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Actin Microfilaments are Involved in Scale Formation of the Chrysomonad Cell *Synura*

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Summary

In the first step of scale formation several small vesicles originating from the Golgi fuse to form a large flattened primary vesicle associated with the surface of one plastid. In a second step this vesicle undergoes several morphogenetic events to form the mold of ornamented scales elaborated in the lumen of the scale forming vesicle (SFV). Thick filaments composed of a stack of actin microfilaments (MFs) settle on the membrane of the SFV turned toward the plastid. They are laterally cross-linked to each other and to the SFV membrane and assembled in a horseshoe figure at the place of the future base-plate of the scale. On the center of the vesicle free of actin MF, where the periplastidial endoplasmic reticulum (PER) and the SFV membranes are glued, a protrusion occurs to form a diverticulum which is to become the future hull or the spine of the finished scale. On the external side of the SFV a microfibrillar network covers the surface of the vesicle. Microtubules (MTs) originating near the kinetosomes change their rectilinear course to follow the two longitudinal margins and the diverticulum of the SFV. MTs are not directly attached to the membrane of the SFV but rather through the microfibrillar network. A set of observations suggest that actin MFs have a structural function in maintaining the shape of the vesicle rather than a role in the migration of the SFV on the surface of the plastid. MTs probably play a role in the migration and in the morphogenesis of the SFV in conjunction with microfibrils. Also, the formation of SFVs and silica deposition are compared to that in diatoms.

Keywords: Actin; Microtubules; Microfibrils; Silica scale; Chrysomonads.

1. Introduction

It is now well established that actin microfilaments (MFs) are one of the components of the cytoskeleton of non-muscle cells (LAZARIDES and REVEL 1979). Their presence has been demonstrated in many groups of animals comprising *Protozoa* since ISHIKAWA *et al.* (1969) devised a method of actin decoration with heavy meromyosin. However, in many cases the exact role of actin is unknown. When associated with myosin, actin plays the same role as in muscle cells in generating movements (CLARKE and SPUDICH 1977). In non-muscle cells actin-G is able to polymerize giving thin filaments of actin-F that cross-link to form bundles, cables and networks which play a structural role (POLLARD 1981, ALLEN 1981). As these microfilaments are made of globular monomers which associate and dissociate by a treadmilling process (WAGNER 1982), actin, like tubulin, may have a dynamic function in cells (COHEN 1979). Moreover, actin binds many other proteins for different functional roles (CRAIG and POLLARD 1982). Here, we have detected the presence of actin in one stage of scale formation in *Synura* cells which can be assimilated to a secretory process. In previous work, scale formation in chrysomonads such as *Synura*, *Mallomonas* and *Paraphysomonas* was compared (MIGNOT and BRUGEROLLE 1982). At the onset elongated flattened vesicle of Golgi origin closely associated with the periplastidial membrane migrates along the external face of one plastid as shown long ago by SCHNEPF and DEICHGRÄBER (1969). This flattened vesicle enlarges and sends out a diverticulum at its center which will later form the hull or the spine of the scale. The definitive shape of the scale is fixed when silica is deposited in the lumen of the vesicle. During this morphogenetic process we have demonstrated the presence of microfibrils (fb) and microfilaments (MFs)

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attached to the membrane of the vesicle. We are now able to demonstrate that microfilaments are composed of actin by heavy meromyosin decoration and to reinvestigate the morphogenetic process of scale formation.

2. Materials and Methods

Culture: Cells of *Synura petersenii* and *S. sphagnicola* were isolated from a pond and cultivated at 14 °C in a medium recommended for *Synura sp.* by GUILLARD and LORENZEN (1972).

Preparation of heavy meromyosin (HMM) and myosin subfragment 1 (S1): Myosin was first extracted from rabbit skeletal muscle according to the method of PERRY (1955). HMM and S 1 were obtained by a chymotrypsin digestion of myosin purified by the method of WEEDS and POPE (1977).

To prepare HMM, soluble myosin was digested at a concentration of 15 to 20 mg/ml in 0.6 M NaCl, 10 mM sodium phosphate pH 7.0, 1 mM dithiothreitol (DTT) and 0.1 mM NaN_3 with chymotrypsin (Sigma) at 0.05 mg/ml for 10–12 minutes. Digestion was stopped by addition of 100 mM phenyl methane sulphonyl fluoride (p.m.s.f.) in ethanol, added to a final concentration of 0.5 mM. After dialysis to 40 mM NaCl, 5 mM sodium phosphate pH 6.5, the soluble fragments were separated from insoluble material by centrifugation at 40,000 × g. HMM concentration was determined by measuring absorbance at 280 nm using an extinction coefficient E 1%/280 nm = 6.47 cm^{-1}.

To prepare myosin subfragment 1 synthetic myosin filaments were prepared by dialysis of myosin to 0.12 M NaCl, 10 mM sodium phosphate pH 7.0, 1 mM D.T.T., 0.1 mM NaN_3. Digestion was carried out at 21–25 °C, at a myosin concentration of 10 to 15 mg/ml with a chymotrypsin concentration of 0.05 mg/ml and 1 mM EDTA was added. The digestion was arrested by addition of 100 mM p.m.s.f. and the proteins were dialysed to 40 mM NaCl, 5 mM sodium phosphate pH 6.5. Soluble products were isolated after centrifugation at 40,000 × g and concentration of subfragment 1 was determined using a value for E 1%/280 nm = 7.50.

Gel electrophoresis: To test for the presence of HMM and S 1 in the chymotrypsin digested myosin solution the digests were solubilized by 2% sodium dodecyl sulphate (SDS) in 0.125 M Tris-HCl solution pH 6.8 with 0.1 M DTT and boiled for 5 minutes. Samples were electrophoresed on 7% SDS polyacrylamide gel according to the method of Laemmli (1970). Gels were fixed and stained with Coomassie blue R 250 (FAIRBANKS *et al.* 1971).

Molecular weights were estimated using standards obtained from Pharmacia and known polypeptides such as myosin, α-actinin, actin, tropomyosin, myosin light chains prepared from mouse muscle according to the procedure of MOOSEKER (1976).

