Review

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The nascent polypeptide-associated complex (NAC) as regulatory hub on ribosomes

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Abstract: The correct synthesis of new proteins is essential for maintaining a functional proteome and cell viability. This process is tightly regulated, with ribosomes and associated protein biogenesis factors ensuring proper protein production, modification, and targeting. In eukaryotes, the conserved nascent polypeptide-associated complex (NAC) plays a central role in coordinating early protein processing by regulating the ribosome access of multiple protein biogenesis factors. NAC recruits modifying enzymes to the ribosomal exit site to process the N-terminus of nascent proteins and directs secretory proteins into the SRP-mediated targeting pathway. In this review we will focus on these pathways, which are critical for proper protein production, and summarize recent advances in understanding the cotranslational functions and mechanisms of NAC in higher eukaryotes.

Keywords: ribosome-associated factors; cotranslational protein modification; methionine aminopeptidases (MetAPs); N-terminal acetyltransferases (NATs); cotranslational protein transport; signal recognition particle (SRP)

1 Introduction

The synthesis of new proteins on ribosomes is crucial for refreshing and adapting the cell's proteome to changing conditions. Ribosomes, complex macromolecular devices composed of ribosomal RNA (rRNA) and ribosomal proteins (r-proteins), decode messenger RNA (mRNA) sequences to synthesize polypeptide chains based on the genetic code. About 60 % of newly made proteins encode cytonuclear proteins while approx. 10 % are imported into mitochondria (Bykov et al. 2020) and approx. 30 % are endomembrane/secreted proteins that are cotranslationally targeted to the endoplasmic reticulum (ER) (Akopian et al. 2013) (Figure 1A). When newly synthesized polypeptides emerge from the ribosomal exit tunnel, they are immediately met by a variety of conserved protein biogenesis factors (Gamerdinger and Deuerling 2024). These factors, often ribosome-associated, guide the nascent chains through the early stages of modification and folding, and, if needed, direct the proteins to their appropriate cellular compartments. Correct protein processing is critical for maintaining a healthy proteome and cellular homeostasis, as improperly folded, modified, or mislocalised proteins can lead to protein aggregation and dysfunction, contributing to various diseases such as neurodegenerative disorders or cancer (McTiernan et al. 2025; Øye et al. 2025).

The vast majority of cytonuclear proteins are modified at their N-terminus as soon as the nascent chain exits the ribosomal tunnel (Figure 1A). Enzymes such as methionine aminopeptidases (MetAPs), N-terminal acetyltransferases (NATs), and N-myristoyltransferases (NMTs) play pivotal roles in modifying nascent polypeptide chains. MetAPs remove the initiating methionine from nascent proteins, a process that facilitates subsequent modification by NATs and NMTs, and determines protein stability, as the amino acid identity and modification status of a proteins' N-terminus has been recognized as a determinant of its half-life in the cell (Varshavsky 2024). NATs acetylate the N-terminus of nascent chains, influencing protein folding (Kang et al. 2012), stability (Hwang et al. 2010), localization (Behnia et al. 2004; Forte et al. 2011; Setty et al. 2004), and protein and membrane interactions (Dikiy and Eliezer 2014; Gao et al. 2016; Scott et al. 2011). NMTs catalyze the attachment of myristoyl groups to specific glycine residues, promoting membrane association and signal transduction (Tate et al. 2024; Traverso et al. 2013). This ensemble of enzymes acts as soon as the N-terminus of a protein emerges from the ribosomal tunnel and prior to other processing pathways such as folding or transport (Kramer et al. 2019). Thus, the activities of these modifying enzymes must be tightly coordinated at the ribosomal exit site in a spatial and timely manner,

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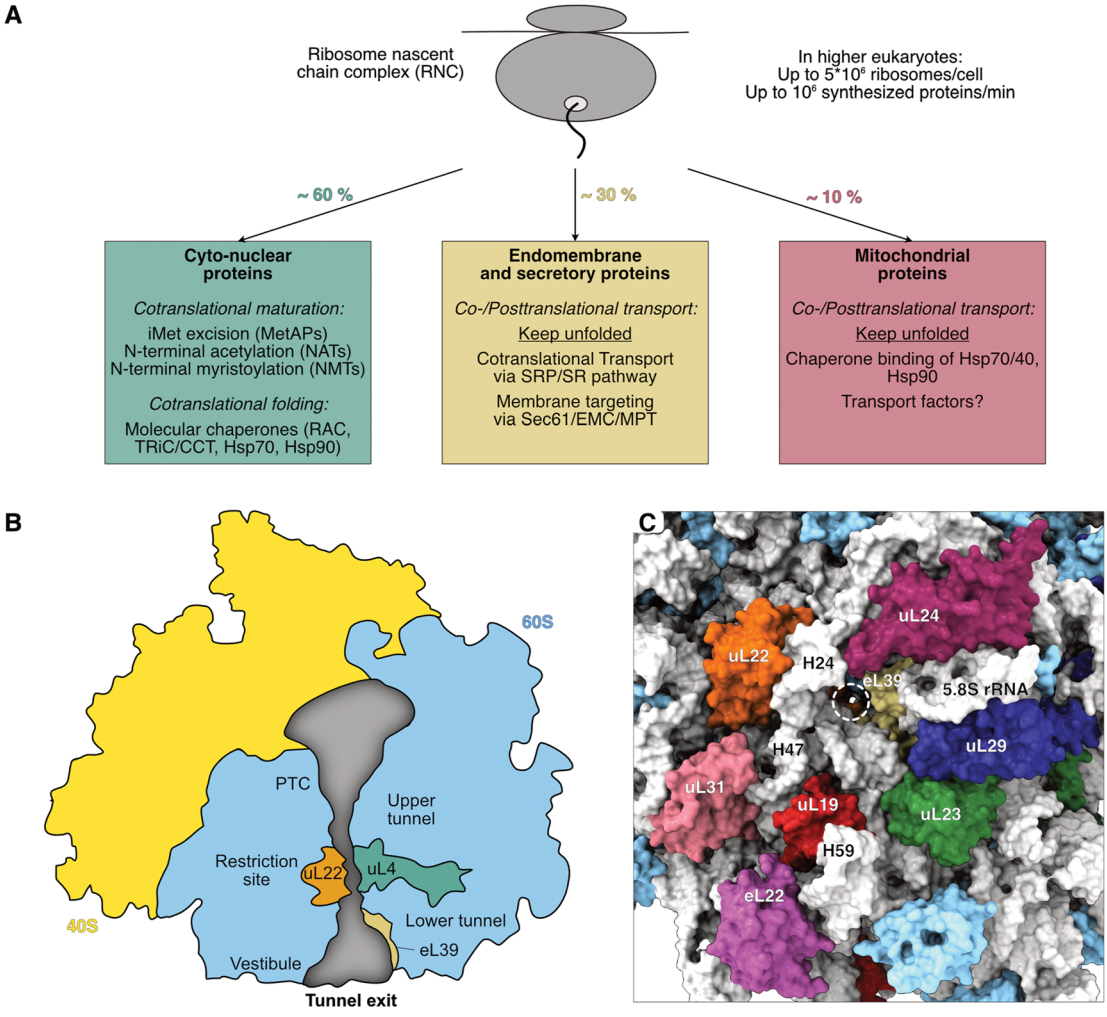

