. Studer, C. Clendinen, G. P. Dann, P. D. Jeffrey and F. M. Hughson (2012). "The structure of Sec12 implicates potassium ion coordination in Sar1 activation." J Biol Chem 287: 43599-43606.

Melnick, J., S. Aviel and Y. Argon (1992). "The endoplasmic reticulum stress protein GRP94, in addition to BiP, associates with unassembled immunoglobulin chains." J Biol Chem 267(30): 21303-21306.

Melnick, J., J. L. Dul and Y. Argon (1994). "Sequential interaction of the chaperones BiP and GRP94 with immunoglobulin chains in the endoplasmic reticulum." Nature 370(6488): 373-375.

Melville, D. B., M. Montero-Balaguer, D. S. Levic, K. Bradley, J. R. Smith, A. K. Hatzopoulos and E. W. Knapik (2011). "The feelgood mutation in zebrafish dysregulates COPII-dependent secretion of select extracellular matrix proteins in skeletal morphogenesis." Dis Model Mech 4(6): 763-776.

Mendelson, K. G., L. R. Contois, S. G. Tevosian, R. J. Davis and K. E. Paulson (1996). "Independent regulation of JNK/p38 mitogen-activated protein kinases by metabolic oxidative stress in the liver." Proc Natl Acad Sci U S A 93: 12908-12913.

Mendoza, M. C., E. E. Er and J. Blenis (2011). "The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation." Trends Biochem Sci 36(6): 320-328.

Menendez, J. A. and R. Lupu (2007). "Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis." Nat Rev Cancer 7(10): 763-777.

Merte, J., D. Jensen, K. Wright, S. Sarsfield, Y. Wang, R. Schekman and D. D. Ginty (2010). "Sec24b selectively sorts Vangl2 to regulate planar cell polarity during neural tube closure." Nat Cell Biol 12(1): 41-46; sup pp 41-48.

Mertens, S., M. Craxton and M. Goedert (1996). "SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases." FEBS Lett 383(3): 273-276.

Miah, M. A., S. E. Byeon, M. S. Ahmed, C. H. Yoon, S. J. Ha and Y. S. Bae (2013). "Egr2 induced during DC development acts as an intrinsic negative regulator of DC immunogenicity." Eur J Immunol 43(9): 2484-2496.

Micaroni, M., G. Perinetti, C. P. Berrie and A. A. Mironov (2010). "The SPCA1 Ca2+ pump and intracellular membrane trafficking." Traffic 11(10): 1315-1333.

Michalak, M., J. Groenendyk, E. Szabo, L. I. Gold and M. Opas (2009). "Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum." Biochem J 417(3): 651-666.

Milgraum, L. Z., L. A. Witters, G. R. Pasternack and F. P. Kuhajda (1997). "Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma." Clin Cancer Res 3(11): 2115-2120.

Miller, E., B. Antonny, S. Hamamoto and R. Schekman (2002). "Cargo selection into COPII vesicles is driven by the Sec24p subunit." EMBO J 21(22): 6105-6113.

Miller, E. A., T. H. Beilharz, P. N. Malkus, M. C. Lee, S. Hamamoto, L. Orci and R. Schekman (2003). "Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles." Cell 114(4): 497-509.

Miller, T. W., B. N. Rexer, J. T. Garrett and C. L. Arteaga (2011). "Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer." Breast Cancer Res 13(6): 224.

Miller, W. E. and R. J. Lefkowitz (2001). "Expanding roles for beta-arrestins as scaffolds and adapters in GPCR signaling and trafficking." Curr Opin Cell Biol 13: 139-145.

Milne, D. M., P. Looby and D. W. Meek (2001). "Catalytic activity of protein kinase CK1 delta (casein kinase 1delta) is essential for its normal subcellular localization." Exp Cell Res 263: 43-54.

Misteli, T. and G. Warren (1994). "COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system." J Cell Biol 125(2): 269-282.

Mizoguchi, T., K. Nakajima, K. Hatsuzawa, M. Nagahama, H. P. Hauri, M. Tagaya and K. Tani (2000). "Determination of functional regions of p125, a novel mammalian Sec23p-interacting protein." Biochem Biophys Res Commun 279(1): 144-149.

Moelleken, J., J. Malsam, M. J. Betts, A. Movafeghi, I. Reckmann, I. Meissner, A. Hellwig, R. B. Russell, T. Söllner, B. Brügger and F. T. Wieland (2007). "Differential localization of coatomer complex isoforms within the Golgi apparatus." Proc Natl Acad Sci U S A 104: 4425-4430.

Mogelsvang, S., N. Gomez-Ospina, J. Soderholm, B. S. Glick and L. A. Staehelin (2003). "Tomographic evidence for continuous turnover of Golgi cisternae in Pichia pastoris." Mol Biol Cell 14(6): 2277-2291.

Mohit, A. A., J. H. Martin and C. A. Miller (1995). "p493F12 kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system." Neuron 14(1): 67-78.

Montegna, E. A., M. Bhave, Y. Liu, D. Bhattacharyya and B. S. Glick (2012). "Sec12 binds to Sec16 at transitional ER sites." PLoS One 7(2): e31156.

Montgomery, T. R., T. Steinkellner, S. Sucic, F. Koban, S. Schuchner, E. Ogris, H. H. Sitte and M. Freissmuth (2014). "Axonal targeting of the serotonin transporter in cultured rat dorsal raphe neurons is specified by SEC24C-dependent export from the endoplasmic reticulum." J Neurosci 34(18): 6344-6351.

Morishima, N., K. Nakanishi, H. Takenouchi, T. Shibata and Y. Yasuhiko (2002). "An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12." J Biol Chem 277(37): 34287-34294.

Morishima, N., K. Nakanishi, K. Tsuchiya, T. Shibata and E. Seiwa (2004). "Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signaling for activation of caspase-12 during ER stress-induced apoptosis." J Biol Chem 279(48): 50375-50381.

Morozova, D., G. Guigas and M. Weiss (2011). "Dynamic structure formation of peripheral membrane proteins." PLoS Comput Biol 7(6): e1002067.

Morrison, D. K. and R. J. Davis (2003). "Regulation of MAP kinase signaling modules by scaffold proteins in mammals." Annu Rev Cell Dev Biol 19: 91-118.

Mossessova, E., L. C. Bickford and J. Goldberg (2003). "SNARE Selectivity of the COPII Coat." Cell 114: 483-495.

Muda, M., A. Theodosious, N. Rodrigues, U. Boschert, M. Camps, C. Gillieron, K. Davies, A. Ashworth and S. Arkinstall (1996). "The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases." J Biol Chem 271: 27205-27208.

Mukhopadhyay, S., C. Bachert, D. R. Smith and A. D. Linstedt (2010). "Manganese-induced trafficking and turnover of the cis-Golgi glycoprotein GPP130." Mol Biol Cell 21(7): 1282-1292.

Mukhopadhyay, S. and A. D. Linstedt (2012). "Manganese blocks intracellular trafficking of Shiga toxin and protects against Shiga toxicosis." Science 335(6066): 332-335.

Mukhopadhyay, S., B. Redler and A. D. Linstedt (2013). "Shiga toxin-binding site for host cell receptor GPP130 reveals unexpected divergence in toxin-trafficking mechanisms." Mol Biol Cell 24(15): 2311-2318.

Müller, J., A. M. Cacace, W. E. Lyons, C. B. McGill and D. K. Morrison (20). "Identification of B-KSR1, a novel brain-specific isoform of KSR1 that functions in neuronal signaling." Mol Cell Biol 20: 5529-5539.

Müller, J., S. Ory, T. D. Copeland, H. Piwnica-Worms and D. K. Morrison (2001). "C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1." Mol Cell 8: 983-993.

Munro, S. and H. R. Pelham (1987). "A C-terminal signal prevents secretion of luminal ER proteins." Cell 48: 899-907.

Muppirala, M., V. Gupta and G. Swarup (2011). "Syntaxin 17 cycles between the ER and ERGIC and is required to maintain the architecture of ERGIC and Golgi." Biol Cell 103(7): 333-350.

Muppirala, M., V. Gupta and G. Swarup (2012). "Tyrosine phosphorylation of a SNARE protein, syntaxin 17: implications for membrane trafficking in the early secretory pathway." Biochim Biophys Acta 1823(12): 2109-2119.

Muppirala, M., V. Gupta and G. Swarup (2013). "Emerging role of tyrosine phosphatase, TCPTP, in the organelles of the early secretory pathway." Biochim Biophys Acta 1833(5): 1125-1132.

Myung, D. S., Y. L. Park, N. Kim, C. Y. Chung, H. C. Park, J. S. Kim, S. B. Cho, W. S. Lee, J. H. Lee and Y. E. Joo (2014). "Expression of early growth response-1 in colorectal cancer and its relation to tumor cell proliferation and apoptosis." Oncol Rep 31: 788-794.

Nagai, N., M. Hosokawa, S. Itohara, E. Adachi, T. Matsushita, N. Hosokawa and K. Nagata (2000). "Embryonic lethality of molecular chaperone hsp47 knockout mice is associated with defects in collagen biosynthesis." J Cell Biol 150(6): 1499-1506.

Nagata, K. (1996). "Hsp47: a collagen-specific molecular chaperone." Trends Biochem Sci 21(1): 22-26.

Nagaya, H., I. Wada, Y. J. Jia and H. Kanoh (2002). "Diacylglycerol kinase delta suppresses ER-to-Golgi traffic via its SAM and PH domains." Mol Biol Cell 13(1): 302-316.

Nakagawa, H., M. Ishizaki, S. Miyazaki, T. Abe, K. Nishimura, M. Komori and S. Matsuo (2012). "Sar1 translocation onto the ER-membrane for vesicle budding has different pathways for promotion and suppression of ER-to-Golgi transport mediated through H89-sensitive kinase and ER-resident G protein." Mol Cell Biochem 366(1-2): 175-182.

Nakagawa, H., S. Miyazaki, T. Abe, H. Umadome, K. Tanaka, K. Nishimura, M. Komori and S. Matsuo (2011). "H89 sensitive kinase regulates the translocation of Sar1 onto the ER membrane through phosphorylation of ER-coupled beta-tubulin." Int J Biochem Cell Biol 43(3): 423-430.

Nakagawa, H., H. Umadome, S. Miyazaki, K. Tanaka, K. Nishimura, M. Komori and S. Matsuo (2011). "ER-resident Gi2 protein controls sar1 translocation onto the ER during budding of transport vesicles." J Cell Biochem 112(9): 2250-2256.

Nakamura, K. and G. L. Johnson (2003). "PB1 domains of MEKK2 and MEKK3 interact with the MEK5 PB1 domain for activation of the ERK5 pathway." J Biol Chem 278(39): 36989-36992.

Nakamura, N., M. Lowe, T. P. Levine, C. Rabouille and G. Warren (1997). "The vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner." Cell 89(3): 445-455.

Nakano, A., D. Brada and R. Schekman (1988). "A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast." J Cell Biol 107(3): 851-863.

Nanao, T., M. Koike, J. Yamaguchi, M. Sasaki and Y. Uchiyama (2015). "Cellular localization and tissue distribution of endogenous DFCP1 protein." Biomed Res 36(2): 121-133.

Nardelli, J., T. J. Gibson, C. Vesque and P. Charnay (1991). "Base sequence discrimination by zinc-finger DNA-binding domains." Nature 349: 175-178.

Nebenführ, A., L. A. Gallagher, T. G. Dunahay, J. A. Frohlick, A. M. Mazurkiewicz, J. B. Meehl and L. A. Staehelin (1999). "Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system." Plant Physiol 121(4): 1127-1142.

Neeli, I., S. A. Siddiqi, S. Siddiqi, J. Mahan, W. S. Lagakos, B. Binas, T. Gheyi, J. Storch and C. M. Mansbach, 2nd (2007). "Liver fatty acid-binding protein initiates budding of pre-chylomicron transport vesicles from intestinal endoplasmic reticulum." J Biol Chem 282(25): 17974-17984.

Negri, S., A. Oberson, M. Steinmann, C. Sauser, P. Nicod, G. Waeber, D. F. Schorderet and C. Bonny (2000). "cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein." Genomics 64: 324-330.

Neuhof, A., M. M. Rolls, B. Jungnickel, K. U. Kalies and T. A. Rapoport (1998). "Binding of signal recognition particle gives ribosome/nascent chain complexes a competitive advantage in endoplasmic reticulum membrane interaction." Mol Biol Cell 9(1): 103-115.

Neumann, N., D. Lundin and A. M. Poole (2010). "Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor." PLoS One 5(10): e13241.

Neve, E. P., K. Svensson, J. Fuxe and R. F. Pettersson (2003). "VIPL, a VIP36-like membrane protein with a putative function

in the export of glycoproteins from the endoplasmic reticulum." Exp Cell Res 288: 70-83.

Ng, M. C., C. H. Tam, W. Y. So, J. S. Ho, A. W. Chan, H. M. Lee, Y. Wang, V. K. Lam, J. C. Chan and R. C. Ma (2010). "Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese." J Clin Endocrinol Metab 95(5): 2418-2425.

Nickel, W., J. Malsam, K. Gorgas, M. Ravazzola, N. Jenne, J. B. Helms and F. T. Wieland (1998). "Uptake by COPI-coated vesicles of both anterograde and retrograde cargo is inhibited by GTPgammaS in vitro." J Cell Sci 111 (Pt 20): 3081-3090.

Nickel, W., K. Sohn, C. Bünning and F. T. Wieland (1997). "p23, a major COPI-vesicle membrane protein, constitutively cycles through the early secretory pathway." Proc Natl Acad Sci U S A 94: 11393-11398.

Nie, Z., M. Boehm, E. S. Boja, W. C. Vass, J. S. Bonifacino, H. M. Fales and P. A. Randazzo (2003). "Specific regulation of the adaptor protein complex AP-3 by the Arf GAP AGAP1." Dev Cell 5(3): 513-521.

