Protoplasma 93, 205–221 (1977)

PROTOPLASMA

Studies on the Biochemistry and Fine Structure of Silicia Shell Formation in Diatoms

VII. Sequential Cell Wall Development in the Pennate *Navicula pelliculosa*

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With 7 Figures

Received April 21, 1977
Accepted June 21, 1977

Summary

The development of the wall of synchronized culture of *N. pelliculosa* is described. The first step, modification of the 3–2 configuration of the girdle bands of the wall during interphase, occurs immediately before mitotic division by the addition of a third girdl band to the hypotheca. Following cytokenesis, the new valve is initiated when a primary central band is formed within a silica deposition vesicle. This band extends the length of the cell and contains a central nodule. Secondary arms extend from the central nodule, join with extensions of the primary central band, and constitute the raphe rib. Mounds or knolls are formed on the central nodule and disappear as the valve matures. Transapical ribs appear on both the primary central band and secondary arms, and cross extensions join to form the sieve plate areas. The wall appears to be released by a joining of the inner silicalemma and the plasmalemma. An organic coat covers the newly released wall. Two girdle bands are formed and released sequentially.

1. Introduction

The structure of mature siliceous walls of the various diatom species, serving as their primary taxonomic characteristic, is well documented, particularly with the availability of the transmission and scanning electron microscopes. Concerning the formation of these walls, some work has been done on the transport of silicic acid into the cell (AZAM *et al.* 1974, SULLIVAN 1976, 1977), and on the chemical composition of the wall (NAKAJIMA and VOLCANI 1969, 1970, HECKY *et al.* 1973), but relatively little is known concerning the actual development of the wall itself.

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STOERMER *et al.* (1965) first described the deposition of silica within a "silica deposition vesicle" in *Amphipleura pellucida*, and REIMANN *et al.* (1966), in *Navicula pelliculosa*, termed the limiting membrane of this vesicle the "silicalemma". Deposition within a silicalemma has also been described briefly in several species (REIMANN *et al.* 1965, LAURITIS *et al.* 1968, PICKETT-HEAPS *et al.* 1975); a more detailed description of wall formation has been presented for some forms of the pennate diatom *Gomphonema parvulum* (DAWSON 1973). In this case as in all others to date, descriptions of wall formation are based upon thin-sectioned material which is difficult to interpret. This paper presents the complete development sequence of the wall of the pennate diatom *N. pelliculosa*, based upon isolated walve preparations as well as upon thin-sectioned material.

2. Materials and Methods

Cultures of *N. pelliculosa* (Bréb.) Hilse (Strain No. 668, Indiana Univ. Culture Collection, Bloomington, Ind., USA) were grown in FWG media and light-dark synchronized as previously described by DARLEY and VOLCANI (1971).

For isolated developing valve preparation, cells were harvested following the dark period. Cells were suspended (1 gr/10 ml) in sucrose-Tris solution (0.2 M sucrose in 0.05 M Tris HCl, pH 8.2) and cracked on a Braun Mechanical Cell Homogenizer (Bronwill Model MSK) with acid washed Ballotini beads (#12). The beads were removed by filtration through a coarse sintered glass funnel and the filtrate was subjected to a series of differential centrifugations at 1,820 × g; 3 × 1 minutes (pellets discarded); 1,265 × g for 5 and 10 minutes successively (pellets of each discarded). The supernatant (from the 10 minutes centrifugation) was centrifuged at 1,265 × g for 15 minutes, and the pellet washed with distilled water to remove the sucrose. A sample, diluted to an appropriate concentration with distilled water, was dropped onto carbon-collodion coated grids, dried at 60 °C and viewed.

Carbon replicas of isolated valves were prepared by shadowing with platinum/carbon at a low angle (< 40°) and with carbon at 90°. Latex spheres (0.1 μm, E.F. Fullam, Inc., Schenectady, N.Y.) were mixed with the wall suspensions as a size reference. Replicas were cleaned by floating on concentrated H_2SO_4, then on commercial hypoclorite bleach and finally on 20% hydrofluoric acid for 1 hour on each solution with thorough rinsing with distilled water between treatments. Replicas were then picked up on uncoated grids.

A series of samples of whole cells were taken during the division cycle of synchronized growth. The pellets were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 2 hours at 4 °C, followed by 1% OsO_4 in the same buffer for 1 hour at room temperature, dehydrated in ethanol series and embedded in Epon 812. Sections were cut with a diamond knife and stained with 5% aqueous uranyl acetate and lead citrate (REYNOLDS

Fig. 1. *a* Isolated mature wall; *CR* = central rib; *RF* = raphe fissure; *CN* = central nodule; *TR* = transapical rib; *SP* = sieve plate; *Gb* = girdle band. ×11,000. *b* Cross section of girdle band area of interphase cell showing 3 girdle bands on epitheca and 2 on hypotheca. ×48,000. *c* Freeze fracture replica of mature wall showing 3 girdle bands of epitheca. ×48,000. *d* Cross section of cell approaching mitotic division with forming third girdle band (arrows); *N* = nucleus; *Nu* = nucleolus; *C* = chloroplast; *M* = mitochondrion; *V* = vacuole; *R* = raphe; *Gb* = girdle bands. ×33,000. *e* Girdle area with third girdle band of hypotheca in place. ×60,000