Figure 1: Overview of protein biogenesis. (A) Overview of protein production pathways and conserved ribosome-associated protein biogenesis factors. (B) Schematic of the ribosomal tunnel, subdivided in the upper tunnel, restriction sites formed by uL22 and uL4, and the lower tunnel and vestibule. Ribosomal protein eL39 decorates the lip of the tunnel exit. PTC: Peptidyl transferase center. (C) Surface representation of molecular model of human 80S ribosome (PDB: 4UG0). Close-up of tunnel exit region with indicated ribosomal proteins and rRNA elements. Remaining 60S r-proteins in light blue, rRNA in grey. Location of ribosomal tunnel is depicted as a dashed white circle.

ensuring that cytonuclear proteins are correctly processed as they are synthesized. How this complex choreography of N-terminal modifications of nascent chains is organized, was enigmatic until recently (see below). Subsequently, molecular chaperones including the ribosome-associated complex (RAC), TRIC/CCT, Hsp70s and Hsp90s can bind cotranslationally to the growing polypeptides to support folding steps to the native state (Kramer et al. 2019).

About 30 % of newly made proteins encode secretory or endomembrane proteins that require appropriate targeting to the endoplasmic reticulum ER (Figure 1A). Transport of newly synthesized proteins carrying a hydrophobic ER-signal sequence (SS) or transmembrane domain (TMD) in the N-terminus is organized by the signal recognition particle (SRP) and the signal recognition particle receptor (SR) at the ER membrane in a GTP-dependent manner. SRP binds to ribosomes and to signal sequences on nascent polypeptides as they emerge from the ribosome and initiates SR-mediated targeting of ribosome-nascent chain complexes (RNCs) to the Sec61 translocase at the ER-membrane (Akopian et al. 2013; Rapoport et al. 2017).

About 10 % of newly synthesized proteins are destined for mitochondria encoded by nuclear DNA and synthesized on cytosolic ribosomes (Bykov et al. 2020) (Figure 1A). Mitochondrial targeting signals can display variation and can be located N-terminally or internally (Bykov et al. 2020). Approximately 60–70 % of nuclear-encoded mitochondrial proteins carry a cleavable mitochondrial targeting sequence (MTS), a short peptide containing positively charged basic residues that facilitates the transport of a protein to the

mitochondria (Roise et al. 1986; Vögtle et al. 2009). That far little is known about specific transport factors for mitochondrial proteins. Albeit cotranslational mitochondrial import is considered to play a role for especially inner mitochondrial membrane proteins (Williams et al. 2014), it is assumed that many of the mitochondrial precursor proteins remain in an unfolded state and are posttranslationally transported to mitochondria with the help of cytosolic chaperones such as Hsp/Hsc70 and Hsp90, which prevent premature folding and aggregation. A very recent study suggests that co-chaperones of Hsc70 (St13 and Stip1) and Hsp90 (p23 and Cdc37) directly bind to the MTS of nascent mitochondrial proteins to facilitate chaperone retention at the mature domain (Juszkiewicz et al. 2025).

2 The ribosomal exit tunnel

Newly synthesized polypeptides exit the ribosome into the cytosol via a tunnel in the large ribosomal subunit (Figure 1B). This tunnel, approximately 100 Å long and 10–20 Å wide, is predominantly lined by core rRNA (28S in higher eukaryotes), with its geometry shaped by extensions of ribosomal proteins uL4 and uL22 forming two narrow constrictions of about 10 Å in eukaryotes (Dao Duc et al. 2019). At the tunnel's rim, where it widens to about 20 Å, ribosomal protein eL39 contributes to its structural features (Nissen et al. 2000) (Figure 1B).

The tunnel can be divided into the upper chamber close to the PTC, the central part with the major constriction sites, and the lower tunnel and vestibule (Samatova et al. 2024). Overall, the ribosomal tunnel possesses an electronegative potential ranging from −8 mV to −22 mV, reflecting its rRNA-dominant composition (Lu et al. 2007). Although the tunnel's geometry prevents extensive protein folding, computational studies suggest that the confinement within the tunnel promotes formation of α-helical structures (Ziv et al. 2005), which have been observed before and after the constriction sites (Agirrezabala et al. 2017; Bhushan et al. 2010; Lu and Deutsch 2005). More complex structures such as zinc-finger domains (Nilsson et al. 2015) and β-hairpin motifs (Kosolapov and Deutsch 2009) have been observed in the lower tunnel and vestibule. The vestibule even allows folding of simple tertiary-structures like partially folded three-helix bundles (Nilsson et al. 2017).

