Signalling in ciliates: long- and short-range signals and molecular determinants for cellular dynamics

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ABSTRACT

In ciliates, unicellular representatives of the bikont branch of evolution, inter- and intracellular signalling pathways have been analysed mainly in *Paramecium tetraurelia*, *Paramecium multimicronucleatum* and *Tetrahymena thermophila* and in part also in *Euplotes raikovi*. Electrophysiology of ciliary activity in *Paramecium* spp. is a most successful example. Established signalling mechanisms include plasmalemmal ion channels, recently established intracellular Ca^{2+} -release channels, as well as signalling by cyclic nucleotides and Ca^{2+} . Ca^{2+} -binding proteins (calmodulin, centrin) and Ca^{2+} -activated enzymes (kinases, phosphatases) are involved. Many organelles are endowed with specific molecules cooperating in signalling for intracellular transport and targeted delivery. Among them are recently specified soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), monomeric GTPases, H^+ -ATPase/pump, actin, etc. Little specification is available for some key signal transducers including mechanosensitive Ca^{2+} -channels, exocyst complexes and Ca^{2+} -sensor proteins for vesicle-vesicle/membrane interactions. The existence of heterotrimeric G-proteins and of G-protein-coupled receptors is still under considerable debate. Serine/threonine kinases dominate by far over tyrosine kinases (some predicted by phosphoproteomic analyses). Besides short-range signalling, long-range signalling also exists, e.g. as firmly installed microtubular transport rails within epigenetically determined patterns, thus facilitating targeted vesicle delivery. By envisaging widely different phenomena of signalling and subcellular dynamics, it will be shown (i) that important pathways of signalling and cellular dynamics are established already in ciliates, (ii) that some mechanisms diverge from higher eukaryotes and (iii) that considerable uncertainties still exist about some essential aspects of signalling.

Key words: ciliates, *Euplotes*, *Paramecium*, protozoa, signalling, *Tetrahymena*, traffic, transport.

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I. INTRODUCTION – AN OVERVIEW

(1) Principles of signalling

As cells became ‘eukaryotic’ by sequestration of an increased genome in a nucleus, a dialogue between the nucleus and the cytoplasm had to be organised. Within an increasing cell body, signalling became important also within the cytoplasm. Signalling implies transfer of information of widely different sorts (Good, Zalatan & Lim, 2011). This can involve many aspects, from soluble messengers to molecular interactions of structure-bound proteins which

may mediate signals *via* step-by-step processes. In its broadest sense, therefore, signalling includes not only primary signals and second messengers, but also molecular components on membrane surfaces which are required as short-range signals for recognition and specific organelle interaction, as well as long-range signals, such as pre-established microtubular rails for directional organelle transport (Fig. 1). Below, I outline this multiple meaning of ‘signalling’ and also the multiplicity of signalling mechanisms and their interactions.

Signalling involves many diverse pathways (Gomperts, Kramer & Tatham, 2009) for which professional terminology

Fig. 1. Long-range and short-range signalling, and molecular cues, as exemplified by vesicle trafficking. Frequently long-range signals and short-range signals are at work consecutively, also including in defined molecular recognition. These three steps are highlighted in blue, green and red, respectively. The example given concerns the many forms of intracellular vesicle trafficking, not only in ciliates, but in man and plants. Left panel: as a long-range signal microtubular guidance helps to target organelles over a range of many micrometres to specific sites. In ciliates, this holds true, e.g. of secretory vesicle docking and of vesicle recycling processes, including guidance of vesicles to a newly forming food vacuole at the cytosome. Middle panel: at the respective target sites, within a sub-micrometre range, molecular recognition and docking of vesicles is enabled by SNARE proteins. Right panel: in a final step, molecular cues provided by SNARE proteins and associated proteins, combined with a local Ca^{2+} signal, act as a short-range signal within the range of tens of nanometres. Abbreviations: mt, microtubule; sto, Ca^{2+} store; tm, target membrane; vm, vesicle membrane. See Appendix (Section XIX) for further definitions.

and standard abbreviations are used, as summarized in a glossary in Appendix (see Section XIX). Molecular key players are Ca^{2+} , cyclic nucleotides, protein kinases and phosphatases and a variety of target proteins.

Examples comparing ciliates with ‘higher’ eukaryotes are found throughout this review. A typical example of long-range signalling is the guidance of secretory vesicles by microtubular rails (see Section IV.1). The same holds for some specific recycling vesicles (see Section V.1). Cyclic nucleotides can be long- and short-range signals depending on the distance between the site of their formation and the target structure. Even Ca^{2+} – normally the prototype of a short-range signal (see Section II.1) – can exceptionally act as a long-range signal in spill-over phenomena (see Section II.1). Signal transfer from an endosymbiont-bearing vacuole to the macronucleus is a typical example of long-range signalling (see Section XIII.2), as is the transfer of maternal genomic information to a newly forming macronucleus in ciliates (Section XV.1). The sequence of long-range signalling and molecular recognition, followed by short-range signalling, e.g. by Ca^{2+} , is exemplified in Fig. 1. For instance, after targetted vesicle transport along microtubules, docking occurs by molecular recognition at a target membrane *via* specific docking proteins, including SNAREs (see Section IV.3). Ca^{2+} is the final short-range signal, essentially causing membrane fusion by a conformational change of a Ca^{2+} -sensing protein

(see Section IV.3). Thus, the transfer of a signal to a remote target can be achieved. Membrane–membrane interactions during vesicle trafficking, including exocytosis, endocytosis, recycling and phagocytosis, are of this category (see Sections IV and V). Similar mechanisms can be performed in widely different situations throughout the cell, although each with specific key players (protein paralogs).

Altogether, work with metazoan cells has established that widely different signalling components and mechanisms may contribute to intracellular signalling and organellar dynamics. Signalling may involve exogenous stimuli (receptor activation, electric activation) and formation of ‘metabolic activators’ derived from metabolic compounds, such as cAMP (from ATP), cGMP from GTP, cyclic adenosine diphosphoribose (cADPR) and nicotinic acid adenine dinucleotidephosphate (NAADP), formed from nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotidephosphate (NADP), respectively. It may also involve Ca^{2+} -influx and/or release, protein phosphorylation and dephosphorylation. Such messages will finally regulate a rearrangement of molecules and the transport of organelles which finally can result in specific molecular interactions.

The temporal range of ‘signalling’ goes from very rapid (sub-millisecond range) to more or less slow processes (minutes, hours). Directional organelle movements and recognition by cognate proteins for docking at another organelle, eventually followed by fusion, is an example of signalling whose steps range from a rather slow (intracellular transport), medium (docking) to extremely fast speed (membrane fusion). These steps all involve molecular cues and some steps require second messengers, all of which apply to eukaryotic cells, from protozoa on.

(2) Background information about signalling from ciliates to man

All this applies to eukaryotic cells, from protozoa to mammals, although information available from protozoa is much more rare than for metazoa. Among protozoa, information comes mainly from *Dictyostelium* spp. (Amoebozoa), whereas data for ciliates (Alveolata) became available only relatively recently. These two taxa are unicellular representatives of the two main branches of ‘higher’ organisms, unikonts and bikonts [also called Amorphea and Diaphoretickes and SA[R]P (He *et al.*, 2014)]. Unikonts are related to metazoa and bikonts to plants (Baldauf *et al.*, 2000; Adl *et al.*, 2005, 2012; Cavalier-Smith, 2010). Considering also the close relationship of ciliates with pathogenic Apicomplexa, such as *Plasmodium* (malaria-causing agent) and *Toxoplasma*, it now is a challenge to review for the first time comprehensively signalling mechanisms in their broadest sense in ciliates.

Analysis of signal transduction in ciliates started with pioneer work using electrophysiology (Naitoh & Eckert, 1969; Eckert & Brehm, 1979; Machemer, 1988a,b; Saimi & Kung, 2002). Voltage changes by de- or hyperpolarisation

can generate distinct signals and, thus, modulate swimming behaviour which involves Ca^{2+} signals (Machemer, 1988b) and generation of cyclic nucleotides (Schultz & Klumpp, 1993; Yang *et al.*, 1997). Thus, electrophysiology with ciliates has brought about paradigms for research up to mammalian cells. By contrast, analysis of signalling inside these cells, e.g. by mobilizing intracellular Ca^{2+} , has lagged behind for a long time.

In all eukaryotes Ca^{2+} can participate in multiple ways in cellular signalling according to the following principles. Metabolic compounds can serve as second messengers. For instance, in metazoan cells, inositol 1,4,5-trisphosphate (InsP_3) generated upon stimulation from phosphatidylinositol 4,5-bisphosphate ($\text{PI}(4,5)\text{P}_2$) is an activator of a subgroup of intracellular Ca^{2+} -release channels, CRCs (Berridge, Bootman & Roderick, 2003), specifically of InsP_3 receptors (IP_3Rs). CRCs of different types can release Ca^{2+} ions from different organelles. Extra- and intracellular Ca^{2+} sources can cooperate in some cells by coordinated activity of Ca^{2+} influx and CRCs, both acting in concert. Thereby Ca^{2+} influx can either precede or follow store depletion (Barritt, 1999; Clapham, 2007). As will be discussed, only the latter mechanism is established in ciliates (see Section VIII). Ca^{2+} can modulate many functions as a second messenger in a cell (Berridge, Bootman & Lipp, 1998; Berridge *et al.*, 2003). Additional signalling processes that use Ca^{2+} as a direct or indirect signal encompass formation of other second messengers and/or protein phosphorylation or dephosphorylation upon stimulation, also in ciliates.

There are many more similarities between ciliates and metazoans (see Appendix). As in some mammalian cells, Ca^{2+} influx in ciliates occurs in concert with mobilisation from cortical pools (alveolar sacs, compartments closely attached to the cell membrane) when exocytosis is stimulated (Plattner & Klauke, 2001). Other organelles release Ca^{2+} in response to InsP_3 which is formed by phospholipase C activity also in ciliates. In metazoans, NAADP and cADPR are additional activators of different Ca^{2+} stores (Lee, 2012) and there is preliminary evidence that this may also hold true for ciliates (Plattner *et al.*, 2012). In response to hyper- and depolarisation, cyclic 3',5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP), respectively, are generated in ciliates (Schultz & Klumpp, 1993; Yang *et al.*, 1997). In the latter case, Ca^{2+} is a primary 'second messenger' provided by influx and causing cGMP formation. Formation of the metabolic second messengers depends on specific cyclases and, also in ciliates, stimulation can result in transient phosphorylation of certain proteins by specific protein kinases; also protein phosphatases may depend on Ca^{2+} as a second messenger. Many of the signal-producing proteins and target proteins, as well as kinases and particularly phosphatases have to be positioned at proper sites to guarantee a fast and specific response (Inagaki *et al.*, 1994). Some are assembled, together with anchoring proteins, as signalling complexes, eventually together with their targets (Good *et al.*, 2011;

Esseltine & Scott, 2013). Thus, a cue for proper positioning may be a prerequisite for proper local signalling from ciliates to mammals – an aspect of paramount importance particularly also for local Ca^{2+} signals arising from specific Ca^{2+} channels. Proper positioning is also important for calcium-binding proteins (CaBPs). Among them are high-affinity/low-capacity types, such as calmodulin (CaM) or synaptotagmin. Low-affinity/high-capacity forms (calreticulin, calsequestrin) are mainly contained in calcium stores. High-affinity/low-capacity CaBPs contain either EF-hand loops or C2 domains and perform extensive conformational change after Ca^{2+} binding, thus transmitting information, as described below. Centrin is cytosolic and possesses domains for both functions, high- and low-affinity Ca^{2+} binding and, thus, can also serve as a sink for Ca^{2+} . For details in *Paramecium tetraurelia*, see Plattner (2014).

One of the well-established short-range signalling mechanisms, from ciliates to man and plants, is the specific recognition of vesicles/membranes of different types by a kind of key–lock mechanism using organelle-specific proteins. This task is essentially executed by specific GTPases (monomeric G-proteins) in conjunction with SNARE proteins (Jahn & Scheller, 2006; Jahn & Fasshauer, 2012). This acronym is derived from 'soluble N-ethylmaleimide sensitive factor [NSF] attachment protein receptors'. Eventually this leads to membrane fusion in dependency of local intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$. As summarised previously (Plattner, 2010b) many, although not all of the molecular key players known from mammalian cells, particularly from neuronal systems (Südhof, 2013), are known also from ciliates. Yet the Ca^{2+} sensor involved in membrane fusion remains enigmatic in ciliates. At the cell membrane, processes such as exo- and endocytosis require a stimulation-induced local increase in $[\text{Ca}^{2+}]_i$ also in ciliates (Plattner & Klauke, 2001). Beyond stimulated signalling, constitutive signalling also occurs, as is the case during intracellular vesicle trafficking. This also requires local availability of Ca^{2+} in the nearby cytosol. Local spontaneous Ca^{2+} signals can be seen with fluorochromes.

Cell functions discussed herein specifically for ciliates range from ciliary beating, different organelle/membrane interactions including exo- and endocytosis and phagocytosis, graviperception, apoptosis-related phenomena as well as interaction between cells by paracrine signalling. They all are accompanied by intracellular signalling processes. In its broadest sense signalling, particularly in ciliates, also has to take into consideration epigenetic phenomena (Nowacki & Landweber, 2009; Chalker, Meyer & Mochizuki, 2013; Simon & Plattner, 2014). As will be discussed, there are also controversies regarding important details of signalling in ciliates. An example is the unsettled issue of phosphorylation of tyrosine residues in proteins. There is also some uncertainty about the existence of heterotrimeric GTP-binding proteins in ciliates.

(3) Some characteristics of the ciliate species under scrutiny

Ciliates are free-living relatives of parasitic Apicomplexa, such as *Plasmodium* and *Toxoplasma*. Ciliates and Apicomplexa both belong to the superphylum Alveolata since their cortex is paved with subplasmalemmal alveoli – flat sacs serving as Ca^{2+} stores. Ciliate species mainly used for signalling studies are *Paramecium tetraurelia* and *Tetrahymena thermophila*, unless indicated otherwise. *P. tetraurelia* is several times bigger ($\sim 40 \times 100 \mu\text{m}$) than *T. thermophila* ($\sim 25 \times 50 \mu\text{m}$) and was, therefore, frequently preferred for techniques such as electrophysiology, microinjection (function repair studies, antibody and Ca^{2+} fluorochrome studies), subcellular fractionation etc. Both species show nuclear dimorphism as they possess a diploid germline micronucleus from which a transcriptionally active ‘somatic’ macronucleus is derived (Orias, Cervantes & Hamilton, 2011; Simon & Plattner, 2014). The micronucleus is transcriptionally inactive and serves for information storage and transfer to a new macronucleus. The macronucleus is polyploid in *P. tetraurelia* (Aury *et al.*, 2006; Duret *et al.*, 2008) where, moreover, most genes are represented by a number of paralogs originating from three rounds of whole-genome duplications (Aury *et al.*, 2006). Such genes are also called ohnologs after geneticist Ohno (1970). When ohnologs originate from the last round of duplication, they may differ only slightly in their nucleotide sequence and, therefore, most likely serve for gene amplification. This also applies to a variety of genes/proteins involved in signalling (Plattner, 2010a,b). Due to their similarities, it is difficult (although mostly not required) to differentiate between some of the most recent ohnologs by methods such as gene silencing or antibody localisation. Example are SNARE ohnologs, as discussed in Section IV.3. Since *T. thermophila* does not have some of these complications (Eisen *et al.*, 2006) it can be a favourable alternative for some problems in cell signalling.

(4) Why study signalling in ciliates?

Signalling in the eukaryotic cell is also fascinating from an evolutionary point of view. In parallel to the evolution of increasingly complex and diversified cells, many mechanisms are present already in protozoans. Nevertheless, knowledge about signalling at this level of evolution is relatively scant. The patchiness of information and remarkable recent progress enabled by molecular biology provided the motivation to summarise current knowledge about signalling in ciliates herein. Among them are species of *Paramecium* and *Tetrahymena* which have served as model systems for many aspects over decades (Simon & Plattner, 2014). As we shall see, some protein molecules and mechanisms involved in signalling are maintained from ciliates up to human brain function where they contribute to ‘wire and fire’ [synaptogenesis and long-term potentiation (learning)]. As will be discussed, this includes CaM, voltage-gated Ca^{2+} channels and their inhibition by forming a Ca^{2+} /CaM complex; it also includes dimeric Ca^{2+} - and CaM-activated

phosphatase 2B (calcineurin). In this we will see specific differences to plants.

There are several reasons to study signalling in ciliates. (i) Concerning one of the most important components of signalling, Ca^{2+} , early classical electrophysiology with ciliates (see Section III) provided basic insights (Machemer, 1988a,b) some of which could be successfully extended to mammalian cells. Conversely, recent progress with multicellular organisms stimulated further work with ciliates. (ii) Ciliates offer the unique possibility to induce synchronous exocytosis and rapid total deciliation, each entailing massive *de novo* organelle formation, based on signalling phenomena (see Section XIII.1). This allows the study of signalling during organelle biogenesis. (iii) In ciliates, a highly regular, largely epigenetically based cell structure allows for precisely localised signalling, thus facilitating structure–function correlation (Simon & Plattner, 2014; Plattner, 2015a). The eggcase-shaped surface of a *Paramecium tetraurelia* cell displays ~ 3000 unit fields (kinetids) with about as many cilia and docking sites for dense core-secretory organelles (trichocysts) in an alternating arrangement. In the smaller genus, *Tetrahymena*, mucocysts substitute for trichocysts, but the cells, although smaller, have the same regular design. (iv) Every ciliate cell contains two different types of nuclei, a germline micronucleus and a transcriptionally active macronucleus, also derived from a micronucleus (Orias *et al.*, 2011; Sperling, 2011). During macronucleus formation, genome reshaping takes place under epigenetic control and the underlying signalling mechanism can be studied (Singh *et al.*, 2014). This is based on epigenetic information transfer *via* scanning RNA (scnRNA) transcripts from the parental macronucleus to the new forming macronucleus (Nowacki, Shetty & Landweber, 2011; Swart *et al.*, 2014). The study of epigenetic inheritance is at the forefront of research in cell biology, and not only in ciliates. (v) Ciliates are at the crossroads of two lineages, unikonts and bikonts. Ciliates, originating about 0.8–1 billion years ago (Parfrey *et al.*, 2011; Dorrell *et al.*, 2013), are assigned to bikonts whose further diversification led to plants (Plattner & Verkhatsky, 2015; Plattner, 2015b). (vi) Finally, ciliates are close relatives of some of the most pernicious apicomplexan parasites, *Plasmodium* and *Toxoplasma*, which are considerably younger than ciliates (Dorrell *et al.*, 2013). Since little is known, e.g. about Ca^{2+} signalling, in the parasitic forms one may expect that comparison with free-living forms may provide seminal insights (Plattner *et al.*, 2012).

II. SUBCOMPARTMENTALISATION OF SIGNALLING PATHWAYS

Local limitation of signalling restricts signals to specific targets. Amongst soluble signals, this is particularly important for Ca^{2+} (Neher, 1998a,b). Stimulus-evoked Ca^{2+} signalling can occur selectively in cilia or along non-ciliary (somatic) regions of the cell membrane, whereas constitutive local intracellular Ca^{2+} signalling requires organelle-specific

Ca^{2+} -release channels. This local regulation allows for efficient, energetically low-cost information transfer and strictly localised signalling avoids inadvertent activation of additional processes. Therefore, many proteins involved in signalling are localised to specific sites, including in ciliates.

(1) Relevance of local Ca^{2+} signalling

The best example of the importance of compartmentalised signalling concerns Ca^{2+} . Ca^{2+} signals are in the form of dissolved (free) Ca^{2+} which is rapidly removed by dilution (diffusion), binding to CaBPs, sequestration into stores and/or extrusion from the cell (Berridge *et al.*, 2003). Importantly, the efficacy of Ca^{2+} signals depends on the very local value of $[\text{Ca}^{2+}]_i$ in a superlinear function (Neher, 1998a). Therefore, Ca^{2+} -influx and Ca^{2+} -release channels require precise localisation with regard to a specific target (Neher, 1998b). In ciliates, depolarisation-sensitive Ca^{2+} -influx channels are found exclusively in the ciliary membrane (Machemer & Ogura, 1979). As is known from CRCs of the superfamily including IP_3 R and ryanodine receptors (RyRs) in *P. tetraurelia*, a multiplicity of different *Pt*CRCs occurs in widely different organelles (Ladenburger & Plattner, 2011). Some occur only in one organelle, others in several, and one organelle may possess one or several types of CRCs of either type [IP_3 R and RyRs (or RyR-like proteins, RyR-LPs)]. They may mediate Ca^{2+} signalling in different – largely unspecified – ways and, thus, locally govern vesicle trafficking.

(2) Local Ca^{2+} signalling in *Paramecium*

The relevance of locally restricted signalling may be exemplified by one of the best-analysed phenomena in ciliates – signalling for trichocyst exocytosis. Upon stimulation, Ca^{2+} release from alveolar sacs initiates additional Ca^{2+} influx *via* unidentified channels in the cell membrane. This sequence is termed store-operated Ca^{2+} entry (SOCE) also in ciliates (Plattner & Klauke, 2001). The influx component can be seen in $^{45}\text{Ca}^{2+}$ flux measurements (Kerboeuf & Cohen, 1990). Together, the internal release and influx of Ca^{2+} , cause a sufficient increase in $[\text{Ca}^{2+}]_i$ in the narrow subplasmalemmal space to stimulate trichocyst exocytosis. This also accelerates subsequent steps leading to exocytosis-coupled endocytosis (Plattner, Braun & Hentschel, 1997), as in different mammalian clear and dense core-secretory vesicle systems such as pituitary (Thomas *et al.*, 1994; Wu *et al.*, 2014) and chromaffin cells (Artalejo *et al.*, 1995).

A $[\text{Ca}^{2+}]_i$ increase was observed in *Paramecium tetraurelia* using Ca^{2+} fluorochromes also when the extracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_o$, was abruptly increased, but this did not induce trichocyst exocytosis (Erxleben *et al.*, 1997). This supports the concept of the requirement for strictly subcompartmentalized, local Ca^{2+} signalling; further spillover beyond the cell cortex did not lead to exocytosis (although it can activate transcriptional activity as discussed in Section XIII).

Normally Ca^{2+} signals in cilia do not spill over into the soma (Husser *et al.*, 2004). Only precisely localised Ca^{2+} signals produce specific responses. This concept of spatially highly restricted signalling has been assessed in great depth for neuronal transmission (Neher, 1998b). All subcompartmentalisation of intracellular Ca^{2+} channels in ciliates serves a similar purpose. As suggested by the distribution of CRCs, in ciliates, this principle may also apply to other trafficking organelles. One example is the phagocytotic pathway (Ladenburger & Plattner, 2011), with its complicated input, retrieval and recycling of vesicles (Fok & Allen, 1993). In metazoans, not only Ca^{2+} , but also ‘metabolic’ second messengers and proteins involved in signalling usually follow the principle of subcompartmentalisation (Barford, 2010). This also applies to ciliates, as outlined in Section VIII.

III. CILIARY BEAT ACTIVITY

Some of the regulation mechanisms for ciliary activity – although not all – are similar in ciliates and mammalian cells. In mammals, cAMP, cGMP and Ca^{2+} have a modulatory effect, predominantly by activating specific kinases (Salathe, 2007). Activation of cAMP-activated protein kinase A (PKA) accelerates ciliary beating just as in ciliates. By contrast, the role of cGMP and cGMP-activated protein kinase (PKG) is less well defined in mammals than in ciliates. In both systems, Ca^{2+} has several regulatory functions. Remarkably, in ciliates, Ca^{2+} influx into cilia takes place *via* voltage-dependent channels that do not occur in animal cilia (although such channels occur in some neurons of the differentiating and differentiated central nervous system of man). In all cells, vesicles delivering proteins to cilia do not flow to the ciliary plasma membrane, but rather fuse in the nearby ‘somatic’ plasma membrane from where proteins dissipate into the ciliary membrane. However, the precise way in which membrane proteins and soluble proteins move into the cilia is not yet known, especially for ciliates. Several molecular cues also regulate the biogenesis of the ciliary axoneme, as discussed in Sections IV.2 and IV.3.

In ciliates, ‘mature’ cilia ready for function are governed by multiple signals. Normal ciliary beat activity depends on hydromechanical coupling of the cilia, as shown in ctenophores (Tamm, 2014) as well as in ciliates (Sleigh, 2014). In ciliates, modification of beat activity can be achieved by electrophysiological signals, i.e. hyper- and depolarisation, respectively (Machemer, 1988a,b). The accompanying signals (ion fluxes) arise at different sites on the cell surface and are followed by formation of the cyclic nucleotides, cAMP and cGMP, respectively (Schultz *et al.*, 1984; Schultz & Klumpp, 1993; Yang *et al.*, 1997). These activate the respective protein kinases for phosphorylation of specific ciliary proteins. A Ca^{2+} /CaM complex participates in the phosphorylation of different dynein proteins and thus affects ciliary beating, as originally shown with *Tetrahymena pyriformis* (Blum *et al.*, 1980).

(1) Aspects pertinent to biogenesis of cilia

Such signalling commences with signals for proper deposition of molecular components, also in cilia. In metazoans, most membrane proteins destined for cilia are thought to be selected at different levels from the trans-Golgi network onwards (Sung & Leroux, 2013). They may be delivered (i) first to the periciliary membrane, (ii) to more lateral parts of the cell membrane for subsequent diffusion into the ciliary membrane, or (iii) to the ciliary pocket (Bloodgood, 2012) by recycling endosomes or other vesicles. In ciliates, the latter are clathrin-coated parasomal sacs. Concomitantly auxiliary proteins contributing to the biogenesis of cilia include coatamer proteins (COPs), adaptor proteins (APs), SNAREs, GTPases including Rab-types, dynein- and kinesin-type motor proteins, intraflagellar transport (IFT) protein complexes, Beadle-Bardet Syndrome (BBS) protein complexes (BBSome) (Valentine *et al.*, 2012), etc. SNAREs [syntaxin (Syx3), synaptosomal associated protein of 25 kDa size (SNAP-25)] and Rab8 are considered mandatory for the formation of a primary cilium in metazoans (Kim & Dynlacht, 2013).

Only selective aspects of targeting are available for ciliates. In ciliates, biogenesis of cilia depends on specific centrin isoforms (Stemm-Wolf *et al.*, 2005; Jerka-Dziadosz *et al.*, 2013). Recently it has been shown that the establishment of ciliary planar polarity (explained in Section III.2) in meningeal ependymal cells also depends on centrin (Ying *et al.*, 2014). BBSome subunits govern channel delivery in *Paramecium* spp. For instance, downregulation of subunits 7, 8 or 9 reduces the delivery of Ca^{2+} -dependent K^{+} channels (serving to reverse the membrane potential after depolarisation) (Valentine *et al.*, 2012), while this does not affect voltage-dependent Ca^{2+} channels (whose transport pathway thus remains open). For electrophysiological details, see Section III.2. Cilia of *Paramecium* spp. contain the GTPase-modulating protein Sec7, i.e. transport protein 7, a guanine nucleotide exchange factor relevant for secretion; this is a ciliary homolog of ADP [adenosine diphosphate]-ribosylation factor nucleotide site opener (ARNO), a guanyl nucleotide exchange factor (GEF) found in the cytoplasm (Nair, Guerra & Satir, 1999). In *Paramecium tetraurelia*, *PtSyx1* is distributed over the entire cell membrane (Kissmehl *et al.*, 2007) and thus may serve as a receptor also for docking of vesicles destined for ciliary biogenesis. Surprisingly the v-/R-SNARE (see Appendix for terminology conventions) *PtSyb10* forms a ring in the periciliary cell membrane and its silencing slows down ciliary activity (cell rotation) (Schilde *et al.*, 2010). It should be noted that generally the designation of *PtSyx* subtypes is comparable with those in mammals; because of wide deviations, including an additional longin domain (see Section IV.2), this is not the case with *PtSyb* subtypes indicated by Arabic numbers. The presence of this v-SNARE in the target membrane may be explained by an excess of vesicle delivery. Normally parasomal sacs are assumed to serve not only for constitutive endocytosis but also for constitutive exocytosis including cell membrane biogenesis

(Flötenmeyer, Momayezi & Plattner, 1999; Capdeville, 2000). In summary, delivery mechanisms and underlying molecular cues and signals may vary for the different ciliary components, although knowledge is restricted, and not only in ciliates.

Dileptus margaritifer possesses sensory cilia on its proboscis (cell body extension used to search for prey) and locomotor cilia on the cell body. There is an enigmatic transformation of cilia when the proboscis is dissected from the cell body; this causes transformation of microtubule singlets to doublets with dynein arms (Golinska, 1982). For cilia, this is considered the first, and still the only known transformation process (Jerka-Dziadosz & Frankel, 2014), but the signals involved remain enigmatic.

(2) Functional aspects of Ca^{2+} increase in cilia

Identical orientation and spatial arrangement of cilia is a prerequisite to their functional coupling in a metachronic beat pattern and this orientation depends on 'planar cell polarity'. As demonstrated in epithelia, this type of polarity requires a newly discovered coiled-coil protein, basal body orientation factor 1 (bbof1), associated with basal bodies (Chien *et al.*, 2013). Whether a homolog exists in ciliates remains to be analysed. In *T. thermophila*, disorientation protein mutant A (DisAp) protein exerts such an effect on the organisation of ciliary arrays (Galati *et al.*, 2014) by its regulatory effect on elongation of striated (kinodesmal) fibres emanating from ciliary basal bodies in an anterior direction. Also in ciliates, functional coupling of cilia does not require any further signals; it occurs by mere hydromechanical (colligative, adhesive) forces when cilia are appropriately spaced, i.e. in the regular, epigenetically predetermined pattern (Beisson & Sonneborn, 1965; Frankel, 1973; Beisson & Jerka-Dziadosz, 1999; Beisson, 2008). This depends on proper positioning of daughter basal bodies during biogenesis which requires specific centrin isoforms (Stemm-Wolf *et al.*, 2005; Jerka-Dziadosz *et al.*, 2013) and some other conserved centrosome/basal body-associated proteins, such as FOR20 [FOP (FGFR1 oncogene partner)-related protein of 20 kDa] (Aubusson-Fleury *et al.*, 2012). In conclusion, proper morphology achieved by epigenetically determined morphogenetic signalling, based on distinct co-assembly of protein components, enables coordinated ciliary beating in ciliates. This is prerequisite to mechanical coupling between cilia.

In ciliates, ciliary beating takes place spontaneously in metachronal waves due to a phase shift between longitudinally arranged, hydrodynamically coupled cilia (Machemer, 1988a). Any change in beat direction and speed requires specific signalling (Machemer, 1988b). Important examples are accelerated forward swimming due to hyperpolarisation or reversal of ciliary beat direction ('ciliary reversal') due to depolarisation (Eckert & Brehm, 1979; Kung & Saimi, 1982). Similarly swimming behaviour can be governed by chemo-attractants and -repellents (Van Houten, 1998). During chemosensory transduction, distinct ion conductances are activated and, thus, yield specific

behavioural/swimming responses (Kung & Saimi, 1982; Saimi & Kung, 1987; Bell *et al.*, 2007).

In ciliates, normal ciliary beat depends on Ca^{2+} (Naitoh & Kaneko, 1972), as does any change in beat activity. This includes not only beat direction (see below), but also beat frequency which, in an extracted model, depends on $[\text{Ca}^{2+}]$ via a Ca^{2+} /CaM complex (Nakaoka, Tanaka & Oosawa, 1984). Remarkably many cation channels of ciliates, ciliary and non-ciliary ('somatic'), are regulated by Ca^{2+} via a Ca^{2+} /CaM complex (Kung *et al.*, 1992; Saimi & Kung, 1994, 2002). During ciliary reversal induction (Fig. 2), $[\text{Ca}^{2+}]$ increases rapidly by influx via voltage-dependent Ca^{2+} channels (Machemer & Ogura, 1979) over the entire length of a cilium in ctenophores (Tamm, 2014) as well as in *Paramecium tetraurelia* (Husser *et al.*, 2004), both systems having been analysed by widely different methods. Interestingly, in ciliates, CaM is distributed over the entire length of cilia (Maible *et al.*, 1981; Momayez *et al.*, 1986) where it exerts different functions. One concerns the plasma membrane Ca^{2+} -ATPase (PMCA). PMCA was demonstrated not only in the somatic plasmalemma (Wright & Van Houten, 1990; Elwess & Van Houten, 1997), but also in cilia of *Paramecium tetraurelia* (Yano *et al.*, 2013). This confirms older biochemical data from *Tetrahymena thermophila* (Dentler, 1988). PMCA may, therefore, possibly belong to the standard inventory of cilia not only in ciliates but also up to mammals. For instance, in cilia of olfactory neurons of mice, PMCA contributes to the downregulation of Ca^{2+} signals (Saidu *et al.*, 2009). It should be noted, however, that no information is available for PMCA in cilia of ctenophores and that cilia in metazoa beyond ctenophores contain other types of Ca^{2+} -influx channels.

In mammals, this pump is activated by subplasmalemmal actin (Dalghi *et al.*, 2013). Actin also occurs in cilia of ciliates (Kissmehl *et al.*, 2004), notably isoforms *PtAct1*, *PtAct2*, *PtAct3*, *PtAct4*, and *PtAct5* (Sehring *et al.*, 2007b). Another function at the cell membrane level is the activation of cation channels pertinent to ciliary activity (Preston *et al.*, 1991).

How can the ciliary beat be changed? A physical signal (anterior or posterior mechanical stimulation) results in an electric signal. Hereby a receptor potential (hyper- or depolarisation) is formed at the level of the somatic cell membrane, followed by an action potential formed at the level of the ciliary membranes (Kung & Saimi, 1982). As described below, this is followed by formation of cyclic nucleotides, activation of protein kinases and phosphorylation of specific proteins. All this prepares the cilium for specific mechanical work.

(a) Hyperpolarisation response

Accelerated forward swimming by hyperpolarisation is triggered not only by touching cells at the rear end, but also by an appropriate change in extracellular ionic conditions or by adding a chemoattractant (Kung & Saimi, 1982; Yang *et al.*, 1997; Van Houten, 1998). The hyperpolarisation-induced receptor potential is carried by a K^{+} outward current (Naitoh & Eckert, 1969) and further on

Fig. 2. Ciliary reversal is an example of spatial signal restriction in ciliates. Depolarisation activates voltage-gated Ca^{2+} channels. The influx of Ca^{2+} is restricted to several hundred ions per cilium by negative feedback via a Ca^{2+} /calmodulin complex (not shown). Ca^{2+} in cilia is downregulated by binding to Ca^{2+} -binding proteins (CaBPs), such as centrin, within the cilium and at its base, thus avoiding signal spread into the cell soma. The occurrence of a plasma membrane Ca^{2+} -ATPase (PMCA) in cilia is thought to be likely. Note that normally cortical calcium stores (alveolar sacs) do not contribute to this ciliary activity.

activates highly Ca^{2+} -selective hyperpolarisation-activated channels in the somatic membrane, peaking within 20–80 ms (Preston, Saimi & Kung, 1992a). This is followed by activation of adenylyl cyclase which forms cyclic AMP (cAMP), a process depending on the hyperpolarising K^{+} conductance (Bonini, Gustin & Nelson, 1986; Schultz & Schönborn, 1994). According to quenched-flow analyses cAMP is formed already within one ciliary stroke (Yang *et al.*, 1997) which lasts ~40 ms. [Note that the minimal analysis time for quenched-flow analyses includes the ~30 ms dead time of the instrument]. Thus, there is a perfect match between an electrical and a chemical signal. This cyclase, that is reportedly Ca^{2+} sensitive (Kudo *et al.*, 1985a; Kudo, Muto & Nozawa, 1985b), is an integral membrane protein coupled to an ion channel domain with a voltage sensor and a K^{+} -pore loop (Weber *et al.*, 2004). Subsequent activation of a PKA in cilia (Hochstrasser & Nelson, 1989) causes phosphorylation of specific ciliary proteins, such as components of dynein, the motor protein responsible for ciliary movement (Hamasaki *et al.*, 1991; Walczak & Nelson, 1993; Kutomi *et al.*, 2012). This chain of events accelerates forward swimming by accelerated dynein activity, before the increased cortical $[\text{Ca}^{2+}]_i$ inactivates the hyperpolarisation-sensitive Ca^{2+} -influx channels (Preston,

Saimi & Kung, 1992b); any participation of CaM in the inactivation of the hyperpolarisation response has not been investigated as yet.

(b) Depolarisation response

Ciliary reversal is induced not only by mechanical stimulation at the anterior part of the cell, e.g. when a cell hits an obstacle, but also by an appropriate change of ionic conditions in the medium, such as increased $[K^+]_o$ causing depolarisation. In the first case, mechanosensitive channels enriched at the anterior part of the somatic cell membrane are activated (Ogura & Machemer, 1980). A receptor potential forms by a Ca^{2+} influx (Naitoh & Eckert, 1969), followed by a K^+ efflux (Satow, Murphy & Kung, 1983), both in the somatic (non-ciliary) part of the cell membrane (Machemer, 1988a,b). This triggers a Ca^{2+} -based action potential by activating depolarisation-activated (voltage-dependent) Ca^{2+} channels that are restricted to the ciliary membrane (Machemer & Ogura, 1979) (Fig. 2). The membrane potential is returned to resting level by a voltage-gated and Ca^{2+} -activated K^+ current – the respective channels also residing in the cilia (Valentine *et al.*, 2012). Most of these data have been collected from *Paramecium tetraurelia*. Remarkably, voltage-gated Ca^{2+} channels are also found in our central nervous system.

Depolarisation entails formation of cGMP (Schultz, Pohl & Klumpp, 1986), by activation of Ca^{2+} /CaM-activated guanylate cyclase (Kudo *et al.*, 1985a,b; Schultz & Klumpp, 1991) and of a PKG, all in cilia (Miglietta & Nelson, 1988). The respective guanylate cyclase is localised to the cilia membrane (Linder *et al.*, 1999). Phosphorylation of a set of proteins somehow causes the reversal of the ciliary beat direction. Inhibition of phosphatase 1 (PP1), sustains backward swimming (Klumpp, Cohen & Schultz, 1990), thus reflecting a role in signal termination. Ca^{2+} influx is short since voltage-dependent Ca^{2+} channels are inactivated by the same Ca^{2+} that they conducted, by forming an inhibitory Ca^{2+} /CaM complex (Brehm & Eckert, 1978). Again the same mechanism is maintained up to the mammalian central nervous system (Levitan, 1999). Here, an additional Ca^{2+} -binding protein, CaBP1, and the Ca^{2+} /CaM complex mutually control each other (Findeisen, Rumpf & Minor, 2013). It remains to be seen whether a similar fine-tuning mechanism also exists in ciliates.

(3) Subsequent regulation steps in cilia

Anywhere in the cell, free Ca^{2+} can be rapidly downregulated by binding to CaBPs. Total calcium (structure-bound + dissolved) and free (dissolved) calcium can be differentially determined by using energy-dispersive X-ray microanalysis (EDX) and fluorochrome analysis, respectively. In *Paramecium* spp., this has been exemplified not only with exocytosis (see Section IV) but also with cilia (Husser *et al.*, 2004). Upon depolarisation, an increase of total and free calcium can be detected in the cilia within one ciliary beat. As seen by EDX, total calcium concentration remains at an increased level, in contrast to free $[Ca^{2+}]$ which

rapidly decays according to fluorochrome measurements. This indicates that Ca^{2+} ions are rapidly bound and trapped inside cilia. Nevertheless, the reversal reaction lasts tens of seconds before normal forward swimming is resumed. Remarkably, this is much longer than the signal of free Ca^{2+} (Husser *et al.*, 2004). The ongoing response may be sustained by the occurrence of additional Ca^{2+} /CaM complexes in the axoneme where CaM also occurs (Momayezi *et al.*, 1986). The actual reversal reaction has tentatively been explained by occupancy of the carboxy-terminal Ca^{2+} -binding loops of CaM by Mg^{2+} (Machemer & Teunis, 1996) – the last of the four hierarchically occupied EF-hand Ca^{2+} -binding loops. Moreover, in cilia differential binding of Ca^{2+} to other Ca^{2+} -dependent and Ca^{2+} -independent CaM binding proteins (CaM-BPs) takes place (Evans & Nelson, 1989), although their respective roles are not precisely known. Not only PKA, PKG and CaM, but also Ca^{2+} -dependent protein kinases (CDPKs), which possess an integrated CaM-like sequence (see Section IX.1), are found in the cilia of *Paramecium tetraurelia* (Kim, Messinger & Nelson, 1998). Collectively these mechanisms can prolong ciliary reaction in some way beyond the actual duration of the free Ca^{2+} signal in cilia.

Thus, a physical signal (hyperpolarisation or depolarisation) results in a Ca^{2+} signal, then in a chemical signal (cyclic nucleotides) which results in the covalent modification (phosphorylation) of ciliary proteins. The spectrum of proteins phosphorylated by PKA and by PKG, respectively, is different (Ann & Nelson, 1995) and they govern ciliary motility in different ways (Bonini & Nelson, 1990). These mechanisms transmit mechanical stimuli to chemical signals which in turn are transmitted to a mechanical response, i.e. accelerated forward swimming or ciliary reversal. Thus, Ca^{2+} can initiate several ciliary responses along different pathways (Machemer & Teunis, 1996) and this is achieved by modification of the phosphorylation state of specific proteins.