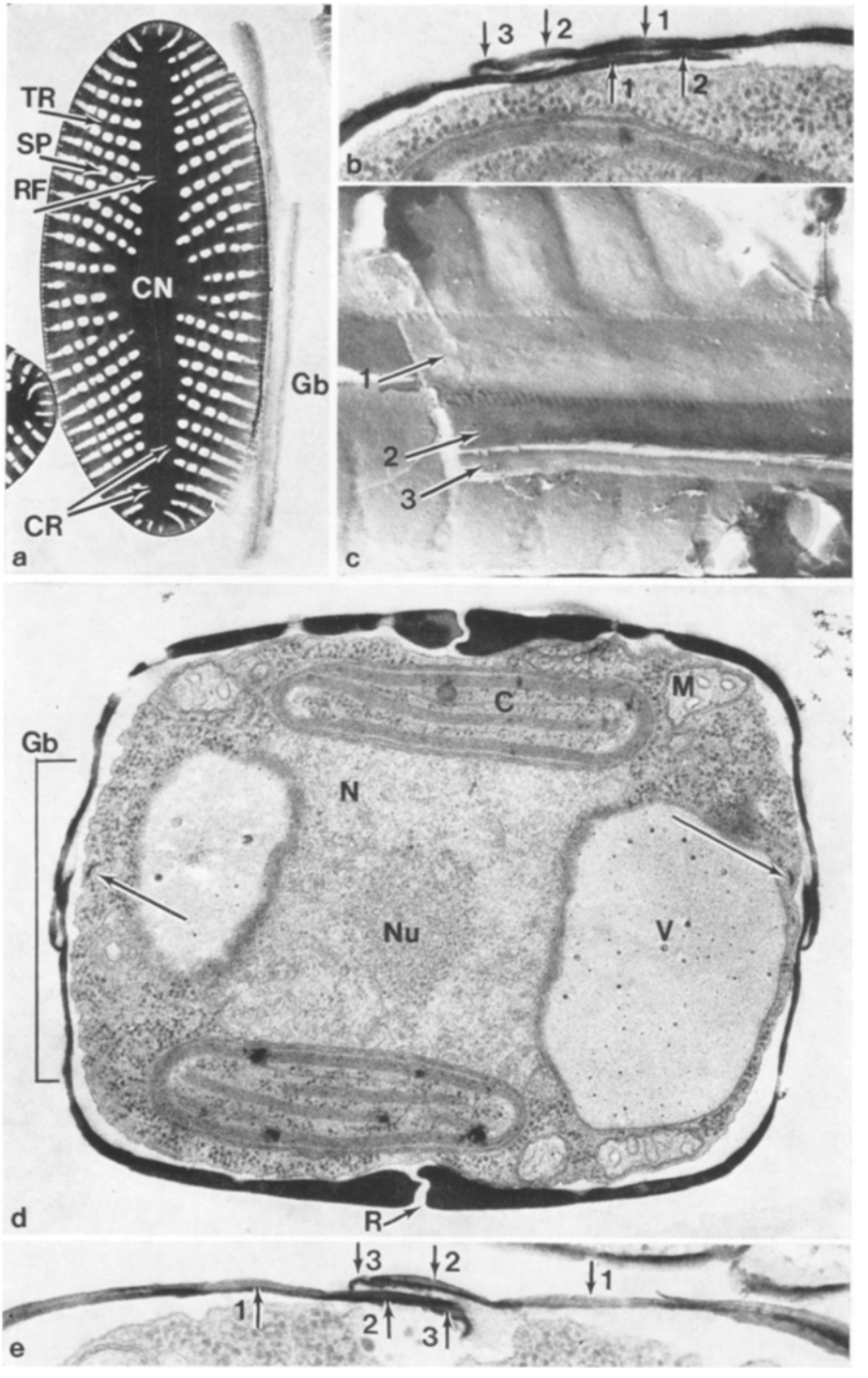

Fig. 1

1963). Some sections were exposed to 48% hydrofluoric acid (HF) vapors for 5 minutes at room temperature for extraction of silica. For freeze fraction, cells were grown in medium with 5% glycerol added. Cells were collected by centrifugation and a drop of suspension frozen onto copper discs by plunging into Freon-22 at liquid nitrogen temperatures. A Balzers freeze-etch apparatus was used for fracturing. Fractures were etched for 1 minute and shadowed with platinum/carbon at 45° and carbon at 90°. Replicas were cleaned as for carbon replicas above. All samples were viewed on a Siemens Elmiskop I electron microscope.

3. Observations

3.1. The Mature Wall

Although the mature wall of *N. pelliculosa* has previously been described (REIMANN *et al.* 1966), a review with several additions and corrections is necessary in order to accurately describe the forming wall.

The mature wall of *N. pelliculosa* is composed of 2 valves, an epitheca and a hypotheca, surrounded by a series of girdle bands. Each valve has a central raphe fissure bordered by the central raphe rib and interruped by the central nodule, radiating transapical ribs and a regular series of sieve plates (Fig. 1 *a;* see also Fig. 3 in REIMANN *et al.* 1966). The girdle region of an interphase cell is composed of a set of 5 girdle bands; *i.e.*, 3 girdle bands on the epitheca and 2 bands on the hypotheca (Figs. 1 *b* and *c*). The first two bands of each valve have been described by REIMANN *et al.* (1966). The third girdle band of the epitheca is very narrow, usually comma-shaped in cross section (Fig. 1 *b*), and has not previously been described. It surrounds the cell at the outer edge of the girdle bands of the epitheca (Fig. 1 *c*). This 3–2 configuration of the girdle bands is constant throughout interphase.

3.2. Preparation for Division

The development of the new "wall" of *N. pelliculosa* technically begins not with the development of a new hypotheca of the two daughter cells, but with the modification of the mother cell hypotheca to become the epitheca of a daughter cell by the addition of the third girdle band. This modification takes place before cell division and is, in fact, a definite indication that cell division is imminent. As the cell nears the end of interphase evident in synchronized culture, the wall of the cell is highly expanded at the girdle region to accommodate cytoplasmic growth (Fig. 1 *d*). The band is formed within a small silica deposition vesicle, which lies within the cytoplasm near the end of the second girdle band of the hypotheca (Fig. 1 *d* arrows). The band is released to the exterior (see later section on girdle bands) and attaches to the outer edge of the second girdle band of the hypotheca (Fig. 1 *e*).

3.3. Valve Formation

The development of the valve in *N. pelliculosa* can be divided into 4 major stages:

