First publl. in: BioEssays 24 (2002), 7, pp. 649-658

My favorite cell—Paramecium

Helmut Plattner

Summary

A *Paramecium* cell has a stereotypically patterned surface, with regularly arranged cilia, dense-core secretory vesicles and subplasmalemmal calcium stores. Less strikingly, there is also a patterning of molecules; for instance, some ion channels are restricted to certain regions of the cell surface. This design may explain very effective and selective responses, such as that to Ca^{2+} upon stimulation. It enables the cell to respond to a Ca^{2+} signal precisely secretion (exocytosis) or by changing its ciliary activity. These responses depend on the location and/or type of signal, even though these two target structures co-exist side-by-side, and normally only limited overlap occurs between the different functions. Furthermore, the patterning of exocytotic sites and the possibility of synchronous exocytosis induction in the sub-second time range have considerably facilitated analyses, and thus led to new concepts of exocytotic membrane fusion. It has been possible to dissect complicated events like overlapping Ca^{2+} fluxes produced from external sources and from internal stores. Since molecular genetic approaches have become available for *Paramecium*, many different gene products have been identified only some of which are known from "higher" eukaryotes. Although a variety of basic cellular functions are briefly addressed to demonstrate the uniqueness of this unicellular organism, this article focuses on exocytosis regulation. *BioEssays* 24:649–658, 2002.

Introduction

In the three introductory sections, I will briefly outline how *Paramecium* became a "maid-of-all work in cell biology" as a model organism, showing that this was due mainly to its regular construction ("designer cell") and the synchrony of some processes. In the subsequent subchapters, the article focuses on the work of my own group, notably on the different aspects of exocytosis regulation, including Ca^{2+} signaling, membrane fusion, extremely fast release of secretory contents (trichocysts), and on novel aspects of exo-endocytosis coupling.

Department of Biology, University of Konstanz, 78457 Konstanz, Germany.
Funding agency: The Deutsche Forschungsgemeinschaft.

Abbreviations used: AED, aminoethyldextran; CaM, calmodulin; CaSR, Ca^{2+}/(polyvalent cation)-sensing receptor; CICR, Ca^{2+}-induced Ca^{2+}-release; CLSM, confocal laser scanning microscope; EDX, energy-dispersive x-ray microanalysis; EM, electron microscope; ER, Endoplasmic Reticulum; ESI, electron spectroscopic imaging; GPI, glycosylphosphatidylinositol (anchor); IMPs, intramembraneous particles; SOC, store-operated Ca^{2+}-influx; SR, Sarcoplasmic Reticulum; svAGs, surface variant antigens.

DOI 10.1002/bies.10112
Published online in Wiley InterScience (www.interscience.wiley.com).

Why *Paramecium*?

30 years ago I was involved in the development of fast freezing methods. Since *Paramecium* is a large cell with unusually high water content I chose it as a difficult test object which could lead us to the limits of cryofixation. Then I showed one of my colleagues, now a leading biochemist, my freeze-fracture pictures of *Paramecium*.[1] Its membranes contained regular aggregates, rings, squares and rows, of intramembraneous particles (IMPs, equivalent to intrinsic membrane proteins) located at different sites. These included the docking sites of dense-core secretory vesicles ("trichocysts") and the base of cilia, as well as a vast subplasmalemmal compartment ("alveolar sacs"). My colleague quickly asked me: "What does all this mean in terms of function?" This question has remained a challenge for me ever since, although I did not know then that much of my future work in cell biology would be dedicated to this unicellular organism and, in particular, its exocytosis regulation. The regular "design" of these beautiful cells turned out to be an excellent basis to address several specific aspects of its biology. In fact, previous workers, notably the geneticist Tracy M. Sonneborn (Bloomington, IND, USA), had already introduces *Paramecium* as an important model in cell biology. The unusual features of *Paramecium*, such as its aberrant codons, were not yet known. Such complications have now been overcome and even gene silencing can be done reliably.[2]

At that time, I was also interested in the cell biology of secretion. Very little was known about the regulation of stimulated exocytosis. Structural implications of membrane interactions obtained by electron microscopy (EM) from chemically fixed material appeared rather vague to me, considering the numerous artifacts we could meanwhile pinpoint by extremely fast cryofixation. Therefore, any structure–function correlation would be far from reliable, whereas in *Paramecium* preformed exocytosis sites presented themselves as clearly structured microdomains, with well-arranged intramembraneous particles (IMPs), as seen after mere physical "fixation". Using artificial membranes, several groups had derived rather simple models of membrane fusion. It was assumed that

cytoskeletal elements would be required to bring membranes into contact and the relevance of a Ca^{2+} signal was already well established. What has survived from these older ideas has turned out to be very complicated. Some of the work with *Paramecium* from different laboratories has contributed to establish new concepts of membrane fusion, as we will see.

Before moving over to the focus of this article, namely exocytosis regulation, I would like to provide a brief introduction to some functional and structural aspects of the *Paramecium* cell.

Paramecium, a maid-of-all-work in cell biology?

Several groups, notably those of the late Roger Eckert, of Ching Kung (Madison, WISC, USA) and of Hans Machemer (Bochum, Germany) have functionally characterized the highly different ion channels in *Paramecium*.[3,4] These channels can be grossly localized either to cilia or somatic (non-ciliary) areas of the cell membrane since it is easy to remove the several thousand cilia by deciliation protocols, a fact exploited by electrophysiologists to study ciliary beat regulation in *Paramecium*. One crucial observation concerned voltage-dependent Ca^{2+}-channels specifically in ciliary membranes and the inactivation of these channels by the very same Ca^{2+} that had passed through them[5]—a regulatory principle involving the Ca^{2+}-binding protein, calmodulin (CaM) that was then without precedent. It would have been difficult to imagine that only 21 years later this "curious finding in a curious organism" would also be detected in neurons.[6]

Subsequent analysis of a broad range of behavioral mutants, frequently based on ciliary channel defects, gave electrophysiologists the opportunity to correlate cellular behavior with channel activity, namely to explain behavior on a molecular scale, and to repair functional defects by microinjection of defective regulators (like CaM) and later by gene transfection of CaM mutants with the wild-type CaM gene.[7]

Paramecia can "smell" bacteria by dissipated metabolites,[8] like folic acid, using anteriorly enriched receptors, and so track down their food which they engulf in an oral region, endowed with a cytostome (cell mouth). From there, membrane-bounded phagosomes are pinched off and rapidly acidified (by fusing acidosomes) to kill bacteria and to make them amenable to digestion by lysosomal enzymes. All this occurs in a well-regulated intracellular cycle.[9] It ends at the cytoproct, a well defined region at the cell surface. This precisely timed ritual facilitates the analysis of specific events occurring during membrane traffic under largely synchronous conditions.

The surface of a *Paramecium* also harbors some other components of interest. In particular, it is fully covered with "surface variant antigens" (svAGs), with only one type being expressed at a time, although cells can be induced to change the type of svAG by heat or pH shock treatment.[10,11] These svAGs are a type of glycosylphosphatidylinositol (GPI)-anchored proteins, i.e., the carboxy-terminal part of the protein is linked to a chain of sugars, followed by a phosphate and an inositol-sugar which, in *Paramecium*, is finally anchored in the cell membrane by a ceramide-type lipid. Quite similar svAGs are of paramount importance in some pathogenic relatives of *Paramecium*, like *Plasmodium* (malaria-causing agent) and *Toxoplasma*, where they act as molecular camouflage to escape the immune system. In *Paramecium*, the degradative pathways of svAGs could be analyzed with some precision on the basis of the established ingestion routes.[12] Some biochemists, therefore, prefer to use *Paramecium*, a harmless cell, to study the biosynthesis of svAGs, although the function of svAGs in *Paramecium* is still enigmatic.