(4) Organismal aspects

During ciliary reversal, normally no Ca^{2+} is spilled into the soma of a *Paramecium tetraurelia* cell (Husser *et al.*, 2004). A spill-over in the reverse direction does occur, however, when massive exocytosis is induced. Then, Ca^{2+} moves from the cell cortex into cilia and, thus, induces ciliary reversal without previous depolarisation (Husser *et al.*, 2004). This also entails cGMP formation (Knoll, Kerboeuf & Plattner, 1992) and, thus, this process proceeds as during ciliary reversal induced by depolarisation. Such automatic coupling of trichocyst exocytosis and ciliary reversal is also triggered when a *Paramecium tetraurelia* cell contacts a predatory cell (Knoll, Haacke-bell & Plattner, 1991b); this is meaningful in the context of the escape mechanism that helps to ward off predators such as *Dileptus margaritifer* (Harumoto & Miyake, 1991). Currently such escape phenomena are known in an increasing number of ciliates (Buonanno, Harumoto & Ortenzi, 2013). During this defence reaction, whose primary chemical signal is unknown, vigorous local trichocyst release first forms a spacer between predator and prey, while

the prey activates the ‘reverse gear’ (Knoll *et al.*, 1991b). This is perfectly mimicked by the standard secretagogue for *Paramecium* spp., aminoethyldextrane (AED). Its local application induces local trichocyst release also in deciliated cells as well as in mutants devoid of voltage-dependent Ca^{2+} channels (Plattner *et al.*, 1984; Plattner, Stürzl & Matt, 1985). In such cells AED also rapidly induces ciliary reversal by spillover of Ca^{2+} from the soma into cilia as documented by fast confocal fluorochrome and EDX analysis (Husser *et al.*, 2004). Since this reaction is short it may easily be overlooked (Harumoto, 1994). Thereby the depolarisation step is bypassed. Lectins contained in the trichocyst tip (Haacke-Bell & Plattner, 1987) may additionally contribute to the defence mechanism.

IV. ORGANELLAR TRAFFICKING SIGNALS – GENERAL RULES AND IMPLICATIONS FOR VESICLE TRANSPORT IN CILIATES

In mammalian cells, the principal pathway of vesicles from the endoplasmic reticulum to the cell membrane was elucidated by Palade (1975) and the underlying molecular machinery by Rothman (2014). The basic principles of trafficking also apply to ciliates.

Long-range guidance of vesicles during trafficking along microtubules has been detected in mammalian gland cells (Lacy *et al.*, 1968) and motoneurons (Gray, 1978). In these cells, as in most others, transport goes from the minus to the plus end of microtubules, i.e. from the cytocentre to the cell periphery. Transport in the reverse direction was first described in *Paramecium tetraurelia* (Plattner, Westphal & Tiggemann, 1982), subsequently in mammalian epithelial cells and finally in immune cells (Griffiths, Tsun & Stinchcombe, 2010). Short-range signals and target proteins for final signal transduction are quite similar in protozoa and metazoa. Such chemical signals inherent to proteins govern the direction of vesicle trafficking, attachment and fusion. A paradigmatic example is neurotransmitter release (Südhof, 2013, 2014).

Short-range signals encompass several proteins mediating vesicle docking and fusion, with the involvement of Ca^{2+} , SNARE (Rothman, 2014; Südhof, 2014) and Rab proteins (Mizuno-Yamasaki, Rivera-Molina & Novick, 2012; Pfeffer, 2013), as specified below. This was detected with mammalian cells and yeast before being explored in ciliates (Bright *et al.*, 2010; Plattner, 2010a,b). These basic aspects are early evolutionary achievements maintained throughout eukaryotic kingdoms (Dacks & Field, 2007; Kloepper, Kienle & Fasshauer, 2008; Stenmark, 2012).

This section contains multiple facets of signalling phenomena as exemplified in Figs 1, 3 and 4 for the secretory pathway and the phagocytotic cycle in *Paramecium*. Surprisingly trichocysts contain signals for their filling state, for their inherent polarity and, thus, for their docking competence. Short-range signals or molecular cues, as known from metazoans, encompass vesicle- (v-) SNAREs

Fig. 3. Molecular cues and short-range signals governing membrane trafficking in ciliates reside in SNARE proteins, small GTPases (Rab proteins) and their modulators, in addition to H^{+} -ATPase molecules. In mammalian cells, luminal acidification has been shown to cause a transmembrane signal by a conformational change in the multimeric H^{+} -ATPase molecule, thus enabling the binding of Rab proteins *via* their modulators and then forming a complex with SNAREs. For membrane fusion to occur as the final signalling step Ca^{2+} has to be provided as a short-range signal.

which, together with additional proteins, have to match with appropriate target- (t-)SNAREs (Jahn & Fasshauer, 2012) (Fig. 3). This also applies to ciliates (Plattner, 2010a,b) (Fig. 4). Upon a local increase of $[\text{Ca}^{2+}]_i$ during the course of stimulation both membranes can fuse. Ca^{2+} flushing from the outside medium through the exocytotic opening into the trichocyst lumen provides an additional signal, required specifically for the explosive decondensation and vigorous release of trichocyst contents (Plattner & Klauke, 2001).

(1) Support by microtubules

Trichocyst transport is saltatory (Aufderheide, 1978), i.e. facilitated and guided by microtubules emanating from ciliary basal bodies, along which they slide in minus-end direction for final docking at the cell membrane (Plattner *et al.*, 1982). The regular arrangement of docking sites between regularly spaced cilia in *Tetrahymena thermophila* and *Paramecium tetraurelia* is mediated by epigenetic signals (Frankel, 1973; Beisson, 2008). Organelles undergoing trafficking encompass not only dense core-secretory organelles, i.e. trichocysts in *Paramecium* spp. and mucocysts in *Tetrahymena* spp., but also clear vesicles for the biogenesis of the plasmalemma (Schilde *et al.*, 2010) and release of acid hydrolases (Kiy *et al.*, 1993) at ill-defined sites. In ciliates, recycling vesicles and phagosomes also travel along defined routes determined by microtubular arrays (Fok & Allen, 1993; Wloga & Frankel, 2012). In

Fig. 4. The membranes of vesicles trafficking along the principal pathways in a *Paramecium* sp. cell (nuclei omitted) contain specific proteins, such as SNAREs. Trafficking routes of the phagocytotic cycle are indicated by dotted red arrows. Subcellular distribution of isoforms of the t-/Q-type SNARE, syntaxin (syx), and of the v-/R-type SNARE, synaptobrevin (syb), are compared. Abbreviations: a = ampullae (on contractile vacuole); as = acidosomes (late endosomes); ci = cilium; cp = cytoproct; cv = contractile vacuole; ds = decorated spongiome (part of contractile vacuole complex); dv = discoidal vesicles (recycling from cytoproct); ee = early endosomes; er = endoplasmic reticulum; fv = food vacuole; ga = Golgi apparatus; gh = ghost (empty trichocyst membrane); oc = oral cavity; pm = plasma membrane; ps = parasomal sacs (clathrin-coated endocytotic vesicles); rv = recycling vesicles; sec22 = transport protein 7 (guanine nucleotide exchange factor) relevant for secretion; ss = smooth spongiome (part of contractile vacuole complex); svcy = small vesicles in cortical cytoplasm; tr = trichocyst; trpc = trichocyst precursor.

T. thermophila microtubules have been shown to contain α -tubulin in association with a variety of different β -tubulins, thus allowing for differential functionalisation (Pucciarelli *et al.*, 2012). Furthermore, a number of microtubules are composed of distinct post-translational modifications of tubulin, in *Paramecium tetraurelia* (Adoutte *et al.*, 1991) as well as in *Tetrahymena thermophila* (Wloga *et al.*, 2008; Wloga & Gaertig, 2010). Such arrays compose the ‘(post)oral fibres’ in *Paramecium multimicronucleatum* and *P. tetraurelia* or the ‘deep fibre’ in *Tetrahymena thermophila*; they guide nascent food vacuoles and drive recycling vesicles back to the cytopharynx (Allen, 1974).

(2) Molecular signals

In mammalian cells, the lumen of all these travelling organelles is more or less acidic due to their endowment with a V-type H^+ -ATPase (Mellman, 1992; Forgac, 2007). Also they store more or less Ca^{2+} inside (see Section VIII). It was also shown with mammalian cells that acidification causes a conformational change in the heterooligomeric H^+ -ATPase molecule which, thus, transmits a signal to the cytoplasmic side. This in turn allows for the docking of small GTPase molecules and their respective modulators (Hurtado-Lorenzo *et al.*, 2006). Rab-type GTPases mediate directional transport, for instance in the exo- and endocytotic pathway by interacting with organelle-specific protein effector molecules.

In higher eukaryotes, the different types of trafficking organelles contain specific GTPases and their respective effector molecules as well as complementary SNAREs on each of the interacting membranes. GTPases are activated by binding GTP which causes a conformational change. Minor changes in amino acids in GTPases can change their specific binding capabilities, whereas effectors usually have no common binding motif (Grosshans, Ortiz & Novick, 2006). Some SNAREs have been recognised as effector partners (Zerial & McBride, 2001). This also applies to longin-type v-SNAREs, variations of ‘synaptobrevins’ containing a longin domain, for some of which binding of small GTPases has been shown (De Franceschi *et al.*, 2014). Although still called ‘synaptobrevins’ longin-type SNAREs are the predominant v-/R-type SNAREs in plants and in ciliates such as *Paramecium tetraurelia* (Schilde *et al.*, 2006, 2010) and *Tetrahymena thermophila* (De Franceschi *et al.*, 2014). Globally this means that in eukaryotes specificity of membrane interaction is determined by several cues.

An example of an effector molecule is early endosome antigen 1 (EEA1), the marker of the early endosomal membrane of metazoan cells (Mishra *et al.*, 2010). (Unfortunately such organelle-specific proteins interacting with GTPases are practically unknown from ciliates.) As mentioned, GTPases contain structural determinants providing interaction with specific effectors (Khan & Ménétrey, 2013). Such proteins enable reversible binding to, and reversible activation of

the next compartment of the trafficking pathway (Pfeffer, 2013). Modulatory proteins include GTPase activating protein (GAP), guanosine nucleotide dissociation inhibitor (GDI) and guanosine nucleotide exchange factor (GEF). Together with organelle-resident SNAREs, firmly installed by a single-pass carboxy-terminal stretch, this complex is the key for docking at the specific target membrane. Finally, in concert with a discrete Ca^{2+} signal and a Ca^{2+} -sensor protein (Rizo, Chen & Arac, 2006), a trans-complex of v- and t-SNAREs can mediate fusion (Jahn & Scheller, 2006). As established in metazoans, this type of signal recognition by molecular cues is essential for delivery of vesicles to their correct targets by matching protein–protein interaction. What is the situation in ciliates?

H^+ -ATPase (Fok *et al.*, 2002; Wassmer *et al.*, 2005, 2006) and SNAREs (Plattner, 2010b) are documented for all trafficking vesicles in *P. tetraurelia*, together with small GTPases in *T. thermophila* (Bright *et al.*, 2010). GTPase modulating proteins GAP, GDI and GEF in ciliates await elucidation. Although the Ca^{2+} content of trafficking vesicles is unknown in ciliates, its presence can be inferred on the one hand from the presence of Ca^{2+} in such organelles in mammalian cells (Section VIII.2) and on the other hand from the presence of CRCs in these organelles in *Paramecium* spp. (Ladenburger & Plattner, 2011).

The situation during the secretory cycle in ciliates is as follows. Dense core-secretory vesicles of ciliates, such as trichocysts (*Paramecium* spp.) and mucocysts (*Tetrahymena* spp.), collectively called extrusomes in the different ciliate species, contain in part similar and in part different contents (Verbsky & Turkewitz, 1998) and they serve different purposes (Hausmann, 1978). Trichocysts are bipolar structures, with a ‘tip’ and a ‘body’. The presence of SNAREs in the *P. tetraurelia* trichocyst membrane, probably *PtSyb5*, is most likely, although not identified conclusively (Schilde *et al.*, 2010). Extrusomes are special in several ways. Trichocysts contain no recognisable H^+ -ATPase (Wassmer *et al.*, 2005, 2006) and the organelles are not recognisably acidic (Lumpert *et al.*, 1992; Wassmer *et al.*, 2009), including trichocyst precursor stages (Garreau de Loubresse, Gautier & Sperling, 1994). No Ca^{2+} is detected inside trichocysts (Hardt & Plattner, 2000). However, low numbers of H^+ -ATPase and low concentrations of Ca^{2+} cannot be excluded. Remarkably a sufficient level of cytosolic Ca^{2+} is required for trichocyst biogenesis (Ladenburger *et al.*, 2009). Possibly Ca^{2+} has to be removed from the lumen at a certain stage of maturation since Ca^{2+} would cause decondensation of the contents after maturation (proteolytic processing), as it does during exocytosis when exogenous Ca^{2+} gets access to the organelle lumen (Bilinski, Plattner & Matt, 1981; Klauke *et al.*, 1998). This sequence of events, Ca^{2+} -insensitive precursor $\rightarrow$ Ca^{2+} -sensitive proteolytically cleaved mature granule lattice protein (pro-Grp1 $\rightarrow$ Grp1), has been verified in detail for *Tetrahymena thermophila* mucocysts (Verbsky & Turkewitz, 1998).

In *T. thermophila*, lysosomal sorting receptors, homologous to sortilin of higher eukaryotes, represent a signal for the

biogenesis of mucocyst contents. This includes proteins relevant for proteolytic processing of some precursors, their eventual aggregation and/or sorting in part in soluble and in part in insoluble form (Briguglio, Kumar & Turkewitz, 2013). In detail, non-aggregating protein, granule tip protein Grt1p, a constituent of the organelle tip, is bound by a specific subtype of sortilin, in contrast to the granule lattice protein, Grl3p; the latter, however, requires a sortilin-mediated mechanism indirectly, as this selects cathepsin 3 which in turn is required for Grl1p processing.

As known from mammalian cells, during maturation of dense core-secretory organelles a proprotein convertase (Steiner, 1998) cleaves the proprotein on the way through the cell (Orci *et al.*, 1985) to generate a mature secretory protein. In *Tetrahymena thermophila* this involves predominantly one form of an aspartyl cathepsin that recognises specific aspartyl residues of mucocyst Grl proteins (Kumar, Briguglio & Turkewitz, 2014); point mutations fail to perform proper self-assembly and to acquire secretory competence.

There is reason to assume a similar mechanism for *Paramecium* spp. In ciliate mutants, extrusomes with non-proteolytically processed contents are not transported to the cell membrane (Pouphile *et al.*, 1986; Tiedtke *et al.*, 1988) – thus suggesting a probable cue for recognition by motor proteins. Previously, selection of secretory proteins by sortilin has been known from mammalian brain where it mediates the delivery of neurotrophic factor to the regulated secretory pathway (Chen *et al.*, 2005).

Trichocysts are transported to the cell membrane according to their inherent polarity, tip first, and their secretory matrix contents provide an additional signal for docking competence (Pouphile *et al.*, 1986). Formation of a ‘tip’ structure is relevant not only in *Paramecium* spp., but also, although structurally less impressively, in *Tetrahymena thermophila*. Here, unilateral assembly of one of the non-Grl proteins and of Grt1p are mandatory for exocytosis competence (Bowman *et al.*, 2005).

When exocytotic membrane fusion is induced in *Paramecium tetraurelia* under conditions inhibiting decondensation/release of trichocyst contents, the trichocyst membrane reseals, the organelles become detached and undergo a new round of docking for successful exocytosis upon stimulation under standard conditions (Klauke & Plattner, 2000). This phenomenon of ‘frustrated exocytosis’ indicates the occurrence of additional signals provided by the filling state that mediate competence for docking of trichocysts at the cell membrane. Such signals can include physical links between the contents of a trichocyst and its membrane, to be visualised only with appropriate fast-freezing methods (Momayezi *et al.*, 1993).

Granule proteins similar to some of the *Tetrahymena* spp. mucocyst proteins are the trichocyst matrix proteins (tnp) of *P. tetraurelia* (Garreau de Loubresse, 1993) and *Pseudomicrothorax dubius* (Peck, Swiderski & Tourmel, 1993). From analyses with *Tetrahymena thermophila* one may conclude that the tip part of extrusomes is probably delivered by vesicles separate from those producing the ‘body/matrix’

(Briguglio *et al.*, 2013). The degree of acidity of the lumen of mucocyst precursors remains to be established, but probably it may not differ from the situation described for *Paramecium* spp.

Considering widely different secretory contents, several cues may govern extrusome biogenesis in the two genera, *Paramecium* and *Tetrahymena*, respectively. Clathrin-mediated budding of some vesicles also requires an adaptor protein complex. For instance, AP3 contributes to mucocyst biogenesis (Briguglio *et al.*, 2013). In *T. thermophila*, a small GTPase, type *TtRabD41* is recognised on docked mucocysts (Bright *et al.*, 2010).

Early endosomes (called ‘terminal cisternae’ in *Paramecium*) and late endosomes [‘acidosomes’ in *Paramecium* (Allen, Ma & Fok, 1993)], phagosomes of different age after pinching off, and diverse recycling vesicles are endowed with a V-type H⁺-ATPase (Wassmer *et al.*, 2005, 2006), with v- and t-type SNAREs (Plattner, 2010b) and with CRCs (Ladenburger & Plattner, 2011). They all vary from organelle to organelle. Participation of the plasmalemmal t-SNARE, *PtSyx1*, in trichocyst exocytosis has been derived from immuno-localisation and gene-silencing experiments (Kissmehl *et al.*, 2007). In *Tetrahymena thermophila* and *Paramecium tetraurelia*, organelles along the endocytotic pathway down to phagosomes and lysosomes dispose of organelle-specific small GTPases (Bright *et al.*, 2010). Thus, signalling follows the established pathway in these organelles, although trichocysts exhibit several features that are unusual for dense core-secretory organelles, i.e. neutral pH and absence of any detectable Ca²⁺, as outlined above.

A tentative identification of a Rab11 effector protein has been presented for *T. thermophila* (Zweifel *et al.*, 2009). The intracellular distribution and dynamics of CDA12 and CDA13 (CDA = cell division protein A) during conjugation and cell division is comparable to family of Rab11-interacting proteins (FIPs) in mammalian cells (Fielding *et al.*, 2005). In particular, Rab11 would be interesting because it is known from mammalian cells to be a master switch for the regulation of cell surface biogenesis, structure and function (Welz, Wellbourne-Wood & Kerkhoff, 2014); this is achieved by regulating widely diversified trafficking pathways.

(3) Support by F-actin, SNAREs and Ca²⁺-dependent proteins

In higher eukaryotes, actin is another regulator of membrane fusion and fission (scission), from exocytosis (Geron, Schejter & Shilo, 2013) and endocytosis (Mooren, Galletta & Cooper, 2012) to phagosome formation and processing (see Section V). With ciliates, such analyses have long been hampered by the insensitivity of some of the actin isoforms to diagnostic fluorescently labelled drugs (Sehring *et al.*, 2007a). Thus, the expected involvement of F-actin in endocytosis *via* parasomal sacs could not be documented. Only by gene silencing studies could *PtAct4* be identified as a key player (Sehring, Reiner & Plattner, 2010) and this isoform, in fact, does not possess normal phalloidin binding signatures (Sehring *et al.*, 2007a).

Finally, Ca²⁺ has to be provided also in ciliates for promoting organelle interactions up to fusion. As specified below, this is achieved by *PtCRCs* specifically localised to, or indirectly associated with the different trafficking compartments (Ladenburger & Plattner, 2011). Briefly, for trichocyst exocytosis Ca²⁺ is provided by release from the cortical stores, the alveolar sacs, and by superimposed influx from the outside medium (Plattner & Klauke, 2001). For further details, see Section VIII. Local Ca²⁺ signals appear indispensable also for other trafficking organelles which also contain *PtCRCs*, although functional scrutiny of such Ca²⁺ signals is still missing for most organelles in ciliates.

For none of the trafficking vesicles of ciliates has any Ca²⁺-sensor protein been ascertained which, in metazoans, is mainly synaptotagmin. This is a single-pass membrane protein with an amino-terminal anchor and two Ca²⁺-binding loops attached to β -barrel motifs called C2A and C2B domains (Rizo *et al.*, 2006). A double C2-domain protein, DOC2 (double C2-like domain containing protein), enables microneme exocytosis for host cell invasion by Apicomplexa, although it lacks a transmembrane domain (Farrell *et al.*, 2012) – in contrast to synaptotagmin. Remarkably the existence of this molecule type has been suggested for *P. tetraurelia* although further scrutiny is required. In the *Paramecium* database only related proteins with eight C2 domains can be found (R. Kissmehl & H. Plattner, unpublished observation). Similar CaBPs with more than two C2 motifs are also established for some metazoan cells where they can substitute for synaptotagmin (Min, Chang & Südhof, 2007; Martens & McMahon, 2008). In addition, in mammalian cells, such ‘extended synaptotagmins’ are also able to connect cortical Ca²⁺ stores [endoplasmic reticulum (ER)] with the plasma membrane (Giordano *et al.*, 2013) – an interesting aspect, considering the likely absence of classical connections by Orai/Stim proteins in ciliates. Despite the potential importance of such C2-domain-carrying proteins for signalling none of these functions has been analysed in ciliates as yet. Docking of trichocysts and assembly of functional exocytosis sites in *Paramecium tetraurelia* requires calmodulin (Kerboeuf *et al.*, 1993) and CaM is enriched in such sites (Plattner & Klauke, 2001).

In multicellular organisms, SNAREs occurring on delivery vesicles (v-SNAREs) usually contain an Arg (R) residue in the central layer of their SNARE domain, whereas target-(t)-SNAREs usually contain a Glu (Q) residue (Fasshauer *et al.*, 1998; Sutton *et al.*, 1998). However, like elsewhere, there are also exceptions to this rule in *Paramecium tetraurelia* (Plattner, 2010a,b). The SNARE domain is essential for pairing of SNAREs in trans-position which allows for subsequent membrane fusion (Jahn & Scheller, 2006; Jahn & Fasshauer, 2012). Whereas the central amino acid is not essential and because of the absence of any stringent cues for the assignment of v-/R- and t-/Q-SNAREs, respectively, to certain organelles, association with organelle-specific small GTPases is essential (Novick & Zerial, 1997; Zerial & McBride, 2001; Grosshans *et al.*, 2006).

In ciliates, syntaxin homologs generally can be attributed more easily to specific membranes, comparable to the situation in different systems; this is not so easy with synaptobrevins. As mentioned above, in *Paramecium tetraurelia* many of the ‘synaptobrevins’ contain an additional longin domain. Examples are *PtSyb1*, *PtSyb2*, *PtSyb3*, *PtSyb6*, *PtSyb7*, *PtSyb8*, and *PtSyb9* (Schilde *et al.*, 2006, 2010). Longins are abundant also in plants where they contribute to organelle targeting (Rossi *et al.*, 2004), but such effects have not been analysed in ciliates as yet. Altogether cues for specific organelle targeting of/by these SNAREs, particularly of ‘synaptobrevins’, are not so obvious, particularly in ciliates. As found by the analysis of exocytotic mutants of *Paramecium tetraurelia*, in the secretory cycle additional proteins play different roles that still have to be specified (Vayssié *et al.*, 2000; Froissard *et al.*, 2004).

Clear vesicles, likely candidates for the biogenesis of the cell membrane and of alveolar sacs, can be recognised only under specific conditions. For instance, vesicle docking is ‘frozen’ when docking is allowed under conditions where fusion with their target membrane is inhibited by silencing the SNARE-specific chaperone, N-ethylmaleimide-sensitive factor (NSF) (Kissmehl *et al.*, 2002; Schilde *et al.*, 2010). This suggests the involvement of SNAREs also in organelle biogenesis and in cell membrane formation by constitutive exocytosis in ciliates. Constitutively active vesicles that contribute to cell membrane formation during cytokinesis can be observed in non-manipulated cells. They contain *PtCRC-V-4* (Ladenburger & Plattner, 2011), whereas in ciliates SNAREs have not yet been identified directly in the cleavage furrow. For good reasons the designation of constitutive exocytosis as ‘non-regulated exocytosis’ in the past has been abandoned more recently. Moreover, NSF silencing induces an impressive amount of autophagic vacuoles, wherein NSF is degraded, while cells continuously decrease in size (Kissmehl *et al.*, 2002). Very recently, autophagic vacuole formation is recognised also to involve specific SNAREs in higher eukaryotes (Shen & Mizushima, 2014).

Empty membranes (‘ghosts’) formed in the course of trichocyst exocytosis-coupled endocytosis are internalised, and this process is also accelerated by increased $[Ca^{2+}]_i$ generated during exocytosis stimulation (Plattner *et al.*, 1997). Under our standard conditions exo–endocytosis coupling is accomplished within 0.35 s (Knoll, Braun & Plattner, 1991a). Comparably rapid coupling has recently been documented, also using quenched-flow/cryofixation methodology, in nerve terminals, also *via* smooth vesicles (Watanabe *et al.*, 2013). In *Paramecium tetraurelia*, this process is different from endocytosis *via* clathrin-coated pits represented by parasomal sacs. Here, endocytosis may be ascribed to the activation of calcineurin, a Ca^{2+} /CaM-activated protein phosphatase (PP2B) that is enriched on *Paramecium*’s parasomal sacs (Momayezi *et al.*, 1996) – the sites of endocytosis by clathrin-coated vesicles. This function is suggested by the scission of clathrin-coated vesicles by dynamin [identified in *Tetrahymena thermophila* (Elde *et al.*, 2005)] which, as

shown in mammalian cells, has to be dephosphorylated by calcineurin to become active (Marks & McMahon, 1998; Lai *et al.*, 1999).

V. THE PHAGOCYTOTIC CYCLE

Phagocytosis of particles and bacteria was detected around 1900 by Metchnikoff in phagocytes from starfish larvae. Soon he extended this discovery to mammalian defence mechanisms against pathogenic microorganisms. In mammals, cues regulating phagocytosis involve actin filaments, acidification (by H^+ -ATPase acquired from endosomes) of phagocytotic vesicles (phagosomes) and fusion with lysosomes. The phagolysosomes thus formed are able to digest foreign materials (Underhill & Goodridge, 2012) and ciliates can, thus, feed on bacteria.

This section outlines the following aspects in ciliates (Fig. 5). As in other systems, there are multiple inputs into the phagosomal system of ciliates (Fok & Allen, 1993). At the cytopharynx, from where phagosomes (food vacuoles) are formed, widely different signals converge. This includes microtubular arrays, called ‘(post)oral fibres’ in *Paramecium* and ‘deep fibres’ in *Tetrahymena*, emanating from here to the cytoproct (the site of spent vacuole exocytosis) and to deeper cytoplasmic regions; they serve to guide recycling vesicles and other vesicle input (Allen, 1974). Late endosomes (‘acidosomes’), vesicles recycling from progressive phagolysosomal stages, and ‘discoidal’ vesicles recycling from the cytoproct after release of undigested material all contribute to food-vacuole formation, in addition to fusion with lysosomes for further maturation (Allen & Fok, 2000). Acidification by the V-type H^+ -ATPase, provided by fusion of acidosomes/late endosomes, is important, as are changing sets of GTPases, SNAREs, actin isoforms and CRCs. Altogether they regulate multiple specific fusion/fission processes.

(1) Role of H^+ -ATPase, GTPases and SNARE proteins

Many signals are required to guide a food vacuole through a ciliate cell (cyclosis) and basic mechanisms may be quite similar in different species. Using monoclonal antibodies (when the molecular biology of ciliates still was in its infancy) the phagosome cycle was scrutinised in *Paramecium multimicronucleatum* (Fok & Allen, 1993; Allen & Fok, 2000). Among recycling vesicles, 100 nm-vesicles were retrieved from advanced stages of food vacuoles and ‘discoidal vesicles’ from the cytoproct, all travelling along cytopharyngeal microtubular ribbons in minus-end direction using dynein as a motor (Ishida *et al.*, 2011). Thus, there are clear-cut long-range signals for specific membrane delivery, supplemented by the following short-range signals. Actin assembly on a nascent phagosome is a signal for pinching off. This is followed by fusion with acidosomes/late endosomes (Allen *et al.*, 1993) endowed with a H^+ -ATPase (Wassmer

Fig. 5. Formation of a food vacuole in a ciliate requires several signalling steps. First, fusion of recycling vesicles causes the cell membrane at the cytopharynx to bulge in, trapping bacteria. Second, fusion of acidosomes (late endosomes) allow for acidification of the phagosome lumen and inactivation of bacteria. Third, fusion with lysosomes enables degradation of bacteria by acid hydrolases in the food vacuole which, thus, has become a phagolysosome. For discussion of involvement of SNAREs, CRCs, etc., see text.

et al., 2005, 2006, 2009). Silencing of some of the H⁺-ATPase subunits inhibits phagosome formation (Wassmer *et al.*, 2005). Acidification is necessary not only for activating lysosomal enzymes after phagosome–lysosome fusion, but is also a signal for travel onwards through the cell. The luminal pH increases steadily to a neutral pH before vesicles reach the cytoproct.

To understand phagosome cyclosis in ciliates, comparison with higher eukaryotic cells may be useful. The latter are paradigmatic for the sequential assembly and role of H⁺-ATPase, actin, small GTPases and SNAREs. For instance, in yeast, vacuoles (acidic lysosome-related compartments), specific SNAREs and Rab proteins are required for the assembly of actin (Karunakaran *et al.*, 2012). In kidney cells, actin contributes to the targeting of some subunits of the H⁺-ATPase to organellar membranes (Beyenbach & Wieczorek, 2006). *Paramecium tetraurelia* has the highest number of genes encoding the α -subunit of the integral V0-part of the H⁺-ATPase, many more than in yeast or mammals (17 *versus* 1 and 4 genes, respectively). Since the V0 part associates reversibly with the catalytic V1 head part *via* the α -subunit one can expect site- and time-variable association of different V1 parts depending on the actual requirements – an aspect suggesting extreme fine-tuning in ciliates. Also unexpected is organelle targeting by the carboxy-terminal half of the α -subunit molecule in *Paramecium tetraurelia* (Wassmer *et al.*, 2006), rather than by the amino-terminal half as in yeast (Kawasaki-Nishi *et al.*, 2001). In kidney proximal tubule cells, acidification of the organellar lumen can cause binding of small GTPases and their modulators (Hurtado-Lorenzo *et al.*, 2006; Brown *et al.*, 2009), which can direct vesicles, from the Golgi apparatus on, in cooperation with the t-SNARE Syx1 (Schwartz *et al.*, 2007). The question arises whether a similar targeting mechanism may be applicable to phagosomes in ciliates.

In *Paramecium tetraurelia*, Rab7, typical of lytic organelles, is bound to vesicles in the oral apparatus and on phagolysosomal membranes (Surmacz, Wiejak & Wyroba, 2006; Osinska *et al.*, 2011); post-transcriptional gene silencing inhibits food vacuole formation. Specific SNAREs type *PtSyx* and *PtSyb* are also exchanged during phagosome cyclosis (Kissmehl *et al.*, 2007; Plattner, 2010a,b), as are H⁺-ATPase subunits (Wassmer *et al.*, 2005, 2006, 2009). Therefore, one may well expect, in ciliates, interaction of specific isoforms of these proteins along the phagocytotic pathway and interdependent signalling for targeting to specific vesicles along this pathway.

Proteome analysis of phagosomes from phagotrophic cells, including many protozoa that obtain energy by digesting phagocytosed microorganisms, revealed several important details (Goyette *et al.*, 2012): (i) membrane composition changes during maturation, (ii) H⁺-ATPase, small GTPases as well as proteins relevant for transmembrane transport processes and trafficking abound, (iii) other types of short-range signalling molecules, although less abundant, also contribute. For instance, in macrophages, Syx7 is an important phagosome SNARE. In *Paramecium tetraurelia*, *PtSyx7* is also one of the phagosome-resident SNAREs, integrated into the phagosome membrane at a later stage of cyclosis (Kissmehl *et al.*, 2007).

Mass spectroscopy of purified phagosomes revealed 453 peptides of which 73 were putative phagosomal proteins and 28 known for their engagement in phagocytosis in other organisms (Jacobs *et al.*, 2006). Among membrane proteins relevant for signalling, Ca²⁺-ATPase, H⁺-ATPase subunits, Rab proteins type 1, 7, and 13 as well as actin-binding proteins were identified, together with fragments of a synaptobrevin-like protein. Functional knock-out studies with *Tetrahymena thermophila* suggest vacuolar protein sorting proteins, *TiVPS13A* and *TiVPS13C*, as accelerators of multiple vesicle fusions with the phago(lyso)some (Samaranayake, Cowan & Klobutcher, 2011). In macrophages, Rab20 is an immune-regulated phagosomal membrane constituent (Pei *et al.*, 2014), whereas in *T. thermophila* *TiRab20* is associated with cytoproct-localised phagosomes (Bright *et al.*, 2010).

(2) Role of actin, Ca²⁺ and Ca²⁺-binding proteins

The occurrence of actin throughout a *Paramecium* cell, with isoforms on specific trafficking organelles (Sehring *et al.*, 2007b), likely reflects important functions. This is also suggested by the situation in higher eukaryotes where F-actin is co-assembled with a selection of proteins at sites of clathrin-mediated endocytosis, sorting endosomes, at the trans-Golgi network, and along the secretory pathway, etc. (Anitei & Hoflack, 2012). Such trafficking pathways can contribute to phagosome formation where requirement of actin is particularly well established (Flannagan, Jaumouille & Grinstein, 2012).

Actin assembly is unequivocally considered crucial for formation of a phagosome, also in ciliates. In *Tetrahymena thermophila* the actin-binding protein ‘actin depolymerising

factor' (ADF)/cofilin binds to the nascent food vacuole where it promotes actin polymerisation (Shiozaki *et al.*, 2013). In *Paramecium tetraurelia* actin isoforms and their arrangement on phagosomes change during the phagosome cycle (Schring *et al.*, 2007b). This can make docking sites available, in a time-variable sequence, for different organelles, such as endosomes and lysosomes. It may also provide cues for fusion and fission processes and perhaps it is also relevant for motor functions to drive cyclosis. In this regard it is interesting to examine how and where actin can interact with some of the many myosin forms found in ciliates (Eisen *et al.*, 2006; Coluccio, 2008). Interaction with some of the cortical myosin species (Garcés, Hoey & Gavin, 1995) has long been considered important for cyclosis. Accordingly, travelling of food vacuoles in *Tetrahymena thermophila* has been shown to depend on actin and myosin type Myo1p (Hosein, Williams & Gavin, 2005).

A role for actin-binding proteins (Watanabe *et al.*, 1998) has been documented in *Tetrahymena pyriformis*. Disruption of F-actin by cytochalasin D or latrunculin A (Zackroff & Hufnagel, 2002) or stabilisation of F-actin by injected phalloidin (Kersken *et al.*, 1986) inhibits food vacuole formation. Silencing of specific actin isoforms disturbs cell morphology and phagosome formation (Schring *et al.*, 2007a,b). However, any specific role of changing actin isoforms in *Paramecium tetraurelia* (Schring *et al.*, 2007b) in the regulation of vesicle docking and fusion/fission processes along this route remains to be established. In fact, in macrophages, actin can either activate or inhibit fusion of phagosomes with lysosomes (Liebl & Griffiths, 2009). Therefore, the role of changing actin isoforms in *Paramecium* spp. may also reflect multiple functions.

Immuno-localisation studies suggest that constitutive Ca^{2+} signalling plays some role during phagosome formation. CaM and CaM-BPs are bound to an early phagosome stage in *Tetrahymena thermophila* (Gonda, Komatsu & Numata, 2000). CaM is associated with food vacuoles also in *Paramecium tetraurelia* (Maihle *et al.*, 1981; Momayezi *et al.*, 1986). The significance of CaM, although well documented for nascent and older stages of food vacuoles in *Paramecium tetraurelia* (Momayezi *et al.*, 1986) and for large (food) vacuoles in *Tetrahymena thermophila* (Maihle *et al.*, 1981), remains to be analysed. Such analyses should include CaM-BPs and it reflects the inhibition of food vacuole formation by the reliable CaM inhibitor, W7 (Gonda *et al.*, 2000). The multiplicity of fusion processes are concordant with variable isoforms and CRCs throughout the phagocytic cycle in *Paramecium* spp. (Ladenburger & Plattner, 2011). For further details about Ca^{2+} signalling in the endo-phago-lysosomal cycle, see Section VIII.

(3) Proteins at the cytoproct

The cytoproct also requires specific signals, or molecular cues, for its function. Here, specific Rab proteins are found in *Tetrahymena thermophila* (Bright *et al.*, 2010): *TtRabD19*, *TtRabD20*, *TtRabD30* and *TtRab4A*, all in the cytoproct region. In *Paramecium tetraurelia*, the same holds for proteins

crossreacting with antibodies against mammalian annexins (Knochel *et al.*, 1996), SNAREs of type *PtSyb6* (Schilde *et al.*, 2006) and *PtSyx1* and *PtSyx4* on membranes surrounding the cytoproct region (Kissmehl *et al.*, 2007), as well as actin isoform *PtAct1* (Schring *et al.*, 2007b).

VI. SIGNALLING IN THE CONTRACTILE VACUOLE COMPLEX

Osmotic and ionic imbalance in freshwater ciliates, resulting from ongoing permeation, requires permanent counteraction by the contractile vacuole complex (Allen & Naitoh, 2002; Allen, Tominaga & Naitoh, 2009). Therefore, many freshwater protozoa and algae contain a contractile vacuole complex (Hausmann, 1978). Most molecular data pertinent to organelle function come from *Paramecium tetraurelia* and *Tetrahymena thermophila*, as summarised recently (Plattner, 2013a). The molecular inventory of this complex organelle, briefly outlined in Fig. 6, includes a V-type H^{+} -ATPase, CRCs, SNAREs, and many other components.

(1) Signals for contractile vacuole biogenesis

Taking into account the numerous additional molecules known from other protozoa, a new hypothetical picture emerges for interaction of components during the biogenesis and rhythmic function of this organelle (Plattner, 2013b). Each of these two aspects is based on multiple signalling events.

Particularly intriguing is the primary signal for formation of a nucleation site enabling *de novo* biogenesis of a new contractile vacuole complex. Strikingly this generally occurs just before cytokinesis and involves components that are relevant also for cytokinesis. As summarised by Plattner (2013b), it starts with the new pore (site of vacuole fusion with the cell membrane for contents release by exocytosis). Here centrin, CaM, γ -tubulin, and NIMA kinase (all known for their relevance to cytokinesis; NIM = never in mitosis) are enriched and, thus, may belong to the cues for nucleation of a pore precursor. The NIMA-related protein kinase family is greatly expanded in *T. thermophila* (Eisen *et al.*, 2006). Growth of microtubules, from the pore to the very tip of radial (collecting) canals may be restricted by 'microtubule destabilizing kinesins' related to kinesin family (kif) proteins. These are kinesin-like proteins that are mainly known from metazoans (Walczak, Gayek & Ohi, 2013), but that are also abundant in *Tetrahymena* spp. (Wickstead & Gull, 2006).

Growing microtubules are accompanied by canal-forming membranes from where a widely branched spongiome emerges (Allen & Naitoh, 2002). The distal part of the spongiome is studded with catalytic V1 parts of the V-type H^{+} -ATPase (Fok *et al.*, 2002). All other parts of the proximal/smooth spongiome, contain InsP_3R -type CRCs, type *PtCRC-II-2* and *PtCRC-II-3*, and the RyR-related *PtCRC-V-4* as well as several t-/Q- and v-/R-SNAREs.

Fig. 6. The microscopic structure of the contractile vacuole complex (lower part of figure) in relation to the molecular constituents of the respective domains, from the pore (site of periodic contents release), via the contractile vacuole, an enlarged domain (ampulla) connecting the vacuole to radial arms, and finally to the end region, the spongiome. Data combined from different species according to Plattner (2013a,b). Six or more arms give the organelle a star-like appearance. The spongiome is endowed with H⁺-ATPase molecules in its outermost part (decorated spongiome) that is continuous with the smooth spongiome. This domain harbours SNARE proteins and Ca²⁺-release channels (CRCs), type *PtCRC-II* (IP₃Rs), serving for the constitutive partial reflux of Ca²⁺ into the cytosol. The cell membrane/vacuole connection as well as the vacuole/ampulla connection are periodically dis- and reconnected in ~10 s intervals, during cycles of systole and diastole, and, thus, require signalling. Presumably this is also the case during restructuring of the spongiome in the course of activity cycles. Accordingly, the presence of CRCs, SNAREs, GTPases, and possibly also of mechanosensitive channels, strongly suggest extensive signalling processes in this organelle. The vacuole filling state may be sensed by (still hypothetical) mechanosensitive channels, directly or indirectly causing Ca²⁺ signal formation and membrane fusion via SNAREs. Biogenesis of the organelle is under epigenetic control and starts with nucleation signals and formation of an anlage at the prospective pore, followed by expansion along microtubules with post-translationally modified tubulins and restriction of radial arm/canal length by a microtubule-destabilizing motor protein related to kif. For further molecular details, see Plattner (2013a,b). The release of water and Ca²⁺ by the vacuole is enabled chemiosmotically by H⁺ sequestered via H⁺-ATPase activity. Although a plasma membrane Ca²⁺-ATPase (PMCA)-type Ca²⁺-ATPase/pump may occur in the organelle, experiments strongly suggest a preponderant role of H⁺/X⁺/Ca²⁺ exchangers (see text). Abbreviations: as, alveolar sac; cm, cell membrane; X, nucleation site. See Appendix (Section XIX) for other abbreviations.

Among them, *PtSyb2* and *PtSyx2* occur also at the pore, together with *PtSyb9* and *PtSyx15* (Plattner, 2013a,b).

(2) Signalling in the mature contractile vacuole complex

The presence of H⁺-ATPase, SNAREs and CRCs (Plattner, 2013a,b), together with the existence of specific Rab-type GTPases, identified in *Tetrahymena* spp. and *Paramecium* spp. (Bright *et al.*, 2010), strongly suggests several roles for SNAREs.

This encompasses membrane dynamics, including (i) ongoing vesicle delivery, (ii) reversible fusion/fission processes within the smooth spongiome and (iii) exocytotic fluid expulsion at the pore. (iv) H⁺ sequestration drives sequestration of water and Ca²⁺ in exchange for H⁺, and (v) some of the Ca²⁺ is released back into the cytosol for fine-tuning and for mediating ongoing membrane fusion processes within the contractile vacuole complex.

