Protoplasma (1996) 193: 144–173

PROTOPLASMA

Pattern morphogenesis in cell walls of diatoms and pollen grains: a comparison

Anna-Maria M. Schmid[1,*], **Roland K. Eberwein**[2], and **Michael Hesse**[2]

[1] Institut für Pflanzenphysiologie, Universität Salzburg, Salzburg and [2] Institut für Botanik, Universität Wien, Vienna

Received April 18, 1996
Accepted June 10, 1996

Dedicated to Prof. Dr. Dr. h.c. Eberhard Schnepf on the occasion of his retirement

Summary. Mechanisms acting in pattern morphogenesis in the cell walls of two distant groups of plants, pollen of spermatophytes and diatoms, are compared in order to discriminate common principles from plant group- and wall material-specific features. The exinous wall in pollen is sequentially deposited on the exocellular side of the plasmalemma, while the siliceous wall in diatoms is formed intracellularly within an expanding silica deposition vesicle (SDV) which is attached to the internal face of the plasmalemma. Two levels of patterning occur in diatom and pollen walls: the overall pattern stabilises the wall mechanically and is apparently initiated in both groups by the parent cell, and a microtubule-dependent aperture and portula pattern created by the new mitotic (diatoms) or meiotic (pollen) cells. The parent wall in diatoms, and also the callosic wall in microspores, functions as anchor surfaces for transient, species-specific patterned adhesions of the plasmalemma to these walls, involved in pattern and shape creation. Patterned adhesion and exocytosis is blocked in pollen walls where the plasmalemma is shielded by the endoplasmic reticulum at the sites of the future apertures. In diatoms, wall patterning is uncoupled from the formation of a siliceous wall per se when the SDV and its wall is formed without contact to the the plasmalemma. Conversely, a blue-print pattern laid out in advance along the plasmalemma can be found in several diatoms. This highlights the key function of the plasmalemma and its associated membrane skeleton (fibrous lamina), and its orchestrated co-operation with elements of the radial filamentous cytoskeleton (actin?) in pattern formation. The role of microtubules during generation of the overall pattern may be primarily a transport and stabilizing function. Auxiliary organelles (spacer vesicles, endoplasmic reticulum, mitochondria) involved in diatoms for shaping the SDV, and a mechanism adhering and disconnecting this SDV together with spacer organelles in a species-specifically controlled sequence to and from the plasmalemma, are unnecessary for pollen wall patterning. The precise positioning of the portula pattern in diatom walls is discussed with respect to their role as permanent anchors of the cytoplasm to its wall, and in providing spatial information for nucelar migration and the next cell division, whereas apertures in pollen are for single use only.

Keywords: Adhesion; Cell wall; Diatoms; Exine; Plasmalemma; Pollen; Silica.

Abbreviations: AF actin filaments; C/Ca callose; CF cleavage furrow; cPL cleavage plasmalemma; DV dense vesicles; ER endoplasmic reticulum; ET epitheca; HT hypotheca; mPL folded plasmalemma; MT microtubules; MTOC microtubule organising centre; PEV primexine (matrix) vesicles; PL plasmalemma; SDV silica deposition vesicle; Si silica; SL SDV-membrane; SPV spacer vesicles.

Introduction

Both diatoms – the most dominant algal group responsible for about a quarter of the global net primary production and oxygen liberation (Werner 1977) – and pollen grains – the carrier for the male gametes of spermatophytes, are characterized by their species-specific ornamentation of the cell wall. The wall is stabilized with amorphous silica in diatoms and sporopollenin in pollen. Pollen grains of related plants usually have similar exine patterns (e.g., Blackmore and Barnes 1991, Traverse 1988) and related diatom species share major characteristics of their

* Correspondence and reprints: Institut für Pflanzenphysiologie, Universität Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

wall microarchitecture (Round et al. 1990). Both wall types are largely resistant to microbial and chemical degradation, and are well preserved in fossil deposits. They are therefore useful tools for reconstructing the histories of aquatic and terrestrial ecosystems (pollen/spores: Faegri and Iversen 1989, Traverse 1988; diatoms: Gersonde and Harwood 1990).

Although there are great differences in the way diatoms and pollen form their walls, the involvement of comparable mechanisms can be expected because both cell types are eucaryotic, possess similar subcellular organelles, and form their cell walls under the same physico-chemical laws. In both cell types, the walls are "light-weight constructions". However, while the pollen wall must have some flexibility to cope with desiccation and rehydration for transport in an air-dried state and imbibition at the stigma (harmomegathic effect; Pacini 1990, Halbritter and Hesse 1993), the typical diatom wall is designed as a rigid "shell" with self-carrying capacity and the ability to bear additional mechanical loads (Schmid 1987b, Steucek and Schmid 1989). The necessity for this becomes apparent, considering that the inextensible wall consists of two overlapping parts (i.e., "thecae"; see Round et al. 1990, Pickett-Heaps et al. 1990, Preisig et al. 1994, for terminology), to accommodate growth and division, and the diatom, as a plant cell, is under turgor. The two thecae are held together by means of various forces, one of which is tension. Tensile forces are transmitted through cytoplasmic strands which are anchored at specific sites in the cell wall (rimoportulae, fultoportulae, raphe ribs, septa) via a specific glycocalyx (Schmid 1986a, 1987a, b, 1994a–c).

The typical angiosperm pollen wall is formed after the microspore or pollen mother cell (microsporocyte = meiocyte) has undergone meiosis (for terminology, development and homology of sporoderm strata, see Punt et al. 1994, Blackmore et al. 1987, Blackmore 1990, Hesse 1991). The microspore tetrad itself and also each of the tetrad cells is first ensheathed by a callosic special wall (Bhandari 1984, Cresti et al. 1992). The pollen wall is then deposited at the external surface of the cell membrane (simultaneously over the entire surface) but inside the callosic layer and consists of a highly elaborate exine (often consisting of tectum, columellae, footlayer, and endexine) and (except in the aperture region where it is more massive) a mostly thin, homogeneous intine (Knox 1984). This concept may be modified in a few taxa, perhaps in relation to some, incompletely understood, phylogenetic and environmental factors.

The walls of the diatoms are formed after vegetative division, or after acytokinetic mitosis (Fig. 1 A, D, E) which is accompanied by transient turgor reduction of the parent cell. One parental theca is inherited by each of the progeny cells, and each of these is now an epitheca, while new hypothecae are formed, back-to-back in the two cells. They are formed intracellularly in a membrane-bound compartment, the silica deposition vesicle (SDV), which is required for controlling the chemistry of silica-polycondensation (Mann 1986), and is initiated as a small, flat, or tubular vesicle at a species-specific site in relation to the daughter nuclei. This part of the SDV will be the first part to become silicified and will thus be preserved as the "pattern centre" of the mature theca. The SDV then enlarges in close association with the internal face of the cell membrane and follows the contours of the developing siliceous ribs, honeycombs, or other structures (Pickett-Heaps et al. 1990; and see below). Upon maturation of the siliceous component, an organic coat is attached to the new wall part which is then exocytosed while the membranes are recycled (Fig. 2). An organic layer, the diatotepum, is ultimately formed in many cells against the inside surface of the siliceous wall, though not at the sites of portulae, sealing chambers and lattices against the cytoplasm (Fig. 5) (Schmid 1984c, 1986a, b, 1987a, b, 1994a; Schmid et al. 1981). During wall formation, the new cells are still enclosed within, and adhered to, the parental wall.

Pattern creation is one of the great unexplained enigmas. We do not know how the wide range of structural diversity is manifested in pollen grains and diatoms, or how the species-specific information is transformed into a three-dimensional, intricate architecture. We are completely ignorant of how the sequential events responsible for wall deposition are started, controlled and stopped. Theoretical biologists, biochemists and physiologists favour pattern assembly by self-organizing processes (Lacalli 1981, Gordon and Drum 1994, Feijó et al. 1995), or propose organic templates (Simkiss 1986); while the involvement of blue-print systems/stencils is suggested by cell biologists who interpret cellular images gained with sophisticated light or electron microscopy (Heslop-Harrison 1963; Dickinson 1970, 1976; references in Hesse 1991, 1995; Schmid 1984c, 1986a, b, 1987a, b, 1994a; Pickett-Heaps et al. 1990). Circumstantial evidence accumulated over the past decades indi-

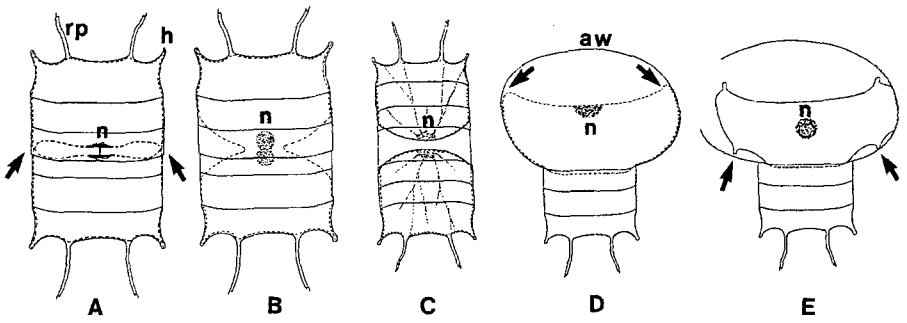


Fig. 1. *Odontella regia*: diagrams showing relation between cell shape and the transient adhesion to the previously formed cell wall (based on Mayer and Schmid 1995, and Schmid et al. unpubl.). **A** Vegetative cell division; adhesion of the protoplast to the equatorial zone of the mother wall (arrows) for moulding the horns (*h*); *n* dividing nucleus; *rp* rimoportulae. **B** Incomplete cleavage and lack of adhesion due to treatment with the anti-MT drug APM. **C** Male cell with two half-spermatogonangia. Lack of adhesion results in two hemispherical, rudimentary valves. **D** Oogonial theca with auxospore. Protoplast adhesion (arrows) to the auxospore wall (*aw*) and contraction of the cell for creating the mold for the rudimentary initial epitheca. **E** Initial cell shaping rudimentary hypotheca by adhesion (arrows) and contraction. Apical axis of auxospore and initial cell aligned with that of the oogonial wall

cates, that all of these mechanisms, and perhaps even others, are operating in an orchestrated action in a way not yet understood.

The main aim of this paper is to compare some morphogenetic mechanisms acting in pattern creation of two completely different systems, pollen and diatoms, as known from our own studies (AMS and MH) and from the literature, in this way discriminating between common principles from group-specific features. Our study will also focus on selected aspects of the creation of shape, where it interferes with wall pattern (especially in diatoms; Fig. 1), and the involvement of subcellular organelles, extracellular matrices, and adhesive and tensile forces, as well as turgor pressure and contractions ("spontaneous plasmolyses"). For reviews on other aspects the reader is referred to Pickett-Heaps et al. (1990), Round et al. (1990), Schmid (1994a), Gordon and Drum (1994) for diatoms, and for pollen grains to Blackmore and Ferguson (1986), Blackmore and Knox (1990) or Blackmore and Barnes (1991).

Creation of shape

Diatoms: circumferential outline and surface form – the role of the parent wall and the sibling cell (adhesions and contractions)

During vegetative division, the new hypothecae are formed intracellularly while the siblings are still enclosed within the parental wall to which the protoplast of many species is adhered by a hitherto unidentified organic adhesive (Figs. 1A, 5–7, 11 and 13). The existing diatom wall thus serves as a mold for the circumferential outline of the new components during morphogenesis. Aberrations in symmetry or notch deformations are perpetuated over many generations in this way (Schmid 1986a).

Because the SDV with the developing siliceous structures (see Introduction) adheres tightly to the internal surface of the plasmalemma created during cleavage (Figs. 2, 3, 12, 14, 20, 21, 26, 27, 30–32, and 36), it is the shape of this cell surface that determines the shape of the new wall. Different mechanisms may be involved in shaping the new wall. For example, the

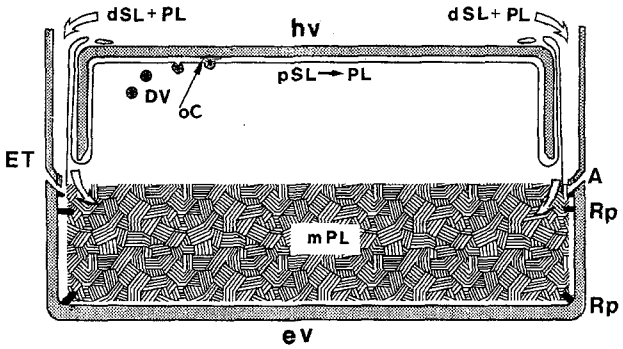
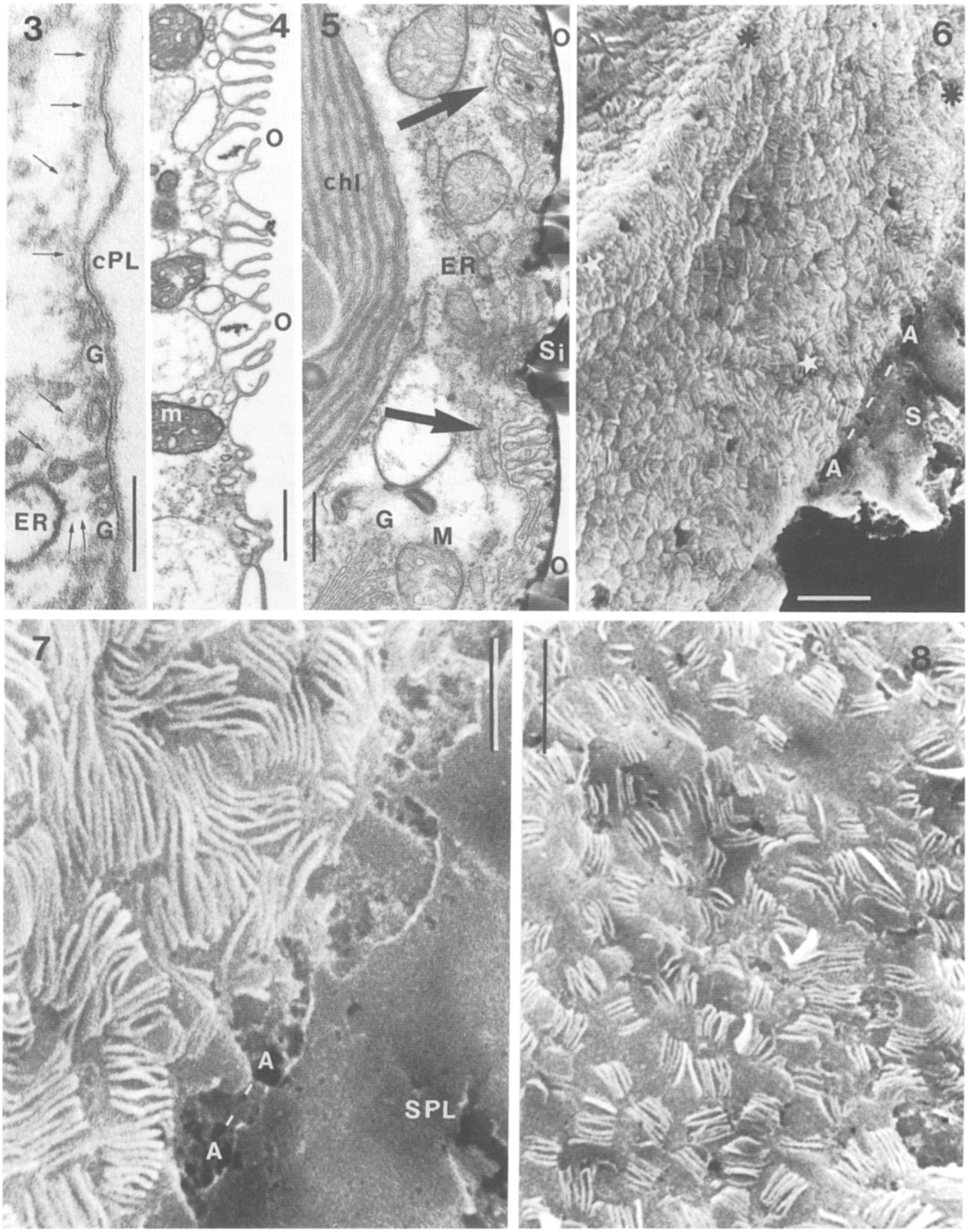
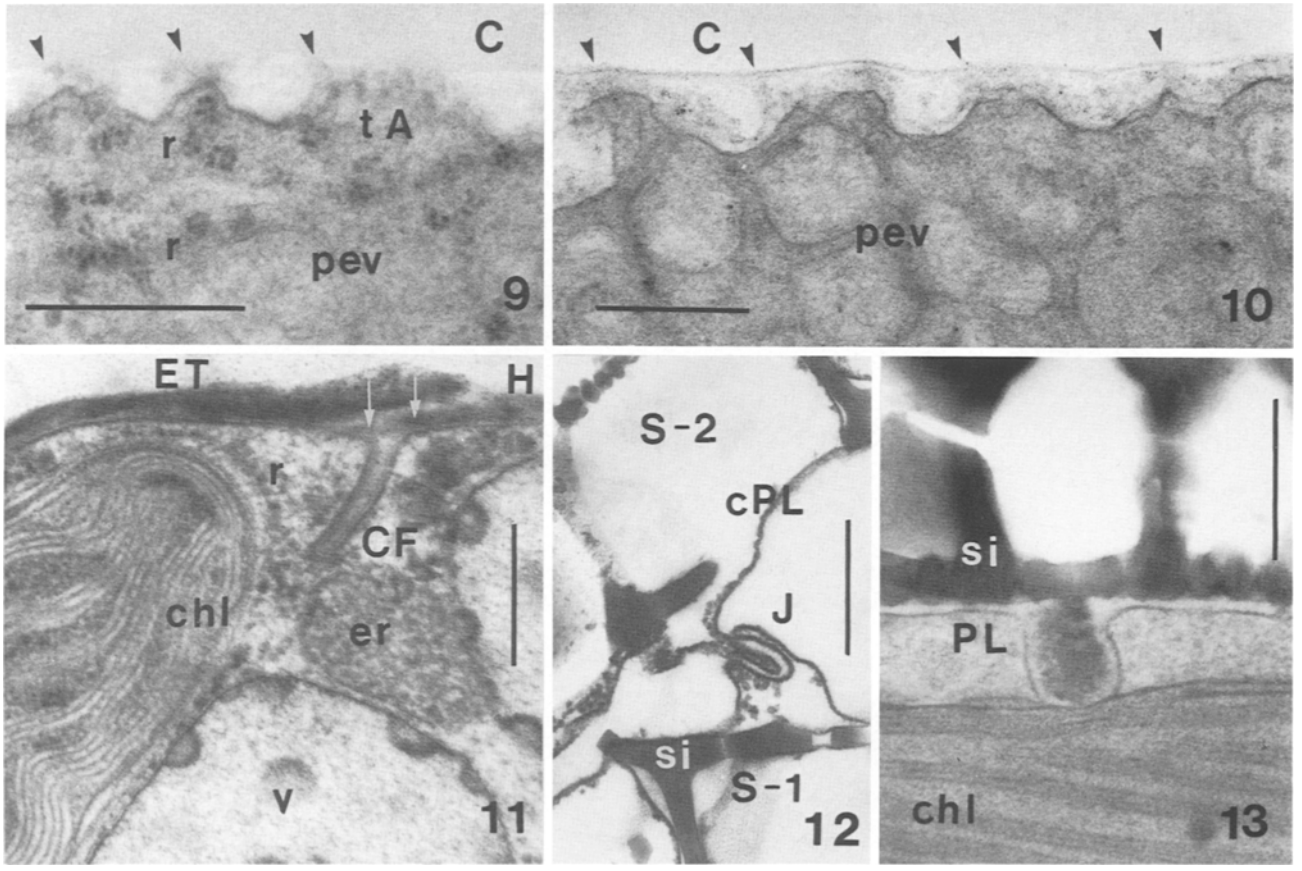