Paramecium—a "designer cell"

The surface of a *Paramecium* displays a highly regular structure, with alternating cilia and trichocysts (Figs. 1 and 2).[13] It has an egg-case-type surface relief, with longitudinal and perpendicular ridges. One unit, a kinetid, is $\sim 1 \times 2$ μm in size and harbors a cilium or two in its central depression. Trichocysts are installed in the middle of perpendicular ridges and, thus, form a line with intermittent cilia (Figs. 1 and 2). There are many parallel rows (kineties) over the entire cell surface. The number of kinetids in *P. tetraurelia* has been

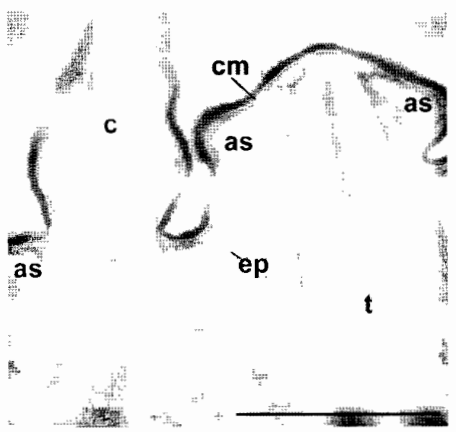

Figure 1. Typical EM aspect of the *Paramecium* cell cortex showing side-by-side a cilium (c) emerging from the surface and a trichocyst (t) docked at the cell membrane (cm) in a position ready for release upon exocytosis stimulation. *Paramecium* cells have an egg case-like surface relief, and cilia are installed at depressions and trichocysts on ridges. Alveolar sacs (as), tightly attached to the cell membrane, flank the onset of both these organelles. The side of the alveolar sacs facing the cell center is enforced by a layer of epiplasm (ep). This, as well as the superficial glycokalyx, appear rather electron dense due to tannic acid and OsO_4 treatment. Bar, 0.5 μm. Unpublished micrograph by C. Westphal and the author.

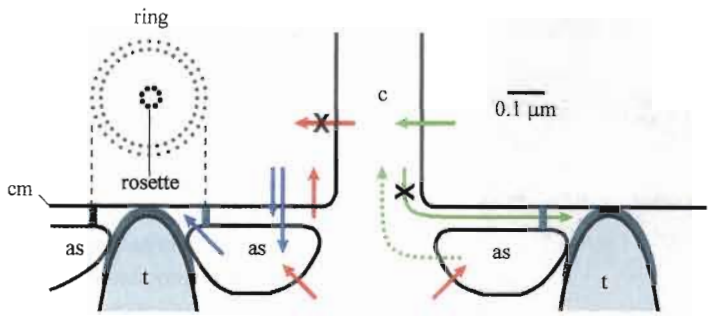

Figure 2. Regular construction of a *Paramecium* cell, with superimposed Ca^{2+}-flux pathways, to be compared with the micrograph presented in Fig. 1. Cilia (c) alternate with trichocyst (t) docking sites at the cell membrane (cm); regions in between are lined by alveolar sacs (as), the cortical calcium stores (~0.1 µm thick, not drawn to scale). As shown by freeze-fracturing, docking sites are lined by a double IMP "ring" and contain a central IMP "rosette" when a docked trichocyst attains exocytosis competence. (To be compared with micrographs of Fig. 3A). Arrows: Ca^{2+} fluxes during exocytosis (blue) or ciliary reversal (green); Ca^{2+} pumps in the cell membrane (except cilia) and SERCA-type Ca^{2+} pump in inner domain of alveolar sacs (red). Note that Ca^{2+} signals from cilia cannot stimulate exocytosis, whereas massive exocytosis induction can cause ciliary reversal (dotted green arrow). Approximate scale bar, 0.1 µm. Scheme based on results compiled in a recent review.[14]

estimated as ~3000, that of trichocyst docking sites as ~1000, that of cilia as ~4000. Close to the basis of cilia, clathrin-coated endocytotic vesicles originate, also arranged in rows. In agreement with Ivonne Capdeville (Gif-sur-Yvette; see citations in Ref. 12), the leading expert on svAGs in *Paramecium*, we consider these the sites not only of replacement of spent svAGs (in addition to phagocytic vacuoles), but also of delivery of new svAGs to the cell surface by constitutive exocytosis.[12]

This spacing of cilia causes their hydrodynamic coupling and, thus, allows a quasi-synchronous beat which is called "metachronic" since it moves like waves over a grain field. As mentioned, the stereotypic character of a *Paramecium* cell also includes the regular arrangement of its trichocysts. Exocytosis, like ciliary beat, is regulated by Ca^{2+}, and hence signal overlap must somehow be avoided. This may be another feature inherent in the regular design of the cell surface, e.g., by keeping different targets at an appropriate distance from the Ca^{2+} channels involved in the different functions.[14] It is unnecessary to emphasize that aspects of this kind are much more amenable to structure–function correlation in such a "designer cell" than in a more "conventional" cell. This holds for surface pattern formation, for example, which quite recently has been shown by Beisson's group to involve novel types of tubulins.[15] My personal interest along these lines, however, has focussed on aspects of exocytosis regulation.

Exocytosis: *Paramecium* as a model system

To understand the value of *Paramecium* as a model system, we have to go back to the early days of exocytosis research. Only very little was known on exocytosis regulation beyond the involvement of Ca^{2+} signaling and the fusion of two membranes. It was generally postulated that proteins have to be eliminated from fusion sites and that Ca^{2+} may mediate fusion by its effect on lipids. At this stage, there were the first hints of an involvement of membrane-integrated and membrane-associated proteins, both before organelle docking (Figs. 2 and 3) and during docking, membrane fusion and resealing.[16,17] Patch-clamping and molecular biology techniques then allowed further progress in understanding exocytosis. However, even now, when we know from other exocytotic systems that dozens of proteins are assembled at an exocytosis site,[18] we are far from identifying them all or knowing how they interact precisely.