The ribosomal surface surrounding the tunnel exit (Figure 1C) functions as a biogenesis factor-binding platform, facilitating efficient handover of nascent chains to processing enzymes, targeting factors, and chaperones. These factors have at least partially overlapping binding sites and compete for timely and specific access to the nascent chain (Gamerdinger and Deuerling 2024; Kramer et al. 2009). Unlike the interior tunnel, the tunnel exit is predominantly composed of ribosomal proteins (universal: uL22, uL23, uL24, uL29; eukaryote-specific: eL19, eL22, eL31 and eL39), with minor contributions of rRNA elements (5.8S rRNA and helices H24, H47, and H59 of 28S rRNA) (Khatter et al. 2015). Ribosomal proteins uL23 and uL29, collectively dubbed the universal ribosome docking site, have been implicated in the binding of various proteins including Sec61 (Beckmann et al. 2001; Voorhees et al. 2014), SRP (Pool et al. 2002; Voorhees and Hegde 2015), NAC (Jomaa et al. 2022; Kramer et al. 2002), and MetAP1 (Gamerdinger et al. 2023; Nyathi and Pool 2015). Additionally, a second adaptor site comprising eL31 and uL22 has been identified as the binding site for RAC (Peisker et al. 2008), NAC (Pech et al. 2010), and the Signal recognition particle receptor (SR) (Halic et al. 2006). Well-regulated binding of factors to the tunnel exit is crucial, as emphasised by the fact, that premature factor association is actively prevented during ribosome biogenesis (Greber et al. 2012).

3 The nascent polypeptide associated complex (NAC)

The nascent polypeptide-associated complex (NAC) is a heterodimer of NACα and NACβ, conserved throughout eukaryotes (Wiedmann et al. 1994). Disruption of NAC gene expression is embryonically lethal in mice (Deng and Behringer 1995), flies (Markesich et al. 2000), and worms (Bloss et al. 2003). NAC is highly abundant and expressed approximately in a 1:1 stoichiometry to ribosomes (Kulak et al. 2014; Raue et al. 2007).

NACα and NACβ show substantial homology and dimerize via their NAC domains, forming a β-barrel-like structure with a hydrophobic core (Figure 2A and B) (Liu et al. 2010; Wang et al. 2010). The two N-termini and the two C-termini protrude as flexible arms from the globular domain. NAC's C-terminals arms act as recruiting devices for protein biogenesis factors (see below). The C-terminal arm of NACα features a three-helix bundle characteristic of ubiquitin-binding (UBA) domains (Figure 2A and B). These domains typically contain a hydrophobic patch on the solvent-accessible surface formed by helices α1-α3, mediating the ubiquitin interaction (Mueller and Feigon 2002). However, the NACα UBA domain does not bind to ubiquitin (Andersen et al. 2007), but rather acts as a general protein-interaction module for recruitment of a subset of ribosome-associated proteins (Jomaa et al. 2022; Lentzsch et al. 2024; Minoia et al. 2024). The two N-terminal NAC arms that protrude from the NAC globular domain are involved in ribosome binding with the N-terminus of NACβ as the major ribosome anchor of this complex (Gamerdinger et al. 2019; Jomaa et al. 2022; Wegrzyn et al. 2006).