Which Ca²⁺ signals are to be expected and why? Irregular, stochastic Ca²⁺ puffs are registered with fluorochromes

along radial arms; they are attributed to constitutive activity of *PtCRC-II/InsP₃R* channels (Ladenburger *et al.*, 2006). The presence of stomatin, which in *Paramecium tetraurelia* is found from the peripheral parts of the radial arms up to the pore (Reuter, Stuermer & Plattner, 2013), suggests the occurrence of mechanosensitive channels since both molecules are always combined with each other in other systems (see Section VIII.2). Therefore, increasing pressure may activate such channels, possibly in conjunction with some of the other CRCs. The contractile vacuole pore region in *Paramecium tetraurelia* contains RyR-type CRCs, type *PtCRC-VI-2* and *PtCRC-VI-3* (Ladenburger & Plattner, 2011). Not only periodic vacuole emptying may thus be regulated, but also periodic de-/recoupling of the ampullae – the swollen end pieces of radial canal – a phenomenon detected by capacitance measurements (Tominaga & Allen, 1998; Grønlien *et al.*, 2002). The periodic transition of flat membranes in diastole to a branched tubular network during systole can hypothetically be mediated by BAR (Bin-amphiphysin-Rvs)-type proteins that are known from mammalian cells to serve this transformation (Mim & Unger, 2012; Shen, Pirruccello & De Camilli, 2012). This function for BAR proteins has been suggested also for the contractile vacuole complex of *Paramecium* spp. (Plattner, 2013a), based on the effect of F-BAR protein in *Dictyostelium discoideum* (Heath & Insall, 2008). Similarly, *Dictyostelium* IBAR-null mutants have been shown to be defective in osmoregulation (Linkner *et al.*, 2014). The signal for this transformation may reside in the decay of internal pressure in the organelle during systole.

Purinergic Ca^{2+} channels, e.g. type P2X, have not yet been identified at a molecular level in ciliates (Burnstock & Verkhratsky, 2009; Coddou *et al.*, 2011; Verkhratsky & Burnstock, 2014). In *Dictyostelium discoideum* cells, in which P2X is associated with Rab11a and a Ca^{2+} -regulated GAP, P2X regulates contractile vacuole activity (Parkinson *et al.*, 2014) and becomes concentrated in those parts of the contractile vacuole membrane where the vacuole is attached to the cell membrane. Here it co-localises with Rab11. Their coassembly serves for the release of Ca^{2+} as a signal required for exocytotic membrane fusion. By contrast, in *Tetrahymena thermophila* Rab11A occurs on endosomes, whereas RabD2, D10 and D14 are seen on the contractile vacuole (Bright *et al.*, 2010). Therefore, in ciliates, Ca^{2+} channels and Rab-GTPases of a type different from those in *Dictyostelium discoideum* may be involved in contractile vacuole discharge, though this requires more scrutiny. InsP_3 does not seem to play a role in contractile vacuole fusion since the CRCs occurring in the pore region, *PtCRC-VI-2* and *PtCRC-VI-3*, are both devoid of an InsP_3 -binding domain (Ladenburger & Plattner, 2011).

The identity and relevance of mechanosensitive channels as well as the occurrence of purinergic receptor-type Ca^{2+} channels in ciliates remain to be settled. So far the occurrence of the latter has been postulated only for the cell surface of *Paramecium tetraurelia*, based on the effects of exogenous GTP, i.e. periodic forward and backward swimming (see Section X.1).

VII. ADDITIONAL DETERMINANTS FOR VESICLE TRAFFICKING IN CILIATES

Most essential molecules in addition to Rab and SNARE proteins have been identified in metazoan cells, from yeast to mammals (see Sections IV.2 and IV.3). Clathrin, APs and COPs, first identified in mammalian cells (Rothman, 2014), serve for vesicle budding from yeast to multicellular organisms. Together with specific subtypes of Rab proteins and SNAREs, subtypes of APs and COPs cooperate at specific sites of vesicle trafficking (Popoff *et al.*, 2011; Bonifacino, 2014) and, thus, jointly help to specify delivery routes. Although COPs and APs also occur in protozoa, molecular details are limited, in some contrast to Rab and SNARE proteins. Also in ciliates numerous molecular recognition and interaction sites, in conjunction with signalling processes, collaborate to bud vesicles, then to put them in motion and finally to dock at a target membrane with which they may fuse. Regrettably, signalling in the Golgi apparatus of ciliates is still poorly understood to date.

A collection of additional proteins relevant for vesicle trafficking, from intracellular vesicle budding to endocytosis, can be identified in the *Paramecium* database (Plattner & Kissmehl, 2003). This includes APs assigned in higher eukaryotes to different local events: AP1 for lysosome budding from the trans-Golgi network, AP2 for endocytosis, AP3 acting on endosomes, AP4 on trans-Golgi network, endosomes and lysosomes; furthermore, COPs for ER to Golgi and intra-Golgi transport (Malkus, Jiang & Schekman, 2002). Clathrin is another molecular filter serving for vesicle budding from the Golgi, from endosomes and at the cell membrane during endocytosis (Arvan & Castle, 1998; Godlee & Kaksonen, 2013; Rothman, 2014). Specific details are known only from *Tetrahymena thermophila* where several APs and eight members of the dynamin family have been found, together with clathrin (Elde *et al.*, 2005). Dynamin is a relatively large GTPase-type protein serving as a ‘pinchase’ for vesicle fission/scission (Hinshaw, 2000), i.e. during pinching off of vesicles. This list is complemented by GTPases of type Rab and Rho (ρ factor) and their regulators, such as GAPs (activation proteins) and GEFs (exchange factors), also found in the *Paramecium* database (Plattner & Kissmehl, 2003). Many of the sequences from this database still require verification, whereas Rab-type GTPases have been more thoroughly analysed and localised in *Tetrahymena thermophila* (Bright *et al.*, 2010).

As to phosphoinositides, the number of molecular species serving as potential cues for specific molecular interactions in general and as substrates for local signalling in particular is remarkably high in ciliates. A *Paramecium tetraurelia* and *Tetrahymena thermophila* genome analysis has revealed the presence of multiple phosphoinositide kinases (Leondaritis *et al.*, 2013). Recall that in higher eukaryotes the rather specific distribution of phosphoinositides in membranes of different trafficking organelles reflects their importance for multiple signalling pathways and molecular recognition cues

(Vicinanza *et al.*, 2008; Michell, 2013). This aspect clearly deserves further clarification in ciliates.

A considerable problem in ciliates is the identification of the Golgi apparatus. Our tentative assignment of *PtSyx5* to the Golgi (Kissmehl *et al.*, 2007) is supported by the assignment of this SNARE to this organelle in higher eukaryotes (Malsam & Söllner, 2011; Hong & Lev, 2014). Moreover, in ciliates, it would be rewarding to analyse e.g. sec22 (SNARE binding to COPII) for identifying the Golgi apparatus. Unfortunately a homologue was found only for *Tetrahymena thermophila* (which was not further pursued), but not for *Paramecium tetraurelia* (Schilde *et al.*, 2006). Attempts to identify the Golgi apparatus by reliable markers established in mammalian cells and indicated in the *Paramecium* database have failed (Schilde *et al.*, 2006). Therefore, currently only *PtSyx5* remains as a tentative marker (Kissmehl *et al.*, 2007). In *Tetrahymena thermophila*, *TtRab6* subtypes A to D have been assigned to the Golgi apparatus, probably for retrograde transport (Bright *et al.*, 2010), and related sequences can be retrieved from the *Paramecium* database.

VIII. WHY THIS MULTIPLICITY OF Ca^{2+} -RELEASE CHANNELS (CRCs) IN CILIATES?

In all eukaryotic cells Ca^{2+} is widely used as a messenger for many processes. This was first recognised for stimulus–contraction coupling in mammalian cells and, from the 1970s on, for stimulus–secretion coupling (Rubin, 1974, 2012). Ca^{2+} regulates many processes even deep inside cells (Berridge *et al.*, 1998, 2003; Clapham, 2007). Therefore, selectively to address deep targets, Ca^{2+} has to be provided at defined sites in microdomains for short periods (Berridge, 2006). This can be achieved anywhere in the cytoplasm by Ca^{2+} release *via* CRCs from stores scattered throughout the cytoplasm. Therefore, different trafficking vesicles are also endowed with CRCs. In ciliates, the only known and most important types of CRCs are IP_3Rs and RyRs , both detected and specified primarily in mammalian cells (Bezprozvanny, 2005; Lanner *et al.*, 2010; Hamada & Mikoshiba, 2012). Only recently have CRCs been identified in ciliates.

This section addresses the molecules required for achieving local, site-specific Ca^{2+} signals throughout a ciliate cell (Fig. 7). In *Paramecium tetraurelia* cells, over 30 CRCs with characteristics of IP_3Rs and/or RyRs have been identified by molecular analysis (Ladenburger & Plattner, 2011). They are consistently distributed over organelles participating in trafficking and, thus, may provide local Ca^{2+} signals for membrane interactions and finally fusion, including exocytosis (Fig. 8). The multitude of CRCs in *Paramecium tetraurelia*, all with a characteristic distribution over specific organelles, suggests differential targeting and the capability for distinct, locally restricted signalling according to local requirements. This reflects the importance of strictly localised Ca^{2+} signalling, also in ciliates, as outlined in Section II.1. In the following we will discuss two CRCs analysed in *Paramecium* spp. in more detail.

(1) InsP_3 - and RyR -type CRCs

PtCRCs encompass subfamilies I–VI, with paralogs (also called ohnologs when attributable to whole-genome duplications) designated by Arabic numbers, e.g. -1, -2 etc., within the subfamilies. Concomitantly, designations usable for molecular databases are, for instance, *PtCRC-II-1* and *PtCRC-II-2* for an IP_3R occurring at the contractile vacuole complex, or *PtCRC-IV-1* and *PtCRC-IV-2* for RyR-LPs occurring in alveolar sacs (Ladenburger *et al.*, 2006, 2009; Ladenburger & Plattner, 2011). Let us exemplify the specific function and localisation of the best analysed *PtCRCs*.

Exocytosis stimulation in *Paramecium* spp. activates Ca^{2+} release from alveolar sacs (Ladenburger *et al.*, 2009) and this is complemented by Ca^{2+} influx from the outside medium (Hardt & Plattner, 2000). Titrating $[\text{Ca}^{2+}]_o$ to values slightly below $[\text{Ca}^{2+}]_i$ determined in unstimulated cells allows one to recognise which occurs first, Ca^{2+} influx or release from internal stores (Klauke & Plattner, 1997; Hardt & Plattner, 2000). Thus it was determined that Ca^{2+} signalling during stimulated exocytosis in *Paramecium tetraurelia* involves a mechanism called store-operated Ca^{2+} influx (SOC, also termed SOCE, store-operated Ca^{2+} entry), as in human skeletal muscle cells. Ca^{2+} mobilisation from the sacs is a first step that triggers influx of additional Ca^{2+} from the outside medium. Whereas the plasmalemmal influx channels have not been identified as yet, localisation and genetic-silencing experiments have identified RyR-LP type *PtCRC-IV* to mediate Ca^{2+} mobilisation from alveolar sacs (Ladenburger *et al.*, 2009). RyR activators, such as caffeine and 4-chloro-*meta*-cresol, have been successfully applied. They all produce a Ca^{2+} signal for trichocyst exocytosis by the SOC(E) mechanism, whereas both phenomena are inhibited after *PtCRC-IV* silencing. The RyR-LP of *Paramecium tetraurelia* is activated not only by the orthodox RyR activators mentioned above, but also by AED (Ladenburger *et al.*, 2009). RyR-LPs in the parts of alveolar sac membranes facing the cell membrane (Ladenburger *et al.*, 2009) provide strictly vectorial dissipation of Ca^{2+} over trichocyst exocytosis sites.

PtIP}_3\text{Rs} bind ^3H -inositol 1,4,5-trisphosphate as demonstrated using the isolated InsP_3 -binding domain after homologous overexpression (Ladenburger *et al.*, 2006). Surprisingly the *PtIP}_3\text{R}* is localised to the contractile vacuole complex where it is constitutively active. Constitutive activity explains why other efforts to monitor InsP_3 increase during stimulation have failed. Formation of InsP_3 generally requires a phospholipase C (PLC) which, in ciliates, occurs in different isoforms (Klöppel *et al.*, 2009; Leondaritis *et al.*, 2011) some of which may fulfill this task. For comparison, phospholipase C subfamilies β , γ , δ , ε , η and ζ occur in animals and only type ζ in plants (Munnik & Testerink, 2009), whereas orthologs in ciliates are addressed in Section IX.1.

Ca^{2+} is sequestered into the contractile vacuole complex dependent on the activity of a V-type H^+ -ATPase/pump (Fok *et al.*, 2002; Wassmer *et al.*, 2005, 2006), probably in conjunction with a hypothetical $\text{Ca}^{2+}/\text{H}^+$ exchanger (Plattner, 2013a,b). *PtIP}_3\text{Rs}* contained in the contractile

Fig. 7. Different isoforms of IP₃R/RyR-type (*PtCRCs*) are distributed over different trafficking organelles of a *Paramecium* cell (nuclei omitted). Some organelles contain more than one type and a given type can occur in several organelles, often of the same trafficking route (red dotted arrows indicating pathways of phagocytotic cycle). Furthermore, a ciliated protozoan cell possesses several ways to eliminate Ca²⁺, be it during constitutive activity or after stimulation. This includes mechanisms indicated by blue solid arrows, i.e. extrusion at the cell surface, sequestration into stores and binding to Ca²⁺-binding proteins (CaBPs). The latter is the most rapid means of Ca²⁺ downregulation after stimulation (e.g. in cilia; see Fig. 2); then, after Ca²⁺ desorption and dissipation, some of the slower processes can follow. Among them, extrusion *via* the contractile vacuole complex appears most effective, although its mode of action is not yet known in detail, secondary active Ca²⁺ export is most likely (Fig. 6). Refilling of alveolar sacs by a Ca²⁺ pump is among the slow processes, as is extrusion by the Ca²⁺ pump of the cell membrane. Abbreviations: a, ampulla; als, alveolar sac; as, acidosomes; ce, centrin; cf, cytopharyngeal fibres; ci, cilium; cp, cytoproct; cv, contractile vacuole; ds, decorated spongiome; dv, discoidal vesicles; ee, early endosome; er, endoplasmic reticulum; fv, food vacuole; ga, Golgi apparatus; gh, 'ghost' (empty trichocyst membrane); oc, oral cavity; pm, plasma membrane; pof, postoral fibres; ps, parasomal sac; rv, recycling vesicles; ss, smooth spongiome; tr, trichocyst; vtv, various trafficking vesicles.

vacuole complex, mainly in its smooth spongiome, allow for stochastic reflux of Ca²⁺ into the nearby cytosol (Ladenburger *et al.*, 2006). As mentioned in Section VI, all this may serve for [Ca²⁺]_i fine-tuning in the cytoplasm, for ongoing vesicle delivery in the course of membrane turnover (biogenesis) and also for restructuring of the densely packed membrane tubules in the smooth spongiome (Plattner, 2013b).

(2) Additional types of CRCs

The occurrence of intracellular mechanosensitive channels in *Paramecium tetraurelia* cells has been postulated on the basis of the presence of stomatin in food vacuoles and in the contractile vacuole complex up to its pore region (Reuter *et al.*, 2013). This scaffolding protein is normally associated with microdomains and, on a molecular level, with mechanosensitive channels for whose activity it is obligatory (Lapatsina *et al.*, 2012). Build up of internal pressure during contractile vacuole diastole (Tani, Allen & Naitoh, 2001) could, thus, be a direct or indirect signal for the activation of mechanosensitive channels and, directly or indirectly, for the release of Ca²⁺ (Plattner, 2013b). Any Ca²⁺ signal could induce the exocytosis of vacuole contents [water and ions, including Ca²⁺ (Allen & Naitoh, 2002; Grønlien *et al.*, 2002;

Stock, Grønlien & Allen, 2002)] in a kiss-and-run mode. The periodic de- and recoupling of radial arms from/to the contractile vacuole during activity cycles, as established by electrophysiological analysis (Grønlien *et al.*, 2002), could be regulated by a similar, pressure-induced signalling pathway.

Although this scenario of intriguing interactions still requires experimental verification, it is in line with other results. Interestingly, ceramide is a component of rafts/microdomains in mammalian cells (Simons & Gerl, 2010) where it can regulate mechanical sensitivity in lipid bilayers (López-Montero *et al.*, 2010). When living *Paramecium tetraurelia* cells are exposed to labelled ceramide, this is endocytosed and transferred to the contractile vacuole system (as well as to food vacuoles where it forms patches) (Iwamoto & Allen, 2004), just like stomatin (Reuter *et al.*, 2013). Microdomain-forming components could coalesce, and this perhaps includes the mechanosensitive channels in the membranes of the contractile vacuole complex. Generally mechanotransduction depends on appropriate lipid microdomains (Anishkin & Kung, 2013).

Two groups of mechanosensitive channels, primarily conducting Ca²⁺ (Hamill & Martinac, 2001), have been detected by database analysis in ciliates, i.e. Piezo (otherwise known from a mammalian neuronal cell line)

Fig. 8. Ca^{2+} signalling mediating trichocyst exocytosis in *Paramecium* spp. This includes a site-directed Ca^{2+} flow, involving steps in the following sequence (indicated by arrows, 1–3). Step 1: release of Ca^{2+} from cortical stores (alveolar sacs); Step 2, a Ca^{2+} influx; Step 3, a second wave of Ca^{2+} release from the sacs in a tightly coupled sequence called SOCE. The release channels involved, type PtCRC-IV, face the cell membrane, whereas the SERCA-type Ca^{2+} pump is localised to the opposite side. The nature of the Ca^{2+} -influx channels involved is not yet established.

(Coste *et al.*, 2010) as well as some transient receptor potential (TRP) channels (Patel & Docampo, 2009; Kung, Martinac & Sukharev, 2010). In the Interpro *Paramecium* database, the Piezo family is represented by proteins of between 2338 and 2765 amino acids (IPRO027272), i.e. comparable to established Piezo-type mechanosensitive ion-channel components 1 and 2 of mammals. Among the large group of TRP channels some are localised to the cell membrane, some to organelles (Patel & Docampo, 2009), including lysosomes. TRP channels contribute to phagosome formation in macrophages (Samie *et al.*, 2013), whereas in ciliates details remain to be established.

In mammalian cells, trafficking organelles store Ca^{2+} to a variable extent (Hay, 2007; Sherwood *et al.*, 2007), from early endosomes (Luzio, Gray & Bright, 2010) to lysosomes (Christensen, Myers & Swanson, 2002). The membranes of some of these organelles are known to contain established CRCs (Zampese & Pizzo, 2012). Concomitantly, Ca^{2+} regulates phagosome formation, fusion of phagosomes with endosomes of different stages and with lysosomes (Jaconi *et al.*, 1990; Hay, 2007; Zampese & Pizzo, 2012). All these examples of CRCs along the endo/lyso/phagosomal pathway make us expect a comparable abundance also in *Paramecium* spp. (Ladenburger & Plattner, 2011).

In *Paramecium tetraurelia*, effects indicative of Ca^{2+} signal formation have been observed after microinjection of cADPR and of NAADP (Plattner *et al.*, 2012). These second messengers are formed from NAD and NADP, respectively, by the same cyclase, CD38 (Lee, 2012). While cADPR is discussed by some as an endogenous activator of some RyRs (Ogunbayo *et al.*, 2011; Lee, 2012; Ernst, Fliegert & Guse, 2013), NAADP is proposed to activate two-pore channels (TPCs) in acidic compartments (Galione *et al.*,

2009; Churamani *et al.*, 2013). However, there is also evidence for co-activation of other types of CRCs (Guse, 2012) and of conductances other than Ca^{2+} (Morgan & Galione, 2014). Thus, the precise mode of action and Ca^{2+} specificity or involvement of TPCs is not yet fully settled even in mammalian cells (Morgan & Galione, 2014). TPCs occur in acidic compartments, e.g. in recycling vesicles, lysosomes and phagolysosomes of animals and plants, whereas only indirect evidence exists for their presence in protozoa (Patel & Cai, 2015). In *Paramecium tetraurelia*, the occurrence of a protein with high-affinity NAADP binding ($K_D = 3.3$ nM) has been ascertained (Plattner *et al.*, 2012), but not investigated further. TPCs are abundant in choanoflagellates (Cai, 2008) – unikont protozoa at the base of early animal evolution; so far TPCs have not been identified at a molecular level in bikonts, such as ciliates. In summary, Ca^{2+} mobilisation by cADPR and NAADP and their receptors in ciliates remains to be scrutinised.

As for RyRs (or RyR-LPs) in bikonts other than ciliates, there are occasionally indirect hints for their occurrence in plants, but no molecular identification is yet available (Blatt, 2000; Munnik & Testerink, 2009). Generally their occurrence is disputed (Edel & Kudla, 2015). In Apicomplexa, important details of Ca^{2+} signalling have not been elucidated yet; for instance, molecular biology suggests the absence of $\text{IP}_3\text{R/RyR}$ -type CRCs (Prole & Taylor, 2011; Plattner *et al.*, 2012). This is in contrast to the persistent observation of InsP_3 effects in these cells (Passos & Garcia, 1998; Lovett *et al.*, 2002; Lourido & Moreno, 2015).

IX. PROTEIN PHOSPHORYLATION INVOLVED IN SIGNALLING

An important signalling mechanism is protein phosphorylation by kinases. In metazoans, this contributes to the regulation of metabolism, cell contraction, intracellular trafficking, secretion, transcriptional activity, apoptosis etc. (Johnson, 2009). Therefore, this section outlines some basic insights into protein kinase and phosphatase activities in ciliates and also covers the controversial question of tyrosine phosphorylation. In metazoans, protein kinases include PKA, PKG (Section III.2), CaM-kinase and protein kinase C (PKC) (see below) etc. Whereas in animal cells phosphorylation of seryl and threonyl residues prevail in cytoplasmic signalling, activation of some cell-surface receptors and some phenomena connected to cell cycle activity mainly involve tyrosyl phosphorylation. In ciliates, Ser and Thr phosphorylation have been confirmed, also by PKA and PKG, whereas PKC (see below) and Tyr phosphorylation by additional kinases are not well established yet.

In principle, protein modifications known from signalling mechanisms in metazoans are also found in ciliates, but they are poorly analysed in the latter. Serine/threonine phosphorylation is common, in contrast to tyrosine phosphorylation which also in ciliates is expected to be restricted to events related to the cell cycle and mitosis. Scrutiny is hampered by

the paucity of biochemical data and subcellular localisation studies with phosphoproteins as well as of protein kinases and phosphatases, but phosphoproteome analysis currently provides an important research focus.

(1) Kinases and the phosphoproteome

Protein kinases contribute 3.8% and ~7% to the genome of *T. thermophila* (Eisen *et al.*, 2006; Tian *et al.*, 2014) and *P. tetraurelia* (Bemm *et al.*, 2009), respectively. Globally the kinome is larger than in mammals, not only in *P. tetraurelia* where a large fraction represents very similar ohnologs from a rather recent whole-genome duplication, but also in *T. thermophila* (Tian *et al.*, 2014). We currently do not know all essential aspects of protein kinases; phosphorylation motifs predicted in *T. thermophila* are shared only in part with mammals. Recent mass spectrometry-based proteome analysis in *T. thermophila* has identified 1384 phosphopeptides and the occurrence of >1000 protein kinases and protein phosphatases (Tian *et al.*, 2014). However, since only a few phosphorylation sites have been specified in these cells, correlation with signalling processes is still difficult. Phospho-Ser/Thr forms largely prevail over the 3.8% phospho-Tyr forms. Six hundred and thirty putative kinases are considered cilia specific (Tian *et al.*, 2014). These predictions require validation.

The field of signalling by protein phosphorylation is not very advanced in ciliates, compared with the manifold signalling modes *via* Ser, Thr and Tyr phosphorylation known in mammalian cells where this takes place in cell membrane, cytosol, organelles, and the nucleus (Good *et al.*, 2011). In ciliates, phosphorylation effects exert a broad activity spectrum ranging from metabolism and ciliary activity to surface morphogenesis. For example, phosphorylation of ciliary rootlet proteins is a signal contributing to the morphogenesis of the *Paramecium tetraurelia* cell cortex (Keryer *et al.*, 1987; Sperling *et al.*, 1991). Mps one binder (MOB1), a regulator of cytokinesis and exit from mitosis (Luca *et al.*, 2001) that is widely distributed from protozoa to plants and mammals (Hergovich, 2011), proved essential for surface pattern formation in the ciliate, *Stentor coeruleus* (Slabodnick *et al.*, 2014). The relevance of mitotic components for the *de novo* biogenesis and proper positioning of new contractile vacuole complexes in the cortex of *Paramecium* spp. has been discussed elsewhere (Plattner, 2013b).

Phosphorylation by specific Ser/Thr kinases, such as PKA, PKG, casein kinase and CDPK, as well as dephosphorylation by protein phosphatases is discussed throughout this review. For instance, for the reversible Thr and Ser phosphorylation of the exocytosis-sensitive phosphoprotein, pp63, see Section XII. Such examples are involved in signalling along different functional cascades. In the *T. thermophila* phosphoproteome analysis, peptide sequences characteristic not only of already known cyclic nucleotide-dependent kinase substrates have been discovered, but interestingly also for PKC types ϵ , δ , ζ , $\alpha/\beta/\gamma$ and μ (Tian *et al.*, 2014). These are valuable suggestions for experimental validation, although they do not yet prove the occurrence of the respective protein kinases.

Remarkably PKC is reportedly absent from plants (Munnik & Testerink, 2009).

Therefore, the question still is open whether ciliates possess an orthodox PI(4,5)P₂ turnover and diacyl glycerol (DAG) formation as a PKC activator. Although this now looks very likely, experimental validation is required. Another putative Ser/Thr kinase in *T. thermophila* is a casein kinase which has been cloned and identified in *P. tetraurelia* (Vetter *et al.*, 2003).

(2) Tyrosyl phosphorylation and mitogen-activated protein kinases in ciliates?

The effect of the phosphorylation of a Tyr residue in a protein is significantly different from the phosphorylation of other amino acid residues. This is due to the fact that the oxygen of the phenol ring sticks out from the protein backbone and, thus, makes a phospho-Tyr site accessible to other binding partners (Hunter, 2014). To date, true signalling *via* Tyr phosphorylation, with dedicated Tyr kinases and Tyr phosphatases, has not been reported from ciliates (Goldberg *et al.*, 2006) or was reported as absent (Liu *et al.*, 2011). Tyr-kinases are known from holozoans, i.e. choanoflagellates and related filastereans (Suga *et al.*, 2014), as well as from amoebocytes (*Dictyostelium* spp.). In *Dictyostelium discoideum*, some kinases phosphorylate transcription factors, Spt6 and Stat, by dual-function kinase activity that is not specific for Tyr (Langenick *et al.*, 2008). Such proteins contain a Src homology (SH2) domain, i.e. a phospho-Tyr binding domain [Src (name derived from sarcoma) designates a non-receptor tyrosine kinase family]. Interestingly, one such protein has been identified in the *T. thermophila* database (Manning *et al.*, 2008; Liu *et al.*, 2011). Here, three phospho-Tyr phosphatases have also been presumed (Manning *et al.*, 2008). In general, however, dedicated, monospecific Tyr kinases are not considered an element of signalling in ciliates, at least not beyond mitotic/nuclear signalling. Only found from choanoflagellates onwards (Hunter, 2014), the non-Tyr-specific Thr/Tyr kinases are considered to represent the origin of metazoan genuine Tyr-kinases.

Remarkably, in mammalian cells, Src, Ras (small GTPase), mitogen-activated protein kinase (MAPK) and kinases following in a signalling cascade (see Section X) operate at many sites, from cell membrane to the transcriptional level, and so can regulate widely different functions (Avraham & Yarden, 2011). This also includes early steps of the secretory pathway at the level of the Golgi apparatus (Farhan *et al.*, 2010). [The latter also holds true for heterotrimeric G-proteins (Jamora *et al.*, 1997; Michaelson *et al.*, 2002; Coria, Masseroni & Diaz Anel, 2014)]. However, very little is known from ciliates as yet, except for some aspects of MAPK-mediated signalling (see Section X).

The ciliate *Euplotes raikovi* releases a water-soluble pheromone that can exert an autocrine effect by activation of Er-MAPK (Vallesi *et al.*, 2010). In mammalian cells, these enzymes have to be double-phosphorylated in their Thr-X-Tyr motifs to achieve full kinase activity, whereby the Tyr residue is autophosphorylated (Fu *et al.*, 2005). It is not yet known whether this double-phosphorylation including Tyr

autokinase activity of this kinase also occurs in *Euplotes raikovi*. Active MAPK typically acts as a Ser/Thr kinase which, therefore, would serve for the transmission of the signal also from the *Er*-MAPK. The receptor–ligand complex is internalised, probably not for its downregulation but rather for signal transfer from inside according to a signalosome function (Vallesi *et al.*, 2005) familiar from mammalian cells. This aspect is followed up in Section X.

Phosphoproteome analysis in *T. thermophila* strongly suggests some Ser/Thr and less Tyr-phosphorylation events along the MAPK and extracellular-signal regulated kinase (MEK), Ras and cyclin-dependent kinase (Cdk) pathways involved in signalling during cell cycle regulation and mitosis (Tian *et al.*, 2014). Experimental verification of one MAPK type, *Ti*MPK3, has been achieved by expression studies in *Tetrahymena thermophila* where further sequences of extracellular-regulated kinase (Erk)-type proteins have also been found (Arslanyolu, 2007). Meanwhile more family members have been identified (Yildiz & Arslanyolu, 2014). In higher eukaryotes, for instance, MEK is a Tyr/Thr kinase. Further support for such signal transduction comes from experiments with *Euplotes raikovi*, including heterologous analyses in mammalian cells (see Section X).

In *Tetrahymena thermophila*, Tyr phosphorylation of an alleged insulin receptor-like protein was derived from immunostaining of Western blots with antibodies that recognise Tyr phosphorylation sites in mammalian cells (Christensen *et al.*, 2003). However, antibodies may also recognise a conformational motif unrelated to phospho-Tyr. Validation by biochemical analysis, e.g. matrix-assisted laser desorption/ionisation (MALDI) analysis etc., would be helpful in such cases.

In summary, no Tyr-protein kinase destined for non-MAPK-related signalling has been experimentally identified at a molecular level in ciliates as yet. This aspect currently awaits experimental scrutiny.

X. SURFACE RECEPTORS FOR INTRACELLULAR SIGNALLING

In metazoans, surface receptors are responsible for many functions, from cell survival to cell division, differentiation to apoptosis, secretion to endocytosis, chemotaxis to immune defence etc. (Gomperts *et al.*, 2009). Most of these functions occur not only in multicellular organisms, but also in yeast and some protozoa, such as *Dictyostelium* spp. Which receptors occur in ciliates?

A crucial finding was signalling in neutrophilic granulocytes by a receptor for formyl-methionyl-leucyl-phenylalanine (fMLP) – the molecular ‘trace’ of bacteria which neutrophils can follow by chemotaxis for subsequent phagocytosis and inactivation by digestion. The fMLP receptor (Quehenberger *et al.*, 1993) is an example of the large family of (trimeric) G-protein-coupled receptors (GPCRs). By combination of different ligands with different GPCR subtypes and different G-protein subunits, many different

signals can be differentially transduced (Katritch, Cherezov & Stevens, 2013). In some cases GPCR activation is followed by PKC activation. GPCRs occur in *Dictyostelium discoideum* and in sponges (Strotmann *et al.*, 2011), whereas occurrence in ciliates requires further scrutiny.

When a chemical signal is released into the medium for stimulating neighbouring cells such ‘paracrine’ effects can be transduced *via* phosphorylation cascades, such as MAPKs. This applies, for instance, not only to mating-inducing pheromones released by yeast cells (Merlini, Dudin & Martin, 2013), but also to developing heart tissue (Noireaud & Andriantsitohaina, 2014). Paracrine signalling by released proteins is currently under scrutiny for ciliates, as discussed below. Signalling by nucleotides such as ATP, released from cells, is established in most animal kingdoms from protozoa onwards (Verkhatsky & Burnstock, 2014). However, in ciliates important details about paracrine signalling by secreted nucleotides remain to be scrutinised.

This section highlights some of the most eminent, although only partially understood questions of signal transduction in ciliates, including the motility response to chemicals (chemokinesis) and directional orientation of motility (chemotaxis), the controversial issues of GPCRs and of MAPKs.

In *Paramecium* spp. and *Tetrahymena* spp. chemosensory transduction can involve attractants and repellents, respectively. For attractants, notably folate, acetate and glutamate, receptors are envisaged; the effects of repellents are more diffuse (Van Houten, 1998). More specifically, in *Euplotes raikovi*, pheromones can act *via* receptors not only as attractants in a paracrine mode, but also induce an autocrine signalling cascade (Vallesi *et al.*, 2010). In *Blepharisma japonica*, two gamones occur: as usual, gamone 1 is a glycoprotein, whereas gamone 2 is a tryptophan derivative (Luporini, Alimenti & Vallesi, 2014). In ciliates, evidence for trimeric G-proteins and receptor coupling is fragmentary – their occurrence is elusive. Another concern envisages purinergic receptors that have been postulated to occur in ciliates. For instance, extracellular GTP (a quite atypical activator) triggers clear-cut periodic ciliary responses, but molecular details are still missing.

(1) Different types of surface receptors

Even normal ciliate cultures require signalling in order to survive. Thus, an isolated ciliated protozoan cell requires a small volume enabling it to condition its medium by secreting proteins that act as survival factors. When transferred into too large a volume of culture medium, cells usually die. In *Tetrahymena thermophila*, survival factors are ~10–22 kDa in size (Rasmussen & Wheatley, 2007). Their signalling pathway remains to be established. When *Tetrahymena thermophila* cells grow in synchronized cultures (not available for *Paramecium*) this stimulates the upregulation of over 1700 genes, among them some of the Rab protein family (Miao *et al.*, 2009).

The bacterial chemoattractant, N-formyl-Met-Leu-Phe, known from its effect on neutrophilic granulocytes and macrophages, is reported to stimulate chemokinesis also

in *Tetrahymena thermophila* (Leick, 1992). A considerable chemokinetic effect, i.e. increased swimming velocity, has been induced by a mixture of 0.2–1.5 kDa-sized oligopeptides released from *Methanococcus* sp. cells at concentrations of $>10^{-6}$ M (Leick & Lindemose, 2007). Any chemotactic effect promoting increased food acquirement has not been analysed.

Ciliates can secrete cell type-specific signalling substances, now called pheromones (previously called gamones or mating-type factors); they are (glyco)proteins of different types. Cell surface-bound pheromones occur in *Paramecium* and *Tetrahymena* species, whereas they are released into the ambient medium as free molecules in *Blepharisma* and *Dileptus* species, also of different species, or finally both bound and free forms occur in different *Euplotes* species (Luporini *et al.*, 2005, 2014; Vallesi *et al.*, 2008). As described in Section IX, in *Euplotes raikovi* a pheromone released into the medium can activate, *via* an autocrine pathway, a mitogenic cascade involving MAPK activity (Vallesi *et al.*, 2010). Important signalling steps are activated when the *Euplotes raikovi* pheromone, Er-1, is applied to human T-cells (Cervia *et al.*, 2013). These data suggest rather extensive effects on the promotion of mitotic activity also in *Euplotes raikovi*.

Depending on the *Euplotes* species, pheromones can also exert paracrine effects by binding to ‘conspecific’ cells to induce mating, i.e. sexual activity (conjugation) (Luporini *et al.*, 2005, 2014; Vallesi *et al.*, 2008). Similar effects are known from *Blepharisma japonicum* where gamone type 2 is secreted by cells of mating type II, but renders cells of mating type I ready for conjugation (Sugiura & Harumoto, 2001). The signalling cascade thus activated must be rather complex, as this activation also includes changes in swimming behaviour and positive chemoresponses that increase the probability of encounters with conjugation partners (Sugiura *et al.*, 2010). Generally, a non-self gamone/pheromone released by a complementary mating type slows down motility and, thus, facilitates partner pairing for conjugation (Ricci, 1996). Sumoylation, i.e. the attachment of a small ubiquitin-like modifier (SUMO), to various proteins is an important signalling process also in ciliates where it is required for cell pairing (conjugation) competence (Nasir *et al.*, 2015) and macronuclear development (Matsuda & Forney, 2006). Electrophysiology demonstrated a reduction of spontaneous depolarisation frequency and an increase in depolarisation- and hyperpolarisation-activated currents in a complex interplay (Stock *et al.*, 1999). Some of these currents are Ca^{2+} activated, but the source of Ca^{2+} could not be established. Along these lines, in *Paramecium bursaria*, microinjection of InsP_3 induces conjugation (Miwa & Wada, 1995), yet, unfortunately, these cells also contain symbiotic *Chlorella* cells which could account for the effect reported. In *Paramecium* spp., no releasable gamones exist. Mating types are maternally inherited, i.e. by the macronucleus. Mating type E depends on the expression of mtA, a membrane-spanning protein. The default type O is formed by the excision of the mtA promotor mediated by scnRNA activity (Singh *et al.*, 2014).

Conjugation (see Section XV) is another process depending on surface receptors and recognition molecules. During conjugation, cell–cell fusion takes place. As in metazoans, this depends on proteins different from SNAREs. In *Tetrahymena thermophila*, HAP2, a transcriptional activator, was identified by green fluorescent protein (GFP)-localisation and gene deletion as a relevant protein (Cole *et al.*, 2014) which is known to mediate gamete fusion in invertebrates. In *T. thermophila*, ASI2 (signal transducers and activators of transcription 2 protein) is considered a putative signal transduction receptor relevant for the transformation of the post-conjugation micronucleus to a macronucleus (Li *et al.*, 2006). Knockout cells are arrested at the stage of macronuclear anlagen development and they die before post-conjugation fission.

When *Paramecium tetraurelia* cells are exposed to extracellular GTP, $\geq 10 \mu\text{M}$, this causes periodic forward and backward swimming (different from ciliary reversal) in ~ 8 s intervals; this is accompanied by oscillating membrane depolarisations (Clark, Hennessey & Nelson, 1993) and by oscillating intracellular Ca^{2+} signals (Sehring & Plattner, 2004) that are consistent with oscillating Ca^{2+} currents (Mimikakis, Nelson & Preston, 1998). This effect is specific to GTP and cannot be induced by any other nucleotide triphosphate in any comparable concentration. By contrast, extracellular signalling by GTP is unusual in higher eukaryotic systems. Here, since the discovery of paracrine effects of nucleotides (Osipchuk & Cahalan, 1992), such signalling has remained restricted essentially to ATP (Verkhatsky & Burnstock, 2014). An extracellular receptor for ATP has recently been found also in *Arabidopsis thaliana* (Choi *et al.*, 2014).

Whereas *Paramecium tetraurelia* is reported usually to react only to GTP (Hennessey, 2005), *Tetrahymena thermophila* displays repetitive back-/forward swimming not only in response to GTP ($10 \mu\text{M}$), but also to ATP ($\sim 500 \mu\text{M}$ for maximal response) (Kim *et al.*, 1999). The occurrence of a receptor is assumed, but the efficiency of drugs on the effects of ATP and GTP, respectively, differ greatly (Hennessey, 2005). Considering the rather problematic pharmacology of ciliates (Plattner *et al.*, 2009) any further interpretation, e.g. on the involvement of trimeric G-proteins (see Section X.2), would greatly benefit from identification of a receptor at a molecular level. This response to nucleotide triphosphates is unexpected insofar as there are no purinergic receptors known from ciliates (Burnstock & Verkhatsky, 2009; Coddou *et al.*, 2011; Verkhatsky & Burnstock, 2014), in contrast to *Dictyostelium discoideum* (Fountain *et al.*, 2007). In *Paramecium tetraurelia* GTP-induced signalling involves release of Ca^{2+} from unidentified internal stores (Wassenberg, Clark & Nelson, 1997), but a stronger first Ca^{2+} peak depends on $[\text{Ca}^{2+}]_o$ (Sehring & Plattner, 2004). The biological significance of these Ca^{2+} oscillations and of the repetitive back/forward movement is not quite clear, but it was suggested that the GTP-induced behaviour counteracts dispersal of the cells (Sehring & Plattner, 2004).

Work with *Paramecium* and *Tetrahymena* species has established their capability for positive and negative chemoresponses to attractants and repellents, respectively (Van Houten, 1998). Examples of attractants are glutamate and folate. Silencing of a gene involved in the biosynthesis of the glycosyl phosphatidylinositol- (GPI-) anchor of surface proteins (*PIG-A*), known from mammalian cells, resulted in reduced chemoattraction by glutamate and folate (Yano, Rachochy & Van Houten, 2003). Since then, the folate receptor was assumed to be a GPI-anchored protein. In mammalian cells there is an ongoing debate about the mechanism of signal transmission by GPI-anchored proteins, considering the absence of a transmembrane domain.

Surface receptors involved in the explosive release of toxic secretory organelles, the toxicysts, are not known. In this crudest form of intercellular ‘communication’ toxicysts are released upon contact with other ciliates serving as prey (Hausmann, 1978). In *Coleps hirtus* the toxins of toxicysts include phytanic acid as well as saturated and unsaturated fatty acids (Buonanno *et al.*, 2014).

(2) G-protein-coupled receptors in ciliates?

In metazoa, many cell surface receptor–ligand interactions transmit intracellular signals *via* heterotrimeric GTP-binding proteins (G-proteins) composed of a dynamically interacting α -subunit that associates reversibly with the β/γ -subunits. Thus, trimeric G-protein-coupled receptors (GPCRs), characterised by seven transmembrane domains, may be important as a first step in signal transduction. Depending on the type of GPCR activated and the α -isoform associated with it, widely different signalling cascades can be activated in higher eukaryotes (Oldham & Hamm, 2008). Trimeric G-proteins occur not only at the cell membrane, but also regulate vesicle trafficking in the Golgi apparatus (Jamora *et al.*, 1997; Michaelson *et al.*, 2002; Coria *et al.*, 2014). The α -subunits are typically ADP-ribosylated by bacterial toxins, such as by Pertussis or Cholera toxin. Among the signalling pathways following GPCR activation are widely different chemical or physical signalling steps, ranging from PI nsP_2 cleavage by PLC to the activation of specific proteins, including some ion channels. PI nsP_2 is cleaved to PI nsP_3 and DAG. Either compound can activate further signalling steps, either by Ca^{2+} mobilisation from stores endowed with IP $_3$ Rs or by phosphorylation of specific proteins by DAG-activated PKC.