Assay for HMM- and S 1-binding on brush-borders: The HMM and S 1 solutions were tested on intestinal mouse brush-borders extracted according to a method from EVANS *et al.* (1971) and modified by MOOSEKER and TILNEY (1975). Extracted brush-borders treated or not with 1% triton X-100 were suspended in a solution containing 60 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 10 mM tosyl arginin methyl ester (TAME, Sigma), 10 mM imidazole buffer pH 7.3, HMM or S 1 at 1 mg/ml for 1 to 2 hours. The brush-borders were then collected by centrifugation at 2,000 × g for 10 minutes and processed for electron microscopy.

Assay for HMM- and S 1-binding on Synura cells: Several procedures were used to make the cell permeable according to the method of ISHIKAWA *et al.* (1969) and of POLLARD *et al.* (1970).

Results were obtained by 20-minutes treatment of the cells by a solution containing 25 or 50% glycerol, 10% DMSO, 60 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 10 mM TAME, 10 mM imidazole buffer pH 7.3. This solution was then replaced by a medium containing HMM or S 1 1 mg/ml in 5% glycerol, 60 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 10 mM TAME, 10 mM imidazole buffer for 2 hours. A control was prepared by omitting HMM or S 1 in the medium.

Electron microscopy: Brush-borders were fixed in 1% v/v glutaraldehyde, 0.2% tannic acid, 0.1 M phosphate buffer pH 7 for 30 minutes and postfixed in 1% OsO_4 in 0.1 M phosphate buffer pH 7 for 1 hour.

Synura cells were fixed in a solution containing 2% glutaraldehyde, 2% OsO_4, 0.1 M phosphate buffer pH 7 for 40 minutes. Then the cells were postfixed in 2% OsO_4 in 0.1 M phosphate buffer for 30 minutes. To improve fixation of microfilaments 0.2% tannic acid was added to the fixing solutions.

Brush-borders or *Synura* cells were then rinsed twiced in distilled water, dehydrated in ethanol, treated with propylene oxide and embedded in epon 812. Thin sections were cut with a diamond knife on a Reichert OMU 2 ultramicrotome and stained with alcoholic uranyl acetate and lead citrate before being viewed with a Siemens Elmiskop I A electron microscope.

3. Observations

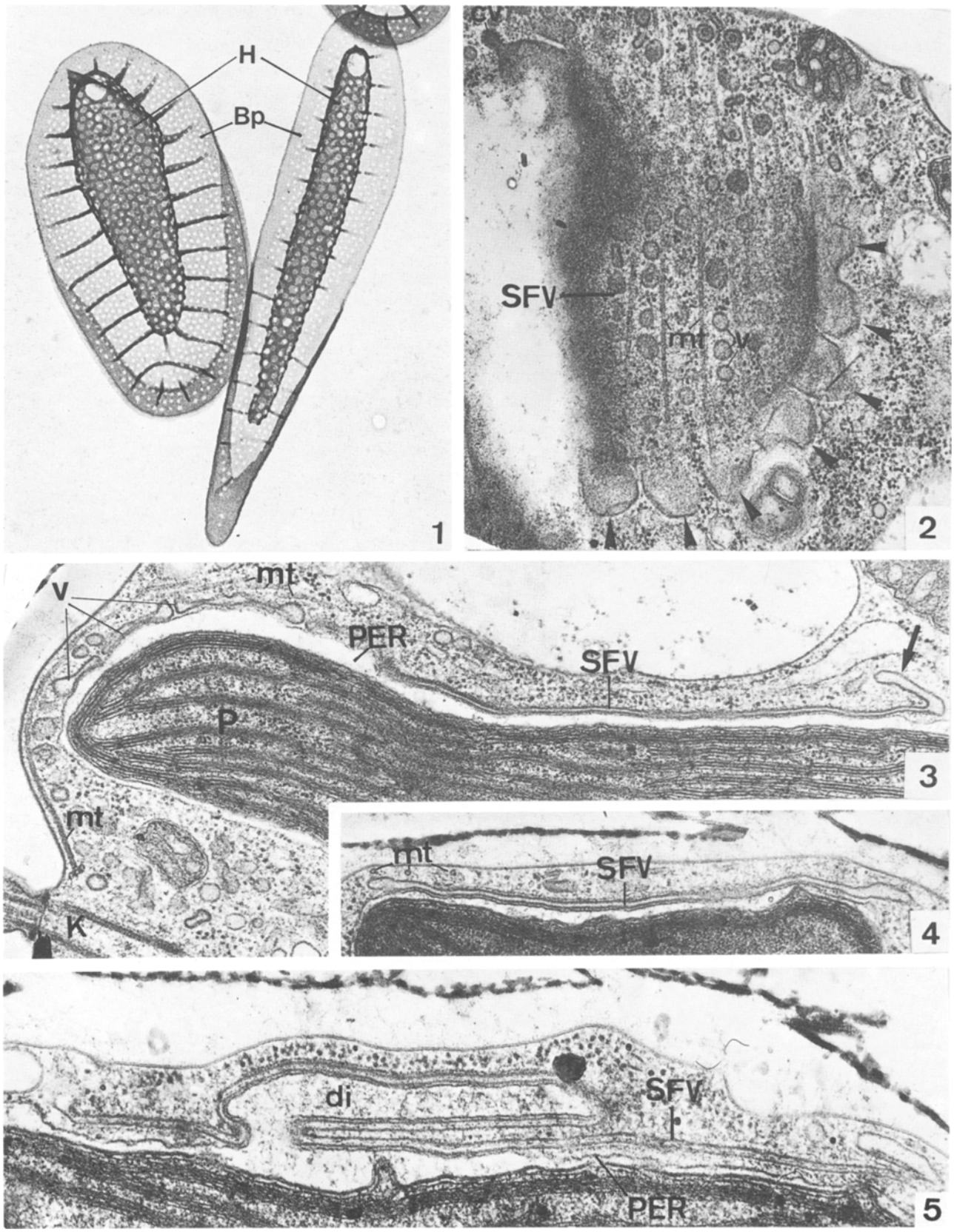
The process of the scale formation was examined in a previous publication (MIGNOT and BRUGEROLLE 1982) and can be divided into three steps: formation of the primary vesicle of the scale molding vesicle, and of the definitive vesicle containing the matured scale. These steps follow an antero-posterior gradient during the synthesis of silica scales.