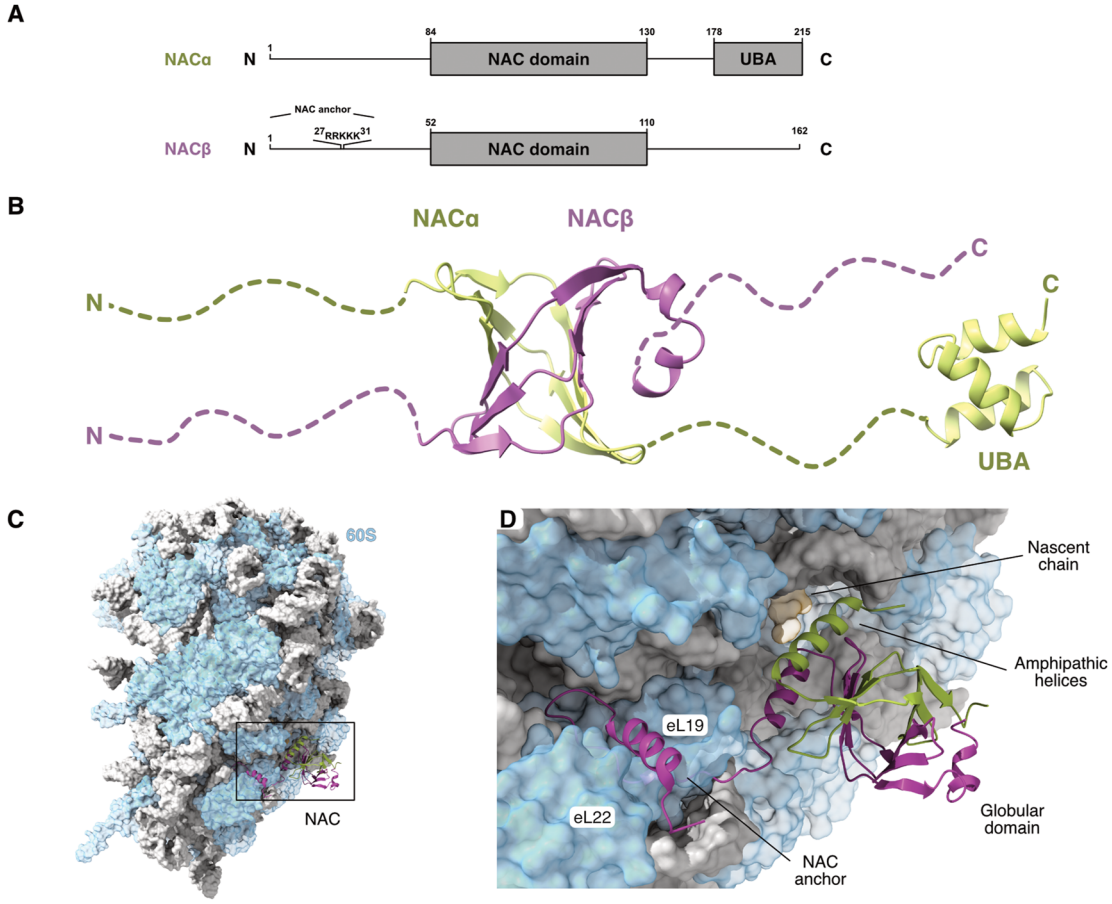

Figure 2: NAC domain architecture, fold, and ribosome-binding mode. (A) Domain architecture of human NAC with annotated ribosome binding motif. In humans, NACβ exists in a prominent short isoform (as depicted here) and a long isoform with an extended N-terminus (not shown). Start and stop of annotated domains according to AlphaFold3 structure prediction below. (B) AlphaFold3 structure prediction (Abramson et al. 2024) of human NAC heterodimer. NACα in light green, NACβ in purple. Prediction of unstructured N- and C-termini not shown. There are depicted as dashed lines. (C) Molecular model of cryo-EM structure of human NAC (NACα in light green, NACβ in purple) bound to an RNC translating an SS-containing nascent chain in an SRP-pre-handover state (PDB: 7QWR). Surface representation of 60S r-proteins in light blue, rRNA in grey, and nascent chain in light orange. (D) Close up of NAC bound to the tunnel exit.

NAC binds in a 1:1 stoichiometry to ribosomes with very high affinity (K_D = 1 nM) (Jomaa et al. 2022). Given its approximately equimolar concentration, the majority of ribosomes are NAC-bound (Kulak et al. 2014; Raue et al. 2007). NAC can interact with empty ribosomes and RNCs displaying nascent chain still buried within the ribosomal tunnel by inserting its NACβ N-terminus into the exit tunnel. Thus, it is assumed that it can contact nascent chains soon after translation initiation (Gamerdinger et al. 2019). In this conformation, NAC blocks the access to the exit tunnel for nascent chain modifying and targeting enzymes, preventing unproductive binding before the nascent chain is exposed (Gamerdinger et al. 2019). As elongation proceeds, NACβ N-terminus is displaced from the tunnel interior by the growing polypeptide and occupies its canonical high-affinity binding site outside the tunnel. Recent cryo-EM data showed that the NACβ N-terminus ("NAC anchor") wraps around eL22 and contacts eL19 and 28S rRNA (Figure 2D) (Jomaa et al. 2022; Lin et al. 2020). This interaction aligns with earlier biochemical data, showing that deletion of the N-terminus or mutations in the conserved RRK(X_n)KK motif located in the N-terminus of NACβ abolish stable ribosome association (Figure 2A) (Pech et al. 2010; Wegrzyn et al. 2006). Additionally, two amphipathic, antiparallel helices, which form only in presence of the ribosome, attach the NAC globular domain to the exit tunnel by forming a low-affinity interaction with the 28S rRNA (Jomaa et al. 2022; Lin et al. 2020) (Figure 2C).

Recent studies in *Caenorhabditis elegans* and human cell lines uncovered NAC's fundamental role and mechanism as a central regulatory hub in protein synthesis by regulating the processing pathways of nascent proteins (Gamerdinger

et al. 2023; Jomaa et al. 2022; Klein et al. 2024a; Lentzsch et al. 2024). NAC not only recognizes the type of nascent proteins produced by ribosomes but also orchestrates their precise modifications and cellular localization by recruiting appropriate cellular factors to ensure the correct fate and function of new proteins.

4 NAC regulates initiator methionine excision (NME) and N-terminal acetylation (NTA) by NatA/E

Newly synthesized proteins initially carry an N-terminal initiator methionine (iMet). This initiator methionine is cleaved cotranslationally, provided the second amino acid is small and uncharged (Ala, Cys, Thr, Ser, Val, Gly or Pro) (Figure 3A). Depending on organism, up to 70 % of proteins undergo NME, a conserved process from bacteria to higher eukaryotes (Giglione et al. 2004). In eukaryotes NME is carried out by two metalloproteases, methionine aminopeptidase 1 and 2 (MetAP1 (Addlagatta et al. 2005) and MetAP2 (Liu et al. 1998)), which have largely overlapping substrate specificities (Xiao et al. 2010) (Figure 3A–C). Both enzymes share a catalytic domain that adopts a characteristic pita-bread fold with conserved metal-binding sites and an active site of similar topology. In contrast to MetAP2, MetAP1 has an extended N-terminus containing a zinc finger domain, which has been implicated in its ribosome binding (Gamerdinger et al. 2023; Vetro and Chang 2002; Zuo et al. 1995). Conversely, MetAP2 features a charged N-terminal extension and an α-helical insertion (insert domain) within its catalytic domain (Figure 3B and C). Cryo-EM studies of MetAP2 (Klein et al. 2024b) and MetAP2-like proteins Arx1 (Greber et al. 2012) and EBP1 (Bhaskar et al. 2021; Kraushar et al. 2021; Wild et al. 2020) have identified the insert domain as the main mediator of ribosome binding. Compared to ribosomes, MetAP1 and MetAP2 are relatively scarce in the cell, present at substoichiometric ratios of at least 1:10 in yeast (Raue et al. 2007) and human cell lines (Geiger et al. 2012; Kulak et al. 2014), precluding quantitative loading of ribosomes of these enzymes for NME. How MetAP1 associates with ribosomes *in vivo* and whether there is any preference for MetAP1 or 2 to bind the translation machinery was unknown until recently. A recent study showed that MetAP1 is the main enzyme associated with ribosomes in human cells and *C. elegans*, while MetAP2 seemingly serves as back up when cells are depleted for MetAP1 function (Gamerdinger et al. 2023).

Another very common cotranslational modification is N-terminal acetylation which neutralises the basic behaviour of the N-terminal primary amine (Figure 3A). The irreversible addition of an acetyl moiety from acetyl-CoA to a substrate occurs in up to 80 % of the proteome, including both cytosolic proteins, as well as transmembrane and organellar proteins (Aksnes et al. 2019). In eukaryotes, a set of five protein complexes called N-terminal acetyltransferases (NATs) - NatA, NatB, NatC, NatD, and NatE - act cotranslationally on cytonuclear proteins, each with a distinct substrate spectrum (Polevoda et al. 2008, 2009) (Figure 3D–H). Like methionine processing enzymes, NATs are relatively low in abundance compared to ribosomes (Geiger et al. 2012; Kulak et al. 2014; Raue et al. 2007).