Fig. 2. *Coscinodiscus wailesii*: diagrammatic summary of the 3 types of plasmamembranes in the genus *Coscinodiscus* at the stage of valve release (*hv*) (only one daughter cell) (based on Schmid 1984c, 1986a, b, 1987a, b). (1) Plasmalemma (*PL*) created during cleavage fuses in the central region with the distal membrane of the SDV (*dSL*) and is retrieved over the margin into the cell (open arrows). (2) Dense Golgi vesicles (*DV*) deliver organic coat material (*oC*) to the SDV. Incorporation of their membrane transforms the proximal face of the SDV (*pSL*) in a mosaic like manner into the new smooth plasmalemma involved in formation of the organic layer. (3) The plasmalemma facing the old valve (*ev*) is ruffled into microplicae (*mPl*) and permanently anchored at the rimoportulae (*Rp*) of the wall. *A* Circumferential adhesion zone (border between smooth and folded plasmalemma) at the valve–girdle junction, *ET* epitheca

protoplast may adhere to the equatorial zone of the parental wall for the duration of cytokinesis and wall formation, and tensioning of the cell membrane may lead to a flat surface (Figs. 2, 11, 18, and 30–32). Lack of adhesion in this region causes hemispherical surfaces of the siblings under a given internal pressure (Fig. 1 B, C) (references in Schmid 1987a, Pickett-Heaps et al. 1990). Creation of more complicated shapes, such as apical elevations (Fig. 1 A) (Mayer and Schmid 1995, Pickett-Heaps 1996), requires precisely-timed transient adhesions to the parent wall and local contraction of the cortical cytoplasm (minimal surfaces). In *Odontella* these temporally and spatially controlled adhesions are specific for the vegetative division but lacking during premeiotic and meiotic divisions, resulting in hemispherical surfaces of egg cells and half-spermatogonangia (Fig. 1 C) (Mayer and Schmid 1995, Schmid et al. unpubl.). The two sibling protoplasts in many diatoms interact in a species-specific way, connected by stable or transient adhesions to each other and to the parent wall. As they develop, the wall surfaces are thus mutually influenced as in *Cymatopleura*, with the undulations of the surface exactly in register (Pickett-Heaps 1991). The marginal spines of *Thalassiosira*, which interdigitate between sibling walls, are the products of very peculiar outfolds of the cell membrane at the final stage of valve formation, where the membranes "crawl" along the parental wall, mediated by adhesions to the parent girdle (Schmid 1984b: figs 12–14) until new spotlike adhesive junctions are established between the two protoplasts. The protoplasts then contract and the cell membranes are in effect lifted tentlike and disconnect from the SDV (Fig. 12), thus providing additional space for the SDV to enlarge and silicify along the suspended plasmalemma. In this way they create hollow or solid spines. The wall pattern is radial at first (proximal wall face) but tensile forces acting during this late phase are distortive and a tangential pattern results at the distal wall face with the spines as the endpoints of tension lines (Schmid 1984a, b, and unpubl.). It is very likely that linking spines of colony forming species, such as *Fragilaria, Aulacoseira*, and others, are formed in a similar manner. Horn formation (Fig. 1 B) (*Attheya*: Schnepf et al. 1980; *Odontella*: Mayer and Schmid 1995, and unpubl.), as well as spine formation (*Thalassiosira*: Schmid 1984a, b), is inhibited with exposure of the cell to anti-MT-drugs prior to cleavage, because adhesion to the parent wall and the sibling plasmalemma is prevented. The role of the MTs in this process is probably transport of the vesicular carriers of the adhesives.

The ultimate control of the circumferential outline of a diatom wall must occur at the stage of initial wall formation in the "auxospore", which may arise vegetatively or as the product of sexual reproduction. Since these stages are rarely observed and not easily induced, information is scarce, but it appears that a combined interaction between osmotic water influx resulting in increasing turgor pressure, and tension-resistant siliceous bands (perizonium) and/or scales, embedded in an organic wall is responsible for complex shapes of auxospores (basically spherical in centric diatoms: Stosch 1982, Stosch et al. 1973, Pickett-Heaps et al. 1990, Round et al. 1990). Their flexible corset then appears to function as an anchor surface for the protoplast during formation of the rigid wall of the initial cell, allowing slight contractions of the protoplast for the creation of surfaces deviating from a hemispherical shape (Fig. 1 D, E). The species-specific shape of the wall is eventually created during the first vegetative division. Recently a specific type of scale ("slit-scale") has been detected in the auxospore wall of *Coscinodiscus granii*. These may be pre-stages of rimoportulae, and perhaps prime candidates for anchoring the auxospore's protoplast (Schmid 1994b). Nothing is known of how these slit scales are localised or how the initial band of a perizonium is determined in free auxospores. In zygotes that remain entirely attached to one theca of the oogonium during their expansion, the orientation of the first siliceous band is always spatially correlated with the oogonial theca. The new apical axis of the auxospore is then either parallel (Fig. 1 D, E) (*Attheya*: Drebes 1976a, b; *Odontella*: (Drebes 1974) or perpendicular (*Chaetoceros*: Stosch et al. 1973, French and Hargraves 1985), or at a defined angle (*Aulacoseira*: Müller 1906; *Ellerbeckia*: Schmid and Crawford unpubl.) to the apical axis of the oogonium. This indicates that morphological features of the oogonial wall to which the protoplast is anchored, serve as spatial determinants which the expanding auxospore might use as a reference for orientation in three dimensions. A fundamental question thus arises, that of "which came first, the chicken or the egg?", since the next oogonial theca has its apical axis aligned with the previous auxospore axis. Such a "structural memory system" has been shown to be present in the genus *Coscinodiscus* also for cell division and sex determination:


3
cPL
G
ER
G
4
O
O
m
5
O
chl
ER
Si
G
M
O
6
A
S
A
7
A
A
SPL
8



Figs. 9 and **10.** *Rosmarinus officinalis* microspores. Species-specific adhesion of the plasmalemma to the callose (*C*) via primexine matrix (arrowheads). *r* Ribosomes; *tA* tubular outfolds of PL; *pev* vesicles delivering primexine and matrix. Ga/pFa/Os/KFeCN; with Thiery in Fig. 10. TEM. Bar: 0.5 μm

Figs. 11–13. Species-specific adhesions in diatoms. TEM

Fig. 11. *Phaeodactylum tricornutum*: adhesion (arrows) at the edge of the hypotheca (*H*) for the cleavage furrow (*CF*). *r* Ribosomes; *er* endoplasmic reticulum; *V* vacuole; *ET* epitheca; *Chl* chloroplast Freeze substitution. Bar: 0.2 μm. (Schmid et al. unpubl.)

Fig. 12. *Thalassiosira eccentrica*: transient junctions (*J*) of the cPL of the two siblings (*S-1, S-2*) for spine formation. *si* Silica. Ga/Os semisimultaneous. Bar: 0.5 μm

Fig. 13. *Planktoniella sol*: exocytosis (*PL*) of adhesive material. Ga/Ta/Os. Bar: 0.3 μm

---

Figs. 3–8. *Coscinodiscus wailesii*: 3 types of plasmamembranes

Fig. 3. cPL involved in the morphogenesis of the SDV and its wall. Fibrous lamina and radial filaments between cPL and ER (arrows). *G* Golgi vesicles for the SDV. Ga/Os/KFeCN; TEM. Bar: 0.1 μm

Fig. 4. Folded mPL, still actively secreting (*o*). Ga/Os simultaneous; TEM. Bar: 0.5 μm

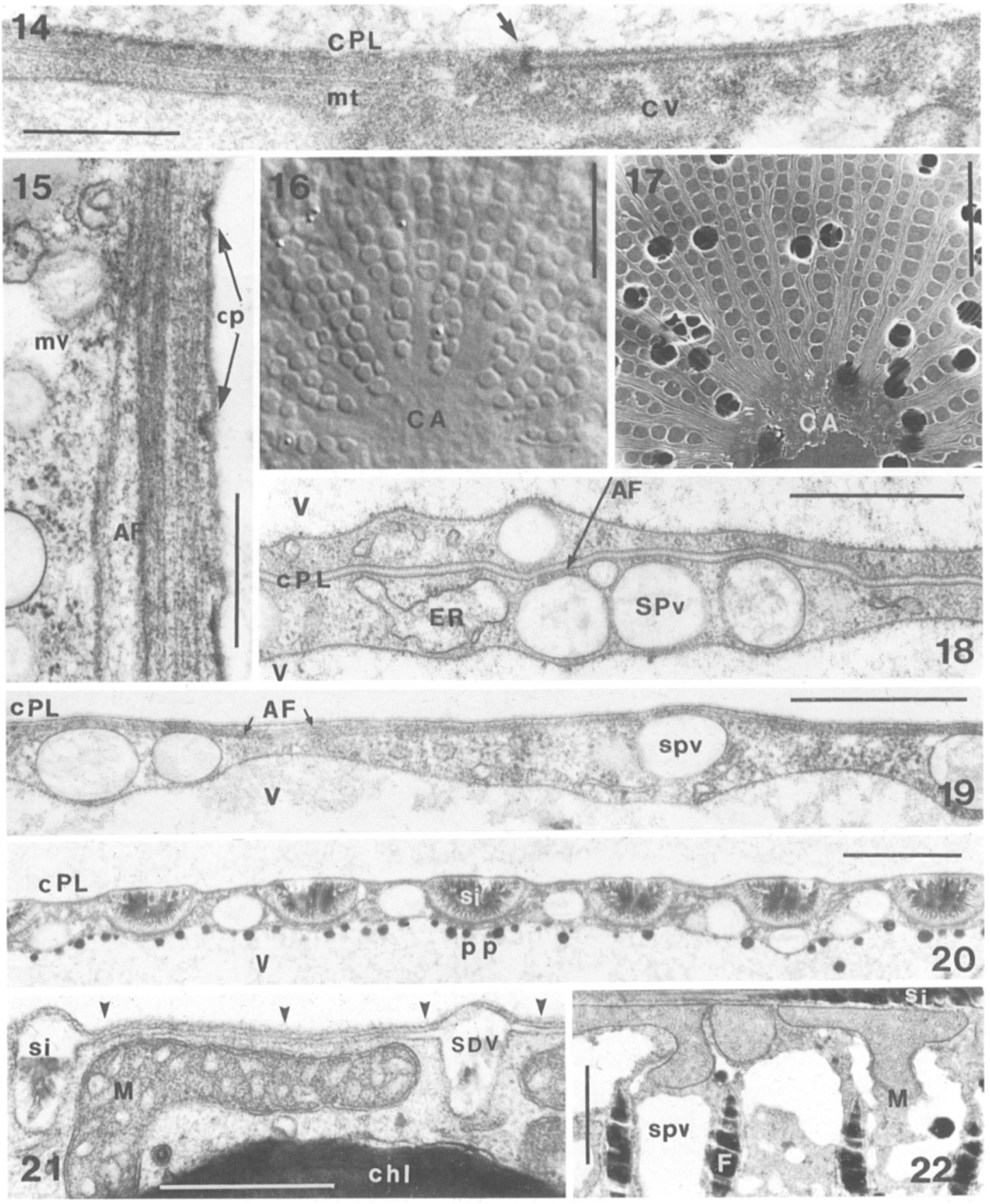
Fig. 5. Folded mPL (arrows) and organic sheet (*o*). *Si* Silica; *G* Golgi; *ER* endoplasmic reticulum. Ga/Ta/Os; TEM. Bar: 0.5 μm

Figs. 6–8. SEM image of the densely folded plasmalemma not involved in morphogenesis; cell wall mechanically removed after Ga/Os-simultaneous fixation and critical point drying

Fig. 6. Overview; dark holes (asterisks) are sites of permanent adhesion to the rimoportulae of the wall; *S* smooth PL; *A–A* circumferential adhesion zone. Bar: 5 μm

Fig. 7. Detail of Fig. 6, showing border between folded and smooth PL (*SPL*), disrupted along the adhesion zone (*A–A*) during preparation. Bar: 1.5 μm

Fig. 8. Early folding stage. Bar: 3 μm


14
CPL
mt
CV
15
mv
cp
AF
16
CA
17
CA
V
AF
cPL
ER
SPv
V
18
cPL
AF
spv
V
19
cPL
si
pp
V
20
si
SDV
M
21
chl
si
spv
M
F
22

two of the rimoportulae arranged in a marginal ring are differentiated as "macro-rimoportulae". They are aligned in both thecae, and thus in the entire clone, 120° apart, in a 4- and 8-o-clock position, act as spatial cues for nuclear migration. The nucleus, which lies at the centre of the epivalve during interphase, is connected to these structures through cytoplasmic strands rich in endoplasmic reticulum. For vegetative mitosis, the nucleus moves invariably to the 12-o-clock location of the girdle equator. For male sex determination, however, it moves to 6-o-clock, whereas in oogonia it moves to the geometric cell centre (Schmid 1994a, c). Structural features of the diatom wall are probably recognised by the adhered, and thus locally deformed, cell membrane and transduced via transmembrane proteins to the cytoskeleton and thus to the nuclear surface (Schmid 1994a). Similar recognition may occur in other plant cells (Gilroy and Trewavas 1990, Traas 1990, Roberts 1990, Schindler 1993).

Microspores (pollen grains before first haploid mitosis) – the role of the callose wall

In microspores, the species-specific tetrahedral, rhomboid, linear or square arrangement of the tetrads is formed when the microsporocyte undergoes meiosis and more or less simultaneous cytokinesis, while it is surrounded by the thick callosic special wall laid down during prophase I of meiosis (Bhandari 1984, Cresti et al. 1992). The orientation of the division plane is evidently controlled by the orientation of the meiotic spindle (Heslop-Harrison 1971a, b; Buchen and Sievers 1981; Dickinson and Sheldon 1990). Spindle orientation in turn, is very sensitive to centrifugation or to application of chemicals. Griseofulvin alters the alignment of spindles and therefore induces T-shaped or linear arranged tetrads instead of (normal) decussate tetrads in *Magnolia* (Brown and Lemmon 1992).

Callose deposition continues after cytokinesis, separating the microspores from each other by centripetal ingrowth of the callosic septum which is continuous with the callosic wall already present around the pollen mother cell (Bhandari 1984). Except for the ingrowing tips the cell membranes are at first smooth below the callosic wall (Cresti et al. 1992). This indicates membrane addition in this region by vesicular activity, probably originating from the Golgi apparatus (Buchen and Sievers 1981). Callose synthase complexes are presumed to be located in the cell membrane (Roberts 1990, Kauss 1996) and, if this is also the case for microspores, then at this stage the entire plasmalemma seems to be uniformly synthesizing callosic wall. This does not, however, suggest a callose synthesis in the Golgi apparatus, but rather, the delivery of a special membrane which is transforming on its way to, and during insertion into, the already existing cell membrane by acquiring β-glucan

---

Figs. 14 and **15.** *Pinnularia*: apical sections. Ga/Ta/Os; TEM. Bar: 0.5 μm

Fig. 14. New surface (*cPL*) close to the raphe ribs. Coated vesicle (arrow) attached to the SDV. *cv* Coated vesicles tangentially sectioned; *mt* microtubules

Fig. 15. Same cell, PL facing old valve: coated pits (*cp*) below the raphe fissure. Actin filaments (*AF*) and mucopolysaccharide vesicles (*mv*) for locomotion

Figs. 16 and **17.** *Coscinodiscus wailesii*: blueprint pattern of spacer vesicles for wall chambers. From Schmid (1984c), with permission. Bars: 10 μm

Fig. 16. Radial arrangement of spacer vesicles along the cPL; *CA* location of nucleus. Ga/pFa; sibling cell lifted off; LM, Nomarski

Fig. 17. Early silicified base layer on a nuclepore filter; *CA* unpatterned central area. Acid cleaned; SEM

Figs. 18–21. *Pinnularia*. Ga/Ta/Os; TEM

Fig. 18. Transversal section through siblings shortly after cleavage: cPL with glycocalyx; spacer vesicles (*spv*) connected via filaments (*AF*); *V* vacuole. Bar: 0.5 μm

Fig. 19. Same stage; apical section: spacer vesicles transported along actin filaments (*AF*). Bar: 0.5 μm

Fig. 20. Apical section: spacer vesicles at their destination. *PP* Polyphosphate. Bar: 1 μm

Fig. 21. Apical section through forming ribs; membranes of the chamber roof-SDV aligned with the cPL (arrowheads). Shape of mitochondrion indicates a non-spacer-function. *chl* Chloroplast. Bar: 0.5 μm

Fig. 22. *Nitzschia sigmoidea*. Apical section through raphe canal at stage of forming fibulae (*F*); shape of mitochondria indicates tight adhesion at their distal side to the raphe forming skeleton; *spv* spacer vesicles for fibulae. Ga/Ta/Os; TEM. Bar: 1 μm

synthase II complexes (Kauss 1996). Significantly, the enzyme is activated by micromolar concentrations of Ca^{2+} ions (Kauss 1996). The frequently observed accumulation of ER-cisternae below the plasma membrane during callose secretion (*Lilium*: Dickinson and Potter 1976, Dickinson and Heslop-Harrison 1977; *Zea mays* megaspores: Russel 1979; *Selaginella*: Buchen and Sievers 1981) might indicate a role in regulation of the Ca^{2+} concentration.