Trimeric G-proteins are also assumed to be involved in chemosensory transduction in ciliates where, however, they have not been identified unambiguously at a molecular level as yet, as outlined below. Therefore, currently *Dictyostelium discoideum* is the only protozoan for which trimeric G-proteins and GPCRs are well established (Manahan *et al.*, 2004). Here, the activation cascade following extracellular cAMP binding is paradigmatic for GPCR activation, followed by activation of Ras and cytosolic adenylate cyclase (Jin, 2011). The current situation in ciliates is rather intriguing. Based on the following observations, clarification will require combining work with molecular biology and biochemistry,

including standard effects of Cholera and Pertussis toxins, and thio-derivatives of GTP and GDP etc.

The autocrine mitogenic effect of *Euplotes raikovi* pheromone activation is assumed to involve a trimeric G-protein, based on the effects of Cholera toxin and γ -thio-GTP (with an enduring activation effect), as well as on recognition of a protein of appropriate size (55 kDa), by antibodies specific for conserved regions of metazoan α -subunits (Ortenzi *et al.*, 2000). Similarly, evidence for the occurrence of trimeric G-proteins in *Paramecium tetraurelia* has been postulated from Western blots using antibodies against established subunits, from binding of GTP- γ -S and effects of stimulatory or inhibitory derivatives of GTP or of GDP as well as from ADP-ribosylation of a putative α -subunit by Pertussis toxin (De Ondarza *et al.*, 2003). Here, interference with trimeric G-protein function reportedly affected swimming behaviour due to effects on Ca^{2+} -influx channels. In *Tetrahymena thermophila*, sequences similar to established genomic sequences of GPCR from other organisms have been used to prepare constructs for functional interference (Lampert, Coleman & Hennessey, 2011). Again Ca^{2+} conductivity, and consequently swimming behaviour, was affected.

Irritatingly comparative genomic and bioinformatics analyses were unable to identify any of the G-protein subunits in ciliates, whereas a GPCR-related protein has been found in some Alveolata (Bradford *et al.*, 2013). Specifically in ciliates, a cAMP receptor of a type restricted to invertebrates is closely related to a rhodopsin-type GPCR (Krishnan *et al.*, 2012), but any function in signal transfer remains open. It remains enigmatic what the presence of a GPCR in the absence of trimeric G-proteins (or, at least, of a catalytic α -subunit) would signify. Alternatively, signalling by a GPCR in a mode independent of trimeric G-proteins has been proposed (Anantharaman *et al.*, 2011), or identification may be obscured by the notorious lack of similarity between GPCRs of different sources (Lima *et al.*, 2014).

In *Paramecium primaurelia* an N-methyl-D-aspartate (NMDA)-like receptor has been envisaged (Ramoino *et al.*, 2006, 2014). If present in ciliates this channel-type receptor could also be coupled to GPCRs and its activation would be interesting with regard to the positive chemotactic effect of glutamate by which its activity is modulated (Zito & Scheuss, 2009). From the *Paramecium* database, partial sequences have been retrieved showing some similarity to important domains and to potentially associated proteins (Ramoino *et al.*, 2014). However, in contrast to expectations (Ramoino *et al.*, 2006) no sequences could be found matching with ionotropic glutamate receptor family members. Again, this would be compatible with the low level of similarity among members of the GPCR superfamily (Lima *et al.*, 2014).

Any concise molecular details about signalling *via* trimeric G-proteins (or its α -subunit) and GPCRs remain to be scrutinised. A stringent combination of experimental approaches and computer modelling would be particularly helpful, as has been used with *Dictyostelium discoideum* (Jin, 2011).

PKC in higher eukaryotes is known to be activated by DAG in consequence of the activation of trimeric G-proteins. In *Paramecium tetraurelia*, partial sequences of a potential substrate (Hinrichsen & Blackshear, 1993) could not be attributed to any PKC function or any underlying molecule. Only recent phosphoproteomic analyses with *T. thermophila* indicated the presence of different PKC paralogs (Tian *et al.*, 2014). To end such basic uncertainty with ciliates, such analyses would be promising when combined with expression studies.

If it occurs in ciliates, a Ca^{2+} /polyvalent cation-sensing receptor would also be a candidate for interaction with a GPCR, as for most such receptors in metazoans (Riccardi & Kemp, 2012). *Paramecium tetraurelia* reacts to polycations, such as AED, by $[\text{Ca}^{2+}]_i$ increase (Klauke & Plattner, 1997) and massive exocytosis (Plattner *et al.*, 1984, 1985). Similarly *Tetrahymena* spp. release mucocysts when exposed to cationic components. The secretagogue effect of AED in *Paramecium* may be comparable to that of compound 48/80 in mast cells, a condensation product from N-methyl-p-methoxyphenethylamine and formaldehyde, and of other cationic–amphiphilic drugs which all act as pseudo-allergens. These are currently assumed to use the machinery of G-protein-coupled receptors or parts thereof (Seifert, 2015). Nevertheless, effects of polycations could also involve polyvalent cation-sensing receptors of a type that is independent of trimeric G-proteins (Bockaert & Pin, 1999) or some unrelated effects on plasmalemmal cation channels (Williams, 1997). This aspect of signalling in ciliates also remains open at present.

XI. GRAVIPERCEPTION AND GRAVIKINESIS/GRAVITAXIS

Early spaceflight experiments revealed the sensitivity of mammalian cells in culture to ‘weightlessness’, i.e. microgravity (Cogoli, Tschopp & Fuchs-Bislin, 1984). Since then unicellular models have frequently been used for such experiments. With ciliates, results are interpreted on the basis of polar mechanosensitive transduction (Machemer, 1988b, 2014), based on electrophysiological recordings in mechanosensation experiments with metazoans (Gillespie & Walker, 2001).

As outlined in this section, ciliates can perceive their orientation in space depending on the gravity field (graviperception). Gravity perception results in gravire-sponses which include gravikinesis (increased motor activity) and graviorientation, jointly also designated as gravitaxis/geotaxis. This can be explained on the basis of gradual polarity of underlying ionic conductances, as outlined below.

Ciliates are able to perceive the earth’s gravity field (Machemer & Teunis, 1996; Hemmersbach & Bräucker, 2002; Hemmersbach & Braun, 2006; Machemer, 2014). Only genera with statocyst-like organelles, such as *Loxodes* (Bräucker, Machemer-Röhnisch & Machemer, 1994; Hemmersbach *et al.*, 1998), display positive gravitaxis,

i.e. they swim downwards. This is in contrast to most other genera (Hemmersbach *et al.*, 1998), such as *Bursaria* (Krause & Bräucker, 2009), *Didinium* (Bräucker *et al.*, 1994), *Paramecium* (Bräucker *et al.*, 1994; Roberts, 2010; Machemer, 2014), *Stylonychia* (Krause, Bräucker & Hemmersbach, 2010) and *Tetrahymena* (Mogami *et al.*, 2004) which all swim upwards. Gravity perception by *Loxodes* is due to an intracellular receptor system involving a structure with density >1.0 . Analysis of systems with negative gravitaxis was much more of a challenge. Analyses included electrophysiological recording of very weak signals; first indications of orientation-dependent sensitivity of ciliates to the gravity field came from membrane potential recordings in *Paramecium caudatum* (Machemer *et al.*, 1998; Gebauer, Watzke & Machemer, 1999). Such studies have since been complemented by micro- and hypergravity experiments.

In cells with negative gravitaxis the cytoplasm exerts pressure onto sensors that are arranged in the cell membrane along a polar gradient. These are depolarising mechanosensitive Ca^{2+} channels and hyperpolarising mechanosensitive K^{+} channels enriched in the anterior and the posterior half of the cell (Section III), respectively, as analysed in the context of gravikinesis (Machemer & Teunis, 1996; Krause & Bräucker, 2009; Krause *et al.*, 2010; Machemer, 2014). Downward movement activates anteriorly enriched depolarisation channels which entails reorientation of the cells in the upward direction, essentially according to the principles described for behavioural reactions (Section III). Increased upward movement is induced and supported by hyperpolarisation due to activation of hyperpolarising channels in the posterior part of the cell. One prerequisite to activation of mechano-channels is that the cytoplasm has a higher density than the surrounding medium. Since the difference is usually very small, the gating energy is only slightly above the thermal noise and, thus, provides only small voltage changes recordable during reorientation of the cells in the gravity field (Machemer *et al.*, 1998; Gebauer *et al.*, 1999; Hemmersbach & Braun, 2006; Machemer, 2014). Support by amplifying structures, such as actin filaments, appears crucial. Concomitantly, current models show such channels in association with the cortical cytoskeleton, specifically with filamentous (F-)actin (Machemer, 2014). For ciliates this is a challenging view, considering that cortical actin has been under considerable debate until quite recently. Support comes from the facts that (i) mechano-channels are consistently considered associated with the cytoskeleton, notably F-actin, in other systems (Lapatsina *et al.*, 2012), this association being mutually interdependent (Kuipers, Middelbeek & van Leeuwen, 2012); (ii) special cortical isoforms, e.g. *PtAct4*, have been identified in *P. tetraurelia* (Sehring *et al.*, 2010), and (iii) subplasmalemmal localisation of actin was shown by immuno-electron microscopy (Kiss-mehl *et al.*, 2004). Therefore, in principle, F-actin associated with the cell membrane is available for amplifying gravity signals.

In summary, during negative gravikinesis an omnipresent physical stimulus is constitutively transduced into an

electrophysiological signal. This in turn, according to a brief note, results in a chemical signal involving the formation of cAMP; conversely manipulation of the cAMP level affects gravikinesis (Hemmersbach *et al.*, 2002). This is in line with the well-documented observation that, in *Paramecium tetraurelia*, cAMP formation is under positive control of hyperpolarising K^+ conductances (Bonini *et al.*, 1986; Schultz & Schönborn, 1994). See Section III.

XII. METABOLIC ASPECTS OF SIGNALLING IN CILIATES

As generally known, all cells rapidly consume and regenerate ATP as their main energy fuel, total turnover taking ~ 1 min or fractions thereof. There are few occasions to study such aspects in detail. During synchronous trichocyst exocytosis in *Paramecium tetraurelia* we registered a significant decay in ATP concentration within seconds (Vilmart-Seuwen *et al.*, 1986). This enables us to dissect this phenomenon and pinpoint more specifically ATP-consuming steps.

This section can be summarised as outlined in Fig. 9. In the context of the ATP decay reported during synchronous trichocyst exocytosis stimulation (Vilmart-Seuwen *et al.*, 1986), essentially eight facets of ATP/energy consumption are discussed, but only a few are elaborated as possibly relevant in quantitative terms.

ATP is present in *Paramecium tetraurelia* cells in a concentration of ~ 1 mM, as in mammalian cells (Balaban, 2009). Within a few seconds of trichocyst exocytosis stimulation, [ATP] decays significantly (Vilmart-Seuwen *et al.*, 1986). Here we discuss possible sources of ATP consumption. Theoretically, one is the termination of Ca^{2+} signalling by activation of Ca^{2+} -ATPases/pumps. Activation of the SNARE-specific chaperone NSF, a triple A-ATPase (AAA-ATPase) (Whiteheart, Schraw & Matveeva, 2001) for the assembly and/or disassembly of SNAREs at membrane fusion sites, e.g. trichocyst exocytosis sites, will also require ATP. This AAA-ATPase function is different from the P-type ATPase activities that hydrolyse ATP for sequestration of Ca^{2+} into stores and extrusion from the cell. Moreover, in *Paramecium tetraurelia*, massive exocytosis induction is superimposed on increased ciliary activity that will require ATP for the formation of second messengers and for the activation of the motor protein, dynein. H^+ -ATPase/pump-coupled $H^+/X^+/Ca^{2+}$ antiporter activity may be an additional route of indirect ATP consumption (secondary active transport), as has been suggested for the activity of the contractile vacuole complex in *Paramecium tetraurelia* (Section VI). The physicochemical disequilibrium between $[Ca^{2+}]$ and $[PO_4^{3-}]$ during a swift cortical $[Ca^{2+}]$ increase will also be discussed below. Finally we ask how excessive ATP consumption occurring in ciliates during synchronous exocytosis can be compensated. In this context, a dramatic event is the reversible dephosphorylation of a 63 kDa phosphoprotein that has been identified as phosphoglucosyltransferase, a key enzyme for access to the glycolytic pathway.

(1) Ca^{2+} -flux, ATP decay and ATP recovery during synchronous trichocyst exocytosis

During AED-induced synchronous trichocyst exocytosis in *P. tetraurelia*, ATP decays significantly by $\sim 20\%$ (averaged from different strains), from between 1.1 and 1.25 mM (Matt, Bilinski & Plattner, 1978; Lumpert, Kersken & Plattner, 1990), within ~ 5 s. ATP content recovers, depending on the strain, during the following ~ 20 – 30 s (Vilmart-Seuwen *et al.*, 1986; Plattner & Kissmehl, 2005). Considering a turnover time of ATP of 1–2 min in mammalian cells under steady-state conditions (Skog, Tribukait & Sundius, 1982; Jauker, Lades & Nowack, 1986), the decay of [ATP] by $\sim 20\%$ within 5 s is difficult to explain. This dramatic decay would appear more feasible if the turnover time was much shorter. In fact, in *Tetrahymena thermophila* this has been estimated as 2 s (Jauker *et al.*, 1986), although this is probably an underestimation. A realistic ATP turnover time in *Paramecium tetraurelia* may be between 10 and 30 s, depending on the strain, as can be estimated from the ATP decay measured during the first second of AED stimulation (Vilmart-Seuwen *et al.*, 1986) in a wildtype (7S: 30 s) and a closely related strain (K401: 10 s), respectively. Such a turnover time would be compatible with the reestablishment of [ATP] in *Paramecium tetraurelia* within ≤ 30 s after massive synchronous exocytosis (Vilmart-Seuwen *et al.*, 1986).

To what extent can ATP hydrolysis be accounted for by Ca^{2+} -ATPase/pump activity and how does this compare with the Ca^{2+} leakage rate? *Paramecium tetraurelia* possesses a very high Ca^{2+} leakage rate under steady-state conditions. In unstimulated cells this is ~ 30 pmole Ca^{2+} per 10^3 cells over 5 min (Kerboeuf & Cohen, 1990). Considering Avogadro's number (6×10^{23}) this corresponds to 3×10^8 Ca^{2+} ions per cell in 5 s, the time interval after which [ATP] is seen to increase again. From the volume of one *Paramecium tetraurelia* cell, i.e. 0.73×10^{-10} l (Erxleben *et al.*, 1997) and an [ATP] of 1.2 mM (7.2×10^{20} molecules l^{-1}), a cell contains a total of 5.6×10^{10} ATP molecules under steady-state conditions. Considering a leakage rate of 0.6×10^8 Ca^{2+} s^{-1} , the ATP supply would suffice to counteract leakage for ~ 15 min if Ca^{2+} /ATP efficiency were 1:1. Since a ratio of 1:3 is more realistic, the ATP supply would be exhausted after 5 min when compensating for Ca^{2+} leakage if not counterbalanced and disregarding ongoing ATP synthesis. Even though for a very restricted time after AED stimulation, the actual Ca^{2+} influx rate is ~ 3 times higher than during steady state (Kerboeuf & Cohen, 1990), ATP expenditure for reestablishment of $[Ca^{2+}]$ homeostasis can only partially explain the [ATP] decay observed, although it cannot be fully neglected.

Based on the following pilot calculations Ca^{2+} flux data may be compatible with a relatively low ATP consumption for primary and secondary active Ca^{2+} transport. Can Ca^{2+} -ATPases/pumps localised to alveolar sacs (Hauser *et al.*, 2000) and to the plasma membrane (Wright & Van Houten, 1990; Elwess & Van Houten, 1997), i.e. SERCA and PMCA, be significant Ca^{2+} regulators over short periods? Interestingly the half-time for refilling of alveolar sacs (with SERCA activity) has been experimentally verified

Fig. 9. Processes responsible for the consumption of ATP observed in *Paramecium* spp. cells during synchronous exocytosis stimulation. Pilot calculations reveal that the relative contribution of some aspects to overall ATP consumption must be very low (e.g. second messenger formation, NSF activity), in contrast to some other processes. This is likely to include mainly Ca^{2+} extrusion via secondary active transport (ion exchangers presumably in the contractile vacuole complex), ciliary reversal superimposed on trichocyst release and kinetic aspects of the interference of Ca^{2+} , phosphate and adenine nucleotides. See Appendix (Section XIX) for definitions of abbreviations.

by three widely different methods, *in situ* and *in vitro*, as 65 min (Mohamed *et al.*, 2003). Assuming that PMCA activity in the cell membrane (occupying about the same area as SERCA in alveolar sacs) has about the same activity, the Ca^{2+} -pump activity would be rather small in relation to the Ca^{2+} load to explain the swift consumption of ATP within 5 s, even if one considers additional SERCA activity in the endoplasmic reticulum. Therefore, a much more rapid and efficient mechanism must be taken into account for the reestablishment of $[\text{Ca}^{2+}]$ homeostasis. Rather than primary active transport, secondary active transport appears to be the dominant $[\text{Ca}^{2+}]$ regulator and ATP consumer. This can be extrapolated from the substantial release of Ca^{2+} by the contractile vacuole (Stock *et al.*, 2002) and the significant retardation effect of the H^+ -ATPase inhibitor, concanamycin B, on the reestablishment of $[\text{Ca}^{2+}]_i$ after strong exocytosis stimulation (Plattner *et al.*, 2012). Under steady-state conditions the contractile vacuole complex can, thus, export its excess of Ca^{2+} caused by steady-state leakage within ~ 5.9 min (Ladenburger *et al.*, 2006). Interestingly this is comparable to the ATP supply that would suffice to counterbalance Ca^{2+} permeation, as discussed above. Increased $[\text{Ca}^{2+}]_i$ can be assumed to activate secondary active extrusion mechanisms, such as $\text{H}^+/\text{X}^+/\text{Ca}^{2+}$ exchangers; although not yet explored in ciliates there are stringent reasons to assume their existence (Section XIV). Another rapid and powerful Ca^{2+} regulator after stimulation is the rapid binding of Ca^{2+} to immobile buffers, such as centrins (Sehring *et al.*, 2009) – independent of ATP. Our calculations consider acute roles of direct or indirect active Ca^{2+} transport which, surprisingly, can account only for a fraction of the ATP consumed during synchronous exocytosis.

(2) ATP decay – unlikely due to second messenger formation, SNARE rearrangement or exocytosis-coupled endocytosis

Some signalling steps depend on metabolites from which ‘metabolic’ second messengers are generated. Formation of

cAMP depends on ATP, that of cGMP on GTP (formed from ATP by GDP transphosphorylation), InsP_3 on PInsP_2 and finally cADPR and NAADP formation depend on NAD(P) (NAD and NADP being H^+ acceptors in bioenergetic processes). However, second messenger formation is unlikely to cause remarkable ATP consumption because of their low concentration, usually nano- to (sub)micromolar. For instance, a $K_d = 3.3$ nM has been determined for NAADP in *Paramecium tetraurelia* homogenates (Plattner *et al.*, 2012).

Could some of the ATP consumption measured during synchronous trichocyst exocytosis be due to NSF activity? ATP-driven disassembly of SNARE complexes consumes ATP and up to six SNARE complexes are usually assumed to occur per exocytosis site, based on data from mammalian cells (Plattner, 2010b). Consider again the availability of 5.6×10^{10} ATP molecules per cell (Section XII.1). The 50 ATP molecules required to disentangle a trans-SNARE complex (Cipriano *et al.*, 2013), the six SNAREs per exocytosis site, and the ~ 1000 trichocyst release sites (Plattner *et al.*, 1985) results in a total of 3×10^5 ATP molecules consumed per cell for the AAA-ATPase function of NSF. The percentage of ATP consumed during synchronous trichocyst exocytosis, therefore, would be only $\sim 0.6 \times 10^{-3}\%$ of the available store. Even assuming 10 times higher numbers of SNAREs per site (Sieber *et al.*, 2007) NSF activity could not explain the ATP consumption actually observed during synchronous trichocyst exocytosis.

Considerable ATP consumption has been assigned to recycling of transmitter vesicles in hippocampal synapses (Rangaraju, Calloway & Ryan, 2014), but this is probably due to steps beyond membrane resealing. In *Paramecium tetraurelia*, retrieval of trichocyst ‘ghosts’ follows synchronous exocytosis after 0.35 s (Knoll *et al.*, 1991a), but takes much longer than any other process envisaged here (Plattner, Knoll & Pape, 1993). Also [ATP] decay observed in exocytosis-incompetent strains (Vilmart-Seuwen *et al.*, 1986) makes this an unlikely explanation for the rapid, transient consumption ATP in the course of exocytosis stimulation.

(3) ATP decay due to superimposed ciliary reversal?

Could ATP be consumed extensively in the course of ciliary reversal which always accompanies exocytosis stimulation as a consequence of Ca^{2+} spill-over into cilia (Plattner *et al.*, 1984, 1985; Section III.4)? Data are available for ATP consumption during normal swimming in *Paramecium* sp., (Gueron & Levit-Gurevich, 1999). The active stroke of one cilium consumes 9×10^{-16} J, the recovery stroke 2×10^{-16} J, giving a total of 11×10^{-16} J consumed per ciliary activity cycle. According to the general rule that 44 kJ are provided per mole ATP and considering Avogadro's number, 1 J would thus be represented by 0.14×10^{20} ATP molecules. Therefore, one beat cycle would consume 0.15×10^5 ATP molecules per cilium. Assuming a cycle of 20 Hz and the involvement of ~ 3000 cilia per cell [derived from the number of unit fields, i.e. kinetids (Erxleben *et al.*, 1997)], $\sim 7.5 \times 10^5$ ciliary beats occur per cell during the 5 s of ATP decay. Therefore, during this time, for normal beat activity, 1.1×10^{10} ATP molecules are consumed, or $0.22 \times 10^{10} \text{ s}^{-1}$ which is equivalent to 4% of the ATP available. This is more than previously estimated for the smaller *Tetrahymena thermophila* cells which have been estimated to consume $\sim 1\%$ of their energy supply for normal swimming activity (Jauker *et al.*, 1986).

To explain the considerable [ATP] decay within 5 s of AED stimulation one should also take into account the occurrence of ciliary reversal which involves intense rotation of the cell. For the following reasons this may require more ATP consumption than normal swimming. First, recall that the active stroke consumes considerably more energy than the recovery stroke and this may also hold for permanent rotation. Second, this expectation is in line with increasing ciliary activity in parallel to $[\text{Ca}^{2+}]$ increase, as recorded with permeabilised cells (Nakaoka *et al.*, 1984); consider that $[\text{Ca}^{2+}]$ in cilia also increases considerably during AED stimulation (Husser *et al.*, 2004). Third, support comes from experiments with *P. tetraurelia* mutants where, in response to AED stimulation, ciliary reversal takes place even in exocytosis-incompetent strains (Plattner *et al.*, 1984, 1985) and they also display ATP decay upon (mock)stimulation with AED (Vilmart-Seuwen *et al.*, 1986). Interestingly, 'pawn' mutants such as strain *d4-500r* (which cannot react to depolarisation by ciliary reversal because of the absence of Ca^{2+} influx *via* defective voltage-dependent Ca^{2+} channels) not only perform ciliary reversal upon (mock)stimulation with AED, but also display the usual [ATP] decay (Vilmart-Seuwen *et al.*, 1986; Müller *et al.*, 2002). As discussed in Section III.4, this is due to Ca^{2+} spillover from the soma into the cilia. In summary, ciliary reversal can be considered to represent an event of extra ATP consumption, assuming that it is increased by intense rotation activity during reversal. Still some additional mechanism may be involved.

(4) Effects of massive exocytosis on ATP synthesis

Some of the [ATP] decay seen upon exocytosis stimulation may be due to inhibited ATP synthesis because of kinetic

considerations resulting from the mass action law. During AED stimulation the dynamic equilibrium between ADP, ATP and PO_4^{3-} will be affected by increased $[\text{Ca}^{2+}]$. When calculated from the contribution of the two Ca^{2+} sources, release from alveolar sacs and influx, and neglecting downregulation phenomena, the global $[\text{Ca}^{2+}]$ would increase to 0.325 mM (Hardt & Plattner, 2000). Still higher values can theoretically be extrapolated for cortical cell layers within short periods, again neglecting rapid downregulation (e.g. by cortical CaBPs; see above and Sections III and V). Massive $[\text{Ca}^{2+}]_i$ increase has to be counteracted not only because of toxic effects of Ca^{2+} , but also because $[\text{Ca}^{2+}]$ could come close to, or even surpass the solubility of $[\text{PO}_4^{3-}]$ in the cells. Excessive $[\text{Ca}^{2+}]$ increase will bind PO_4^{3-} and, if not transiently forming highly insoluble calcium phosphates (see <http://butane.chem.uiuc.edu/pshapley/GenChem2/Tables.html>), it could reduce the availability of phosphate for the $\text{ADP} \rightarrow \text{ATP}$ rephosphorylation processes. Considering the rapid turnover of ATP (Section XII.1) the cells may be energetically compromised by this kinetic effect before the excess of Ca^{2+} is downregulated.

Therefore, the kinetics of ATP generation is crucial. In the cell cortex, the availability of ATP is expected to be restricted within narrow spaces, such as cilia, and the narrow space between plasmalemma and alveolar sacs. In large protozoan species, to improve availability of dissolved O_2 , mitochondria are frequently enriched in cortical layers (Fenchel, 2014). This has been observed in *P. tetraurelia* (H. Plattner, unpublished observation). In *Paramecium tetraurelia*, when analysed *in vitro*, [ATP] within cilia is only ~ 0.15 mM and, thus, only ~ 12 – 15% of the whole-cell value (Noguchi, Sawada & Akazawa, 2001). For sufficient energy supply *in vitro*, phosphoarginine has to be provided at concentrations of ~ 0.4 mM (Noguchi *et al.*, 2001). This serves for the rephosphorylation of ADP to ATP, the substrate of the dynein ATPase that drives ciliary beating, and involves phosphoarginine provided by a ciliary arginine kinase which in *Tetrahymena pyriformis* is 40 kDa in size (Michibata *et al.*, 2014). Generally, phosphagenes help to maintain the ATP level in narrow spaces, as discussed previously (Plattner & Kissmehl, 2005). In muscle, compensation mechanisms include phosphocreatine (in mammals) or phosphoarginine in crustaceans as a store of sufficient free enthalpy. In ciliates rapid restoration of energy supply can, thus, be achieved for the ciliary subcompartment (Noguchi *et al.*, 2001) and most likely also in the narrow space between the plasmalemma and alveolar sac. This mechanism requires positioning of some key enzymes precisely at relevant cortical regions (see below). The compensating effects of the phosphagenes may not suffice sufficiently to counteract the abrupt [ATP] decay in narrow spaces and the ciliate cell therefore activates glycolytic substrates, as follows.

How can a *Paramecium* cell avoid being compromised by such ATP decay, particularly considering its rapid turnover? In *Paramecium tetraurelia*, a phosphoprotein of 63 kDa (pp63) is dephosphorylated during trichocyst exocytosis and was, therefore, called 'parafusin' (Gilligan & Satir, 1982). Its dephosphorylation actually occurs during the 80 ms required

for AED-induced synchronous exocytosis (Höhne-Zell *et al.*, 1992), followed by rephosphorylation during ~ 10 – 30 s (Ziesenis & Plattner, 1985). When the ‘non-discharge’ mutant strain *nd9-28°C* (Gilligan & Satir, 1982) or any of the other exocytosis-incompetent strains (Ziesenis & Plattner, 1985) are (mock)stimulated for exocytosis, parafusin/pp63 is not dephosphorylated. [Recall that exocytosis-incompetent strains generate the same Ca^{2+} transient as wildtype cells (Klaue & Plattner, 1997) and the same ciliary response (Plattner *et al.*, 1984, 1985)]. Evidently the Ca^{2+} signal cannot be transmitted to the defective fusion machinery and none of the subsequent steps (membrane resealing, detachment of ghosts, i.e. surface restructuring) take place.

Yet parafusin/pp63 has been identified as phosphoglucosyltransferase (Hauser *et al.*, 1997; Müller *et al.*, 2002). According to MALDI analysis, the molecule is phosphorylated on Ser and Thr residues (Kusmann *et al.*, 1999). *In vitro* it is dephosphorylated by calcineurin/PP2B (Kissmehl *et al.*, 1996), cloned and characterised also in *Paramecium tetraurelia* (Fraga *et al.*, 2010). It is rephosphorylated by a Ca^{2+} -inhibited casein kinase, also known in *Paramecium tetraurelia* (Vetter *et al.*, 2003). Thus, a Ca^{2+} signal may induce and maintain the dephosphorylated state and cause activation of phosphoglucosyltransferase activity to funnel substrate into the glycolytic pathway within the narrow subplasmalemmal space where it is localised (Plattner & Kissmehl, 2005). This activation is assumed to depend on the desorption of dephospho-parafusin/(p)p63 from structures to which it is normally bound in its phosphorylated form. Soluble enzymes are known to have frequently a higher activity state in the free form, e.g. due to more enzyme/substrate ‘encounters’ and lack of steric constraints.

Thus, *Paramecium tetraurelia* cells reversibly dephosphorylate parafusin/pp63 for <30 s (Ziesenis & Plattner, 1985) and regain their normal ATP level within ~ 20 – 30 s (Vilmart-Seuwen *et al.*, 1986) after AED stimulation. Remarkably, restoration of the ATP pool is greatly retarded in cells unable to dephosphorylate parafusin/pp63 (Müller *et al.*, 2002; Plattner & Kissmehl, 2005). Considering an ATP turnover time of <30 s (see above) the viability of such cells could be compromised [although in knockout experiments with *Tetrahymena thermophila*, mucocyst exocytosis can still be performed (Chilcoat & Turkewitz, 1997)]. In fact, Ca^{2+} homeostasis is compromised in yeast cells after disruption of the phosphoglucosyltransferase gene (Fu *et al.*, 2000). Activation of phosphoglucosyltransferase can increase NADH and ATP production in cytosol and mitochondria and, thus, avoid compromising the cells energetically during massive exocytosis stimulation.

In addition, in *Paramecium tetraurelia* ATP consumption will in part be counteracted by stimulation of mitochondria. Upon induction of synchronous trichocyst exocytosis, $[\text{Ca}^{2+}]$ instantaneously increases in mitochondria (Hardt & Plattner, 2000). This requires a uniporter of a type detected in *Tetrahymena thermophila* (Bick, Calvo & Mootha, 2012). Comparison of signals measured in mitochondria for whole and free calcium Ca^{2+} using X-ray microanalysis (Hardt & Plattner, 2000) and fluorochrome analysis (Plattner *et al.*,

2012), respectively, reveals that only a small fraction of Ca^{2+} entering a mitochondrion is retained in the organelle. Consider that in muscle the entire cascade of oxidative phosphorylation (Glancy *et al.*, 2013) as well as ATP synthase activity is activated (Balaban, 2009) and only some of the mitochondrial matrix dehydrogenases (Denton, 2009). Therefore, a similar ATP-restoring effect can be reasonably expected to accompany exocytosis in *Paramecium* spp. Additional components of a mitochondrial Ca^{2+} uniporter have been identified in mammalian cells (Sancak *et al.*, 2013). Here, subunits type 1 and 2 of mitochondrial calcium uptake (MICU), the pore-forming subunit mitochondrial calcium uniporter (MCU) in conjunction with the MCU regulator, essential MCU regulator (EMRE), mediate interaction of subunits to form a functional channel (Kamer & Mootha, 2014). Note that MICUs are EF-hand CaBPs. In *Tetrahymena thermophila*, MCU and MICU1 have been identified, in contrast to EMRE, while MICU2 was not the focus of that study (Sancak *et al.*, 2013). Thus, important aspects are already in place to support the hypothesis that ATP decay is immediately counteracted by resynthesis, also in mitochondria.

In summary there remain several possible explanations for the ATP decay seen during massive exocytosis induction. (i) Ciliary reversal (assuming that it consumes more ATP than normal forward swimming). (ii) A secondary active Ca^{2+} transport coupled directly or indirectly to a H^+ -ATPase is likely to be more important than a primary active Ca^{2+} transport. (iii) A swift (5 s) reduction in ATP synthesis is expected for kinetic reasons, i.e. $\text{Ca}^{2+}/\text{PO}_4^{3-}$ disequilibrium due to a significant cortical $[\text{Ca}^{2+}]$ increase. The activation of parafusin/pp63 by dephosphorylation and the activation of mitochondrial ATP synthesis are considered able to counteract ATP decay efficiently, so that [ATP] can be restored within ~ 20 – 30 s without compromising the vitality of the cells even after massive exocytosis stimulation.

XIII. FEEDBACK FROM CYTOPLASMIC FUNCTIONAL STATES, FROM MITOCHONDRIA AND FROM ENDOSYMBIONTS TO THE MACRONUCLEUS

Generally cells control the production of proteins according to functional needs, based on a feedback system (Fig. 10). Mucocyst biogenesis in *Tetrahymena thermophila* has served as a paradigm in the field (Haddad & Turkewitz, 1997) and the underlying mechanisms may be of medical interest (Hutton, 1997). In ciliates, feedback has to reach the macronucleus, but how signalling takes place in molecular terms remains to be explored. Remarkably, in *Tetrahymena thermophila*, the micro- and macronucleus have different subsets of nucleoporins which may mediate selective transport of signals into the macronucleus (Iwamoto *et al.*, 2009). In *Paramecium tetraurelia*, corresponding genes are upregulated after massive exocytosis, as is also the case after radical deciliation. A genome-wide microarray analysis of gene expression in *Tetrahymena thermophila* revealed the upregulation of an unexpectedly

Fig. 10. A variety of signals feed back into the transcriptionally active macronucleus of ciliates. This occurs after massive exocytosis or after deciliation; other signals emerge from endosymbiotic bacteria and green algae (similarly to signals arising from mitochondria).

large number of specific genes also during conjugation (Miao *et al.*, 2009). Endosymbionts exert a similar feedback to transcription (Fig. 10). In higher eukaryotes, nuclear feedback is particularly well established for endosymbiont-derived organelles, such as mitochondria, but data for ciliates are not yet very detailed.

(1) Feedback from the cytoplasm to the macronucleus

Some signalling molecules are transferred from the cytosol into the nucleus to influence transcription. A longstanding example is the transcription factor, 'nuclear factor of activated T-cells' (NFAT), that is activated *via* dephosphorylation by PP2B and then activates T-lymphocytes (Baksh & Burakoff, 2000). Interestingly this phosphatase also occurs in *Paramecium tetraurelia* according to evidence from cell biology (Momayezi *et al.*, 1987), biochemistry (Kissmehl *et al.*, 1997) and molecular biology (Fraga *et al.*, 2010). However, nuclear signalling by calcineurin has not yet been explored in ciliates.

In ciliates, feedback from the cytosol to the transcriptionally active macronucleus does take place. This has been shown in connection with massive mucocyst release in *Tetrahymena thermophila* (Haddad & Turkewitz, 1997) as well as in *Paramecium tetraurelia* after massive trichocyst exocytosis (Arnaiz *et al.*, 2010). The same occurs in the course of reciliation following deciliation (Arnaiz *et al.*, 2010). Genes encoding components of the respective organelles, which have to be synthesized *de novo*, are most intensely transcribed, but many others are also upregulated. Not all translation products can easily be fitted into the spectrum one would expect based on current knowledge about signalling from cilia to the nucleus in mammalian cells (Basten & Giles, 2013) and about biosynthesis and function of cilia and of dense core-secretory organelles. Although unexplored, in ciliates, PP2B/calcineurin may play a role in organelle regeneration by activation of gene transcription. In metazoans, different specific transcription factors were identified as crucial for ciliary biogenesis and function (Choksi *et al.*, 2014).

In higher eukaryotes nuclear actin is capable of remodelling chromatin (Kapoor & Shen, 2014) and can regulate the transcriptional activity of RNA polymerases (Treisman,

2013). This may also be applicable to ciliates, as *PtAct4* localises to the transcriptionally active macronucleus in regions adjacent to heterochromatin domains (Sehring *et al.*, 2010). Its monomeric nuclear form (Kapoor & Shen, 2014) would not have been detected in studies with fluorescent drugs, such as phalloidin, for which this actin paralog has no binding motifs.

(2) Feedback from mitochondria and endosymbionts

In general terms, feedback between endosymbiont and nuclear genomes is important in several regards. Early in evolution symbiosis led to the transformation of bacteria to mitochondria. With pathogenic Apicomplexa the interplay between a vestigial genome of a plastid-derived endosymbiont with mitochondria and cytosol has received attention as an example of recent evolution, with the potential for medical exploitation (Howe & Purton, 2007). Currently the interplay between *Legionella pneumophila* and amoebae is also raising medical interest (Escoll *et al.*, 2013). In *Paramecium* spp., bacterial endosymbionts provide a feedback to the nuclear genome. Studies about endosymbionts in ciliates may yield hints to mechanisms for establishing obligatory endosymbiosis.

(a) Mitochondria

Data about signalling between mitochondria and the macronucleus in ciliates, including *Paramecium* spp., are limited (Sainsard-Chanet & Cummings, 1988; Beale & Preer, 2008). As expected from other organisms, the biosynthesis of important enzymes depends on proper interaction between the nuclear and the mitochondrial genome. Wildtype mitochondria microinjected into *Paramecium tetraurelia* mutants or into related species, are degraded due to incompatibility (Sainsard-Chanet & Cummings, 1988), thus suggesting fine-tuning between the two compartments. Remarkably, in humans, transfer of mitochondria from healthy donors, combined with *in vitro* fertilisation, is currently under public discussion as a means to overcome mitochondriopathies.

The mitochondrial genome of several ciliates, i.e. species of *Euplotes*, *Paramecium* and *Tetrahymena*, is now fully sequenced. How is crosstalk between the mitochondrial and the nuclear genome effected? 44 protein-coding genes are found in the mitochondrial DNA of *T. pyriformis* (Barbrook *et al.*, 2010), but only 13 in man (Schon, DiMauro & Hirano, 2012). According to mass spectrometry of mitochondria isolated from *Tetrahymena thermophila*, 545 of a total of 573 identified mitochondrial proteins are imported (Smith *et al.*, 2007). In *Tetrahymena thermophila*, compared to man, fewer mitochondrial tRNAs (Brunk *et al.*, 2003) and mitochondrial ribosomal RNAs are encoded by mitochondrial DNA (Barbrook *et al.*, 2010). This is in agreement with a tendency for decreasing autonomy of mitochondria during evolution. In the future, genomics and proteomics should allow us to delve more deeply into the signalling between mitochondrial and macronuclear genomes in ciliates.

(b) *Symbionts*

Among ciliates, *Paramecium* species are particularly liable to the symbiotic integration of bacteria, e.g. *Holospira* species, as obligatory symbionts in their cytoplasm or macronucleus. A periplasmic protein of 89 kDa supports host cell invasion (Fujishima & Kodama, 2012). This alters gene transcription and lowers stress sensitivity. Signalling processes required for the escape from phagosomes and the establishment of symbiosis may now be more easily amenable to systematic study with genetically altered *Escherichia coli* cells, as shown with *Tetrahymena pyriformis* (Siegmund *et al.*, 2013).

Some ciliate species (e.g. *Paramecium bursaria*) can establish permanent symbiosis with *Chlorella* algae. Experimental infection revealed distinct steps (Kodama & Fujishima, 2010): (i) phagocytosis of the algae, (ii) fusion with acidosomes, (iii) fusion with lysosomes; (iv) some of the surviving *Chlorella* cells bud from the phagolysosomes and, enclosed in a membrane, (v) accumulate at the cell periphery. Signals for these distinct steps, also including resistance to the fusion of stage iv-symbionts with lysosomes, remain to be elucidated. It became evident from mRNA profiling that, after subtraction of *Chlorella* sequences, the transcription of many proteins is selectively enhanced in symbiont-bearing *P. bursaria* cells (Kodama *et al.*, 2014). The propagation of the algae is synchronised with *Paramecium*'s cell cycle by an unknown signal (Gerashchenko, 2010).

XIV. DOWNREGULATION OF THE MOST COMMON ACTIVATION MECHANISMS

In many cases, activation of a cell should be transient, occasionally even very short. A good example is Ca^{2+} (Berridge *et al.*, 2003) of which some aspects in *Paramecium* spp. have been discussed in preceding sections. Ca^{2+} signals in particular have to be rapidly downregulated, not only to avoid toxic effects, but also to minimise energy expenditure and to restrict the signal to very local effects near precisely positioned channels (Sections I, II, VIII and XII). In all eukaryotic cells inactivation mechanisms can include feedback by downregulation of Ca^{2+} influx or mobilisation, by binding to CaBPs, and by sequestration or extrusion from the cell. Metabolic messengers, such as cyclic nucleotides are broken down by phosphodiesterases (few details being known for ciliates). Similarly protein phosphorylation can be reversed by phosphatases of which ciliates possess different types. Ciliates have examples of all these mechanisms, as summarised in Fig. 7 for downregulation of Ca^{2+} signals by primary active and possibly also by secondary active transporters.