NatA, a heterodimer consisting of catalytic subunit Naa10 and ribosome-binding subunit Naa15 (Figure 3D), acts downstream of NME by MetAPs and acetylates neo-N-termini starting with Ala, Cys, Thr, Ser, Val or Gly (~40 % of human proteome) (Arnesen et al. 2005, 2009). Interestingly, in higher eukaryotes NatA activity is regulated by Huntingtin Interacting Protein K (HYPK) (Arnesen et al. 2010). HYPK contains an N-terminal ubiquitin associated (UBA) domain, that binds Naa15 with high affinity, and inhibits NatA through its C-terminal domain, which sterically blocks the catalytic centre of Naa10 (Gottlieb and Marmorstein 2018; Weyer et al. 2017). NatD is a highly specific NAT that acetylates SGRG-starting N-termini found in histones H2A and H4 (Hole et al. 2011). If the nascent protein maintains the iMet, the N-termini are acetylated by NatB, NatC, and NatE depending on the identity of the second amino acid. Glx-Asx-type N-termini (MD-, ME-, MN- and MQ-starting), which comprise approximately 20 % of the human proteome, are processed by NatB, a heterodimer of the catalytic subunit Naa20 and the ribosome-binding subunit Naa25 (Polevoda et al. 2003) (Figure 3E). Together, NatC and NatE account for the remaining methionine-starting N-termini (canonical substrates: ML-, MI-, MF-, MY-, MK-starting), adding up to about 20 % of the human proteome. Proteomics studies, however, suggest that these enzymes may acetylate a broader range of N-termini (MM-, MS-, MT-, MA-, MV-, MH-, MW-starting) (Van Damme et al. 2011, 2016, 2023). NatC is a heterotrimer composed of the catalytic subunit Naa30, the ribosome-binding subunit Naa35, and an additional auxiliary subunit Naa38 (Deng et al. 2023) (Figure 3F). In contrast, Naa50 serves as the catalytic subunit of NatE and binds the ribosome by attaching to the NatA complex (Deng et al. 2019) (Figure 3H). Naa50 can co-bind with HYPK to NatA but, unlike Naa10, is not fully inhibited by HYPK's C-terminus (Deng et al. 2020a). All Nat catalytic subunits share a conserved GCN5-related-N-acetyltransferase (GNAT) fold (Figure 3I). Substrate recognition is determined by the geometry and