In diatoms, callose has been identified so far only in the genus *Pinnularia* (Waterkeyn and Bienfait 1987), where it is thought to function as a flexible joint sealing the two thecae during interphase, persisting through cell division, and vanishing, once the new cells form their own callosic strip. Screening of about 30 other genera has been fruitless (Schmid 1994a, and unpubl.).

Comparing the morphogenesis of the topography of pollen grain walls with that of diatom walls, it seems likely that the callosic wall, which is formed in most microspores, serves a function similar to the parental wall in diatoms, in providing anchor surfaces for patterned adhesions and contractions, and thus patterned ektexine deposition (Figs. 9, 10, and 36 a). In *Ceratophyllum* (Takahashi 1995c) and other submerged flowering plants the lack of an ektexine is correlated with a lack of callose synthesis; the thin, structureless mature exine is considered to consist of only an endexine. Furthermore, not only is failure in callose dissolution one of the various features causing male sterility (Graybosch and Palmer 1985a, b), but premature callose digestion also results in disturbances in the ektexine correlated with sterility, as suggested for transgenic tobacco (Worall et al. 1992, Kauss 1996). These findings could, of course, be interpreted in many ways, but could also indicate that an adhesive surface for wall formation is lacking. In hepatics (Brown and Lemmon 1987, and R. C. Brown pers. comm.), the callose serves as a framework rather than as a mold. The latter has often been suggested to be the usual function for the callose in Spermatophyta (for review, see Hesse 1995), but we believe this to be an erroneous interpretation. Possible exceptions are realised in echinolophate pollen, where the differential deposition of callose is reported to cause the angular shape of the microspores in *Scorzonera* (Barnes and Blackmore 1986) and in *Tragopogon* (Blackmore and Barnes 1987). In *Catananche*, pockets of callose displace the plasma membrane and define the sites of the lacunae between the ridges (Blackmore and Barnes 1988), while in *Tradescantia* shape control is clearly under the influence of microtubules (Tiwari 1989). In *Helianthus* and *Farfugium*, the inner surface of the callose wall does not possess an echinate pattern before exine deposition begins, whereas later, as the forming spines thrust into the callose wall, their imprints are clearly visible (Horner and Pearson 1978, Takahashi 1989a). These examples demonstrate, that the callose wall does not serve as a "mold" for the exine patterning, but as a surface for patterned adhesions of the plasmalemma and early wall deposits (Figs. 9, 10, 36 a, and 41).

Cell adhesion as one of the key mechanisms for motility, cell division, morphogenesis and intercellular communication, has been well studied in animal cells, and only more recently considered to be of fundamental importance also in higher plants (references in Gilroy and Trewavas 1990; Roberts 1990, 1994; Traas 1990; Schindler 1993; Reuzeau and Pont-Lezica 1995). Recently Wang et al. (1994) were able to show cross-reactivity of a 55 kDa protein in the vegetative and floral organs, i.e., also in anthers, of *Lilium longiflorum* with rabbit antihuman vitronectin antibodies, and also the presence of vitronectin-like sequences in the genome using a vitronectin cDNA probe. Vitronectin is known as extracellular matrix glycoprotein involved in cell adhesion and movement in animals by interaction with plasma membrane receptors such as integrins, which in turn are intricately connected to the cytoskeleton (Hay 1991, Hynes 1992). A tissue-specific localisation of this protein in the anther of *Lilium* has not yet been undertaken, and in diatoms none of the substrate adhesion substances visible in TEM (Figs. 5, 12, and 13) (Edgar and Pickett-Heaps 1984; Schmid 1984a, 1994a) has yet been identified. A thorough study, therefore, would be rewarding.

Creation of pattern

While in diatoms the pattern was generally assumed to be created (and controlled?) by the new cell formed during division (Pickett-Heaps et al. 1990, Round et al. 1990), in pollen grains, wall pattern is thought to be under gametophytic, sporocytic, or even sporophytic (tapetum) control and to be the result of prepatterning events, or of purely physical phenomena (e.g., Heslop-Harrison 1971a, b; Sheldon and Dickinson 1983, 1986; Dickinson and Sheldon 1984; Skvarla and Rowley 1987; Blackmore and Barnes 1991; van Uffelen 1991; Southworth and Jernstedt 1995).

As Dickinson and Sheldon (1986) have pointed out, two levels of patterning are found in the pollen wall architecture, and this holds true also for diatoms (Schmid 1979, 1986a, 1994a, b; Mayer and Schmid 1995). Thus two complex systems of pattern exist with respect to the wall composition in pollen and diatoms:
1. the overall microarchitecture that mechanically stabilizes the wall (= primary pattern), i.e., exine ornamentation and topography in pollen, and rib- and honeycomb systems in diatoms; and
2. the system of apertures and portulae, i.e., colpi and pori (colpori) in pollen grains and the raphe slit in pennate diatoms, rimoportulae (= labiate processes) and fultuportulae (= strutted processes) in centric diatoms. While in pollen grains the function of the apertures is clearly defined as the exit pore for the pollen tube, in diatoms, the ports to the cellular environment have various physiological functions, such as secretion of group-specific organic matter involved in motility, adhesion, control of sinking, excretion of waste products, and perhaps also uptake of nutrients and silicon (Fig. 15). It is also very likely that chemical signals are transmitted for intercellular communication, e.g., pheromones. In addition, in the mature wall, the portulae serve a mechanical function as anchor sites for the cytoskeleton (Figs. 2, 6, and 24) and as exact reference points for intracellular orientation. Conversely, in pollen grains the spatial correlation between the initiation of the pollen tube and the position of the porus/colpus appears to be less precise, since in monocolpate pollen such as *Lilium*, the pollen tube can be initiated either at one pole of the colpus or at its centre. It is not known how the exit-pore for the tube is determined in pantoporate pollen possessing up to 50 apertures per cell (G. Obermeyer pers. comm.).

The primary pattern

In pollen grains and diatoms, the overall (= primary) pattern is initiated first, in pollen over the entire cell surface and in diatoms growing centrifugally from a species-specific site (see above). In pollen it is laid out before meiosis (see below), while at first the definite aperture pattern lags behind – as in diatoms. Later it exerts visible effects on the primary pattern during development in many species, causing localised rearrangements or distortions (Sheldon and Dickinson 1983; Dickinson and Sheldon 1986, 1990; Schmid 1979; Pickett-Heaps et al. 1990; Mayer and Schmid 1995).

Pollen exines are initiated as patterned primexines. They are composed of polysaccharides secreted by Golgi vesicles, proteins (accumulations of ribosomes are often found directly below the plasmalemma of the future probaculae), and cellulose (Figs. 9, 10, 36 a, and 41 a–c) (Dickinson and Heslop-Harrison 1977, Buchen and Sievers 1981, Porter et al. 1983), and later become impregnated with more resistant materials (Heslop-Harrison 1963, Blackmore and Barnes 1991). Thereby a change from electron translucent to an electron dense matrix occurs, surrounding the probacula (Figs. 36 a, 38, and 41) (Takahashi 1989a, b). In *Farfugium* (Takahashi 1989a), the primexine is a fibrous layer with discrete globular elements that coincide with the future probacula. A similar developmental process has been observed in *Hibiscus* (Takahashi and Kouchi 1988). After primexine deposition is terminated, (proto-) sporopollenin precipitation is initiated at specific sites and probaculae are formed. The ultimate pattern of sporopollenin deposition becomes established at this stage (Figs. 36 a and 41) (Cresti et al. 1992, Gabarayeva and Rowley 1994).
The initial exine pattern, established during the tetrad stage, may be modified during the free microspore stage in various ways, and thus create the enormous complexity and diversity in pollen exine architecture. The final striate pattern of mature *Ipomopsis* pollen is such an example (Takahashi and Skvarla 1991b). In *Hibiscus* (Takahashi and Kouchi 1988) and other pollen grains with supratectate elements, these elements are formed exogenously after the microspores have been released into the loculus, and after the callose wall is degraded (Takahashi 1995b). In *Pinus*, sacci inflation and final exine sculpturing takes place at this stage (Dickinson and Bell 1970).
Conversely, in diatoms, the siliceous part of the cell wall is completed within the cell, inside the SDV, and the pattern develops progressively as silicification occurs. Because of the chemical environment needed for silica polycondensation (Mann 1986), there is no possibility of wall material being added after exocytosis except as a deposit of organic matter on the wall interior, roughly comparable to the deposition of the intine in pollen (Schmid 1986a). Therefore, the siliceous pattern of the diatom wall cannot become modified, as soon as it is extracellular.

The primary pattern – a microtubule-based pattern?

In diatoms, administration of microtubule (= MT) inhibitors (colchicine, amiprophosmethyl) after cyto-

kinesis, in concentrations sufficient to affect portulae pattern, shows only negligible effects on the primary pattern (Oey and Schnepf 1970; Schnepf et al. 1980; Schmid 1980, 1984b,1986a, and unpubl.). Centrifugation of microspores and treatment with colchicine at the early tetrad phase likewise affects the aperture pattern, but not the primary pattern (Heslop-Harrison 1971a, b; Sheldon and Dickinson 1983, 1986; Owens et al. 1990; all for *Lilium*), although MTs were clearly absent following colchicine treatment and the pollen grains' shape was modified (*Lilium*: Sheldon and Dickinson 1986, *Tradescantia*: Tiwari and Gunning 1986, Tiwari 1989). Sheldon and Dickinson (1983) have shown, that the phase most sensitive to disrupting the primary pattern by centrifugation is the early meiotic prophase. This indicates that the mechanism for establishing the primary pattern is operative *before* meiotic division (Dickinson and Heslop-Harrison 1977, Dickinson and Sheldon 1990). By analogy in diatoms, the rib and areolar pattern is disturbed by antimicrotubule agents only when incubated into the anti-MT drug prior to mitosis (Oey and Schnepf 1970; Schmid 1980, 1984a). Application of fluorescent tagged antibodies directed to tubulin, revealed that the cytoplasmic MTs of diatoms are depolymerized during mitosis in prometaphase, perhaps to supplement the tubulin pool for the formation of the spindle, and do not begin re-synthesis from the new MTOCs before completion of cleavage (Wordemann 1992; Schmid 1994a, and unpubl.). Consequently the cytoplasmic interphase symmetry breaks down, and results in a random mixture of all organelles, but is restored after the re-appearance of cytoplasmic MTs (Schmid 1994a). Such a cyclic breakdown and re-synthesis of MTs also occurs during the sequential mitoses and final meiotic division during spermiogenesis, as expressed in *Coscinodiscus* (accompanied by a reduction of organelles and eventually extrusion of chloroplasts; Schmid 1994c). It may in addition be a mechanism to effect equal distribution of cellular constituents during cell division.

The situation in pollen meiosis appears very dramatic too: in *Lilium* a major part of the parental ribosomes are eliminated until diplotene (Dickinson and Heslop-Harrison 1977), and correspond to a decrease in total cytoplasmic RNA, as demonstrated with in situ hybridisation (Porter et al. 1983). This reduction is preceded by an increase in acid phosphatase activity, as shown for *Cosmos* (Dickinson and Heslop-Harrison 1977). The authors maintain that this degradation in the sporocyte cytoplasm is a prerequisite for the transition from the vegetative to the sexual phase. Restoration of the cytoplasmic ribosome population in the microspores may involve either the activity of supernumerary nucleoli synthesized in the late prophase of the parent nucleus and released into the cytoplasm at the end of the meiotic division, or inheritance of small areas of ribosome-containing premeiotic cytoplasm encircled by double membranes (perhaps ER) that sometimes form several layers. The membranes are supposed to be directly involved in the synthesis of the wall (Dickinson and Heslop-Harrison 1977). Also chloroplasts, mitochondria, and membranes undergo a dramatic change in correlation with the ribosome cycle (Dickinson and Heslop-Harrison 1977). Furthermore, Dickinson and Sheldon (1984) and Sheldon and Dickinson (1986) report that, by application of immunofluorescence and EM methods, the only MTs visible throughout the meiotic division stages are those of the spindle, and a new MT-cytoskeleton starts to radiate out from nuclear associated MTOCs in the tetrad only after cytokinesis. The authors claim that the reappearance of the MTs is clearly far too late to influence the primary pattern of the exine (Dickinson and Sheldon 1990). This holds true also for diatoms (Schmid 1980, 1984b, 1986a, 1994a; Mayer and Schmid 1995).

Microtubules and turgor pressure. Southworth and Jernstedt (1995) and Muñoz et al. (1995) reinvestigated the role of MTs in exine patterning, also using immunofluorescence labelling of tubulin, but came to controversial conclusions to explain the pattern in pollen grains of two *Vigna* species. Southworth and Jernstedt (1995) speculated that in *V. unguiculata* a spatial congruence of exine pattern and MTs is caused by the developing exine, which, while growing, would rearrange the radiating MTs according to the mechanism of "cellular tensegrity" (i.e., cellular architecture is based on the tensional integrity of the cytoskeleton; Ingber 1993). The consequence drawn from this by the authors is a pattern generated "as a response to tensile and rigid properties of the cytoskeleton and to osmotic pressure [the authors probably mean turgor pressure] in the microspore, balanced against the pressure and volume of the newly secreted matrix". Therefore, there would also be no necessity for a cytoplasmic preplan (Southworth and Jernstedt 1995). While we believe the "tensegrity" mechanism to operate on a larger scale than micro-

patterns (compare Fig. 1 A with B and C), the model of Southworth and Jernstedt is also difficult to reconcile with the results from previous work gained from centrifugation experiments and treatment with anti-MT drugs (e.g., Heslop-Harrison 1971a, b; Sheldon and Dickinson 1983, 1986). Moreover, it is also clearly contradicted by the work on *V. vexillata*, issued from the same laboratory one month later (Muñoz et al. 1995), where the authors interpret the spatial congruence between exine pattern and MT-arrangement as "strongly suggestive of MT involvement in pollen wall pattern determination in this taxon". This model too, is in conflict with previous experimental approaches. Furthermore, considering the fact that the surfaces of the two *Vigna* pollen look almost identical in SEM (cf. Southworth and Jernstedt 1995: fig. 1, Muñoz et al. 1995: fig. 1), it is difficult to imagine that two such different mechanisms, which exclude each other entirely, might be operative in related species and leading to the same result.

Measurements of turgor pressure in microspores have not yet been published (G. Obermeyer pers. comm.). Obermeyer and Bentrup (1996) review data gained on mature and germinating pollen using osmometric methods and incipient plasmolysis. The recorded values of pollen tubes were around 12 bar, while the values of ungerminated, but mature *Lilium* pollen were between 8 and 10 bar. However, turgor values gained with these methods may be too high, as indicated by recent measurements using the turgor pressure probe and *Lilium* pollen tubes (R. Benkert et al. unpubl.). These revealed a mean turgor value of ca. 2 bars, independent of the stage of growth, and a "burst pressure" between 3 and 5 bars, where the tip wall of the pollen tube burst due to an increase in hydrostatic pressure caused by microinjection of silicon oil. We may speculate that the turgor value in the microspore is much lower (G. Obermeyer pers. comm.), and turgor increase is coupled to K^+ and, subsequently, water influx at the stage of vacuolisation of the free microspore and its expansion (Obermeyer and Blatt 1995). This coincides with the view of Dickinson and Bell (1970), and Dickinson and Sheldon (1986), who consider even slight plasmolysis to occur during exine formation.

In diatoms, cell expansion is restricted to preprophase accompanied by girdle band formation, and/or posttelophase, coupled to valve release and formation of the first girdle band, whereas during mitosis, cytokinesis and wall formation, active turgor reduction appears to take place (Stosch et al. 1973; references in Schmid 1987b). One cause for this might be that the cytoplasmic strands running from the nucleus to the cell wall-anchors (rimoportulae, fultoportulae, ribs, septa; Schmid 1987b, 1994a) and connecting the two thecae during interphase in a controlled fashion are depolymerised in prometaphase (Schmid 1994a, Franz and Schmid 1994, Mayer and Schmid 1995), and the adhesive substance between the overlaping thecae might not be sufficient in its strength to avoid accidental opening of the wall during division. Another reason may be seen in the wall-forming machinery itself, which is clearly malfunctioning when turgor is increased during wall formation, i.e., by slight (20 mOsmol) dilution of a marine medium. In *Coscinodiscus* this leads to premature release of spacer organelles and as a result, to a cell wall lacking a self-carrying capacity (see below) (cf. Pickett-Heaps et al. 1990: fig. 96 a, b; normal vs. reduced thickness of cell wall in *C. wailesii*). Shaping mechanisms mediated by local adhesions of the protoplast to the mother-wall and local contractions, such as are operative in *Biddulphiopsis* (Franz and Schmid 1994), or *Odontella* (Fig. 1 A) (Mayer and Schmid 1995), may be possible only under reduced turgor, and we believe this to be necessary also in pollen wall formation.

