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The origin of the silica deposition vesicle of diatoms

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Abstract. The origin of the silica deposition vesicle (SDV) in a centric diatom, *Ditylum brightwellii*, has been investigated using transmission electron microscopy and inhibitors, to study the involvement of the endoplasmic reticulum, Golgi apparatus, and microfilaments in the origin and organization of the SDV. Dividing cells contain enough small vesicles to build the SDV to about one half of its final size; inhibition of protein glycosylation in the endoplasmic reticulum and Golgi apparatus prevents the further expansion of the half-formed SDV; and microfilaments are involved in organizing the small vesicles to form the SDV.

Key words: *Ditylum brightwellii*; Endoplasmic reticulum; Golgi apparatus; Microfilaments; Silica deposition vesicles.

Introduction

Siliceous structures are formed in a great variety of protista including diatoms and protozoa ranging from flagellates to sarcodinids. They are produced within the lacuna of intracellular silica deposition vesicles (SDVs) and are expelled when mature (for a review, see Simpson and Volcani, 1981). In a testate amoeba, *Netzlia tuberculata*, small vesicles secreted by the Golgi apparatus with distinct fibrilar contents fuse with each other or with digestive vacuoles to form the SDV (Anderson, 1988). In centric diatoms, as silica deposition progresses, the SDV gradually expands by the coalescence of small vesicles (Dawson, 1973; Schmid and Schulz, 1979; Schnepf *et al.*, 1980; Li and Volcani, 1984, 1985a). Since the small vesicles in diatoms are usually translucent and have no distinctive morphological characteristics, their origin has been controversial. Thus it has been suggested that the SDV (or the small vesicles) in diatoms may derive from Golgi apparatus activity (Reimann *et al.*, 1966; Dawson, 1973; Schnepf *et al.*, 1980), or possibly from the endoplasmic reticulum (Dawson, 1973; Hoops and Floyd, 1979), or even from a combined interaction between Golgi vesicles, endoplasmic reticulum and plasmalemma (Chiappino and Volcani, 1977). Pickett-Heaps *et al.* (1975) have pointed out that there is no convincing evidence for the involvement of the Golgi apparatus and endoplasmic reticulum in diatom wall formation and suggested that the SDV is equivalent to a giant highly evolved "Golgi" vesicle which can expand itself.

Furthermore there is no information about how the small vesicles are organized to form the SDV. The fact that they always fuse to the expanding margin of the SDV, not its ventral side (Li and Volcani, 1985a), indicates that they are controlled by a well organized system. Recently we successfully labeled the SDV of a centric diatom, *Ditylum brightwellii* (West) Grunow with a fluorescent dye, rhodamine 123 (Li *et al.*, 1989); however, the small vesicles are too small to be seen in fluorescence microscopy, making it impossible to trace their origin and transportation.

In the present study morphological changes and secretory activities of the Golgi apparatus during the cell cycle of *D. brightwellii* were examined by transmission electron microscopy; the relationships among the SDV, Golgi apparatus, and endoplasmic reticulum were

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determined using the inhibitors, 1-deoxynojirimycin and swansonine; and the involvement of microfilaments in SDV formation was determined using the microfilament inhibitor, cytochalasin B.

Meterials and Methods

Cell Culture

A unialgal culture of *D. brightwellii* was maintained in F/2 medium (McLachlan, 1973) under cool-white fluorescent light (55-60 microeinsteins m^{-2} sec^{-1}) at 18-20°C, and partial synchronization of cell division was achieved in an 8 hours light: 16 hours dark cycle as described by Eppley *et al.* (1967).

Inhibitor Treatments

For each experiment, 20 cells with embryonic valves at various developing stages were selected for the inhibitor treatment. Each inhibitor treatment dish contained 4 ml of F/2 medium with one of the following inhibitors: 1-deoxynojirimycin (0.5, 0.8, or 1.2 mg mL^{-1}), swansonine (0.25, 0.5, or 1.0 μg mL^{-1}), and cytochalasin B (0.5, 1.5, or 3.0 μg mL^{-1}). At intervals, the progress of valve developments was examined under an inverted differential interference contrast microscope. After 30, 45, and 60 min of incubation, aliquots of the cells were transferred to 4 ml of fresh normal culture medium and allowed to recover from the drug treatment.

Electron Microscopy

For scanning electron microscopy, cells were killed with 1N HCI and boiled in 50% sulphuric acid for 10 min on a depression slide. The embryonic valves were then transferred to an open Beam capsule and rinsed with distilled water. After drying, the Nuclepore filter with the embryonic valves was attached to an aluminum stage, coated with gold in a sputter coater, and examined in a Hitachi S-2300 electron microscope.