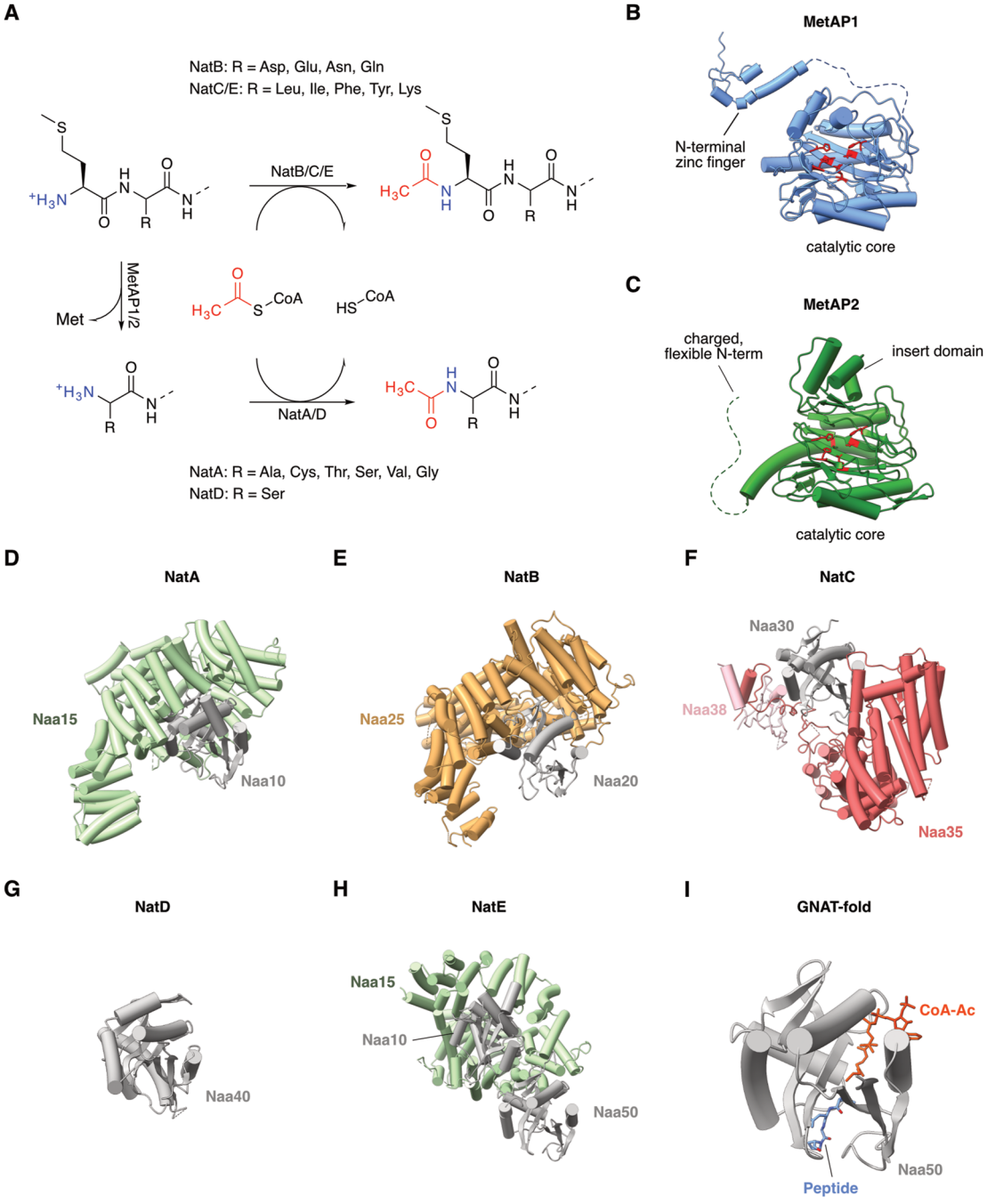

Figure 3: The human set of N-terminal modification enzymes acting cotranslationally on nascent polypeptides. (A) MetAPs cleave the iMet of cyto-nuclear nascent polypeptides, provided the second amino acid is small and uncharged (R = Ala, Cys, Thr, Ser, Val, Gly, Pro). Using Ac-CoA as a coenzyme, NatA acetylates these N-termini (R = Ala, Cys, Thr, Ser, Val, Gly). NatD specifically acetylates the N-termini of histones H2A and H4 (SGRG-starting). Methionine-retaining N-termini are acetylated cotranslationally by a combination of NatB, NatC, and NatE. (B and C) molecular models of (B) human MetAP1 (in blue, PDB: 2B3K) and (C) human MetAP2 (in green, PDBN: 1BN5). Crystal structures are aligned to their common catalytic core. Key, conserved catalytic and metal-binding residues are depicted in red. MetAP1 N-terminal zinc-finger domain is visualized via AlphaFold3 modelling (Abramson et al. 2024). (D–H) Molecular models of human NATs acting cotranslationally aligned to their catalytic subunit, which is accessible for substrates from below. (D) Crystal structure of NatA (ribosome-binding subunit Naa15 in light green, catalytic subunit Naa10 in grey; PDB: 6C9M). (E) Cryo-EM structures of NatB (ribosome-binding subunit Naa25 in brown, catalytic subunit Naa20 in grey; PDB: 6VP9). (F) Cryo-EM structure of NatC (ribosome-binding subunit Naa35 in red, auxiliary subunit Naa38 in pink, and catalytic subunit Naa30 in grey; PDB: 7MX2). (G) Crystal structure of NatD (Naa40) in grey (PDB: 4U9W). (H) Cryo-EM structure of NatE (ribosome-binding subunit Naa15 in light green, and catalytic subunits Naa10 and Naa50 in grey; PDB: 6PPL). Structure is aligned to Naa50. (I) Close-up of human Naa50 (grey) to illustrate GNAT fold of NATs with substrate peptide (blue) and Ac-CoA (red) in catalytic site (PDB: 3TFY).

composition of the substrate binding pocket (Deng et al. 2020b, 2021, 2023; Grunwald et al. 2020; Liszczak et al. 2013; Magin et al. 2015).

Approximately 40 % of the mammalian proteome undergoes N-terminal methionine removal by MetAPs and subsequent acetylation by NatA in a strictly cotranslational and sequential manner. Recent studies revealed that NAC is essential to coordinate the function of both enzymes (Figure 4A). NAC employs its long and flexible C-terminal arms to recruit MetAP1 and NatA to the ribosome tunnel exit to form the quaternary complex, enabling their substrate-specific cotranslational N-terminal maturation activity (Figure 4A–D). Cryo-EM studies showed that NAC forms a multienzyme complex with MetAP1 and NatA early in translation, positioning their active sites to facilitate efficient sequential processing of the nascent protein (Gamerdinger et al. 2023; Klein et al. 2024a; Lentzsch et al. 2024). Additionally, NAC alleviates inhibitory interactions from the NatA regulatory protein HYPK, activating NatA on the ribosome and ensuring cotranslational Nt-acetylation (Lentzsch et al. 2024). The quaternary complex of NAC, MetAP1, and NatA forms a semicircular arrangement around the ribosome tunnel exit, with the catalytic sites of the processing enzymes directed to the tunnel exit with distances of 5 nm and 6 nm respectively (Figure 4A). This spatial organisation is well-suited for initiator methionine excision and subsequent acetylation.

The long, flexible C-terminal arm of NACβ (Figure 2B) actively recruits MetAP1 to ribosomes to form a methionine excision complex for nascent proteins (Figure 4A–D). Structural studies have identified a conserved hydrophobic motif (^{146}VPDLV^{150} in human NACβ) at the end of the NACβ arm, which specifically interacts with the N-terminal zinc-finger domain of MetAP1 (Gamerdinger et al. 2023). Experimental data from *in vivo* models confirm that this interaction is essential for the enzyme's ribosome binding and for facilitating N-terminal methionine excision by MetAP1 in cells. Additionally, the association of NAC's globular domain with the ribosomal tunnel exit is critical for proper enzyme docking (Figure 4D) (Gamerdinger et al. 2023). The NACα C-terminal UBA domain and a helix (H2) following the NAC globular domain play distinct roles in NatA/E recruitment (Figure 4A–C). The UBA domain captures the enzyme complex. Notably, the binding to NACα H2 releases the inhibitory block of HYPK from the Naa10 active site while both UBA domains of NAC and HYPK remain anchored to distinct locations on Naa15 (Figure 4A).

The high-affinity interactions between NACβ's C-terminal hydrophobic motif with MetAP1 and NACα's C-terminal UBA domain with NatA/E facilitate efficient ribosome association of these N-terminal maturation enzymes (Figure 4A–D). This highlights the importance of precise spatial organisation of processing enzymes at the tunnel for efficient N-terminal maturation. Consistent with the distances of the catalytic sites, *in vitro* reconstitution of this process demonstrated that NME occurs at a nascent chain length of approximately 60 amino acids, while N-terminal acetylation by NatA takes place at around 95 amino acids (Lentzsch et al. 2024). Moreover, *in vitro* analyses suggest a significant co-dependency of MetAP1 and NatA for optimal catalytic activity showing that NatA activity was enhanced in the presence of MetAP1 in an *in vitro* reconstitution assay (Lentzsch et al. 2024). These findings support the notion that the quaternary complex organized by NAC offers a unique N-terminal processing environment, where the position and activity of the respective enzymes are finetuned by each other's presence.

Interestingly, N-terminal processing of cyto-nuclear proteins differs between yeast and higher eukaryotes. Yeast MetAP1 and NatA both rely on the extension segment ES27L$_a$ for correct positioning on the ribosome which is prerequisite for their cotranslational activity (Fujii et al. 2018; Knorr et al. 2019, 2023; Shankar et al. 2020). Moreover, NAC knock-out in yeast is viable and does not result in a growth phenotype (Reimann et al. 1999) suggesting plasticity of cotranslational protein biosynthesis pathways in lower eukaryotic organisms.