Both, pollen and diatom walls have in common that they are formed while they are still confined within their mother wall, i.e., thick callose in pollen, and rigid silica enveloped by an organic coat in diatoms. The diameter of the tetrads enclosed in the callose does not increase until release into the loculus (Pérez-Muñoz et al. 1993b). Nor does the pervalvar-axis of the diatom parent-cell increase during formation of the new walls until the final stage is reached (Schmid 1987a, b; Franz and Schmid 1994, Mayer and Schmid 1995). Thus, to allow the new wall to thicken and to form elaborate chambers or probaculae and spines within a given external boundary, the volume of the cytoplasm of each of the cells must decrease. This is hardly possible under a high turgor pressure, which is supposed to be one of the driving forces in the model of Southworth and Jernstedt (1995). The morphogenesis of the sacci during exine formation in *Pinus* is, perhaps, the best example to demonstrate that turgor pressure during wall formation within the tetrad is lacking: Dickinson and Bell (1970) found the development of a space between the inner face of the primexine and the plasmalemma as the first indication of the future sac-

ci. This space fills with fibrillar matrix delivered by Golgi-derived vesicles, and extends deeply (ca. 10 μm) into the microspore cytoplasm. This size increase of the sacci causes negligible or no distortion of the callose wall (Dickinson and Bell 1970), whereas if the cells were under turgor, the callose wall would bulge outwards at the sites of sacci development. Dramatic volume increase of the microspore cytoplasm and inflation of the sacci occurs not before release into the loculus (Dickinson and Bell 1970), perhaps coupled to a similar mechanism of water influx as described for *Lilium* (Obermeyer and Blatt 1995).

Furthermore, several observations in diatoms suggest that an increase in intracellular hydrostatic pressure interferes with cytoskeletal function. Lowering the external salinity by 50 to 60 mOsmol causes an immediate (seconds) depolymerisation of cytoplasmic MTs in the marine *Biddulphiopsis*, and as a consequence contraction of the tensile cortical net due to the lack of stabilizing MTs. The protoplast shrinks to form an X-shaped body, anchored to the poles of the cell wall at the rimoportulae with an organic adhesive (Schmid 1994a: fig. 3, and unpubl.). Considering the susceptibility of the cytoplasmic microtubules to a variety of factors, we believe it to be a great risk for a cell to depend entirely on such a sensitive system during the formation of its "life-vest".

The primary pattern – a membrane-based pattern?

Coated vesicles; Golgi-vesicles; reaction-diffusion patterns. Based on the abundance of coated vesicles close to the new meiotic cell membrane, Sheldon and Dickinson (1983) and Dickinson and Sheldon (1986) have suggested that the exine pattern is generated in the plasmalemma by organised segregation of membrane components. Material synthesized prior to meiosis in the endoplasmic reticulum would be progressively inserted into the plasmalemma by coated vesicles during meiotic division. Also, in some diatoms (e.g., *Synedra ulna, Navicula oblonga, Achnanthes longipes*: Schmid unpubl.) clathrin-coated vesicles and coated pits (CPs) are seen at random intervals during prophase in those membrane domains that later become integrated into the cleavage surface (Schmid 1994a). After cleavage, CPs are situated next to the outgrowing edge(s) of the silica deposition vesicle in *S. ulna* (Schmid unpubl.). However, the modern concept of clathrin-coated vesicles sees their role in an involvement in endocytotic processes rather than in exocytosis, and with a transport function from the trans-Golgi-reticulum to different kinds of cytoplasmic compartments (e.g., Robinson and Depta 1988, Robinson and Hillmer 1990). Coated vesicles have been found by Schnepf and Deichgräber (1969) and Brugerolle and Bricheux (1984) to be associated with the early scale-SDV in *Synura*, and recently a similar association was observed in the diatom *Pinnularia* (Fig. 14). The plasmalemma below the raphe fissure of the old valve of the same cell was densely covered with coated pits (Fig. 15), suggesting perhaps a mechanism for taking up important constituents (Si?) needed for SDV-function.

Vesicles have been seen by many authors coalescing with the plasma membrane in meiocytes (e.g., Dickinson 1976; Buchen and Sievers 1981; Dickinson and Sheldon 1986; Cresti et al. 1992; Pérez-Muñoz et al. 1993a, b). Perhaps the new vesicle membranes gradually replace the existing plasmalemma which was specialised to perform callose-synthesis, and thus comprise the new plasmalemma, now specialised in exine formation, in a patterned array (see below, for membrane-turnover in diatoms). Therefore, the model of Sheldon and Dickinson (1983) appears to be still valid in a modified way, and encompassing the Golgi-apparatus on the route from the ER to the cell membrane. Their proposed membrane platelets ("islands") in the plasmalemma, either created by biophysical reorganisation (by addition of hydrophobic constituents) or by reaction-diffusion patterns (Lacalli 1981; see also Feijó et al. 1995), would eventually meet and their interfaces would assume a reticulate lattice, as they compress together, providing a prepattern for the mural reticulum (Dickinson and Sheldon 1986, 1990).

The periodic patterns in diatoms have also been subjected to theoretical models. A pattern based on the solidification properties of the amorphous silica itself has been proposed by Gordon and Brodland (1990) and Gordon and Drum (1994), but is inconsistent with the fact that the membrane of the SDV tightly surrounds the condensing silica throughout the development. There are exceptions to this rule (e.g., *Pinnularia, Catacombas*), which are discussed below. The regular spacing of transversal ribs along the axial rib (or along the raphe-ribs) in pennate diatoms, or the dichotomous branching involved in the maintenance of the regular spacing whenever the ribs diverge as a result of their radial growth in centric and some pennate diatoms, were interpreted by Lacalli (1981) as "two-dimensional consequences of a one dimensional spacing mechanism operating along the growing

edge". A reaction-diffusion mechanism would impose a spatial periodicity on the initiation step, leading eventually to the patterns expressed in diatoms (Lacalli 1981). At present, insufficient information exists to prove or disprove this model but there is increasingly circumstantial evidence to suggest that the plasmalemma (PL), created during cleavage (cPL) and most probably evolved from Golgi vesicles, carries morphogenetic information for the new valve (Schmid 1984c, 1994a; and see below) together with its glycocalyx and its membrane skeleton (= fibrous lamina). In diatoms, dictyosomes replicate in preprophase (clearly visible in small species that possess a single dictyosome during interphase, such as *Phaeodactylum*: Schmid et al. unpubl.). At least part of the cPL of the daugther cells stems from parent Golgi vesicles, in this way complying with the postulate of Schnepf that membranes represent elements of inheritance (Schnepf 1984).

Plasmalemma domains and patterned adhesions. The two parts of the heteropolar cell wall of diatoms, epitheca and hypotheca, are of different age. The epitheca is at least one cell cycle older than the hypotheca, due to the specific division mode (Pickett-Heaps et al. 1990, Round et al. 1990), but can theoretically, be a hundred cell cycles older or even more, because the newly created wall part during division is always the hypotheca. In many diatoms, heteropolarity is expressed also in the protoplast, visible in light microscope by the species-specific location of the interphase nucleus (references in Schmid 1987b, 1994a). The model organism to demonstrate heteropolarity in diatoms is *Coscinodiscus*. In the whole genus, not only the interphase nucleus is associated with the epitheca, but there is also a radial MT-skeleton emanating from the nucleus-associated MTOC (Schmid 1987b, and unpubl.) and a peculiar plasmalemma domain, which has yet to be discovered in other diatoms (Fig. 2) (Schmid 1987a, b). The specific PL-domains are stabilised by adhesion of defined zones of the heteropolar protoplast to defined zones of the heteropolar parent wall, reflecting a functional membrane turnover during the cell cycle (Figs. 2–8) (Schmid 1984c, 1986b, 1987a, b; Pickett-Heaps et al. 1990).

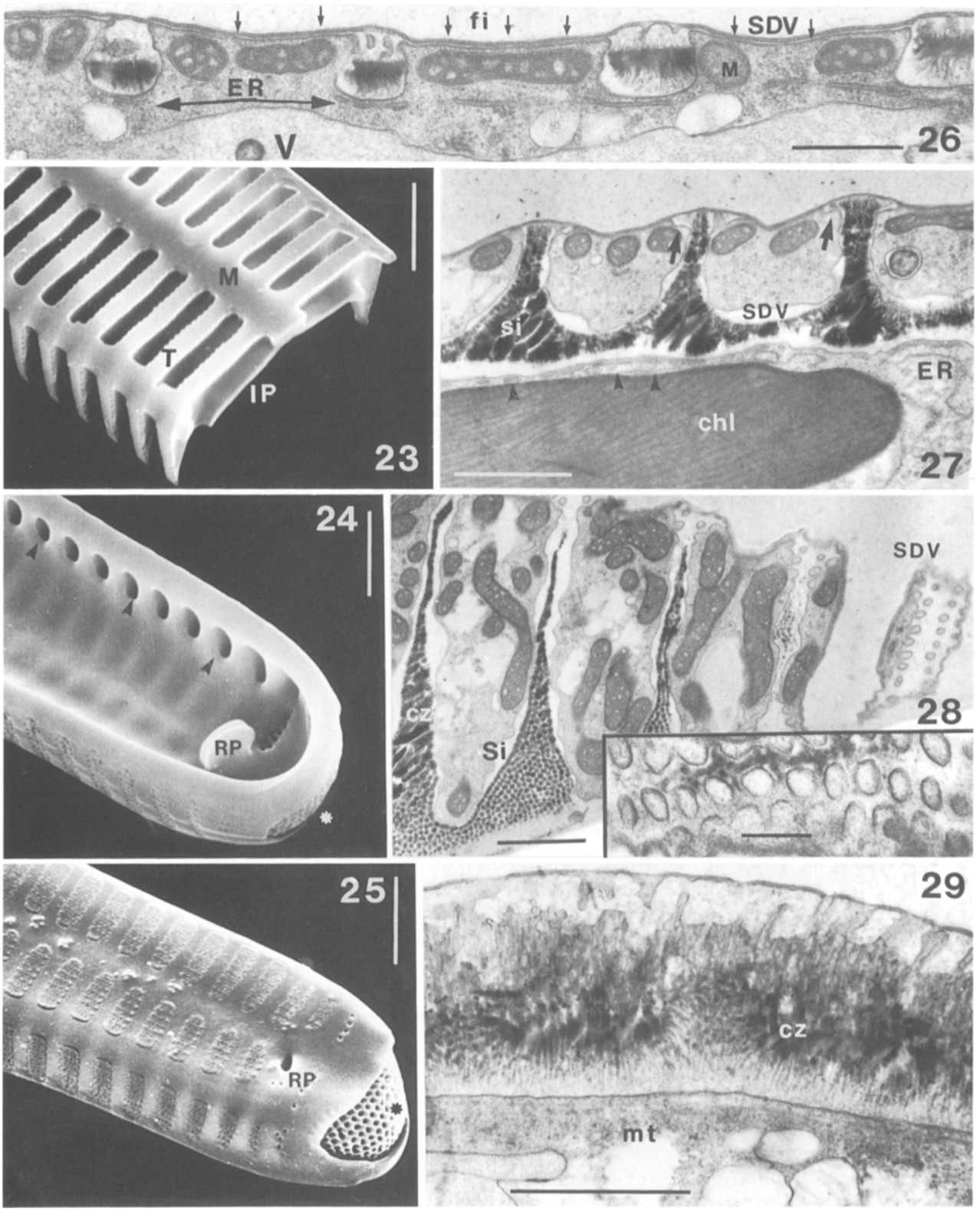
1. There is the smooth cleavage PL with its fibrous lamina acting as a moulding surface for the SDV and the silicifying wall inside, which is tightly attached to this cPL until wall-extrusion (Figs. 2 and 3).

2. At exocytosis, the new PL is formed by incorporation of a novel type of Golgi-vesicle (dense vesicle, DV) into the proximal membrane of the SDV (pSL), which, in a mosaic-like manner is probably transformed into the new, smooth PL (sPL), while the outer membranes (distal part of the SDV and cleavage PL) fuse, to liberate the wall, and are then retracted into the cell and recycled (Figs. 2, 6, and 7) (Schmid 1984c, 1986b, 1987a, b, 1988; Pickett-Heaps et al. 1990: figs. 30 c and 97). This new sPL is involved in the formation of an organic sheet (= diatotepum), which is firmly glued to the siliceous wall, and separates it from the cytoplasm except at the sites of the portulae.

3. The PL-domain facing the old theca, not involved in morphogenesis, i.e., the metabolically active PL, is ruffled into a patterned array of microplicae. It is sharply demarcated against the smooth PL-regions by a circumferential adhesion zone to the valve–girdle junction of the parental wall (Figs. 2 and 4–8) (Schmid 1987a, b)

There is circumstantial evidence that in microspores too, the PL created during meiotic cytokinesis is different to the PL that is already present in the microspore mother cell. Dickinson and Sheldon (1986) report that centrifugation at particular stages of meiotic division influences "only" the patterning formed at the new crosswalls within the tetrad, but not that area where the young microspore inherits the PL from the mother cell (Sheldon and Dickinson 1983), indicating that the patterned array of the PL is created during its growth by insertion of material into the PL (Dickinson and Sheldon 1986). Lateral displacement of patterning elements in the PL of the meiocyte is, perhaps, impaired by already existing anchor zones of the meiocyte PL to the callosic wall. Careful TEM investigation involving plasmolysis experiments should help to evaluate this possibility.

The PL domain of diatoms most interesting for comparison with the PL function in pollen exine formation, is the cleavage PL and its fibrous lamina (Figs. 3 and 11), which is involved, analogous to a spectrin-ankyrin coat, in the adhesion and expansion of the SDV and also in the transient adhesion of spacer organelles (Figs. 3, 14, 16, 20, 21, 26, 30–32, and 36) (Schmid 1984c; and see below). The cPL and its glycocalyx performs transient connections to the sibling cell (Fig. 12) (Schmid 1984b). The fibrous lamina intricately connects also to the fibrous dense domains surrounding the growing tips of the SDV, and to other


fi
SDV
M
ER
V
26
M
T
IP
23
si
SDV
ER
chl
27
24
RP
SDV
cz
Si
28
25
RP
29
cz
mt

cytoskeletal elements guiding vesicle traffic (Figs. 3, 18, and 19). Together, they seem to provide a master copy for the cell wall pattern (areolae, ribs, etc.) as suggested by the presence of a blue-print for the SDV in *Coscinodiscus* (Figs. 16 and 17) (Schmid 1984c, 1987a, b, 1994a), which appears to transmit its information in a sequential manner to the growing SDV. The SDV as a membranous reaction vessel for silica polycondensation, and in *Coscinodiscus* originating from the coalescence of Golgi vesicles (Schmid 1984c, 1988; Pickett-Heaps et al. 1990: fig. 30), does not have moulding capability per se. Modelling of the SDV is achieved by application of external forces, beautifully demonstrated also in the shaping of the scale vesicle in *Synura* (Schnepf and Deichgräber 1969, Brugerolle and Bricheux 1984), which is accomplished by the local expansion of the underlying outer envelope of the chloroplast (substituting for the cleavage PL in diatoms) and cytoskeletal elements. The complexity of the auxiliary machinery needed for this process in diatoms, depends on how the wall is formed (see below).

Uncoupling of pattern creation and wall formation in diatoms. Another indication for the key role of the cPL in pattern formation is provided with pennate diatoms such as *Pinnularia* or *Catacombas*, where elongated wall-chambers are serially arranged transversally to the raphe (*Pinnularia*; Figs. 20, 21, 26–28, and 30–32), or to the median rib (*Catacombas*; Figs. 23–25), and the centric *Cyclotella*, where similar chambers radiate to the margin. They are covered with a perforated roof to the exterior (Fig. 25) and with a large unstructured "internal plate" with large openings to the inside (Figs. 23, 24, and 27). The sequence of wall formation in these species is from the exterior towards the interior (Figs. 21, 23, 26, and 27). When the internal plate is formed, laid out in advance by the endoplasmic reticulum (Figs. 26 and 27), there exists no direct connection between the plate SDV and the cPL, since the SDV for the chamber roofs is already present and tightly adhered to the internal surface of the cell membrane and covered at its internal face by mitochondria. The mitochondria are connected to the roof SDV via a bristle coat similar in appearance and function to the filaments connecting the scale vesicles of *Synura* to the chloroplast-ER (Figs. 21 and 26) (Brugerolle and Bricheux 1984, Schmid 1994a). All endoskeletons (e.g., the "craticula" in *Navicula cuspidata*; Schmid 1979) are unstructured because they are formed without contact with the plasmamembrane, whereas the internal wall-faces of diatoms like *Thalassiosira* (Fig. 36) (Schmid and Schulz 1979) or *Stephanopyxis* (Pickett-Heaps et al. 1990: fig. 83 f, g) do have a porous pattern, because the sequence of valve formation here is from the interior to the exterior of the valve, and the SDV is closely associated with the cPL at all stages of growth.