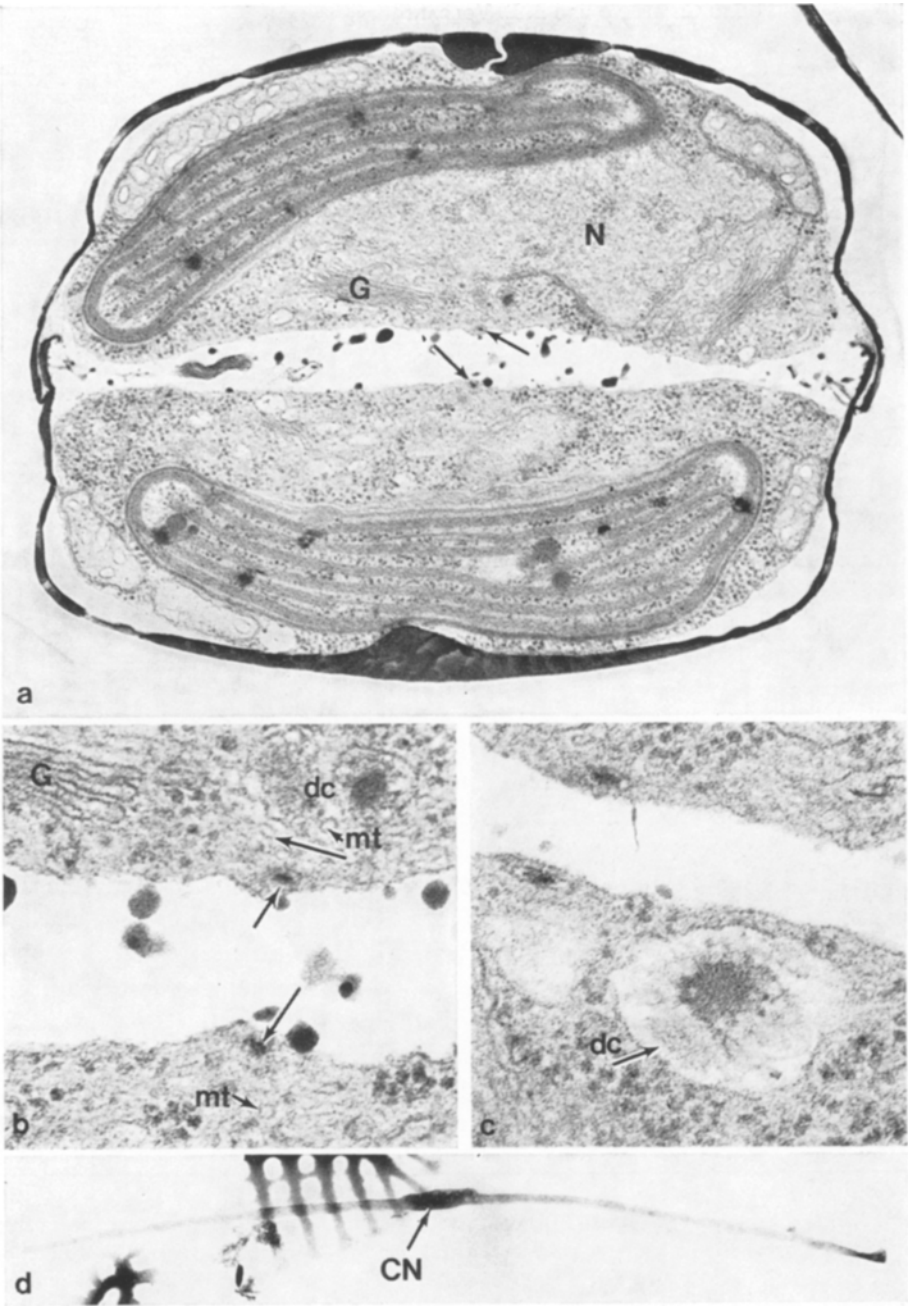

Fig. 2. Stage 1 of development. *a* Cell immediately following cytokenesis with initial silica deposition vesicles (arrows); *G* = Golgi apparatus. ×30,000. *b* High magnification of the silica deposition vesicles delimited by a silicalemma (arrows); *dc* = "dense complex"; *mt* = microtubules. ×105,000. *c* Dense complex: membrane bound vesicle containing a rounded mass of dense material which is resistant to HF extraction. ×90,000. *d* Isolated developing valve preparation; the primary central band with enlarged "primordial" central nodule (*CN*). ×20,000

3.3.1. Stage One

The first evidence of a developing valve in *N. pelliculosa* appears immediately following the completion of cytokinesis while the two daughter cells are still enclosed within the highly expanded, modified wall of the mother cell (Fig. 2 *a*). Just below the plasmalemma, at the midpoint of each of the daughter cells, a minute silica deposition vesicle appears delimited by the silicalemma; at the earliest recognizable stage, a very small amount of polymerized silica is already present (Fig. 2 *a*, arrows). Serial sections indicate that even at this early stage, the vesicle extends along the entire length of the cell. In the area beneath the initial vesicle is a series of small vesicles (Fig. 2 *b*), which in serial section prove to be a series of microtubules associated with a "dense complex" (Figs. 2 *b*, and *c*) located at the center of the cell.

In isolated developing valve preparations, the earliest valve seen was a single siliceous band (Fig. 2 *d*) approximately 6 μm long, which corresponds to the approximate length of the raphe of the mature valve (REIMANN *et al.* 1966). This single band will be termed the "primary central band." It is uniform in structure along its entire length except for a slightly enlarged area in the central region (Fig. 2 *d*). This enlarged area is the primordial central nodule.

3.3.2. Stage Two

The second stage of valve development is characterized by growth of the ends of the primary central band and by differentiation of the primordial central nodule.

In isolated preparations at this stage, as well as in stage one, developing valves appear to be highly flexible and are often seen contorted in various postures (Fig. 3 *a*). The ends of the primary central band, already reaching the ends of the cell, continue to elongate and then make a U-turn and begin to extend toward the center of the cell (Fig. 3 *a*, arrows).

The differentiation of the primordial central nodule area begins with the growth of side arms from each end of the nodule (Figs. 3 *a–c*). These "secondary arms" cross through the midplane and turn toward the ends of

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Fig. 3. Stage 2 of development. *a* Isolated valve preparation showing flexibility of the valve and in-turning of the ends of the primary central band (*pCB*) (arrows); *CN* central nodule; *TR* transapical ribs; *sa* secondary arms. ×37,000. *b* Differentiation of the central nodule, with dense areas and formation of secondary arms *sa* ×32,000. *c* Slightly later stage than *b* with transapical ribs (*TR*), central nodule and secondary arms more developed. Ends of the primary central bands have been broken during isolation. ×22,000. *d* Central nodule area of isolated valve showing continuation of dense areas along the primary central band (*pCB*)) and the secondary arm (*sa*). ×43,000. *e* Cross section of central nodule area showing dense areas to be mounds or knolls of silica (arrows). ×69,000. *f–g* Serial section of *d* with appearance and gradual disappearance of the secondary arm (*sa*); *mt* = microtubules. ×69,000