Nie, Z. and P. A. Randazzo (2006). "Arf GAPs and membrane traffic." J Cell Sci 119(Pt 7): 1203-1211.

Nishimoto, S. and E. Nishida (2006). "MAPK signalling: ERK5 versus ERK1/2." EMBO Rep 7(8): 782-786.

Nishitoh, H., A. Matsuzawa, K. Tobiume, K. Saegusa, K. Takeda, K. Inoue, S. Hori, A. Kakizuka and H. Ichijo (2002). "ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats." Genes Dev 16(11): 1345-1355.

Nishitoh, H., M. Saitoh, Y. Mochida, K. Takeda, H. Nakano, M. Rothe, K. Miyazono and H. Ichijo (1998). "ASK1 is essential for JNK/SAPK activation by TRAF2." Mol Cell 2(3): 389-395.

Niu, T. K., A. C. Pfeifer, J. Lippincott-Schwartz and C. L. Jackson (2005). "Dynamics of GBF1, a Brefeldin A-sensitive Arf1 exchange factor at the Golgi." Mol Biol Cell 16(3): 1213-1222.

Niu, X., C. Gao, L. Jan Lo, Y. Luo, C. Meng, J. Hong, W. Hong and J. Peng (2012). "Sec13 safeguards the integrity of the endoplasmic reticulum and organogenesis of the digestive system in zebrafish." Dev Biol 367(2): 197-207.

Niu, X., J. Hong, X. Zheng, D. B. Melville, E. W. Knapik, A. Meng and J. Peng (2014). "The nuclear pore complex function of Sec13 protein is required for cell survival during retinal development." J Biol Chem 289(17): 11971-11985.

Niwa, M., C. Sidrauski, R. J. Kaufman and P. Walter (1999). "A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response." Cell 99(7): 691-702.

Noble, A. J., Q. Zhang, J. O'Donnell, H. Hariri, N. Bhattacharya, A. G. Marshall and S. M. Stagg (2013). "A pseudoatomic model of the COPII cage obtained from cryo-electron microscopy and mass spectrometry." Nat Struct Mol Biol 20(2): 167-173.

Nonhoff, U., M. Ralser, F. Welzel, I. Piccini, D. Balzereit, M. L. Yaspo, H. Lehrach and S. Krobitsch (2007). "Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules." Mol Biol Cell 18(4): 1385-1396.

Novick, P., C. Field and R. Schekman (1980). "Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway." Cell 21(1): 205-215.

Novoa, I., H. Zeng, H. P. Harding and D. Ron (2001). "Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha." J Cell Biol 153(5): 1011-1022.

Nufer, O., S. Mitrovic and H. P. Hauri (2003). "Profile-based data base scanning for animal L-type lectins and characterization of VIPL, a novel VIP36-like endoplasmic reticulum protein." J Biol Chem 278: 15886-15896.

Nyfeler, B., V. Reiterer, M. W. Wendeler, E. Stefan, B. Zhang, S. W. Michnick and H. P. Hauri (2008). "Identification of ERGIC-53 as an intracellular transport receptor of alpha1-antitrypsin." J Cell Biol 180(4): 705-712.

O'Donovan, K. J. and J. M. Baraban (1999). "Major Egr3 isoforms are generated via alternate translation start sites and differ in their abilities to activate transcription." Mol Cell Biol 19: 4711-4718.

O'Donovan, K. J., Y. Levkovitz, D. Ahn and J. M. Baraban (2000). "Functional comparison of Egr3 transcription factor isoforms: identification of an activation domain in the N-terminal segment absent from Egr3beta, a major isoform expressed in brain." J Neurochem 75: 1352-1357.

O'Donovan, K. J., W. G. Tourellotte, J. Millbrandt and J. M. Baraban (1999). "The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience." Trends Neurosci 22: 167-173.

Ogawa, N. and K. Mori (2004). "Autoregulation of the HAC1 gene is required for sustained activation of the yeast unfolded protein response." Genes Cells 9(2): 95-104.

Ohashi, Y. and S. Munro (2010). "Membrane delivery to the yeast autophagosome from the Golgi-endosomal system." Mol Biol Cell 21(22): 3998-4008.

Ohisa, S., K. Inohaya, Y. Takano and A. Kudo (2010). "sec24d encoding a component of COPII is essential for vertebra formation, revealed by the analysis of the medaka mutant, vbi." Dev Biol 342(1): 85-95.

Ohmachi, M., C. E. Rocheleau, D. Church, E. Lambie, T. Schedl and M. V. Sundaram (2002). "C. elegans ksr-1 and ksr-2 have both unique and redundant functions and are required for MPK-1 ERK phosphorylation." Curr Biol 12: 427-433.

Okada, T., M. Miyashita, J. Fukuhara, M. Sugitani, T. Ueno, M. E. Samson-Bouma and L. P. Aggerbeck (2011). "Anderson's disease/chylomicron retention disease in a Japanese patient with uniparental disomy 7 and a normal SAR1B gene protein coding sequence." Orphanet J Rare Dis 6: 78.

Okada, T., H. Yoshida, R. Akazawa, M. Negishi and K. Mori (2002). "Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response." Biochem J 366(Pt 2): 585-594.

Olzmann, J. A., R. R. Kopito and J. C. Christianson (2013). "The mammalian endoplasmic reticulum-associated degradation system." Cold Spring Harb Perspect Biol 5.

Ong, Y. S., B. L. Tang, L. S. Loo and W. Hong (2010). "p125A exists as part of the mammalian Sec13/Sec31 COPII subcomplex to facilitate ER-Golgi transport." J Cell Biol 190(3): 331-345.

Orci, L., M. Ravazzola, P. Meda, C. Holocomb, H. P. Moore, L. Hicke and R. Schekman (1991). "Mammalian Sec23p homologue is restricted to the endoplasmic reticulum transitional cytoplasm." Proc Natl Acad Sci U S A 88(19): 8611-8615.

Otsu, W., T. Kurooka, Y. Otsuka, K. Sato and M. Inaba (2013). "A new class of endoplasmic reticulum export signal PhiXPhiXPhi for transmembrane proteins and its selective interaction with Sec24C." J Biol Chem 288(25): 18521-18532.

Otte, S. and C. Barlowe (2002). "The Erv41p-Erv46p complex: multiple export signals are required in trans for COPII-dependent transport from the ER." EMBO J. 21: 6095-6104.

Otte, S. and C. Barlowe (2004). "Sorting signals can direct receptor-mediated export of soluble proteins into COPII vesicles." Nat Cell Biol 6: 1189-1194.

Otteken, A. and B. Moss (1996). "Calreticulin interacts with newly synthesized human immunodeficiency virus type 1 envelope glycoprotein, suggesting a chaperone function similar to that of calnexin." J Biol Chem 271(1): 97-103.

Ou, W. J., P. H. Cameron, D. Y. Thomas and J. J. Bergeron (1993). "Association of folding intermediates of glycoproteins with calnexin during protein maturation." Nature 364(6440): 771-776.

Owens, D. M. and S. M. Keyse (2007). "Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases." Oncogene 26: 3203-3213.

Oz-Levi, D., B. Ben-Zeev, E. K. Ruzzo, Y. Hitomi, A. Gelman, K. Pelak, Y. Anikster, H. Reznik-Wolf, I. Bar-Joseph, T. Olender, A. Alkelai, M. Weiss, E. Ben-Asher, D. Ge, K. V. Shianna, Z. Elazar, D. B. Goldstein, E. Pras and D. Lancet (2012). "Mutation in TECPR2 reveals a role for autophagy in hereditary spastic paraparesis." Am J Hum Genet 91(6): 1065-1072.

Oz-Levi, D., A. Gelman, Z. Elazar and D. Lancet (2013). "TECPR2: a new autophagy link for neurodegeneration." Autophagy 9(5): 801-802.

Ozcan, U., Q. Cao, E. Yilmaz, A. H. Lee, N. N. Iwakoshi, E. Ozdelen, G. Tuncman, C. Gorgun, L. H. Glimcher and G. S. Hotamisligil (2004). "Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes." Science 306(5695): 457-461.

Pagano, A., F. Letourneur, D. Garcia-Estefania, J. L. Carpentier, L. Orci and J. P. Paccaud (1999). "Sec24 proteins and sorting at the endoplasmic reticulum." J Biol Chem 274(12): 7833-7840.

Pagel, J. I. and E. Deindl (2011). "Early growth response 1--a transcription factor in the crossfire of signal transduction cascades." Indian J Biochem Biophys 48: 226-235.

Palmer, D. J., J. B. Helms, C. J. Beckers, L. Orci and J. E. Rothman (1993). "Binding of coatomer to Golgi membranes requires ADP-ribosylation factor." J Biol Chem 268(16): 12083-12089.

Palmer, K. J., J. E. Konkel and D. J. Stephens (2005). "PCTAIRE protein kinases interact directly with the COPII complex and modulate secretory cargo transport." J Cell Sci 118(Pt 17): 3839-3847.

Papanikou, E. and B. S. Glick (2014). "Golgi compartmentation and identity." Curr Opin Cell Biol 29: 74-81.

Paris, N., C. M. Stanley, R. L. Jones and J. C. Rogers (1996). "Plant cells contain two functionally distinct vacuolar compartments." Cell 85(4): 563-572.

Paroutis, P., N. Touret and S. Grinstein (2004). "The pH of the secretory pathway: measurement, determinants, and regulation." Physiology (Bethesda) 19: 207-215.

Parra, E., J. Ferreira and A. Ortega (2011). "Overexpression of EGR-1 modulates the activity of NF-kappaB and AP-1 in prostate carcinoma PC-3 and LNCaP cell lines." Int J Oncol 39(2): 345-352.

Pathre, P., K. Shome, A. Blumental-Perry, A. Bielli, C. J. Haney, S. Alber, S. C. Watkins, G. Romero and M. Aridor (2003). "Activation of phospholipase D by the small GTPase Sar1p is required to support COPII assembly and ER export." EMBO J 22(16): 4059-4069.

Patwardhan, S., A. Gashler, M. G. Siegel, L. C. Chang, L. J. Joseph, T. B. Shows, M. M. Le Beau and V. P. Sukhatme (1991). "EGR3, a novel member of the Egr family of genes encoding immediate-early transcription factors." Oncogene 6: 917-928.

Pearce, L. R., X. Huang, J. Boudeau, R. Pawlowski, S. Wullschleger, M. Deak, A. F. Ibrahim, R. Gourlay, M. A. Magnuson and D. R. Alessi (2007). "Identification of Protor as a novel Rictor-binding component of mTOR complex-2." Biochem J 405(3): 513-522.

Pearce, L. R., E. M. Sommer, K. Sakamoto, S. Wullschleger and D. R. Alessi (2011). "Protor-1 is required for efficient mTORC2-mediated activation of SGK1 in the kidney." Biochem J 436(1): 169-179.

Pecot, M. Y. and V. Malhotra (2004). "Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells." Cell 116(1): 99-107.

Pelham, H. R. (1988). "Evidence that luminal ER proteins are sorted from secreted proteins in a post-ER compartment." EMBO J. 7: 913-918.

Peng, W. X., F. Y. Pan, X. J. Liu, S. Ning, N. Xu, F. L. Meng, Y. Q. Wang and C. J. Li (2010). "Hypoxia stabilizes microtubule networks and decreases tumor cell chemosensitivity to anticancer drugs through Egr-1." Anat Rec 293: 414-420.

Penke, Z., E. Morice, A. Veyrac, A. Gros, C. Chagneau, P. LeBlanc, N. Samson, K. Baumgärtel, I. M. Mansuy, S. Davis and S. Laroche (2013). "Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory." Philos Trans R Soc Lond B Biol Sci 369: 20130159.

Pepperkok, R., J. A. Whitney, M. Gomez and T. E. Kreis (2000). "COPI vesicles accumulating in the presence of a GTP restricted arf1 mutant are depleted of anterograde and retrograde cargo." J Cell Sci 113 (Pt 1): 135-144.

Peterson, J. R., A. Ora, P. N. Van and A. Helenius (1995). "Transient, lectin-like association of calreticulin with folding intermediates of cellular and viral glycoproteins." Mol Biol Cell 6(9): 1173-1184.

Peterson, T. R., M. Laplante, C. C. Thoreen, Y. Sancak, S. A. Kang, W. M. Kuehl, N. S. Gray and D. M. Sabatini (2009). "DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival." Cell 137(5): 873-886.

Pio, R., Z. Jia, V. T. Baron and D. Mercola (2013). "Early growth response 3 (Egr3) is highly over-expressed in non-relapsing prostate cancer but not in relapsing prostate cancer." PLoS One 8(1): e54096.

Plemper, R. K., S. Bohmler, J. Bordallo, T. Sommer and D. H. Wolf (1997). "Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation." Nature 388(6645): 891-895.

Plotnikov, A., E. Zehorai, S. Procaccia and R. Seger (2011). "The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation." Biochim Biophys Acta 1813: 1619-1633.

Poderoso, C., D. P. Converso, P. Maloberti, A. Duarte, I. Neuman, S. Galli, F. Cornejo Maciel, C. Paz, M. C. Carreras, J. J. Poderoso and E. J. Podesta (2008). "A mitochondrial kinase complex is essential to mediate an ERK1/2-dependent phosphorylation of a key regulatory protein in steroid biosynthesis." PLoS One 3(1): e1443.

Poirier, R., H. Cheval, C. Mailhes, S. Garel, P. Charnay, S. Davis and S. Laroche (2008). "Distinct functions of egr gene family members in cognitive processes." Front Neurosci 2: 47-55.

Porstmann, T., B. Griffiths, Y. L. Chung, O. Delpuech, J. R. Griffiths, J. Downward and A. Schulze (2005). "PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP." Oncogene 24(43): 6465-6481.