Originally, it was believed that proteins had no role in Ca^{2+}-signaling during exocytosis. It was assumed that membrane fusion occurred by local lipid phase transition. The occurrence of IMP aggregates (integral membrane proteins, as defined above), like "rosettes" (of the type shown in Fig. 3A) precisely over trichocyst exocytosis sites and their re-arrangement, not before, but only during/after fusion,[1,16] however, argued against such protein-free models. Important support came from work in Janine Beisson's laboratory (CNRS, Gif-sur-Yvette, F) showing the absence of rosettes from docking sites in a trichocyst-free "trichless" mutant and in "non-discharge" (*nd*) mutants of *P. tetraurelia*, although these exocytosis-incompetent strains contained trichocysts at docking sites.[19] As we showed later, these are held in place in *nd* mutants by attachment to the cortical calcium stores, rather than to the cell membrane.[20] To achieve exocytosis-competence, trichocyst attachment ("docking") requires the assembly of "connecting material" between trichocyst and cell membrane, as we found in collaboration with our French colleagues by analyzing different strains.[21,22] Ongoing collaboration with the group at Gif, now headed by Jean Cohen, has led to the discovery in

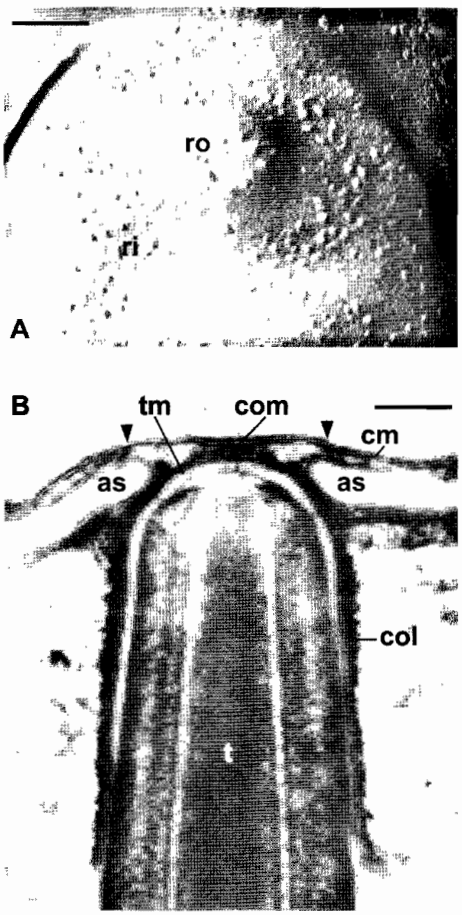

Figure 3. Docking site of an exocytosis-competent trichocyst (t) in a freeze-fracture replica (A) and in an ultrathin section (B). Note "rosette" IMPs (ro) centered in the fusogenic area, delineated by a double IMP "ring" (ri), presence of "connecting material" (com) between trichocyst membrane (tm) and cell membrane (cm), as well as close attachment of alveolar sacs (as) to the cell membrane. The exocytotic site proper is delineated by arrowheads, coinciding with electron-dense connections between the borders of alveolar sacs (B) and the size of a "ring" (A). Several proteins contained in "connecting material" are identified,[24] including calmodulin.[25,26] Upon stimulation, membrane fusion would occur, with restructuring of rosettes, formation of a fusion pore and its enlargement, followed by vigorous discharge of trichocyst contents, all within ~0.1 seconds.[30,37] Bar, 0.1 μm. Adapted from Plattner H, Lumpert CJ, Knoll G, Kissmehl R, Höhne B, Momayezi M, Glas-Albrecht R. Eur J Cell Biol 1991;55:3–16 with permission of the publishers.

Paramecium of the same molecular docking machinery (Froissard et al. and Kissmehl et al., unpublished data) as known from other cellular systems.[18]

How is it that proteins were not initially considered important for exocytosis regulation? One reason was that one relied too much on—undoubtedly very precise—biophysical measurements with artificial membranes, with the prediction that protein-free domains would be required for fusion. The other reason was that EM images were often only believed when they documented precisely this postulate. As it turned out, some of these images reflected inadequate preparation. Only fast freezing, with the avoidance of any chemical treatment showed the true state of the membrane at fusion (for summary, see Ref. 17). John Heuser's analyses of synapses, mast cells and other cells, as well as our own work (not only with *Paramecium* but also with chromaffin cells) showed that IMPs do not move out of fusion zones; a strict positive correlation between the requirement for proteins and exocytosis competence was achieved with *Paramecium* cells.[17,22]

Most colleagues working with "higher" eukaryotes considered such results with *Paramecium* peculiar to protozoa—nothing more. It was necessary to demonstrate these structures to be proteins and one of our publications carried the then provocative title "Membrane-integrated proteins at preformed exocytosis sites".[23] While this is widely accepted now, we *Paramecium* workers still have to admit that we currently know much less about distinct proteins involved in docking and fusion than those working with "higher" eukaryotes. However, in the last few years Jean Cohen's group in Gif has found several novel proteins involved in organelle docking and/or membrane fusion.[24] Furthermore, our previous EM studies, showing CaM at exocytosis sites,[25] have been substantiated by molecular analyses. A "CaM-minus" mutant, unable to properly assemble exocytosis sites over docked, but non-extrudable trichocysts, can be structurally and functionally repaired by transfection with the wild-type CaM gene.[26] Only very recently has CaM been re-introduced into the assembly line of secretory organelle docking proteins in mammalian cells.[27] Another aspect, which at the time was novel, was highlighted by the title "ATP keeps exocytosis sites in a primed state but is not required for membrane fusion"[28]—another fact that is now widely accepted following establishment in mammalian cells.

After having been pilloried for the heretical statement that proteins would be required for docking and membrane fusion, I sensed a total change in attitude after an invited talk at a conference on membrane fusion, 1987, in Buffalo. After my talk, several colleagues reassured me that they would take a close look into a role for proteins in exocytosis regulation in other cells. When one now compares reviews from around 1990[29,30] with recent work,[18,24,31] one can see the great progress that has been made in this field.

Trichocyst expulsion—the fastest dense-core vesicle secretion known

With *Paramecium*, we had to overcome some very simple problems, in particular that of appropriate exocytosis stimulation. (The picric acid stimulation used particularly by geneticists killed the cells.) We introduced aminoethyldextran (AED),

an impermeable large polycation as a secretagogue.[32,33] AED-stimulated exocytosis entails the vigorous synchronous expulsion of trichocyst contents within a small fraction of a second. Only in the last few years have we learned that *Paramecium*, like many eukaryotic cells, may have a sensor for such compounds on their surface,[14,34] with the complicated name "Ca^{2+}/(polyvalent cation)-sensing receptor", CaSR.[35] In *Paramecium*, AED causes immediate, rapid, synchronous exocytosis within less than a second and new trichocysts are synthesized and installed within ~8 hours.[36] AED causes the same cell reactions as seen during exocytosis under "natural" conditions, as discussed below.

To analyze this rapid synchronous process and to exploit its amplification effect, Gerd Knoll and Claudia Braun in my laboratory have expanded a previously established cryofixation method, i.e., spray-freezing, to a time-resolved quenched-flow method.[37] Cells and stimulant (and eventually another solution, e.g., a chelator to adjust extracellular Ca^{2+} to different levels) are contained in separate containers and rapidly mixed in a small chamber. Thus, stimulation occurs under well-defined conditions. After different stimulation times, from 30 milliseconds on (deadtime) up to 1 second, cells are shot into melting propane (−187°C). Precisely triggered samples can be used for further analysis by biochemical or (ultra-)structural methods. Any mechanical impairment was excluded (while tentative use of commercial devices mostly resulted in homogenates).