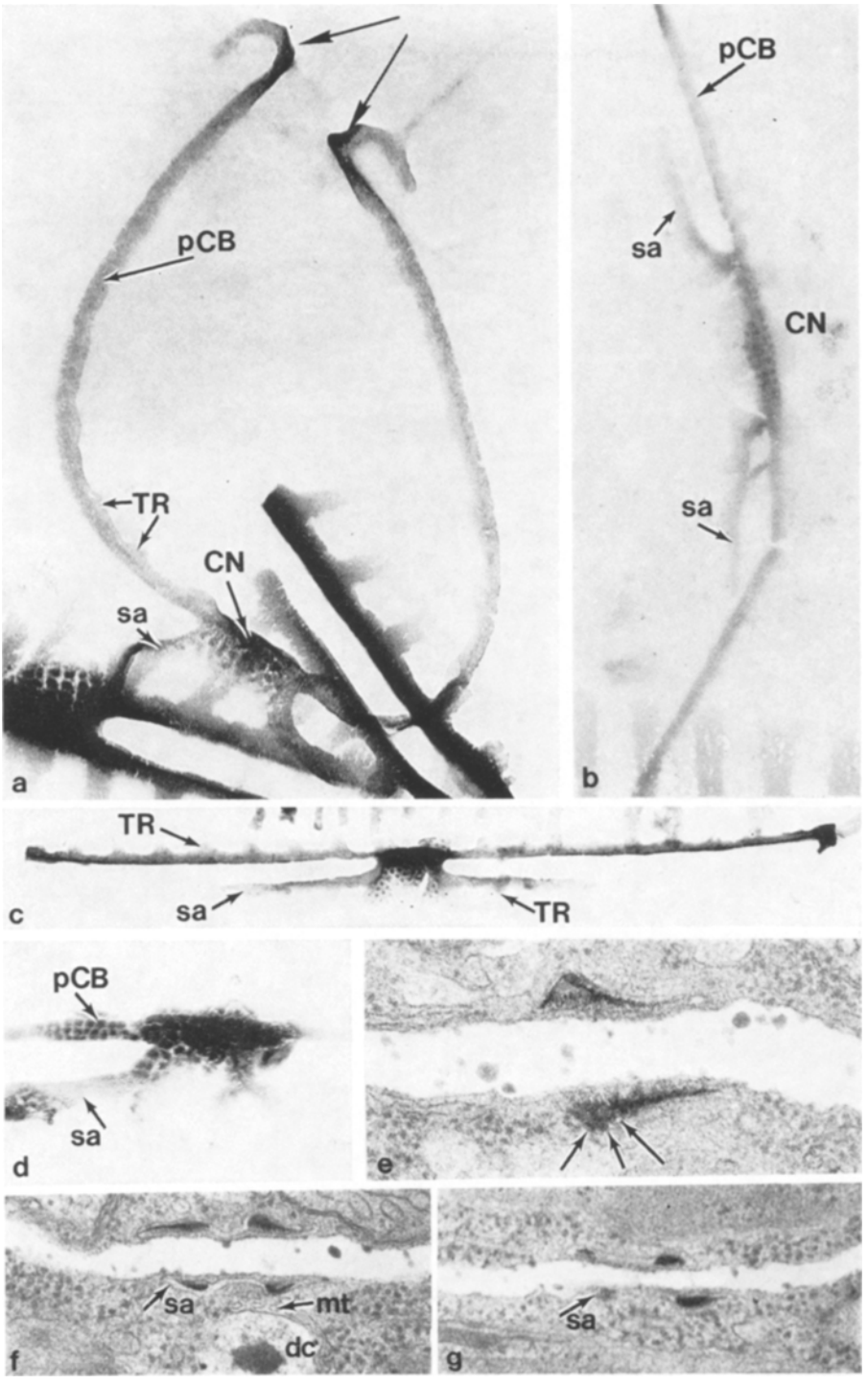

Fig. 3

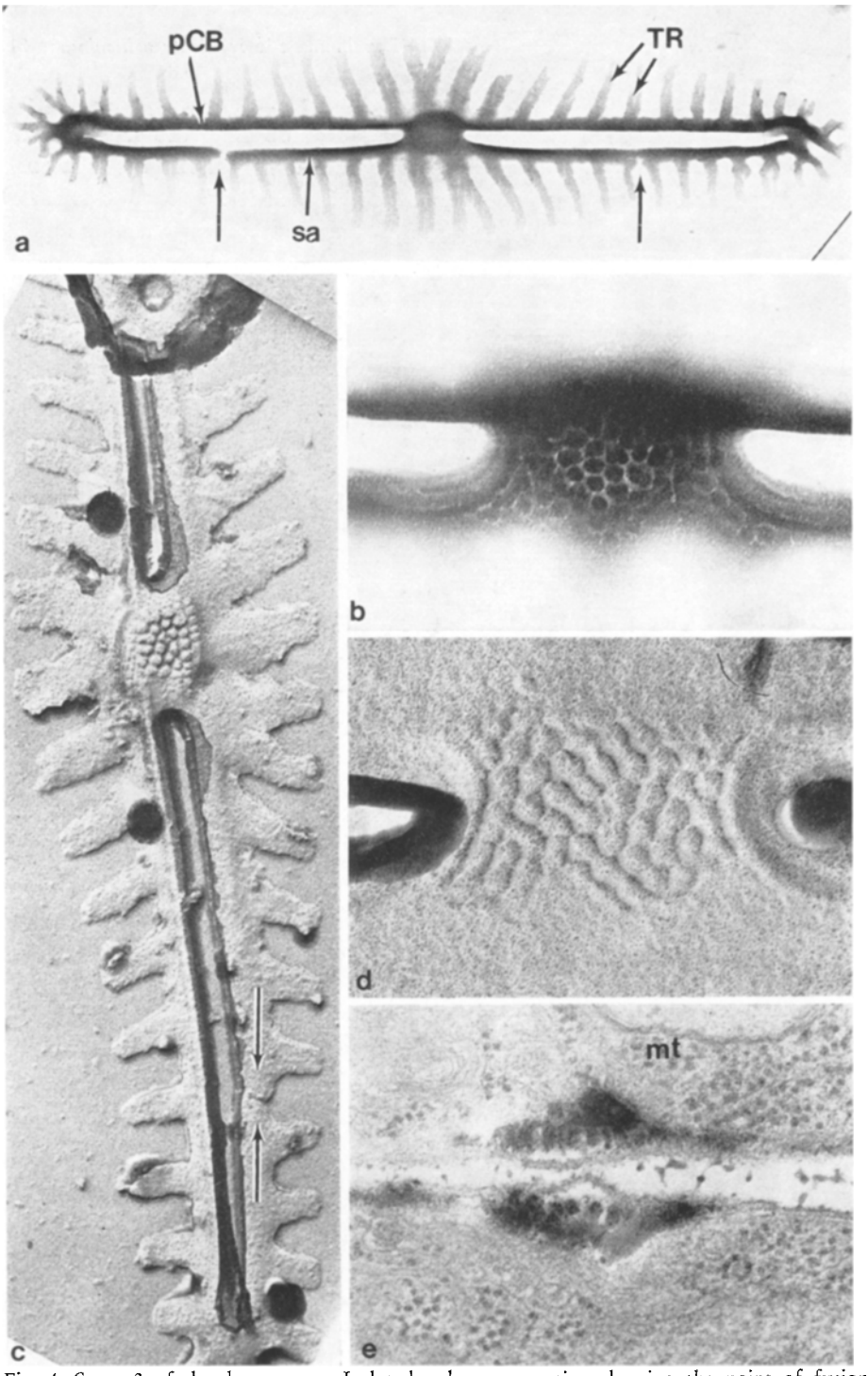

Fig. 4. Stage 3 of development. *a* Isolated valve preparation showing the point of fusion of the primary central band (*pCB*) and the secondary arms (*sa*); *TR* = transapical ribs. ×20,000. *b* High magnification of central nodule with array of electron dense areas. ×67,000. *c* C-Pt replica of the inner face of an isolated developing valve showing the mounds or knolls of silica of the central nodule. Also note the point of fusion of the primary central band and the secondary arms (arrows). ×40,000. *d* C-Pt replica of central nodule area of the exterior face of the developing valve. ×80,000. *e* Central nodule area in cross section. Note array of microtubules (*mt*). ×60,000