For Ca^{2+} signals there are different routes of inactivation (Berridge *et al.*, 2003). Negative feedback by the inhibitory Ca^{2+} /CaM complex can be used to inactivate ciliary voltage-dependent Ca^{2+} -channels during ciliary reversal (Brehm & Eckert, 1978), as outlined in Section III.2. Binding to high-capacity/low-affinity CaBPs can also terminate

a Ca^{2+} signal. This is achieved, for instance, by cortical centrin after massive trichocyst exocytosis stimulation (Sehring *et al.*, 2009), but may also take place at the base of cilia to terminate ciliary Ca^{2+} signals (Gonda, Oami & Takahashi, 2007). Slow dissipation of Ca^{2+} from the low-affinity binding sites of CaBPs may subsequently allow its removal by extrusion. In *Paramecium* spp. this probably involves a secondary active transporter (putative H^+ / Ca^{2+} and/or a similar antiporter) for Ca^{2+} sequestration in acidic compartments, such as the contractile vacuole complex (Plattner *et al.*, 2012; Plattner, 2013a) and periodic release via the contractile vacuole pore (Stock *et al.*, 2002). This can be concluded from the effect of the H^+ -ATPase inhibitor, concanamycin B (Grønlien *et al.*, 2002), which inhibits $[\text{Ca}^{2+}]_i$ downregulation after exocytosis stimulation (Plattner *et al.*, 2012), as outlined in Sections VI and XII. An abundance of primary and secondary active transporters and channels have been implied by bioinformatic analysis of the genomic databases of *P. tetraurelia*, *T. thermophila* and of the fish pathogen, *Ichthyophthirius multifiliis* (Kumar & Saier, 2015).

Ciliates possess primary active Ca^{2+} transporters, including the sarcoplasmic/endoplasmic reticulum- (SERCA-) type and the PMCA. Both are P-type ATPases, i.e. forming a phospho-intermediate. In ciliates, the kinetics of the SERCA is much slower than required for rapid Ca^{2+} downregulation (Mohamed *et al.*, 2003) and the same may hold true for the PMCA (Plattner & Klauke, 2001; Section XII). For instance, alveolar sacs are refilled with $t_{1/2} = 65$ min (Mohamed *et al.*, 2003). Therefore, secondary active transport systems are probably more important (Plattner, 2014), although reliable data are still missing. In ciliates, primary active Ca^{2+} transport appears more appropriate for general household functions rather than for even moderately rapid Ca^{2+} dynamics.

Many of the signals regulating membrane trafficking involve several specific steps and, therefore, are transient. This applies, e.g. to the reversible binding of successive ligands, as is the case with small GTPases. Also the phosphorylation state of proteins may change in signalling cascades. In cilia protein phosphatase 1 (PP1) and PP2C are localised to the peripheral part of the axoneme (Momayezi *et al.*, 1996; Grothe *et al.*, 1998) and thus could contribute to reversing the state of phosphorylation, i.e. of activation. For instance, termination of ciliary reversal is inhibited by inhibition of PP1 activity (Klump *et al.*, 1990). The importance of the dephosphorylation of dynamin – a well-established prerequisite for clathrin-mediated endocytosis in other systems – is suggested by the association of PP2B/calcineurin with parasomal sacs in *Paramecium tetraurelia* (Momayezi, Kissmehl & Plattner, 2000).

The behavioural response to exogenous nucleotide triphosphates, i.e. repetitive back- and forward swimming, in *Tetrahymena* spp. and *Paramecium* spp. (Section X) is spontaneously attenuated and ended upon longer exposure time, probably by receptor adaptation (Kim *et al.*, 1999; Hennessey, 2005).

XV. EPIGENETIC SIGNALLING, ENCYSTMENT AND 'PROGRAMMED NUCLEAR DEATH' IN CILIATES

Epigenetic signalling by non-DNA-encoded signals is the focus of current biomedical research, particularly since the discovery of prion proteins (PrPs). In mammals, the normal conformation PrP^c can be misfolded to PrP^{sc} ('c' and 'sc' standing for cellular and scrapie disease, respectively). The PrP^{sc} form propagates based on epigenetic information transfer onto PrP^c molecules – until the abundance of PrP^{sc} acts as a pathogen causing brain degeneration (Prusiner, 2004). Remarkably, the concept of epigenetic information transfer is based on seminal observations with fungi and ciliates (Beisson, 2008). The regular design of the ciliate cell surface (Section I.4), its surface antigens, macronuclear differentiation etc. are all epigenetically determined, thus making ciliates attractive models (Simon & Plattner, 2014). Phenomena thus controlled range from tailoring of the new macronuclear genome (Duharcourt, Lepère & Meyer, 2009) when old macronuclei are disrupted ('nuclear death', see Section XV.2) and non-Mendelian expression of surface molecules (Sonneborn, 1949) to cell surface pattern formation (Frankel, 1973; Beisson, 2008) and even change of cell size and shape. The information may come from the macronucleus, the cell surface, or from soluble material (kairomones) in the medium. Several important molecular key players have recently been detected in ciliates and are discussed below in the context of epigenetic signalling.

The second aspect discussed here concerns apoptosis-like phenomena. In metazoans, apoptosis is the most prominent form of programmed cell death which is regulated by a complicated network. This includes 'DNA ladder' formation by fragmentation to nucleosome-size DNA pieces and multiples thereof, mitochondrial degeneration and participation of nuclear, mitochondrial and cytosolic proteins. This includes apoptosis-inducing factors, protease-type caspases, endonucleases, etc. (Norberg, Orrenius & Zhivotovsky, 2010). Elements of the apoptotic machinery can be traced back to unicellular unikonts, such as choanoflagellates (Zmasek & Godzik, 2013), although important questions remain open with all protozoa.

While ciliate cells can divide over long periods, for 'quasi-eternal' life, they can rejuvenate their macronuclei by formation of new ones from the micronucleus (corresponding to soma and germline, respectively, in metazoa). There are two ways, (i) conjugation (cell pairing with exchange of dividing micronuclei) and (ii) autogamy (internal 'fertilization' by fusion of dividing micronuclei within one cell) – each of these processes resulting in the formation of a new macronucleus per cell, serving for gene transcription. Degradation of the old macronucleus is paralleled by formation of a 'DNA ladder' and the abundance of autophagy, including mitochondria, with this respect closely resembling genuine apoptosis (Endoh & Kobayashi, 2006). Is it justified to consider these details as sufficient criteria for programmed cell death – or rather 'programmed nuclear death'?

(1) Feedback from intracellular epigenetic signals

Regulation of surface pattern formation has to consider not only the specific localisation of components in anterior/posterior, top/bottom and left/right dimensions, but also pattern formation on a variable scale. Examples range from the polar arrangement of somatic ion channels, sites of contractile vacuole and extrusion sites of spent food vacuoles (cytoproct), arrangement of cilia for rotational movement to organisation of kinetids. All are epigenetically controlled (Beisson & Sonneborn, 1965; Frankel, 1973; Beisson & Jerka-Dziadosz, 1999; Beisson, 2008). Many factors cooperate, as derived from the analysis of mutants of different *Paramecium* and *Tetrahymena* species. Whole-genomic analysis of covariation in mutants or during knockout of selective components (see below) would appear feasible, considering these widely different components.

In *Paramecium tetraurelia* cells, surface pattern formation depends on protein phosphorylation processes (Keryer *et al.*, 1987; Sperling *et al.*, 1991). Another factor is the self-assembly of epiplasmins (Aubusson-Fleury *et al.*, 2013). Alveolins, proteins with charged repeat motifs, appear relevant for regular surface pattern formation since knock-down entails disorganised cortical pattern in misshapen cells, as demonstrated with *Tetrahymena thermophila* (El-Haddad *et al.*, 2013). Such effects are also observed after knock-down of other cortical components, e.g. of a cortical actin isoform, PtAct4 (Sehring *et al.*, 2010) and a variety of SNAREs and PtCRCs (Simon & Plattner, 2014). Since γ -tubulin and η -tubulin are required for basal body duplication in *Paramecium tetraurelia* (Ruiz *et al.*, 1999, 2000) their proper positioning may be another critical factor, together with centrin, in *Paramecium* spp. (Ruiz *et al.*, 2005) as well as in *Tetrahymena thermophila* (Stemm-Wolf *et al.*, 2005). So far, the role of cortical actin in cortex formation has not been explored in ciliates although it is known from mammalian cells to contribute together with tubulin (Li & Gundersen, 2008). In addition to these examples many more factors may come into play. When ciliary biogenesis has been analysed in mutants by light and electron microscopy, as recently summarised by Pearson (2014), multiple basal body formation in proper arrangement depends on asymmetrically spaced scaffolds. These include transiently visible structures, such as the 'anterior left filament' of infraciliary lattice filaments (ILF) (Jerka-Dziadosz *et al.*, 2013) and centrin isoforms -2 and -3 (Ruiz *et al.*, 2005; Stemm-Wolf *et al.*, 2005), together with their binding partners, e.g. members of the Sfi protein family (Stemm-Wolf, Meehl & Winey, 2013). Transient asymmetry thus forms and determines the mutual arrangement of newly forming basal bodies (Pearson, 2014).

Epigenetic signalling observed during formation of a new macronucleus after conjugation or autogamy is a consequence of nuclear dimorphism. Transcripts from the meiotic micronucleus pair with gene sequences from the old macronucleus to generate sequences destined for the newly forming macronucleus (Duharcourt *et al.*, 2009; Nowak *et al.*, 2011). This process results in the elimination of precisely defined 'internal eliminated sequences' (IES).

Originally this probably evolved as a mechanism for the elimination of transposons (Klobutcher & Herrick, 1995; Chalker & Yao, 2011), but later, in ciliates, this mechanism has been integrated into normal, epigenetically controlled cell development. IES elimination is executed by different transposases; in oligotrichs, such as *Paramecium* and *Tetrahymena*, it is a domesticated PiggyBack transposase (Vogt & Mochizuki, 2013) operating at sites determined by specific nucleotide flanks. Additional proteins required for IES elimination are 'protein involved in development of the somatic genome' (Pdsg) found in *Paramecium* (Arambasic *et al.*, 2014) and 'defective in excision' *PtDIE5* and *TtDIE5*, found in *P. tetraurelia* and *T. thermophila*, respectively (Matsuda *et al.*, 2010). Heterochromatin is formed on IES sites before excision and this depends on methylation of Lys residue 9 in histone 3 (Liu *et al.*, 2011). End-joining of excision sites involves a ligase of type IV of the non-homologous end-joining (NHEJ) pathway (Marmignon *et al.*, 2014).

Another example, epigenetic regulation of differential gene transcription under varying external or internal/physiological conditions, has been analysed in *Tetrahymena thermophila* by a combined bioinformatic and proteomic approach (Zhang *et al.*, 2014). Covariation of some specific post-translational histone modifications, including acetylation, methylation and mono-ubiquitination, indicates molecular crosstalk.

Other signalling mechanisms are under epigenetic control. For instance, mutually exclusive expression of isoforms of variant surface antigens (vsAG, 'serotypes' recognised by antibodies) in most strains of *P. tetraurelia* ensures the presence of one single kind of vsAG (GPI-anchored proteins) per cell at any time (Preer, Preer & Rudman, 1981; Preer *et al.*, 1987). This is regulated by a feedback mechanism (Gilley *et al.*, 1990; Matsuda & Forney, 2005). When a vsAG is removed, an alternative vsAG isoform can be implanted on the cell surface – all under the control of differential transcription (Leeck & Forney, 1996). Change of culture temperature (Antony & Capdeville, 1989; Benwakrim *et al.*, 1998) or application of antibodies against the specific serotype actually expressed both induce the removal of the old vsAGs and transcription of an alternative serotype protein (Juergensmeyer, 1969). The mutually exclusive expression of vsAGs in *Paramecium tetraurelia* depends on an epigenetic signal; only subtelomeric genes are engaged in the formation of RNAi-mediated silencing of the silent vsAG species (Baranasic *et al.*, 2014). Thus, the considerable extent of chromosome fragmentation in these ciliate cells becomes important.

The physiological function of vsAGs and details of their regulation in ciliates remain enigmatic (Simon & Schmidt, 2007). A signalling function in the sense of defence against predators has been proposed (Harumoto & Miyake, 1993; Clark & Forney, 2003). In this case, the sense, or precise function that vsAG variation remains to be answered.

Oxytrichia species are a special case where chromosome fragmentation is much more extensive than in other ciliates, followed by rearrangement of DNA during formation of a new macronucleus from a micronuclear precursor

(Nowacki & Landweber, 2009). In *Oxytrichia* spp., during nuclear transformation achieved by transiently expressed macronuclear RNA templates, some mutations occurring in the scnRNA can be transferred to the new macronucleus, while the micronuclear (germline) DNA is unchanged (Nowacki & Landweber, 2009). In this case the macronuclear genome can, therefore, be called an 'epigenome'.

(2) Feedback from extracellular epigenetic signals, encystment and 'programmed nuclear death'

(a) Epigenetics

Epigenetic signals can reach some ciliates from the surrounding medium. GPI-anchored proteins, such as vsAGs, when released from the cell surface of different ciliates, can potentially provide signalling phenomena (Simon & Kusch, 2013). Another example is chemical signalling among species by release of substances called kairomones. *Tetrahymena vorax* is known for its capability to adapt its oral cavity to the size of the prey available. Buhse (1967) named the responsible kairomone 'stomatin'. Note that such 'stomatins' are different from the scaffolding SPFH-family proteins (SPFH means stomatin, prohibitin, flotillin and HflK/C superfamily). Previous attempts to establish intracellular signal transduction mechanisms in *T. vorax* by inhibitor studies (Ryals, Bae & Patterson, 1999) suffer from the widely aberrant pharmacology of ciliates (Plattner *et al.*, 2009). The kairomone stomatin has been identified as a complex of ferrous iron and some nucleic acid catabolites (Smith-Somerville *et al.*, 2000). Other examples are anti-predator defences in planktonic ciliates (Lass & Spaak, 2003; Lynn, 2010), as has been detected with *Euplotes octocarinatus* (Kusch & Heckmann, 1992). These cells can react to the presence of a predatory species, *Lembadion bullinum*, by enlargement of body size and formation of body extensions to make them less prone to engulfment. A kairomone of 31.5 kDa, called L-factor, was isolated, and after cloning of the gene, a Cys-rich protein was recombinantly expressed in *E. coli* (Peters-Regehr, Kusch & Heckmann, 1997). The cDNA-derived protein was of slightly higher molecular weight, but exerted the same kairomone function as the natural L-factor, although at somewhat higher concentration (10^{-10} versus 10^{-12} M).

As in other eukaryotes, gene expression in ciliates can be regulated epigenetically. Many examples of such epigenetic signalling, e.g. by histone modifications, mutually exclusive expression of paralogs etc., are discussed elsewhere (Chalker *et al.*, 2013; Simon & Plattner, 2014).

(b) Cysts

Some ciliates, with the exception of *Paramecium* spp., react to different stress factors by the formation of cysts. Stress includes adverse environmental conditions, such as starvation or high cell density (Tomaru, 2002). Cysts are forms of latent life (parabiosis) with the capability of 'reanimation'. Although analysed in numerous morphological studies, biochemical

analysis by protein profiling has begun only recently with *Colpoda cucullus* (Sogame *et al.*, 2014). The expression of a heat shock protein and of specific actin isoforms is enhanced. In *Euplotes encysticus*, cyst formation induced by starvation and low temperature triggers the expression of a variety of proteins, in part of unknown function and in part related to gene and RNA regulation, protein degradation, oxidation resistance, stress response, transport and cytoskeletal organisation including a keratin-like protein (Chen *et al.*, 2014).

(c) Programmed nuclear death

The final signal an old macronucleus receives after conjugation or autogamy is that for its breakdown and elimination – a process resembling apoptosis. In ciliates, ‘programmed cell death’ is restricted to the degradation of the old (parental) macronucleus and, therefore, should rather be designated ‘programmed nuclear death’. Programmed nuclear death is accompanied by morphological features familiar from apoptotic metazoan cells, but there are also essential differences. In *Tetrahymena thermophila*, the macronucleus undergoes autophagy in the following steps (Akematsu, Pearlman & Endoh, 2010): (i) loss of nuclear pores in parallel to nuclear condensation; (ii) transformation of the outer nuclear membrane into an autophagosome-like membrane, with glycosylation sites and phosphatidylserine residues exposed to the cytosolic side; (iii) disintegration of the inner nuclear membrane; (iv) wrapping of mitochondria with a membrane to which lysosomes become attached; (v) stepwise fusion of these two organelles with the modified macronucleus, and finally (vi) autophagosomal digestion of nuclear and mitochondrial contents. This morphological sequence differs from apoptosis in metazoans where nuclei become heavily fragmented. It is unclear why only macronuclei are degraded; this may be due to differential permeation properties of nuclear pores in the macro- and micronuclei (Iwamoto *et al.*, 2009, 2015).

Marginal notes published in the context of work with protozoa provide few indications of molecular regulators of programmed nuclear death in ciliates (Srivastava *et al.*, 2010; Smirlis & Soteriadou, 2011). In *Tetrahymena thermophila* this is initiated by an apoptosis-inducing factor and accompanied by formation of a DNA ladder (Lu & Wolfe, 2001; Akematsu & Endoh, 2010), as is characteristic of apoptosis in metazoans. Two genes encoding homologs of apoptosis-inducing factor are found in the *Tetrahymena thermophila* genome (Akematsu & Endoh, 2010). Knock-out of an apoptosis-inducing factor gene gave ambiguous results (Akematsu *et al.*, 2012); either knock-out was incomplete, only one gene out of several ohnologs was knocked-out (when not very similar), or the effect was compensated by a mechanism yet to be determined. Unexpectedly the nuclease involved is of mitochondrial origin, as found with *Tetrahymena thermophila*; this enzyme, TMN1 (*Tetrahymena* mitochondrial nuclease), also occurs in *Paramecium tetraurelia* (Osada *et al.*, 2014). Probing *Tetrahymena* extracts with caspase substrates and caspase inhibitors was compatible with the presence of some

form of caspase activity (Ejercito & Wolfe, 2003; González *et al.*, 2008). However, no gene for caspase proper has been found so far (Akematsu *et al.*, 2012). Molecular identification and characterisation of caspase-like proteins, similar to the paracaspase of *Dictyostelium discoideum* or the metacaspase of *Plasmodium falciparum* and *Arabidopsis thaliana* (Uren *et al.*, 2000), would be crucial.

In summary, some gross morphological traits and DNA ladder formation are in common for programmed cell death in metazoans and ‘programmed nuclear death’ in ciliates where, however, some essential features are either different or still require elucidation.

XVI. CONCLUSIONS

(1) Signalling implies long- and short-range information transfer, to achieve specific targeting and specific responses. Signalling regulates many aspects of subcellular dynamics. In principle, ciliates use similar, although not all, mechanisms for signalling and trafficking as metazoans, including Ca^{2+} and cyclic nucleotides, protein kinases and phosphatases, etc.

(2) Microtubules serve as long-range signals in different parts of the ciliate cell. Short-range signals include cues for molecular interactions, as is the case with SNARE proteins and small GTPases.

(3) Signalling by Ca^{2+} is elaborate in ciliates, including different influx and intracellular release channels. A local Ca^{2+} signal probably is required for vesicle docking and certainly for eventual vesicle/membrane fusion. The signal-transducing Ca^{2+} sensor remains unidentified in ciliates.

(4) Different, specifically localised influx channels regulate ciliary responses. Depolarisation *via* channels in the non-ciliary membrane activates voltage-dependent Ca^{2+} channels in the cilia and thereby ciliary beat reversal. Hyperpolarisation accelerates forward swimming.

(5) Ca^{2+} , cyclic nucleotides and protein phosphorylation cooperate in the regulation of ciliary beating. Ser/Thr phosphorylation prevails over Tyr phosphorylation. In ciliates, dedicated Tyr-kinases probably do not contribute to signalling beyond a MAPK activity chain. Much work remains to be done.

(6) In ciliates, specific isoforms of CRCs related to IP_3Rs and RyR-related Ca^{2+} -release channels are localised to specific vesicles undergoing trafficking. This allows for very locally restricted signalling and mutually independent activation of widely different processes. Probably cADPR and NAADP are additional second messengers for activating CRCs in some Ca^{2+} stores of ciliates.

(7) Stimulated exocytosis is initiated by Ca^{2+} release from cortical stores (alveolar sacs), superimposed on store-operated Ca^{2+} -entry, SOC(E). This is an old mechanism conserved in lineages up to man, e.g. in skeletal muscle. Molecules serving for coupling of the store with

the outside medium are unknown and possibly different in ciliates.

(8) Important aspects still to be elaborated, or even to be detected in ciliates are trimeric GTP-binding proteins and associated receptors, GPCRs being important signal transducers in metazoans. The same is true of organelle-specific targeting and effectors, e.g. of molecular cues mediating organelle specificity of SNAREs and of small GTPases.

(9) The identity and distribution of mechanosensitive channels, established in the plasmalemma, but expected also for food vacuoles and the contractile vacuole complex, have to be settled on the basis of the molecular candidates available from ciliate databases. Also to be scrutinised are details concerning the mechanism of transmission of chemical information to mechanical work in cilia.

(10) Epigenetic signalling, since its discovery in fungi and ciliates, is a particularly rewarding field for future research.

(11) One can now compare the molecular background of signalling in representatives of the two main evolutionary lineages, Uni- and Bikonta (Plattner & Verkhatsky, 2015). This includes choanoflagellates (Cai *et al.*, 2015) and animals (Cai, 2008; Cai *et al.*, 2015) on the one hand and ciliates (Plattner, 2015b) and plants (Edel & Kudla, 2015) on the other. Such a comparison reveals the following interesting features.

(12) Some signalling elements have been maintained throughout eukaryotes, unikonts and bikonts. Among them are Ca^{2+} , calmodulin, cyclic nucleotide-activated protein kinases, H^{+} -ATPase/pump, Rab proteins and SNAREs.

(13) Some functions are specific to bikonts. For example, Ca^{2+} /CaM-activated protein kinase of unikonts is replaced by CDPK in bikonts (Sections III, IX). Also in ciliates ‘synaptobrevins’ are longins, as in plants (Section IV.2).

(14) Some other functions occurring in ciliates are found in unikonts, while they are reduced or abolished in plants. For instance, the function of calcineurin is greatly reduced in plants, with only the B-subunit being conserved. In plants it serves for the regulation of channel protein phosphorylation to defend against stress (Edel & Kudla, 2015). By contrast, ciliates express both subunits, A and B, probably with pleiotropic functions (Sections XII.3 and XII.4). Dimeric calcineurin also occurs in unikonts, from myxamoebae to man. Both subunits are used by *Dictyostelium discoideum* for stress control (Thewes *et al.*, 2014) and, in man, for immune responses as well as for signal transduction and information storage in the central nervous system. Voltage-dependent Ca^{2+} -influx channels are absent from plants, as are IP_3R - and RyR -type CRCs. These are present in ciliates as well as in unikonts up to mammals (Plattner & Verkhatsky, 2012, 2015).

(15) There are examples of the presence of some signalling pathways in animals and plants, but with fragmentary evidence for ciliates. There are only suggestions for the occurrence of trimeric G-proteins in ciliates, although they are well documented for unikonts, from *Dictyostelium discoideum* (Manahan *et al.*, 2004) to man, and for ‘higher’

bikonts, such as plants (Ishida *et al.*, 2014). One may expect their identification also in ciliates. No synaptotagmin has been found in ciliates, in contrast to animals (Section IV.3 and IX.1) and plants (Craxton, 2004). However, ciliates contain synaptotagmin-like proteins, comparable to the ‘extended synaptotagmins’ of some animal cells, which might serve as a Ca^{2+} sensor, e.g. during exocytosis.

(16) The use of Tyr phosphorylation for signalling is now attributed not only to metazoans but increasingly also to plants (De la Fuente van Bentem & Hirt, 2008; Ghelis, 2011) – in contrast to ciliates, where it contributes to the MAPK signalling pathway (Section IX.2).

(17) In summary, in the signalling machinery of ciliates we observe traits in common to all eukaryotes, or in common with either other bikonts or unikonts. For some proteins common precursor molecules are likely to occur in protozoa, e.g. InsP_3 - and RyR s in ciliates (Plattner & Verkhatsky, 2012, 2015), whose descendants may have further differentiated in unikonts and been lost in plants. A strict separation of the two lineages on the basis of signalling is not possible. Thus, with regard to signalling, ciliates appear to be at the crossroads between uni- and bikonts. Further investigations may contribute to the ongoing controversies about the validity of deep-rooting phylogeny.

(18) The golden thread running through signalling in ciliates is that crucial pathways appeared early in evolution and have been maintained, with variations on a basic theme, over a billion years.

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XVIII. REFERENCES

- ADL, S. M., SIMPSON, A. G., FARMER, M. A., ANDERSEN, R. A., ANDERSON, O. R., BARTA, J. R., BOWSER, S. S., BRUGEROLLE, G., FENSOME, R. A., FREDERICQ, S., JAMES, T. Y., KARPOV, S., KUGRENS, P., KRUG, J., LANE, C. E., LEWIS, L. A., LODGE, J., LYNN, D. H., MANN, D. G., MCCOURT, R. M., MENDOZA, L., MOESTRUP, O., MOZLEY-STANDRIDGE, S. E., NERAD, T. A., SHEARER, C. A., SMIRNOV, A. V., SPIEGEL, F. W. & TAYLOR, M. F. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* **52**, 399–451.
- ADL, S. M., SIMPSON, A. G. B., LANE, C. E., LUKES, J., BASS, D., BOWSER, S. S., BROWN, M. W., BURKI, F., DUNTHORN, M., HAMPL, V., HEISS, A., HOPPENRATH, M., LARA, E., LE GALL, L., LYNN, D. H., McMANUS, H., MITCHELL, E. A. D., MOZLEY-STANDRIDGE, S. E., PARFREY, L. W., PAWLOWSKI, J., RUECKERT, S., SHADWICK, L., SCHOCH, C. L., SMIRNOV, A. & SPIEGEL, F. W. (2012). The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology* **59**, 429–493.
- ADOUTTE, A., DELGADO, P., FLEURY, A., LEVILLIERS, N., LAINÉ, M. C., MARTY, M. C., BOISVIEUX-ULRICH, E. & SANDOZ, D. (1991). Microtubule diversity in ciliated cells: evidence for its generation by post-translational modification in the axonemes of *Paramecium* and quail oviduct cells. *Biology of the Cell* **71**, 227–245.
- AKEMATSU, T. & ENDOH, H. (2010). Role of apoptosis-inducing factor (AIF) in programmed nuclear death during conjugation in *Tetrahymena thermophila*. *BMC Cell Biology* **11**, 13.
- AKEMATSU, T., KOBAYASHI, T., OSADA, E., FUKUDA, Y., ENDOH, H. & PEARLMAN, R. E. (2012). Programmed nuclear death and its relation to apoptosis and autophagy during sexual reproduction in *Tetrahymena thermophila*. *Japanese Journal of Protozoology* **45**, 1–15.

- AKEMATSU, T., PEARLMAN, R. E. & ENDOH, H. (2010). Gigantic macroautophagy in programmed nuclear death of *Tetrahymena thermophila*. *Autophagy* **6**, 901–911.
- ALLEN, R. D. (1974). Food vacuole membrane growth with microtubule-associated membrane transport in *Paramecium*. *Journal of Cell Biology* **63**, 904–922.
- ALLEN, R. D. & FOK, A. K. (2000). Membrane trafficking and processing in *Paramecium*. *International Review of Cytology* **198**, 277–318.
- ALLEN, R. D., MA, L. & FOK, A. K. (1993). Acidosomes: recipients of multiple sources of membrane and cargo during development and maturation. *Journal of Cell Science* **106**, 411–422.
- ALLEN, R. D. & NAITOH, Y. (2002). Osmoregulation and contractile vacuoles of protozoa. *International Review of Cytology* **215**, 351–394.
- ALLEN, P. D., TOMINAGA, T. & NAITOH, Y. (2009). The contractile vacuole complex and cell volume control in protozoa. In *Osmotic and Ionic Regulation: Cells and Animals* (ed. D. H. EVANS), pp. 69–106. CRC Press, Taylor and Francis Group, Boca Raton.
- ANANTHARAMAN, V., ABHIMAN, S., DE SOUZA, R. F. & ARAVIND, L. (2011). Comparative genomics uncovers novel structural and functional features of the heterotrimeric GTPase signaling system. *Gene* **475**, 63–78.
- ANISHKIN, A. & KUNG, C. (2013). Stiffened lipid platforms at molecular force foci. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 4886–4892.
- ANITEI, M. & HOFLACK, B. (2012). Bridging membrane and cytoskeleton dynamics in the secretory and endocytic pathways. *Nature Cell Biology* **14**, 11–19.
- ANN, K. S. & NELSON, D. L. (1995). Protein substrates for cGMP-dependent protein phosphorylation in cilia of wild type and atalanta mutants of *Paramecium*. *Cell Motility and the Cytoskeleton* **30**, 252–260.
- ANTONY, C. & CAPDEVILLE, Y. (1989). Uneven distribution of surface-antigens during antigenic variation in *Paramecium primaurelia*. *Journal of Cell Science* **92**, 205–215.
- ARAMBASIC, M., SANDOVAL, P. Y., HOEHENER, C., SINGH, A., SWART, E. C. & NOWACKI, M. (2014). Pds1 and Pds2, novel proteins involved in developmental genome remodelling in *Paramecium*. *PLoS One* **9**, e112899.
- ARNAIZ, O., GOÛT, J. F., BÉTERMIER, M., BOUHOUCHE, K., COHEN, J., DURET, L., KAPUSTA, A., MEYER, E. & SPERLING, L. (2010). Gene expression in a paleopolyploid: a transcriptome resource for the ciliate *Paramecium tetraurelia*. *BMC Genomics* **11**, 547.
- ARSLANYOLU, M. (2007). Cloning and partial characterization of *Tetrahymena thermophila* mitogen activated protein kinase 3 (TtMPK3) gene. *Journal of Applied Biological Sciences* **1**, 1–11.
- ARTALEJO, C. R., HENLEY, J. R., MCNIVEN, M. A. & PALFREY, H. C. (1995). Rapid endocytosis coupled to exocytosis in adrenal chromaffin cells involves Ca^{2+} , GTP, and dynamin but not clathrin. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 8328–8332.
- ARVAN, P. & CASTLE, D. (1998). Sorting and storage during secretory granule biogenesis: looking backward and looking forward. *Biochemical Journal* **332**, 593–610.
- AUBUSSON-FLEURY, A., BRICHEUX, G., DAMAJ, R., LEMULLOIS, M., COFFE, G., DONNADIEU, F., KOLL, F., VIGUES, B. & BOUCHARD, P. (2013). Epilasmis and epiplasm in *Paramecium*: the building of a submembranous cytoskeleton. *Protist* **164**, 451–469.
- AUBUSSON-FLEURY, A., LEMULLOIS, M., DE LOUBRESSE, N. G., LALIGNE, C., COHEN, J., ROSNET, O., JERKA-DZIADOSZ, M., BEISSON, J. & KOLL, F. (2012). The conserved centrosomal protein FOR20 is required for assembly of the transition zone and basal body docking at the cell surface. *Journal of Cell Science* **125**, 4395–4404.
- AUFDERHEIDE, K. J. (1978). Motility events of trichocyst insertion in *Paramecium tetraurelia*. *Journal of Protozoology* **25**, 362–365.
- AURY, J.-M., JAILLON, O., DURET, L., NOEL, B., JUBIN, C., PORCEL, B. M., SÉGURENS, B., DAUBIN, V., ANTHOUARD, V., AIACH, N., ARNAIZ, O., BILLAUT, A., BEISSON, J., BLANC, I., BOUHOUCHE, K., CAMARA, F., DUHARCOURT, S., GUIGO, R., GODENDEAU, D., KATINKA, M., KELLER, A.-M., KISSMEHL, R., KLOTZ, C., KOLL, F., LEMOUEL, A., LEPÈRE, G., MALINSKY, S., NOWACKI, M., NOWAK, J., PLATTNER, H., POULAIN, J., RUIZ, F., SERRANO, V., ZAGULSKI, M., DESSEN, P., BÉTERMIER, M., WEISSENBACH, J., SCARPELLI, C., SCHÄCHTER, V., SPERLING, L., MEYER, E., COHEN, J. & WINCKER, P. (2006). Global trends of whole genome duplications revealed by the genome sequence of the ciliate *Paramecium tetraurelia*. *Nature* **444**, 171–178.
- AVRAHAM, R. & YARDEN, Y. (2011). Feedback regulation of EGFR signalling: decision making by early and delayed loops. *Nature Reviews Molecular Cell Biology* **12**, 104–117.
- BAKSH, S. & BURAKOFF, S. J. (2000). The role of calcineurin in lymphocyte activation. *Seminars in Immunology* **12**, 405–415.
- BALABAN, R. S. (2009). The role of Ca^{2+} signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochimica et Biophysica Acta* **1787**, 1334–1341.
- BALDAUF, S. L., ROGER, A. J., WENK-SIEFERT, I. & DOOLITTLE, W. F. (2000). A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**, 972–977.
- BARANASIC, D., OPPERMAN, T., CHEAIB, M., CULLUM, J., SCHMIDT, H. & SIMON, M. (2014). Genomic characterization of variable surface antigens reveals a telomer position effect as a prerequisite for RNA interference-mediated silencing in *Paramecium tetraurelia*. *mBio* **5**(6), e01328-14.
- BARBROOK, A. C., HOWE, C. J., KURNIAWAN, D. P. & TARR, S. J. (2010). Organization and expression of organellar genomes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **365**, 785–797.
- BARFORD, D. (2010). The structure and topology of protein serine/threonine phosphatases. In *Handbook of Cell Signaling* (Volume 2, eds R. A. BRADSHAW and E. A. DENNIS), pp. 677–681. Elsevier, New York.
- BARRITT, G. J. (1999). Receptor-activated Ca^{2+} inflow in animal cells: a variety of pathways tailored to meet different intracellular Ca^{2+} signalling requirements. *Biochemical Journal* **337**, 153–169.
- BASTEN, S. G. & GILES, R. H. (2013). Functional aspects of primary cilia in signaling, cell cycle and tumorigenesis. *Cilia* **2**, 6.
- BEALE, G. H. & PREE, J. R. JR. (2008). *Paramecium Genetics and Epigenetics*. CRC Press, Boca Raton.
- BEISSON, J. (2008). Preformed cell structure and cell heredity. *Prion* **2**, 1–8.
- BEISSON, J. & JERKA-DZIADOSZ, M. (1999). Polarities of the centriolar structure: morphogenetic consequences. *Biology of the Cell* **91**, 367–378.
- BEISSON, J. & SONNEBORN, T. M. (1965). Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. *Proceedings of the National Academy of Science of the United States of America* **53**, 275–282.
- BELL, W. E., PRESTON, R. R., YANO, J. & VAN HOUTEN, J. L. (2007). Genetic dissection of attractant-induced conductances in *Paramecium*. *Journal of Experimental Biology* **210**, 357–365.
- BEMM, F., SCHWARZ, R., FORSTER, F. & SCHULTZ, J. (2009). A kinome of 2600 in the ciliate *Paramecium tetraurelia*. *Federation of European Biological Societies Letters* **583**, 3589–3592.
- BENWAKRIM, A., TREMOLIERE, A., LABARRE, J. & CAPDEVILLE, Y. (1998). The lipid moiety of the GPI-anchor of the major plasma membrane proteins in *Paramecium primaurelia* is a ceramide: variation of the amide-linked fatty acid composition as a function of growth temperature. *Protist* **149**, 39–50.
- BERRIDGE, M. J. (2006). Calcium microdomains: organization and function. *Cell Calcium* **40**, 405–412.
- BERRIDGE, M. J., BOOTMAN, M. D. & LIPP, P. (1998). Calcium – a life and death signal. *Nature* **395**, 645–648.
- BERRIDGE, M. J., BOOTMAN, M. D. & RODERICK, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nature Reviews Molecular Cell Biology* **4**, 517–529.
- BEYENBACH, K. W. & WIECZOREK, H. (2006). The V-type H^{+} ATPase: molecular structure and function, physiological roles and regulation. *Journal of Experimental Biology* **209**, 577–589.
- BEZPROZVANNY, I. (2005). The inositol 1,4,5-trisphosphate receptor. *Cell Calcium* **38**, 261–272.
- BICK, A. G., CALVO, S. E. & MOOTHA, V. K. (2012). Evolutionary diversity of the mitochondrial calcium uniporter. *Science* **336**, 886.
- BILINSKI, M., PLATTNER, H. & MATT, H. (1981). Secretory protein decondensation as a distinct, Ca^{2+} -mediated event during the final steps of exocytosis in *Paramecium* cells. *Journal of Cell Biology* **88**, 179–188.
- BLATT, M. R. (2000). Cellular signaling and volume control in stomatal movements in plants. *Annual Review of Cell and Developmental Biology* **16**, 221–241.
- BLOODGOOD, R. A. (2012). The future of ciliary and flagellar membrane research. *Molecular Biology of the Cell* **23**, 2407–2411.
- BLUM, J. J., HAYES, A., JAMIESON, G. A. & VANAMAN, T. C. (1980). Calmodulin confers calcium sensitivity on ciliary dynein ATPase. *Journal of Cell Biology* **87**, 386–397.
- BOCKAERT, J. & PIN, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *European Molecular Biology Organisation Journal* **18**, 1723–1729.
- BONIFACINO, J. S. (2014). Adaptor proteins involved in polarized sorting. *Journal of Cell Biology* **204**, 7–17.
- BONINI, N. M., GUSTIN, M. C. & NELSON, D. L. (1986). Regulation of ciliary motility by membrane potential in *Paramecium*: a role for cyclic AMP. *Cell Motility and the Cytoskeleton* **6**, 256–272.
- BONINI, N. M. & NELSON, D. L. (1990). Phosphoproteins associated with cyclic nucleotide stimulation of ciliary motility in *Paramecium*. *Journal of Cell Science* **95**, 219–230.
- BOWMAN, G. R., ELDE, N. C., MORGAN, G., WINEY, M. & TURKEWITZ, A. P. (2005). Core formation and the acquisition of fusion competence are linked during secretory granule maturation in *Tetrahymena*. *Traffic* **6**, 303–323.
- BRADFORD, W., BUCKHOLZ, A., MORTON, J., PRICE, C., JONES, A. M. & URANO, D. (2013). Eukaryotic G protein signaling evolved to require G protein-coupled receptors for activation. *Science Signaling* **6**, ra37.
- BRÄUCKER, R., MACHEMER-RÖHNISCH, S. & MACHEMER, H. (1994). Gravid responses in *Paramecium caudatum* and *Didinium nasutum* examined under varied hypergravity conditions. *Journal of Experimental Biology* **197**, 271–294.
- BREHM, P. & ECKERT, R. (1978). Calcium entry leads to inactivation of calcium channel in *Paramecium*. *Science* **202**, 1203–1206.
- BRIGHT, L. J., KAMBESIS, N., NELSON, S. B., JEONG, B. & TURKEWITZ, A. P. (2010). Comprehensive analysis reveals dynamic and evolutionary plasticity of Rab GTPases and membrane traffic in *Tetrahymena thermophila*. *PLoS Genetics* **6**, e1001155.
- BRIGUGLIO, J. S., KUMAR, S. & TURKEWITZ, A. P. (2013). Lysosomal sorting receptors are essential for secretory granule biogenesis in *Tetrahymena*. *Journal of Cell Biology* **203**, 537–550.