Fig. 1. From tip to back, the shape of the scales vary from large and stumpy to elongated and slender in the cell of *Synura petersenii*. Hull (*H*), base-plate (*Bp*). × 18,000

Fig. 2. Tangential section of a primary vesicle showing the festooned margin (arrow-heads) and a coated vesicle (*cv*) fusing to it. Several parallel microtubules (*mt*) and trains of small vesicles (*v*) follow the surface of the primary vesicle (*SFV*). × 29,000

Figs. 3 and 4. Sections showing train of flattened vesicles (*v*) between the peripheral microtubules (*mt*) forming the cap and the surface of the plastid (*P*). Closely associated to the *PER* membrane, a primary vesicle (*SFV*) begins to fold back its margin (arrow). × 32,000 and 39,000

Fig. 5. The diverticulum (*di*) protrudes at the site of the future hull of the scale; membranes of the *PER* and of the *SFV* are glued together in the diverticulum. × 50,000.



Figs. 1–5

3.1. The Primary Vesicle Formation

We have demonstrated that a train of flat and relatively small vesicles probably originating from the Golgi body migrate toward the external surface of one plastid (Fig. 3). They move along the periplastidial endoplasmic reticulum (PER) and are probably guided by the cap-like set of cortical microtubules (MT) originating from a microtubule organizing center (MTOC) around the pair of kinetosomes. These vesicles probably fuse to form a large "primary vesicle" closely applied on the PER surface (Fig. 4). These primary vesicles are flattened with festooned edges (Fig. 2), which could indicate that they enlarge by a fusion of pheripheral vesicles. Moreover, small, coated vesicles have been seen to fuse with the edges of this primary vesicle (Fig. 2). The membrane of the primary vesicles has a clear tripartite structure and presents a positive reaction to polysaccharides, differing in these two respects from the PER membrane (see MIGNOT and BURGEROLLE 1982). When the lower edge of the primary vesicle folds back, thin microfibrils are set along this elevated edge while microfilaments appear between the edge of the vesicle and the PER (Figs. 3, 5, and 6).

3.2. The Scale-Forming Vesicle (SFV)

During this step the primary vesicles undergoes several morphogenetic changes to constitute the mold in which silica is deposited to form definitive mineralized scale.

3.2.1. Vesicle Morphogenesis

At the center of the SFV the membrane facing the plastid is still glued to the PER membrane and is devoid of microfilaments. This area of the vesicle protrudes to form a diverticulum which later will constitute the hull of the scale (Fig. 5) or the spine (Fig. 16). During this process PER membrane is drawn into the diverticulum implying PER membrane growth as well as growth of the membrane of the SFV.

3.2.2. Intervention of Microfilaments

Improvement of the fixation and use of tannic acid have helped to distinguish thick filaments under the molding vesicle (Figs. 6 and 7). They are attached to the membrane of the vesicle facing the plastid which is unglued from the PER membrane at this place. Tangential sections show that these filaments run in parallel with a periodicity of about 32 nm forming a figure like a horse-shoe in large scale vesicles (Fig. 8) or figures like two longitudinal brackets in elongated vesicles (Fig. 9). Some sections show that parallel thick filaments are composed by a stack of about 5 to 10 thin filaments 7 nm thick (Fig. 11). Treatment of the cells by HMM or S 1 clearly decorates these filaments which are made of actin (Figs. 12–15). These actin microfilaments (MFs) appear cross-linked and sometimes disorganized and detached from the membrane which has been partly solubilized by the treatment.

3.2.3. Intervention of Microfibrils and Microtubules

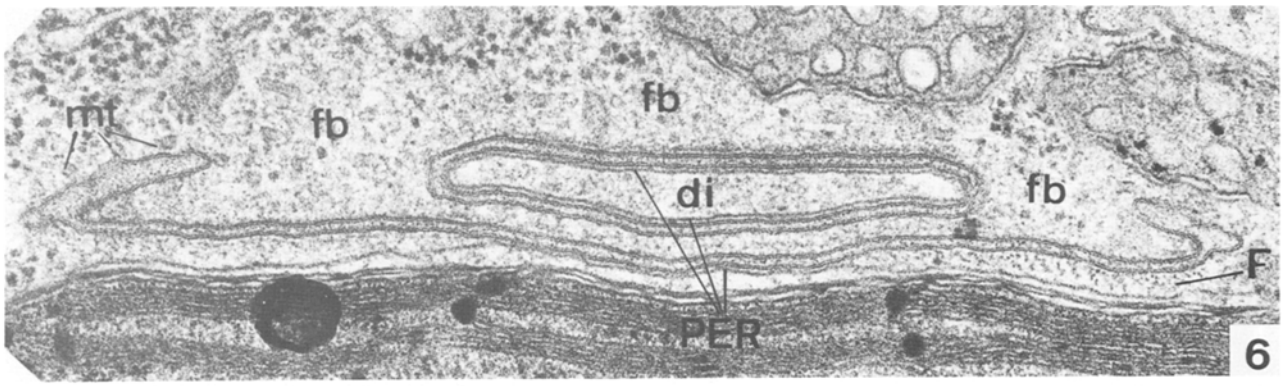
At the same time as the setting-up of microfilaments on one face of the SFV, microfibrils (fb) accumulate on the outer face (Fig. 6). They appear firstly on the folded edges of the vesicle then cover the diverticulum and form a microfibrillar network where the ribosomes and small vesicle are excluded (Fig. 6). These microfibrils are ill-preserved by the fixations and nearly disappear in the presence of tannic acid (Fig. 7). They are very similar and can be composed of the same components of the microfibrils which invade the cytoplasm of the stalks forming the cell colony (not shown).