For transmission electron microscopy, cells were fixed in 1% glutaraldehyde and 0.2% OsO_4 simultaneously (Tippit and Pickett-Heaps, 1977) in F/2 medium for 20 min at room temperature, dehydrated in a continuous ethanol series, and flat-embedded in Spurr's resin (Spurr, 1969). Sections were cut with a diamond knife, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined in a Hitachi H-600-3 electron microscope.

Results

The exponentially growing cells of *D. brightwellii* divided once per day in the dark light cycle, Prior to mitosis, the central cytoplasmic mass with the nucleus migrates laterally to the midgirdle region and stays there for about one hour. Cytokinesis takes about 4-6 min, and valve formation is usually completed within 1.5 h. The sequential formation of the SDV and wall components in this diatom has already been described in detail (Li and Volcani, 1985a).

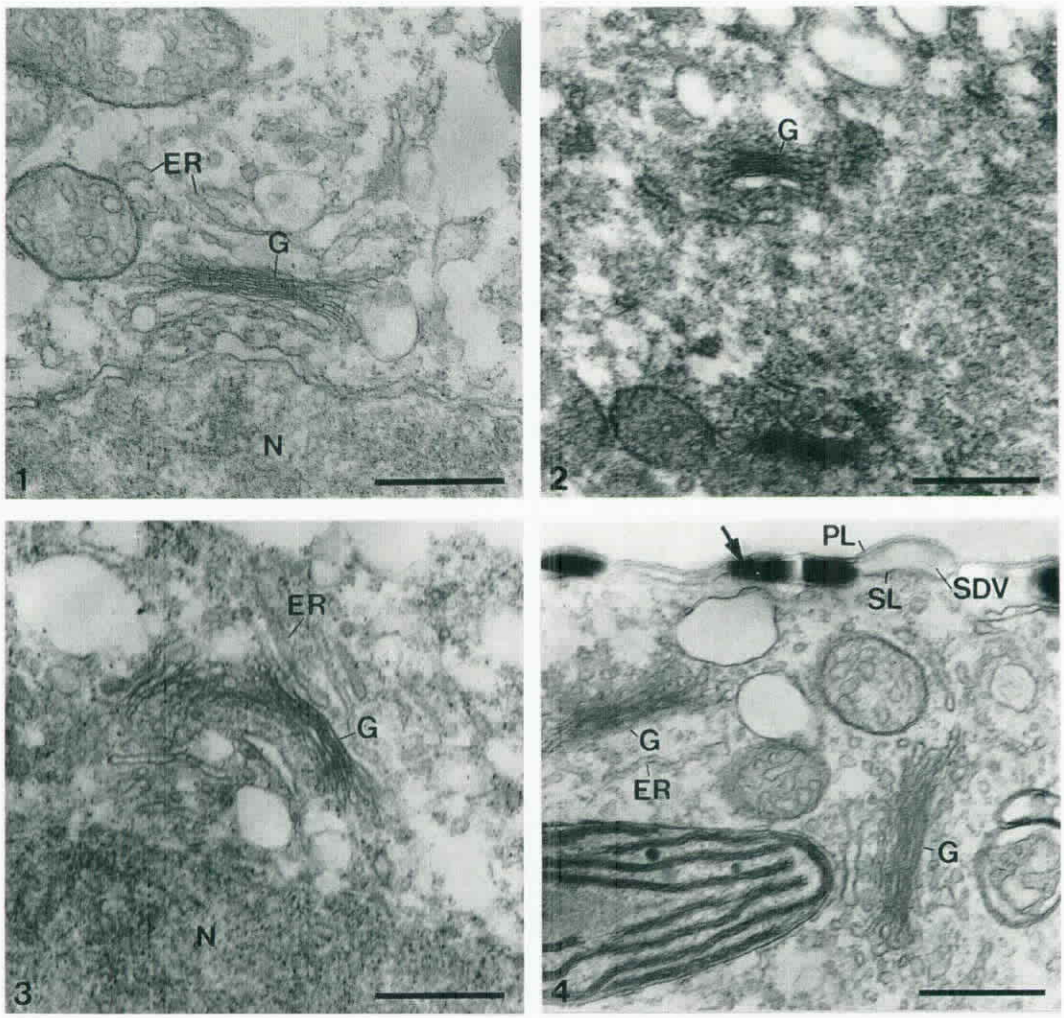
Variations of Golgi Apparatus in the Cell Cycle