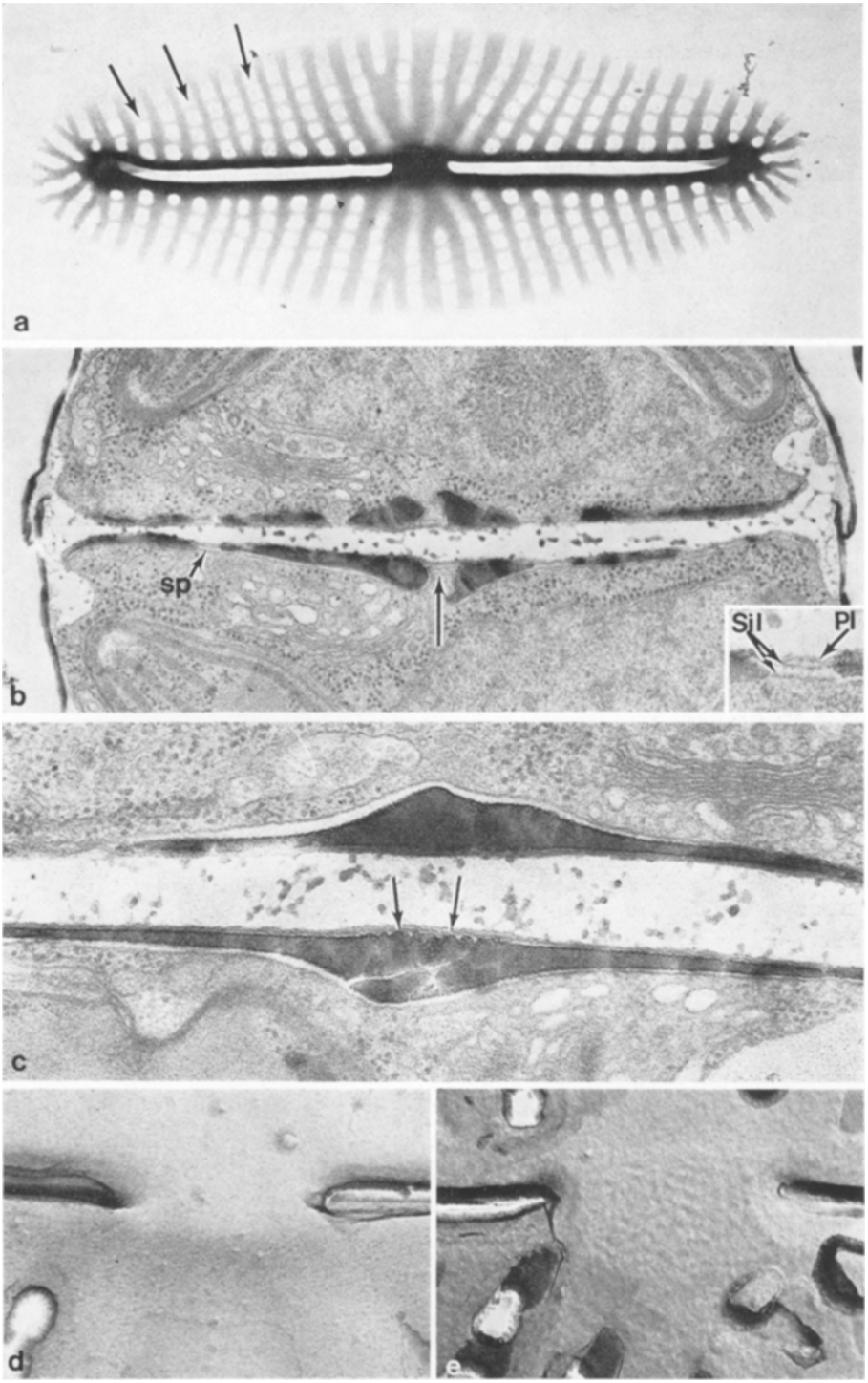

Fig. 5. Stage 4 of development. *a* Isolated valve preparation. Cross extensions between transapical ribs have fused (arrows). ×16,000. *b* Cross section of cell taken along the raphe showing in-turning of the margin of the developing valve, the continuity of the silicalemma across the raphe fissure (arrow), and formation of the sieve plate (*sp.*). ×34,000. Inset, high magnification of developing sieve plate within the silicalemma (*Sil*); *Pl* plasmalemma. ×120,000. *c* Cross section of central nodule region showing shallow knolls on exterior face (arrows) and loss of knolls on the interior face. ×60,000. *d* C-Pt replica of interior face of the central nodule with no evidence of the knolls. ×52,000. *e* C-Pt replica of exterior face of the central nodule showing shallow knolls still present. ×58,800

the cell. Gradually, the area between the second arms is filled, forming a "fan-shaped" central nodule (Figs. 3 *a* and *c*). Conspicuous on this fan-shaped structure is a pattern of electron dense areas (Figs. 3 *a–c*), usually confined to the fan-shaped structure, but in a few cases, extending for a short distance along the primary central band and the secondary arms (Fig. 3 *d*).

In a cross-section, the dense areas of the central region are small mounds or knolls (Fig. 3 *e*, arrows). At this stage the silicalemma can be seen more distinctly. Serial sections (Figs. 3 *f* and *g*) show the silicalemma to be one continuous vesicle, and the appearance (Fig. 3 *f*) and gradual disappearance (Fig. 3 *g*) of the secondary arms can be followed. The complex array of microtubules and dense complex described above is apparent within the cytoplasm (Fig. 3 *f*).

Also during stage two, the first indication of the transapical ribs appear as small projections regularly spaced along the outer edges of the primary central band of the secondary arms (Figs. 3 *a* and *c*).

For simplicity we will follow the development of the wall of only one of the daughter cells enclosed within the mother wall. However, it must be noted that the developmental sequence is concurrently progressing in each daughter cell and that throughout the sequence, particularly during early stages, the developing valves of the two daughter cells are mirror images of each other.

3.3.3. Stage Three

By the third stage of development, the valve begins to take on the basic outline of the mature valve (Fig. 4 *a*). The secondary arms, extending from the central nodule, approach and ultimately fuse with the forward extensions of the primary central band (Figs. 4 *a* and *c*, arrows). This fusion forms the "central rib" and "raphe fissure" as described in the mature cell (REIMANN *et al.* 1966). The transapical ribs are now very distinct along the entire length of the central rib (Figs. 4 *a* and *c*).

At this stage the mounds or knolls are very prominent on the central nodule (Figs. 4 *b–e*). Carbon-platinum replicas show that the mounds occur on both sides of the central nodule and rise independently from a common base. The

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Fig. 6. *a* Point of contact (arrows) of the silicalemma and the plasmalemma. The outer silicalemma and plasmalemma cannot be resolved; *Gb* = old girdle bands. ×77,000. *b* Thin section of newly released wall following extraction of silica with HF. An organic coat can be seen to cover the exterior and interior face of the valve (arrows). ×95,000. *c* Formation of the first girdle band of the newly formed hypothecae within a silicalemma. ×55,000. *d* Release of a girdle band from the cell. ×55,000. *e* Daughter cells in the process of separation and the initiation of the second girdle band (arrows). ×23,000. High magnification of *e* showing involvement of Golgi apparatus (upper inset) and endoplasmic reticulum (lower inset) in girdle band deposition. ×66,000