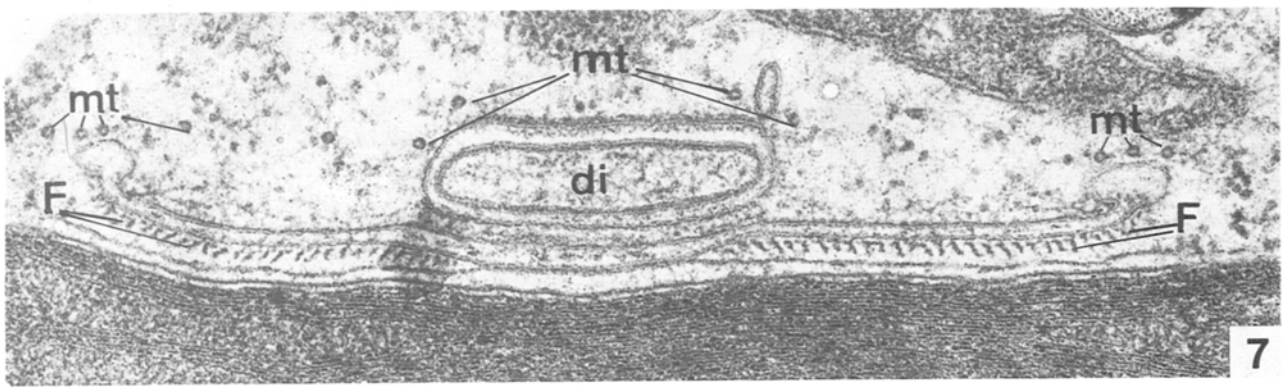
In transverse sections of SFV, MTs are positioned near the folded edges of the vesicle and along the diverticulum (Fig. 7). They are never directly connected to the membrane of the SFV. Tangential sections show the same MT distribution (Figs. 8 and 10). Some MT seem to change their linear course to take the curved pathway of the margin of the SFV (Fig. 10).

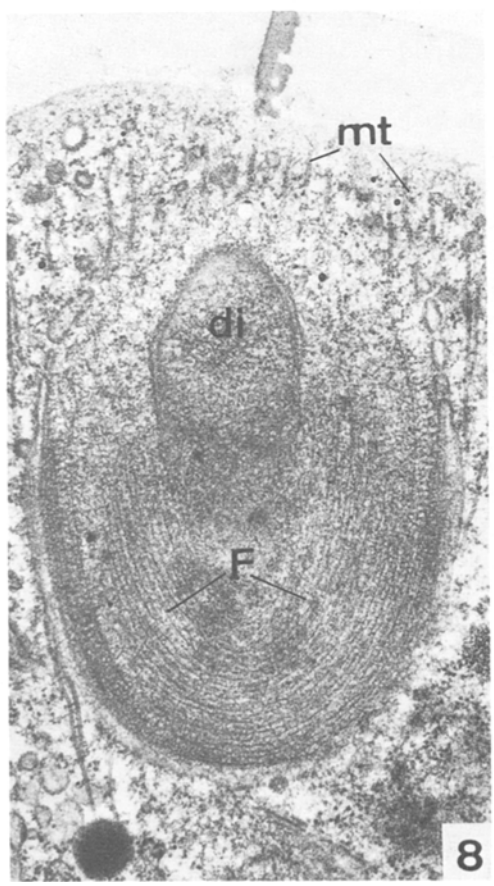
Figs. 6 and 7. In this transverse section of the SFV, thick filaments (*F*) settle on the membrane facing the plastid except in the diverticulum (*di*) where membranes are glued. On the external side of the SFV, MTs are seen near the membrane in a microfibrillar network (*fb*) covering this SFV face. In Fig. 7 the cell is fixed in the presence of tannic acid. The lumen of the SFV contains granulo-fibrillar material at this stage. × 56,000 and 57,000

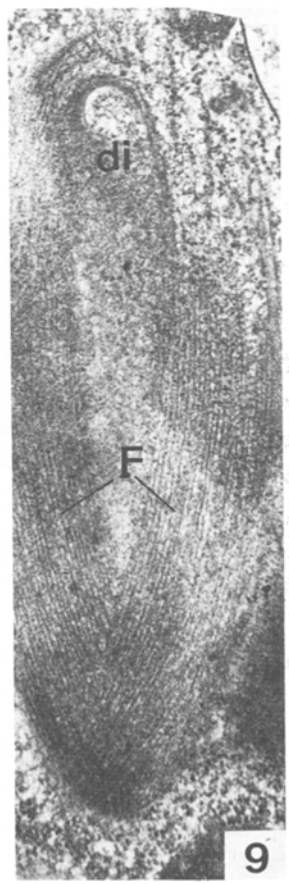
Figs. 8 and 9. Sections tangential to the SFV showing the horse-shoe arrangement of thick filaments (*F*) around the diverticulum (*di*) in a stumpy scale (Fig. 8) while in the slender scale (Fig. 9) filaments are arranged like two brackets on each side of the diverticulum. × 28,000 and 27,000

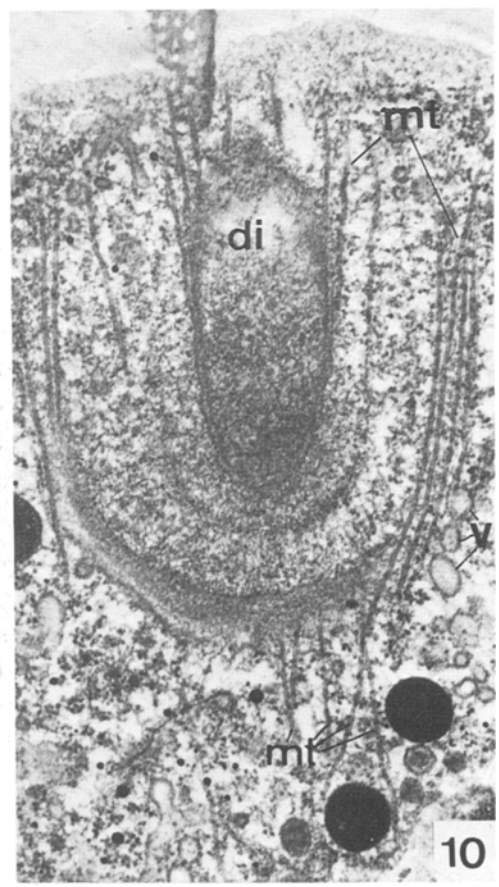
Fig. 10. This section tangential to the SFV shows MTs lining the diverticulum (*di*) and the curved margins of the SFV where small vesicles (*v*) are also seen. × 26,000











Figs. 6–10

3.2.4. Silica Deposit

During the morphogenetic process the lumen of the flattened vesicles seems to contain a granulo-fibrillar material (Figs. 5 and 6). Small patches and thin plaques of a dense deposit then appear in the lumen (Fig. 11), and when this forms a continuous sheet the vesicle detaches from the surface of the plastid (Fig. 17). Notice that in *Synura sphagnicola* the wall of the spine is delicately striated (Fig. 16).