- BROWN, D., PAUNESCU, T. G., BRETON, S. & MARSHANSKY, V. (2009). Regulation of the V-ATPase in kidney epithelial cells: dual role in acid-base homeostasis and vesicle trafficking. *Journal of Experimental Biology* **212**, 1762–1772.
- BRUNK, C. F., LEE, L. C., TRAN, A. B. & LI, J. (2003). Complete sequence of the mitochondrial genome of *Tetrahymena thermophila* and comparative methods for identifying highly divergent genes. *Nucleic Acids Research* **31**, 1673–1682.
- BUHSE, H. E. JR. (1967). Microstome-macrotope transformation in *Tetrahymena vorax* strain V2 type S induced by a transforming principle, stomatin. *Journal of Protozoology* **14**, 608–613.
- BUONANNO, F., ANESI, A., GUELLA, G., KUMAR, S., BHARTI, D., LA TERZA, A., QUASSINTI, L., BRAMUCCI, M. & ORTENZI, C. (2014). Chemical offense by means of toxicysts in the freshwater ciliate, *Coleps hirtus*. *Journal of Eukaryotic Microbiology* **61**, 293–304.
- BUONANNO, F., HARUMOTO, T. & ORTENZI, C. (2013). The defensive function of trichocysts in *Paramecium tetraurelia* against metazoan predators compared with the chemical defense of two species of toxin-containing ciliates. *Zoological Science* **30**, 255–261.
- BURNSTOCK, G. & VERKHATSKY, A. (2009). Evolutionary origins of the purinergic signalling system. *Acta Physiologica (Oxford)* **195**, 415–447.
- CAI, X. (2008). Unicellular Ca^{2+} signaling 'toolkit' at the origin of metazoa. *Molecular Biology and Evolution* **25**, 1357–1361.
- CAI, X., WANG, X., PATEL, S. & CLAPHAM, D. E. (2015). Insights into the early evolution of animal calcium signaling machinery: a unicellular point of view. *Cell Calcium* **57**, 166–173.
- CAPDEVILLE, Y. (2000). *Paramecium* GPI proteins: variability of expression and localization. *Protist* **151**, 161–169.
- CAVALIER-SMITH, T. (2010). Deep phylogeny, ancestral groups and the four ages of life. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **365**, 111–132.
- CERVIA, D., CATALANI, E., BELARDINELLI, M. C., PERROTTA, C., PICCHIETTI, S., ALIMENTI, C., CASINI, G., FAUSTO, A. M. & VALLESI, A. (2013). The protein pheromone Er-1 of the ciliate *Euplotes raikovi* stimulates human T-cell activity: involvement of interleukin-2 system. *Experimental Cell Research* **319**, 56–67.
- CHALKER, D. L., MEYER, E. & MOCHIZUKI, K. (2013). Epigenetics of ciliates. *Cold Spring Harbor Perspectives in Biology* **5**, a017764.
- CHALKER, D. L. & YAO, M. C. (2011). DNA elimination in ciliates: transposon domestication and genome surveillance. *Annual Review of Genetics* **45**, 227–246.
- CHEN, J., GAO, X., WANG, B., CHEN, F., WU, N. & ZHANG, Y. (2014). Proteomic approach to reveal the proteins associated with encystment of the ciliate *Euplotes encysticus*. *PLoS One* **9**, e97362.
- CHEN, Z. Y., IERACI, A., TENG, H., DALL, H., MENG, C. X., HERRERA, D. G., NYKJAER, A., HEMPSTEAD, B. L. & LEE, F. S. (2005). Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *Journal of Neuroscience* **25**, 6156–6166.
- CHIEN, Y. H., WERNER, M. E., STUBBS, J., JOENS, M. S., LI, J., CHIEN, S., FITZPATRICK, J. A., MITCHELL, B. J. & KINTNER, C. (2013). Bbpf1 is required to maintain cilia orientation. *Development* **140**, 3468–3477.
- CHILCOAT, N. D. & TURKEWITZ, A. P. (1997). In vivo analysis of the major exocytosis-sensitive phosphoprotein in *Tetrahymena*. *Journal of Cell Biology* **139**, 1197–1207.
- CHOI, J., TANAKA, K., CAO, Y., QI, Y., QIU, J., LIANG, Y., LEE, S. Y. & STACEY, G. (2014). Identification of a plant receptor for extracellular ATP. *Science* **343**, 290–294.
- CHOKSI, S. P., LAUTER, G., SWOBODA, P. & ROY, S. (2014). Switching on cilia: transcriptional networks regulating ciliogenesis. *Development* **141**, 1427–1441.
- CHRISTENSEN, S. T., GUERRA, C. F., AWAN, A., WHEATLEY, D. N. & SATIR, P. (2003). Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Current Biology* **13**, R50–R52.
- CHRISTENSEN, K. A., MYERS, J. T. & SWANSON, J. A. (2002). pH-dependent regulation of lysosomal calcium in macrophages. *Journal of Cell Science* **115**, 599–607.
- CHURAMANI, D., HOOPER, R., RAHMAN, T., BRAILOIU, E. & PATEL, S. (2013). The N-terminal region of two-pore channel 1 regulates trafficking and activation by NAADP. *Biochemical Journal* **453**, 147–151.
- CIPRIANO, D. J., JUNG, J., VIVONA, S., FENN, T. D., BRUNGER, A. T. & BRYANT, Z. (2013). Processive ATP-driven substrate disassembly by the N-ethylmaleimide-sensitive factor (NSF) molecular machine. *Journal of Biological Chemistry* **288**, 23436–23445.
- CLAPHAM, D. E. (2007). Calcium signaling. *Cell* **131**, 1047–1058.
- CLARK, T. G. & FORNEY, J. D. (2003). Free-living and parasitic ciliates. In *Antigenic Variation* (eds A. CRAIG and R. SCHERF), pp. 375–402. Elsevier, London.
- CLARK, K. D., HENNESSEY, T. M. & NELSON, D. L. (1993). External GTP alters the motility and elicits an oscillating membrane depolarization in *Paramecium tetraurelia*. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 3782–3786.
- CODDOU, C., YAN, Z., OBSIL, T., HUIDOBRO-TORO, J. P. & STOJILKOVIC, S. S. (2011). Activation and regulation of purinergic P2X receptor channels. *Pharmacological Reviews* **63**, 641–683.
- COGOLI, A., TSCHOPP, A. & FUCHS-BISLIN, P. (1984). Circumnutation observed without a significant gravitational force in spaceflight. *Science* **225**, 229–231.
- COLE, E. S., CASSIDY-HANLEY, D., PINELLO, J. F., ZENG, H., HSUEH, M., KOLBIN, D., OZZELLO, C., GIDDINGS, T., WINEY, M. & CLARK, T. G. (2014). Function of the male-gamete-specific fusion protein HAP2 in a seven-sexed ciliate. *Current Biology* **24**, 2168–2173.
- COLUCCIO, L. M. E. (2008). *Myosin. A Superfamily of Molecular Motors*. Springer, Dordrecht.
- CORIA, A. S., MASSERONI, M. L. & DIAZ ANEL, A. M. (2014). Regulation of PKD1-mediated Golgi to cell surface trafficking by Gαq subunits. *Biology of the Cell* **106**, 30–43.
- COSTE, B., MATHUR, J., SCHMIDT, M., EARLEY, T. J., RANADE, S., PETRUS, M. J., DUBIN, A. E. & PATAPOUTIAN, A. (2010). Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **330**, 55–60.
- CRAXTON, M. (2004). Synaptotagmin gene content of the sequenced genomes. *BMC Genomics* **5**, 43 (doi: 10.1186/1471-2164-5-43).
- DACKS, J. B. & FIELD, M. C. (2007). Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. *Journal of Cell Science* **120**, 2977–2985.
- DALGHI, M. G., FERNANDEZ, M. M., FERREIRA-GOMES, M., MANGIALAVORI, I. C., MALCHIODI, E. L., STREHLER, E. E. & ROSSI, J. P. (2013). Plasma membrane calcium ATPase activity is regulated by actin oligomers through direct interaction. *Journal of Biological Chemistry* **288**, 23380–23393.
- DE FRANCESCHI, N., WILD, K., SCHLACHT, A., DACKS, J. B., SINNING, I. & FILIPPINI, F. (2014). Longin and GAF domains: structural evolution and adaptation to the subcellular trafficking machinery. *Traffic* **15**, 104–121.
- DE LA FUENTE VAN BENTEM, S. & HIRT, H. (2008). Protein tyrosine phosphorylation in plants: more abundant than expected? *Trends in Plant Science* **14**, 71–76.
- DENTLER, W. L. (1988). Fractionation of *Tetrahymena* ciliary membranes with Triton S-114 and the identification of a ciliary membrane ATPase. *Journal of Cell Biology* **107**, 2679–2688.
- DENTON, R. M. (2009). Regulation of mitochondrial dehydrogenases by calcium ions. *Biochimica et Biophysica Acta* **1787**, 1309–1316.
- DE ONDARZA, J., SYMINGTON, S. B., VAN HOUTEN, J. L. & CLARK, J. M. (2003). G-protein modulators alter the swimming behavior and calcium influx of *Paramecium tetraurelia*. *Journal of Eukaryotic Microbiology* **50**, 349–355.
- DORRELL, R. G., BUTTERFIELD, E. R., NISBET, R. E. R. & HOWE, C. J. (2013). Evolution: unveiling early aeolotes. *Current Biology* **23**, R1093–R1096.
- DUHARCOURT, S., LEPÈRE, G. & MEYER, E. (2009). Developmental genome rearrangements in ciliates: a natural genomic subtraction mediated by non-coding transcripts. *Trends in Genetics* **25**, 344–350.
- DURET, L., COHEN, J., JUBIN, C., DESSEN, P., GOÛT, J.-F., MOUSSET, S., AURY, J.-M., JAILLON, O., NOËL, B., ARNAIZ, O., BÉTERMIER, M., WINCKER, P. & MEYER, E. (2008). Analysis of the sequence variability in the macronuclear DNA of *Paramecium tetraurelia*. *Genome Research* **18**, 585–596.
- ECKERT, R. & BREHM, P. (1979). Ionic mechanisms of excitation in *Paramecium*. *Annual Review of Biophysics and Bioengineering* **8**, 353–383.
- EDEL, K. H. & KUDLA, J. (2015). Increasing complexity and versatility: how the calcium signaling toolkit was shaped during plant land colonization. *Cell Calcium* **57**, 231–246.
- EISEN, J. A., COYNE, R. S., WU, M., WU, D., THIAGARAJAN, M., WORTMAN, J. R., BADGER, J. H., REN, Q., AMEDEO, P., JONES, K. M., TALLON, L. J., DELCHER, A. L., SALZBERG, S. L., SILVA, J. C., HAAS, B. J., MAJOROS, W. H., FARZAD, M., CARLTON, J. M., SMITH, R. K. JR., GARG, J., PEARLMAN, R. E., KARRER, K. M., SUN, L., MANNING, G., ELDE, N. C., TURKEWITZ, A. P., ASAI, D. J., WILKES, D. E., WANG, Y., CAI, H., COLLINS, K., STEWART, B. A., LEE, S. R., WILAMOWSKA, K., WEINBERG, Z., RUZZO, W. L., WLOGA, D., GAERTIG, J., FRANKEL, J., TSAO, C. C., GOROVSKY, M. A., KEELING, P. J., WALLER, R. F., PATRON, N. J., CHERRY, J. M., STOVER, N. A., KRIEGER, C. J., DEL TORO, C., RYDER, H. F., WILLIAMSON, S. C., BARBEAU, R. A., HAMILTON, E. P. & ORIAS, E. (2006). Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. *PLoS Biology* **4**, e286.
- EJERCITO, M. & WOLFE, J. (2003). Caspase-like activity is required for programmed nuclear elimination during conjugation in *Tetrahymena*. *Journal of Eukaryotic Microbiology* **50**, 427–429.
- ELDE, N. C., MORGAN, G., WINEY, M., SPERLING, L. & TURKEWITZ, A. P. (2005). Elucidation of clathrin-mediated endocytosis in *Tetrahymena* reveals an evolutionarily convergent recruitment of dynamin. *PLoS Genetics* **1**, e52.
- EL-HADDAD, H., PRZYBORSKI, J. M., KRAFT, L. G., MCFADDEN, G. I., WALLER, R. F. & GOULD, S. B. (2013). Characterization of TtALV2, an essential charged repeat motif protein of the *Tetrahymena thermophila* membrane skeleton. *Eukaryotic Cell* **12**, 932–940.
- ELWESS, N. L. & VAN HOUTEN, J. L. (1997). Cloning and molecular analysis of the plasma membrane Ca^{2+} -ATPase gene in *Paramecium tetraurelia*. *Journal of Eukaryotic Microbiology* **44**, 250–257.
- ENDOH, H. & KOBAYASHI, T. (2006). Death harmony played by nucleus and mitochondria. Nuclear apoptosis during conjugation of *Tetrahymena*. *Autophagy* **2**, 129–131.
- ERNST, I. M., FLIEGERT, R. & GUSE, A. H. (2013). Adenine dinucleotide second messengers and T-lymphocyte calcium signaling. *Frontiers in Immunology* **4**, 259.

- ERXLEBEN, C., KLAUKE, N., FLÖTENMEYER, M., BLANCHARD, M. P., BRAUN, C. & PLATTNER, H. (1997). Microdomain Ca^{2+} activation during exocytosis in *Paramecium* cells. Superposition of local subplasmalemmal calcium store activation by local Ca^{2+} influx. *Journal of Cell Biology* **136**, 597–607.
- ESCOLL, P., ROLANDO, M., GOMEZ-VALERO, L. & BUCHRIESER, C. (2013). From amoeba to macrophages: exploring the molecular mechanisms of *Legionella pneumophila* infection in both hosts. *Current Topics in Microbiology and Immunology* **376**, 1–34.
- ESSELTINE, J. L. & SCOTT, J. D. (2013). AKAP signaling complexes: pointing towards the next generation of therapeutic targets? *Trends in Pharmacological Sciences* **34**, 648–655.
- EVANS, T. C. & NELSON, D. L. (1989). The cilia of *Paramecium tetraurelia* contain both Ca^{2+} -dependent and Ca^{2+} -inhibitable calmodulin-binding proteins. *Biochemical Journal* **259**, 385–396.
- FARHAN, H., WENDELER, M. W., MITROVIC, S., FAVA, E., SILBERBERG, Y., SHARAN, R., ZERIAL, M. & HAURI, H. P. (2010). MAPK signaling to the early secretory pathway revealed by kinase/phosphatase functional screening. *Journal of Cell Biology* **189**, 997–1011.
- FARRELL, A., THIRUGANAM, S., LORESTANI, A., DVORIN, J. D., EIDELL, K. P., FERGUSON, D. J., ANDERSON-WHITE, B. R., DURAISINGH, M. T., MARTH, G. T. & GUBBELS, M. J. (2012). A DOC2 protein identified by mutational profiling is essential for apicomplexan parasite exocytosis. *Science* **335**, 218–221.
- FASSHAUER, D., SUTTON, R. B., BRUNGER, A. T. & JAHN, R. (1998). Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q_2 - and R-SNAREs. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 15781–15786.
- FENCHEL, T. (2014). Respiration in heterotrophic unicellular eukaryotic organisms. *Protist* **165**, 485–492.
- FIELDING, A. B., SCHONTEICH, E., MATHESON, J., WILSON, G., YU, X., HICKSON, G. R. X., SRIVASTAVA, S., BALDWIN, S. A., PREKERIS, R. & GOULD, G. W. (2005). Rab11-FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. *European Molecular Biology Organisation Journal* **24**, 3389–3399.
- FINDEISEN, F., RUMPF, C. H. & MINOR, D. L. Jr. (2013). Apo states of calmodulin and CaBP1 control CaV1 voltage-gated calcium channel function through direct competition for the IQ domain. *Journal of Molecular Biology* **425**, 3217–3234.
- FLANNAGAN, R. S., JAUMOUILLE, V. & GRINSTEIN, S. (2012). The cell biology of phagocytosis. *Annual Review of Pathology* **7**, 61–98.
- FLÖTENMEYER, M., MOMAYEZI, M. & PLATTNER, H. (1999). Immunolabeling analysis of biosynthetic and degradative pathways of cell surface components (glycocalyx) in *Paramecium* cells. *European Journal of Cell Biology* **78**, 67–77.
- FOK, A. K. & ALLEN, P. D. (1993). Membrane flow in the digestive cycle of *Paramecium*. In *Membrane Traffic in Protozoa* (ed. H. PLATTNER), pp. 311–337. JAI Press, Greenwich, London.
- FOK, A. K., YAMAUCHI, K., ISHIHARA, A., AIHARA, M. S., ISHIDA, M. & ALLEN, R. D. (2002). The vacuolar-ATPase of *Paramecium multimicronucleatum*: gene structure of the B subunit and the dynamics of the V-ATPase-rich osmoregulatory membranes. *Journal of Eukaryotic Microbiology* **49**, 185–196.
- FORGAC, M. (2007). Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nature Reviews Molecular Cell Biology* **8**, 917–929.
- FOUNTAIN, S. J., PARKINSON, K., YOUNG, M. T., CAO, L., THOMPSON, C. R. & NORTH, R. A. (2007). An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. *Nature* **448**, 200–203.
- FRAGA, D., SEHRING, I. M., KISSMEHL, R., REISS, M., GAINES, R., HINRICHSSEN, R. & PLATTNER, H. (2010). Protein phosphatase 2B (PP2B, calcineurin) in *Paramecium*: partial characterization reveals that two members of the unusually large catalytic subunit family have distinct roles in calcium-dependent processes. *Eukaryotic Cell* **9**, 1049–1063.
- FRANKEL, J. (1973). Dimensions of control of cortical patterns in *Euplotes* – Role of preexisting structure, clonal life-cycle, and genotype. *Journal of Experimental Zoology* **183**, 71–94.
- FROISSARD, M., KELLER, A. M., DEDIEU, J. C. & COHEN, J. (2004). Novel secretory vesicle proteins essential for membrane fusion display extracellular-matrix domains. *Traffic* **5**, 493–502.
- FU, L., MISETA, A., HUNTON, D., MARCHASE, R. B. & BEDWELL, D. M. (2000). Loss of the major isoform of phosphoglucosyltransferase results in altered calcium homeostasis in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **275**, 5431–5440.
- FU, Z., SCHROEDER, M. J., SHABANOWITZ, J., KALDIS, P., TOGAWA, K., RUSTGI, A. K., HUNT, D. F. & STURGILL, T. W. (2005). Activation of a nuclear Cdc2-related kinase within a mitogen-activated protein kinase-like TDY motif by autophosphorylation and cyclin-dependent protein kinase-activating kinase. *Molecular Cell* **25**, 6047–6064.
- FUJISHIMA, M. & KODAMA, Y. (2012). Endosymbionts in *Paramecium*. *European Journal of Protistology* **48**, 124–137.
- GALATI, D. F., BONNEY, S., KRONENBERG, Z., CLARISSA, C., YANDELL, M., ELDE, N. C., JERKA-DZIADOSZ, M., GIDDINGS, T. H., FRANKEL, J. & PEARSON, C. G. (2014). DisA-dependent striated fiber elongation is required to organize ciliary arrays. *The Journal of Cell Biology* **207**, 705–715.
- GALIONE, A., EVANS, A. M., MA, J., PARRINGTON, J., ARREDOUANI, A., CHENG, X. & ZHU, M. X. (2009). The acid test: the discovery of two-pore channels (TPCs) as NAADP-gated endolysosomal Ca^{2+} release channels. *Pflügers Archiv European Journal of Physiology* **458**, 869–876.
- GARCÉS, J. A., HOEY, J. G. & GAVIN, R. H. (1995). Putative myosin heavy and light chains in *Tetrahymena*: co-localization to the basal body-cage complex and association of the heavy chain with skeletal muscle actin filaments in vitro. *Journal of Cell Science* **108**, 869–881.
- GARREAU DE LOUBRESSE, N. (1993). Early steps of the secretory pathway in *Paramecium*: ultrastructural, immunochemical, and genetic analysis of trichocyst biogenesis. In *Membrane Traffic in Protozoa* (ed. H. PLATTNER), pp. 27–59. JAI Press, Greenwich, London.
- GARREAU DE LOUBRESSE, N., GAUTIER, M. C. & SPERLING, L. (1994). Immature secretory granules are not acidic in *Paramecium*: implications for sorting to the regulated pathway. *Biology of the Cell* **82**, 139–147.
- GEBAUER, M., WATZKE, D. & MACHEMER, H. (1999). The gravikinetic response of *Paramecium* is based on orientation-dependent mechanotransduction. *Naturwissenschaften* **86**, 352–356.
- GERASHCHENKO, B. I. (2010). Proliferation of green *Paramecium bursaria*: a vision through flow cytometry. *Cytometry. Part A* **77**, 111–112.
- GERON, E., SCHEJTER, E. D. & SHILO, B. Z. (2013). Directing exocrine secretory vesicles to the apical membrane by actin cables generated by the formin mDial. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 10652–10657.
- GHELLIS, T. (2011). Signal processing by protein tyrosine phosphorylation in plants. *Plant Signaling and Behavior* **6**, 942–951.
- GILLESPIE, P. G. & WALKER, R. G. (2001). Molecular basis of mechanosensory transduction. *Nature* **413**, 194–202.
- GILLEY, D., RUDMAN, B. M., PREER, J. R. Jr. & POLISKY, B. (1990). Multilevel regulation of surface antigen gene expression in *Paramecium tetraurelia*. *Molecular Cell Biology* **10**, 1538–1544.
- GILLIGAN, D. M. & SATIR, B. H. (1982). Protein phosphorylation/dephosphorylation and stimulus-secretion coupling in wild type and mutant *Paramecium*. *Journal of Biological Chemistry* **257**, 13903–13906.
- GIORDANO, F., SAHEKI, Y., IDEVALL-HAGREN, O., COLOMBO, S. F., PIRRUCCELLO, M., MILOSEVIC, I., GRACHEVA, E. O., BAGRIANTSEV, S. N., BORGESE, N. & DE CAMILLI, P. (2013). $\text{PI}(4,5)\text{P}_2$ -dependent and Ca^{2+} -regulated ER-PM interactions mediated by the extended synaptotagmins. *Cell* **153**, 1494–1509.
- GLANCY, B., WILLIS, W. T., CHESSE, D. J. & BALABAN, R. S. (2013). Effect of calcium on the oxidative phosphorylation cascade in skeletal muscle mitochondria. *Biochemistry* **52**, 2793–2809.
- GODLEE, C. & KAKSONEN, M. (2013). Review series: from uncertain beginnings: initiation mechanisms of clathrin-mediated endocytosis. *Journal of Cell Biology* **203**, 717–725.
- GOLDBERG, J. M., MANNING, G., LIU, A., FEY, P., PILCHER, K. E., XU, Y. & SMITH, J. L. (2006). The *Dictyostelium* kinome—analysis of the protein kinases from a simple model organism. *PLoS Genetics* **2**, e38.
- GOLINSKA, K. (1982). Regulation of ciliary pattern in *Dileptus* (Ciliata). 1. Sensory cilia and their conversion into locomotor cilia. *Journal of Embryology and Experimental Morphology* **68**, 99–114.
- GOMPERTS, B. D., KRAMER, I. M. & TATHAM, P. E. R. (2009). *Signal Transduction*. Second Edition (J. Elsevier, London, New York).
- GONDA, K., KOMATSU, M. & NUMATA, O. (2000). Calmodulin and Ca^{2+} /calmodulin-binding proteins are involved in *Tetrahymena thermophila* phagocytosis. *Cell Structure and Function* **25**, 243–251.
- GONDA, K., OAMI, K. & TAKAHASHI, M. (2007). Centrin controls the activity of the ciliary reversal-coupled voltage-gated Ca^{2+} channels Ca^{2+} -dependently. *Biochemical and Biophysical Research Communications* **362**, 170–176.
- GONZÁLEZ, A. M., DÍAZ, S., GALLEGO, A. & GUTIÉRREZ, J. C. (2008). Programmed nuclear death and other apoptotic-like phenomena in ciliated protozoa. In *Programmed Cell Death in Protozoa* (ed. J. M. PÉREZ MARTÍN), pp. 143–159. Landes Bioscience and Springer Bioscience + Business Media, Georgetown.
- GOOD, M. C., ZALATAN, J. G. & LIM, W. A. (2011). Scaffold proteins: hubs for controlling the flow of cellular information. *Science* **332**, 680–686.
- GOYETTE, G., BOULAIS, J., CARRUTHERS, N. J., LANDRY, C. R., JUTRAS, I., DUCLOS, S., DERMINE, J. F., MICHNICK, S. W., LABOISSIERE, S., LAJOIE, G., BARREIRO, L., THIBAUT, P. & DESJARDINS, M. (2012). Proteomic characterization of phagosomal membrane microdomains during phagolysosome biogenesis and evolution. *Molecular and Cellular Proteomics* **11**, 1365–1377.
- GRAY, E. G. (1978). Synaptic vesicles and microtubules in frog motor endplates. *Proceedings of the Royal Society of London B: Biological Sciences* **203**, 219–227.
- GRIFFITHS, G. M., TSUN, A. & STINCHCOMBE, J. C. (2010). The immunological synapse: a focal point for endocytosis and exocytosis. *Journal of Cell Biology* **189**, 399–406.
- GRÖNLIEN, H. K., STOCK, C., AIHARA, M. S., ALLEN, R. D. & NAITOH, Y. (2002). Relationship between the membrane potential of the contractile vacuole complex and its osmoregulatory activity in *Paramecium multimicronucleatum*. *Journal of Experimental Biology* **205**, 3261–3270.

- GROSSHANS, B. L., ORTIZ, D. & NOVICK, P. (2006). Rabs and their effectors: achieving specificity in membrane traffic. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 11821–11827.
- GROTHE, K., HANKE, C., MOMAYEZI, M., KISSMEHL, R., PLATTNER, H. & SCHULTZ, J. E. (1998). Functional characterization and localization of protein phosphatase type 2C from *Paramecium*. *Journal of Biological Chemistry* **273**, 19167–19172.
- GUERON, S. & LEVIT-GUREVICH, K. (1999). Energetic considerations of ciliary beating and the advantage of metachronal coordination. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 12240–12245.
- GUSE, A. H. (2012). Linking NAADP to ion channel activity: a unifying hypothesis. *Science Signaling* **5**, pe18.
- HAACKE-BELL, B. & PLATTNER, H. (1987). Secretory lectins contained in trichocyst tips of *Paramecium*. *European Journal of Cell Biology* **44**, 1–9.
- HADDAD, A. & TURKEWITZ, A. P. (1997). Analysis of exocytosis mutants indicates close coupling between regulated secretion and transcription activation in *Tetrahymena*. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 10675–10680.
- HAMADA, K. & MIKOSHIBA, K. (2012). Revisiting channel allostery: a coherent mechanism in IP₃ and ryanodine receptors. *Science Signaling* **5**, pe24.
- HAMASAKI, T., BARKALOW, K., RICHMOND, J. & SATIR, P. (1991). cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium*. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 7918–7922.
- HAMILL, O. P. & MARTINAC, B. (2001). Molecular basis of mechanotransduction in living cells. *Physiological Reviews* **81**, 685–740.
- HARDT, M. & PLATTNER, H. (2000). Sub-second quenched-flow/X-ray microanalysis shows rapid Ca²⁺ mobilization from cortical stores paralleled by Ca²⁺ influx during synchronous exocytosis in *Paramecium* cells. *European Journal of Cell Biology* **79**, 642–652.
- HARUMOTO, T. (1994). The role of trichocyst discharge and backward swimming in escaping behavior of *Paramecium* from *Dileptus margaritifer*. *Journal of Eukaryotic Microbiology* **41**, 560–564.
- HARUMOTO, T. & MIYAKE, A. (1991). Defensive function of trichocysts in *Paramecium*. *Journal of Experimental Zoology* **260**, 84–92.
- HARUMOTO, T. & MIYAKE, A. (1993). Possible participation of surface antigens of *Paramecium* in predator-prey interaction. *Journal of Eukaryotic Microbiology* **40**, 27A.
- HAUSER, K., KISSMEHL, R., LINDER, J., SCHULTZ, J. E., LOTTSPREICH, F. & PLATTNER, H. (1997). Identification of isoforms of the exocytosis-sensitive phosphoprotein PP63/parafusin in *Paramecium tetraurelia* and demonstration of phosphoglucosylase activity. *Biochemical Journal* **323**, 289–296.
- HAUSER, K., PAVLOVIC, N., KLAUKE, N., GEISSINGER, D. & PLATTNER, H. (2000). Green fluorescent protein-tagged sarco(endo)plasmic reticulum Ca²⁺-ATPase overexpression in *Paramecium* cells: isoforms, subcellular localization, biogenesis of cortical calcium stores and functional aspects. *Molecular Microbiology* **37**, 773–787.
- HAUSMANN, K. (1978). Extrusive organelles in protists. *International Review of Cytology* **52**, 197–276.
- HAY, J. C. (2007). Calcium: a fundamental regulator of intracellular membrane fusion? *European Molecular Biology Organisation Reports* **8**, 236–240.
- HE, D., FIZ-PALACIOS, O., FU, C. J., FEHLING, J., TSAI, C. C. & BALDAUF, S. L. (2014). An alternative root for the eukaryote tree of life. *Current Biology* **24**, 465–470.
- HEATH, R. J. & INSALL, R. H. (2008). *Dictyostelium* MEGAPs: F-BAR domain proteins that regulate motility and membrane tubulation in contractile vacuoles. *Journal of Cell Science* **121**, 1054–1064.
- HEMMERSBACH, R. & BRÄUCKER, R. (2002). Gravity-related behaviour in ciliates and flagellates. *Advances in Space Biology and Medicine* **8**, 59–75.
- HEMMERSBACH, R. & BRAUN, M. (2006). Gravity-sensing and gravity-related signaling pathways in unicellular model systems of protists and plants. *Signal Transduction* **6**, 432–442.
- HEMMERSBACH, R., VOORMANN, R., BROMEIS, B., SCHMIDT, N., RABEN, H. & IVANOVA, K. (1998). Comparative studies of the graviresponses of *Paramecium* and *Loxodes*. *Advances in Space Research* **21**, 1285–1289.
- HEMMERSBACH, R., WILCZEK, M., STIEBER, C., BRÄUCKER, R. & IVANOVA, K. (2002). Variable acceleration influences cyclic AMP levels in *Paramecium biaurelia*. *Journal of Gravitational Physiology* **9**, P267–268.
- HENNESSEY, T. M. (2005). Responses of the ciliates *Tetrahymena* and *Paramecium* to external ATP and GTP. *Purinergic Signalling* **1**, 101–110.
- HERGOVICH, A. (2011). MOB control: reviewing a conserved family of kinase regulators. *Cellular Signalling* **23**, 1433–1440.
- HINRICHSSEN, R. D. & BLACKSHEAR, P. J. (1993). Regulation of peptide-calmodulin complexes by protein kinase C in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 1585–1589.
- HINSHAW, J. E. (2000). Dynamin and its role in membrane fission. *Annual Review of Cell and Developmental Biology* **16**, 483–519.
- HOCHSTRASSER, M. & NELSON, D. L. (1989). Cyclic AMP-dependent protein kinase in *Paramecium tetraurelia*. Its purification and the production of monoclonal antibodies against both subunits. *Journal of Biological Chemistry* **264**, 14510–14518.
- HÖHNE-ZELL, B., KNOLL, G., RIEDEL-GRAS, U., HOFER, W. & PLATTNER, H. (1992). A cortical phosphoprotein ('PP63') sensitive to exocytosis triggering in *Paramecium* cells. Immunolocalization and quenched-flow correlation of time course of dephosphorylation with membrane fusion. *Biochemical Journal* **286**, 843–849.
- HONG, W. & LEV, S. (2014). Tethering the assembly of SNARE complexes. *Trends in Cell Biology* **24**, 35–43.
- HOSEIN, R. E., WILLIAMS, S. A. & GAVIN, R. H. (2005). Directed motility of phagosomes in *Tetrahymena thermophila* requires actin and Myo1p, a novel unconventional myosin. *Cell Motility and the Cytoskeleton* **61**, 49–60.
- HOWE, C. J. & PURTON, S. (2007). The little genome of apicomplexan plastids: its raison d'être and a possible explanation for the 'delayed death' phenomenon. *Protist* **158**, 121–133.
- HUNTER, T. (2014). The genesis of tyrosine phosphorylation. *Cold Spring Harbor Perspectives in Biology* **6**, a020644.
- HURTADO-LORENZO, A., SKINNER, M., EL ANNAN, J., FUTAI, M., SUN-WADA, G. H., BOURGOIN, S., CASANOVA, J., WILDEMAN, A., BECHOUA, S., AUSIELLO, D. A., BROWN, D. & MARSHANSKY, V. (2006). V-ATPase interacts with ARNO and Arf6 in early endosomes and regulates the protein degradative pathway. *Nature Cell Biology* **8**, 124–136.
- HUSSER, M. R., HARDT, M., BLANCHARD, M. P., HENTSCHEL, J., KLAUKE, N. & PLATTNER, H. (2004). One-way calcium spill-over during signal transduction in *Paramecium* cells: from the cell cortex into cilia, but not in the reverse direction. *Cell Calcium* **36**, 349–358.
- HUTTON, J. C. (1997). *Tetrahymena*: the key to the genetic analysis of the regulated pathway of polypeptide secretion? *Proceedings of the National Academy of Sciences of the United States of America* **94**, 10490–10492.
- INAGAKI, N., ITO, M., NAKANO, T. & INAGAKI, M. (1994). Spatiotemporal distribution of protein kinase and phosphatase activities. *Trends in Biochemical Sciences* **19**, 448–452.
- ISHIDA, M., AIHARA, M. S., ALLEN, R. D. & FOK, A. K. (2011). The native structure of cytoplasmic dynein at work translocating vesicles in *Paramecium*. *European Journal of Cell Biology* **90**, 81–92.
- ISHIDA, T., TABATA, R., YAMADA, M., AIDA, M., MITSUMASU, K., FUJIWARA, M., YAMAGUCHI, K., SHIGENOBU, S., HIGUCHI, M., TSUJI, H., SHIMAMOTO, K., HASEBE, M. & SAWA, S. (2014). Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in *Arabidopsis*. *European Molecular Biology Organisation Reports* **15**, 1202–1209.
- IWAMOTO, M. & ALLEN, R. D. (2004). Uptake and rapid transfer of fluorescent ceramide analogues to acidosomes (late endosomes) in *Paramecium*. *Journal of Histochemistry and Cytochemistry* **52**, 557–565.
- IWAMOTO, M., KOUJIN, T., OSAKADA, H., MORI, C., KOJIDANI, T., MATSUDA, A., ASAKAWA, H., HIRAOKA, Y. & HARAGUCHI, T. (2015). Biased assembly of the nuclear pore complex is required for somatic and germline nuclear differentiation in *Tetrahymena*. *Journal of Cell Science* **128**, 1812–1823.
- IWAMOTO, M., MORI, C., KOJIDANI, T., BUNAI, F., HORI, T., FUKAGAWA, T., HIRAOKA, Y. & HARAGUCHI, T. (2009). Two distinct repeat sequences of Nup98 nucleoporins characterize dual nuclei in the binucleated ciliate *Tetrahymena*. *Current Biology* **19**, 843–847.
- JACOBS, M. E., DESOUSA, L. V., SAMARANAYAKE, H., PEARLMAN, R. E., SIU, K. W. & KLOBUTCHER, L. A. (2006). The *Tetrahymena thermophila* phagosome proteome. *Eukaryotic Cell* **5**, 1990–2000.
- JACONI, M. E., LEW, D. P., CARPENTIER, J. L., MAGNUSSON, K. E., SJOGREN, M. & STENDAHL, O. (1990). Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils. *Journal of Cell Biology* **110**, 1555–1564.
- JAHN, R. & FASSHAUER, D. (2012). Molecular machines governing exocytosis of synaptic vesicles. *Nature* **490**, 201–207.
- JAHN, R. & SCHELLER, R. H. (2006). SNAREs – engines for membrane fusion. *Nature Reviews Molecular Cell Biology* **7**, 631–643.
- JAMORA, C., TAKIZAWA, P. A., ZAAROUR, R. F., DENESVRE, C., FAULKNER, D. J. & MALHOTRA, V. (1997). Regulation of Golgi structure through heterotrimeric G proteins. *Cell* **91**, 617–626.
- JAUKER, F., LADES, S. & NOWACK, T. (1986). The energy budget of *Tetrahymena* and the material fluxes into and out of the adenylate pool. *Experimental Cell Research* **166**, 161–170.
- JERKA-DZIADOSZ, M. & FRANKEL, J. (2014). In memoriam: Krystyna Golinska (1933–2013). *Journal of Eukaryotic Microbiology* **61**, 328–332.
- JERKA-DZIADOSZ, M., KOLL, F., WLOGA, D., GOGENDEAU, D., GARREAU DE LOUBRESSE, N., RUIZ, F., FABCZAK, S. & BEISSON, J. (2013). A centrin3-dependent, transient, appendage of the mother basal body guides the positioning of the daughter basal body in *Paramecium*. *Protist* **164**, 352–368.
- JIN, T. (2011). GPCR-controlled chemotaxis in *Dictyostelium discoideum*. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine* **3**, 717–727.
- JOHNSON, L. N. (2009). The regulation of protein phosphorylation. *Biochemical Society Transactions* **37**, 627–641.
- JUERGENSMEYER, E. B. (1969). Serotype expression and transformation in *Tetrahymena pyriformis*. *Journal of Protozoology* **16**, 344–352.

- KAMER, K. J. & MOOTHA, V. K. (2014). MICU1 and MICU2 play nonredundant roles in the regulation of the mitochondrial calcium uniporter. *European Molecular Biology Organisation Reports* **15**, 299–307.
- KAPOOR, P. & SHEN, X. (2014). Mechanisms of nuclear actin in chromatin-remodeling complexes. *Trends in Cell Biology* **24**, 238–246.
- KARUNAKARAN, S., SASSER, T., RAJALEKSHMI, S. & FRATTI, R. A. (2012). SNAREs, HOPS and regulatory lipids control the dynamics of vacuolar actin during homotypic fusion in *S. cerevisiae*. *Journal of Cell Science* **125**, 1683–1692.
- KATRITCH, V., CHEREZOV, V. & STEVENS, R. C. (2013). Structure-function of the G protein-coupled receptor superfamily. *Annual Review of Pharmacology and Toxicology* **53**, 531–556.
- KAWASAKI-NISHI, S., BOWERS, K., NISHI, T., FORGAC, M. & STEVENS, T. H. (2001). The amino-terminal domain of the vacuolar proton-translocating ATPase a subunit controls targeting and in vivo dissociation, and the carboxyl-terminal domain affects coupling of proton transport and ATP hydrolysis. *Journal of Biological Chemistry* **276**, 47411–47420.
- KERBOEUF, D. & COHEN, J. (1990). A Ca^{2+} influx associated with exocytosis is specifically abolished in a *Paramecium* exocytotic mutant. *Journal of Cell Biology* **111**, 2527–2535.
- KERBOEUF, D., LE BERRE, A., DEDIEU, J. C. & COHEN, J. (1993). Calmodulin is essential for assembling links necessary for exocytotic membrane fusion in *Paramecium*. *European Molecular Biology Organisation Journal* **12**, 3385–3390.
- KERSKEN, H., MOMAYEZI, M., BRAUN, C. & PLATTNER, H. (1986). Filamentous actin in *Paramecium* cells: functional and structural changes correlated with phalloidin affinity labeling in vivo. *Journal of Histochemistry and Cytochemistry* **34**, 455–465.
- KERYER, C., DAVIS, F. M., RAO, P. N. & BEISSON, J. (1987). Protein phosphorylation and dynamics of cytoskeletal structures associated with basal bodies in *Paramecium*. *Cell Motility and the Cytoskeleton* **8**, 44–54.
- KHAN, A. R. & MÉNÉTREY, J. (2013). Structural biology of Arf and Rab GTPases' effector recruitment and specificity. *Structure* **21**, 1284–1297.
- KIM, S. & DYNLACHT, B. D. (2013). Assembling a primary cilium. *Current Opinion in Cell Biology* **25**, 506–511.
- KIM, M. Y., KURUVILLA, H. G., RAGHU, S. & HENNESSEY, T. M. (1999). ATP reception and chemosensory adaptation in *Tetrahymena thermophila*. *Journal of Experimental Biology* **202**, 407–416.
- KIM, K., MESSINGER, L. A. & NELSON, D. L. (1998). Ca^{2+} -dependent protein kinases of *Paramecium* – cloning provides evidence of a multigene family. *European Journal of Biochemistry* **251**, 605–612.
- KISSMEHL, R., FROISSARD, M., PLATTNER, H., MOMAYEZI, M. & COHEN, J. (2002). NSF regulates membrane traffic along multiple pathways in *Paramecium*. *Journal of Cell Science* **115**, 3935–3946.
- KISSMEHL, R., SCHILDE, C., WASSMER, T., DANZER, C., NUEHSE, K., LUTTER, K. & PLATTNER, H. (2007). Molecular identification of 26 syntaxin genes and their assignment to the different trafficking pathways in *Paramecium*. *Traffic* **8**, 523–542.
- KISSMEHL, R., SEHRING, I. M., WAGNER, E. & PLATTNER, H. (2004). Immunolocalization of actin in *Paramecium* cells. *Journal of Histochemistry and Cytochemistry* **52**, 1543–1559.
- KISSMEHL, R., TREPTAU, T., HOFER, H. W. & PLATTNER, H. (1996). Protein phosphatase and kinase activities possibly involved in exocytosis regulation in *Paramecium tetraurelia*. *Biochemical Journal* **317**, 65–76.
- KISSMEHL, R., TREPTAU, T., KOTTWITZ, B. & PLATTNER, H. (1997). Occurrence of a para-nitrophenyl phosphate-phosphatase with calcineurin-like characteristics in *Paramecium tetraurelia*. *Archives of Biochemistry and Biophysics* **344**, 260–270.
- KIY, T., VOSSKÜHLER, C., RASMUSSEN, L. & TIEDTKE, A. (1993). Three pools of lysosomal enzymes in *Tetrahymena thermophila*. *Experimental Cell Research* **205**, 286–292.
- KLAUKE, N., KISSMEHL, R., PLATTNER, H., HAGA, N. & WATANABE, T. (1998). An exocytotic mutant of *Paramecium caudatum*: membrane fusion without secretory contents release. *Cell Calcium* **23**, 349–360.
- KLAUKE, N. & PLATTNER, H. (1997). Imaging of Ca^{2+} transients induced in *Paramecium* cells by a polyamine secretagogue. *Journal of Cell Science* **110**, 975–983.
- KLAUKE, N. & PLATTNER, H. (2000). “Frustrated exocytosis” – a novel phenomenon: membrane fusion without contents release, followed by detachment and reattachment of dense core vesicles in *Paramecium* cells. *Journal of Membrane Biology* **176**, 237–248.
- KLOBUTCHER, L. A. & HERRICK, G. (1995). Consensus inverted terminal repeat sequence of *Paramecium* IESs: resemblance to termini of Tc1-related and *Euplotes* Tec transposons. *Nucleic Acids Research* **23**, 2006–2013.
- KLOPPER, T. H., KLENLE, C. N. & FASSHAUER, D. (2008). SNAREing the basis of multicellularity: consequences of protein family expansion during evolution. *Molecular Biology of the Cell* **25**, 2055–2068.
- KLÖPPEL, C., MÜLLER, A., MARKER, S. & SIMON, M. (2009). Two isoforms of eukaryotic phospholipase C in *Paramecium* affecting transport and release of GPI-anchored proteins in vivo. *European Journal of Cell Biology* **88**, 577–592.
- KLUMPP, S., COHEN, P. & SCHULTZ, J. E. (1990). Okadaic acid, an inhibitor of protein phosphatase 1 in *Paramecium*, causes sustained Ca^{2+} -dependent backward swimming in response to depolarizing stimuli. *European Molecular Biology Organisation Journal* **9**, 685–689.
- KNOCHEL, M., KISSMEHL, R., WISSMANN, J. D., MOMAYEZI, M., HENTSCHEL, J., PLATTNER, H. & BURGOYNE, R. D. (1996). Annexins in *Paramecium* cells. Involvement in site-specific positioning of secretory organelles. *Histochemistry and Cell Biology* **105**, 269–281.
- KNOLL, G., BRAUN, C. & PLATTNER, H. (1991a). Quenched flow analysis of exocytosis in *Paramecium* cells: time course, changes in membrane structure, and calcium requirements revealed after rapid mixing and rapid freezing of intact cells. *Journal of Cell Biology* **113**, 1295–1304.
- KNOLL, G., HAACKE-BELL, B. & PLATTNER, H. (1991b). Local trichocyst exocytosis provides an efficient escape mechanism for *Paramecium* cells. *European Journal of Protistology* **27**, 381–385.
- KNOLL, G., KERBOEUF, D. & PLATTNER, H. (1992). A rapid calcium influx during exocytosis in *Paramecium* cells is followed by a rise in cyclic GMP within 1 s. *Federation of European Biological Sciences Letters* **304**, 265–268.
- KODAMA, M. & FUJISHIMA, M. (2010). Induction of secondary symbiosis between the ciliate *Paramecium* and the green alga *Chlorella*. In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (ed. A. MÉNDEZ-VILAS), pp. 95–102. Formatex Research Center, Badajoz.
- KODAMA, Y., SUZUKI, H., DOHRA, H., SUGI, M., KITAZUME, T., YAMAGUCHI, K., SHIGENOBU, S. & FUJISHIMA, M. (2014). Comparison of gene expression of *Paramecium bursaria* with and without *Chlorella variabilis* symbionts. *BMC Genomics* **15**, 183.
- KRAUSE, M. & BRÄUCKER, R. (2009). Gravitaxis of *Bursaria truncatella*: electrophysiological and behavioural analyses of a large ciliate cell. *European Journal of Protistology* **45**, 98–111.
- KRAUSE, M., BRÄUCKER, R. & HEMMERSBACH, R. (2010). Gravitaxis in *Stylonychia mytilus* is based on membrane potential changes. *Journal of Experimental Biology* **213**, 161–171.
- KRISHNAN, A., ALMEN, M. S., FREDRIKSSON, R. & SCHIOTH, H. B. (2012). The origin of GPCRs: identification of mammalian like rhodopsin, adhesion, glutamate and frizzled GPCRs in fungi. *PLoS One* **7**, e29817.
- KUDO, S., MUTO, Y., INAGAKI, M., HIDAKA, H. & NOZAWA, Y. (1985a). Interaction of calcium-binding proteins with calmodulin-dependent guanylate cyclase in *Tetrahymena* plasma membrane. *Comparative Biochemistry and Physiology. B* **80**, 495–498.
- KUDO, S., MUTO, Y. & NOZAWA, Y. (1985b). Regulation by calcium of hormone-insensitive adenylate cyclase and calmodulin-dependent guanylate cyclase in *Tetrahymena* plasma membrane. *Comparative Biochemistry and Physiology. B* **80**, 813–816.
- KUIPERS, A. J., MIDDELBEEK, J. & VAN LEEUWEN, F. N. (2012). Mechanoregulation of cytoskeletal dynamics by TRP channels. *European Journal of Cell Biology* **91**, 834–846.
- KUMAR, S., BRIGUGLIO, J. S. & TURKEWITZ, A. P. (2014). An aspartyl cathepsin, CTH3, is essential for proprotein processing during secretory granule maturation in *Tetrahymena thermophila*. *Molecular Biology of the Cell* **25**, 2444–2460.
- KUMAR, U. & SAIER, M. H. Jr. (2015). Comparative genomic analysis of integral membrane transport proteins in ciliates. *Journal of Eukaryotic Microbiology* **62**, 167–187.
- KUNG, C., MARTINAC, B. & SUKHAREV, S. (2010). Mechanosensitive channels in microbes. *Annual Review of Microbiology* **64**, 313–329.
- KUNG, C., PRESTON, R. R., MALEY, M. E., LING, K. Y., KANABROCKI, J. A., SEAVEY, B. R. & SAIMI, Y. (1992). In vivo *Paramecium* mutants show that calmodulin orchestrates membrane responses to stimuli. *Cell Calcium* **13**, 413–425.
- KUNG, C. & SAIMI, Y. (1982). The physiological basis of taxes in *Paramecium*. *Annual Review of Physiology* **44**, 519–534.
- KUSCH, J. & HECKMANN, K. (1992). Isolation of the lembadion-factor, a morphogenetically active signal, that induces *Euplotes* cells to change from their ovoid form into a larger lateral winged morph. *Developmental Genetics* **13**, 241–246.
- KUSSMANN, M., HAUSER, K., KISSMEHL, R., BREED, J., PLATTNER, H. & ROEPSTORFF, P. (1999). Comparison of in vivo and in vitro phosphorylation of the exocytosis-sensitive protein PP63/parafusin by differential MALDI mass spectrometric peptide mapping. *Biochemistry* **38**, 7780–7790.
- KUTOMI, O., HORI, M., ISHIDA, M., TOMINAGA, T., KAMACHI, H., KOLL, F., COHEN, J., YAMADA, N. & NOGUCHI, M. (2012). Outer dynein arm light chain 1 is essential for controlling the ciliary response to cyclic AMP in *Paramecium tetraurelia*. *Eukaryotic Cell* **11**, 645–653.
- LACY, P. E., HOWELL, S. L., YOUNG, D. A. & FINK, C. J. (1968). New hypothesis of insulin secretion. *Nature* **219**, 1177–1179.
- LADENBURGER, E. M., KORN, I., KASIELKE, N., WASSMER, T. & PLATTNER, H. (2006). An $\text{Ins}(1,4,5)\text{P}_3$ receptor in *Paramecium* is associated with the osmoregulatory system. *Journal of Cell Science* **119**, 3705–3717.
- LADENBURGER, E. M. & PLATTNER, H. (2011). Calcium-release channels in *Paramecium*. Genomic expansion, differential positioning and partial transcriptional elimination. *PLoS One* **6**, e27111.
- LADENBURGER, E. M., SEHRING, I. M., KORN, I. & PLATTNER, H. (2009). Novel types of Ca^{2+} release channels participate in the secretory cycle of *Paramecium* cells. *Molecular and Cellular Biology* **29**, 3605–3622.
- LAI, M. M., HONG, J. J., RUGGIERO, A. M., BURNETT, P. E., SLEPNEV, V. I., DE CAMILLI, P. & SNYDER, S. H. (1999). The calcineurin-dynamin 1 complex as a calcium sensor for synaptic vesicle endocytosis. *Journal of Biological Chemistry* **274**, 25963–25966.