In most diatoms, raphe ribs are initiated much narrower than they appear at maturity, and "homogeneous central and axial areas" are formed during wall thickening. Figures 30–32 demonstrate that this may be due to the action of the endoplasmic reticulum which shields the rib SDV from cytoplasm (and initially also from the cPL). It thus inhibits radial connections

---

Figs. 23–25. *Catacombas gaillonii.* Valves. SEM. Bar: 2 μm

Fig. 23. Developmental stage with complete median (*M*) and transversal ribs (*T*), chamber side walls and internal plate (*IP*)

Fig. 24. Internal view, complete: structureless internal plate (formed along the ER as in *Pinnularia*); chamber openings (arrowheads). *RP* Rimoportula; asterisk, apical porefield

Fig. 25. External view, complete: patterned roof-plates formed along the cPL

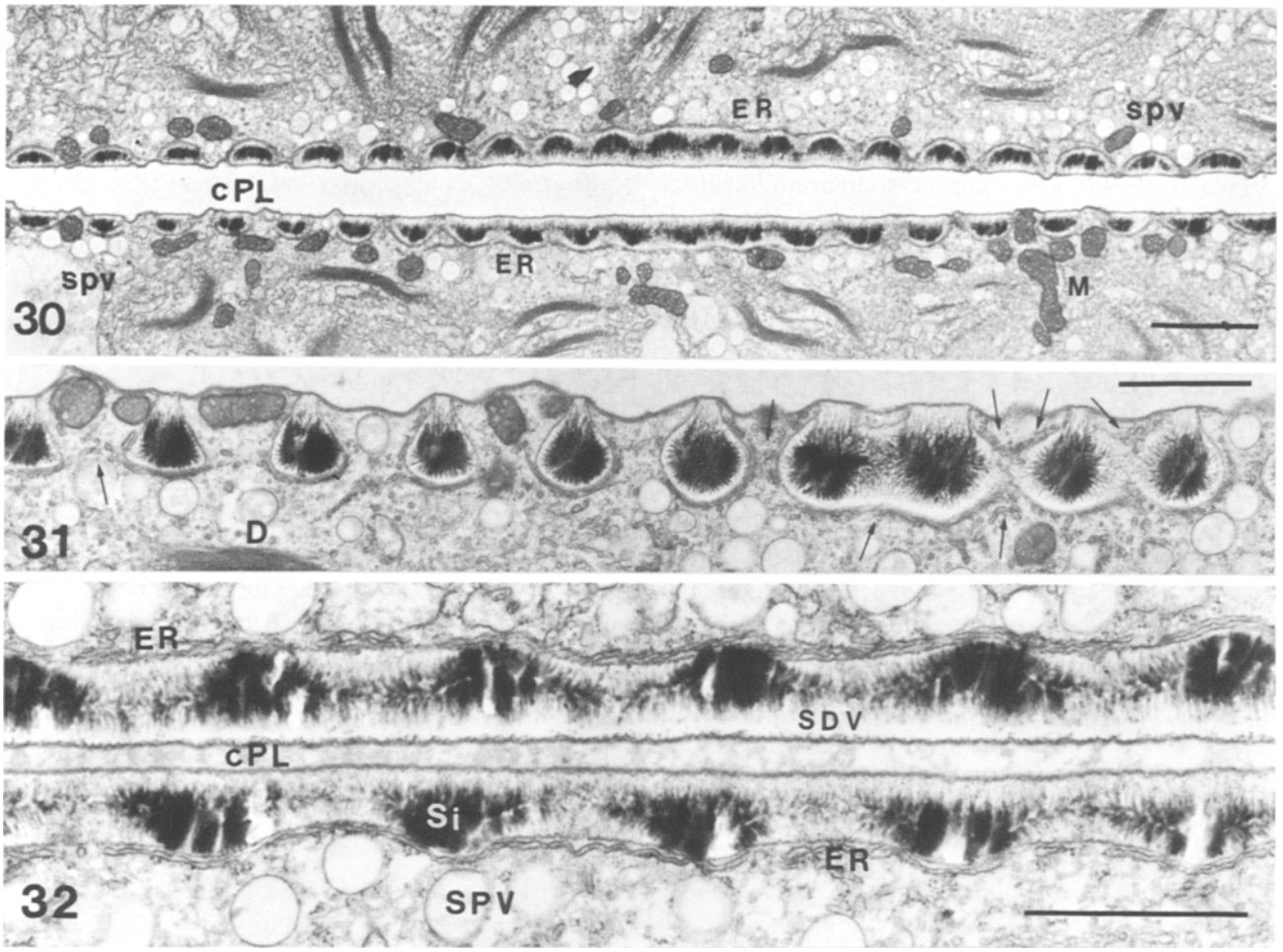
Figs. 26–29. *Pinnularia.* Ga/Ta/Os; TEM

Fig. 26. Apical section through forming chambers; parallel membranes of the SDV for the future roofs attached to the cPL. Fibrous elements (*fi*, arrows) between SDV and mitochondria (*M*). Transversal ribs capped by ER; ER also below mitochondria, foreshadowing the internal plate. Bar: 0.5 μm

Fig. 27. Apical section through forming chambers at a stage similar to *Catacombas* (Fig. 23). Chloroplast ER (arrowheads) can substitute for normal ER. SDV for chamber roofs starts to silicify from the sidewalls (arrows). Bar: 1 μm

Fig. 28. Oblique valvar plane section. Differentiation of patterned chamber roofs: distal membrane of the SDV forms tubular invaginations (right). New silica (*Si*) in columns distal to the compacting zone (*cz*). Bar: 1 μm. **Inset** Detail of roof SDV. Bar: 0.2 μm

Fig. 29. Apical section, close to the raphe rib: distinct polarity of the SDV with tubular invaginations distal, and proximal smooth membrane with subjacent MTs. Silicifying columns distal from the compacting zone (*cz*) thicker than the proximal ones. Bar: 1 μm



Figs. 30–32. *Pinnularia*, Ga/Ta/Os; TEM apical sections: Prepattern of ER for future SDV

Fig. 30. Overview of siblings at the stage where spacer vesicles (*spv*) give way to mitochondria (*M*); thickening of the central area and raphe ribs by filling the space between transverse ribs. Bar: 2 μm

Fig. 31. Detail showing fusion of rib-SDV (right) as a result of continuous ER (arrows). *D* Dictyosome. Bar: 1 μm

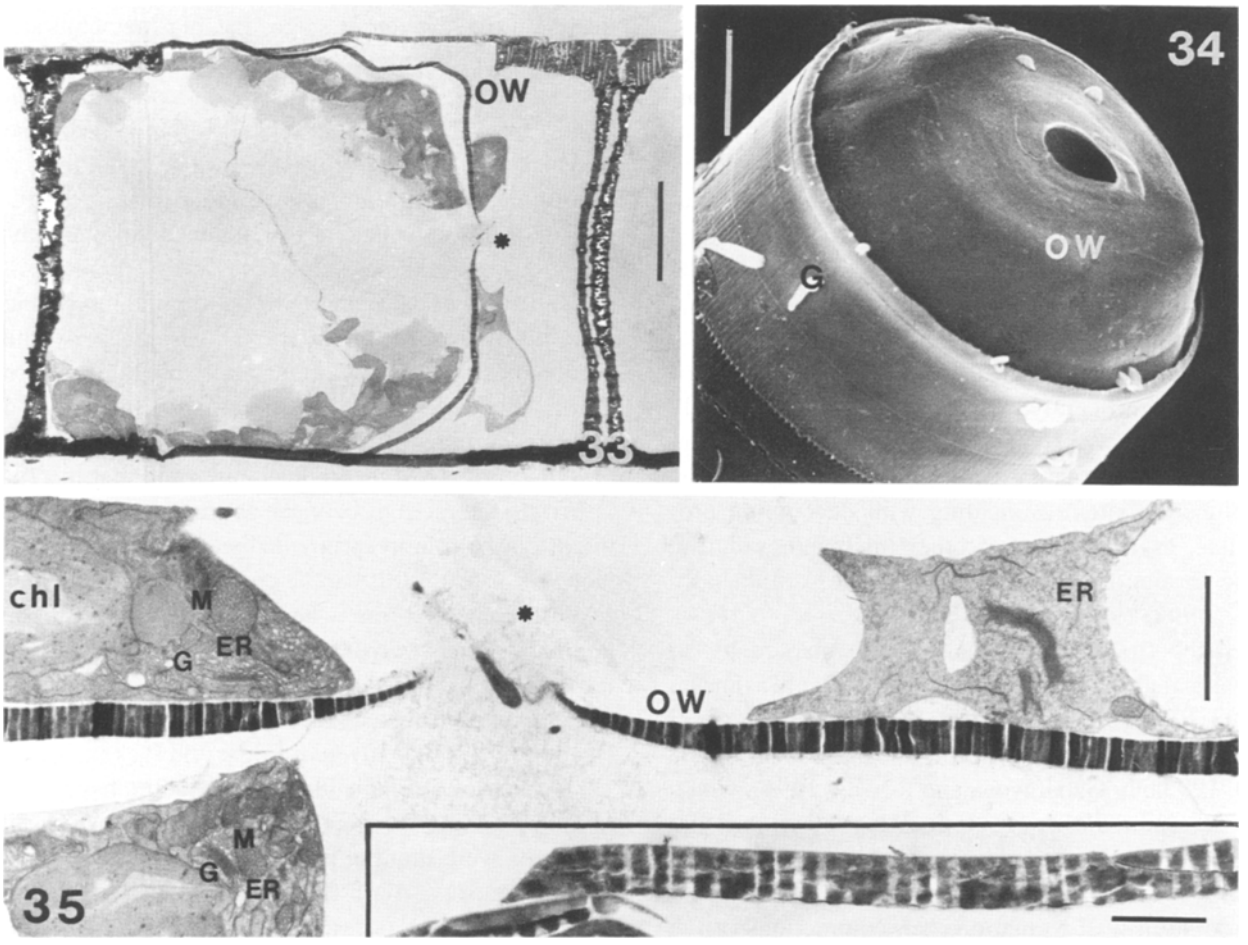
Fig. 32. Detail of a similar stage of raphe rib thickening, with all former transverse ribs and interspaces accompanied by ER-cisternae. Bar: 1 μm

between the membrane skeleton (fibrous lamina) and other cytoskeletal elements and, in turn, causes the SDV to fuse. This results in a silicification of the former interspaces. Compaction of the radial siliceous strings in *Coscinodiscus* (Schmid and Volcani 1983) occurs in the same way (Schmid 1984c, 1986a, b). Also, the unstructured central area in this genus (Figs. 16 and 17) precisely coincides with the area where the SDV is shielded during wall formation by the telophase nucleus (Schmid 1984c, 1986a, b), which makes a transport of spacer vesicles (see below) into this region impossible. A similar blocade results in the creation of the "central nodule" in raphe-bearing pennate diatoms. This central occlusion of the raphe slit coincides with the location of the MTOC and associated ER during wall formation (Pickett-Heaps et al. 1979, Edgar and Pickett-Heaps 1984, Pickett-Heaps et al. 1990, Schmid unpubl.). In *Hantzschia spectabilis* the presence of the MTOC at this site is very transient, and the raphe slit in this species runs without interruption from pole to pole (Edenhofner and Schmid, unpubl.). Also, the mode of silica deposition within the SDV at the side covered by the ER differs from that apposed to the cPL (Fig. 29) (Pickett-Heaps et al. 1979).

A further striking example for the role of the PL in patterning is the "oogonial wall" of *Ellerbeckia*, which is completely structureless (Figs. 33–35)

(Schmid and Crawford unpubl.), and which is formed inside the cytoplasm in a similar manner to the structureless cyst walls in chrysophytes (Preisig 1994), even with a similar function of the central pore (for the external cytoplasm to retract into the interior). Thus, silicification per se can clearly be uncoupled from pattern formation in wall elements, providing additional arguments against a pattern based on the solidification properties of silica (Gordon and Brodland 1990, Gordon and Drum 1994), and substantiation of the hypothesis, that patterns are created in close association with the plasmamembrane, i.e., the cell surface, in cooperation with radial elements of the cytoskeleton. In Synurophyceae the chloroplast-ER partly substitutes for the PL in moulding the scales (Schnepf and Deichgräber 1969, Brugerolle and Bricheux 1984, Preisig 1994), however, the patterned arrangement of scales at the cell surface still needs the involvement of the plasmalemma and its skeleton to build a fully integrated cell covering (Brugerolle and Bricheux 1984).

The role of the plasmalemma in exine patterning: local protrusions, or patterned adhesions to the callosic wall? For creation of pollen ornamentation too, the decisive role of the plasmalemma has now been accepted by many researchers, especially since it was impossible to find any particular organelles in the



Figs. 33–35. *Ellerbeckia arenaria*: structureless oogonial wall (*OW*) formed without contact with the plasmalemma. Ga/Os-simultaneous fixation (Schmid and Crawford unpubl.)

Fig. 33. Overview: section through forming oogonium with cytoplasm still outside the wall. TEM; asterisk marks same site in Fig. 35. Bar: 10 μm

Fig. 34. SEM image of structureless oogonium wall with central hole. Bar: 10 μm

Fig. 35. Detail of Fig. 33: structureless wall; blocks are sectioning artifacts due to the brittle silica. Cytoplasm outside the wall with Golgi-ER-mitochondrion-units, chloroplasts. Bar: 2 μm. **Inset** Detail of Fig. 33. Normal, patterned girdle-bands. Bar: 1 μm

cytoplasm associated with the sites of the forming extracellular structures (Dickinson and Heslop-Harrison 1968; Rowley and Skvarla 1975; Horner and Pearson 1978; Dickinson and Sheldon 1986, 1990; Skvarla and Rowley 1987; Takahashi 1989a, b, 1992, 1993, 1995b; Owens et al. 1990; Takahashi and Skvarla 1991a; Uehara and Kurita 1991; Scott 1994). Also, considering the chemical composition of the sequentially deposited material, it is generally assumed that cellulosic components are synthesized from enzyme complexes located in the plasmalemma, whereas other wall precursors may be delivered from the dictyosomes by vesicular activity to the PL and there be exocytosed (Figs. 9, 10, and 41) (Dickinson 1976, Dickinson and Heslop-Harrison 1968, Dickinson and Bell 1970, Cresti et al. 1992, Luegmayr 1994).

An example of a very localised activation of the PL is, in our opinion, provided with *Nuphar*, where the normal exinous spines develop directly on the plasmamembrane, and thrust into the callosic wall prior to the appearance of any other exinous structures (Takahashi 1992). In contrast to Takahashi's conclusion (1992), we interpret this situation as evidence for the involvement of the PL, such that the smooth PL area, surrounding the spine-producing domains, is either masked or not yet activated for deposition of primexine. Significantly, this PL area, as it eventually becomes involved in secreting the protectum, assumes an undulating profile in thin sections, its "protuberant sites" coinciding with developing probaculae, as found also in other microspores during exine formation (Figs. 36 a, 38, and 41) (Takahashi 1992, 1993, 1995a, b).

Often the first signs of patterns are marked by an "invaginated plasmamembrane" in profile (Takahashi 1989a, b, 1992, 1993, 1995a; Takahashi and Kouchi 1988; Takahashi and Skvarla 1991a; Pérez-Muñoz et al. 1993a, b; Gabarayeva and Rowley 1994), which were interpreted as being "reticulate protrusions" that correspond to the mature exine ornamentation (Takahashi 1993, 1995a). However, from what we know from diatom wall formation, i.e., contractions of the entire cell necessary to allow the growth of external spines or exit-tubes (*Ditylum*: Pickett-Heaps et al. 1988; *Corethron*: Crawford and Hinz 1995), and the importance of the adhesion of the PL to a surface (parent wall) for shape control, we are inclined to interpret the reported "protrusions" as being patterned sites of adhesions of the PL to the callosic wall. The areas between them would be retractions from this anchor surface (Figs. 9, 10, 36 a, 38, and 41). Thus we substantiate and elaborate an earlier view of Dickinson and Sheldon (1986), that the PL is anchored at the callosic wall at certain sites, which in some species may be characterised by "transient tubular accumulations" (cf. Fig. 9), while in others these characteristic structures may be absent from the adhesion zones, or have been overlooked (Dickinson and Sheldon 1986). In our opinion, the micrograph providing the clearest evidence for this modern concept is Dickinson's (1976: fig. 1 B), showing patterned adhesion of the PL to the callosic wall in *Pinus*, although at that time it was interpreted in a different way. Significantly, in *Ceratophyllum demersum*, where a callosic wall is not synthesized and exine initiation therefore does not occur during the tetrad stage, the plasmamembrane shows no conformational changes, i.e., there are no "protrusions". The view, that the callose wall might be important for exine patterning is shared now also by Takahashi (1995b). He re-investigated wall formation in *Lilium* and found a localised retreat of the PL from the callose while it was in contact with it at other regions. He regarded this as the first morphological indication of exine pattern formation and suggested that this mosaic differentiation takes place by the involvement of "microfilaments", lying subjacent to the invaginated PL and illustrated with the aid of a high resolution SEM (Takahashi 1995b). Tiwari (1989), using TEM techniques, proposed that in *Tradescantia* interactions between actin filaments and PL might play a role in (prim-) exine pattern formation. How far, if at all, this can be correlated with the situation in some diatoms, where the PL and its fibrous lamina interacts with the fibrous dense domains in forming the patterned growth of the SDV (see above) is not known. In pennate diatoms (*Pinnularia*: Pickett-Heaps et al. 1979; *Navicula*: Edgar and Pickett-Heaps 1984) bundles of filaments, probably actin, have been found parallel to the edge of the growing SDV. These filaments were thought to be involved in the expansion of the SDV. Actin filaments play the major role in the moulding process of scale vesicles in chrysophytes (Brugerolle and Brichieux 1984), and are functionally important for diatoms in transporting spacer vesicles to their destination (Figs. 18 and 19) (see below).

In *Lilium*, the regularly retracted regions of the PL correspond to the future lumina of the exine reticulum, and the spaces between the PL and the callose

wall are filled at this early stage with fibrous material (Dickinson and Sheldon 1986, Takahashi 1995b). Fibrous threads would accumulate and get entangled at the "protuberant sites" [i.e., at sites of adhesion to the callose wall and probably comparable to the zones where the "tubular accumulations" sensu Dickinson and Sheldon (1986) adhere the PL to the callose (Fig. 9), and later to the primary fibrillar layer (Figs. 37 and 38)]. Granular material would precipitate onto these threads as in *Caesalpinia* (Takahashi 1993) and develop into the protectum. In *Canna* (Rowley and Skvarla 1975) and in *Artemisia* (Rowley and Dahl 1977) a mucopolysaccharide glycocalyx is reported to be formed as the first precursor for the outer tectum. These precursors subsequently differentiate the probaculae between protectum and the "protruded" sites of the PL (Figs. 36 a, 37, and 38). We interpret the stage presented in Pérez-Muñoz et al. (1993a: fig. 12) showing the same situation in a particularly clear way, because the cell had shrunk during fixation and dehydration. The specific anchor sites of the cell membrane *and* the protectum to the callose wall are very prominent, exhibiting concave surfaces between them. The growth of the probaculae then pushes also the PL at their bases away from the callose wall and the primary fibrillar layer (Figs. 36 a, 38, and 41), permitting the PL to re-assume a more even profile (Dickinson and Sheldon 1986).

To create the exine pattern specific for a certain taxon, the adhesion pattern must be species-specific as it is in diatoms where it is controlled also by other factors, such as "sex-hormones", since adhesion is not effected during differentiation of male and female cells (Fig. 1 A–C) (*Odontella*: Mayer and Schmid 1995), or already existing adhesions are dissolved in the process of spermiogenesis (*Coscinodiscus*: Schmid 1994c).