In the interphase cells all the Golgi apparatus, about 50 in total, are evenly distributed over the surface of the nucleus. Each Golgi apparatus usually consists of 5 cisternae, about 1 μm in diameter, and the distance between its *cis* face and the nuclear envelope is about 0.1 μm (Fig. 1). Prior to mitosis the Golgi apparatus moves with the migrating nucleus to the mid-girdle region. During mitosis the Golgi apparatus and endoplasmic reticulum partially fragment, and each Golgi apparatus becoming only 0.3 μm in diameter (Fig. 2). The reassembly of the Golgi apparatus starts during telophase and the reformation of the interphase Golgi apparatus is completed prior to the completion of cytokinesis (Fig. 3); however, most of them remain irregularly dispersed until the new valve is half-formed (Fig. 4).

Since the size of the small vesicles which coalesce with the SDV is fairly constant (30-40 μm in diameter) at all stages of valve formation in *D. brightwellii,* we counted the number of these vesicles that appeared in the vicinity of each Golgi apparatus during the cell cycle. The data were obtained from serial section analysis of cells at various stages (Table 1, Figs. 1-4), and two cells were selected for each stage.

1-deoxynojirimycin Inhibits Silicification

1-deoxynojirimycin, a derivative of nojirimycin and a specific inhibitor of the endoplasmic reticulum, inhibits the α-glucosidase that participates in glycoprotein processing and catalyses the cleavage of glucose units from $Glc_3Man_9GlcNac_2$ side chain of glycoproteins in the endoplasmic reticulum (Gross *et al.,* 1983; Saunier *et al.,* 1982). In 0.5 mg mL^{-1} 1-deoxynojirimycin, the valves of the treated post-telophase cells



Figs. 1–4. Sections of *D. brightwellii* at different stages in the cell cycle in TEM, showing the Golgi apparatus (G) lies close to the nucleus (N) in interphase (Fig. 1), a remainder of the fragmented Golgi apparatus in mitosis (Fig. 2), reassembling Golgi apparatus during cytokinesis (Fig. 3), and two irregularly dispersed Golgi apparatus when the new valve (arrow) is about one third of its final size (Fig. 4); ER, endoplasmic reticulum; PL, plasmalemma; SL, silicalemma; SDV, silica deposition vesicle. Scale bar = 0.5 μm.

Table 1. *The estimated number of the small vesicles near each Golgi apparatus at various stages in the cell cycle of* ***Ditylum brightwellii***

Cell stage	Number of small vesicles/Golgi apparatus
Interphase	50
Anaphase	40
Cytokinesis	90
Half valve is formed	70

that just completed cytokinesis were formed as fast as those in the normal culture (Fig. 5). In 0.8 mg mL^{-1} 1-deoxynojirimycin , valve formation in post-telophase cells and developing cells with tiny embryonic valves (Fig. 6) progressed slowly and stopped at about one half of the mature size after 2 h (Figs. 5, 7), whereas valve formation in the developing cells with embryonic valves larger than half of the mature size could not proceed. In 1.2 mg mL^{-1} 1-deoxynojirimycin, valve formation was stopped in cells at any stage of development and the cells lysed within 1.5 h (Fig. 5).

If the inhibitor containing medium (0.8 mg mL^{-1} 1-deoxynojirimycin) was removed and replaced with fresh normal medium within 30 min, embryonic valves of the treated cells could expand to their final size at a normal rate (Fig. 8). Once the incubation time exceeded