3.3. Scale Finishing and Transport onto the Cell Surface

When the SFV is detached from the PER surface actin microfilaments and microfibrils are progressively lost while microtubules are pushed away from the outer surface of the vesicle before disappearing. As the vesicles are drawn near the cell surface the scale wall thickens. After the SFV has been detached from the PER the hull of the scale still contains a cytoplasmic diverticulum which disappears before the scale is discharged on the cell surface (Fig. 17). It is noteworthy that the tripartite structure of the membrane of the SFV is less visible at this terminal stage of scale formation than it was in the preceding stages and than the plasma membrane. It is very unlikly that the membrane of the vesicle is incorporated into the plasma membrane when the scale is discharged on the cell surface. More likely the membrane of the remaining flattened vesicle would be recycled. The scale covering of the cell is generally separated by a space from the plasma membrane after fixation of the cells. Each scale is associated to the neighboring scales by thin microfibrils containing polysaccharides, which also probably serve to attach the scales to the plasma membrane in the living cells.

4. Discussion

In a previous publication (MIGNOT and BRUGEROLLE 1982) we described and compared the process of scale formation in three different species of chrysomonads. Here, we add new observations and comments.

The first is the demonstration of thick filaments associated with the SFV. Using tannic acid mixed with the fixative these filaments are thicker and better preserved; they resembled those found in *Mallomonas* but not seen in *Paraphysomonas.* They are in fact composed of a stack 7 nm actin microfilaments since they are decorated by HMM or S 1 myosin subfragments. This test is generally considered best for the identification of F-actin, and controls performed on intestinal brush-borders have given an excellent decoration of these well known actin MFs (BEGGS *et al.* 1978). Photographs show that actin MFs are laterally cross-linked to form thick filaments. It has been demonstrated that actin MF can cross-link by self association (GRIFFITH and POLLARD 1981) or by an intermediate protein in brush-border microvilli (MOOSEKER and TILNEY 1975, MOOSEKER *et al.* 1980) and form cables or bundles of MFs in *Nitella* (KERSEY and WESSELS, 1976, HIGASHI-FUJIME 1980). In *Synura* these thick filaments are regularly arranged and linked to the membrane of the SFV. Their horse-shoe pattern following the shape of the future scale base-plate suggests that they have a structural role in maintaining in place the molding membrane where they are attached. This hypothesis is corroborated by the fact that they are only present in areas where the membrane does not change its shape. They are absent from the surface of the diverticulum. Their presence is transient, they appear during the molding of the vesicle and disappear once the scale is mineralized.

MFs are laterally attached to the membrane of the SFV. Such a linkage to the membrane has been demonstrated in actin MFs of intestinal brush-border microvilli (MOOSEKER and TILNEY 1975) where actin MFs have a structural role and are implicated in the terminal web movements (HIROKAWA *et al.* 1983).

When associated with myosin MFs, actin MFs are generally involved in movements of cellular organelles such as chloroplasts in *Nitella* (SHEETZ and SPUDICH 1983) or lysosomes in polymorphonuclear leukocytes

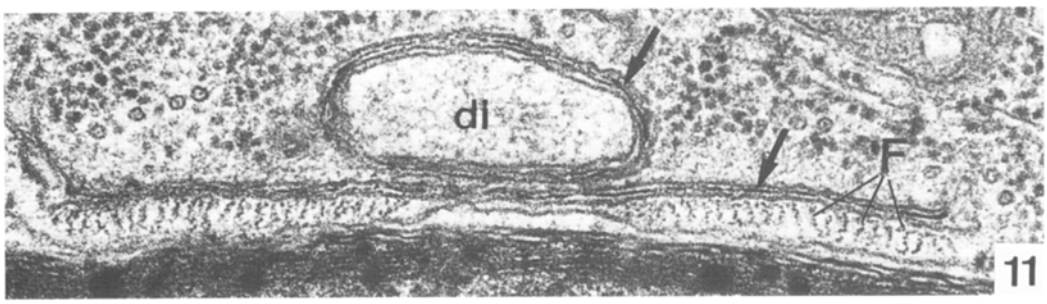
Fig. 11. This transverse section of the SFV shows that thick filaments (*F*) are composed of stacks of microfilaments. At this stage the lumen of the SFV contains several patches of dense siliceous material forming a fairly complete sheet (arrows). × 60,000

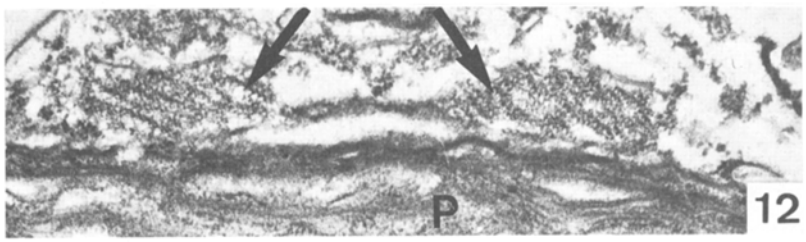
Figs. 12 and 13. Oblique and transverse sections through the HMM decorated actin MFs (arrows) attached to a SFV (Fig. 12) and on SFV already containing siliceous material. Notice the absence of decorated MF on the external side of the SFV. × 36,000 and 40,000