5 NAC's role in SRP-dependent ER targeting

Approximately one third of the proteome is targeted to the ER, with the majority relying on the cotranslational action of the signal recognition particle (SRP) (Akopian et al. 2013). SRP is a universally conserved ribonucleoprotein composed of six protein subunits (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) and a 7S SRP RNA in eukaryotes (Walter and Blobel 1980, 1982). Notably, SRP is much less abundant in the cytosol compared to ribosomes, with a ratio of at least 1:10 (Kulak et al. 2014). When a hydrophobic targeting signal of a protein destined to the ER, either an N-terminal SS or TMD, emerges from the ribosomal tunnel exit, it is recognized and bound by SRP54 (Voorhees and Hegde 2015). Once bound by SRP, the RNC is delivered to the Sec61 translocon in ER-membrane via the ER-membrane associated SRP receptor (SR) in a GTP-dependent manner. SRP then dissociates from SR, completing the targeting cycle (Jomaa et al. 2021; Kobayashi et al. 2018; Lee et al. 2018).

The SS/TMD recognition is a critical step in ER targeting, However, *in vitro* SRP also binds to signal-less ribosome nascent chain complexes (RNCs) suggesting a regulatory mechanism at the ribosome *in vivo* (Chartron et al. 2016; Flanagan et al. 2003; Kalies et al. 1994). Two studies provided

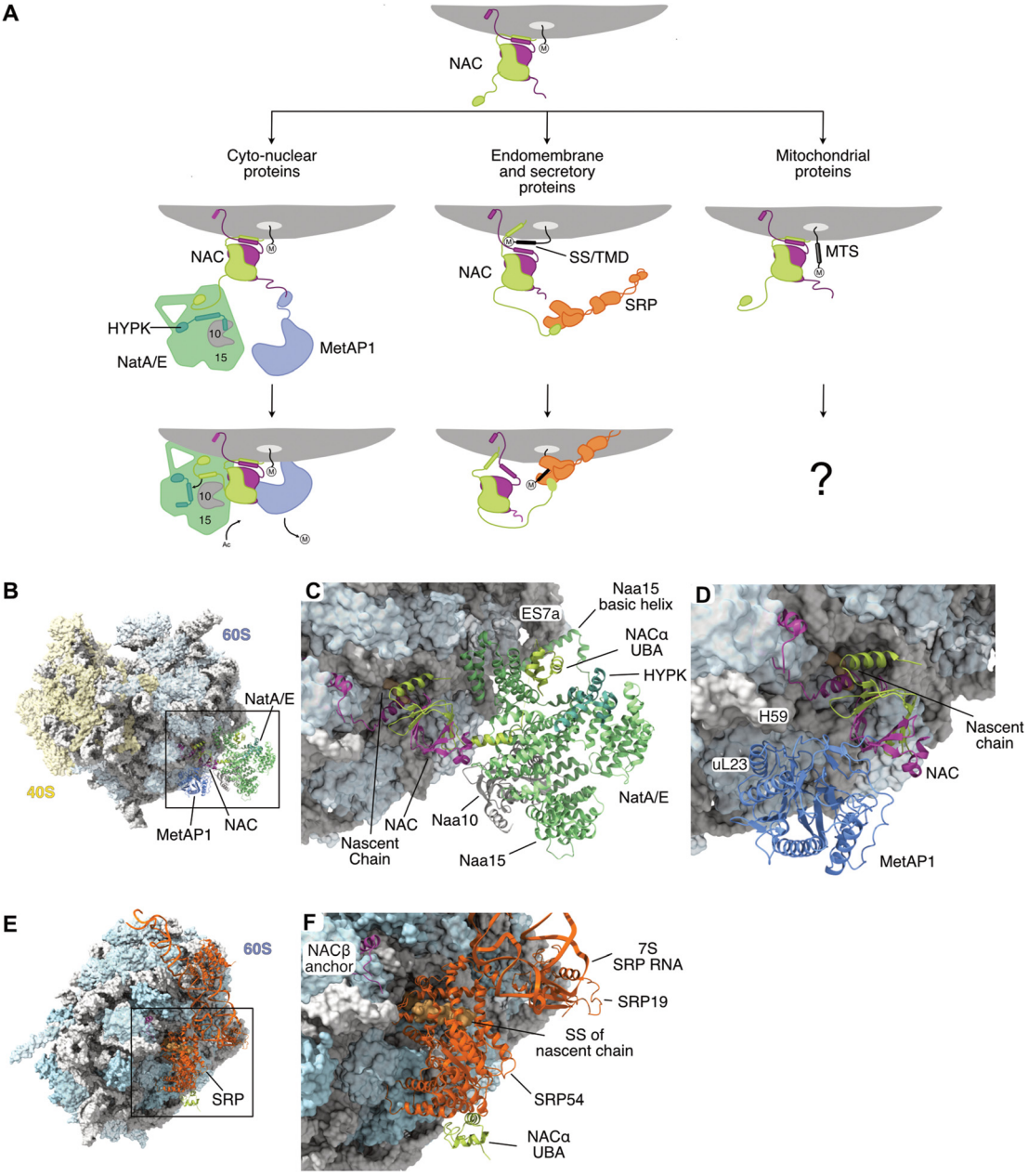

Figure 4: NAC regulates cotranslational protein processing and targeting. (A) Model of NAC (NACα in light green, NACβ in purple) as a regulatory hub on the ribosome, orchestrating cotranslational protein processing. MetAP1 in blue, Naa15 in green, Naa10 in grey, HYPK in cyan, SRP in orange. (B–D) Molecular model of cryo-EM structure of quaternary complex of NAC, MetAP1, NatA/E•HYPK, and RNC displaying a model protein (PDB: 9F1D). Surface representations of 40S r-proteins in light yellow, 60S r-proteins in light blue, rRNA in grey, and nascent chain in light brown. Naa50 is not depicted for clarity. (C) Close-up of NAC and NatA/E•HYPK. (D) Close-up of NAC and MetAP1. (E) Molecular model of cryo-EM structure of NAC (NACα in light green, NACβ in purple), SRP (orange), and RNC displaying a signal sequence in post-handover state of the nascent chain (PDB: 7QWQ). Surface representations of 60S r-proteins in light blue, rRNA in grey, nascent chain in light brown. NAC globular domain is not resolved in this structure. (F) Close-up of NAC and SRP at the ribosomal tunnel exit.