Quantitative freeze-fracture analysis showed that exocytosis occurs within 80 milliseconds, followed by slightly slower membrane resealing, all within ~350 ms.[37] These values, obtained under standard conditions, are for all events in all cells analyzed, while individual events are much faster. Thus, exocytosis in *Paramecium* operates much faster than any other dense core-vesicle exocytosis.[38]

Long before these experiments, we had established that exocytosis operates by "focal membrane fusion",[17] with a 10 nm large fusion pore formed within an estimated 1 millisecond period, and we were aware that real values may be well below our spatial and temporal resolution. In fact, patch-clamp analyses with mammalian cells[39] justified our caution—real values turned out to be ~10 times below our resolution limits.[40]

During synchronous trichocyst exocytosis, using quenched-flow analysis, we found no increase of cyclic nucleotides (in contrast to manipulations of ciliary activity and chemokinesis stimulation, Ref. 41) or of any other potential second messenger besides Ca^{2+}.[14,42] We then learned to process samples by freeze-substitution under conditions of calcium retention.[43] Calcium distribution could then be analyzed after different conditions of AED stimulation in specially equipped electron microscopes (see below). Together with quantitative ultrastructural analyses, we could thus establish a time scale in the sub-second range.

Calcium signaling during exocytosis

Ca^{2+} was established as a second messenger in exocytosis—and in a variety of other cell functions—over 40 years ago (for recent review, see Ref. 44). During this time, accurate measurements of intracellular calcium in ionic, dissolved form, $[Ca^{2+}]_i$, have been obtained in many cells, but nobody had succeeded with *Paramecium* for a variety of reasons. Ca^{2+}-fluorochrome indicators were not easily taken up and, if so, rapidly sequestered into large vacuoles. In addition, double wavelength (2λ) analysis, required to correct for any cell shape change during stimulation, was hampered by rapid swimming and the jerky recoil during massive trichocyst release. Norbert Klauke in my laboratory has tackled all these problems.[45] To get access to Ca^{2+} signals within 80 milliseconds, we also established fast 1λ-analyses in a confocal laser scanning microscope (CLSM) equipped with an opto-acoustic beam deflection system. In fact, $[Ca^{2+}]_i$ was seen to rise within 80 ms, the time required for exocytosis.[46] This was corroborated by Christian Erxleben from our Biology Department, by recording Ca^{2+}-activated currents over the cell membrane in the whole cell-patching mode.[46,47] The smallest current peaks correlated with release of single trichocysts. The current signal increased in parallel with the extent of exocytotic activity.

It was important then to obtain spatially resolved information on the Ca^{2+} signal. Clearly, microdomain regulation was already well established in muscle as well as in neuronal and from gland cells.[48] *Paramecium* offered the advantage of its clear, repetitive structural situation. Meanwhile the group of André Adoutte, then at the Université de Paris à Orsay, had isolated alveolar sacs from *Paramecium* and characterized them as subplasmalemmal calcium storage compartments.[49] Alveolar sacs are firmly attached at a distance of only 15 nm to the cell membrane which they flank almost entirely. Jean Cohen's group found a strong $^{45}Ca^{2+}$ influx during AED stimulation,[50] as we confirmed.[51] How would endogenous and exogenous components interact?

First we analyzed, in situ as well as with cortex fragments and with isolated alveolar sacs[52]—an in vitro system equivalent to that derived from sea urchin eggs, the effect of many of the second messengers reported in other systems, but we found none.[42] It was then reasonable to analyze whether Ca^{2+} itself might be the causative agent of Ca^{2+} signaling. Two such phenomena are known from other systems, i.e., a "Ca^{2+}-induced Ca^{2+}-release" (CICR) or a "store-operated Ca^{2+} influx" (SOC).[44] With CICR, such as is seen in cardiac muscle cells, the first step is a Ca^{2+}-influx from the outside, which rapidly mobilizes Ca^{2+} from subplasmalemmal stores (e.g., Sarcoplasmic Reticulum, SR). In a second step, SOC is characterized by the opposite chain of events, with a primary mobilization of Ca^{2+} from subplasmalemmal stores, followed by a rapid influx from the outside. Various details of these signaling mechanisms still remain unsettled, also with other systems. It seemed advisable to go

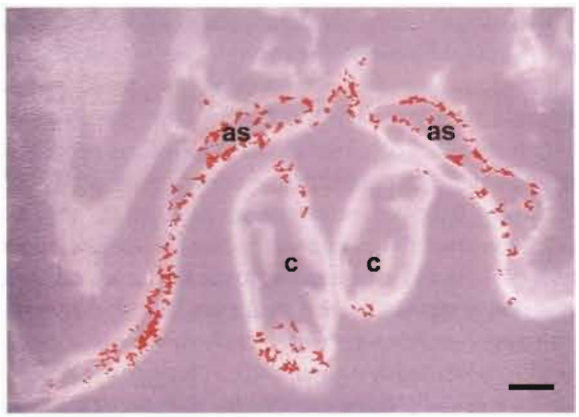

Figure 4. ESI localization of calcium in a cross-section though a *Paramecium* cell prepared by fast freezing, freeze-substitution with a calcium-binding agent, and ultrathin section analysis by ESI. Red dots along the outlines of alveolar sacs (as) and along the membrane of cilia (c) are false color signals for calcium. Bar, 0.1 μm. Unpublished micrograph from Ref. 43.

back to the structural context within the cell. With some special EM instrumentation, by electron spectroscopic imaging (ESI, Fig. 4) and by energy-dispersive X-ray microanalysis (EDX) applied to quench-frozen materials, we could pinpoint calcium in alveolar sacs and document its rapid redistribution during exocytosis stimulation.[43] We thus could combine widely different methods, such as freeze-fracturing, electrophysiology, confocal fluorochrome analysis, ESI and EDX, all in the sub-second time range. (Unfortunately more detailed patch-clamp analyses on this aspect have not been possible so far with these large and rigid cells—this remains an interesting option for future work.) Only much later has calcium been localized by ESI in neurons.[53]

Then Martin Hardt joined my laboratory to specialize in EDX. Based on calibrated standards, EDX allows local (< 70 nm) concentrations of total calcium, [Ca], i.e., free and bound, to be measured. (In most cells, ~0.1 to 0.01% of calcium is in free form before stimulation, and this percentage may increase locally ~10- to > 100-times upon stimulation.) We found in alveolar sacs, which are ~100 nm wide, a [Ca] of 43 mM.[54,55] This is almost identical to that in SR, with which alveoli share the occurrence of a similar Ca^{2+}-binding protein.[56] Stimulation during chelation of extracellular Ca^{2+} revealed that ~40% of the calcium is released from alveoli during 80 ms stimulation. Stimulation in the presence of extracellular Ca^{2+} resulted in a delayed depletion of stores, as if stores were being replenished during release of their Ca^{2+}. Concomitantly, when $Sr^{2+}$was substituted for Ca^{2+} in the medium briefly before stimulation, Sr X-ray signals were clearly registered from inside the stores.[55]

We are now confronted with the problem of which mechanism is involved in the rapid entry of Ca^{2+} (or its substitute, Sr^{2+}) into the alveolar sacs. Uptake by the organellar Ca^{2+}-pump seems unlikely from its kinetics.[14] However, this aspect is not yet fully settled for many other secretory systems. The same holds for the unexpectedly rapid transient Ca^{2+} uptake into mitochondria[55]—another aspect found later on in neuronal cells by analytical EM analysis[57]—although it is known from cardiac cells that mitochondrial Ca^{2+} oscillates with every heart beat.[58]