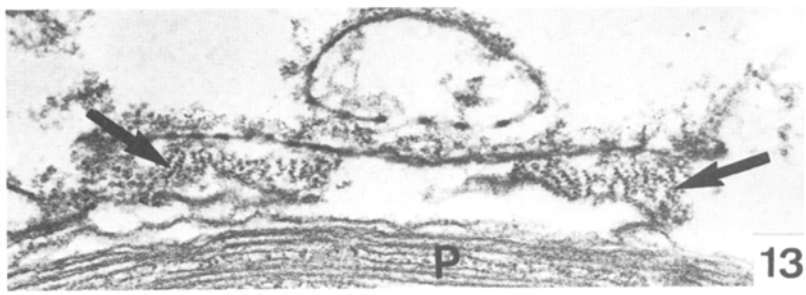
Figs. 14 and 15. Tangential and longitudinal sections showing HMM decorated actin MFs lateraly linked together (arrows). × 61,000 and 55,000

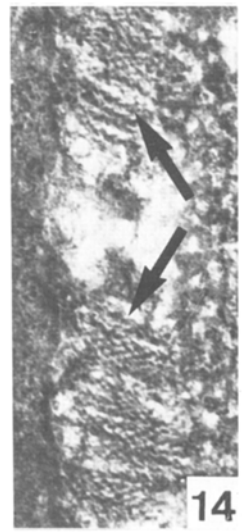
Fig. 16. Section through the SFV of *Synura sphagnicola* where the protruding diverticulum (*di*) forms the future spine of the scale. The siliceous wall in the spine appears delicately striated (arrows) (*Bp*) base-plate of the future scale. × 33,000

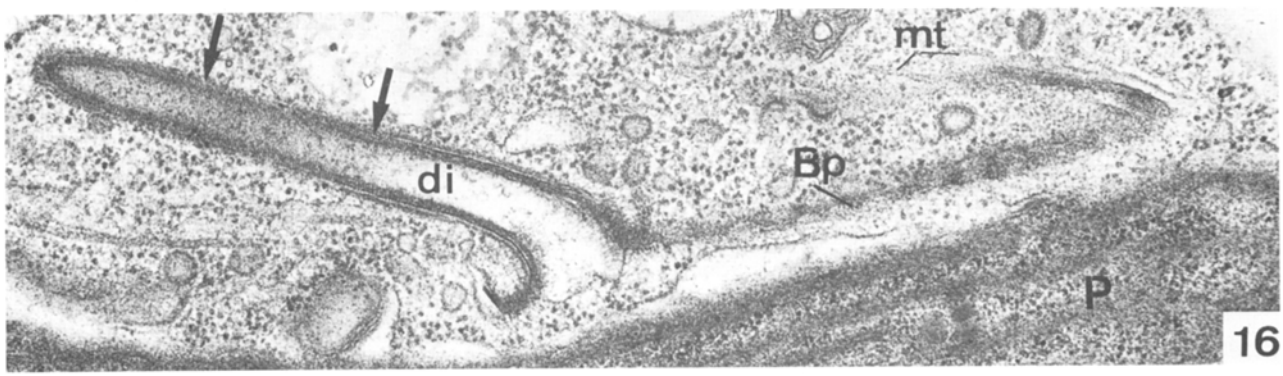
Fig. 17. Different steps of scale formation. Actin MFs (*F*), MTs (*mt*) and microfibrils (*fb*) disappear when a continuous siliceous wall is formed in the SFV. The cytoplasm entrapped in the diverticulum (*di*) disappears as the wall thickens (arrow). The membrane of the mature SFV is thinner than the plasma membrane (*pM*). × 35,000

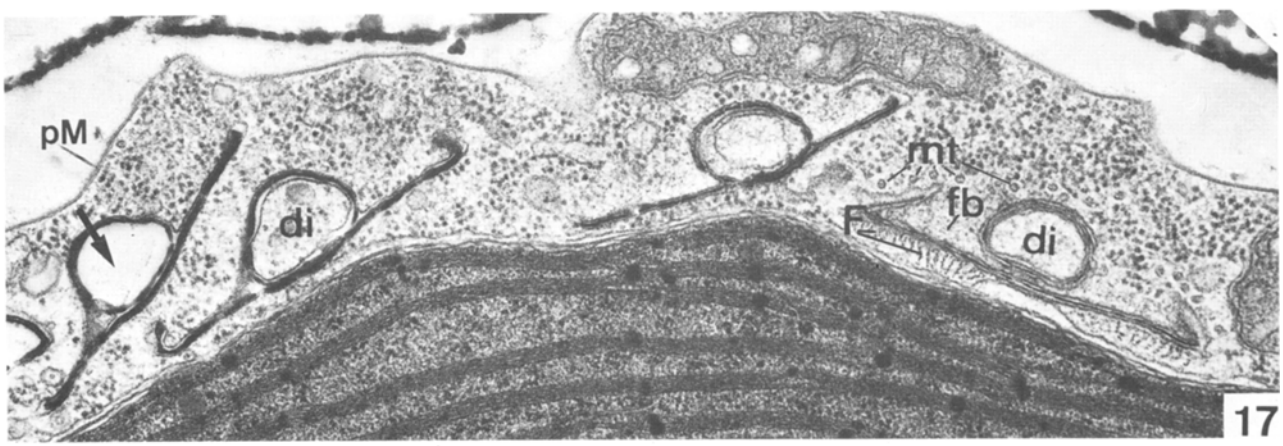












Figs. 11–17

(MOORE *et al.* 1976). Since actin MFs are involved in cell motility or in transport we must envisage such a function in *Synura*. Primary vesicles and SFVs migrate first along the plastidial face and matured vesicles reach the cell surface when they are discharged. We have not seen any actin MFs under the primary vesicles. They are only present during the molding when the SFVs acquire their definitive shape; afterwards they disappear. Moreover, the horse-shoe shape of the actin MFs, seems not to be the most favorable arrangement for pulling the SFVs and actin MFs seem not to be involved in the migration of SFVs.