The pattern generation of Thalassiosira as a model for comparison with angiosperm pollen with tectate exine. Morphogenesis of the patterned wall in *Thalassiosira* (Schmid and Schulz 1979; Schmid 1984a, b) serves as a paradigm for the importance of the cPL and its skeleton in pattern formation, and in our opinion provides the best example for comparison with patterned pollen wall formation, but on a different cytoplasmic level, i.e., the callosic side of the PL in pollen (Fig. 36 a) and the cytoplasmic side of the PL in diatoms (Fig. 36 b). In *Thalassiosira*, wall chambers are created without the involvement of spacer vesicles, possible due to the chamber architecture in combination with the direction of SDV-growth, which

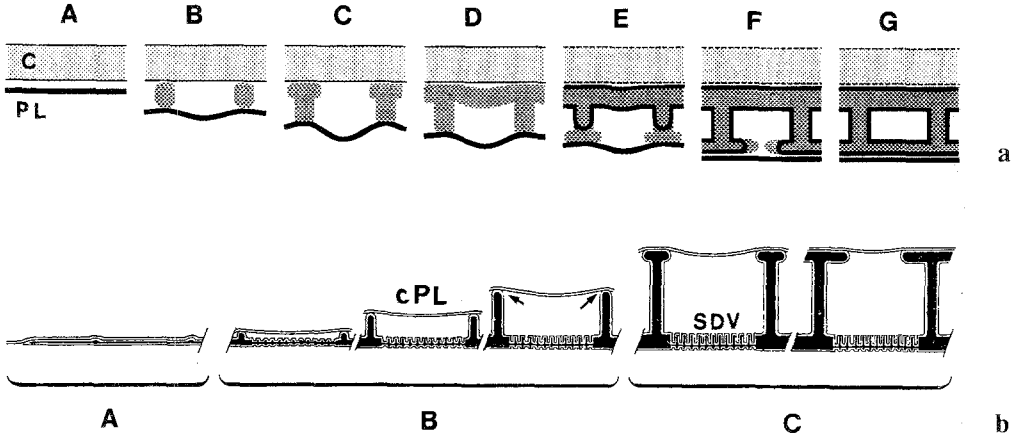


Fig. 36 a, b. Diagrammatic comparison of pollen and diatom wall formation. **a** Schematic summary of tectate ectexine formation of some angiosperm pollen at the tetrad-stage. A Smooth plasmalemma (*PL*) during callose (*C*) synthesis. *B–D* Patterned "undulation" of the PL; formation of probaculae (dotted columns) at the former adhesion sites of the PL to the callose. Formation of protectum by lateral accumulation of primexine along the callose. *E* and *F* Formation of the footlayer by secretion of primexine along the PL; begin of impregnation with sporopollenin (indicated as dark line around the dotted area). *F* and *G* Maturation of ectexine by completion of footlayer and impregnation with sporopollenin; smoothening of PL; start of callose degradation (interrupted lines). **b** *Thalassiosira eccentrica* (based on Schmid and Schulz 1979; Schmid 1984b). Formation of chamber pattern without involvement of spacer vesicles. *A* Radial expansion of siliceous meshwork within the SDV, along the cPL. *B* Differentiation of pseudopore-plates and chamber side walls. Addition of material at the cPL side (arrows). *C* Second horizontal differentiation: formation of the outer layer

guarantees that the growth-region of the SDV is in close contact with the cPL at all stages of development (Fig. 36 b). Figure 36 b first summarizes the formation of the base layer, the expansion of radial ribs forming a siliceous reticulum within the SDV. The pseudo-poreplates are then set into the meshes, and the side walls differentiate, forming hexagonal chambers. Addition of wall material by vesicular activity takes place only at the sites where the SDV is tightly adhered to the internal surface of the cPL (Fig. 36 b), thus pushing the solidifying base layer away from the cPL. Secretion of swelling mucous substances, visible when fixed with Alcian Blue added to the glutaraldehyde (Schmid 1984b), would substitute in this case for spacer vesicles (see below), since the base layer is a closed plate in *Thalassiosira* and separates the cytoplasm from the cPL. This situation is perhaps analogous to the patterned periplasmic space between the PL and the callose wall in some angiosperm microspore walls with a tectate exine (Fig. 36 a). As in diatoms, material is added at the PL-side, enlarging the gap between the PL and the callosic wall. The hexagonal pattern for the chamber side walls in diatoms must be the result of the localised interaction between cPL and its skeleton with the distal membrane of the SDV; while the reticulate patterns of pollen exines may be the result of the localised adhesion of the PL to the callose wall. In diatoms, the second lateral expansion of the SDV along the cPL leaves either round openings in the outer wall (Fig. 36 b), or differentiates a real pore plate instead of the opening (*Planktoniella sol*; Schmid unpubl.). The analogous stage in pollen wall formation is the secretion of the foot-layer (Fig. 36 a).

[A complication specific for the *Thalassiosira* family, but not relevant for comparison with pollen wall formation is the fact, that each pore of the pseudo poreplates is bridged by a silicified plate and the SDV is already continuous over the whole new surface except for the sites where the future portulae are formed. Thus material is transported through these portulae and via the SDV-margin (Schmid 1984a).]

The primary pattern – auxiliary mechanisms

Diatoms. In the centric diatom *Coscinodiscus*, the primary pattern of the cell wall is laid out in advance by a cytoplasmic blue-print consisting of spacer vesicles and endoplasmic reticulum attached to the cPL (cf. Figs. 16 and 17) (Schmid 1984c, 1986a, b, 1987a, b, 1994a; Pickett-Heaps et al. 1990). The growing SDV was shown to be then sandwiched between the cPL and the ER which gives way to the SDV, and remains attached to its internal face until valve release. A homogeneous lateral expansion of the SDV is clearly inhibited by the presence of the immovable spacer vesicles, spacing out the internal openings of the chambers. Vertical growth is accomplished by addition of small Golgi vesicles to the SDV, again at the sites where it attaches to the cPL, i.e., direction of wall thickening is here also from proximal to distal. Golgi-origin of vesicles could be determined, since in this genus (and in *Ellerbeckia*: Fig. 35) the dictyosomes do not form a perinuclear shell as in most diatoms, but are, together with a mitochondrion and an ER-cisterna, interposed between them, distributed throughout the cytoplasm and thus also in the close vicinity of the new forming wall (Schmid 1984c, 1988). Vesicles may be simply contributing membrane to the expanding SDV and silicon enters the SDV by passing across its membrane in soluble form. Conversely, it is also possible that silicon is aquired by the vesicles as soon as they leave the nucleus-associated Golgi stack in more conventional diatoms (Schmid and Schulz 1979).

Modelling of the chambered wall is eventually the result of a complex interaction of adhesion and disconnection of spacer vesicles and SDV to and from the cPL. Unlike the situation in *Thalassiosira*, where the mechanism is not clear, in *Coscinodiscus* the height of the chambers, and thus the wall thickness, is determined by how long the spacer vesicles adhere to the cPL while the SDV is growing vertically. As soon as they disconnect, the SDV expands again laterally along the cPL and forms the outer layer of the chambers. Once more, there is competition for the adhesion sites in the cPL and its fibrous lamina, this time between the SDV and small ER-vesicles, that results in a perforated outer wall. A premature disconnection of the spacer vesicles (e.g., under experimental turgor increase) leads to a lack of chamber sidewalls, thus to thin flexible walls that cannot perform the required mechanical functions (see above). The key function of the cPL and its associated skeleton in the patterning process is evidenced by the fact that only the plasmalemma involved in wall formation is smooth in this genus (Figs. 2 and 3), whereas the other part of the PL is ruffled into microplicae (Figs. 2 and 4–8) (Schmid 1987a, b, 1994a).

Spacer vesicles have also been found in several pennate diatoms, *Pinnularia* sp., *Catacombas gaillonii*,

Nitzschia sigmoidea, and *Achnanthes longipes*, and could in these species be traced back to the Golgi apparatus (Schmid 1994a, and unpubl.). Spacer vesicles may be present in all cases where wall thickening occurs at least partly form the outside to the inside (e.g., *Pinnularia, Catacombas*, Figs. 23, 26, and 27), or, when SDV-growth is similar as in *Coscinodiscus*, with the cytoplasm filling the chambers during formation. Whether these vesicles are purely physical spacers or provide chemical signals in addition is not known. In the pennate *Pinnularia* the spacer vesicles are identical in ultrastructure and origin with the polysaccharide vesicles used for locomotion (cf. Figs. 15, 18, and 19), and are replaced by mitochondria at a later stage (Figs. 21, 26–28, 30, and 31) (Schmid 1994a). The presence of mitochondria between the transverse ribs was discovered first by Pickett-Heaps et al. (1979), but that they follow the spacer vesicles, was revealed only during re-investigation of *Pinnularia*, as was the observation that the SDV for the outer wall is already attached to the cPL at this early stage, and the mitochondria, in turn, are adhered to its internal face (Figs. 21 and 26) (Schmid 1994a). Since this outer part of the SDV is silicified and molded last, i.e., after the internal plate has been formed (Figs. 27–29), a mechanism preventing silicification must exist. Whether mitochondria play a role in this process is not known. On the other hand, mitochondria appear to function as "primary spacers" in the formation of raphe canals in *Nitzschia, Hantzschia,* and *Surirella*, pennate diatoms which have their locomotion slit, the raphe, raised on a keel (Pickett-Heaps et al. 1990, Schmid 1994a). Again, they are held in in the tight grip of a fibrous skeleton (Fig. 22), this time involved in raphe formation. And they also act in scale-forming protists as modelling organelles (Preisig 1994). Conversely, mitochondria could function during wall formation in providing energy for morphogenesis and perhaps silica uptake, and thus their localised arrangement could reflect an optimal strategic position.

Unlike higher plant cells, diatoms possess a centralised cytoskeleton, i.e., radial cytoplasmic MTs focusing in the nucleus associated interphase-MC (MTOC: Pickett-Heaps et al. 1990), and actin filaments are closely aligned (Schmid unpubl.). The cytoplasmic architecture is dependent on this intact cytoskeleton. Its depolymerisation during mitosis results in random distribution of subcellular organelles, and the species-specific interphase architecture becomes established as the new cytoskeleton radiates out again (cf. Schmid 1994a: figs 14 and 15). During formation of the blue-print pattern in *Pinnularia*, filaments (probably actin) have been found involved in the transport of spacer vesicles (Figs. 18 and 19) (Schmid unpubl.), which become stabilised by the fibrous lamina. The filament bundles lining the edges of the new SDV in another *Pinnularia,* which were interpreted to be involved in the SDVs expansion (Pickett-Heaps et al. 1979), could have a similar function. During interphase, the ER appears dependent either on MTs or MT-dependent actin filaments (Schmid 1994a), whereas during pre-pattern formation only filaments have been found so far (Figs. 18 and 19; and unpubl.). In a later stage in *Coscinodiscus* when the tubular SDV with the silicifying ribs reaches the margin, MTs run parallel to each rib, next to the ER (identified with immunofluorescence and EM studies; Schmid unpubl.). In pennate diatoms, MTs mainly lie next to the middle rib during wall formation (Pickett-Heaps et al. 1979, Edgar and Pickett-Heaps 1984), regardless of whether this rib is penetrated by a raphe slit or not (Pickett-Heaps et al. 1990). In the needle-shaped *Synedra ulna* and *Nitzschia sigmoidea*, the MTs, during valve formation, form bundles which are stabilised by cross bridges (Schmid 1994a, and unpubl.). The evolution of these cross bridges may have been the prerequisite for the evolution of pennate diatoms. Aside from their function as transport rails for wall and adhesive material, the role of MTs in diatom wall formation may be rather a stabilizing one (see also below), whereas (actin) filaments and their associated proteins, in cooperation with the cPL and its skeleton, appear to play a key role in the patterning process itself. In synurophytes, actin filaments as well as MTs are involved in scale modelling (Schnepf and Deichgräber 1969, Brugerolle and Bricheux 1984); but while MTs seem responsible primarily for transport of the primary vesicles to the chloroplast, and stabilisation of the scale vesicle's margin, actin filaments cover this vesicle entirely during the moulding process and connect it to the chloroplast envelope, indicating a structural role in maintaining the vesicle membrane in place (Brugerolle and Bricheux 1984). This coincides with the situation in some diatoms (e.g., *Pinnularia*: Figs. 21 and 26) (Schmid 1994a).

The role of the ER for the diatom wall and chrysophyte-scale formation is not clear. In diatoms, the ER occurs in a patterned array during the entire morphogenetic event (Pickett-Heaps et al. 1979; Schmid

1984c, 1987a, b, 1994a). It might be involved in the formation of an organic template onto which silica precipitates in the SDV (Pickett-Heaps et al. 1979, Schmid 1994a); or in protein formation for the fibrous lamina of the cPL; or may play a role in Ca^{++} regulation for assisting either polymerisation of silica (McConnaughey 1989) or membrane fusion; or may have another, yet unknown function.

Microspores. If our concept is correct, then auxiliary spacers are not required in patterning the pollen-exine (cf. Fig. 36 a, b). However, the early view of Heslop-Harrison (1963), that the location of the probaculae may be correlated with the previous disposition of the endoplasmic reticulum in the young microspore, was discussed as a possibility by Buchen and Sievers (1981) and Dickinson and Sheldon (1990), and re-emphasized by Pérez-Muñoz et al. (1993a, b), who clearly state that ER is involved in pollen wall patterning (i.e., the primary pattern). However, in none of the examples used for documentation is the relationship as convincing as it is for the pollen aperture location (Figs. 37 and 41), or for the involvement in diatom wall formation (see above). Thus a high degree of uncertainty still exists concerning involvement of subcellular organelles in the creation of the primary pattern in pollen.

The aperture and portulae patterns (secondary patterns)

The aperture pattern and the role of the spindle and the ER

The final geometry of the microspore including the arrangement of apertures is determined by the orientation of the meiotic spindle microtubules (Heslop-Harrison 1971a, Buchen and Sievers 1981, Dickinson and Sheldon 1986). Aperture location is disturbed by centrifugation and colchicine treatment and, depending on the stage of application, apertures are either absent or appear with random orientation and location (Heslop-Harrison 1971a; Buchen and Sievers 1981; Sheldon and Dickinson 1983, 1986). After this MT-dependent layout of the future apertures they are spaced out in the primexine wall by a regularly arranged endoplasmic reticulum throughout the tetrad stage (Figs. 37 and 41) (Heslop-Harrison 1963). MTs radiating from the nuclear region (Dickinson and Sheldon 1990) could be involved in locating this ER. The PL forms a "flattened eminence" supported by this ER, which forms either sheets (Fig. 41) (Heslop Harrison 1963, Horner and Pearson 1978) or discontinuous cisternae apposed to the PL (Fig. 37) (Dickinson and Potter 1976, Takahashi 1989, Luegmayr 1994, Ubera et al.1996, Frosch unpubl.). These ER cisternae possibly block vesicle transport to this site and exocytosis (Fig. 41) (Heslop-Harrison 1963, Horner and Pearson 1978, Buchen and Sievers 1981), such that no wall material is laid down over these areas, and primexine is thin (Luegmayr 1994) or absent (Shivanna and Johri 1985). Therefore, this area lies closer to the callose wall (Figs. 37 and 41). Aperture positioning can also be determined by multiple layers of unidentified tripartite lamellae arranged parallel to the plasmamembrane by external or internal apposition (Figs. 39 and 40).