Is the rapid Ca^{2+} release in *Paramecium* a CICR- or a SOC-type mechanism? The following data support a SOC mechanism. (i) Membrane fusion occurs to a considerable extent also when the secretagogue, AED, is applied together with a rapid Ca^{2+} chelator.[43,59] (ii) Under such conditions, considerable Ca^{2+} mobilization from subplasmalemmal stores is seen by EDX.[55] (iii) Finally, Ihab Mohamed, my recent collaborator from Ain-Shams University in Cairo, has, in collaboration with Jean Cohen, analyzed a double mutant, *pwA/nd12*, which has no Ca^{2+} influx when grown at 35°C. These cells show that part of the Ca^{2+} signaling originates from inside.[60] As in wild-type cells, AED probably activates a CaSR[34] which, by an as yet unknown mechanism, activates Ca^{2+} release from alveoli, without any influx. While Ca^{2+} released from stores, therefore, is probably the primary component, clearly the system works optimally only when this endogenous signal is enforced by massive Ca^{2+} influx from outside.[50,59] The type of Ca^{2+}-channels in alveoli of *Paramecium* is unknown, but we know these channels do not respond to any of the metabolic activators known from other systems.[14] They may be of the ryanodine receptor-type, as in the SR, since Ca^{2+} can be released from alveolar sacs by the SR activators, caffeine and 4-chloro-m-cresol.[34,60] Influx channels are low specificity somatic cation-channels,[34] perhaps of the type described in *Paramecium* by Saitow et al.[61]

What does the extremely rapid substitution, during stimulation, of exogenous for endogenous Ca^{2+} in subplasmalemmal stores imply? For the following reasons, we assume that Ca^{2+} pump y and y release channel molecules are positioned at remote places and that the pump is the much slower regulatory component. After activation, due to kinetic properties, calcium in the cytosol can be downregulated only over longer time periods, by the plasmalemmal Ca^{2+}-ATPase[8] and by the SERCA (Sarcoplasmic-Endoplasmic Reticulum Ca^{2+}-ATPase-)-type pump in alveolar sacs.[62,63] Only that domain of alveolar sacs that faces the cell center is densely studded with Ca^{2+}-pump molecules, mediating slow ATP-dependent Ca^{2+} re-uptake.[64] The dense packing of the Ca^{2+} pump allows accommodation of any Ca^{2+}-release channels only in the remaining domain of alveoli, i.e., that facing trichocyst docking sites and the cell membrane, respectively. Under such conditions, Ca^{2+} primarily released from alveoli, as well as secondarily from superimposed influx from the outside

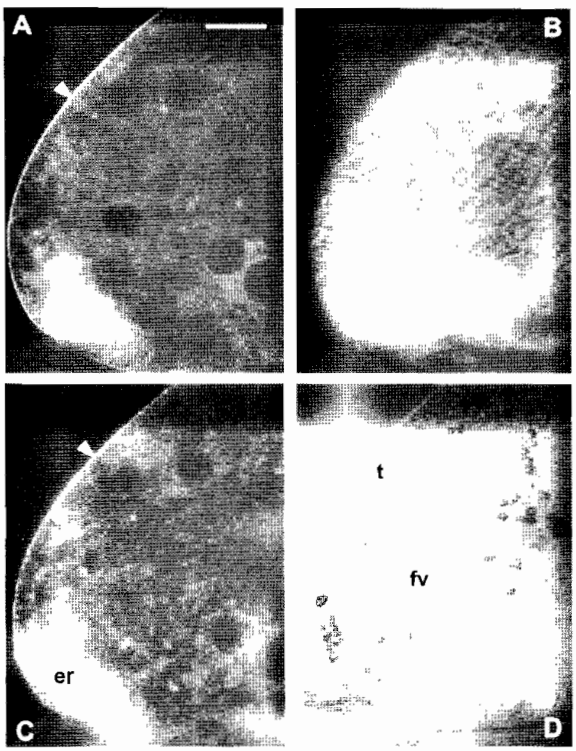

Figure 5. Confocal images of a cell overexpressing a fusion protein of the SERCA-type Ca^{2+} pump (cloned in *Paramecium*[62]) and green fluorescent protein (GFP). In the different planes, fluorescence images show intense cortical labeling (arrowheads in median (**A**) and close-top-median section (**C**)), which corresponds to the outlines of alveolar sacs seen en face in **B**. **C:** Labeling of some peripheral endoplasmic reticulum (er) indicates the biogenetic pathway of the Ca^{2+} pump, which finally is strongly concentrated in alveolar sacs. **D:** A transmitted light image through a median plane, with peripherally docked trichocysts (t) and food vacuoles (fv). Bar, 10 μm. Reproduced from Hauser K, Pavlovic N, Klauke N, Geissinger D, Plattner H. Mol Microbiol 2000;37:773–787 with permission of the publishers.

medium and further efflux from alveoli, would move directly towards trichocyst exocytosis sites (Fig. 2). Concomittant microdomains of Ca^{2+} activation are recognized by fast confocal analysis.[46] Such site-directed Ca^{2+}-flux is not known from other secretory systems.

Actual $[Ca^{2+}]_i$ at exocytosis sites during activation may be ≤ 10 μM, as estimated from Ca^{2+}-chelator (buffer) injection studies.[45] According to pilot calculations, this would represent only $\leq 0.1\%$ of all Ca^{2+} set in motion during stimulation.[14] This is not unusual since, as in other cells,[65] most Ca^{2+} is rapidly bound to Ca^{2+}-binding proteins with which the *Paramecium* cortex abounds.[14] This SERCA has been cloned and tagged with green fluorescent protein (Fig. 5) by my postdoctoral coworker Karin Hauser.[62,66] We could thus analyze for the first time the biogenetic pathway of a cortical calcium store in a secretory system. The SERCA of alveolar sacs is assembled in the ER and then largely delivered to alveolar sacs which do not display any continuity with the ER.[66]

Release of secretory contents

The content of trichocysts consists of a quasi-crystalline assembly of similar proteins ("trichynins") encoded by a ≈100 genes family grouped into 4–8 genes, with > 85% nucleotide identity, as found by Linda Sperling's group in Gif.[67] Interestingly, mature trichocyst proteins decondense as soon as they "see" > 1 μM Ca^{2+},[68] as it automatically occurs in vivo after formation of an exocytotic opening. This allows rapid re-crystallization, possibly in a cooperative way, causing vigorous expulsion, during which trichocyst contents stretch to several times their original length.

The high sensitivity of trichocyst contents to Ca^{2+} is in contrast to the high calcium content in many other secretory organelles.[69] It also poses the question, how are trichynins folded, since normally folding in the ER involves the assistance of Ca^{2+}-dependent chaperones. These are a kind of molecular midwife that helps a nascent polypeptide chain to fold properly. In fact, we have evidence of the occurrence of similar chaperones in the rough ER of *Paramecium*,[56] as in mammalian cells. The answer to this kind of "Ca^{2+}-paradox" may be that Ca^{2+} sensitivity emerges only after pre-trichynins are post-translationally cleaved before their paracrystalline assembly. Another unusual feature of trichocysts is that they are not remarkably acidic compartments.[70] This exemplifies the fact that, despite the widely different cargo in different secretory organelles, the control mechanism of docking and membrane fusion may be very similar, although release of the contents may depend on some properties of the actual contents. In fact, a *P. caudatum* mutant, *tnd1*, can perform membrane fusion without contents release, due to aberrant Ca^{2+}-binding.[71]