- LAMPERT, T. J., COLEMAN, K. D. & HENNESSEY, T. M. (2011). A knockout mutation of a constitutive GPCR in *Tetrahymena* decreases both G-protein activity and chemoattraction. *PLoS One* **6**, e28022.
- LANGENICK, J., ARAKI, T., YAMADA, Y. & WILLIAMS, J. G. (2008). A *Dictyostelium* homologue of the metazoan Cbl proteins regulates STAT signalling. *Journal of Cell Science* **121**, 3524–3530.
- LANNER, J. T., GEORGIU, D. K., JOSHI, A. D. & HAMILTON, S. L. (2010). Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harbor Perspectives in Biology* **2**, a003996.
- LAPATSINA, L., BRAND, J., POOLE, K., DAUMKE, O. & LEWIN, G. R. (2012). Stomatin-domain proteins. *European Journal of Cell Biology* **91**, 240–245.
- LASS, S. & SPAAK, P. (2003). Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia* **491**, 221–239.
- LEE, H. C. (2012). Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. *Journal of Biological Chemistry* **287**, 31633–31640.
- LEECK, C. L. & FORNEY, J. D. (1996). The 5' coding region of *Paramecium* surface antigen genes controls mutually exclusive transcription. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 2838–2843.
- LEICK, V. (1992). Chemotactic properties, cellular-binding and uptake of peptides and peptide derivatives – studies with *Tetrahymena thermophila*. *Journal of Cell Science* **103**, 565–570.
- LEICK, V. & LINDEMOSSE, S. (2007). Chemokinesis by *Tetrahymena* in response to bacterial oligopeptides. *Journal of Eukaryotic Microbiology* **54**, 271–274.
- LEONARITIS, G., SARRI, T., DAFNIS, I., EFSTATHIOU, A. & GALANOPOULOU, D. (2011). Biochemical and genetic evidence for the presence of multiple phosphatidylinositol- and phosphatidylinositol 4,5-bisphosphate-specific phospholipases C in *Tetrahymena*. *Eukaryotic Cell* **10**, 412–422.
- LEONARITIS, G., SIOKOS, J., SKARIPA, I. & GALANOPOULOU, D. (2013). Genome-wide analysis of the phosphoinositide kinome from two ciliates reveals novel evolutionary links for phosphoinositide kinases in eukaryotic cells. *PLoS One* **8**, e78848.
- LEVITAN, I. B. (1999). It is calmodulin after all! Mediator of the calcium modulation of multiple ion channels. *Neuron* **22**, 645–648.
- LI, R. & GUNDERSEN, G. G. (2008). Beyond polymer polarity: how the cytoskeleton builds a polarized cell. *Nature Reviews Molecular Cell Biology* **9**, 860–873.
- LI, S., YIN, L., COLE, E. S., UDANI, R. A. & KARRER, K. M. (2006). Progeny of germ line knockouts of ASI2, a gene encoding a putative signal transduction receptor in *Tetrahymena thermophila*, fail to make the transition from sexual reproduction to vegetative growth. *Developmental Biology* **295**, 633–646.
- LIEBL, D. & GRIFFITHS, G. (2009). Transient assembly of F-actin by phagosomes delays phagosome fusion with lysosomes in cargo-overloaded macrophages. *Journal of Cell Science* **122**, 2935–2945.
- LIMA, W. C., BALESTRINO, D., FORESTIER, C. & COSSON, P. (2014). Two distinct sensing pathways allow recognition of *Klebsiella pneumoniae* by *Dictyostelium* amoebae. *Cellular Microbiology* **16**, 311–323.
- LINDER, J. U., ENGEL, P., REIMER, A., KRÜGER, T., PLATTNER, H., SCHULTZ, A. & SCHULTZ, J. E. (1999). Guanylyl cyclases with the topology of mammalian adenylyl cyclases and an N-terminal P-type ATPase-like domain in *Paramecium*, *Tetrahymena* and *Plasmodium*. *European Molecular Biology Organisation Journal* **18**, 4222–4232.
- LINKNER, J., WITTE, G., ZHAO, H., JUNEMANN, A., NORDHOLZ, B., RUNGE-WOLLMANN, P., LAPPALAINEN, P. & FAIX, J. (2014). The inverse BAR domain protein IBARa drives membrane remodeling to control osmoregulation, phagocytosis and cytokinesis. *Journal of Cell Science* **127**, 1279–1292.
- LIU, B. A., SHAH, E., JABLONOWSKI, K., STERGACHIS, A., ENGELMANN, B. & NASH, P. D. (2011). The SH2 domain-containing proteins in 21 species establish the provenance and scope of phosphotyrosine signaling in eukaryotes. *Science Signaling* **4**, ra83.
- LÓPEZ-MONTERO, I., MONROY, F., VELEZ, M. & DEVAUX, P. F. (2010). Ceramide: from lateral segregation to mechanical stress. *Biochimica et Biophysica Acta* **1798**, 1348–1356.
- LOURIDO, S. & MORENO, S. N. J. (2015). The calcium signalling toolkit of the apicomplexan parasites *Toxoplasma gondii* and *Plasmodium spp.* *Cell Calcium* **57**, 186–193.
- LOVETT, J. L., MARCHESINI, N., MORENO, S. N. & SIBLEY, L. D. (2002). *Toxoplasma gondii* microneme secretion involves intracellular Ca^{2+} release from inositol 1,4,5-trisphosphate IP_3 /ryanodine-sensitive stores. *Journal of Biological Chemistry* **277**, 25870–25876.
- LU, E. & WOLFE, J. (2001). Lysosomal enzymes in the macronucleus of *Tetrahymena* during its apoptosis-like degradation. *Cell Death and Differentiation* **8**, 289–297.
- LUCA, F. C., MODY, M., KURISCHKO, C., ROOF, D. M., GIDDINGS, T. H. & WINEY, M. (2001). *Saccharomyces cerevisiae* Mob1p is required for cytokinesis and mitotic exit. *Molecular and Cellular Biology* **21**, 6972–6983.
- LUMPERT, C. J., GLAS-ALBRECHT, R., EISENMANN, E. & PLATTNER, H. (1992). Secretory organelles of *Paramecium* cells (trichocysts) are not remarkably acidic compartments. *Journal of Histochemistry and Cytochemistry* **40**, 153–160.
- LUMPERT, C. J., KERSKEN, H. & PLATTNER, H. (1990). Cell surface complexes ('cortices') isolated from *Paramecium tetraurelia* cells as a model system for analysing exocytosis in vitro in conjunction with microinjection studies. *Biochemical Journal* **269**, 639–645.
- LUPORINI, P., ALIMENTI, C., ORTENZI, C. & VALLESI, A. (2005). Ciliate mating types and their specific protein pheromones. *Acta Protozoologica* **44**, 89–101.
- LUPORINI, P., ALIMENTI, C. & VALLESI, A. (2014). Ciliate mating types and pheromones. In *Cilia and Flagella, Ciliates and Flagellates* (eds K. HAUSMANN and R. RADEK), pp. 95–118. Schweizerbart Science Publishers, Stuttgart.
- LUZIO, J. P., GRAY, S. R. & BRIGHT, N. A. (2010). Endosome-lysosome fusion. *Biochemical Society Transactions* **38**, 1413–1416.
- LYNN, D. H. (2010). *The Ciliated Protozoa*. Third Edition (). Springer-Verlag, Dordrecht, Heidelberg, London, New York.
- MACHEMER, H. (1988a). Electrophysiology. In *Paramecium* (ed. H.-D. GÖRTZ), pp. 185–215. Springer-Verlag, Berlin, Heidelberg.
- MACHEMER, H. (1988b). Motor control of cilia. In *Paramecium* (ed. H.-D. GÖRTZ), pp. 216–235. Springer-Verlag, Berlin, Heidelberg.
- MACHEMER, H. (2014). How do protists keep up? In *Cilia and Flagella, Ciliates and Flagellates* (eds K. HAUSMANN and R. RADEK), pp. 133–146. Schweizerbart Science Publishers, Stuttgart.
- MACHEMER, H., BRÄUCKER, R., MACHEMER-RÖHNISCH, S., NAGEL, U., NEUGEBAUER, D. C. & WESKAMP, M. (1998). The linking of extrinsic stimuli to behaviour: roles of cilia in ciliates. *European Journal of Protistology* **34**, 254–261.
- MACHEMER, H. & OGURA, A. (1979). Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *Journal of Physiology* **296**, 49–60.
- MACHEMER, H. & TEUNIS, P. F. M. (1996). Sensory motor coupling and motor responses. In *Ciliates. Cells and Organisms* (eds K. HAUSMANN and P. C. BRADBURY), pp. 379–402. Gustav Fischer Verlag, Stuttgart.
- MAHLE, N. J., DEDMAN, J. R., MEANS, A. R., CHAFOULEAS, J. G. & SATIR, B. H. (1981). Presence and indirect immunofluorescent localization of calmodulin in *Paramecium tetraurelia*. *Journal of Cell Biology* **89**, 695–699.
- MALKUS, P., JIANG, F. & SCHEKMAN, R. (2002). Concentrative sorting of secretory cargo proteins into COPII-coated vesicles. *Journal of Cell Biology* **159**, 915–921.
- MALSAM, J. & SÖLLNER, T. H. (2011). Organization of SNAREs within the Golgi stack. *Cold Spring Harbor Perspectives in Biology* **3**, a005249.
- MANAHAN, C. L., IGLESIAS, P. A., LONG, Y. & DEVREOTES, P. N. (2004). Chemoattractant signaling in *Dictyostelium discoideum*. *Annual Review of Cell and Developmental Biology* **20**, 223–253.
- MANNING, G., YOUNG, S. L., MILLER, W. T. & ZHAI, Y. (2008). The protist, *Monosiga brevicollis*, has a tyrosine kinase signaling network more elaborate and diverse than found in any known metazoan. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 9674–9679.
- MARKS, B. & McMAHON, H. T. (1998). Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. *Current Biology* **8**, 740–749.
- MARMIGNON, A., BISCHEROUR, J., SILVE, A., FOJCIK, C., DUBOIS, E., ARNAIZ, O., KAPUSTA, A., MALINSKY, S. & BÉTERMIER, M. (2014). Ku-mediated coupling of DNA cleavage and repair during programmed genome rearrangements in the ciliate *Paramecium tetraurelia*. *PLoS Genetics* **10**(8), e1004552.
- MARTENS, S. & McMAHON, H. T. (2008). Mechanisms of membrane fusion: disparate players and common principles. *Nature Reviews Molecular Cell Biology* **9**, 543–556.
- MATSUDA, A. & FORNEY, J. D. (2005). Analysis of *Paramecium tetraurelia* A-51 surface antigen gene mutants reveals positive-feedback mechanisms for maintenance of expression and temperature-induced activation. *Eukaryotic Cell* **4**, 1613–1619.
- MATSUDA, A. & FORNEY, J. D. (2006). The SUMO pathway is developmentally regulated and required for programmed DNA elimination in *Paramecium tetraurelia*. *Eukaryotic Cell* **5**, 806–815.
- MATSUDA, A., SHIEH, A. W., CHALKER, D. L. & FORNEY, J. D. (2010). The conjugation-specific Die5 protein is required for development of the somatic nucleus in both *Paramecium* and *Tetrahymena*. *Eukaryotic Cell* **9**, 1087–1099.
- MATT, H., BILINSKI, M. & PLATTNER, H. (1978). Adenosinetriphosphate, calcium and temperature requirements for the final steps of exocytosis in *Paramecium* cells. *Journal of Cell Science* **32**, 67–86.
- MELLMAN, I. (1992). The importance of being acid: the role of acidification in intracellular membrane traffic. *Journal of Experimental Biology* **172**, 39–45.
- MERLINI, L., DUDIN, O. & MARTIN, S. G. (2013). Mate and fuse: how yeast cells do it. *Open Biology* **3** (doi: 10.1098/rsob.130008).
- MIAO, W., XIONG, J., BOWEN, J., WANG, W., LIU, Y., BRAGUINET, O., GRIGULL, J., PEARLMAN, R. E., ORIAS, E. & GOROVSKY, M. A. (2009). Microarray analyses of gene expression during the *Tetrahymena thermophila* life cycle. *PLoS One* **4**, e4429.
- MICHAELSON, D., AHEARN, I., BERGO, M., YOUNG, S. & PHILIPS, M. (2002). Membrane trafficking of heterotrimeric G proteins via the endoplasmic reticulum and Golgi. *Molecular Biology of the Cell* **13**, 3294–3302.
- MICHELL, R. H. (2013). Inositol lipids: from an archaic origin to phosphatidylinositol 3,5-bisphosphate faults in human disease. *Federation of European Biological Societies Journal* **280**, 6281–6294.
- MICHIBATA, J., OKAZAKI, N., MOTOMURA, S., UDA, K., FUJIWARA, S. & SUZUKI, T. (2014). Two arginine kinases of *Tetrahymena pyriformis*: characterization and localization. *Comparative Biochemistry and Physiology, Part B* **171**, 34–41.
- MIGLIETTA, L. A. & NELSON, D. L. (1988). A novel cGMP-dependent protein kinase from *Paramecium*. *Journal of Biological Chemistry* **263**, 16096–16105.

- MIM, C. & UNGER, V. M. (2012). Membrane curvature and its generation by BAR proteins. *Trends in Biochemical Sciences* **37**, 526–533.
- MIMIKAKIS, J. L., NELSON, D. L. & PRESTON, R. R. (1998). Oscillating response to a purine nucleotide disrupted by mutation in *Paramecium tetraurelia*. *Biochemical Journal* **330**, 139–147.
- MIN, S. W., CHANG, W. P. & SÜDHOF, T. C. (2007). E-syts, a family of membranous Ca^{2+} -sensor proteins with multiple C2 domains. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 3823–3828.
- MISHRA, A., EATHIRAJ, S., CORVERA, S. & LAMBRIGHT, D. G. (2010). Structural basis for Rab GTPase recognition and endosome tethering by the C2H2 zinc finger of early endosomal autoantigen 1 (EEA1). *Proceedings of the National Academy of Sciences of the United States of America* **107**, 10866–10871.
- MIWA, I. & WADA, T. (1995). Light-pulses and injection of Ip_3 induce mating ability in *Paramecium bursaria*. *Journal of Experimental Zoology* **272**, 338–344.
- MIZUNO-YAMASAKI, E., RIVERA-MOLINA, F. & NOVICK, P. (2012). GTPase networks in membrane traffick. *Annual Review of Biochemistry* **81**, 637–659.
- MOGAMI, Y., YAMANE, A., GINO, A. & BABA, S. A. (2004). Bioconvective pattern formation of *Tetrahymena* under altered gravity. *Journal of Experimental Biology* **207**, 3349–3359.
- MOHAMED, I., HUSSER, M., SEHRING, I., HENTSCHEL, J., HENTSCHEL, C. & PLATTNER, H. (2003). Refilling of cortical calcium stores in *Paramecium* cells: in situ analysis in correlation with store-operated calcium influx. *Cell Calcium* **34**, 87–96.
- MOMAYEZY, M., HABERMANN, A. W., SOKOLOVA, J. J., KISSMEHL, R. & PLATTNER, H. (1993). Ultrastructural and antigenic preservation of a delicate structure by cryopreparation: identification and immunogold localization during biogenesis of a secretory component (membrane-matrix connection) in *Paramecium trichocysts*. *Journal of Histochemistry and Cytochemistry* **41**, 1669–1677.
- MOMAYEZY, M., KERSKEN, H., GRAS, U., VILMART-SEUWEN, J. & PLATTNER, H. (1986). Calmodulin in *Paramecium tetraurelia*: localization from the in vivo to the ultrastructural level. *Journal of Histochemistry and Cytochemistry* **34**, 1621–1638.
- MOMAYEZY, M., KISSMEHL, R. & PLATTNER, H. (2000). Quantitative immunogold localization of protein phosphatase 2B (calcineurin) in *Paramecium* cells. *Journal of Histochemistry and Cytochemistry* **48**, 1269–1281.
- MOMAYEZY, M., LUMPERT, C. J., KERSKEN, H., GRAS, U., PLATTNER, H., KRINKS, M. H. & KLEF, C. B. (1987). Exocytosis induction in *Paramecium tetraurelia* cells by exogenous phosphoprotein phosphatase in vivo and in vitro: possible involvement of calcineurin in exocytotic membrane fusion. *Journal of Cell Biology* **105**, 181–189.
- MOMAYEZY, M., WLOGA, D., KISSMEHL, R., PLATTNER, H., JUNG, G., KLUMPP, S. & SCHULTZ, J. E. (1996). Immunolocalization of protein phosphatase type 1 in *Paramecium* cells using antibodies against recombinant protein and peptides. *Journal of Histochemistry and Cytochemistry* **44**, 891–905.
- MOOREN, O. L., GALLETTA, B. J. & COOPER, J. A. (2012). Roles for actin assembly in endocytosis. *Annual Review of Biochemistry* **81**, 661–686.
- MORGAN, A. J. & GALIONE, A. (2014). Two-pore channels (TPCs): current controversies. *Bioessays* **36**, 173–183.
- MÜLLER, S., DIEDERICH, K., BREED, J., KISSMEHL, R., HAUSER, K., PLATTNER, H. & WELTE, W. (2002). Crystal structure analysis of the exocytosis-sensitive phosphoprotein, pp63/parafusin (phosphoglucosyltransferase), from *Paramecium* reveals significant conformational variability. *Journal of Molecular Biology* **315**, 141–153.
- MUNNIK, T. & TESTERINK, C. (2009). Plant phospholipid signaling: 'in a nutshell'. *Journal of Lipid Research* **50**(Suppl), S260–S265.
- NAIR, S., GUERRA, C. & SATIR, P. (1999). A Sec7-related protein in *Paramecium*. *Federation of the American Societies of Experimental Biology Journal* **13**, 1249–1257.
- NAITOH, Y. & ECKERT, R. (1969). Ionic mechanisms controlling behavioral responses of *Paramecium* to mechanical stimulation. *Science* **164**, 963–965.
- NAITOH, Y. & KANEKO, H. (1972). Reactivated triton-extracted models of *Paramecium*: modification of ciliary movement by calcium ions. *Science* **176**, 523–524.
- NAKAOKA, Y., TANAKA, H. & OOSAWA, F. (1984). Ca^{2+} -dependent regulation of beat frequency of cilia in *Paramecium*. *Journal of Cell Science* **65**, 223–231.
- NASIR, A. M., YANG, Q., CHALKER, D. L. & FORNEY, J. D. (2015). SUMOylation is developmentally regulated and required for cell pairing during conjugation in *Tetrahymena thermophila*. *Eukaryotic Cell* **14**, 170–181.
- NEHER, E. (1998a). Usefulness and limitations of linear approximations to the understanding of Ca^{++} signals. *Cell Calcium* **24**, 345–357.
- NEHER, E. (1998b). Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release. *Neuron* **20**, 389–399.
- NOGUCHI, M., SAWADA, T. & AKAZAWA, T. (2001). ATP-regenerating system in the cilia of *Paramecium caudatum*. *Journal of Experimental Biology* **204**, 1063–1071.
- NOIREAUD, J. & ANDRIANTSITOHAINA, R. (2014). Recent insights in the paracrine modulation of cardiomyocyte contractility by cardiac endothelial cells. *BioMed Research International* **2014** (doi: 10.1155/2014/923805).
- NORBERG, E., ORRENIUS, S. & ZHIVOTOVSKY, B. (2010). Mitochondrial regulation of cell death: processing of apoptosis-inducing factor (AIF). *Biochemical and Biophysical Research Communications* **396**, 95–100.
- NOVICK, P. & ZERIAL, M. (1997). The diversity of Rab proteins in vesicle transport. *Current Opinion in Cell Biology* **9**, 496–504.
- NOWACKI, M. & LANDWEBER, L. F. (2009). Epigenetic inheritance in ciliates. *Current Opinion in Microbiology* **12**, 638–643.
- NOWACKI, M., SHETTY, K. & LANDWEBER, L. F. (2011). RNA-mediated epigenetic programming of genome rearrangements. *Annual Review of Genomics and Human Genetics* **12**, 367–389.
- NOWAK, J. K., GROMADKA, R., JUSZCZUK, M., JERKA-DZIADOSZ, M., MALISZEWSKA, K., MUCCHIELLI, M.-H., GOUT, J.-F., ARNAIZ, O., AGIER, N., TANG, T., AGGERBECK, L. P., COHEN, J., DELACROIX, H., SPERLING, L., HERBERT, C. J., ZAGULSKI, M. & BÉTERMIER, M. (2011). Functional study of genes essential for autogamy and nuclear reorganization in *Paramecium*. *Eukaryotic Cell* **10**, 363–372.
- OGUNBAYO, O. A., ZHU, Y., ROSSI, D., SORRENTINO, V., MA, J., ZHU, M. X. & EVANS, A. M. (2011). Cyclic adenosine diphosphate ribose activates ryanodine receptors, whereas NAADP activates two-pore domain channels. *Journal of Biological Chemistry* **286**, 9136–9140.
- OGURA, A. & MACHEMER, H. (1980). Distribution of mechanoreceptor channels in the *Paramecium* surface-membrane. *Journal of Comparative Physiology* **135**, 233–242.
- OHNO, S. (1970). *Evolution by Gene Duplication*. George Allen and Unwin, London.
- OLDHAM, W. M. & HAMM, H. E. (2008). Heterotrimeric G protein activation by G-protein-coupled receptors. *Nature Reviews Molecular Cell Biology* **9**, 60–71.
- ORCI, L., RAVAZZOLA, M., AMHERDT, M., MADSEN, O., VASSALLI, J. D. & PERRELET, A. (1985). Direct identification of prohormone conversion site in insulin secreting cells. *Cell* **42**, 671–681.
- ORIAS, E., CERVANTES, M. D. & HAMILTON, E. P. (2011). *Tetrahymena thermophila*, a unicellular eukaryote with separate germline and somatic genomes. *Research in Microbiology* **162**, 578–586.
- ORTENZI, C., ALIMENTI, C., VALLESI, A., DI PRETORO, B., TERZA, A. L. & LUPORINI, P. (2000). The autocrine mitogenic loop of the ciliate *Euplotes raikovi*: the pheromone membrane-bound forms are the cell binding sites and potential signaling receptors of soluble pheromones. *Molecular Biology of the Cell* **11**, 1445–1455.
- OSADA, E., AKEMATSU, T., ASANO, T. & ENDOH, H. (2014). A novel mitochondrial nuclease-associated protein: a major executor of the programmed nuclear death in *Tetrahymena thermophila*. *Biology of the Cell* **106**, 97–109.
- OSINSKA, M., WIEJAK, J., WYPYCH, E., BILSKI, H., BARTOSIEWICZ, R. & WYROBA, E. (2011). Distinct expression, localization and function of two Rab7 proteins encoded by paralogous genes in a free-living model eukaryote. *Acta Biochimica Polonica* **58**, 597–607.
- OSIPCHUK, Y. & CAHALAN, M. (1992). Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. *Nature* **359**, 241–244.
- PALADE, G. E. (1975). Intracellular aspects of the process of protein synthesis. *Science* **189**, 347–358.
- PARFREY, L. W., LAHR, D. J., KNOLL, A. H. & KATZ, L. A. (2011). Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 13624–13629.
- PARKINSON, K., BAINES, A. E., KELLER, T., GRUENHEIT, N., BRAGG, L., NORTH, R. A. & THOMPSON, C. R. (2014). Calcium-dependent regulation of Rab activation and vesicle fusion by an intracellular P2X ion channel. *Nature Cell Biology* **16**, 87–98.
- PASSOS, A. P. & GARCÍA, C. R. (1998). Inositol 1,4,5-trisphosphate induced Ca^{2+} release from chloroquine-sensitive and -insensitive intracellular stores in the intracythotrophic stage of the malaria parasite *P. chabaudi*. *Biochemical and Biophysical Research Communications* **245**, 155–160.
- PATEL, S. & CAI, X. (2015). Evolution of acidic Ca^{2+} stores and their resident Ca^{2+} -permeable channels. *Cell Calcium* **57**, 222–230.
- PATEL, S. & DOCAMPO, R. (2009). In with the TRP channels: intracellular functions for TRPM1 and TRPM2. *Science Signaling* **2**, pe69.
- PEARSON, C. G. (2014). Choosing sides – asymmetric centriole and basal body assembly. *Journal of Cell Science* **127**, 2803–2810.
- PECK, R. K., SWIDERSKI, B. & TOURMEL, A.-M. (1993). Involvement of the trans-Golgi network coated vesicles, vesicle fusion, and secretory product condensation in the biogenesis of *Pseudomicrothorax* trichocysts. In *Membrane Traffic in Protozoa* (ed. H. PLATTNER), pp. 1–25. JAI Press, Greenwich, London.
- PEI, G., REPNIK, U., GRIFFITHS, G. & GUTIERREZ, M. G. (2014). Identification of an immune-regulated phagosomal Rab cascade in macrophages. *Journal of Cell Science* **127**, 2071–2082.
- PETERS-REGEHR, T., KUSCH, J. & HECKMANN, K. (1997). Primary structure and origin of a predator released protein that induces defensive morphological changes in *Euplotes*. *European Journal of Protistology* **33**, 389–395.
- PFEFFER, S. R. (2013). Rab GTPase regulation of membrane identity. *Current Opinion in Cell Biology* **25**, 414–419.
- PLATTNER, H. (2010a). How to design a highly organized cell: an unexpectedly high number of widely diversified SNARE proteins positioned at strategic sites in the ciliate, *Paramecium tetraurelia*. *Protist* **161**, 497–516.
- PLATTNER, H. (2010b). Membrane trafficking in protozoa SNARE proteins, H^{+} -ATPase, actin, and other key players in ciliates. *International Review of Cell and Molecular Biology* **280**, 79–184.
- PLATTNER, H. (2013a). Contractile vacuole complex-its expanding protein inventory. *International Review of Cell and Molecular Biology* **306**, 371–416.
- PLATTNER, H. (2013b). The contractile vacuole complex of protists – new cues to function and biogenesis. *Critical Reviews in Microbiology* **41**, 218–227 (doi: 10.3109/1040841X2013.821650).

- PLATTNER, H. (2014). Calcium regulation in the protozoan model, *Paramecium tetraurelia*. *Journal of Eukaryotic Microbiology* **61**, 95–114.
- PLATTNER, H. (2015a). Calcium signalling in the ciliated protozoan model, *Paramecium*: strict signal localisation by epigenetically controlled positioning of different Ca^{2+} -channels. *Cell Calcium* **57**, 203–213.
- PLATTNER, H. (2015b). Molecular aspects of calcium signalling at the crossroads of unikont and bikont eukaryotic evolution – the ciliated protozoan *Paramecium* in focus. *Cell Calcium* **57**, 174–185.
- PLATTNER, H., BRAUN, C. & HENTSCH, J. (1997). Facilitation of membrane fusion during exocytosis and exocytosis-coupled endocytosis and acceleration of “ghost” detachment in *Paramecium* by extracellular calcium. A quenched-flow/freeze-fracture analysis. *Journal of Membrane Biology* **158**, 197–208.
- PLATTNER, H. & KISSMEHL, R. (2003). Molecular aspects of membrane trafficking in *Paramecium*. *International Review of Cytology* **232**, 185–216.
- PLATTNER, H. & KISSMEHL, R. (2005). Molecular aspects of rapid, reversible, Ca^{2+} -dependent de-phosphorylation of pp63/parafusin during stimulated exo-endocytosis in *Paramecium* cells. *Cell Calcium* **38**, 319–327.
- PLATTNER, H. & KLAUKE, N. (2001). Calcium in ciliated protozoa: sources, regulation, and calcium-regulated cell functions. *International Review of Cytology* **201**, 115–208.
- PLATTNER, H., KNOLL, G. & PAPE, R. (1993). Synchronization of different steps of the secretory cycle in *Paramecium tetraurelia*: trichocyst exocytosis, exocytosis-coupled endocytosis and intracellular transport. In *Membrane Traffic in Protozoa* (ed. H. PLATTNER), pp. 123–148. JAI Press, Greenwich, London.
- PLATTNER, H., MATT, H., KERSKEN, H., HAACK, B. & STÜRZL, R. (1984). Synchronous exocytosis in *Paramecium* cells. I. A novel approach. *Experimental Cell Research* **151**, 6–13.
- PLATTNER, H., SEHRING, I. M., MOHAMED, I. K., MIRANDA, K., DE SOUZA, W., BILLINGTON, R., GENAZZANI, A. & LADENBURGER, E. M. (2012). Calcium signaling in closely related protozoan groups (Alveolata): non-parasitic ciliates (*Paramecium*, *Tetrahymena*) vs. parasitic Apicomplexa (*Plasmodium*, *Toxoplasma*). *Cell Calcium* **51**, 351–382.
- PLATTNER, H., SEHRING, I. M., SCHILDE, C. & LADENBURGER, E. M. (2009). Pharmacology of ciliated protozoa–drug (in)sensitivity and experimental drug (ab)use. *International Review of Cell and Molecular Biology* **273**, 163–218.
- PLATTNER, H., STÜRZL, R. & MATT, H. (1985). Synchronous exocytosis in *Paramecium* cells. IV. Polyamino compounds as potent trigger agents for repeatable trigger-redocking cycles. *European Journal of Cell Biology* **36**, 32–37.
- PLATTNER, H. & VERKHATSKY, A. (2012). Ca^{2+} signalling early in evolution – all but primitive. *Journal of Cell Science* **126**, 2141–2150.
- PLATTNER, H. & VERKHATSKY, A. (2015). The ancient roots of calcium signalling evolutionary tree. *Cell Calcium* **57**, 123–132.
- PLATTNER, H., WESTPHAL, C. & TIGGEMANN, R. (1982). Cytoskeleton-secretory vesicle interactions during the docking of secretory vesicles at the cell membrane in *Paramecium tetraurelia* cells. *Journal of Cell Biology* **92**, 368–377.
- POPOFF, V., ADOLF, F., BRÜGGER, B. & WIELAND, F. (2011). COPI budding within the Golgi stack. *Cold Spring Harbor Perspectives in Biology* **3**, a005231.
- POUPHILE, M., LEFORTTRAN, M., PLATTNER, H., ROSSIGNOL, M. & BEISSON, J. (1986). Genetic dissection of the morphogenesis of exocytosis sites in *Paramecium*. *Biology of the Cell* **56**, 151–161.
- PREER, J. R., JR., PREER, L. B. & RUDMAN, B. M. (1981). mRNAs for the immobilization antigens of *Paramecium*. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 6776–6778.
- PREER, J. R., JR., PREER, L. B., RUDMAN, B. & BARNETT, A. (1987). Molecular biology of the genes for immobilization antigens in *Paramecium*. *Journal of Protozoology* **34**, 418–423.
- PRESTON, R. R., KINK, J. A., HINRICHSSEN, R. D., SAIMI, Y. & KUNG, C. (1991). Calmodulin mutants and Ca^{2+} -dependent channels in *Paramecium*. *Annual Review of Physiology* **53**, 309–319.
- PRESTON, R. R., SAIMI, Y. & KUNG, C. (1992a). Calcium current activated upon hyperpolarization of *Paramecium tetraurelia*. *Journal of General Physiology* **100**, 233–251.
- PRESTON, R. R., SAIMI, Y. & KUNG, C. (1992b). Calcium-dependent inactivation of the calcium current activated upon hyperpolarization of *Paramecium tetraurelia*. *Journal of General Physiology* **100**, 253–268.
- PROLE, D. L. & TAYLOR, C. W. (2011). Identification of intracellular and plasma membrane calcium channel homologues in pathogenic parasites. *PLoS One* **6**, e26218.
- PRUSINER, S. B. (2004). *Prion Biology and Diseases*. Second Edition. Cold Spring Harbor Laboratories, Cold Spring Harbor.
- PUCCIARELLI, S., BALLARINI, P., SPARVOL, D., BARCHETTA, S., YU, T., DETRICH, H. W. & MICELI, C. (2012). Distinct functional roles of beta-tubulin isoforms in microtubule arrays of *Tetrahymena thermophila*, a model single-celled organism. *PLoS One* **7**, e39694.
- QUEHENBERGER, O., PROSSNITZ, E. R., CAVANAGH, S. L., COLCHERANE, C. G. & YE, R. D. (1993). Multiple domains of the N-formylpeptide receptor are required for high-affinity ligand binding. Construction and analysis of chimeric N-formyl peptide receptors. *Journal of Biological Chemistry* **268**, 18167–18175.
- RAMOINO, P., CANDIANI, S., PITTALUGA, A. M., USAI, C., GALLUS, L., FERRANDO, S., MILANESE, M., FAIMALI, M. & BONANNO, G. (2014). Pharmacological characterization of NMDA-like receptors in the single-celled organism *Paramecium primaurelia*. *Journal of Experimental Biology* **217**, 463–471.
- RAMOINO, P., GALLUS, L., BELTRAME, F., DIASPRO, A., FATO, M., RUBINI, P., STIGLIANI, S., BONANNO, G. & USAI, C. (2006). Endocytosis of GABA_A receptors modulates membrane excitability in the single-celled organism *Paramecium*. *Journal of Cell Science* **119**, 2056–2064.
- RANGARAJU, V., CALLOWAY, N. & RYAN, T. A. (2014). Activity-driven local ATP synthesis is required for synaptic function. *Cell* **156**, 825–835.
- RASMUSSEN, M. I. & WHEATLEY, D. N. (2007). Purification and characterisation of cell survival factor 1 (TCSF1) from *Tetrahymena thermophila*. *Journal of Cell Communication and Signaling* **1**, 185–193.
- REUTER, A. T., STUERMER, C. A. & PLATTNER, H. (2013). Identification, localization, and functional implications of the microdomain-forming stomatin family in the ciliated protozoan *Paramecium tetraurelia*. *Eukaryotic Cell* **12**, 529–544.
- RICCARDI, D. & KEMP, P. J. (2012). The calcium-sensing receptor beyond extracellular calcium homeostasis: conception, development, adult physiology, and disease. *Annual Review of Physiology* **74**, 271–297.
- RICCI, N. (1996). Ethology of ciliates. In *Ciliates, Cells as Organisms* (eds K. HAUSMANN and P. C. BRADBURY), pp. 403–416. Gustav Fischer Verlag, Stuttgart, Jena, New York.
- RIZO, J., CHEN, X. & ARAC, D. (2006). Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends in Cell Biology* **16**, 339–350.
- ROBERTS, A. M. (2010). The mechanics of gravitaxis in *Paramecium*. *Journal of Experimental Biology* **213**, 4158–4162.
- ROSSI, V., BANFIELD, D. K., VACCA, M., DIETRICH, L. E., UNGERMANN, C., D’ESPOSITO, M., GALLI, T. & FILIPPINI, F. (2004). Longins and their longin domains: regulated SNAREs and multifunctional SNARE regulators. *Trends in Biochemical Sciences* **29**, 682–688.
- ROTHMAN, J. E. (2014). The principle of membrane fusion in the cell (Nobel lecture). *Angeordnete Chemie International Edition* **53**, 12676–12694.
- RUBIN, R. (1974). *Calcium and the Secretory Process*. Plenum Press, New York.
- RUBIN, R. P. (2012). *Calcium and Cellular Secretion*. Springer, Heidelberg, New York, London.
- RUIZ, F., BEISSON, J., ROSSIER, J. & DUPUIS-WILLIAMS, P. (1999). Basal body duplication in *Paramecium* requires γ -tubulin. *Current Biology* **9**, 43–46.
- RUIZ, F., GARREAU DE LOUBRESSE, N., KLOTZ, C., BEISSON, J. & KOLL, F. (2005). Centrin deficiency in *Paramecium* affects the geometry of basal-body duplication. *Current Biology* **15**, 2097–2106.
- RUIZ, F., KRZYWICKA, A., KLOTZ, C., KELLER, A., COHEN, J., KOLL, F., BALAVOINE, G. & BEISSON, J. (2000). The SM19 gene, required for duplication of basal bodies in *Paramecium*, encodes a novel tubulin, η -tubulin. *Current Biology* **10**, 1451–1454.
- RYALS, P. E., BAE, S. & PATTERSON, C. E. (1999). Evidence for early signaling events in stomatin-induced differentiation of *Tetrahymena vorax*. *Journal of Eukaryotic Microbiology* **46**, 77–83.
- SAIDU, S. P., WEERARATNE, S. D., VALENTINE, M., DELAY, R. & VAN HOUTEN, J. L. (2009). Role of plasma membrane calcium ATPases in calcium clearance from olfactory sensory neurons. *Chemical Senses* **34**, 349–358.
- SAIMI, Y. & KUNG, C. (1987). Behavioral genetics of *Paramecium*. *Annual Review of Genetics* **21**, 47–65.
- SAIMI, Y. & KUNG, C. (1994). Ion channel regulation by calmodulin binding. *Federation of European Biochemical Societies Letters* **350**, 155–158.
- SAIMI, Y. & KUNG, C. (2002). Calmodulin as an ion channel subunit. *Annual Review of Physiology* **64**, 289–311.
- SAINARD-CHANET, A. & CUMMINGS, D. (1988). Mitochondria. In *Paramecium* (ed. H.-D. GÖRTZ), pp. 167–184. Springer-Verlag, Berlin, Heidelberg.
- SALATHE, M. (2007). Regulation of mammalian ciliary beating. *Annual Review of Physiology* **69**, 401–422.
- SAMARANAYAKE, H. S., COWAN, A. E. & KLOBUTCHER, L. A. (2011). Vacuolar protein sorting protein 13A, TiVPS13A, localizes to the *Tetrahymena thermophila* phagosome membrane and is required for efficient phagocytosis. *Eukaryotic Cell* **10**, 1207–1218.
- SAMIE, M., WANG, X., ZHANG, X., GOSCHKA, A., LI, X., CHENG, X., GREGG, E., AZAR, M., ZHUO, Y., GARRITY, A. G., GAO, Q., SLAUGENHAUPT, S., PICKEL, J., ZOLOV, S. N., WEISMAN, L. S., LENK, G. M., TITUS, S., BRYANT-GENEVIER, M., SOUTHAL, N., JUAN, M., FERRER, M. & XU, H. (2013). A TRP channel in the lysosome regulates large particle phagocytosis via focal exocytosis. *Developmental Cell* **26**, 511–524.
- SANCAK, Y., MARKHARD, A. L., KITAMI, T., KOVACS-BOGDAN, E., KAMER, K. J., UDESHI, N. D., CARR, S. A., CHAUDHURI, D., CLAPHAM, D. E., LI, A. A., CALVO, S. E., GOLDBERGER, O. & MOOTHY, V. K. (2013). EMRE is an essential component of the mitochondrial calcium uniporter complex. *Science* **342**, 1379–1382.
- SATOW, Y., MURPHY, A. D. & KUNG, C. (1983). The ionic basis of the depolarizing mechanoreceptor potential of *Paramecium tetraurelia*. *Journal of Experimental Biology* **103**, 253–264.
- SCHILDE, C., SCHÖNEMANN, B., SEHRING, I. M. & PLATTNER, H. (2010). Distinct subcellular localization of a group of synaptobrevin-like SNAREs in *Paramecium tetraurelia* and effects of silencing SNARE-specific chaperone NSF. *Eukaryotic Cell* **9**, 288–305.