The portulae pattern and the role of the spindle and the cytoskeleton

Specific cytoskeletal organelles are involved in morphogenesis of the portulae pattern in diatoms, apparently under the control of the cell's MTOC-spindle-complex (Schmid 1994a, and unpubl.). This would seem to be logical, since portulae serve a variety of striking mechanical functions in the interphase cell, besides their role in physiology. In pennate diatoms, raphe ribs bordering the raphe slit serve as the main stabilizing elements of the elongated wall and as anchor-zones for the cytoplasm. In centric diatoms,

---

Figs. 37–40. Angiosperm microspores in early tetrad stage (callose stage = C). Ga/pFa/Os/KFeCN-fix., TEM

Figs. 37 and **38.** *Monophyllaea horsfieldii* (Gesneriaceae) (modified from Luegmair 1994, with permission). Bar: 1 μm

Fig. 37. ER-profiles in cross-section (arrow) mark the future aperture. Primexine matrix (asterisks) is very thin in this region. Modified Thiéry-PATAg-reaction

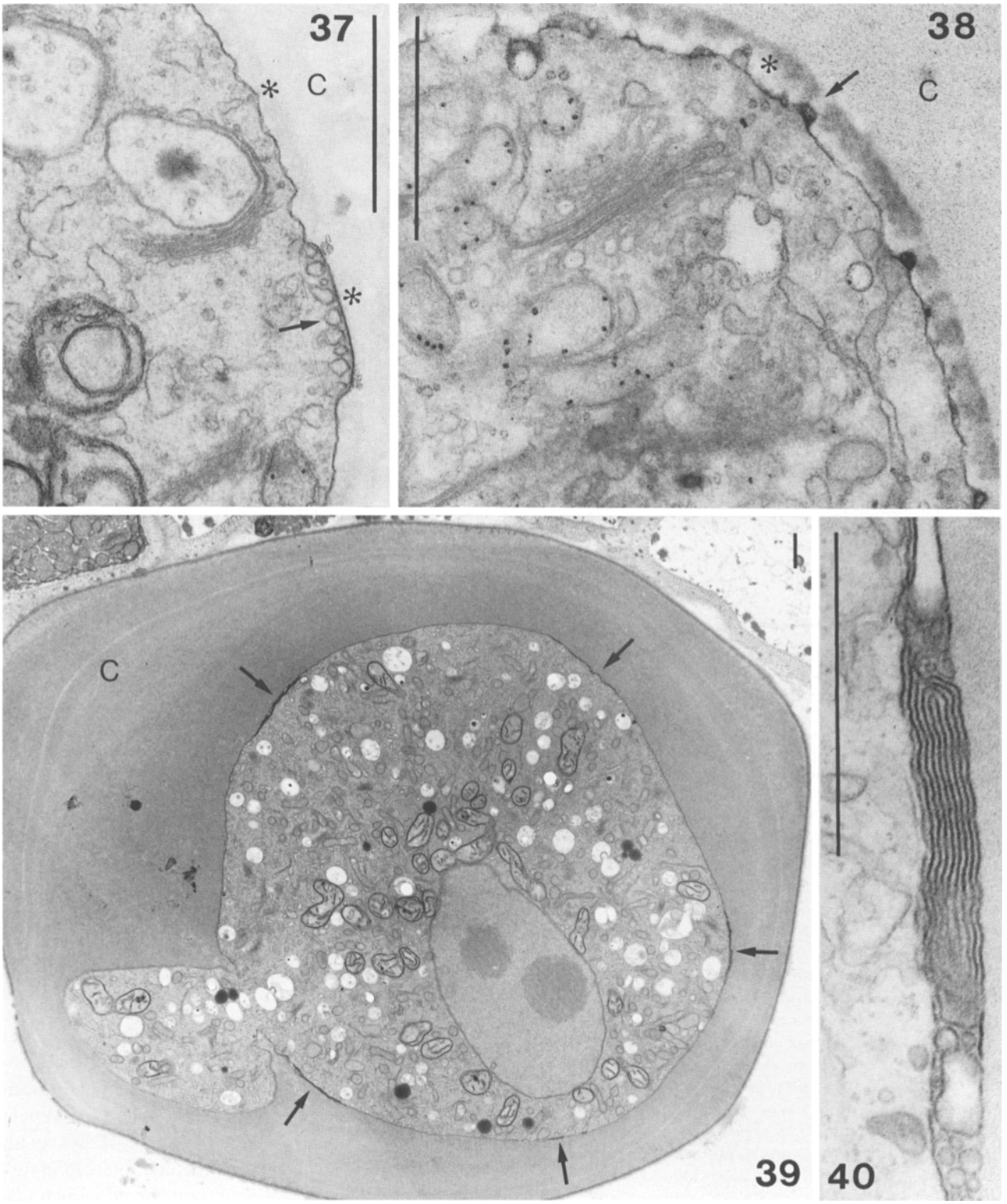
Fig. 38. Probacula (arrow) under the primexine matrix (asterisks) separating the plasmamembrane from the callose (*C*)

Figs. 39 and **40.** *Rosmarinus officinalis* (Lamiaceae) (courtesy of Dr. J.L. Ubera-Jimenez). Bar: 1 μm

Fig. 39. Slightly oblique section of a microspore within callose; left, another member of the tetrad; five sites (arrows) of folded membranous lamellae (ER?) marking the future apertures

Fig. 40. Detail of Fig. 39: membranous lamellae (ER?)

portulae form tubular wall projections to the cell interior, i.e., rimoportulae (labiate processes) and fultoportulae (strutted processes), which (1) provide permanent attachment sites for adhering the turgid protoplast to wall shapes deviating from hemispherical surfaces (Figs. 2 and 6) (Schmid 1994a: figs. 2–4); (2) they help to center the interphase nucleus and to anchor it via cytoplasmic strands and its cytoskeleton



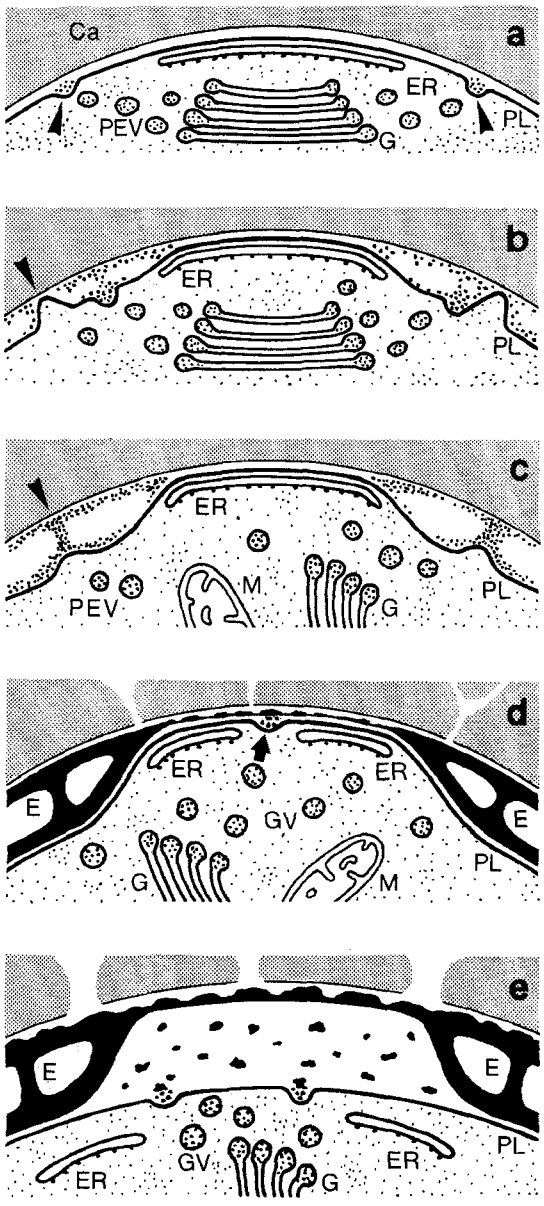


Fig. 41. Schematic summary of aperture formation in a tectate pollen exine. Role of the endoplasmic reticulum (*ER*) in preventing exocytosis (modified after Heslop-Harrison 1963). **a** Cell after meiotic division. Smooth PL below callose (*Ca*); ER-cisterna aligned with the PL at the future site of aperture. *PEV* Golgi vesicles with primexine matrix, exocytosed at specific sites of the PL; vesicle fusion inhibited at the region where the PL is shielded by the ER. **b** Later stage in development; localised adhesions of the PL to the callose (arrowhead); exocytosis of Golgi vesicles except in the ER-covered area. External accumulation of material causes retreat of the PL from the callose. **c** Later stage. Formation of probaculae at the former sites of PL-adhesion to callose, and protectum; flattened eminence where ER shields PL. **d** Ectexine almost mature; ER-cisternae start to disconnect from the PL in aperture region allowing vesicle fusion and exocytosis of wall material (arrow). Callose begins to degrade (cracks in shaded area). **e** ER shield has disappeared; deposition of exine material in the aperture area

through the plasmalemma to the rigid wall. Anchorage is secured by an adhesive glycocalyx, and the two thecae are thus connected in a controlled fashion (Schmid 1994a: figs. 2–4, 10 and 11, Franz and Schmid 1994, Mayer and Schmid 1995); (3) anchorage allows the generation of tensile forces necessary for normal cleavage, spermiogenesis, and initial cell formation (Figs. 1 A, D, E and 11) [if the protoplast becomes disconnected from the wall the diatom cell is unable to divide (for higher plants, see Schindler 1993; Reuzeau and Pont-Lezica 1995). Further, auxospore-walls are probably not sufficient as abuttment for the cleavage furrow, such that the first two thecae formed within the auxospore cover are each formed after an acytokinetic mitosis]; (4) portulae provide spatial cues for intracellular orientations, necessary for example in nuclear migration during cell division and for sex determination (Schmid 1994a, c). When cells are plasmolysed or contracted, protoplasts always remain adhered to the portulae (Schmid 1987b, 1994a, c).

The hypothesis proposing that the MT-skeleton influences location and orientation of the portulae is testable with drugs interfering with microtubule activity (Schmid 1980, 1984a, 1986a, 1994a). The following cytoskeletal structures involved in positioning and shaping of the portulae arise after SDV initiation in all cases investigated.

The raphe fiber: The raphe fiber is an electron dense fibrillar structure with undefined substructure apparently dependent on the MTOC and responsible for keeping the raphe fissure open during formation of the raphe ribs (Pickett-Heaps et al. 1979, Edgar and Pickett-Heaps 1984, Schmid unpubl.). The entire fiber is stabilized by cortical MTs (e.g., Pickett-Heaps et al. 1979), and exposure to anti-MT drugs during morphogenesis causes fragmentation of the raphe fissure (Schmid 1980).

The labiate process apparatus: This is a fibrous structure involved in the formation of the rimoportulae (labiate processes; Schnepf et al. 1980, Li and Volcani 1985, Pickett-Heaps et al. 1988) and to date undefined in most species. Anti-MT drugs applied during wall formation cause misshaping, dislocation and clustering (Schmid 1986a, b, 1987a, b, 1994a). In *Ditylum*, a dense, ill defined material extends from the spindle pole towards the SDV, where the labiate process apparatus is formed (Pickett-Heaps et al. 1988). In *Coscinodiscus* the structure involved in formation of the rimoportulae resembles ultrastructural-

ly the MTOC-spindle-precursor complex of this cell, only on a smaller scale, and both resemble a rhizoplast (i.e., striated flagellar root; Schmid 1994a). In *Coscinodiscus*, the rudiments of rimoportulae are already present in the auxospore wall, distinguishable from the normal wall-scales by their larger size and the clear slit in their centre (Schmid 1994b, c).

The strutted process apparatus: This is another dense fibrillar structure that is involved in the formation of fultoportulae (strutted processes; Schmid and Schulz 1979, Schmid 1986a), i.e., anchor-handles restricted to the *Thalassiosira* family and are otherwise involved in chitin fibril formation (Herth and Schnepf 1982) and likewise disturbed during their formation with MT inhibitors (Schmid 1984a).

No diatotepum (organic sheet) is present across the portulae in centric diatoms or the raphe in pennate diatoms.

Concluding remarks

With our study we have tried to document the current level of understanding in pattern formation in two distant groups of plant cells, pollen and diatoms. With this conglomerate of facts, circumstantial evidence, interpretations, and speculation, we begin to see a picture, that demonstrates the complexity and dynamics of an orchestrated interaction between, on the one hand, the plasmalemma with its associated fibrous lamina and on the other a coherent network of cytoskeleton, where temporal events are translated into structure and pattern. Although there are great differences in the application of cytoplasmic tools and mechanisms for the patterning process, based mainly on the differences in the chemical composition of the material used for wall formation, there are surprising resemblances. By comparing these two cell systems, we offer the possibility that adhesion and retraction, as one of the key mechanisms for morphogenesis in diatoms, may play a similar crucial role in the patterning of pollen walls. It appears that in both cell types species-specific physical connections exist between the previously deposited wall (diatom parent wall/callose wall) and the plasmalemma, along which the new wall is formed as a lightweight construction. The principle of the "chainline", a tension-model for optimising compression-stability of bows, vaults and cupulas, which was discovered by architects in the Baroque period (Otto 1982), is realised on a micro-scale in the walls of many diatoms (Schmid 1984c, 1987b; Steucek and Schmid 1988), and might have been applied also in some pollen for stabilizing the wall.

Both cell types appear to inherit wall-design and material for their initial pattern from the maternal cytoplasm (in *Attheya*, embryonic SDVs for both future cells are already present in anaphase: Schnepf et al. 1980; the same was found for *Ditylum* by Pickett-Heaps et al. 1988) – a selective advantage perhaps, which might have persisted over evolutionary time, ensuring protection of the new protoplasts until the young progeny cells have their cytoplasm reorganized after the break down of the cytoskeleton during mitosis (Schmid 1994a) and meiosis (Sheldon and Dickinson 1986). Aperture and portulae pattern on the other hand, are clearly dependent on the intact cytoskeleton, which is polymerised only after cytokinesis is complete, and this may be the cause for the delay in their appearance during morphogenesis and their susceptibility to MT-inhibitors (e.g., Schmid 1980, 1986a). Microtubule action in turn, might imply vigorous dynamics, exerting visible effects on the primary pattern already present in the vicinity of apertures (Dickinson and Sheldon 1990) and that of portulae. The forces acting during formation of the rimoportulae in *Odontella regia*, a bipolar centric diatom (Fig. 1), are so dynamic, that all valves become diagonally distorted during morphogenesis (Mayer and Schmid 1995).

Positioning of the portulae pattern in diatoms requires much greater accuracy than that of the apertures, because the latter are formed for single use only, whereas the portulae, as the permanent anchor sites of the diatom protoplast to its wall, must provide precise spatial information for the cytoskeleton for the next mitotic division, i.e., for the next spindle, and for positioning the next set of portulae. This "structural memory" system appears to provide a kind of inheritance which is not yet understood, and to unravel how it started in diatoms we must go back to auxospores. Scientific development will hopefully provide us with a firm basis of future research on this speculative issue.

Acknowledgements

MH would like to thank Dr. E. Luegmayr for providing Figs. 37 and 38, Dr. J. L. Ubera-Jimenez for providing Figs. 39 and 40, Doz. M. Weber for valuable discussion, and Dr. H. Halbritter for darkroom assistance. AMS wishes to thank Prof. H. Kauss for permission to use his unpublished manuscript, Prof. J. Pickett-Heaps for the gift of a video-copy of his unpublished video-disc on diatoms, and Drs. G. Obermeyer and R. Benkert for useful comments on various aspects of pollen physiology. The correction of the English by Dr. R. M. Crawford and the helpful advice by the anonymous reviewers are greatly appreciated.

References

Barnes SH, Blackmore S (1986) Some functional features in pollen development. In: Blackmore S, Ferguson IK (eds) Pollen and spores: form and function. Academic Press, London, pp. 71–80 (Linnean Society symposium series, vol 12)

Bhandari NN (1984) The microsporangium. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin Heidelberg New York Tokyo, pp 53–121

Blackmore S (1990) Sporoderm homologies and morphogenesis of *Echinops sphaerocephala* (Compositae). Plant Syst Evol Suppl 5: 1–12

– Barnes SH (1987) Pollen wall morphogenesis in *Tragopogon porrifolius* (Compositae: Lactuceae) and its taxonomic significance. Rev Palaeobot Palynol 52: 233–246

– – (1988) Pollen ontogeny in *Catananche caerulea* L. (Compositae: Lactuceae) I. Premeiotic phase to establishment of tetrads. Ann Bot 62: 605–614

– – (eds) (1991) Pollen and spores: patterns of diversification. Clarendon Press, Oxford, pp. 1–387 (The Systematics Association special volume 44)

– Ferguson IK (eds) (1986) Pollen and spores: form and function. Academic Press, London (Linnean Society symposium series, vol 12)

– Knox RB (eds) (1990) Microspores: evolution and ontogeny. Academic Press, London

– McConchie CA, Knox RB (1987) Phylogenetic analysis of the male ontogenetic program in aquatic and terrestrial monocotyledons. Cladistics 3: 333–347

Brown RC, Lemmon BE (1987) Involvement of callose in determination of exine patterning in three hepatics of the subclass Jungermanniidae. Mem N Y Bot Garden 45: 11–121

– – (1992) Control of division plane in normal and griseofulvin-treated microsporocytes of *Magnolia*. J Cell Sci 103: 1031–1038

Brugerolle G, Bricheux G (1984) Actin microfilaments are involved in scale formation of the chrysomonad cell *Synura*. Protoplasma 123: 203–212

Buchen B, Sievers A (1981) Sporogenesis and pollen grain formation. In: Kiermayer O (ed) Cytomorphogenesis in plants. Springer, Wien New York, pp 349–376 (Alfert M et al (eds) Cell biology monographs, vol 8]

Crawford, RM, Hinz F (1995) The spines of the centric diatom *Corethron criophilum*: light microscopy of vegetative cell division. Eur J Phycol 30: 95–105

Cresti M, Blackmore S, Went JL van (1992) Atlas of sexual reproduction in flowering plants. Springer, Berlin Heidelberg New York Tokyo, pp 1–249

Dickinson HG (1970) Ultrastructural aspects of primexine formation in the microspore tetrad of *Lilium longiflorum*. Cytobiologie 1: 437–449

– (1976) Common factors in exine deposition. In: Ferguson IK, Muller J (eds) The evolutionary significance of the exine. Academic Press, London, pp 27–38 (Linnean Society symposium series, vol 1)

– Bell PR (1970) The development of the sacci during pollen formation in *Pinus banksiana*. Grana 10: 101–108

– Heslop-Harrison J (1977) Ribosomes, membranes and organelles during meiosis in angiosperms. Philos Trans R Soc Lond Biol 277: 327–342

– – (1968) Common mode of deposition for the sporopollenin of sexine and nexine. Nature 220: 926–927

– Potter UJ (1976) The development of patterning in the alveolar sexine of *Cosmos bipinnatus*. New Phytol 76: 543–550

– Sheldon JM (1984) A radial system of microtubules extending between the nuclear envelope and the plasma membrane during early male haplophase in flowering plants. Planta 161: 86–90

– – (1986) The generation of patterning at the plasma membrane of the young microspores in *Lilium*. In: Blackmore S, Ferguson IK (eds) Pollen and spores: form and function. Academic Press. London, pp 1–17 (Linnean Society symposium series, vol 12)

– – (1990) The cell biological basis of exine formation in *Lilium* sp. In: Blanca G, Diaz de la Guardia C, Fernandez MC, Garrido M, Rodriguez-Garcia MI, Romero Garcia AT (eds) Polen, esporas y sus aplicaciones. VII Simposio de Palinologia, Granada, 1988. Universidad de Granada, Granada, pp 17–29

Drebes G (1974) Marines Phytoplankton. Eine Auswahl der Helgoländer Planktonalgen (Diatomeen, Peridineen). Thieme, Stuttgart

– (1976a, b) *Attheya decora* (Centrales). Vegetative Vermehrung und geschlechtliche Fortpflanzung. b/w 16mm cine films. Institut für Wissenschaftlichen Film, Göttingen

Edgar L, Pickett-Heaps JD (1984) Valve morphogenesis in the pennate diatom *Navicula cuspidata*. J Phycol 20: 47–61

Faegri K, Iversen J (1989) Textbook of pollen analysis, 4th edn. Wiley, Chichester

Feijó JA, Malhó R, Obermeyer G (1995) Ion dynamics and its possible role during in vitro germination and tube growth. Protoplasma 187: 155–167