To be or not to be—that is the question: function of trichocysts

Why should the cell bother to produce 1000 large packages of para-crystalline secretory products, with the investment of ~40% of its protein contents? Surely this points toward a vital function. Under laboratory conditions, some *Paramecium* strains live well without any extrudable trichocysts. Pioneer studies by Harumoto and Miyake,[72] then at the University of Camerino, Italy, revealed that the discharge of trichocysts is a very effective defence against some predators. When we then had a closer look in the microscope into such "dangerous encounters" of a *Paramecium* with a predatory *Dileptus* cell, we saw the explosive local release of trichocysts, which formed a space-keeper, while the cell was pushed backward.[73] With a slight delay, cells activated their "back-gear",

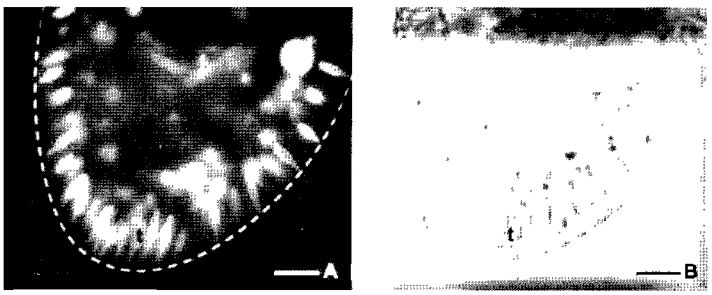

Figure 6. "Frustrated exocytosis" shown by incorporation of the fluorescent dye, FM1-43, into the membranes of trichocysts (t). **A:** Fluorescence, **B:** transmitted light image. The dye was added to the *Paramecium* culture and incorporated spontaneously into the cell membrane (dotted line). Membrane fusion allowed the dye to diffuse into the trichocyst membrane, while it was washed out again from the cell membrane after occurrence membrane fusion and resealing (yet without contents release) was accomplished. Such trichocysts can be internalized and redocked, just like normal trichocysts (not shown), and probed, e.g., for the time required for the assembly of a functional docking site. Bar, 5 μm. Reproduced from Klauke N, Plattner H. J Membr Biol 2000;176:237–248 with permission of the publishers.

i.e., they performed ciliary reversal. As in the experiments of our Japanese colleagues, the escape rate was 100%, while none of the paramecia survived when they were unable to release any trichocysts. Therefore this may justify the high investment in a complicated secretory organelle, like a trichocyst. In the course of the defensive response, ciliary reversal is probably triggered by a spill-over of the Ca^{2+} signal generated during exocytosis since the same is seen during local AED application, even in *pawn* mutants, which lack any functional Ca^{2+} channels in their cilia.[32] The entire defence reaction is identical to that seen with AED,[73] as are the Ca^{2+} signals under such conditions.[74]

Exocytosis-coupled endocytosis and redocking of new trichocysts

Exocytotic openings are rapidly resealed during ~350 milliseconds.[37] Empty "ghosts" are then removed within ~20 to 30 minutes or so.[36] What remains is a "plug"—a knot of proteins. New organelles are formed in the classical assembly line and installed at the preformed docking sites. These undergo a very characteristic ultrastructural transformation and thus acquire fusion capacity.

Since this maturation of an exocytotic site is normally difficult to analyze, we looked for a way to synchronize trichocyst docking. Norbert Klauke in my laboratory has achieved this to some extent by what we call "frustrated exocytosis"; Fig. 6.[75] In the presence of the fluorescent fusion indicator, FM1-43, membrane fusion can occur, while trichocyst contents discharge can be inhibited by lowering $[Ca^{2+}]$ in the medium close to internal resting levels. Cells do recognize—by an unknown signal—that membranes had fused, but not that their trichocysts were not emptied. They reseal their membranes as they normally do with "ghosts", and fluorescent trichocyts are internalized, followed by rather fast re-installation at the cell surface. A second stimulus applied after different re-docking periods indicates that fusion capacity may be acquired in minutes or less. To what extent docking is reversible is a standing issue also with other cells.[76] But the situation in *Paramecium* is unique, inasmuch as normally all docked trichocysts are exocytosis competent, but only ~50%, e.g., in chromaffin cells.

Perspectives

Recent analyses on vacuole fusion in yeast showed the involvement of several components that previously were considered for *Paramecium*, i.e., Ca^{2+}, CaM, dispersal of intrinsic oligomeric proteins and a protein phosphatase.[31] This demonstrates that such model systems can still deliver important clues. There is also an increasing interest in *Paramecium* by some parasitologists. In fact, Apicomplexans, like *Plasmodium* (the malaria-causing agent) and *Toxoplasma* (causing fetal deformations), contain not only secretory organelles serving host cell invasion, but also structures resembling alveolar sacs from where Ca^{2+} may be mobilized during invasion.[77] Although of paramount medical importance, their analysis in Apicomplexans may be more difficult, however, as it is with the intracellular pathways of svAGs. The establishment of a *Paramecium* genome project[78] will greatly help us to bring many of the observations onto a molecular level. So far, fewer than 1000 genes have been cloned, a few by my postdoctoral coworkers, Karin Hauser and recently Roland Kissmehl, and many more by an increasingly collaborating international consortium. Given the current funding situation, this may be the ultimate survival chance for those dedicating their work to this harmless but fascinating "lower" eukaryote.

Acknowledgments

I thank all my collaborators for their dedicated work and Ms. Doris Bliestle for artwork.

References

1. Plattner H, Miller F, Bachman L. Membrane specializations in the form of regular membrane-to-membrane attachment sites in *Paramecium*. A correlated freeze-etching and ultrathin-sectioning analysis. J Cell Sci 1973;13:687–719.
2. Ruiz F, Vayssié L, Klotz C, Sperling L, Madeddu L. Homology-dependent gene silencing in *Paramecium*. Mol Biol Cell 1998;9:931–943.