Porstmann, T., C. R. Santos, B. Griffiths, M. Cully, M. Wu, S. Leevers, J. R. Griffiths, Y. L. Chung and A. Schulze (2008). "SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth." Cell Metab 8(3): 224-236.

Porstmann, T., C. R. Santos, C. Lewis, B. Griffiths and A. Schulze (2009). "A new player in the orchestra of cell growth: SREBP activity is regulated by mTORC1 and contributes to the regulation of cell and organ size." Biochem Soc Trans 37(Pt 1): 278-283.

Poschl, E., U. Schlotzer-Schrehardt, B. Brachvogel, K. Saito, Y. Ninomiya and U. Mayer (2004). "Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development." Development 131(7): 1619-1628.

Potter, C. J., L. G. Pedraza and T. Xu (2002). "Akt regulates growth by directly phosphorylating Tsc2." Nat Cell Biol 4(9): 658-665.

Prescott, A. R., T. Farmaki, C. Thomson, J. James, J. P. Paccaud, B. L. Tang, W. Hong, M. Quinn, S. Ponnambalam and J. Lucocq (2001). "Evidence for prebudding arrest of ER export in animal cell mitosis and its role in generating Golgi partitioning intermediates." Traffic 2(5): 321-335.

Presley, J. F., N. B. Cole, T. A. Schroer, K. Hirschberg, K. J. Zaal and J. Lippincott-Schwartz (1997). "ER-to-Golgi transport visualized in living cells." Nature 389(6646): 81-85.

Preuss, D., J. Mulholland, A. Franzusoff, N. Segev and D. Botstein (1992). "Characterization of the Saccharomyces Golgi complex through the cell cycle by immunoelectron microscopy." Mol Biol Cell 3(7): 789-803.

Pryde, J. G., T. Farmaki and J. M. Lucocq (1998). "Okadaic acid induces selective arrest of protein transport in the rough endoplasmic reticulum and prevents export into COPII-coated structures." Mol Cell Biol 18(2): 1125-1135.

Pryer, N. K., N. R. Salama, R. Schekman and C. A. Kaiser (1993). "Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro." J Cell Biol 120(4): 865-875.

Puhka, M., M. Joensuu, H. Vihinen, I. Belevich and E. Jokitalo (2012). "Progressive sheet-to-tubule transformation is a general mechanism for endoplasmic reticulum partitioning in dividing mammalian cells." Mol Biol Cell 23(13): 2424-2432.

Puhka, M., H. Vihinen, M. Joensuu and E. Jokitalo (2007). "Endoplasmic reticulum remains continuous and undergoes sheet-to-tubule transformation during cell division in mammalian cells." J Cell Biol 179(5): 895-909.

Puig, A., M. M. Lyles, R. Noiva and H. F. Gilbert (1994). "The role of the thiol/disulfide centers and peptide binding site in the chaperone and anti-chaperone activities of protein disulfide isomerase." J Biol Chem 269(29): 19128-19135.

Pullikuth, A., E. McKinnon, H. J. Schaeffer and A. D. Catling (2005). "The MEK1 scaffolding protein MP1 regulates cell spreading by integrating PAK1 and Rho signals." Mol Cell Biol 25: 5119-5133.

Pulvirenti, T., M. Giannotta, M. Capestrano, M. Capitani, A. Pisanu, R. S. Polishchuk, E. San Pietro, G. V. Beznoussenko, A. A. Mironov, G. Turacchio, V. W. Hsu, M. Sallese and A. Luini (2008). "A traffic-activated Golgi-based signalling circuit coordinates the secretory pathway." Nat Cell Biol 10(8): 912-922.

Puri, C., M. Renna, C. F. Bento, K. Moreau and D. C. Rubinsztein (2014). "ATG16L1 meets ATG9 in recycling endosomes: additional roles for the plasma membrane and endocytosis in autophagosome biogenesis." Autophagy 10(1): 182-184.

Puthenveedu, M. A. and A. D. Linstedt (2001). "Evidence that Golgi structure depends on a p115 activity that is independent of the vesicle tether components giantin and GM130." J Cell Biol 155(2): 227-238.

Quan, H., G. Fan and C. C. Wang (1995). "Independence of the chaperone activity of protein disulfide isomerase from its thioredoxin-like active site." J Biol Chem 270(29): 17078-17080.

Quilty, D., F. Gray, N. Summerfeldt, D. Cassel and P. Melancon (2014). "Arf activation at the Golgi is modulated by feed-forward stimulation of the exchange factor GBF1." J Cell Sci 127(Pt 2): 354-364.

Rabinovich, E., A. Kerem, K. U. Frohlich, N. Diamant and S. Bar-Nun (2002). "AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation." Mol Cell Biol 22(2): 626-634.

Radulescu, A. E., S. Mukherjee and D. Shields (2011). "The Golgi protein p115 associates with gamma-tubulin and plays a role in Golgi structure and mitosis progression." J Biol Chem 286(24): 21915-21926.

Raingeaud, J., S. Gupta, J. S. Rogers, M. Dickens, J. Han, R. J. Ulevitch and R. J. Davis (1995). "Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine." J Biol Chem 270(13): 7420-7426.

Rambourg, A., Y. Clermont, L. Ovtracht and F. Képès (1995). "Three-dimensional structure of tubular networks, presumably Golgi in nature, in various yeast strains: a comparative study." Anat Rec 243(3): 283-293.

Randazzo, P. A., Z. Nie, K. Miura and V. W. Hsu (2000). "Molecular aspects of the cellular activities of ADP-ribosylation factors." Sci STKE 2000(59): re1.

Randow, F. and B. Seed (2001). "Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability." Nat Cell Biol 3(10): 891-896.

Ravikumar, B., K. Moreau, L. Jahreiss, C. Puri and D. C. Rubinsztein (2010). "Plasma membrane contributes to the formation of pre-autophagosomal structures." Nat Cell Biol 12(8): 747-757.

Ravikumar, B., K. Moreau and D. C. Rubinsztein (2010). "Plasma membrane helps autophagosomes grow." Autophagy 6(8): 1184-1186.

Reinhard, C., M. Schweikert, F. T. Wieland and W. Nickel (2003). "Functional reconstitution of COPI coat assembly and disassembly using chemically defined components." Proc Natl Acad Sci U S A 100(14): 8253-8257.

Reiterer, V., S. Maier, H. H. Sitte, A. Kriz, M. A. Ruegg, H. P. Hauri, M. Freissmuth and H. Farhan (2008). "Sec24- and ARFGAP1-dependent trafficking of GABA transporter-1 is a prerequisite for correct axonal targeting." J Neurosci 28(47): 12453-12464.

Reiterer, V., B. Nyfeler and H. P. Hauri (2010). "Role of the lectin VIP36 in post-ER quality control of human alpha1-antitrypsin." Traffic 11(8): 1044-1055.

Ren, X., G. G. Farias, B. J. Canagarajah, J. S. Bonifacino and J. H. Hurley (2013). "Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1." Cell 152(4): 755-767.

Rena, G., S. Guo, S. C. Cichy, T. G. Unterman and P. Cohen (1999). "Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B." J Biol Chem 274(24): 17179-17183.

Ri, M., E. Tashiro, D. Oikawa, S. Shinjo, M. Tokuda, Y. Yokouchi, T. Narita, A. Masaki, A. Ito, J. Ding, S. Kusumoto, T. Ishida, H. Komatsu, Y. Shiotsu, R. Ueda, T. Iwawaki, M. Imoto and S. Iida (2012). "Identification of Toyocamycin, an agent cytotoxic for multiple myeloma cells, as a potent inhibitor of ER stress-induced XBP1 mRNA splicing." Blood Cancer J 2(7): e79.

Ritter, C. and A. Helenius (2000). "Recognition of local glycoprotein misfolding by the ER folding sensor UDP-glucose:glycoprotein glucosyltransferase." Nat Struct Biol 7(4): 278-280.

Ritter, C., K. Quirin, M. Kowarik and A. Helenius (2005). "Minor folding defects trigger local modification of glycoproteins by the ER folding sensor GT." EMBO J 24(9): 1730-1738.

Robbins, D. J. and M. H. Cobb (1992). "Extracellular signal-regulated kinases 2 autophosphorylates on a subset of peptides phosphorylated in intact cells in response to insulin and nerve growth factor: analysis by peptide mapping." Mol Biol Cell 3(3): 299-308.

Roberg, K. J., M. Crotwell, P. Espenshade, R. Gimeno and C. A. Kaiser (1999). "LST1 is a SEC24 homologue used for selective export of the plasma membrane ATPase from the endoplasmic reticulum." J Cell Biol 145(4): 659-672.

Robinson, D. G., F. Brandizzi, C. Hawes and A. Nakano (2015). "Vesicles versus Tubes: is ER-Golgi Transport in Plants Fundamentally Different to other Eukaryotes ?" Plant Physiol.

Roghi, C. and V. J. Allan (1999). "Dynamic association of cytoplasmic dynein heavy chain 1a with the Golgi apparatus and intermediate compartment." J Cell Sci 112: 4673-4685.

Ron, D. and J. F. Habener (1992). "CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription." Genes Dev 6(3): 439-453.

Rossanese, O. W., J. Soderholm, B. J. Bevis, I. B. Sears, J. O'Connor, E. K. Williamson and B. S. Glick (1999). "Golgi structure correlates with transitional endoplasmic reticulum organization in Pichia pastoris and Saccharomyces cerevisiae." J Cell Biol 145(1): 69-81.

Rossi, M., D. Colecchia, C. Iavarone, A. Strambi, F. Piccioni, A. Verrotti di Pianella and M. Chiariello (2011). "Extracellular signal-regulated kinase 8 (ERK8) controls estrogen-related receptor alpha (ERRalpha) cellular localization and inhibits its transcriptional activity." J Biol Chem 286(10): 8507-8522.

Roux, P. P., B. A. Ballif, R. Anjum, S. P. Gygi and J. Blenis (2004). "Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase." Proc Natl Acad Sci U S A 101(37): 13489-13494.

Roy, F., G. Laberge, M. Douziech, D. Ferland-McCollough and M. Therrien (2002). "KSR is a scaffold required for activation of the ERK/MAPK module." Genes Dev 16(4): 427-438.

Rubartelli, A., F. Cozzolino, M. Talio and R. Sitia (1990). "A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence." EMBO J 9(5): 1503-1510.

Rubinfeld, H., T. Hanoch and R. Seger (1999). "Identification of a cytoplasmic-retention sequence in ERK2." J Biol Chem 274: 30349-30352.

Rubinfeld, H. and R. Seger (2005). "The ERK cascade: a prototype of MAPK signaling." Mol Biotechnol 31: 151-174.

Rubinsztein, D. C., T. Shpilka and Z. Elazar (2012). "Mechanisms of autophagosome biogenesis." Curr Biol 22(1): R29-34.

Ruggiano, A., O. Foresti and P. Carvalho (2014). "Quality control: ER-associated degradation: protein quality control and beyond." J Cell Biol 204(6): 869-879.

Russell, R. C., H. X. Yuan and K. L. Guan (2014). "Autophagy regulation by nutrient signaling." Cell Res 24(1): 42-57.

Russo, R., C. Langella, M. R. Esposito, A. Gambale, F. Vitiello, F. Vallefuoco, T. Ek, E. Yang and A. Iolascon (2013). "Hypomorphic mutations of SEC23B gene account for mild phenotypes of congenital dyserythropoietic anemia type II." Blood Cells Mol Dis 51(1): 17-21.

Rutkowski, D. T. and R. J. Kaufman (2003). "All roads lead to ATF4." Dev Cell 4(4): 442-444.

Rutkowski, D. T. and R. J. Kaufman (2007). "That which does not kill me makes me stronger: adapting to chronic ER stress." Trends Biochem Sci 32(10): 469-476.

Saelzler, M. P., C. C. Spackman, Y. Liu, L. C. Martinez, J. P. Harris and M. K. Abe (2006). "ERK8 down-regulates transactivation of the glucocorticoid receptor through Hic-5." J Biol Chem 281(24): 16821-16832.

Safford, M., S. Collins, M. A. Lutz, A. Allen, C. T. Huang, J. Kowalski, A. Blackford, M. R. Horton, C. Drake, R. H. Schwartz and J. D. Powell (2005). "Egr-2 and Egr-3 are negative regulators of T cell activation." Nat Immunol 6: 472-480.

Saito-Nakano, Y. and A. Nakano (2000). "Sed4p functions as a positive regulator of Sar1p probably through inhibition of the GTPase activation by Sec23p." Genes Cells 5(12): 1039-1048.

Saito, K., M. Chen, F. Bard, S. Chen, H. Zhou, D. Woodley, R. Polischuk, R. Schekman and V. Malhotra (2009). "TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites." Cell 136(5): 891-902.

Saito, K., K. Yamashiro, Y. Ichikawa, P. Erlmann, K. Kontani, V. Malhotra and T. Katada (2011). "cTAGE5 mediates collagen secretion through interaction with TANGO1 at endoplasmic reticulum exit sites." Mol Biol Cell 22(13): 2301-2308.

Saito, K., K. Yamashiro, N. Shimazu, T. Tanabe, K. Kontani and T. Katada (2014). "Concentration of Sec12 at ER exit sites via interaction with cTAGE5 is required for collagen export." J Cell Biol 206(6): 751-762.

Salama, N. R., J. S. Chuang and R. W. Schekman (1997). "Sec31 encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum." Mol Biol Cell 8(2): 205-217.

Salama, N. R., T. Yeung and R. W. Schekman (1993). "The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins." EMBO J 12(11): 4073-4082.

Sancak, Y., C. C. Thoreen, T. R. Peterson, R. A. Lindquist, S. A. Kang, E. Spooner, S. A. Carr and D. M. Sabatini (2007). "PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase." Mol Cell 25(6): 903-915.