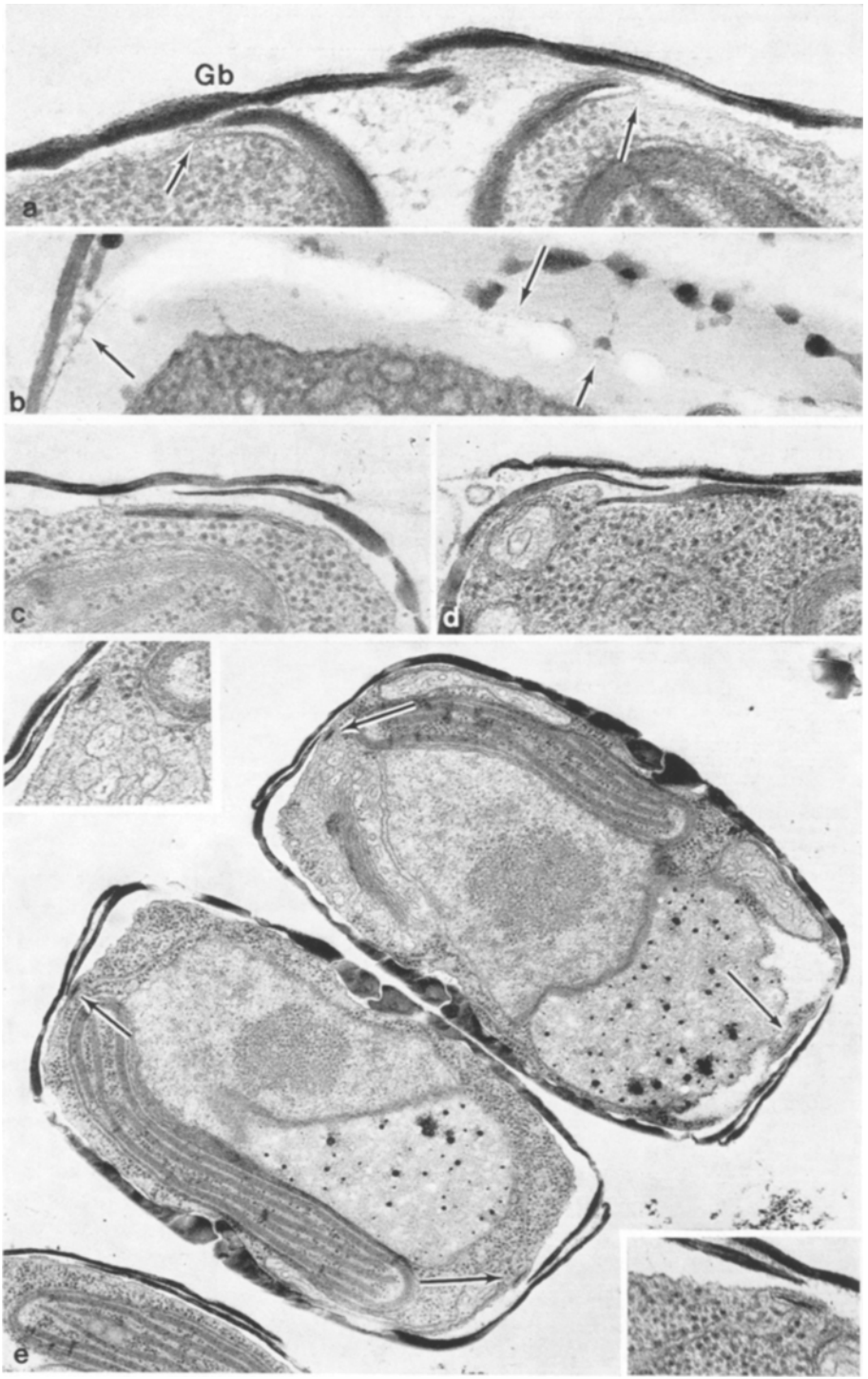

Fig. 6

mounds of the interior surface (*i.e.*, toward the interior of the cell) are very pronounced, approximately 300–400 Å in diameter and 500 Å high (Fig. 4 *c*). The mounds of the exterior surface are shallow (Fig. 4 *d*). The mounds can also be seen in thin section (Fig. 4 *e*), but sectioning artifacts and poor orientation tend to obscure their true configuration.

3.3.4. Stage Four

Following the completion of the central rib, cross extensions between the transapical ribs appear along their length (Fig. 5 *a*, arrows). These extensions are laid down successively, the first appearing at the origin of each rib (*i.e.*, near the central rib and central nodule). The extensions of adjacent ribs directly appose each other, and with continued growth, they ultimately meet and fuse, becoming bridges. Within the rounded areas formed by the fusion of these bridges, the sieve plates described by REIMANN *et al.* (1966) are formed (Fig. 5 *b*, inset).

By this final stage of valve development, the knolls of the central nodule have become less distinct. It appears from thin sections that the interstitial spaces between the knolls are gradually filled until the knolls are completely obliterated (Fig. 5 *c*, arrows). The knolls of the interior surface, even though more pronounced at earlier stages, disappear (Figs. 5 *c* and *d*) before those of the exterior which can be detected, even though very shallow, for some time after the others have disappeared (Figs. 5 *c* and *e*). When the valve reaches maturity the knolls, both interior and exterior, have completely disappeared and there is no evidence, either in isolated preparations or thin section, that they ever existed.

As the valve reaches maturity, its edges turn inward and it assumes the classic shape of a pennate diatom (Fig. 5 *b*). The extreme edges of the silica deposition vesicle approach the plasmalemma (Fig. 6 *a*, arrows), and the valve is released from the cell. At this stage a new plasmalemma is seen underneath the valve. We were unable to establish whether it was derived from the silicalemma as was described in *G. parvulum* (DAWSON 1973), or from the extension of the old plasmalemma surrounding the cell. The outer silicalemma and the plasmalemma, which are well defined up to this stage, are no longer delineated after the release of the valve, as is also the case in the mature valve (REIMANN *et al.* 1966), but the organic coat surrounding the valve is evident from HF treated sections (Fig. 6 *b*, arrows). The coat is possibly derived from the silicalemma and old plasmalemma on the outer side and/or secretions of the silicalemma on the inner side.