3. Preston RR, Saimi Y. Calcium ions and the regulation of motility in *Paramecium*. In: Bloodgood RA, editor. Ciliary and Flagellar Membranes. New York, London: Plenum Press; 1990. p. 173–200.
4. Machemer H, Teunis PFM. Sensory-motor coupling and motor responses. In: Hausmann K, Bradbury PC, editors. Ciliates. Cells and Organisms. Stuttgart: Gustav Fischer Verlag; 1996. p. 379–402.
5. Brehm P, Eckert R. Calcium entry leads to inactivation of calcium channel in *Paramecium*. Science 1978;202:1203–1206.
6. Levitan IB. It is calmodulin after all! Mediator of the calcium modulation of multiple ion channels. Neuron 1999;22:645–648.
7. Saimi Y, Kung C. Ion channel regulation by calmodulin binding. FEBS Lett 1994;350:155–158.
8. Van Houten J. Chemosensory transduction in *Paramecium*. Eur J Protistol 1998;34:301–307
9. Allen RD, Fok AK. Membrane trafficking and processing in *Paramecium*. Int Rev Cytol 2000;198:277–317.
10. Capdeville Y. *Paramecium* GPI proteins: variability of expression and localization. Protist 2000;151:161–169.
11. Kusch J, Schmidt HG. Genetically controlled expression of surface variant antigens in free-living protozoa. J Membr Biol 2001;180:101–109.
12. Flötenmeyer M, Momayezi M, Plattner H. Immunolabeling analysis of biosynthetic and degradative pathways of cell surface components (glycocalyx) in *Paramecium* cells. Eur J Cell Biol 1999;78:67–77.
13. Allen RD. Cytology In: Görtz HD, editor. *Paramecium*. Berlin, Heidelberg: Springer Verlag; 1988. p. 4–40.
14. Plattner H, Klauke N. Calcium in ciliated protozoa: Sources, regulation, and calcium-regulated cell functions. Int Rev Cytol 2001;201:115–208.
15. Ruiz F, Krzywicka A, Klotz C, Keller AM, Cohen J, Koll F, Balavoine G, Beisson J. The SM19 gene, required for duplication of basal bodies in *Paramecium*, encodes a novel tubulin, η-tubulin. Curr Biol 2000;10: 1451–1454.
16. Plattner H. Intramembraneous changes on cationophore-triggered exocytosis in *Paramecium*. Nature 1974;252:722–724.
17 Plattner H. Membrane behaviour during exocytosis. Cell Biol. Int. Rep 1981;5:435–459.
18. Jahn R, Südhof TC. Membrane fusion and exocytosis. Annu Rev Biochem 1999;68:863–911.
19. Beisson J, Lefort-Tran M, Pouphile M, Rossignol M, Satir B. Genetic analysis of membrane differentiation in *Paramecium*. Freeze-fracture study of the trichocyst cycle in wild-type and mutant strains. J Cell Biol 1976;69:126–143.
20. Pape R, Plattner H. Secretory organelle docking at the cell membrane in *Paramecium* cells: Dedocking and synchronized redocking of trichocysts. Exp Cell Res 1990;191:263–272.
21. Plattner H, Reichel K, Matt H, Beisson J, Lefort-Tran M, Pouphile M. Genetic dissection of the final exocytosis steps in *Paramecium* cells: Cytochemical localization of Ca^{2+}-ATPase activity over preformed exocytosis sites. J Cell Sci 1980;46:17–40.
22. Pouphile M, Lefort-Tran M, Plattner H, Rossignol M. Beisson J. Genetic dissection of the morphogenesis of exocytosis sites in *Paramecium*. Biol Cell 1986;56:151–162.
23. Vilmart J, Plattner H. Membrane-integrated proteins at preformed exocytosis sites. J Histochem Cytochem 1983;31:626–632.
24. Vayssié L, Skouri F, Sperling L, Cohen J. Molecular genetics of regulated secretion in *Paramecium*. Biochimie (Paris) 2000;82:269–288.
25. Momayezi M, Kersken H, Gras U, Vilmart-Seuwen J, Plattner H. Calmodulin in *Paramecium*: Localization from the in vivo to the ultrastructural level. J Histochem Cytochem 1986;34:1621–1638.
26. Kerboeuf D, LeBerre A, Dedieu JC, Cohen J. Calmodulin is essential for assembling links necessary for exocytotic membrane fusion in *Paramecium*. EMBO J 1993;12:3385–3390.
27. Quetglas S, Leveque C, Miquelis R, Sato K, Seagar M. Ca^{2+}-dependent regulation of synaptic SNARE complex assembly via a calmodulin- and phospholipid-binding domain of synaptobrevin. Proc Natl Acad Sci USA 2000;97:9595–9700.
28. Vilmart-Seuwen J, Kersken H, Stürzl R, Plattner H. ATP keeps exocytosis sites in a primed state but is not required for membrane fusion. An anlysis with *Paramecium* cells in vivo and in vitro. J Cell Biol 1986;103: 1279–1288.
29. Plattner H. Regulation of membrane fusion during exocytosis. Int Rev Cytol 1989;119:197–286.
30. Plattner H, Lumpert CJ, Knoll G, Kissmehl R, Höhne B, Momayezi M, Glas-Albrecht R. Stimulus-secretion coupling in *Paramecium* cells. Eur J Cell Biol 1991;55:3–16.
31. Peters C, Bayer MJ, Bühler S, Andersen JS, Mann M, Mayer A. Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. Nature 2001;409:581–588.
32. Plattner H, Matt H, Kersken H, Haacke B, Stürzl R. Synchronous exocytosis in *Paramecium* cells. I. A novel approach. Exp Cell Res 1984; 151:6–13.
33. Plattner H, Stürzl R, Matt H. Synchronous exocytosis in *Paramecium* cells. IV. Polyamino-compounds as potent trigger agents for repeatable trigger-redocking cycles. Eur J Cell Biol 1985;36:32–37
34. Klauke N, Blanchard MP, Plattner H. Polyamine triggering of exocytosis in *Paramecium* involves an extracellular Ca^{2+}/(polyvalent cation)-sensing receptor, subplasmalemmal Ca-store mobilization and store-operated Ca^{2+}-influx via unspecific cation channels. J Membr Biol 2000;174:141–156.
35. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev 2001;81:239–297.
36. Plattner H, Knoll G, Pape R. Synchronization of different steps of the secretory cycle in *Paramecium*: Trichocyst exocytosis, exocytosis-coupled endocytosis and intracellular transport. In: Plattner H, editor. Membrane Traffic in Protozoa. Greenwich, CT, USA, London, GB: JAI Press; 1993. p. 123–148.
37. Knoll G, Braun C, Plattner H. 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. J Cell Biol 1991a;113:1295–1304.
38. Kasai H. Comparative biology of Ca^{2+}-dependent exocytosis: Implications of kinetic diversity for secretory function. Trends Neurosci 1999;22: 88–93.
39. Neher E, Marty A. Discrete changes of cell membrane capacitance observed under conditions of enhanced secretion in bovine adrenal chromaffin cells. Proc Natl Acad Sci USA 1982;79:6712–6716.
40. Almers W. Breckenridge LJ, Spruce AE. The mechanism of exocytosis during secretion in mast cells. In: Oxford GS, Armstrong CM, editors. Secretion and its Control. New York: Rockefeller University Press; 1989. p. 269–282.
41. Yang WQ, Braun C, Plattner H, Purvee J, Van Houten JL. Cyclic nucleotides in glutamate chemosensory signal transduction of *Paramecium*. J Cell Sci 1997;110:2567–2572.
42. Länge S, Klauke N, Plattner H. Subplasmalemmal Ca^{2+} stores of probable relevance for exocytosis in *Paramecium*. Alveolar sacs share some but not all characteristics with sarcoplasmic reticulum. Cell Calcium 1995 17:335–344.
43. Knoll G, Grässle A, Braun C, Probst W, Höhne-Zell B, Plattner H. A calcium influx is neither strictly associated with nor necessary for exocytotic membrane fusion in *Paramecium* cells. Cell Calcium 1993;14: 173–183.
44. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nature Rev Cell Biol 2000;1:11–21.
45. Klauke N, Plattner H. Imaging of Ca^{2+} transients induced in *Paramecium* cells by a polyamine secretagogue. J Cell Sci 1997;110:975–983.
46. Erxleben C, Klauke N, Flötenmeyer M, Blanchard MP, Braun C, Plattner H. Microdomain Ca^{2+} activation during exocytosis in *Paramecium* cells. Superposition of local subplasmalemmal calcium store activation by local Ca^{2+} influx. J Cell Biol 1997;136:597–607.
47 Erxleben C, Plattner H. Ca^{2+} release from subplasmalemmal stores as primary event during exocytosis in *Paramecium* cells. J Cell Biol 1994;127:935–945.
48. Neher E. Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 1998;20:389–399.
49. Stelly N, Mauger JP, Keryer G, Claret M, Adoutte A. Cortical alveoli of *Paramecium*: A vast submembranous calcium storage compartment. J Cell Biol 1991;113:103–112.
50. Kerboeuf D, Cohen J. A Ca^{2+} influx associated with exocytosis is specifically abolished in a *Paramecium* exocytotic mutant. J Cell Biol 1990;111:2527–2535.