Sánchez-Fernández, G., S. Cabezudo, C. García-Hoz, A. B. Tobin, F. J. Mayor and C. Ribas (2013). "ERK5 activation by Gq-coupled muscarinic receptors is independent of receptor internalization and β-arrestin recruitment." PLoS One 8: e84174.

Sanchez-Wandelmer, J., N. T. Ktistakis and F. Reggiori (2015). "ERES: sites for autophagosome biogenesis and maturation?" J Cell Sci 128(2): 185-192.

Sane, A., E. Seidman, S. Spahis, V. Lamantia, C. Garofalo, A. Montoudis, V. Marcil and E. Levy (2015). "New Insights in

Intestinal Sar1B GTPase Regulation and Role in Cholesterol Homeostasis." J Cell Biochem.

Sannerud, R., M. Marie, C. Nizak, H. A. Dale, K. Pernet-Gallay, F. Perez, B. Goud and J. Saraste (2006). "Rab1 defines a novel pathway connecting the pre-Golgi intermediate compartment with the cell periphery." Mol Biol Cell 17: 1514-1526.

Santarpia, L., S. M. Lippman and A. K. El-Naggar (2012). "Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy." Expert Opin Ther Targets 16(1): 103-119.

Saraste, J., H. A. Dale, S. Bazzocco and M. Marie (2009). "Emerging new roles of the pre-Golgi intermediate compartment in biosynthetic-secretory trafficking." FEBS Lett 583(23): 3804-3810.

Sarbassov, D. D., S. M. Ali, D. H. Kim, D. A. Guertin, R. R. Latek, H. Erdjument-Bromage, P. Tempst and D. M. Sabatini (2004). "Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton." Curr Biol 14(14): 1296-1302.

Sarmah, S., A. Barrallo-Gimeno, D. B. Melville, J. Topczewski, L. Solnica-Krezel and E. W. Knapik (2010). "Sec24D-dependent transport of extracellular matrix proteins is required for zebrafish skeletal morphogenesis." PLoS One 5(4): e10367.

Sato, K. and A. Nakano (2005). "Dissection of COPII subunit-cargo assembly and disassembly kinetics during Sar1p-GTP hydrolysis." Nat Struct Mol Biol 12(2): 167-174.

Sato, K. and A. Nakano (2007). "Mechanisms of COPII vesicle formation and protein sorting." FEBS Lett 581: 2076-2082.

Sato, K., S. Nishikawa and A. Nakano (1995). "Membrane protein retrieval from the Golgi apparatus to the endoplasmic reticulum (ER): characterization of the RER1 gene product as a component involved in ER localization of Sec12p." Mol Biol Cell 6(11): 1459-1477.

Sato, M., K. Sato and A. Nakano (1996). "Endoplasmic reticulum localization of Sec12p is achieved by two mechanisms: Rer1p-dependent retrieval that requires the transmembrane domain and Rer1p-independent retention that involves the cytoplasmic domain." J Cell Biol 134: 279-293.

Sato, M., K. Sato and A. Nakano (2002). "Evidence for the intimate relationship between vesicle budding from the ER and the unfolded protein response." Biochem Biophys Res Commun 296(3): 560-567.

Scales, S. J., R. Pepperkok and T. E. Kreis (1997). "Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI." Cell 90(6): 1137-1148.

Schaeffer, H. J., A. D. Catling, S. T. Eblen, L. S. Collier, A. Krauss and M. J. Weber (1998). "MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade." Science 281: 1668-1671.

Scheuner, D., B. Song, E. McEwen, C. Liu, R. Laybutt, P. Gillespie, T. Saunders, S. Bonner-Weir and R. J. Kaufman (2001). "Translational control is required for the unfolded protein response and in vivo glucose homeostasis." Mol Cell 7(6): 1165-1176.

Schimmöller, F., B. Singer-Krüger, S. Schröder, U. Krüger, C. Barlowe and H. Riezman (1995). "The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi." EMBO J. 14: 1329-1339.

Schindler, A. J. and R. Schekman (2009). "In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles." Proc Natl Acad Sci U S A 106(42): 17775-17780.

Schlacht, A. and J. B. Dacks (2015). "Unexpected ancient paralogues and an evolutionary model for the COPII coat complex." Genome Biol Evol.

Schmidt, K., F. Cavodeassi, Y. Feng and D. J. Stephens (2013). "Early stages of retinal development depend on Sec13 function." Biol Open 2(3): 256-266.

Schrag, J. D., J. J. Bergeron, Y. Li, S. Borisova, M. Hahn, D. Y. Thomas and M. Cygler (2001). "The Structure of calnexin, an ER chaperone involved in quality control of protein folding." Mol Cell 8(3): 633-644.

Schröder-Köhne, S., F. Letourneur and H. Riezman (1998). "Alpha-COP can discriminate between distinct, functional di-lysine signals in vitro and regulates access into retrograde transport." J Cell Sci 111: 3459-3470.

Schwaller, M., B. Wilkinson and H. F. Gilbert (2003). "Reduction-reoxidation cycles contribute to catalysis of disulfide isomerization by protein-disulfide isomerase." J Biol Chem 278(9): 7154-7159.

Schwarz, K., A. Iolascon, F. Verissimo, N. S. Trede, W. Horsley, W. Chen, B. H. Paw, K. P. Hopfner, K. Holzmann, R. Russo, M. R. Esposito, D. Spano, L. De Falco, K. Heinrich, B. Joggerst, M. T. Rojewski, S. Perrotta, J. Denecke, U. Pannicke, J. Delaunay, R. Pepperkok and H. Heimpel (2009). "Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II." Nat Genet 41(8): 936-940.

Schweizer, A., J. A. Fransen, T. Bächi, L. Ginsel and H. P. Hauri (1988). "Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus." J Cell Biol 107: 1643-1653.

Scorrano, L., S. A. Oakes, J. T. Opferman, E. H. Cheng, M. D. Sorcinelli, T. Pozzan and S. J. Korsmeyer (2003). "BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis." Science 300(5616): 135-139.

Scott, D. C. and R. Schekman (2008). "Role of Sec61p in the ER-associated degradation of short-lived transmembrane proteins." J Cell Biol 181(7): 1095-1105.

Sealey-Cardona, M., K. Schmidt, L. Demmel, T. Hirschmugl, T. Gesell, G. Dong and G. Warren (2014). "Sec16 determines the size and functioning of the Golgi in the protist parasite, Trypanosoma brucei." Traffic 15(6): 613-629.

Sears, R., F. Nuckolls, E. Haura, Y. Taya, K. Tamai and J. R. Nevins (2000). "Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability." Genes Dev 14(19): 2501-2514.

Seger, R., N. G. Ahn, J. Posada, E. S. Munar, A. M. Jensen, J. A. Cooper, M. H. Cobb and E. G. Krebs (1992). "Purification and characterization of mitogen-activated protein kinase activator(s) from epidermal growth factor-stimulated A431 cells." J Biol Chem 267(20): 14373-14381.

Sepulveda, M. R., J. Vanoevelen, L. Raeymaekers, A. M. Mata and F. Wuytack (2009). "Silencing the SPCA1 (secretory pathway Ca2+-ATPase isoform 1) impairs Ca2+ homeostasis in the Golgi and disturbs neural polarity." J Neurosci 29(39): 12174-12182.

Serafini, T., L. Orci, M. Amherdt, M. Brunner, R. A. Kahn and J. E. Rothman (1991). "ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein." Cell 67(2): 239-253.

Settles, E. I., A. F. Loftus, A. N. McKeown and R. Parthasarathy (2010). "The vesicle trafficking protein Sar1 lowers lipid membrane rigidity." Biophys J 99(5): 1539-1545.

Shaffer, A. L., M. Shapiro-Shelef, N. N. Iwakoshi, A. H. Lee, S. B. Qian, H. Zhao, X. Yu, L. Yang, B. K. Tan, A. Rosenwald, E. M. Hurt, E. Petroulakis, N. Sonenberg, J. W. Yewdell, K. Calame, L.

H. Glimcher and L. M. Staudt (2004). "XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation." Immunity 21(1): 81-93.

Sharma, C., T. Vomastek, A. Tarcsafalvi, A. D. Catling, H. J. Schaeffer, S. T. Eblen and M. J. Weber (2005). "MEK partner 1 (MP1): regulation of oligomerization in MAP kinase signaling." J Cell Biochem 94: 708-719.

Sharpe, L. J., W. Luu and A. J. Brown (2011). "Akt phosphorylates Sec24: new clues into the regulation of ER-to-Golgi trafficking." Traffic 12(1): 19-27.

Shaul, Y. D. and R. Seger (2006). "ERK1c regulates Golgi fragmentation during mitosis." J Cell Biol 172(6): 885-897.

Shaywitz, D. A., P. J. Espenshade, R. E. Gimeno and C. A. Kaiser (1997). "COPII subunit interactions in the assembly of the vesicle coat." J Biol Chem 272(41): 25413-25416.

Shaywitz, D. A., L. Orci, M. Ravazzola, A. Swaroop and C. A. Kaiser (1995). "Human SEC13Rp functions in yeast and is located on transport vesicles budding from the endoplasmic reticulum." J Cell Biol 128(5): 769-777.

She, Q. B., N. Chen and Z. Dong (2000). "ERKs and p38 kinase phosphorylate p53 protein at serine 15 in response to UV radiation." J Biol Chem 275(27): 20444-20449.

Shen-Orr, S. S., R. Milo, S. Mangan and U. Alon (2002). "Network motifs in the transcriptional regulation network of Escherichia coli." Nat Genet 31(1): 64-68.

Shen, Y. and L. M. Hendershot (2005). "ERdj3, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates." Mol Biol Cell 16(1): 40-50.

Shen, Y., L. Meunier and L. M. Hendershot (2002). "Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress." J Biol Chem 277(18): 15947-15956.

Shenoy, S. K., M. T. Drake, C. D. Nelson, D. A. Houtz, K. Xiao, S. Madabushi, E. Reiter, R. T. Premont, O. Lichtarge and R. J. Lefkowitz (2006). "beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor." J Biol Chem 281: 1261-1273.

Shi, Y., K. M. Vattem, R. Sood, J. An, J. Liang, L. Stramm and R. C. Wek (1998). "Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control." Mol Cell Biol 18(12): 7499-7509.

Shibata, H., T. Kanadome, H. Sugiura, T. Yokoyama, M. Yamamuro, S. E. Moss and M. Maki (2015). "A new role for annexin A11 in the early secretory pathway via stabilizing Sec31A protein at the endoplasmic reticulum exit sites (ERES)." J Biol Chem 290(8): 4981-4993.

Shibayama, S., R. Shibata-Seita, K. Miura, Y. Kirino and K. Takishima (2002). "Identification of a C-terminal region that is required for the nuclear translocation of ERK2 by passive diffusion." J Biol Chem 277: 37777-37782.

Shima, D. T., K. Haldar, R. Pepperkok, R. Watson and G. Warren (1997). "Partitioning of the Golgi apparatus during mitosis in living HeLa cells." J Cell Biol 137(6): 1211-1228.

Shima, D. T., S. J. Scales, T. E. Kreis and R. Pepperkok (1999). "Segregation of COPI-rich and anterograde-cargo-rich domains in endoplasmic-reticulum-to-Golgi transport complexes." Curr Biol 9(15): 821-824.

Shimoi, W., I. Ezawa, K. Nakamoto, S. Uesaki, G. Gabreski, M. Aridor, A. Yamamoto, M. Nagahama, M. Tagaya and K. Tani (2005). "p125 is localized in endoplasmic reticulum exit sites and involved in their organization." J Biol Chem 280(11): 10141-10148.

Shimoni, Y., T. Kurihara, M. Ravazzola, M. Amherdt, L. Orci and R. Schekman (2000). "Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in Saccharomyces cerevisiae." J Cell Biol 151(5): 973-984.

Shin, S. Y., C. G. Kim and Y. H. Lee (2013). "Egr-1 regulates the transcription of the BRCA1 gene by etoposide." BMB Rep 46: 92-96.

Shin, S. Y., J. H. Kim, J. H. Lee, Y. Lim and Y. H. Lee (2012). "2'-Hydroxyflavanone induces apoptosis through Egr-1 involving expression of Bax, p21, and NAG-1 in colon cancer cells." Mol Nutr Food Res 56: 761-774.

Shindiapina, P. and C. Barlowe (2010). "Requirements for transitional endoplasmic reticulum site structure and function in Saccharomyces cerevisiae." Mol Biol Cell 21(9): 1530-1545.

Shohat, M., G. Janossy and R. R. Dourmashkin (1973). "Development of rough endoplasmic reticulum in mouse splenic lymphocytes stimulated by mitogens." Eur J Immunol 3(11): 680-687.

Short, B., C. Preisinger, R. Körner, R. Kopajtich, O. Byron and F. A. Barr (2001). "A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic." J Cell Biol 155: 877-883.

Shorter, J. and G. Warren (2002). "Golgi architecture and inheritance." Annu Rev Cell Dev Biol 18: 379-420.

Shoulders, C. C., D. J. Stephens and B. Jones (2004). "The intracellular transport of chylomicrons requires the small GTPase, Sar1b." Curr Opin Lipidol 15(2): 191-197.

Shugrue, C. A., E. R. Kolen, H. Peters, A. Czernik, C. Kaiser, L. Matovcik, A. L. Hubbard and F. Gorelick (1999). "Identification of the putative mammalian orthologue of Sec31P, a component of the COPII coat." J Cell Sci 112 (Pt 24): 4547-4556.

Siddiqi, S. and C. M. Mansbach, 2nd (2012). "Phosphorylation of Sar1b protein releases liver fatty acid-binding protein from multiprotein complex in intestinal cytosol enabling it to bind to endoplasmic reticulum (ER) and bud the pre-chylomicron transport vesicle." J Biol Chem 287(13): 10178-10188.

Siddiqi, S. and C. M. Mansbach, 2nd (2015). "Dietary and biliary phosphatidylcholine activates PKCzeta in rat intestine." J Lipid Res 56(4): 859-870.