3.4. Girdle Band Formation

The initiation of girdle bands occurs immediately following the release of the newly formed valve. The first girdle band appears within a deposition

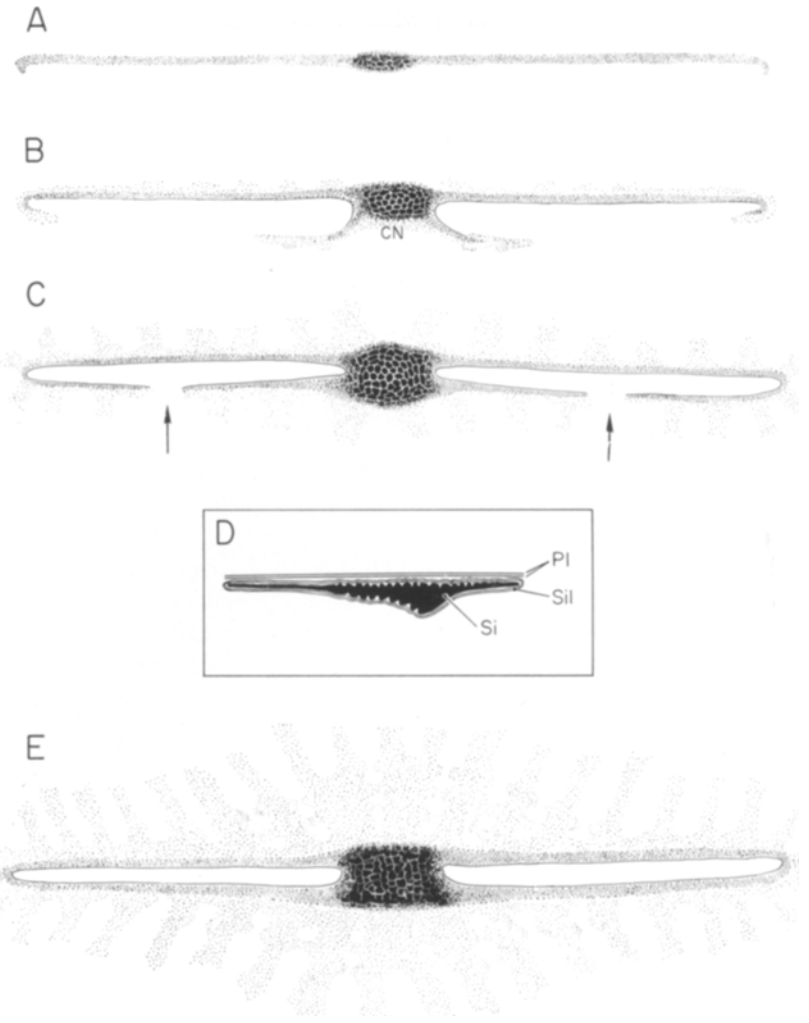

Fig. 7. Schematic drawing summarizing valve development. *A* First stage of development: the primary central band. *B* Second stage of development: the in-turning of the primary central band and differentiation of the central nodule (*CN*). *C* Third stage of development: approach of the primary central band extensions and secondary arms which ultimately fuse forming the raphe rib; growth of transapical ribs. *D* Cross section of central nodule at the third stage of development with mounds or knolls on inner and outer surface; *Si* silica; *Sil* silicalemma; *Pl* plasmalemma. *E* Fourth stage of development: growth of cross bridges and sieve plates and loss of mounds of central nodule

vesicle as a narrow siliceous band which surrounds the cell just within the cytoplasm, in the area near the ends of the new valve (Fig. 6 *c*). At this time the two daughter cells are still attached to each other. When the girdle band is fully formed it is released as described for the valve and attaches to the end of the valve (Figs. 6 *c* and *d*) by an extension of the organic coat.

The formation of the second girdle band (Fig. 6 *e*, arrows) is not begun until the first is released, and the mechanism of formation appears to be the same as in the first. Cross sections show that although the Golgi apparatus is active near the forming girdle band in certain regions of the cell (Fig. 6 *e*, upper inset), the endoplasmic reticulum is involved in other areas (Fig. 6 *e*, lower inset). By this time, the two daughter cells may or may not be still attached. With the release of the second girdle band, the 3–2 configuration of girdle bands of the interphase cell and wall formation is complete.

4. Discussion

With the advantage of observing isolated valves at various stages of development, it has become evident that in *N. pelliculosa* valve formation follows a definite and sequential pattern of an orderly augmentation of an original, single central band. As shown in Fig. 7, this continuity of growth always extends from the central raphe rib to the outer edge of the cell at the girdle area. This is in contrast to valve formation reported in *Nitzschia alba* (LAURITIS *et al.* 1968) where the central raphe is reported to be deposited last and in *Gomphonema parvulum* (DAWSON 1973) where the outer edges of the valve are reported to be deposited before the intervening area between the edge and the central raphe. Recent data (CHIAPPINO *et al.* 1977), however, show that *N. alba* does indeed follow a sequential development starting with the formation of the raphe rib similar to *N. pelliculosa.*

In *N. pelliculosa* the silica deposition vesicle, although sequentially enlarged as the valve is formed, is one continuous vesicle. Although the vesicle follows the configuration of the valve closely during very early stages of central band and raphe rib formation, cross sections of later stages always show the deposition vesicle to be continuous not only across the raphe canal, but also when cut tangentially across several transapical ribs. Cross sections, however, do show constrictions of this vesicle across the raphe canal and in the areas where the sieve plates are formed. Whether the vesicle acts as a "mold" as suggested by STOERMER *et al.* (1965), during early stages of development or whether there is an involvement of an organic matrix within the silica *per se* which controls the precise formation of valve has yet to be determined. On the basis of our observations, the origin and source of the silicalemma in *N. pelliculosa* is still conjectural. It has been thought that the Golgi apparatus may be the source of the silicalemma (REIMANN 1964, DAWSON 1973).

Although vesicles of Golgi origin are found concentrated near the center of the cell (mid-body) in *N. pelliculosa*, the initial silica deposition vesicle is much smaller than those of the Golgi. Attempts to detect the origin of the vesicle in *N. pelliculosa* are hindered by the small size of the cell. The first discernible vesicle already contains a small amount of silica approximately the same size and electron density as that of the ribosomes. A series of permanganate fixations were attempted to eliminate this problem, but artifacts and membrane alterations known to be produced by permanganate fixation (ROSENBLUTH 1963, DOGGENWEILER and HEUSER 1967) were so great that no reliable information could be obtained. On the basis of our observations we agree with PICKETT-HEAPS *et al.* (1975) that caution in interpretation is advisable and that the origin and growth of the silicalemma could involve a complex interaction of Golgi vesicles, endoplasmic reticulum and/or microtubules. Even an involvement of the plasmalemma, as has been proposed in sponge spicule formation (SIMPSON and VACCARO 1974), should not be disregarded.

Some species of diatoms produce girdle bands (and/or intercalary bands) throughout their life cycle (ROUND 1972). The 3–2 girdle band configuration in *N. pelliculosa*, however, remains constant throughout interphase. Strictly speaking, the initiation of the third girdle band and the preparation of the old hypotheca to become the new epitheca represents the beginning or first stage of cell division in this diatom. This necessitates a refinement of the theory (GEITLER 1963, v. STOSCH and KOWALLIK 1969) that valve formation is dependent upon prior mitotic division, normal or abnormal. Although the theory refers to the formation of the two daughter hypothecae, mitosis does not trigger silica deposition in this diatom but is only part of a continuous and orderly cycle of genetically controlled events.

The orderly deposition of the girdle bands of the hypothecae of the daughter cells has previously been reported in *G. parvulum* (DAWSON 1973). The order of formation and the mechanism of release from the cytoplasm appears to be the same in *N. pelliculosa*. However, in this case the origin of the deposition vesicle seems to involve the endoplasmic reticulum in addition to Golgi activities rather than the coalescing of Golgi vesicles alone (DAWSON 1973).