- SCHILDE, C., WASSMER, T., MANSFELD, J., PLATTNER, H. & KISSMEHL, R. (2006). A multigene family encoding R-SNAREs in the ciliate *Paramecium tetraurelia*. *Traffic* **7**, 440–455.
- SCHON, E. A., DiMAURO, S. & HIRANO, M. (2012). Human mitochondrial DNA: roles of inherited and somatic mutations. *Nature Reviews Genetics* **13**, 878–890.
- SCHULTZ, J. E., GRÜNEMUND, R., VON HIRSCHHAUSEN, R. & SCHONEFELD, U. (1984). Ionic regulation of cyclic AMP levels in *Paramecium tetraurelia* in vivo. *Federation of European Biochemical Societies Letters* **167**, 113–116.
- SCHULTZ, J. E. & KLUMPP, S. (1991). Calcium-regulated guanylyl cyclases from *Paramecium* and *Tetrahymena*. *Methods in Enzymology* **195**, 466–474.
- SCHULTZ, J. E. & KLUMPP, S. (1993). Cyclic nucleotides and calcium signaling in *Paramecium*. *Advances in Second Messenger and Phosphoprotein Research* **27**, 25–46.
- SCHULTZ, J. E., POHL, T. & KLUMPP, S. (1986). Voltage-gated Ca^{2+} entry into *Paramecium* linked to intracellular increase in cyclic-GMP. *Nature* **322**, 271–273.
- SCHULTZ, J. E. & SCHÖNBORN, C. (1994). Cyclic AMP formation in *Tetrahymena pyriformis* is controlled by a K^{+} -conductance. *Federation of European Biochemical Societies Letters* **356**, 322–326.
- SCHWARTZ, J. H., LI, G., YANG, Q., SURI, V., ROSS, J. J. & ALEXANDER, E. A. (2007). Role of SNAREs and H^{+} -ATPase in the targeting of proton pump-coated vesicles to collecting duct cell apical membrane. *Kidney International* **72**, 1310–1315.
- SEHRING, I. M., KLOTZ, C., BEISSON, J. & PLATTNER, H. (2009). Rapid downregulation of the Ca^{2+} -signal after exocytosis stimulation in *Paramecium* cells: essential role of a centrin-rich filamentous cortical network, the infraciliary lattice. *Cell Calcium* **45**, 89–97.
- SEHRING, I. M., MANSFELD, J., REINER, C., WAGNER, E., PLATTNER, H. & KISSMEHL, R. (2007a). The actin multigene family of *Paramecium tetraurelia*. *BMC Genomics* **8**, 82.
- SEHRING, I. M., REINER, C., MANSFELD, J., PLATTNER, H. & KISSMEHL, R. (2007b). A broad spectrum of actin paralogs in *Paramecium tetraurelia* cells display differential localization and function. *Journal of Cell Science* **120**, 177–190.
- SEHRING, I. M. & PLATTNER, H. (2004). Ca^{2+} oscillations mediated by exogenous GTP in *Paramecium* cells: assessment of possible Ca^{2+} sources. *Cell Calcium* **36**, 409–420.
- SEHRING, I. M., REINER, C. & PLATTNER, H. (2010). The actin subfamily PtAct4, out of many subfamilies, is differentially localized for specific local functions in *Paramecium tetraurelia* cells. *European Journal of Cell Biology* **89**, 509–524.
- SEIFERT, R. (2015). How do basic secretagogues activate mast cells? *Naunyn-Schmiedeberg's Archives of Pharmacology* **388**, 279–281.
- SHEN, H. M. & MIZUSHIMA, N. (2014). At the end of the autophagic road: an emerging understanding of lysosomal functions in autophagy. *Trends in Biochemical Sciences* **39**, 61–71.
- SHEN, H., PIRRUCCELLO, M. & DE CAMILLI, P. (2012). SnapShot: membrane curvature sensors and generators. *Cell* **150**, 1300.e1–e2.
- SHERWOOD, M. W., PRIOR, I. A., VORONINA, S. G., BARROW, S. L., WOODSMITH, J. D., GERASIMENKO, O. V., PETERSEN, O. H. & TEPIKIN, A. V. (2007). Activation of trypsinogen in large endocytic vacuoles of pancreatic acinar cells. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 5674–5679.
- SHIOZAKI, N., NAKANO, K., KUSHIDA, Y., NOGUCHI, T. Q., UYEDA, T. Q., WLOGA, D., DAVE, D., VASUDEVAN, K. K., GAERTIG, J. & NUMATA, O. (2013). ADF/cofilin is not essential but is critically important for actin activities during phagocytosis in *Tetrahymena thermophila*. *Eukaryotic Cell* **12**, 1080–1086.
- SIEBER, J. J., WILLIG, K. I., KUTZNER, C., GERDING-REIMERS, C., HARKE, B., DONNERT, G., RAMMNER, B., EGGELING, C., HELL, S. W., GRUBMÜLLER, H. & LANG, T. (2007). Anatomy and dynamics of a supramolecular membrane protein cluster. *Science* **317**, 1072–1076.
- SIEGMUND, L., BURMEISTER, A., FISCHER, M. S. & WOSTEMEYER, J. (2013). A model for endosymbiosis: interaction between *Tetrahymena pyriformis* and *Escherichia coli*. *European Journal of Protistology* **49**, 552–563.
- SIMON, M. C. & KUSCH, J. (2013). Communicative functions of GPI-anchored surface proteins in unicellular eukaryotes. *Critical Reviews in Microbiology* **39**, 70–78.
- SIMON, M. & PLATTNER, H. (2014). Unicellular eukaryotes as models in cell and molecular biology: critical appraisal of their past and future value. *International Review of Cell and Molecular Biology* **309**, 141–198.
- SIMON, M. C. & SCHMIDT, H. J. (2007). Antigenic variation in ciliates: antigen structure, function, expression. *Journal of Eukaryotic Microbiology* **54**, 1–7.
- SIMONS, K. & GERL, M. J. (2010). Revitalizing membrane rafts: new tools and insights. *Nature Reviews Molecular Cell Biology* **11**, 688–699.
- SINGH, D. P., SAUDEMONT, B., GUGLIELMI, G., ARNAIZ, O., GOÛT, J. F., PRAJER, M., POTEKHIN, A., PRZYBOS, E., AUBUSSON-FLEURY, A., BHULLAR, S., BOUHOUCHE, K., LHUILLIER-AKAKPO, M., TANTY, V., BLUGEON, C., ALBERTI, A., LABADIE, K., AURY, J. M., SPERLING, L., DUHARCOURT, S. & MEYER, E. (2014). Genome-defence small RNAs exapted for epigenetic mating-type inheritance. *Nature* **509**, 447–452.
- SKOG, S., TRIBUKAIT, B. & SUNDIUS, G. (1982). Energy metabolism and ATP turnover time during the cell cycle of Ehrlich ascites tumour cells. *Experimental Cell Research* **141**, 23–29.
- SLABODNICK, M. M., RUBY, J. G., DUNN, J. G., FELDMAN, J. L., DERISI, J. L. & MARSHALL, W. F. (2014). The kinase regulator Mob1 acts as a patterning protein for *Stentor* morphogenesis. *PLoS Biology* **12**(5), e1001861.
- SLEIGH, M. A. (2014). Encounters with cilia. In *Cilia and Flagella, Ciliates and Flagellates* (eds K. HAUSMANN and R. RADEK), pp. 121–131. Schweizerbart Science Publishers, Stuttgart.
- SMIRLIS, D. & SOTERIADOU, K. (2011). Trypanosomatid apoptosis: 'apoptosis' without the canonical regulators. *Virulence* **2**, 253–256.
- SMITH, D. G. S., GAWRYLUK, R. M. R., SPENCER, D. F., PEARLMAN, R. E., SIU, K. W. M. & GRAY, M. W. (2007). Exploring the mitochondrial proteome of the ciliate protozoan *Tetrahymena thermophila*: direct analysis by tandem mass spectrometry. *Journal of Molecular Biology* **374**, 837–863.
- SMITH-SOMERVILLE, H. E., HARDMAN, J. K., TIMKOVICH, R., RAY, W. J., ROSE, K. E., RYALS, P. E., GIBBONS, S. H. & BUHSE, H. E. Jr. (2000). A complex of iron and nucleic acid catabolites is a signal that triggers differentiation in a freshwater protozoan. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 7325–7330.
- SOGAME, Y., KOJIMA, K., TAKESHITA, T., KINOSHITA, E. & MATSUOKA, T. (2014). Identification of differentially expressed water-insoluble proteins in the encystment process of *Colpoda cucullus* by two-dimensional electrophoresis and LC-MS/MS analysis. *Journal of Eukaryotic Microbiology* **61**, 51–60.
- SONNEBORN, T. M. (1949). Beyond the gene. *American Scientist* **37**, 33–59.
- SPERLING, L. (2011). Remembrance of things past retrieved from the *Paramecium* genome. *Research in Microbiology* **162**, 587–597.
- SPERLING, L., KERYER, G., RUIZ, F. & BEISSON, J. (1991). Cortical morphogenesis in *Paramecium*: a transcellular wave of protein phosphorylation involved in ciliary rootlet disassembly. *Developmental Biology* **148**, 205–218.
- SRIVASTAVA, M., SIMAKOV, O., CHAPMAN, J., FAHEY, B., GAUTHIER, M. E., MITROS, T., RICHARDS, G. S., CONACO, C., DACRE, M., HELLSTEN, U., LARROUX, C., PUTNAM, N. H., STANKE, M., ADAMSKA, M., DARLING, A., DEGNAN, S. M., OAKLEY, T. H., PLACHETZKI, D. C., ZHAI, Y., ADAMSKI, M., CALCINO, A., CUMMINS, S. F., GOODSTEIN, D. M., HARRIS, C., JACKSON, D. J., LEYS, S. P., SHU, S., WOODCROFT, B. J., VERVOORT, M., KOSIK, K. S., MANNING, G., DEGNAN, B. M. & ROKHSAR, D. S. (2010). The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature* **466**, 720–726.
- STEINER, D. F. (1998). The proprotein convertases. *Current Opinion in Chemical Biology* **2**, 37–39.
- STEMM-WOLF, A. J., MEEHL, J. B. & WINEY, M. (2013). Sfr13, a member of a large family of asymmetrically localized Sfr1-repeat proteins, is important for basal body separation and stability in *Tetrahymena thermophila*. *Journal of Cell Science* **126**, 1659–1671.
- STEMM-WOLF, A. J., MORGAN, G., GIDDINGS, T. H. Jr., WHITE, E. A., MARCHIONE, R., McDONALD, H. B. & WINEY, M. (2005). Basal body duplication and maintenance require one member of the *Tetrahymena thermophila* centrin gene family. *Molecular Biology of the Cell* **16**, 3606–3619.
- STENMARK, H. (2012). The Rabs: a family at the root of metazoan evolution. *BMC Biology* **10**, 68.
- STOCK, C., GRÖNLIN, H. K. & ALLEN, R. D. (2002). The ionic composition of the contractile vacuole fluid of *Paramecium* mirrors ion transport across the plasma membrane. *European Journal of Cell Biology* **81**, 505–515.
- STOCK, C., KRÜPPEL, U. T., KEY, G. & LUEKEN, W. (1999). Sexual behaviour in *Euplotes raikovi* is accompanied by pheromone-induced modifications of ionic currents. *Journal of Experimental Biology* **202**, 475–483.
- STROTMANN, R., SCHRÖCK, K., BÖSELT, I., STÄUBERT, C., RUSS, A. & SCHÖNEBERG, T. (2011). Evolution of GPCR: change and continuity. *Molecular and Cellular Endocrinology* **331**, 170–178.
- SÜDHOF, T. C. (2013). Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron* **80**, 675–690.
- SÜDHOF, T. C. (2014). The molecular machinery of neurotransmitter release (Nobel lecture). *Angewandte Chemie International Edition* **53**, 12696–12717.
- SUGA, H., TORRUELLA, G., BURGER, G., BROWN, M. W. & RUIZ-TRILLO, I. (2014). Earliest holozoan expansion of phosphotyrosine signaling. *Molecular Biology of Evolution* **31**, 517–528.
- SUGIURA, M. & HARUMOTO, T. (2001). Identification, characterization, and complete amino acid sequence of the conjugation-inducing glycoprotein (blepharmon) in the ciliate *Blepharisma japonicum*. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 14446–14451.
- SUGIURA, M., SHIOTANI, H., SUZAKI, T. & HARUMOTO, T. (2010). Behavioural changes induced by the conjugation-inducing pheromones, gamone 1 and 2, in the ciliate *Blepharisma japonicum*. *European Journal of Protistology* **46**, 143–149.
- SUNG, C. H. & LEROUX, M. R. (2013). The roles of evolutionarily conserved functional modules in cilia-related trafficking. *Nature Cell Biology* **15**, 1387–1397.
- SURMACZ, L., WIEJAK, J. & WYROBA, E. (2006). Cloning of two genes encoding Rab7 in *Paramecium*. *Acta Biochimica Polonica* **53**, 149–156.
- SUTTON, R. B., FASSHAUER, D., JAHN, R. & BRUNGER, A. T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature* **395**, 347–353.
- SWART, E. C., WILKES, C. D., SANDOVAL, P. Y., ARAMBASIC, M., SPERLING, L. & NOWACKI, M. (2014). Genome-wide analysis of genetic and epigenetic control of programmed DNA deletion. *Nucleic Acids Research* **42**, 8970–8983.

- TAMM, S. L. (2014). Ctenophores and termites – systems for motility. In *Cilia and Flagella, Ciliates and Flagellates* (eds K. HAUSMANN and R. RADEK), pp. 147–171. Schweizerbart Science Publishers, Stuttgart.
- TANI, T., ALLEN, R. D. & NAITOH, Y. (2001). Cellular membranes that undergo cyclic changes in tension: direct measurement of force generation by an in vitro contractile vacuole of *Paramecium multimicronucleatum*. *Journal of Cell Science* **114**, 785–795.
- THEWES, S., SCHUBERT, S. K., PARK, K. & MUTZEL, R. (2014). Stress and development in *Dictyostelium discoideum*: the involvement of the catalytic A subunit. *Journal of Basic Microbiology* **54**, 607–613.
- THOMAS, P., LEE, A. K., WONG, J. G. & ALMERS, W. (1994). A triggered mechanism retrieves membrane in seconds after Ca^{2+} -stimulated exocytosis in single pituitary cells. *Journal of Cell Biology* **124**, 667–675.
- TIAN, M., CHEN, X., XIONG, Q., XIONG, J., XIAO, C., GE, F., YANG, F. & MIAO, W. (2014). Phosphoproteomic analysis of protein phosphorylation networks in *Tetrahymena thermophila*, a model single-celled organism. *Molecular and Cellular Proteomics* **13**, 503–519.
- TIEDTKE, A., HUNSELER, P., FLORIN-CHRISTENSEN, J. & FLORIN-CHRISTENSEN, M. (1988). Exocytosis, endocytosis and membrane recycling in *Tetrahymena thermophila*. *Journal of Cell Science* **89**, 515–520.
- TOMARU, A. (2002). Encystment-inducing factors in the ciliate *Euplotes elegans*. *Zoological Science* **19**, 741–746.
- TOMINAGA, T. & ALLEN, R. (1998). Electrophysiology of the in situ contractile vacuole complex of *Paramecium* reveals its membrane dynamics and electrogenic site during osmoregulatory activity. *Journal of Experimental Biology* **201**, 451–460.
- TREISMAN, R. (2013). Shedding light on nuclear actin dynamics and function. *Trends in Biochemical Sciences* **38**, 376–377.
- UNDERHILL, D. M. & GOODRIDGE, H. S. (2012). Information processing during phagocytosis. *Nature Reviews Immunology* **12**, 492–502.
- UREN, A. G., O'ROURKE, K., ARAVIND, L., PISABARRO, M. T., SESHAGIRI, S., KOONIN, E. V. & DIXIT, V. M. (2000). Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Molecular Cell* **6**, 961–967.
- VALENTINE, M. S., RAJENDRAN, A., YANO, J., WEERARATNE, S. D., BEISSON, J., COHEN, J., KOLL, F. & VAN HOUTEN, J. (2012). *Paramecium* BBS genes are key to presence of channels in cilia. *Cilia* **1**, 16.
- VALLESI, A., BALLARINI, P., DI PRETORO, B., ALIMENTI, C., MICELI, C. & LUPORINI, P. (2005). Autocrine, mitogenic pheromone receptor loop of the ciliate *Euplotes raikovi*: pheromone-induced receptor internalization. *Eukaryotic Cell* **4**, 1221–1227.
- VALLESI, A., DI PRETORO, B., BALLARINI, P., APONE, F. & LUPORINI, P. (2010). A novel protein kinase from the ciliate *Euplotes raikovi* with close structural identity to the mammalian intestinal and male-germ cell kinases: characterization and functional implications in the autocrine pheromone signaling loop. *Protist* **161**, 250–263.
- VALLESI, A., GIUSEPPE, G. D., DINI, F. & LUPORINI, P. (2008). Pheromone evolution in the protozoan ciliate, *Euplotes*: the ability to synthesize diffusible forms is ancestral and secondarily lost. *Molecular Phylogenetics and Evolution* **47**, 439–442.
- VAN HOUTEN, J. (1998). Chemosensory transduction in *Paramecium*. *European Journal of Protistology* **34**, 301–307.
- VAYSSIÉ, L., SKOURI, F., SPERLING, L. & COHEN, J. (2000). Molecular genetics of regulated secretion in *Paramecium*. *Biochimie* **82**, 269–288.
- VERBSKY, J. W. & TURKEWITZ, A. P. (1998). Proteolytic processing and Ca^{2+} -binding activity of dense-core vesicle polypeptides in *Tetrahymena*. *Molecular Biology of the Cell* **9**, 497–511.
- VERKHRATSKY, A. & BURNSTOCK, G. (2014). Biology of purinergic signalling: its ancient evolutionary roots, its omnipresence and its multiple functional significance. *BioEssays* **36**, 697–705.
- VETTER, D., KISSMEHL, R., TREPTAU, T., HAUSER, K., KELLERMANN, J. & PLATTNER, H. (2003). Molecular identification of a calcium-inhibited catalytic subunit of casein kinase type 2 from *Paramecium tetraurelia*. *Eukaryotic Cell* **2**, 1220–1233.
- VICINANZA, M., D'ANGELO, G., DI CAMPLI, A. & DE MATTEIS, M. A. (2008). Function and dysfunction of the PI system in membrane trafficking. *European Molecular Biology Organisation Journal* **27**, 2457–2470.
- VILMART-SEUWEN, J., KERSKEN, H., STÜRZL, R. & PLATTNER, H. (1986). ATP keeps exocytosis sites in a primed state but is not required for membrane fusion: an analysis with *Paramecium* cells in vivo and in vitro. *Journal of Cell Biology* **103**, 1279–1288.
- VOGT, A. & MOCHIZUKI, K. (2013). A domesticated *PiggyBac* transposase interacts with heterochromatin and catalyzes reproducible DNA elimination in *Tetrahymena*. *PLoS Genetics* **9**(12), e1004032.
- WALCZAK, C. E., GAYEK, S. & OHI, R. (2013). Microtubule-depolymerizing kinesins. *Annual Review of Cell and Developmental Biology* **29**, 417–441.
- WALCZAK, C. E. & NELSON, D. L. (1993). In vitro phosphorylation of ciliary dyneins by protein kinases from *Paramecium*. *Journal of Cell Science* **106**, 1369–1376.
- WASSENBERG, J. J., CLARK, K. D. & NELSON, D. L. (1997). Effect of SERCA pump inhibitors on chemoresponses in *Paramecium*. *Journal of Eukaryotic Microbiology* **44**, 574–581.
- WASSMER, T., FROISSARD, M., PLATTNER, H., KISSMEHL, R. & COHEN, J. (2005). The vacuolar proton-ATPase plays a major role in several membrane-bounded organelles in *Paramecium*. *Journal of Cell Science* **118**, 2813–2825.
- WASSMER, T., KISSMEHL, R., COHEN, J. & PLATTNER, H. (2006). Seventeen a-subunit isoforms of *Paramecium* V-ATPase provide high specialization in localization and function. *Molecular Biology of the Cell* **17**, 917–930.
- WASSMER, T., SEHRING, I. M., KISSMEHL, R. & PLATTNER, H. (2009). The V-ATPase in *Paramecium*: functional specialization by multiple gene isoforms. *Pflügers Archiv European Journal of Physiology* **457**, 599–607.
- WATANABE, A., KURASAWA, Y., WATANABE, Y. & NUMATA, O. (1998). A new *Tetrahymena* actin-binding protein is localized in the division furrow. *Journal of Biochemistry* **123**, 607–613.
- WATANABE, S., ROST, B. R., PÉREZ, M. C., DAVIS, M. W., KIELCZYNSKI, B. S., ROSENEMUND, D. & JORGENSEN, E. M. (2013). Ultrafast endocytosis at mouse hippocampal synapses. *Nature* **504**, 242–247.
- WEBER, J. H., VISHNYAKOV, A., HAMBACH, K., SCHULTZ, A., SCHULTZ, J. E. & LINDER, J. U. (2004). Adenylyl cyclases from *Plasmodium*, *Paramecium* and *Tetrahymena* are novel ion channel/enzyme fusion proteins. *Cellular Signalling* **16**, 115–125.
- WELZ, T., WELLBOURNE-WOOD, J. & KERKHOFF, E. (2014). Orchestration of cell surface proteins by Rab11. *Trends in Cell Biology* **24**, 407–415.
- WHITEHEART, S. W., SCHRAW, T. & MATVEEVA, E. A. (2001). N-ethylmaleimide sensitive factor (NSF) structure and function. *International Review of Cytology* **207**, 71–112.
- WICKSTEAD, B. & GULL, K. (2006). A “holistic” kinesin phylogeny reveals new kinesin families and predicts protein functions. *Molecular Biology of the Cell* **17**, 1734–1743.
- WILLIAMS, K. (1997). Interactions of polyamines with ion channels. *Biochemical Journal* **325**, 289–297.
- WLOGA, D. & FRANKEL, J. (2012). From molecules to morphology: cellular organization of *Tetrahymena thermophila*. *Methods in Cell Biology* **109**, 83–140.
- WLOGA, D. & GAERTIG, J. (2010). Post-translational modifications of microtubules. *Journal of Cell Science* **123**, 3447–3455.
- WLOGA, D., ROGOWSKI, K., SHARMA, N., VAN DIJK, J., JANKE, C., EDDÉ, B., BRÉ, M. H., LEVILLIERS, N., REDEKER, V., DUAN, J., GOROVSKY, M. A., JERKA-DZIADOSZ, M. & GAERTIG, J. (2008). Glutamylation on alpha-tubulin is not essential but affects the assembly and functions of a subset of microtubules in *Tetrahymena thermophila*. *Eukaryotic Cell* **7**, 1362–1372.
- WRIGHT, M. V. & VAN HOUTEN, J. L. (1990). Characterization of a putative Ca^{2+} -transporting Ca^{2+} -ATPase in the pellicles of *Paramecium tetraurelia*. *Biochimica et Biophysica Acta* **1029**, 241–251.
- WU, L. G., HAMID, E., SHIN, W. & CHIANG, H. C. (2014). Exocytosis and endocytosis: modes, functions, and coupling mechanisms. *Annual Review of Physiology* **76**, 301–331.
- YANG, W. Q., BRAUN, C., PLATTNER, H., PURVEE, J. & VAN HOUTEN, J. L. (1997). Cyclic nucleotides in glutamate chemosensory signal transduction of *Paramecium*. *Journal of Cell Science* **110**, 2567–2572.
- YANO, J., RACHOCHY, V. & VAN HOUTEN, J. L. (2003). Glycosyl phosphatidylinositol-anchored proteins in chemosensory signaling: antisense manipulation of *Paramecium tetraurelia* PIG-A gene expression. *Eukaryotic Cell* **2**, 1211–1219.
- YANO, J., RAJENDRAN, A., VALENTINE, M. S., SAHA, M., BALLIF, B. A. & VAN HOUTEN, J. L. (2013). Proteomic analysis of the cilia membrane of *Paramecium tetraurelia*. *Journal of Proteomics* **78**, 113–122.
- YILDIZ, M. T. & ARSLANYOLU, M. (2014). In silico identification and characterization of the MAPK family members of unicellular model eukaryote *Tetrahymena thermophila*. *European Journal of Protistology* **50**, 538–550.
- YING, G., AVASTHI, P., IRWIN, M., GERSTNER, C. D., FREDERICK, J. M., LUCERO, M. T. & BAEHR, W. (2014). Centrin 2 is required for mouse olfactory ciliary trafficking and development of ependymal cilia planar polarity. *Journal of Neuroscience* **34**, 6377–6388.
- ZACKROFF, R. V. & HUFNAGEL, L. A. (2002). Induction of anti-actin drug resistance in *Tetrahymena*. *Journal of Eukaryotic Microbiology* **49**, 475–477.
- ZAMPESE, E. & PIZZO, P. (2012). Intracellular organelles in the saga of Ca^{2+} homeostasis: different molecules for different purposes? *Cellular and Molecular Life Sciences* **69**, 1077–1104.
- ZERIAL, M. & MCBRIDE, H. (2001). Rab proteins as membrane organizers. *Nature Reviews Molecular Cell Biology* **2**, 107–117.
- ZHANG, C., GAO, S., MOLASCON, A. J., WANG, Z., GOROVSKY, M. A., LIU, Y. & ANDREWS, P. C. (2014). Bioinformatic and proteomic analysis of bulk histones reveals PTM crosstalk and chromatin features. *Journal of Proteome Research* **13**, 3330–3337.
- ZIESENISS, E. & PLATTNER, H. (1985). Synchronous exocytosis in *Paramecium* cells involves very rapid (≤ 1 s), reversible dephosphorylation of a 65-kD phosphoprotein in exocytosis-competent strains. *Journal of Cell Biology* **101**, 2028–2035.
- ZITO, K. & SCHEUSS, V. (2009). NMDA receptor function and physiological modulation. In *Encyclopedia of Neuroscience* (Volume 6, ed. L. R. SQUIRE), pp. 1157–1164. Academic Press, Oxford.
- ZMASEK, C. M. & GODZIK, A. (2013). Evolution of the animal apoptosis network. *Cold Spring Harbor Perspectives in Biology* **5**, a008649.
- ZWEIFEL, E., SMITH, J., ROMERO, D., GIDDINGS, T. H., WINEY, M., HONTS, J., DAHLSEID, J., SCHNEIDER, B. & COLE, E. S. (2009). Nested genes CDA12 and CDA13 encode proteins associated with membrane trafficking in the ciliate *Tetrahymena thermophila*. *Eukaryotic Cell* **8**, 899–912.

XIX. APPENDIX:

BIOLOGICAL AND TECHNICAL TERMS: GLOSSARY, AND ABBREVIATIONS USED

AAA-ATPase: triple-A ATPase: a chaperone (protein mediating correct conformation of another protein). For example NSF is a chaperone specific for SNARE proteins, occurring in protozoa, animals and plants; **ADF:** actin depolymerising factor; **AED:** aminoethyl dextrane: secretagogue stimulating trichocyst exocytosis in *Paramecium* spp; **Alveolar sacs:** flat, sac-like cortical Ca^{2+} stores closely attached to the cell membrane in ciliates and other Alveolata; **AP:** adaptor protein: APs mediate self-assembly of coat proteins on vesicles budding from the cell membrane or from specific organelles. Found in protozoa, animals and plants; **ARNO:** ADP [adenosine diphosphate]-ribosylation factor nucleotide site opener, a guanosine nucleotide exchange factor; **ASI2:** signal transducer and activator of transcription 2 protein; **BAR:** Bin-amphiphysin-Rvs; **bbofl:** basal body orientation factor 1; **BBS:** Beadle-Bardet Syndrome: molecular defect of ciliary function in man, with an ortholog of BBS-protein in ciliates; **C2 domain:** protein domain with β -barrel conformational motif and an extending loop appropriate for low-capacity/high-sensitivity Ca^{2+} binding. Found in protozoa, animals and plants; **$[\text{Ca}^{2+}]_i$:** intracellular free (dissolved, not protein-bound) Ca^{2+} concentration; **$[\text{Ca}^{2+}]_o$:** extracellular Ca^{2+} concentration; **Ca^{2+} -ATPase:** Ca^{2+} pump for primary active Ca^{2+} transport, forming a phosphorylated intermediate (in contrast to V-H^{+} -ATPase). Found in protozoa, animals and plants; **cADPR:** cyclic adenosine diphosphoribose. Second messenger; **cAMP:** cyclic adenosine monophosphate (AMP); **CaBP:** Ca^{2+} -binding protein: high capacity/low affinity (in cytosol and Ca^{2+} storage organelles) or low capacity/high affinity (in cytosol). Found in all kingdoms; **CaM:** calmodulin: Ca^{2+} -binding protein with four EF-hand motifs (each usually made of 12 amino acids) enabling Ca^{2+} binding with low capacity/high affinity. Found in all kingdoms; **CaM-BP:** calmodulin-binding protein; **CDA11, 12:** cell division (protein) A11, 12; **Cdk:** cyclin-dependent kinase; **CDPK:** Ca^{2+} -dependent protein kinase: a protein kinase with integrated calmodulin-like motif, specific to plants and some protozoa such as ciliates; **Contractile vacuole complex:** osmoregulatory organelle with rhythmic pulsations, serving for the extrusion of an excess of water (and ions) in some freshwater protozoa; **COP:** coatamer protein: ubiquitous non-clathrin-type polymeric protein assembly mediating vesicle budding, e.g. from Golgi apparatus; **CRC:** Ca^{2+} -release channel: see IP_3R , RyR and RyR-LP ; recently identified in protozoa (ciliates and trypanosomatids), but absent from plants; **DAG:** diacyl glycerol: protein kinase C-activating product formed (together with inositol 1,4,5-trisphosphate) by phospholipase C-mediated cleavage of phosphatidyl inositol 4,5-bisphosphate; occurrence not established for ciliates; **DIE:** defective in excision; **DisAp:** disorientation protein mutant A; **DOC2:** double C2-like domain containing protein 2; **EDX:** energy-dispersive X-ray microanalysis:

electron microscopic analysis of local element composition by element-specific X-ray fluorescence generated by the electron beam used for image formation. Chemical signals can be assigned to specific subcellular structures, such as Ca^{2+} stores. The method can be applied to cells rapidly frozen under defined functional states (quenched-flow/rapid freezing). EDX allows for quantitation, e.g. measuring changes in total Ca^{2+} content in an organelle during stimulation; **EEA1:** early endosome antigen 1; **EF-hand motif:** helix-loop-helix motif of CaBPs for high affinity Ca^{2+} binding; **EMRE:** essential MCU regulator; **ER:** endoplasmic reticulum; **Er-1:** *Euplotes raikovi* pheromone; **Erk:** extracellular signal-regulated kinase; **FIPs:** family of RabII-interacting proteins; **fMLP:** formyl-methionyl-leucyl-phenylalanine; **FOR20:** FOP (FGFR1 oncogene partner)-related protein of 20 kDa size; **GAP:** GTPase-activating protein: activator of small (monomeric) GTPases ('G-proteins'); **GDI:** guanosine nucleotide dissociation inhibitor: inhibits GTP/GDP exchange on small GTPases; **GEF:** guanosine nucleotide exchange factor: stimulates GTP/GDP exchange on small GTPases, i.e. monomeric guanosine triphosphate (GTP) binding proteins with GTPase (GTP hydrolysing) function; **GFP:** green fluorescent protein; **GPCR:** G-protein-coupled receptor: receptors mainly on cell surface coupled to trimeric G-proteins (with α -, β - and γ -subunits); occurrence in ciliates likely, but not definitely established; **GPI-anchor:** glycosyl phosphatidylinositol-anchor: attachment of a protein to a membrane *via* a carboxyterminal anchor formed by glycosyl residues complemented by a phosphate and an inositol group. Found in protozoa, animals and plants; **G-proteins:** proteins binding guanosine nucleotides. Specifically GTPase molecules [small (monomeric) G-proteins involved, e.g. in vesicle trafficking] and trimeric G-proteins (involved in signalling from the cell membrane); **GrIp:** granule lattice protein; **Grtp:** granule tip protein; **GTPase:** small monomeric G-proteins involved, e.g. in vesicle trafficking. Found in protozoa, animals and plants; **HAP2:** a transcriptional activator; **IES:** internal eliminated sequence: defined stretches of micronuclear DNA (probably derived from old transposons) occurring in ciliates; they are eliminated under epigenetic control, i.e. by scanning RNA (scnRNA) during formation of a new macronuclear genome from a postmitotic micronuclear genome; **IFT:** intraflagellar transport: this also includes intraciliary transport of macromolecule aggregates; in protozoa and animals; **ILF:** infraciliary lattice filaments; **InsP₃:** inositol 1,4,5-trisphosphate (InsP_3): formed (together with diacylglycerol) by phospholipase C-mediated cleavage of phosphatidyl inositol 4,5-bisphosphate; activates InsP_3 receptors (CRCs). InsP_3 effects reported from all kingdoms, but formation in plants is unlikely; **IP_3R :** inositol 1,4,5-trisphosphate receptor: InsP_3 -receptor-type CRCs; established from protozoa onwards, including ciliates and trypanosomatids, and animals; expected also for choanoflagellates; **kif:** kinesin family; **-LP:** -like protein: expressing similarity, e.g. between calmodulin and calmodulin-like sequences in some proteins; **MALDI:** matrix-assisted laser desorption/ionisation: mass spectrometric analysis of

biomolecules, based on binding to a support matrix, laser irradiation for fragmentation and desorption from the matrix and ionisation, coupled to acceleration of ionised fragments for mass recording in a spectrometer. Phosphorylation sites in proteins can thus be determined; **MAPK**: mitogen-activated protein kinase: protein kinase activated during mitosis and some other functions; probably in all eukaryotic kingdoms, but little studied in ciliates; **MCU**: mitochondrial calcium uniporter; **MEK**: MAP and ERK kinase (ERK = extracellular-signal regulated kinase): protein kinase involved in intracellular signalling after MAPK activation; probably in all eukaryotic kingdoms, but little studied in ciliates; **MICU**: mitochondrial calcium uptake; **MOB1**: Mps One Binder; **mtA**: name of a promotor; **Muocysts**: dense core-secretory organelles of *Tetrahymena* spp; **Myo**: myosin; **NAADP**: nicotinic acid adenine dinucleotide-phosphate. Second messenger; **NFAT**: nuclear factor for activation of T-cells; **NHEJ**: non-homologous end-joining; **NIMA**: never in mitosis A; **NMDA**: N-methyl-D-aspartate; **NSF**: N-ethylmaleimide sensitive factor: SNARE-specific chaperone (see AAA-ATPase) serving for proper conformational arrangement of SNARE proteins. Found in all kingdoms; **Pdsg**: *Paramecium* protein involved in development of the somatic genome; **PIInsP₂**: phosphatidylinositol 4,5-bisphosphate; **PKA**: cAMP-activated protein kinase A: activated by cyclic 3',5'-adenosinemonophosphate. Found in all kingdoms; **PKC**: protein kinase C: activated by diacyl glycerol; present in animals, but not identified at a molecular level in plants, ciliates and other protozoa; **PKG**: cGMP-activated protein kinase: activated by cyclic 3',5'-guanosinemonophosphate. Found in all kingdoms; **PLC**: phospholipase C; **PMCA**: plasma membrane Ca²⁺-ATPase: Ca²⁺ pump in the cell membrane. Found in all kingdoms; **PP1**: protein phosphatase 1; **PP2B**: protein phosphatase 2B: dimeric protein identical with calcineurin. Described in *Dictyostelium* and *Paramecium* spp., also occurs in animals whereas in plants only subunit B is found; **PrP**: prion protein; **PtAct**, **PtCRC**, **PtSyb**, **PtSyx**, **PtSNARE etc.**: *Pt* designates proteins from *P. tetraurelia*, such as actin etc. Subfamilies are indicated with roman numbers and individual paralogs (including ohnologs) by Arabic numbers; e.g. *PtCRC*-II-1 and -2 indicate specific Ca²⁺-release channels; **Rab**: 'Ras-related in brain': small GTPase family involved in directing intracellular vesicle traffic in all kingdoms; **Rho**: ρ factor, a GTPase; **RyR**: ryanodine receptor: type of Ca²⁺-release channel previously characterised by its response to the plant toxin ryanodine; typical for sarcoplasmic reticulum in muscle cells, but also occurring in many other animal cells. Not established in plant cells. A prototype

occurs in ciliates; **RyR-LP**: ryanodine receptor-like protein: a ryanodine receptor-type Ca²⁺-release channel found in *Paramecium* spp; **scnRNA**: scanning RNA: recognises internal eliminated sequences (IES), i.e. defined stretches of micronuclear DNA (probably derived from old transposons) in ciliates, to be eliminated under epigenetic control during formation of a new macronuclear genome; **Sec7**: transport protein 7: a guanine nucleotide exchange factor, relevant for secretion; **SERCA**: sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase: Ca²⁺ pump in the sarcoplasmic reticulum of muscle cells and in the endoplasmic reticulum of protozoa, plants and animals; **SH2**: Src homology domain 2; **SNAP-25**: synaptosomal associated protein of 25 kDa size; **SNAREs**: soluble N-ethylmaleimide sensitive factor (NSF) attachment protein receptors: single-span membrane proteins of specific types distributed over membranes undergoing interaction (docking) for final fusion; see scheme in Fig. 1. The differentiation between t-SNAREs (t = target) and v-SNAREs (v = vesicle) may not reflect the real topology. Therefore, alternative designations are Q-SNAREs and R-SNARE, for t- and v-SNAREs, respectively, according to the predominant 'central' amino acid in the SNARE domain. Found in all kingdoms; **SOC(E)**: store-operated Ca²⁺(entry): also called capacitative Ca²⁺ influx. SOC (E) superimposes mobilisation of Ca²⁺ from a cortical store as a first step. Found from *Paramecium* to man, but probably not in plants; **SPFH**: stomatin, prohibitin, flotillin and HflK/C superfamily; **Spt6**: transcription activation factor; **Src**: name derived from sarcoma; a non-receptor tyrosine kinase family; **Stat**: transcription activation factor; **SUMO**: small ubiquitin-like modifier; **Syb**: synaptobrevin: v-/R-SNARE (see SNARE), e.g. on trafficking vesicles. Universal distribution, although as 'longin' forms in ciliates and plants; **Syx**: syntaxin: t-/Q-SNARE (see SNARE), e.g. at the cell membrane and on trafficking vesicles. Found in all kingdoms; **TMN1**: *Tetrahymena* mitochondrial nuclease; **tmp**: trichocyst matrix protein; **TPC**: two-pore channel: cation (Ca²⁺) channels with dimeric design of a split voltage-gated Ca²⁺/Na⁺ channel, typically occurring in acidic compartments. Found in acidic compartments of plants and animals, whereas only indirect evidence is available from ciliates; **Trichocysts**: dense core-secretory organelles of *Paramecium* spp; **TRP**: transient receptor potential channels; **t-SNARE/Q-SNAREs**: see SNAREs; **VPS**: vacuolar protein sorting protein; **vsAG**: variant surface antigens: GPI-anchored glycoprotein components of the ciliate cell surface, with mutually exclusive expression under epigenetic control. Found in all kingdoms; **v-SNARE/R-SNARE**: see SNAREs.