Siddiqi, S., U. Saleem, N. A. Abumrad, N. O. Davidson, J. Storch, S. A. Siddiqi and C. M. Mansbach, 2nd (2010). "A novel multiprotein complex is required to generate the prechylomicron transport vesicle from intestinal ER." J Lipid Res 51(7): 1918-1928.

Siddiqi, S. A., F. S. Gorelick, J. T. Mahan and C. M. Mansbach, 2nd (2003). "COPII proteins are required for Golgi fusion but not for endoplasmic reticulum budding of the pre-chylomicron transport vesicle." J Cell Sci 116(Pt 2): 415-427.

Siddiqi, S. A. and C. M. Mansbach, 2nd (2008). "PKC zeta-mediated phosphorylation controls budding of the pre-chylomicron transport vesicle." J Cell Sci 121(Pt 14): 2327-2338.

Sidrauski, C. and P. Walter (1997). "The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response." Cell 90(6): 1031-1039.

Simoes, A. E., D. M. Pereira, S. E. Gomes, H. Brito, T. Carvalho, A. French, R. E. Castro, C. J. Steer, S. N. Thibodeau, C. M. Rodrigues and P. M. Borralho (2015). "Aberrant MEK5/ERK5

signalling contributes to human colon cancer progression via NF-kappaB activation." Cell Death Dis 6: e1718.

Simpson, J. C., B. Joggerst, V. Laketa, F. Verissimo, C. Cetin, H. Erfle, M. G. Bexiga, V. R. Singan, J. K. Heriche, B. Neumann, A. Mateos, J. Blake, S. Bechtel, V. Benes, S. Wiemann, J. Ellenberg and R. Pepperkok (2012). "Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway." Nat Cell Biol 14(7): 764-774.

Siniossoglou, S., C. Wimmer, M. Rieger, V. Doye, H. Tekotte, C. Weise, S. Emig, A. Segref and E. C. Hurt (1996). "A novel complex of nucleoporins, which includes Sec13p and a Sec13p homolog, is essential for normal nuclear pores." Cell 84(2): 265-275.

Slack, D. N., O. M. Seternes, M. Gabrielsen and S. M. Keyse (2001). "Distinct binding determinants for ERK2/p38alpha and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1." J Biol Chem 276: 16491-16500.

Sluss, H. K., T. Barrett, B. Dèrijard and R. J. Davis (1994). "Signal transduction by tumor necrosis factor mediated by JNK protein kinases." Mol Cell Biol 14: 8376-8384.

Soderholm, J., D. Bhattacharyya, D. Strongin, V. Markovitz, P. L. Connerly, C. A. Reineke and B. S. Glick (2004). "The transitional ER localization mechanism of Pichia pastoris Sec12." Dev Cell 6(5): 64959.

Sood, R., A. C. Porter, K. Ma, L. A. Quilliam and R. C. Wek (2000). "Pancreatic eukaryotic initiation factor-2alpha kinase (PEK) homologues in humans, Drosophila melanogaster and Caenorhabditis elegans that mediate translational control in response to endoplasmic reticulum stress." Biochem J 346 Pt 2: 281-293.

Sorgjerd, K., B. Ghafouri, B. H. Jonsson, J. W. Kelly, S. Y. Blond and P. Hammarstrom (2006). "Retention of misfolded mutant transthyretin by the chaperone BiP/GRP78 mitigates amyloidogenesis." J Mol Biol 356(2): 469-482.

Soung, Y. H., J. W. Lee, S. W. Nam, J. Y. Lee, N. J. Yoo and S. H. Lee (2006). "Mutational analysis of AKT1, AKT2 and AKT3 genes in common human carcinomas." Oncology 70(4): 285-289.

Sousa, M. and A. J. Parodi (1995). "The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase." EMBO J 14(17): 4196-4203.

Spacek, J. and K. M. Harris (1997). "Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat." J Neurosci 17(1): 190-203.

Spang, A., K. Matsuoka, S. Hamamoto, R. Schekman and L. Orci (1998). "Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes." Proc Natl Acad Sci U S A 95(19): 11199-11204.

Sparkes, I. A., T. Ketelaar, N. C. de Ruijter and C. Hawes (2009). "Grab a Golgi: laser trapping of Golgi bodies reveals in vivo interactions with the endoplasmic reticulum." Traffic 10(5): 567-571.

Sprangers, J. and C. Rabouille (2015). "SEC16 in COPII coat dynamics at ER exit sites." Biochem Soc Trans 43: 97-103.

Stagg, S. M., C. Gurkan, D. M. Fowler, P. LaPointe, T. R. Foss, C. S. Potter, B. Carragher and W. E. Balch (2006). "Structure of the Sec13/31 COPII coat cage." Nature 439(7073): 234-238.

Stagg, S. M., P. LaPointe, A. Razvi, C. Gurkan, C. S. Potter, B. Carragher and W. E. Balch (2008). "Structural basis for cargo regulation of COPII coat assembly." Cell 134(3): 474-484.

Stamnes, M. A., M. W. Craighead, M. H. Hoe, N. Lampen, S. Geromanos, P. Tempst and J. E. Rothman (1995). "An integral membrane component of coatomer-coated transport vesicles defines a family of proteins involved in budding." Proc Natl Acad Sci U S A 92: 8011-8015.

Stankewich, M. C., P. R. Stabach and J. S. Morrow (2006). "Human Sec31B: a family of new mammalian orthologues of yeast Sec31p that associate with the COPII coat." J Cell Sci 119(Pt 5): 958-969.

Stanley, P. (2011). "Golgi glycosylation." Cold Spring Harb Perspect Biol 3: a005199.

Stephens, D. J. (2003). "De novo formation, fusion and fission of mammalian COPII-coated endoplasmic reticulum exit sites." EMBO Rep 4(2): 210-217.

Stephens, D. J. and R. Pepperkok (2002). "Imaging of procollagen transport reveals COPI-dependent cargo sorting during ER-to-Golgi transport in mammalian cells." J Cell Sci 115(Pt 6): 1149-1160.

Steringer, J. P., S. Bleicken, H. Andreas, S. Zacherl, M. Laussmann, K. Temmerman, F. X. Contreras, T. A. Bharat, J. Lechner, H. M. Muller, J. A. Briggs, A. J. Garcia-Saez and W. Nickel (2012). "Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2)-dependent oligomerization of fibroblast growth factor 2 (FGF2) triggers the formation of a lipidic membrane pore implicated in unconventional secretion." J Biol Chem 287(33): 27659-27669.

Stewart, S., M. Sundaram, Y. Zhang, J. Lee, M. Han and K. L. Guan (1999). "Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization." Mol Cell Biol 19(8): 5523-5534.

Strambi, A., M. Mori, M. Rossi, D. Colecchia, F. Manetti, F. Carlomagno, M. Botta and M. Chiariello (2013). "Structure prediction and validation of the ERK8 kinase domain." PLoS One 8(1): e52011.

Strating, J. R. and G. J. Martens (2009). "The p24 family and selective transport processes at the ER-Golgi interface." Biol Cell 101: 495-509.

Stronge, V. S., Y. Saito, Y. Ihara and D. B. Williams (2001). "Relationship between calnexin and BiP in suppressing aggregation and promoting refolding of protein and glycoprotein substrates." J Biol Chem 276(43): 39779-39787.

Sturgill, T. W. and L. B. Ray (1986). "Muscle proteins related to microtubule associated protein-2 are substrates for an insulin-stimulatable kinase." Biochem Biophys Res Commun 134: 565-571.

Sucic, S., A. El-Kasaby, O. Kudlacek, S. Sarker, H. H. Sitte, P. Marin and M. Freissmuth (2011). "The serotonin transporter is an exclusive client of the coat protein complex II (COPII) component SEC24C." J Biol Chem 286(18): 16482-16490.

Sucic, S., F. Koban, A. El-Kasaby, O. Kudlacek, T. Stockner, H. H. Sitte and M. Freissmuth (2013). "Switching the clientele: a lysine residing in the C terminus of the serotonin transporter specifies its preference for the coat protein complex II component SEC24C." J Biol Chem 288(8): 5330-5341.

Suda, Y. and A. Nakano (2012). "The Yeast Golgi Apparatus." Traffic 13(4): 505-510.

Sukhatme, V. P., X. M. Cao, L. C. Chang, C. H. Tsai-Morris, D. Stamenkovich, P. C. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows and T. Curran (1988). "A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization." Cell 53: 37-43.

Sun, T., H. Tian, Y. G. Feng, Y. Q. Zhu and W. Q. Zhang (2013). "Egr-1 promotes cell proliferation and invasion by increasing

β-catenin expression in gastric cancer." Dig Dis Sci 58: 423-430.

Sun, W., K. Kesavan, B. C. Schaefer, T. P. Garrington, M. Ware, N. L. Johnson, E. W. Gelfand and G. L. Johnson (2001). "MEKK2 associates with the adapter protein Lad/RIBP and regulates the MEK5-BMK1/ERK5 pathway." J Biol Chem 276(7): 5093-5100.

Sun, Z., F. Anderl, K. Frohlich, L. Zhao, S. Hanke, B. Brugger, F. Wieland and J. Bethune (2007). "Multiple and stepwise interactions between coatomer and ADP-ribosylation factor-1 (Arf1)-GTP." Traffic 8(5): 582-593.

Sundaram, M. and M. Han (1995). "The C. elegans ksr-1gene encodes a novel Raf-related kinase involvedin Ras-mediated signal transduction." Cell 83: 889-901.

Supek, F., D. T. Madden, S. Hamamoto, L. Orci and R. Schekman (2002). "Sec16p potentiates the action of COPII proteins to bud transport vesicles." J Cell Biol 158(6): 1029-1038.

Sütterlin, C., C. Y. Lin, Y. Feng, D. K. Ferris, R. L. Erikson and V. Malhotra (2001). "Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis." Proc Natl Acad Sci U S A 98(16): 9128-9132.

Suzuki, K., M. Akioka, C. Kondo-Kakuta, H. Yamamoto and Y. Ohsumi (2013). "Fine mapping of autophagy-related proteins during autophagosome formation in Saccharomyces cerevisiae." J Cell Sci 126(Pt 11): 2534-2544.

Suzuki, T., A. Inoue, Y. Miki, T. Moriya, J. Akahira, T. Ishida, H. Hirakawa, Y. Yamaguchi, S. Hayashi and H. Sasano (2007). "Early growth responsive gene 3 in human breast carcinoma: a regulator of estrogen-meditated invasion and a potent prognostic factor." Endocr Relat Cancer 14(2): 279-292.

Svaren, J., T. Ehrig, S. A. Abdulkadir, M. U. Ehrengruber, M. A. Watson and J. Milbrandt (2000). "EGR1 target genes in prostate carcinoma cells identified by microarray analysis." J Biol Chem 275(49): 38524-38531.

Swanton, E., S. High and P. Woodman (2003). "Role of calnexin in the glycan-independent quality control of proteolipid protein." EMBO J 22(12): 2948-2958.

Swaroop, A., T. L. Yang-Feng, W. Liu, L. Gieser, L. L. Barrow, K. C. Chen, N. Agarwal, M. H. Meisler and D. I. Smith (1994). "Molecular characterization of a novel human gene, SEC13R, related to the yeast secretory pathway gene SEC13, and mapping to a conserved linkage group on human chromosome 3p24-p25 and mouse chromosome 6." Hum Mol Genet 3(8): 1281-1286.

Swart, J. M., D. M. Bergeron and T. C. Chiles (2000). "Identification of a membrane Ig-induced p38 mitogen-activated protein kinase module that regulates cAMP response element binding protein phosphorylation and transcriptional activation in CH31 B cell lymphomas." J Immunol 164(5): 2311-2319.

Swiatek, P. J. and T. Gridley (1993). "Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20." Genes Dev 7: 2071-2084.

Szegezdi, E., S. E. Logue, A. M. Gorman and A. Samali (2006). "Mediators of endoplasmic reticulum stress-induced apoptosis." EMBO Rep 7(9): 880-885.

Szul, T., R. Garcia-Mata, E. Brandon, S. Shestopal, C. Alvarez and E. Sztul (2005). "Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor GBF1." Traffic 6(5): 374-385.

Szul, T. and E. Sztul (2011). "COPII and COPI traffic at the ER-Golgi interface." Physiology (Bethesda) 26(5): 348-364.

Tabata, K. V., K. Sato, T. Ide, T. Nishizaka, A. Nakano and H. Noji (2009). "Visualization of cargo concentration by COPII minimal machinery in a planar lipid membrane." EMBO J 28(21): 3279-3289.

Takagi, J., L. Renna, H. Takahashi, Y. Koumoto, K. S. Tamura, G., Y. Fukao, M. Kondo, M. Nishimura, T. Shimada, F. Brandizzi and I. Hara-Nishimura (2013). "MAIGO5 functions in protein export from Golgi-associated endoplasmic reticulum exit sites in Arabidopsis." Plant Cell 25(11): 4658-4675.

Takashima, M., W. Ogawa, A. Emi and M. Kasuga (2009). "Regulation of SREBP1c expression by mTOR signaling in hepatocytes." Kobe J Med Sci 55(2): E45-52.

Takeda, A. N., T. K. Oberoi-Khanuja, G. Glatz, K. Schulenburg, R. P. Scholz, A. Carpy, B. Macek, A. Remenyi and K. Rajalingam (2014). "Ubiquitin-dependent regulation of MEKK2/3-MEK5-ERK5 signaling module by XIAP and cIAP1." EMBO J 33(16): 1784-1801.

Takida, S., Y. Maeda and T. Kinoshita (2008). "Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane." Biochem J 409: 555-562.

Tan, D., Y. Cai, J. Wang, J. Zhang, S. Menon, H. T. Chou, S. Ferro-Novick, K. M. Reinisch and T. Walz (2013). "The EM structure of the TRAPPIII complex leads to the identification of a requirement for COPII vesicles on the macroautophagy pathway." Proc Natl Acad Sci U S A 110(48): 19432-19437.