Franz SM, Schmid AM (1984) Cell-cycle and phenotypes of *Biddulphiopsis titiana*. Diatom Res 9: 265–288

French FW, Hargraves PE (1985) Spore formation in the life cycles of the diatoms *Chaetoceros diadema* and *Leptocylindrus danicus*. J Phycol 21: 447–483

Gabarayeva NI, Rowley JR (1994) Exine development in *Nymphaea colorata* (Nymphaeaceae). Nord. J Bot 14: 671–691

Gersonde R, Harwood DM (1990) Lower cretaceous diatoms from ODP LEG 113 site 693 (Weddel Sea). Part 1: Vegetative cells. In: Barker PF, Kennett JP (eds) Proceedings of the ocean drilling program, scientific results, vol 113. Texas A&M University, College Station, TX, pp 403–425

Gilroy S, Trewavas A (1990) Signal sensing and signal transduction across the plasmamembrane. In: Larsson C, Moller IM (eds) The plant plasma membrane: structure, function and molecular biology. Springer, Berlin Heidelberg New York Tokyo, pp 203–232

Gordon R, Brodland GW (1990) On square holes in pennate diatoms. Diatom Res 5: 409–413

– Drum RW (1994) The chemical basis of diatom morphogenesis. Int Rev Cytol 150: 243–372

Graybosch RA, Palmer RG (1985a) Male sterility in soybean (*Glycine max*). I. Am J Bot 72: 1738–1750

– (1985b) Male sterility in soybean (*Glycine max*). II. Am J Bot 72: 1751–1764

Halbritter H, Hesse M (1993) Sulcus morphology in some monocot families. Grana 32: 87–99

Hay ED (1991) Cell biology of extracellular matrix. Plenum, New York

Herth W, Schnepf E (1982) Chitin-fibril formation in algae. In: Brown RM (ed) Cellulose and other natural products. Plenum, New York, pp 185–206

Heslop-Harrison J (1963) An ultrastructural study of pollen wall ontogeny in *Silene pendula*. Grana Palynol 4: 7–24

– (1971a) Wall pattern formation in angiosperm microsporogenesis. Symp Soc Exp Biol 25: 277–300
– (1971b) The pollen wall: structure and development. In: Heslop-Harrison J (ed) Pollen: development and physiology. Butterworth, London, pp 75–98
Hesse M (1991) Cytology and morphogenesis of pollen and spores. Prog Bot 52: 19–34
– (1995) Cytology and morphogenesis of pollen and spores. Prog Bot 56: 33–55
Horner HT, Pearson CB (1978) Pollen wall and aperture development in *Helianthus annus* (Compositae: Heliantheae). Am J Bot 65: 293–309
Hynes RO (1992) Integrins: versatility, modulation, and signalling in cell adhesion. Cell 69: 11–25
Ingber DE (1993) Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. J Cell Sci 104: 613–627
Kauss H (1996) Callose synthesis. In: Smallwood M, Knox P, Bowles DJ (eds) Membranes: specialized functions in plant cells. Bios Scientific Publishers, Oxford (in press)
Knox RB (1984) The pollen grain. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin Heidelberg New York Tokyo, pp 197–271
Lacalli TC (1981) Dissipative structures and morphogenetic pattern in unicellular algae. Philos Trans R Soc Lond Biol 294: 547–588
Li CW, Volcani BE (1985) Studies on the biochemistry and fine structure of silica shell formation in diatoms. VII. Morphogenesis of the cell wall in a centric diatom, *Ditylum brightwellii*. Protoplasma 124: 10–29
Luegmayr E (1994) Pollenmorphologische und pollenontogenetische Untersuchungen an ausgewählten Gesneriaceae. PhD thesis, Universität Wien, Vienna, Austria
McConnaughey T (1989) Biomineralization mechanisms. In: Crick RE (ed) Origin, evolution, and modern aspects of biomineralization in plants and animals. Plenum, New York, pp 57–73
Mann S (1986) Biomineralization in lower plants and animals – chemical perspectives. In: Leadbeater BSC, Riding R (eds) Biomineralization in lower plants and animals. Clarendon Press, Oxford, pp 39–54 (Systematics Association special volume 30)
Mayer C, Schmid AM (1995) Morphology, cell-cycle and growth rates of *Odontella regia*. Diatom Res 10: 299–320
Müller O (1906) Pleomorphismus, Auxosporen und Dauersporen bei Melosira-Arten. Jahrb Wiss Bot 43: 49–88
Muñoz CA, Webster BD, Jernstedt JA (1995) Spatial congruence between exine pattern, microtubules and endomembranes in *Vigna* pollen. Sex Plant Reprod 8: 147–151
Obermeyer G, Bentrup FW (1996) Developmental physiology: regulation of polar cell growth and morphogenesis. Prog Bot 57: 54–67
– Blatt MR (1995) Electrical properties of intact pollen grains of *Lilium longiflorum*: characteristics of the non-germinating pollen grain. J Exp Bot 46: 803–813
Oey JL, Schnepf E (1970) Über die Auslösung der Valvenbildung bei der Diatomee *Cyclotella cryptica*. Versuche mit Colchicin, Actinomycin-D, und Fluodesoxyuridin (FUDR). Arch Microbiol 17: 199–213
Otto F (1982) Natürliche Konstruktionen. Deutsche Verlagsanstalt, Stuttgart
Owens SJ, Sheldon, JM, Dickinson HG (1990) The microtubular cytoskeleton during pollen development. Plant Syst Evol Suppl 5: 31–37
Pacini E (1990) Harmomegathic characters of pteridophyta spores and spermatophyta pollen. Plant Syst Evol Suppl 5: 1–12
Pérez-Muñoz CA, Jernstedt JA, Webster BD (1993a) Pollen wall development in *Vigna vexillata* I. Characterization of wall layers. Am J Bot 80: 1183–1192
– – – (1993b) Pollen wall development in *Vigna vexillata* II. Ultrastructural studies. Am J Bot 80: 1193–1202
Pickett-Heaps JD (1991) Post-mitotic cellular reorganisation in the diatom *Cymatopleura solea*: the role of microtubules and the microtubule centre. Cell Motil Cytoskeleton 18: 279–292
– (1996) Cell division in the centric diatoms *Odontella* (cf. *mobiliensis*) & *O. sinensis*. In: Pickett-Heaps JD, Pickett-Heaps J (eds) Diatoms: Cell division, motility and sexual reproduction. Video-disc. Cytographics Production, Sinauer, Sunderland, MA (in press)
– Tippit DH, Andreozzi JA (1979) Cell division in the pennate diatom *Pinnularia*. IV. Valve morphogenesis. Biol Cell 35: 199–206
– Wetherbee R, Hill DRA (1988) Cell division and morphogenesis of the labiate process in the centric diatom *Ditylum brightwellii*. Protoplasma 143: 139–149
– Schmid AM, Edgar L (1990) The cell biology of diatom valve formation. Prog Phycol Res 7: 1–168
Porter EK, Parry D, Dickinson HG (1983) Changes in poly(A)$^+$ RNA during male meiosis in *Lilium*. J Cell Sci 62: 177–186
Preisig HR (1994) Siliceous structures and silicification in flagellated protists. Protoplasma 181: 29–42
– Anderson OR, Corliss JO, Moestrup O, Powell MJ, Roberson RW, Wetherbee R (1994) Terminology and nomenclature of protist cell surface structures. Protoplasma 181: 1–28
Punt W, Blackmore S, Nilsson S, LeThomas A (1994) Glossary of pollen and spore terminology. LPP Foundation, Utrecht (LPP contributions series, vol 1)
Reuzeau C, Pont-Lezica RF (1995) Comparing plant and animal extracellular matrix cytoskeleton connections – are they alike? Protoplasma 186: 113–121
Roberts K (1990) Structures at the plant cell surface. Curr Opin Cell Biol 2: 920–928
– (1994) The plant extracellular matrix: in a new expansive mood. Curr Opin Cell Biol 6: 688–694
Robinson DG, Depta H (1988) Coated vesicles. Annu Rev Plant Physiol Plant Mol Biol 39: 53–99
– Hillmer S (1990) Endocytosis in plants. Physiol Plant 79: 96–104
Round FE, Crawford RM, Mann DG (1990) The diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge
Rowley JR, Dahl AO (1977) Pollen development in *Artemisia vulgaris* with special reference to glycocalyx material. Pollen Spores 19: 169–284
– Skvarla JJ (1975) The glycocalyx and initiation of exine spinules on microspores of *Canna*. Am J Bot 62: 479–485
Russel SD (1979) Fine structure of megagametophyte development in *Zea mays*. Can J Bot 57: 1093–1110
Schindler M (1993) Das neue Bild der Zellwand. Biologie Unserer Zeit 23: 113–120
Schmid AM (1979) Influence of environmental factors on the development of the valve of diatoms. Protoplasma 99: 99–115

Schmid AM (1980) Valve morphogenesis in diatoms: a pattern-related filamentous system in pennates and the effect of APM, colchicine and osmotic pressure. Nova Hedwigia 33: 811–847
– (1984a) Wall morphogenesis in *Thalassiosira eccentrica*: comparison of auxospore formation and the effect of MT-inhibitors. In: Mann DG (ed) Proceedings of the 7th International Symposium on Diatoms, Philadelphia, PA, 1982, pp 47–70
– (1984b) Tricornate spines in *Thalassiosira eccentrica* as a result of valve modelling. In: Mann DG (ed) Proceedings of the 7th International Symposium on Diatoms, Philadelphia, PA, 1982, pp 71–95
– (1984c) Valve morphogenesis in diatoms. In: Bach K, Burckhardt B (eds) Diatoms I. Shells in nature and technics. Cramer, Stuttgart, pp 300–317 (Communications of the Institute of Lightweight Structures, vol 28)
– (1986a) Organization and function of cell-structures in diatoms and their morphogenesis. In: Ricard M (ed) Proceedings of the 8th International Symposium on Diatoms, Paris, 1984, pp 271–292
– (1986b) Wall morphogenesis in *Coscinodiscus wailesii*. II. Cytoplasmic events of valve morphogenesis. In: Ricard M (ed) Proceedings of the 8th International Symposium on Diatoms, Paris, 1984, pp 293–314
– (1987a) Wall morphogenesis in centric diatoms. In: Wiessner W, Robinson D, Starr RC (eds) Algal development: molecular and cellular aspects. Springer, Berlin Heidelberg New York Tokyo, pp 34–41
– (1987b) Morphogenetic forces in diatom wall formation. In: Bereiter-Hahn J, Anderson OR, Reif WE (eds) Cytomechanics: the mechanical basis of cell form and structure. Springer, Berlin Heidelberg New York Tokyo, pp 183–199
– (1988) The special Golgi-ER-mitochondrium unit in the diatom genus *Coscinodiscus*. Plant Syst Evol 158: 211–223
– (1994a) Aspects of morphogenesis and function of diatom cell walls with implications for taxonomy. Protoplasma 181: 43–60
– (1994b) Slit scales in the auxospore scale case of *Coscinodiscus granii*: the rudiments of rimoportulae? Diatom Res 9: 371–375
– (1994c) Sexual reproduction in *Coscinodiscus granii* Gough in culture. In: Marino D (ed) Proceedings of the 13th International Symposium on Diatoms, Italy, 1994, pp 139–159
– Schulz D (1979) Wall morphogenesis in diatoms: deposition of silica by cytoplasmic vesicles. Protoplasma 100: 267–288
– Volcani BE (1983) Wall morphogenesis of *Coscinodiscus wailesii* (Gran) Angst. I. Valve morphology and development of its architecture. J Phycol 19: 387–402
– Borowitzka MA, Volcani BE (1981) Morphogenesis and biochemistry of diatom cell walls. In: Kiermayer O (ed) Cytomorphogenesis in plants. Springer, Wien New York, pp 63–97 [Alfert M et al (eds) Cell biology monographs, vol 8]
Schnepf E (1984) The cytological viewpoint of functional compartmentation. In: Wiessner W, Robinson DG, Starr RC (eds) Compartments in algal cells and their interaction. Springer, Berlin Heidelberg New York Tokyo, pp 1–10
– Deichgräber G (1969) Über die Feinstruktur von *Synura petersenii* unter besonderer Berücksichtigung der Morphogenese ihrer Kieselschuppen. Protoplasma 68: 85–106
– Drebes G (1980) Morphogenetic process in *Attheya decora* (Bacillariophyceae, Biddulphiinae). Plant Syst Evol 135: 265–277
Scott RJ (1994) Pollen exine – the sporopollenin enigma and the physics of pattern. In: Scott RJ, Stead AD (eds) Molecular and cellular aspects of plant reproduction. Cambridge University Press, Cambridge, pp 49–82
Sheldon JM, Dickinson, HG (1983) Determination of patterning in the pollen wall of *Lilium henryi*. J Cell Sci 63: 191–208
– (1986) Pollen wall formation in *Lilium*: the effect of chaotropic agents, and the organization of the microtubluar cytoskeleton during pattern development. Planta 186: 11–23
Shivanna KR, Johri BM (1985) The angiosperm pollen: structure and function. Wiley Eastern, New Delhi
Simkiss K (1986) The process of biomineralization in lower plants and animals – an overview. In: Leadbeater BSC, Riding R (eds) Biomineralization in lower plants and animals. Clarendon Press, Oxford, pp 19–37 (Systematics Association special volume 30)
Skvarla JJ, Rowley JR (1987) Ontogeny of pollen in *Poinciana* (Leguminosae). I. Development of exine template. Rev Palaeobot Palynol 50: 293–311
Southworth D, Jernstedt JA (1995) Pollen exine development precedes microtubule rearrangement in *Vigna unguiculata* (Fabaceae): a model for pollen wall patterning. Protoplasma 187: 79–87
Steucek GL, Schmid AM (1989) Leight weight architecture of the diatom *Thalassiosira*. In: Reiner R, Wirth H (eds) Contributions to the 1st International Symposium "Natürliche Konstruktionen: Leichtbau in Architektur und Natur", SFB 230, Stuttgart, 1988, pp 195–203
Stosch HA von (1982) On auxospore envelopes in diatoms. Bacillaria 5: 127–156
– Theil G, Kowallik K (1973) Entwicklungsgeschichtliche Untersuchungen an Diatomeen. V. Bau und Lebenszyklus von *Chatoceros didymum*, mit Beobachtungen über einige andere Arten der Gattung. Helgol Wiss Meeresunters 25: 384–445
Takahashi M (1989a) Development of the echinate pollen wall in *Farfugium japonicum* (Compositae: Senecioneae). Bot Mag Tokyo 102: 219–234
– (1989b) Pattern determination of the exine in *Caesalpinia japonica* (Leguminosae: Caesalpinioideae). Am J Bot 76: 1615–1626
– (1992) Development of spinous exine in *Nuphar japonicum* DeCandolle (Nymphaeaceae). Rev Palaeobot Palynol 75: 317–322
– (1993) Exine initiation and substructure in pollen of *Caesalpinia japonica* (Leguminosae: Caesalpinioideae). Am J Bot 80: 192–197
– (1995a) Exine development in *Aucuba japonica* Thunberg (Cornaceae). Rev Palaeobot Palynol 85: 199–205
– (1995b) Three-dimensional aspects of exine initiation and development in *Lilium longiflorum* (Liliaceae). Am J Bot 82: 847–854
– (1995c) Development of structure-less pollen wall in *Ceratophyllum demersum* L. (Ceratophyllaceae). J Plant Res 108: 205–208
– Kouchi J (1988) Ontogenetic development of spinous exine in *Hibiscus syriacus* (Malvaceae). Am J Bot 75: 1549–1558
– Skvarla JJ (1991a) Exine pattern formation by plasma membrane in *Bougainvillea spectabilis* Willd. (Nyctaginaceae). Am J Bot 78: 1063–1069
– (1991b) Development of striate exine in *Ipomopsis rubura* (Polemoniaceae). Am J Bot 78: 1724–1731

Tiwari SC (1989) Cytoskeleton during pollen development in *Tradescantia virginiana*: a study employing chemical fixation, freeze-substitution, immunofluorescence, and colchicine administration. Can J Bot 67: 1244–1253

– Gunning BES (1986) Colchicine inhibits plasmodium formation and disrupts pathways of sporopollenin secretion in the anther tapetum of *Tradescantia virginiana* L. Protoplasma 133: 115–128

Traas JA (1990) The plasmamembrane associated cytoskeleton. In: Larson C, Moller IM (eds) The plant plasma membrane. Springer, Berlin Heidelberg New York Tokyo, pp 269–292

Traverse A (1988) Paleopalynology. Unwin Hyman, Boston

Ubera Jimenez JL, Hidalgo Fernandez P, Schlag MG, Hesse M (1996) Pollen and tapetum development in male fertile *Rosmarinus officinalis* L. (Lamiaceae). Grana 34: 305–316

Uehara K, Kurita S (1991) Ultrastructural study on spore wall morphogenesis in *Lycopodium clavatum* (Lycopodiaceae). Am J Bot 78: 24–36

Uffelen GA van (1991) The control of spore wall formation. In: Blackmore S, Barnes SH (eds) Pollen and spores: patterns of diversification. Clarendon Press, Oxford, pp 89–102 (The Systematics Association special volume 44)

Wang C-S, Walling LL, Gu YQ, Ware CF. Lord EM (1994) Two classes of proteins and mRNAs in *Lilium longiflorum* L. identified by human vitronectin probes. Plant Physiol. 104: 711–717

Waterkeyn L, Bienfait A (1987) Localisation et rôle des β-1,3-glucanes (callose et chrysolaminarine) dans le genre *Pinnularia* (diatomées). Cellule 74: 199–226

Werner D (1977) Introduction with a note on taxonomy. In: Werner D (ed) The biology of diatoms. Bot Monogr 13: 1–17

Wordemann L (1992) The cytoskeleton in diatoms. The mitotic spindle and the cell cycle dependent organization In: Menzel D (ed) The cytoskeleton of the algae. CRC Press, Boca Raton, pp 39–57

Worrall D, Hird DL, Hodge R, Paul W, Draper J, Scott R (1992) Premature dissolution of the microspore callose wall causes male sterility in transgenic tobacco. Plant Cell 4: 759–771