evidence *in vivo* that the binding of SRP to secretory nascent chains is tightly regulated by NAC (Gamerdinger et al. 2015; Jomaa et al. 2022) (Figure 4A). Via globular domain docking, NAC prevents incorrect association of SRP to RNCs without SS by sterically blocking SRP access to the ribosomal exit site. For ribosomes engaged in secretory protein translation, the release of NAC's globular domain from the tunnel exit is necessary to allow access of SRP (Figure 4A). This detachment is triggered by hydrophobic ER-targeting signals in the growing polypeptide which interact with a hydrophobic

pocket of NACs globular domain beneath the two α-helices that tether the globular domain to the ribosome exit site (Figure 2C). This interaction destabilizes the ribosome-binding platform, causing the NAC globular domain to detach, thereby eliminating its antagonistic effect on SRP (Figure 4A). However, NAC remains associated with the ribosome via its N-terminal NACβ anchor, which binds outside the SRP docking region (Figure 4E and F). Importantly, there is a second activity of NAC on SRP binding. Structural and biochemical analyses revealed that the UBA domain of NACα specifically interacts with the NG-domain of SRP54 for SRP recruitment (Jomaa et al. 2022) (Figure 4A, E and F). It is suggested that this enhances the local concentration of SRP, which is about 10-fold less abundant than ribosomes (Kulak et al. 2014), and allows SRP to scan the ribosomal exit site for binding access and the nascent chains for the presence of a signal sequence.

Notably, proteins destined for the endoplasmic reticulum (ER) appear to be exempt from NME and NTA, as N-terminal processing disfavours translocation (Forte et al. 2011). This phenomenon can be explained by the conformational change of NAC's globular domain induced by hydrophobic ER targeting signals. Signal sequence exposure and interactions with the hydrophobic pocket in NACs globular domain detaches this domain from the ribosomal tunnel exit (Figure 4A) and thereby disrupts efficient MetAP1 binding and subsequent acetylation of nascent proteins.

6 Other roles of NAC

Yeast NAC has also been linked to cotranslational targeting to mitochondria, acting as a stimulatory factor for import. Deletion of NAC genes is possible in yeast and cells display mild mitochondrial targeting defects, consistent with decreased levels of mitochondria-associated ribosomes (Alamo et al. 2011; Fünfschilling and Rospert 1999; George et al. 1998, 2002; Yogev et al. 2007). OM14, a yeast-specific outer mitochondrial membrane protein, serves as an NAC receptor, enhancing cotranslational import and interacting with the TOM complex to facilitate nascent polypeptide handover to the translocation pore (Lesnik et al. 2014; Schulte et al. 2023). Similar mechanisms remain to be found in other eukaryotic species (Figure 4A). In *C. elegans,* NAC depletion triggers the mitochondrial stress response, at least partially due to mistargeting of ER-bound substrates to mitochondria (Gamerdinger et al. 2015). A CRISPRi screen in human cell lines implicated NAC in the biogenesis of polytopic outer mitochondrial membrane proteins (Muthukumar et al. 2024).

Moreover, NAC has long been considered a ribosome-associated chaperone (Deuerling et al. 2019; Shen et al. 2019). Knockout of NAC and RAC in yeast results in a synthetic cell viability defect associated with wide-spread aggregation, particularly of ribosomal proteins (Koplin et al. 2010; Ott et al. 2015). Cotranslational ubiquitylation is also elevated in NAC-depleted cells, primarily for aggregation-prone proteins, membrane-targeted, and mitochondrial species, indicative of a protective role during translation (Duttler et al. 2013; Wang et al. 2013). NAC also exhibits chaperone activity off the ribosome, colocalizing with aggregates during aging and after heat shock. Additionally, overexpression of NAC delays polyQ aggregation and facilitates luciferase refolding in worms (Kirstein-Miles et al. 2013; Shen et al. 2019). *In vitro*, NAC prevents aggregation of substrates such as polyQ, firefly luciferase, Aβ-40, and α-synuclein in an ATP-independent manner, characteristic of holdase-type chaperone. For polyQ- and luciferase-type aggregates, this activity was partly attributed to charged interactions with the NACβ N-terminus, suggesting that NAC can shuttle between ribosome-associated and posttranslational chaperone roles (Kirstein-Miles et al. 2013; Martin et al. 2018; Shen et al. 2019). However, the precise mechanism of NAC's chaperone activity and substrate recognition remains unexplored.

7 Concluding remarks

Positioned at the forefront of protein production, NAC plays an essential role in maintaining proteome integrity in eukaryotes by regulating the access of different protein biogenesis factors including MetAP1 for N-terminal methionine excision, NatA/E for N-terminal acetylation, and SRP for transport of nascent proteins to the ER. It is unclear if NAC is also involved in the binding of other NATs. Moreover, NAC's role in regulating further cotranslational modifications including N-terminal myristoylation by N-terminal myristoyl transferases (NMT1 and NMT2) is unknown (Duronio et al. 1989; Wilcox et al. 1987; Yang et al. 2005). N-terminal myristoylation occurs downstream of iMet cleavage by MetAP1/2 and directly competes with NatA for its substrates (Castrec et al. 2018). It is tempting to speculate that NAC coordinates also NMT1/2 binding to ensure efficient and specific processing of nascent chains. Further open questions are (i) regarding the purpose and functionality of the NAC's tunnel insertion, and how this relates to NAC's function as a molecular control hub at the ribosome tunnel exit, (ii) NAC's role in the handling of mitochondrial proteins, and (iii) if NAC's chaperone activity aids folding of nascent proteins given its ability to efficiently suppress protein aggregation *in vivo* and *in vitro*.

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