Tan, Y., N. Dourdin, C. Wu, T. De Veyra, J. S. Elce and P. A. Greer (2006). "Ubiquitous calpains promote caspase-12 and JNK activation during endoplasmic reticulum stress-induced apoptosis." J Biol Chem 281(23): 16016-16024.

Tang, B. L., J. Kausalya, D. Y. Low, M. L. Lock and W. Hong (1999). "A family of mammalian proteins homologous to yeast Sec24p." Biochem Biophys Res Commun 258(3): 679-684.

Tang, B. L., S. H. Wong, X. L. Qi, S. H. Low and W. Hong (1993). "Molecular cloning, characterization, subcellular localization and dynamics of p23, the mammalian KDEL receptor." J Cell Biol 120: 325-338.

Tang, B. L., T. Zhang, D. Y. Low, E. T. Wong, H. Horstmann and W. Hong (2000). "Mammalian homologues of yeast sec31p. An ubiquitously expressed form is localized to endoplasmic reticulum (ER) exit sites and is essential for ER-Golgi transport." J Biol Chem 275(18): 13597-13604.

Tang, D., K. Mar, G. Warren and Y. Wang (2008). "Molecular mechanism of mitotic Golgi disassembly and reassembly revealed by a defined reconstitution assay." J Biol Chem 283(10): 6085-6094.

Tang, D., H. Yuan, O. Vielemeyer, F. Perez and Y. Wang (2012). "Sequential phosphorylation of GRASP65 during mitotic Golgi disassembly." Biol Open 1(12): 1204-1214.

Tang, E. D., G. Nunez, F. G. Barr and K. L. Guan (1999). "Negative regulation of the forkhead transcription factor FKHR by Akt." J Biol Chem 274(24): 16741-16746.

Tang, X., L. Deng, H. Xiong, G. Li, J. Lin, J. Liu, F. Kong, G. Tu, H. Peng and S. Liang (2014). "Expression profile of mitrogen-activated protein kinase (MAPK) signaling genes in the skeletal muscle & liver of rat with type 2 diabetes: Role in disease pathology." Indian J Med Res 140: 744-755.

Tani, K., T. Mizoguchi, A. Iwamatsu, K. Hatsuzawa and M. Tagaya (1999). "p125 is a novel mammalian Sec23p-interacting protein with structural similarity to phospholipid-modifying proteins." J Biol Chem 274(29): 20505-20512.

Tani, K., M. Tagaya, S. Yonekawa and T. Baba (2011). "Dual function of Sec16B: Endoplasmic reticulum-derived protein

secretion and peroxisome biogenesis in mammalian cells." Cell Logist 1(4): 164-167.

Tanoue, T., T. Moriguchi and E. Nishida (1999). "Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5." J Biol Chem 274: 19949-19956.

Tanoue, T., T. Yamamoto, R. Maeda and E. Nishida (2001). "A Novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 alpha and beta MAPKs." J Biol Chem 276: 26629-26639.

Tao, J., M. Zhu, H. Wang, S. Afelik, M. P. Vasievich, X. W. Chen, G. Zhu, J. Jensen, D. Ginsburg and B. Zhang (2012). "SEC23B is required for the maintenance of murine professional secretory tissues." Proc Natl Acad Sci U S A 109(29): E2001-2009.

Tao, W., J. F. Shi, Q. Zhang, B. Xue, Y. J. Sun and C. J. Li (2013). "Egr-1 enhances drug resistance of breast cancer by modulating MDR1 expression in a GGPPS-independent manner." Biomed Pharmacother 67: 197-202.

Taymans, J. M. and V. Baekelandt (2014). "Phosphatases of alpha-synuclein, LRRK2, and tau: important players in the phosphorylation-dependent pathology of Parkinsonism." Front Genet 5: 382.

Teal, S. B., V. W. Hsu, P. J. Peters, R. D. Klausner and J. G. Donaldson (1994). "An activating mutation in ARF1 stabilizes coatomer binding to Golgi membranes." J Biol Chem 269(5): 3135-3138.

Tee, A. R., D. C. Fingar, B. D. Manning, D. J. Kwiatkowski, L. C. Cantley and J. Blenis (2002). "Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling." Proc Natl Acad Sci U S A 99(21): 13571-13576.

Teis, D., W. Wunderlich and L. A. Huber (2002). "Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction." Dev Cell 3: 803-814.

Terasawa, K., K. Okazaki and E. Nishida (2003). "Regulation of c-Fos and Fra-1 by the MEK5-ERK5 pathway." Genes Cells 8(3): 263-273.

Thedieck, K., P. Polak, M. L. Kim, K. D. Molle, A. Cohen, P. Jeno, C. Arrieumerlou and M. N. Hall (2007). "PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis." PLoS One 2(11): e1217.

Therrien, M., H. C. Chang, N. M. Solomon, F. D. Karim, D. A. Wassarman and G. M. Rubin (1995). "KSR, a novel protein kinase required for RAS signal transduction." Cell 83: 879-888.

Therrien, M., N. R. Michaud, G. M. Rubin and D. K. Morrison (1996). "KSR modulates signal propagation within the MAPK cascade." Genes Dev 10: 2684-2695.

Thigpen, A. E., K. M. Cala, J. M. Guileyardo, K. H. Molberg, J. D. McConnell and D. W. Russell (1996). "Increased expression of early growth response-1 messenger ribonucleic acid in prostatic adenocarcinoma." J Urol 155(3): 975-981.

Thuerauf, D. J., N. D. Arnold, D. Zechner, D. S. Hanford, K. M. DeMartin, P. M. McDonough, R. Prywes and C. C. Glembotski (1998). "p38 Mitogen-activated protein kinase mediates the transcriptional induction of the atrial natriuretic factor gene through a serum response element. A potential role for the transcription factor ATF6." J Biol Chem 273(32): 20636-20643.

Thyberg, J. and S. Moskalewski (1992). "Reorganization of the Golgi complex in association with mitosis: redistribution of mannosidase II to the endoplasmic reticulum and effects of brefeldin A." J Submicrosc Cytol Pathol 24(4): 495-508.

Tidyman, W. E. and K. A. Rauen (2009). "The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation." Curr Opin Genet Dev 19: 230-236.

Tillmann, K. D., V. Millarte and H. Farhan (2013). "Regulation of traffic and organelle architecture of the ER-Golgi interface by signal transduction." Histochem Cell Biol 140(3): 297-306.

Tirasophon, W., A. A. Welihinda and R. J. Kaufman (1998). "A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells." Genes Dev 12(12): 1812-1824.

Tisdale, E. J. (1999). "A Rab2 mutant with impaired GTPase activity stimulates vesicle formation from pre-Golgi intermediates." Mol Biol Cell 10(6): 1837-1849.

Tisdale, E. J. (2000). "Rab2 requires PKC iota/lambda to recruit beta-COP for vesicle formation." Traffic 1(9): 702-712.

Tisdale, E. J. (2001). "Glyceraldehyde-3-phosphate dehydrogenase is required for vesicular transport in the early secretory pathway." J Biol Chem 276(4): 2480-2486.

Tisdale, E. J. (2002). "Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase Ciota /lambda and plays a role in microtubule dynamics in the early secretory pathway." J Biol Chem 277(5): 3334-3341.

Tisdale, E. J. (2003). "Rab2 interacts directly with atypical protein kinase C (aPKC) iota/lambda and inhibits aPKCiota/lambda-dependent glyceraldehyde-3-phosphate dehydrogenase phosphorylation." J Biol Chem 278(52): 52524-52530.

Tisdale, E. J. and C. R. Artalejo (2006). "Src-dependent aprotein kinase C iota/lambda (aPKCiota/lambda) tyrosine phosphorylation is required for aPKCiota/lambda association with Rab2 and glyceraldehyde-3-phosphate dehydrogenase on pre-golgi intermediates." J Biol Chem 281(13): 8436-8442.

Tisdale, E. J. and C. R. Artalejo (2007). "A GAPDH mutant defective in Src-dependent tyrosine phosphorylation impedes Rab2-mediated events." Traffic 8(6): 733-741.

Tisdale, E. J., F. Azizi and C. R. Artalejo (2009). "Rab2 utilizes glyceraldehyde-3-phosphate dehydrogenase and protein kinase C{iota} to associate with microtubules and to recruit dynein." J Biol Chem 284(9): 5876-5884.

Tisdale, E. J. and W. E. Balch (1996). "Rab2 is essential for the maturation of pre-Golgi intermediates." J Biol Chem 271(46): 29372-29379.

Tisdale, E. J. and M. R. Jackson (1998). "Rab2 protein enhances coatomer recruitment to pre-Golgi intermediates." J Biol Chem 273(27): 17269-17277.

Tohgo, A., E. W. Choy, D. Gesty-Palmer, K. L. Pierce, S. Laporte, R. H. Oakley, M. G. Caron, R. J. Lefkowitz and L. M. Luttrell (2003). "The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation." J Biol Chem 278: 6258-6267.

Tohgo, A., K. L. Pierce, E. W. Choy, R. J. Lefkowitz and L. M. Luttrell (2002). "beta-Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation." J Biol Chem 277: 9429-9436.

Topham, M. K. and S. M. Prescott (1999). "Mammalian diacylglycerol kinases, a family of lipid kinases with signaling functions." J Biol Chem 274(17): 11447-11450.

Topilko, P., S. Schneider-Maunoury, G. Levi, A. Trembleau, D. Gourdji, M. A. Driancourt, C. V. Rao and P. Charnay (1998). "Multiple pituitary and ovarian defects in Krox-24 (NGFIA/Egr-1) targeted mice." Mol Endocrinol 12: 107-122.

Torii, S., M. Kusakabe, T. Yamamoto, M. Maekawa and E. Nishida (2004). "Sef is a spatial regulator for Ras/MAP kinase signaling." Dev Cell 7: 33-44.

Tourtellotte, W. G., C. Keller-Peck, J. Milbrandt and J. Kucera (2001). "The transcription factor Egr3 modulates sensory axon-myotube interactions during muscle spindle morphogenesis." Dev Biol 232: 388-399.

Tourtellotte, W. G. and J. Milbrandt (1998). "Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3." Nat Genet 20: 87-91.

Tourtellotte, W. G., R. Nagarajan, A. Auyeung, C. Mueller and J. Milbrandt (1999). "Infertility associated with incomplete spermatogenic arrest and oligozoospermia in Egr4-deficient mice." Development 126: 5061-5071.

Townley, A. K., Y. Feng, K. Schmidt, D. A. Carter, R. Porter, P. Verkade and D. J. Stephens (2008). "Efficient coupling of Sec23-Sec24 to Sec13-Sec31 drives COPII-dependent collagen secretion and is essential for normal craniofacial development." J Cell Sci 121: 3025-3034.

Townley, A. K., K. Schmidt, L. Hodgson and D. J. Stephens (2012). "Epithelial organization and cyst lumen expansion require efficient Sec13-Sec31-driven secretion." J Cell Sci 125(Pt 3): 673-684.

Travers, K. J., C. K. Patil, L. Wodicka, D. J. Lockhart, J. S. Weissman and P. Walter (2000). "Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation." Cell 101(3): 249-258.

Tsai, J. C., L. Liu, J. Guan and W. C. Aird (2000). "The Egr-1 gene is induced by epidermal growth factor in ECV304 cells and primary endothelial cells." Am J Physiol Cell Physiol 279(5): C1414-1424.

Tsai, J. C., L. Liu, J. Zhang, K. C. Spokes, J. N. Topper and W. C. Aird (2001). "Epidermal growth factor induces Egr-1 promoter activity in hepatocytes in vitro and in vivo." Am J Physiol Gastrointest Liver Physiol 281(5): G1271-1278.

Tsvetanova, N. G. (2013). "The secretory pathway in control of endoplasmic reticulum homeostasis." Small GTPases 4(1): 28-33.

Tsvetanova, N. G., D. P. Riordan and P. O. Brown (2012). "The yeast Rab GTPase Ypt1 modulates unfolded protein response dynamics by regulating the stability of HAC1 RNA." PLoS Genet 8(7): e1002862.

Tyedmers, J., M. Lerner, M. Wiedmann, J. Volkmer and R. Zimmermann (2003). "Polypeptide-binding proteins mediate completion of co-translational protein translocation into the mammalian endoplasmic reticulum." EMBO Rep 4(5): 505-510.

Uemura, T., M. Yamamoto, A. Kametaka, Y. S. Sou, A. Yabashi, A. Yamada, H. Annoh, S. Kametaka, M. Komatsu and S. Waguri (2014). "A cluster of thin tubular structures mediates transformation of the endoplasmic reticulum to autophagic isolation membrane." Mol Cell Biol 34(9): 1695-1706.

Urano, F., X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H. P. Harding and D. Ron (2000). "Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1." Science 287(5453): 664-666.

van Zuylen, W. J., P. Doyon, J. F. Clement, K. A. Khan, L. M. D'Ambrosio, F. Do, M. St-Amant-Verret, T. Wissanji, G. Emery, A. C. Gingras, S. Meloche and M. J. Servant (2012). "Proteomic profiling of the TRAF3 interactome network reveals a new role for the ER-to-Golgi transport compartments in innate immunity." PLoS Pathog 8(7): e1002747.

Vander Haar, E., S. I. Lee, S. Bandhakavi, T. J. Griffin and D. H. Kim (2007). "Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40." Nat Cell Biol 9(3): 316-323.

Vembar, S. S. and J. L. Brodsky (2008). "One step at a time: endoplasmic reticulum-associated degradation." Nat Rev Mol Cell Biol 9(12): 944-957.