The constant presence of MTs on the external face of the SFVs suggests that they could guide the SFVs during their migration along the plastid. These MTs follow the two longitudinal margins of the SFVs and are also seen along the diverticulum. They run at a short distance from the SFV membrane and do not contact the membrane itself. As they change their linear course to follow a curved path in the vicinity of the SFVs we may suppose that they are indirecly linked to them. Microfibrils probably effect this linkage because actin MFs are not present in this area. When the vesicles containing matured scales migrate toward the cell surface, MTs progressively pull away from the SFVs and rarefy to finally disappear along with the microfibrils. The appearance of a microfibrillar matrix in the external face of the SFVs is concomitant with that of actin MFs on the internal face. These microfibrils are thinner than actin MFs and they are attached to the membrane of the SFVs. They resemble the microfibrillar network connected to the plasma membrane forming the intercellular junctions between the stalks of the colonial cells. Microfibrils could link together MTs and the membrane of SFVs. The involvement of MTs in movement of cell organelles is well known (see STEPHENS and EDDS 1976, DUSTIN 1978, HYAMS and STEBBINGS 1979). Generally MTs are associated with myosin or actin microfilaments although some examples of organelle motion occurs only in the presence of MTs and microfibrils. This is the case in the reticulopodia of *Allogromia* (TRAVIS and ALLEN 1981), as for the transport of pigment granules in crayfish retinula cells (FRIXIONE 1983) or in the pigment granule motion in erythrophores (BECKERLE and PORTER 1983). It is of interest that in diatoms such as *Pinnularia,* microtubules and microfilaments are also involved in the formation of the silicified valves since they have been seen in the vicinity of the "silica deposition vesicle" (PICKETT-HEAPS *et al.* 1979, SCHMID *et al.* 1981). To our knowledge these microfilaments have not been identified in diatoms but their size indicates that they might be actin MFs.

It is the first time that actin has been demonstrated in chrysomonads and we may suppose that other species of this group, especially those which phagocytate or which form filopods contain actin MFs. Among flagellates, choanoflagellates possess actin MFs in their tentacles which are similar to those of brush-border microvilli; actin MFs are also present under the plasma membrane of the stalk (LEADBEATER 1983). In other *Protozoa* actin MFs have been demonstrated in amoeba (POLLARD *et al.* 1970) where they play a role in amoeboid movement and in certain ciliates such as *Tetrahymena* (MÉTÉNIER 1982) while in others such as *Spirostomum* they seem to be lacking (HOBBS *et al.* 1983).

Now, if we consider the formation of the molding vesicle, the most intriguing process is the protrusion of a diverticulum in the center of the SFV. It occurs at a precise site when the PER membrane is glued to the SFV membrane in a zone where actin MFs are absent. The extensive increase of the PER membrane surface as well as of that of the SFVs in the diverticulum necessitates a synthesis and a growth of membrane. It is difficult to find out what the promotor is in this morphogenesis. We can suppose that cytoskeletal elements; microtubules and microfibrils, are involved rather than the PER. The mechanism by which the diverticulum is formed must be very precise to reproduce the same mold for each scale of a species.

Though the scales have the same pattern decoration in a given species there is a progressive variation of their shape from the anterior to the posterior part of the cell. Those from the top are large and stumpy whereas those from the stalk are elongated and slender (Fig. 1 see also MIGNOT and BRUGEROLLE 1982). To explain this apparent selection we think that the stumpy scales are elaborated in the upper part of the cell while the slender ones are formed from SFVs which have migrated far back along the plastid. We also must assume an action of the plasma membrane in the positioning of the scales to build a harmonious cell covering.

The actual role of the cytoskeletal elements might be ascertained by the use of drugs directed against microtubules, such as colchicine or nocodazole, or against actin, such as cytochalasins. Unfortunately, at present these drugs seem not to be active on this model and/or difficult to employ at the required concentrations on these very delicate organism; although experimentation is being continued in this direction.

The membrane of the SFV is equivalent to the silica-

lemma of diatoms. We have shown previously that these membranes originate from the Golgi and contains polysaccharide moieties (MIGNOT and BURGEROLLE 1982). Neither the way in which silica is carried in the vesicle nor the process by which silica is assembled to build the delicate architecture of the scale wall is known. In several mineralized scales silica is deposited in an organic framework (see ROMANOVICZ 1981) that could correspond to the material seen in the lumen of the young SFV. In *Synura* SFVs, the framework seems not to be directly organized in relation to the actin microfilaments since the scale ornaments do not coincide with the actin MF arrangement. The process of silica deposition seems different in *Synura* from that in diatoms where small dark silica spheres are carried by small SFVs vesicles which fuse to the "silica deposition vesicle" (SCHMID and SCHULZ 1979, SCHMID *et al.* 1981. In *Synura* as in other chrysomonads the transport of silica elements is very discreet (HIBBERD 1977) and we have only seen some coated vesicles fusing with the margin at the SFV.

References

ALLEN, R. D., 1981: Motility. J. Cell Biol. **91**, 148 s—155 s.

BECKERLE, M. C., PORTER, K. R., 1983: Analysis of the role of microtubules and actin in erythrophore intracellular motility. J. Cell Biol. **96**, 354—362.

BEGG, D. A., RODEWALD, R., REBHUN, L. I., 1978: The visualization of actin filaments polarity in thin sections. J. Cell Biol. **79**, 846—852.

CLARKE, M., SPUDICH, J. A., 1977: Non-muscle contractile proteins: the role of actin and myosin in cell motility and shape determination. Ann. Rev. Biochem. **46**, 797—822.

COHEN, C., 1979: Cell architecture and morphogenesis. I. The cytoskeletal proteins. Trends in Biochem. Sci. **4**, 73—76.

CRAIG, S. W., POLLARD, T. D., 1982: Acting binding proteins. Trends in Biochem. Sci. **7**, 88—91.

DUSTIN, P., 1978: Microtubules. Berlin-Heidelberg-New York: Springer.

FAIRBANKS, G., STECK, T. L., WALLACH, D. F. H., 1971: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**, 2606—2617.