51. Knoll G, Kerboeuf D, Plattner H. A rapid calcium influx during exocytosis in *Paramecium* cells is followed by a rise in cyclic GMP within 1 s. FEBS Lett 1992;304:265–268.
52. Lumpert CJ, Kersken H, Plattner H. Cell surface complexes ('cortices') isolated from *Paramecium* cells as a model system for analysing exocytosis in vitro in conjunction with microinjection studies. Biochem J 1990;269:639–645.
53. Grohovaz F, Bossi M, Pezzati R, Meldolesi J, Tarelli FT. High resolution ultrastructural mapping of total calcium: Electron spectroscopic imaging/electron energy loss spectroscopy analysis of a physically/chemically processed nerve-muscle preparation. Proc Natl Acad Sci USA 1996;93: 4799–4803.
54. Hardt M, Plattner H. Quantitative energy-dispersive x-ray microanalysis of calcium dynamics in cell suspensions during stimulation on a subsecond time scale: preparative and analytical aspects as exemplified with *Paramecium* cells. J Struct Biol 1999;128:187–199.
55. Hardt M, Plattner H. Sub-second quenched-flow/X-ray microanalysis shows rapid Ca^{2+} mobilization from cortical stores paralleled by Ca^{2+} influx during synchronous exocytosis in *Paramecium* cells. Eur J Cell Biol 2000;79:642–652.
56. Plattner H, Habermann A, Kissmehl R, Klauke N, Majoul I, Söling HD. Differential distribution of calcium-stores in *Paramecium* cells. Occurrence of a subplasmalemmal store with a calsequestrin-like protein. Eur J Cell Biol 1997b;72:297–306.
57. Pezzati R, Meldolesi J, Grohovaz F. Ultra rapid calcium events in electrically stimulated frog nerve terminals. Biochem Biophys Res Commun 2001;285:724–727.
58. Duchen MR, Leyssens A, Crompton M. Transient mitochondrial depolarizations reflect focal sarcoplasmic reticular calcium release in single rat cardiomyocytes. J Cell Biol 1998;142:975–988.
59. Plattner H, Braun C, Hentschel J. Facilitation of membrane fusion during exocytosis and exocytosis-coupled endocytosis and acceleration of "ghost" detachment in *Paramecium* by extracellular calcium. J Membr Biol 1997a;158:197–208.
60. Mohamed I, Klauke N, Hentschel J, Cohen J, Plattner H. Functional and fluorochrome analysis of an exocytotic mutant yields evidence of store-operated Ca^{2+} influx in *Paramecium*. J Membr Biol (in press).
61. Saitow F, Nakaoka Y, Oosawa Y A calcium-activated, large conductance and non-selective cation channel in *Paramecium* cell. Biochim Biophys Acta 1997;1327:52–60.
62. Hauser K, Pavlovic N, Kissmehl R, Plattner H. Molecular characterization of a sarco(endo)plasmic reticulum Ca^{2+}-ATPase gene from *Paramecium* and localization of its gene product to sub-plasmalemmal calcium stores. Biochem J 1998;334:31–38.
63. Kissmehl R, Huber S, Kottwitz B, Hauser K, Plattner H. Subplasmalemmal Ca-stores in *Paramecium*. Identification and characterisation of a sarco(endo)plasmic reticulum-like Ca^{2+}-ATPase by phosphoenzyme intermediate formation and its inhibition by caffeine. Cell Calcium 1998; 24:193–203.
64. Plattner H, Flötenmeyer M, Kissmehl R, Pavlovic N, Hauser K, Momayezi M, Braun N, Tack J, Bachmann L. Microdomain arrangement of the SERCA-type Ca^{2+} pump (Ca^{2+}-ATPase) in subplasmalemmal calcium stores of *Paramecium* cells. J Histochem Cytochem 1999;47:841–854.
65. Mogami H, Gardner J, Gerasimenko OV, Camello P, Petersen OH, Tepikin AV. Calcium binding capacity of the cytosol and endoplasmic reticulum of mouse pancreatic acinar cells. J Physiol 1999:518:463–467.
66. Hauser K, Pavlovic N, Klauke N, Geissinger D, Plattner H. Green fluorescent protein-tagged sarco(endo)plasmic reticulum Ca^{2+}-ATPase overexpression in *Paramecium* cells: isoforms, subcellular localization, biogenesis of cortical calcium stores and functional aspects. Mol Microbiol 2000;37:773–787
67. Gautier MC, Sperling L, Madeddu L. Cloning and sequence analysis of genes coding for *Paramecium* secretory granule (trichocyst) proteins. A unique protein fold for a family of polypeptides with different primary structures. J Biol. Chem 1996;271:10247–10255.
68. Bilinski M, Plattner H, Matt H. Secretory protein decondensation as a distinct Ca^{2+}-mediated event during the final steps of exocytosis in *Paramecium* cells. J Cell Biol 1981;88:179–188.
69. Chanat E, Huttner WB. Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. J Cell Biol 1991;115:1505–1519.
70. Lumpert CJ, Glas-Albrecht R, Eisenmann E, Plattner H. Secretory organelles of *Paramecium* cells (trichocysts) are not remarkably acidic compartments. J Histochem Cytochem 1992;40:153–160.
71. Klauke N, Kissmehl R, Plattner H, Haga N, Watanabe T. An exocytotic mutant of *Paramecium*: Membrane fusion without secretory contents release. Cell Calcium 1998;23:349–360.
72. Harumoto T, Miyake A. Defensive function of trichocysts in *Paramecium*. J Exp Zool 1991;260:84–92.
73. Knoll G, Haacke-Bell B, Plattner H. Local trichocyst exocytosis provides an efficient escape mechanism for *Paramecium* cells. Eur J Protistol 1991b;27:381–385.
74. Iwadate Y, Katoh K, Asai H, Kikuyama M. Simultaneous recording of cytosolic Ca^{2+} levels in *Didinium* and *Paramecium* during a *Didinium* attack on *Paramecium*. Protoplasma 1997; 117–127
75. Klauke N, Plattner H. "Frustrated exocytosis"—a novel phenomenon: membrane fusion without contents release, followed by detachment and reattachment of dense core vesicles in *Paramecium* cells. J Membr Biol 2000;176:237–248.
76. Elhamdani A, Palfrey HC, Artalejo CR. Quantal size is dependent on stimulation frequency and calcium entry in calf chromaffin cells. Neuron 2001;31:819–830.
77. Carruthers VB, Sibley LD. Mobilization of intracellular calcium stimulates microneme discharge in *Toxoplasma gondii*. Mol Microbiol 1999;31: 421–428.
78. Dessen P, Zagulski M, Gromadka R, Plattner H, Kissmehl R, Meyer E, Bétermier M, Schultz JE, Linder U, Pearlman E. et al. *Paramecium* genome survey: a pilot project. Trends Genet 2001;17:306–308.