The valves of various living diatom species are reported to be made up of amorphous silica gel (KAMATANI 1971). Amorphous silica occurs in various forms depending upon the conditions under which it is formed (ILER 1955). These conditions include differences in pH, silicic acid concentrations, and/or possible interactions with organic compounds. The mechanism of silicic acid polymerization in diatoms is not known. The mounds or knolls of the central nodule of *N. pelliculosa* correspond in size (300–400 Å in diameter) to the "primary" particles described in precious opal, another form of amorphous silica (DARRAGH *et al.* 1966). Opal phytoliths found in a variety of higher plants, and tabashir, a type of opal found in bamboo, are made up of similar

basic particles (JONES *et al.* 1966). Opal has also been described in radiolaria (CACHON and CACHON 1972) and marine gastropods (LOWENSTAM 1971). Although the mounds or knolls of *N. pelliculosa* are transient and ultimately engulfed, the similarity in their appearance to that of various opals of organic and inorganic origin is intriguing. Whether these mounds are formed by other diatoms during valve formation has yet to be determined.

Acknowledgements

The authors wish to thank Dr. F. AZAM for the isolation of the developing valve preparations. Dr. L. DYCK for help given in C-Pt replica preparation, to Dr. H. BAUER and Dr. H. MATSUDO, for preparing the freeze-fracture replicas, and to JUTA KIETHE for the technical assistance. This study was supported by grant GM-08229-13, 14, 16 from the National Institutes of Health, USPHS.

References

AZAM, F., B. B. HEMMINGSEN, and B. E. VOLCANI, 1974: Role of silicon in diatom metabolism. V. Silic acid transport and metabolism in the heterotrophic diatom *Nitzschia alba*. Arch. Microbiol. **97**, 103—114.

CACHON, P. J., et M. CACHON, 1972: Les modalites du dépôt de la silice chez les radiolaires. Arch. Protistenk. **114**, 1—13.

CHIAPPINO, M. L., F. AZAM, and B. E. VOLCANI, 1977: Effect of germanic acid on developing cell walls of diatoms. Protoplasma **93**, 191—204.

DARLEY, W. M., and B. E. VOLCANI, 1971: Synchronized cultures: diatoms. In: Methods in enzymology (SAN PIETRO, A., ed.), Vol. XXIII, Part A, pp. 85—96. New York: Academic Press.

DARRAGH, P. J., A. J. GASKIN, B. C. TERRELL, and J. V. SANDERS, 1966: Origin of precious opal. Nature **209**, 13—16.

DAWSON, P., 1973: Observations on the structure of some forms of *Gomphonema parvulum* Kutz. III. Frustule formation. J. Phycol. **9**, 353—365.

DOGGENWEILER, C. F., and J. E. HEUSER, 1967: Ultrastructure of the prawn nerve sheaths. Role of fixative and osmotic pressure in vesiculation of thin cytoplasmic laminae. J. Cell Biol. **34**, 407—420.

GEITLER, L., 1963: Alle Schalenbildungen der Diatomeen treten als Folge von Zell- oder Kernteilungen auf. Ber. dts. bot. Ges. **75**, 393—396.

HECKY, R. E., K. MOPPER, P. KILHAM, and E. T. DEGENS, 1973: The amino acid and sugar composition of diatom cell walls. Mar. Biol. **19**, 323—331.

ILER, R. K., 1955: The colloid chemistry of silica and the silicates. New York: Cornell Univ. Press.

JONES, L. H. P., A. A. MILNE, and J. V. SANDERS, 1966: Tabashir: an opal of plant origin. Science **151**, 464—466.

KAMATANI, A., 1971: Physical and chemical characteristics of biogenous silica. Mar. Biol. **8**, 89—95.

LAURITIS, J. A., J. COOMBS, and B. E. VOLCANI, 1968: Studies on the biochemistry and fine structure of silica shell formation in diatoms. IV. Fine structure of the apochlorotic diatom *Nitzschia alba* Lewin and Lewin. Arch. Mikrobiol. **62**, 1—16.

LOWENSTAM, H. A., 1971: Opal precipitation by marine gastropods (*Mollusca*). Science **171**, 487—490.

NAKAJIMA, T., and B. E. VOLCANI, 1969: 3,4-Dihydroxyproline: a new amino acid in diatom cell walls. Science **164**, 1400—1401.

— 1970: ε-N-trimethyl-L-δ-hydroxylysine phosphate and its nonphosphorylated compound in diatom cell walls. Biochem. biophys. Res. Commun. **39**, 28—33.

PICKETT-HEAPS, J. D., K. L. MCDONALD, and D. H. TIPPIT, 1975: Cell division in the pennate diatom *Diatoma vulgare*. Protoplasma **86**, 205—242.

REIMANN, B. E. F., 1964: Deposition of silica inside a diatom cell. Exp. Cell Res. **34**, 605—608.

— J. C. LEWIN, and B. E. VOLCANI, 1965: Studies on the biochemistry and fine structure of silica shell formation in diatoms. I. The structure of the cell wall of *Cylindrotheca fusiformis* Reimann and Lewin. J. Cell Biol. **24**, 39—55.

— 1966: Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. The structure of the cell wall of *Navicula pelliculosa* (Brèb.) Hilse. J. Phycol. **2**, 74—84.

REYNOLDS, E. S., 1963: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208—212.

ROSENBLUTH, J., 1963: Contrast between osmium-fixed and permanganate-fixed toad spinal ganglia. J. Cell Biol. **16**, 143—157.

ROUND, F. E., 1972: The formation of girdle, intercalary bands and septa in diatoms. Nova Hedwigia XXIII, 449—463.

SIMPSON, T. L., and C. A. VACCARO, 1974: An ultrastructural study of silica deposition in the freshwater sponge *Spongilla lacustris*. J. Ultrastruct. Res. **47**, 296—309.

STOERMER, E. F., H. S. PANKRATZ, and C. C. BOWEN, 1965: Fine structure of the diatom *Amphipleura pellucida*. II. Cytoplasmic fine structure and frustule formation. Amer. J. Bot. **52**, 1067—1078.

STOSCH, H. A. v., and K. KOWALLIK, 1969: Der von L. GEITLER aufgestellte Satz über die Notwendigkeit einer Mitose für jede Schalenbildung von Diatomeen. Beobachtungen über die Reichweite und Überlegungen zu seiner zellmechanischen Bedeutung. Österr. bot. Z. **116**, 454—474.

SULLIVAN, C. W., 1976: Diatom mineralization of silicic acid. I. $Si(OH)_4$ transport characteristics in *Navicula pelliculosa*. J. Phycol. **12**, 390—396.

— 1977: Diatom mineralization of silicic acid. II. Regulation of $Si(OH)_4$ transport rates during the cell cycle of *Navicula pelliculosa*. J. Phycol. **13**, 89—91.

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