FRIXIONE, E., 1983: Firm structural association between migratory pigment granules and microtubules in crayfish retinula cells. J. Cell Biol. **96**, 1258—1265.

GRIFFITH, L. M., POLLARD, T. D., 1982: Cross-linking of actin filaments networks by self-association and actin-binding macromolecules. J. biol. Chem. **257**, 9135—9142.

GUILLARD, R. R., LORENZEN C. J., 1972: Yellow-green algae with chlorophyllide c. J. Phycol. **8**, 10—14.

HIBBERD, D. J., 1977: Ultrastructure of cyst formation in *Ochromonas tuberculata* (*Chrysophyceae*). J. Phycol. **13**, 309—320.

HIGASHI-FUJIME, S., 1980: Active movement *in vitro* of bundles of microfilaments isolated from *Nitella* cell. J. Cell Biol. **87**, 569—578.

HIROKAWA, N., KELLER III, T. C. S., CHASAN, R., MOOSEKER, M. S., 1983: Mechanism of brush-border contractility studied by the quick-freeze deep-etch method. J. Cell Biol. **96**, 1325—1336.

HOBBS, U. S., JENKINS, R. A., BAMBURG, J. R., 1983: Evidence for the lack of actin involvement in mitosis and in the contractile process in *Spirostomum teres*. J. Cell Sci. **60**, 169—179.

HYAMS, J. S., STEBBINGS, H., 1979: Microtubule associated cytoplasmic transport. In: Microtubules (ROBERTS, K., HYAMS, J. S., eds.). London: Academic Press. pp. 497—530.

ISHIKAWA, H., BISCHOFF, R., HOLTZER, H., 1969: Formation of arrow-head complexes with heavy meromyosin in a variety of cell types. J. Cell Biol. **43**, 312—328.

KERSEY, Y. M., WESSELS, N. K:, 1976: Localization of actin filaments in internodal cells of Characean algea. J. Cell Biol. **68**, 264—275.

LAEMMLI, U. K., 1970: Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature **227**, 670—685.

LAZARIDES, E., REVEL, J. P., 1979: The molecular basis of cell movement. Scientific American **240**, 89—101.

LEADBEATER, B. S. C., 1983: Distribution and chemistry of microfilaments in choanoflagellates, with special reference to the collar and tentacle systems. Protistologica **19**, 157—166.

MÉTÉNIER, G., 1982: Mise en evidence de filaments "actin-like" chez *Tetrahymena paravorax*. J. Protozool. **29**, 308—309.

MIGNOT, J.P., BRUGEROLLE, G., 1982: Scale formation in chrysomonad flagellates. J. Ultrastruct. Res. **81**, 13—26.

MOORE, P. L., BANK, H. L., BRISSIE, N. T., SPICER, S. S., 1976: Association of microfilaments bundles with lysosomes in polymorphonuclear leukocytes. J. Cell Biol. **71**, 659—666.

MOOSEKER, M. S., 1976: Brush-border mobility. Microvillar contraction in triton treated brush-borders isolated from intestinal epithelium. J. Cell Biol. **71**, 417—433.

— TILNEY, L., 1975: Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. J. Cell Biol. **67**, 725—743.

— GROWES, T. A., WHARTON, K. A., FALCO, N., HOWE, C. L., 1980: Regulation of microvillus structure: calcium-dependent solution and cross-binding of actin filaments in the microvilli of intestinal epithelial cells. J. Cell Biol. **87**, 809-822.

PERRY, S. V., 1955: Myosin adenosine triphosphatase. In: Methods in enzymology II, pp. 512—584.

PICKETT-HEAPS, J. D., TIPPIT, D. M., ANDREOZZI, J. A., 1979: Cell division in the penate diatom *Pinnularia*. The valve and associated cytoplasmic organelles. Biol. Cell. **35**, 195—198.

— — — 1979: Cell division in the penate diatom *Pinnularia*. IV. Valve morphogenesis. Biol. Cell. **35**, 199—203.

POLLARD T. D., SHELTON, E., WEIHING, R. R., KORN, E. D., 1970: Ultrastructural characterization of F-actin from *Acanthamoeba castellannii* and identification of cytoplasmic filaments as F-actin by reaction with rabbit HMM. J. mol. Biol. **50**, 91—97.

— 1981: Cytoplasmic contractile protein. J. Cell Biol. **91**, 156s—165s.

ROMANOVICZ, D. K., 1981: Scale formation in flagellates, pp. 27—62. In: Cytomorphogenesis in plants (KIERMAYER, O., ed.). Wien-New York: Springer.

SCHMID, A. M., SCHULZ, D., 1979: Wall morphogenesis in diatoms: Deposition of silica by cytoplasmic vesicles. Protoplasma **100**, 267—288.

— BOROWITZKA, M. A., VOLCANI, B. E., 1981: Morphogenesis and biochemistry of diatom cell walls. In: Cytomorphogenesis in plants (KIERMAYER, O., ed.), pp. 63—97. Wien-New York: Springer.

SCHNEPF, E., DEICHGRÄBER, G., 1969: Über die Feinstructur von

Synura petersenii unter besonderer Berücksichtigung der Morphogenese ihrer Kieselschuppen. Protoplasma **68**, 85—106.

SHEETZ, M. P., SPUDICH, J. A., 1983: Movement of myosin-coated fluorescent beads on actin cables *in vitro*. Nature **303** (5912), 31—34.

STEPHENS, R. E., EDDS, K. T., 1976: Microtubules: structure, chemistry, and function. Physiol. Rev. **56**, 709—777.

TRAVIS, T. L., ALLEN, R. D., 1981: Study on the motility of *Foraminifera*. I. Ultrastructure of the reticulopodial network of *Allogromia laticollaris*. J. Cell Biol. **90**, 211—221.

WAGNER, A., 1982: Treadmilling of actin of physiological salt concentrations. An analysis of the critical concentrations of actin filaments. J. mol. Biol. **161**, 607—615.

WEEDS, A. G., POPE, B., 1977: Studies on the chymotryptic digestion of myosin. Effects of divalent cations on proteolytic susceptibility. J. mol. Biol. **111**, 129—157.