Venditti, R., T. Scanu, M. Santoro, G. Di Tullio, A. Spaar, R. Gaibisso, G. V. Beznoussenko, A. A. Mironov, A. Mironov, Jr., L. Zelante, M. R. Piemontese, A. Notarangelo, V. Malhotra, B. M. Vertel, C. Wilson and M. A. De Matteis (2012). "Sedlin controls the ER export of procollagen by regulating the Sar1 cycle." Science 337(6102): 1668-1672.

Venditti, R., C. Wilson and M. A. De Matteis (2014). "Exiting the ER: what we know and what we don't." Trends Cell Biol 24(1): 9-18.

Virolle, T., A. Krones-Herzig, V. Baron, G. De Gregorio, E. D. Adamson and D. Mercola (2003). "Egr1 promotes growth and survival of prostate cancer cells. Identification of novel Egr1 target genes." J Biol Chem 278(14): 11802-11810.

Vjestica, A., X. Z. Tang and S. Oliferenko (2008). "The actomyosin ring recruits early secretory compartments to the division site in fission yeast." Mol Biol Cell 19(3): 1125-1138.

von Heijne, G. (1983). "Patterns of amino acids near signal-sequence cleavage sites." Eur J Biochem 133(1): 17-21.

von Heijne, G. (1984). "Analysis of the distribution of charged residues in the N-terminal region of signal sequences: implications for protein export in prokaryotic and eukaryotic cells." EMBO J. 3(10): 2315-2318.

Von Heijne, G. (1985). "Signal sequences. The limits of variation." J Mol Biol 184(1): 99-105.

Von Heijne, G. (1986). "Towards a comparative anatomy of N-terminal topogenic protein sequences." J Mol Biol 189(1): 239-242.

Wallings, R., C. Manzoni and R. Bandopadhyay (2015). "Cellular processes associated with LRRK2 function and dysfunction." FEBS J.

Wang, B. T., G. S. Ducker, A. J. Barczak, R. Barbeau, D. J. Erle and K. M. Shokat (2011). "The mammalian target of rapamycin regulates cholesterol biosynthetic gene expression and exhibits a rapamycin-resistant transcriptional profile." Proc Natl Acad Sci U S A 108(37): 15201-15206.

Wang, J., D. Tan, Y. Cai, K. M. Reinisch, T. Walz and S. Ferro-Novick (2014). "A requirement for ER-derived COPII vesicles in phagophore initiation." Autophagy 10(4): 708-709.

Wang, L., T. E. Harris, R. A. Roth and J. C. Lawrence, Jr. (2007). "PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding." J Biol Chem 282(27): 20036-20044.

Wang, L., R. Ma, R. A. Flavell and M. E. Choi (2002). "Requirement of mitogen-activated protein kinase kinase 3 (MKK3) for activation of p38alpha and p38delta MAPK isoforms by TGF-beta 1 in murine mesangial cells." J Biol Chem 277(49): 47257-47262.

Wang, Q., L. Li and Y. Ye (2008). "Inhibition of p97-dependent protein degradation by Eeyarestatin I." J Biol Chem 283(12): 7445-7454.

Wang, X., S. Pesakhov, J. S. Harrison, M. Danilenko and G. P. Studzinski (2014). "ERK5 pathway regulates transcription factors important for monocytic differentiation of human myeloid leukemia cells." J Cell Physiol 229(7): 856-867.

Wang, X. S., K. Diener, C. L. Manthey, S. Wang, B. Rosenzweig, J. Bray, J. Delaney, C. N. Cole, P. Y. Chan-Hui, N. Mantlo, H. S. Lichenstein, M. Zukowski and Z. Yao (1997). "Molecular

cloning and characterization of a novel p38 mitogen-activated protein kinase." J Biol Chem 272(38): 23668-23674.

Wang, X. Z., H. P. Harding, Y. Zhang, E. M. Jolicoeur, M. Kuroda and D. Ron (1998). "Cloning of mammalian Ire1 reveals diversity in the ER stress responses." EMBO J 17(19): 5708-5717.

Wang, X. Z. and D. Ron (1996). "Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP Kinase." Science 272(5266): 1347-1349.

Wang, Y. and J. Seemann (2011). "Golgi biogenesis." Cold Spring Harb Perspect Biol 3(10): a005330.

Wang, Y., J. Seemann, M. Pypaert, J. Shorter and G. Warren (2003). "A direct role for GRASP65 as a mitotically regulated Golgi stacking factor." EMBO J 22(13): 3279-3290.

Wansleeben, C., H. Feitsma, M. Montcouquiol, C. Kroon, E. Cuppen and F. Meijlink (2010). "Planar cell polarity defects and defective Vangl2 trafficking in mutants for the COPII gene Sec24b." Development 137(7): 1067-1073.

Ware, F. E., A. Vassilakos, P. A. Peterson, M. R. Jackson, M. A. Lehrman and D. B. Williams (1995). "The molecular chaperone calnexin binds Glc1Man9GlcNAc2 oligosaccharide as an initial step in recognizing unfolded glycoproteins." J Biol Chem 270(9): 4697-4704.

Warren, G., C. Featherstone, G. Griffiths and B. Burke (1983). "Newly synthesized G protein of vesicular stomatitis virus is not transported to the cell surface during mitosis." J Cell Biol 97(5 Pt 1): 1623-1628.

Watson, P., A. K. Townley, P. Koka, K. J. Palmer and D. J. Stephens (2006). "Sec16 defines endoplasmic reticulum exit sites and is required for secretory cargo export in mammalian cells." Traffic 7(12): 1678-1687.

Webber, J. L. and S. A. Tooze (2010). "Coordinated regulation of autophagy by p38alpha MAPK through mAtg9 and p38IP." EMBO J 29(1): 27-40.

Wei, H., S. Ahn, W. G. Barnes and R. J. Lefkowitz (2004). "Stable interaction between beta-arrestin 2 and angiotensin type 1A receptor is required for beta-arrestin 2-mediated activation of extracellular signal-regulated kinases 1 and 2." J Biol Chem 279: 48255-48261.

Wei, J. H. and J. Seemann (2009). "Remodeling of the Golgi structure by ERK signaling." Commun Integr Biol 2(1): 35-36.

Wei, M. C., W. X. Zong, E. H. Cheng, T. Lindsten, V. Panoutsakopoulou, A. J. Ross, K. A. Roth, G. R. MacGregor, C. B. Thompson and S. J. Korsmeyer (2001). "Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death." Science 292(5517): 727-730.

Weidberg, H., E. Shvets and Z. Elazar (2011). "Biogenesis and cargo selectivity of autophagosomes." Annu Rev Biochem 80: 125-156.

Weissman, J. T., H. Plutner and W. E. Balch (2001). "The mammalian guanine nucleotide exchange factor mSec12 is essential for activation of the Sar1 GTPase directing endoplasmic reticulum export." Traffic 2(7): 465-475.

Wendeler, M. W., J. P. Paccaud and H. P. Hauri (2007). "Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum." EMBO Rep 8(3): 258-264.

Westrate, L. M., J. E. Lee, W. A. Prinz and G. K. Voeltz (2015). "Form Follows Function: The Importance of Endoplasmic Reticulum Shape." Annu Rev Biochem 84.

Whitmarsh, A. J. (2006). "The JIP family of MAPK scaffold proteins." Biochem Soc Trans 34: 828-832.

Whitmarsh, A. J., J. Cavanagh, C. Tournier, J. Yasuda and R. J. Davis (1998). "A mammalian scaffold complex that selectively mediates MAP kinase activation." Science 281: 1671-1674.

Whittle, J. R. and T. U. Schwartz (2010). "Structure of the Sec13-Sec16 edge element, a template for assembly of the COPII vesicle coat." J Cell Biol 190(3): 347-361.

Wieland, F. T., M. L. Gleason, T. A. Serafini and J. E. Rothman (1987). "The rate of bulk flow from the endoplasmic reticulum to the cell surface." Cell 50: 289-300.

Wiest, D. L., J. K. Burkhardt, S. Hester, M. Hortsch, D. I. Meyer and Y. Argon (1990). "Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately." J Cell Biol 110(5): 1501-1511.

Wild, P., D. G. McEwan and I. Dikic (2014). "The LC3 interactome at a glance." J Cell Sci 127(Pt 1): 3-9.

Williams, D. B. (2006). "Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum." J Cell Sci 119(Pt 4): 615-623.

Williams, J., M. Dragunow, P. Lawlor, S. Mason, W. C. Abraham, J. Leah, R. Bravo, J. Demmer and W. Tate (1995). "Krox20 may play a key role in the stabilization of long-term potentiation." Brain Res Mol Brain Res 28: 87-93.

Willoughby, E. A., G. R. Perkins, M. K. Collins and A. J. Whitmarsh (2003). "The JIP-1 scaffold protein targets MKP-7 to dephosphorylate JNK." J Biol Chem 278: 10731-10736.

Wilson, D. W., M. J. Lewis and H. R. Pelham (1993). "pH-dependent binding of KDEL to its receptor in vitro." J Biol Chem 268: 7465-7468.

Wisniewski, J. R., P. Ostasiewicz, K. Dus, D. F. Zielinska, F. Gnad and M. Mann (2012). "Extensive quantitative remodeling of the proteome between normal colon tissue and adenocarcinoma." Mol Syst Biol 8: 611.

Witte, K., A. L. Schuh, J. Hegermann, A. Sarkeshik, J. R. Mayers, K. Schwarze, J. R. Yates, 3rd, S. Eimer and A. Audhya (2011). "TFG-1 function in protein secretion and oncogenesis." Nat Cell Biol 13(5): 550-558.

Wolf, I., H. Rubinfeld, S. Yoon, G. Marmor, T. Hanoch and R. Seger (2001). "Involvement of the activation loop of ERK in the detachment from cytosolic

anchoring." J Biol Chem 276: 24490-24497.

Woo, S. M., K. J. Min, S. Kim, J. W. Park, D. E. Kim, K. S. Chun, Y. H. Kim, T. J. Lee, S. H. Kim, Y. H. Choi, J. S. Chang and T. K. Kwon (2014). "Silibinin induces apoptosis of HT29 colon carcinoma cells through early growth response-1 (EGR-1)-mediated non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) up-regulation." Chem Biol Interact 211: 36-43.

Wooten, M. W., M. L. Vandenplas, M. L. Seibenhener, T. Geetha and M. T. Diaz-Meco (2001). "Nerve growth factor stimulates multisite tyrosine phosphorylation and activation of the atypical protein kinase C's via a src kinase pathway." Mol Cell Biol 21(24): 8414-8427.

Worley, P. F., R. V. Bhat, J. M. Baraban, C. A. Erickson, B. L. McNaughton and C. A. Barnes (1993). "Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement." J Neurosci 13: 4776-4786.

Wortzel, I. and R. Seger (2011). "The ERK Cascade: Distinct Functions within Various Subcellular Organelles." Genes Cancer 2: 195-209.

Wu, J., Y. Dang, W. Su, C. Liu, H. Ma, Y. Shan, Y. Pei, B. Wan, J. Guo and L. Yu (2006). "Molecular cloning and characterization of rat LC3A and LC3B--two novel markers of autophagosome." Biochem Biophys Res Commun 339(1): 437-442.

Wu, J., D. T. Rutkowski, M. Dubois, J. Swathirajan, T. Saunders, J. Wang, B. Song, G. D. Yau and R. J. Kaufman (2007). "ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress." Dev Cell 13(3): 351-364.

Wu, S., F. Hong, D. Gewirth, B. Guo, B. Liu and Z. Li (2012). "The molecular chaperone gp96/GRP94 interacts with Toll-like receptors and integrins via its C-terminal hydrophobic domain." J Biol Chem 287(9): 6735-6742.

Wunderlich, W., I. Fialka, D. Teis, A. Alpi, A. Pfeiffer, R. G. Parton, F. Lottspeich and L. A. Huber (2001). "A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mp1 on a late endosomal/lysosomal compartment." J Cell Biol 152: 765-776.

Xiang, L., E. Etxeberria and W. Van den Ende (2013). "Vacuolar protein sorting mechanisms in plants." FEBS J 280(4): 979-993.

Xiang, Y. and Y. Wang (2010). "GRASP55 and GRASP65 play complementary and essential roles in Golgi cisternal stacking." J Cell Biol 188(2): 237-251.

Xiang, Y., X. Zhang, D. B. Nix, T. Katoh, K. Aoki, M. Tiemeyer and Y. Wang (2013). "Regulation of protein glycosylation and sorting by the Golgi matrix proteins GRASP55/65." Nat Commun 4: 1659.

Xing, H., K. Kornfeld and A. J. Muslin (1997). "The protein kinase KSR interacts with 14-3-3 protein and Raf." Curr Biol 7: 294-300.

Xu, Y. M., F. Zhu, Y. Y. Cho, A. Carper, C. Peng, D. Zheng, K. Yao, A. T. Lau, T. A. Zykova, H. G. Kim, A. M. Bode and Z. Dong (2010). "Extracellular signal-regulated kinase 8-mediated c-Jun phosphorylation increases tumorigenesis of human colon cancer." Cancer Res 70(8): 3218-3227.

Yamada, K., D. J. Gerber, Y. Iwayama, T. Ohnishi, H. Ohba, T. Toyota, J. Aruga, Y. Minabe, S. Tonegawa and T. Yoshikawa (2007). "Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia." Proc Natl Acad Sci U S A 104(8): 2815-2820.

Yamagata, K., W. E. Kaufmann, A. Lanahan, M. Papapavlou, C. A. Barnes, K. I. Andreasson and P. F. Worley (1994). "Egr3/Pilot, a zinc finger transcription factor, is rapidly regulated by activity in brain neurons and colocalizes with Egr1/zif268." Learn Mem 1: 140-152.

Yamaguchi, H. and H. G. Wang (2004). "CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells." J Biol Chem 279(44): 45495-45502.

Yamamoto, H., S. Kakuta, T. M. Watanabe, A. Kitamura, T. Sekito, C. Kondo-Kakuta, R. Ichikawa, M. Kinjo and Y. Ohsumi (2012). "Atg9 vesicles are an important membrane source during early steps of autophagosome formation." J Cell Biol 198(2): 219-233.

Yamamoto, K., T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada and K. Mori (2007). "Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1." Dev Cell 13(3): 365-376.

Yamasaki, A., K. Tani, A. Yamamoto, N. Kitamura and M. Komada (2006). "The Ca2+-binding protein ALG-2 is recruited to endoplasmic reticulum exit sites by Sec31A and stabilizes the localization of Sec31A." Mol Biol Cell 17(11): 4876-4887.

Yang, J. S., S. Y. Lee, M. Gao, S. Bourgoin, P. A. Randazzo, R. T. Premont and V. W. Hsu (2002). "ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat." J Cell Biol 159(1): 69-78.

Yang, J. Y., C. S. Zong, W. Xia, H. Yamaguchi, Q. Ding, X. Xie, J. Y. Lang, C. C. Lai, C. J. Chang, W. C. Huang, H. Huang, H. P. Kuo, D. F. Lee, L. Y. Li, H. C. Lien, X. Cheng, K. J. Chang, C. D. Hsiao, F. J. Tsai, C. H. Tsai, A. A. Sahin, W. J. Muller, G. B. Mills, D. Yu, G. N. Hortobagyi and M. C. Hung (2008). "ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation." Nat Cell Biol 10(2): 138-148.

Yang, S. H., A. Galanis and A. D. Sharrocks (1999). "Targeting of p38 mitogen-activated protein kinases to MEF2 transcription factors." Mol Cell Biol 19(6): 4028-4038.

Yang, S. Z. and S. A. Abdulkadir (2003). "Early growth response gene 1 modulates androgen receptor signaling in prostate carcinoma cells." J Biol Chem 278(41): 39906-39911.

Yang, S. Z., I. A. Eltoum and S. A. Abdulkadir (2006). "Enhanced EGR1 activity promotes the growth of prostate cancer cells in an androgen-depleted environment." J Cell Biochem 97(6): 1292-1299.

Yang, X. Y., X. Y. Zhou, Q. Q. Wang, H. Li, Y. Chen, Y. P. Lei, X. H. Ma, P. Kong, Y. Shi, L. Jin, T. Zhang and H. Y. Wang (2013). "Mutations in the COPII vesicle component gene SEC24B are associated with human neural tube defects." Hum Mutat 34(8): 1094-1101.

Yao, Z. and R. Seger (2009). "The ERK signaling cascade--views from different subcellular compartments." Biofactors 35: 407-416.

Yasuda, J., A. J. Whitmarsh, J. Cavanagh, M. Sharma and R. J. Davis (1999). "The JIP group of mitogen-activated protein kinase scaffold proteins." Mol Cell Biol 19: 7245-7254.

Ye, J., R. B. Rawson, R. Komuro, X. Chen, U. P. Dave, R. Prywes, M. S. Brown and J. L. Goldstein (2000). "ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs." Mol Cell 6(6): 1355-1364.

Ye, Y., H. H. Meyer and T. A. Rapoport (2001). "The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol." Nature 414(6864): 652-656.

Yeong, F. M. (2013). "Multi-step down-regulation of the secretory pathway in mitosis: a fresh perspective on protein trafficking." Bioessays 35(5): 462-471.

Yi, P., D. T. Nguyen, A. Higa-Nishiyama, P. Auguste, M. Bouchecareilh, M. Dominguez, R. Bielmann, S. Palcy, J. F. Liu and E. Chevet (2010). "MAPK scaffolding by BIT1 in the Golgi complex modulates stress resistance." J Cell Sci 123(Pt 7): 1060-1072.

Yla-Anttila, P., H. Vihinen, E. Jokitalo and E. L. Eskelinen (2009). "3D tomography reveals connections between the phagophore and endoplasmic reticulum." Autophagy 5(8): 1180-1185.

Yonekawa, S., A. Furuno, T. Baba, Y. Fujiki, Y. Ogasawara, A. Yamamoto, M. Tagaya and K. Tani (2011). "Sec16B is involved in the endoplasmic reticulum export of the peroxisomal membrane biogenesis factor peroxin 16 (Pex16) in mammalian cells." Proc Natl Acad Sci U S A 108(31): 12746-12751.

Yoon, S., M. Y. Lee, S. W. Park, J. S. Moon, Y. K. Koh, Y. H. Ahn, B. W. Park and K. S. Kim (2007). "Up-regulation of acetyl-CoA carboxylase alpha and fatty acid synthase by human epidermal growth factor receptor 2 at the translational level in breast cancer cells." J Biol Chem 282(36): 26122-26131.

Yorimitsu, T. and K. Sato (2012). "Insights into structural and regulatory roles of Sec16 in COPII vesicle formation at ER exit sites." Mol Biol Cell 23(15): 2930-2942.

Yosef, N., E. Zalckvar, A. D. Rubinstein, M. Homilius, N. Atias, L. Vardi, I. Berman, H. Zur, A. Kimchi, E. Ruppin and R. Sharan (2011). "ANAT: a tool for constructing and analyzing functional protein networks." Sci Signal 4(196): pl1.

Yoshida, H., K. Haze, H. Yanagi, T. Yura and K. Mori (1998). "Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors." J Biol Chem 273(50): 33741-33749.

Yoshida, H., T. Matsui, A. Yamamoto, T. Okada and K. Mori (2001). "XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor." Cell 107(7): 881-891.

Yoshida, H., T. Okada, K. Haze, H. Yanagi, T. Yura, M. Negishi and K. Mori (2000). "ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response." Mol Cell Biol 20(18): 6755-6767.

Yoshida, H., T. Okada, K. Haze, H. Yanagi, T. Yura, M. Negishi and K. Mori (2001). "Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response." Mol Cell Biol 21(4): 1239-1248.

Yoshihisa, T., C. Barlowe and R. Schekman (1993). "Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum." Science 259(5100): 1466-1468.

Yoshimura, S., K. Yoshioka, F. A. Barr, M. Lowe, K. Nakayama, S. Ohkuma and N. Nakamura (2005). "Convergence of cell cycle regulation and growth factor signals on GRASP65." J Biol Chem 280(24): 23048-23056.

Young, A. R., E. Y. Chan, X. W. Hu, R. Kochl, S. G. Crawshaw, S. High, D. W. Hailey, J. Lippincott-Schwartz and S. A. Tooze (2006). "Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes." J Cell Sci 119(Pt 18): 3888-3900.

Yu, S. and M. G. Roth (2002). "Casein kinase I regulates membrane binding by ARF GAP1." Mol Biol Cell 13(8): 2559-2570.

Yu, S., A. Satoh, M. Pypaert, K. Mullen, J. C. Hay and S. Ferro-Novick (2006). "mBet3p is required for homotypic COPII vesicle tethering in mammalian cells." J Cell Biol 174: 359-368.

Yu, W., W. J. Fantl, G. Harrowe and L. T. Williams (1998). "Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK." Curr Biol 8(1): 56-64.

Yung, Y., Z. Yao, T. Hanoch and R. Seger (2000). "ERK1b, a 46-kDa ERK isoform that is differentially regulated by MEK." J Biol Chem 275(21): 15799-15808.

Zaal, K. J., C. L. Smith, R. S. Polishchuk, N. Altan, N. B. Cole, J. Ellenberg, K. Hirschberg, J. F. Presley, T. H. Roberts, E. Siggia, R. D. Phair and J. Lippincott-Schwartz (1999). "Golgi membranes are absorbed into and reemerge from the ER during mitosis." Cell 99(6): 589-601.

Zacharogianni, M., A. A. Gomez, T. Veenendaal, J. Smout and C. Rabouille (2014). "A stress assembly that confers cell viability by preserving ERES components during amino-acid starvation." Elife 3.

Zacharogianni, M., V. Kondylis, Y. Tang, H. Farhan, D. Xanthakis, F. Fuchs, M. Boutros and C. Rabouille (2011). "ERK7 is a negative regulator of protein secretion in response to amino-acid starvation by modulating Sec16 membrane association." EMBO J 30(18): 3684-3700.

Zanetti, G., K. B. Pahuja, S. Studer, S. Shim and R. Schekman (2012). "COPII and the regulation of protein sorting in mammals." Nat Cell Biol 14(1): 20-28.

Zanetti, G., S. Prinz, S. Daum, A. Meister, R. Schekman, K. Bacia and J. A. Briggs (2013). "The structure of the COPII transport-vesicle coat assembled on membranes." Elife 2: e00951.

Zarubin, T. and J. Han (2005). "Activation and signaling of the p38 MAP kinase pathway." Cell Res(11-8).

Zer, C., G. Sachs and J. M. Shin (2007). "Identification of genomic targets downstream of p38 mitogen-activated protein kinase pathway mediating tumor necrosis factor-alpha signaling." Physiol Genomics 31(2): 343-351.

Zeuschner, D., W. J. Geerts, E. van Donselaar, B. M. Humbel, J. W. Slot, A. J. Koster and J. Klumperman (2006). "Immuno-electron tomography of ER exit sites reveals the existence of free COPII-coated transport carriers." Nat Cell Biol 8(4): 377-383.

Zhan, X., T. S. Kaoud, S. Kook, K. N. Dalby and V. V. Gurevich (2013). "JNK3 enzyme binding to arrestin-3 differentially affects the recruitment of upstream mitogen-activated protein (MAP) kinase kinases." J Biol Chem 288: 28535-28547.

Zhang, C., J. Kawauchi, M. T. Adachi, Y. Hashimoto, S. Oshiro, T. Aso and S. Kitajima (2001). "Activation of JNK and transcriptional repressor ATF3/LRF1 through the IRE1/TRAF2 pathway is implicated in human vascular endothelial cell death by homocysteine." Biochem Biophys Res Commun 289(3): 718-724.

Zhang, C. J., A. G. Rosenwald, M. C. Willingham, S. Skuntz, J. Clark and R. A. Kahn (1994). "Expression of a dominant allele of human ARF1 inhibits membrane traffic in vivo." J Cell Biol 124(3): 289-300.

Zhang, F. and G. Du (2012). "Dysregulated lipid metabolism in cancer." World J Biol Chem 3(8): 167-174.

Zhang, H., C. Y. Koo, J. Stebbing and G. Giamas (2013). "The dual function of KSR1: a pseudokinase and beyond." Biochem Soc Trans 41: 1078-1082.

Zhang, R., S. Lu, L. Meng, Z. Min, J. Tian, R. K. Valenzuela, T. Guo, L. Tian, W. Zhao and J. Ma (2012). "Genetic evidence for the association between the early growth response 3 (EGR3) gene and schizophrenia." PLoS One 7: e30237.

Zhang, Y. C., Y. Zhou, C. Z. Yang and D. S. Xiong (2009). "A review of ERGIC-53: its structure, functions, regulation and relations with diseases." Histol Histopathol 24: 1193-1204.

Zhao, X., A. Claude, J. Chun, D. J. Shields, J. F. Presley and P. Melancon (2006). "GBF1, a cis-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic." J Cell Sci 119(Pt 18): 3743-3753.

Zhou, G., Z. Q. Bao and J. E. Dixon (1995). "Components of a new human protein kinase signal transduction pathway." J Biol Chem 270(21): 12665-12669.

Zhu, J., J. Blenis and J. Yuan (2008). "Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1." Proc Natl Acad Sci U S A 105(18): 6584-6589.

Zhu, Y., L. M. Traub and S. Kornfeld (1998). "ADP-ribosylation factor 1 transiently activates high-affinity adaptor protein complex AP-1 binding sites on Golgi membranes." Mol Biol Cell 9(6): 1323-1337.

Zimmermann, R., S. Eyrisch, M. Ahmad and V. Helms (2011). "Protein translocation across the ER membrane." Biochim Biophys Acta 1808(3): 912-924.

Zinszner, H., M. Kuroda, X. Wang, N. Batchvarova, R. T. Lightfoot, H. Remotti, J. L. Stevens and D. Ron (1998). "CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum." Genes Dev 12(7): 982-995.

Zipfel, P. F., E. L. Decker, C. Holst and C. Sherka (1997). "The human zinc finger protein EGR-4 acts as autoregulatory transcriptional repressor." Biochim Biophys Acta 1354: 134-144.

Zong, W. X., C. Li, G. Hatzivassiliou, T. Lindsten, Q. C. Yu, J. Yuan and C. B. Thompson (2003). "Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis." J Cell Biol 162(1): 59-69.

Zong, W. X., T. Lindsten, A. J. Ross, G. R. MacGregor and C. B. Thompson (2001). "BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak." Genes Dev 15(12): 1481-1486.

Acknowledgements

After I had interviewed for this PhD position four years ago, I realized that I would have a great combination of a supportive and enthusiastic supervisor, an interesting project, a great working atmosphere, and beautiful surroundings. I am very lucky to say that looking back, I was right.

In addition to being a very supportive supervisor with regard to the project, Hesso sees his role also as an educator, and actively supports the development of other skills. I admire this and am very grateful for learning so much during these last four years. Besides my supervisor, I would like to thank the members of my thesis committee, past (Prof. Dr. Christof Hauck) and present (Prof. Dr. Daniel Legler).

A special thank-you also goes to Veronika, whom I want to thank for her tremendous support and never ending patience in the lab, and for being a great role model with regards to her amazing problem-solving skills!

At the institute, I have experienced a fantastic working atmosphere. I want to thank my fellow PhD students and colleagues, many of which have become great friends, for making the lab a place I always enjoyed working at. I am grateful for all of your support during difficult times, as well as for being so open with your thoughts and quirks and the ensuing fun. Also for sharing in pointless, ridiculous as well as serious discussions, about anything. These lift your spirits when the experiments fail! To un-fail experiments, I could always ask for help, either technical assistance or thoughts on a problem; and I have learned a lot from you.

Lastly, I want to thank my family for their continuous support, and for just listening and understanding.