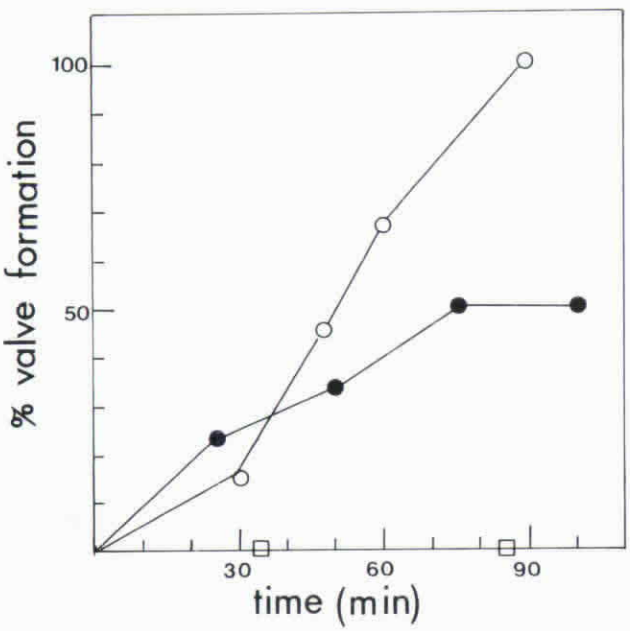


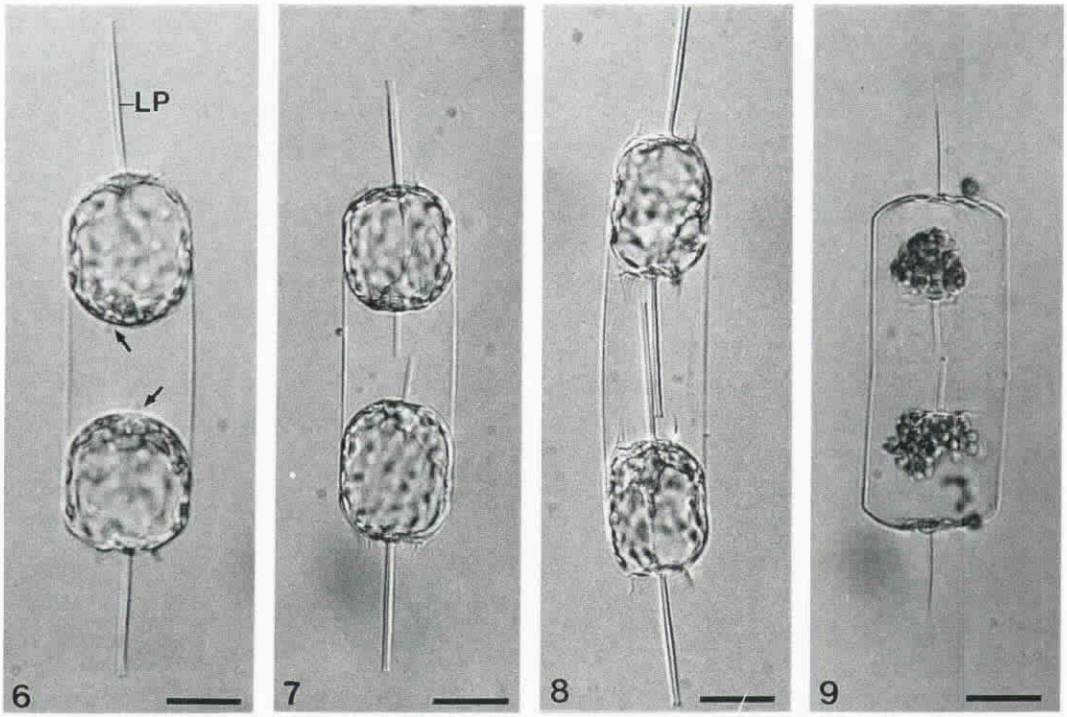
Fig. 5. Valve formation of post-telophase cells incubated in 1-deoxynojirimycin (○, 0.5 mg mL^{-1}; ●, 0.8 mg mL^{-1}; □, 1.2 mg mL^{-1}).

30 min, however, the inhibitor-treated cells could not recover and usually lysed within 2.5 h (Fig. 9).

Swansonine Inhibits Silicification

Swansonine is used as an inhibitor of Golgi mannosidase II in the biosynthesis of complex glycoproteins (Kang and Elbein, 1983; Tulsiani *et al.*, 1982; Tulsiani and Touster, 1983). In 0.25 μg mL^{-1} or 0.5 μg mL^{-1} swansonine, valve formation in post-telophase cells and developing cells with tiny embryonic valves progressed slower than in controls and stopped at about one half of the mature size; whereas valve formation in cells where the embryonic valves were already nearly half the mature size was almost completedly arrested (Fig. 10). Silicification was severely inhibited in 1.0 μg mL^{-1} swansonine: both in post-telophase cells and in developing cells with tiny embryonic valves. Valve formation progressed very slowly and stopped at about one third of the mature size after 1.5 h incubation (Fig. 11). All the treated cells lysed within 2.5 h.

If the inhibitor-containing medium (0.25 μg mL^{-1} or 0.5 μg mL^{-1}) swansonine was removed and replaced



Figs. 6–9. Sibling cells treated with 1-deoxynojirimycin in differential interference contrast microscopy. Scale bar = 25 μm. Fig. 6. Sibling cells with developing embryonic valves, showing a minute outer tube (arrows) of the labiate process (LP) is just formed; Fig. 7. New valves of the sibling cells incubated in 0.8 mg mL^{-1} 1-deoxynojirimycin can only reach half of their final size; Fig. 8. Recovered sibling cells in fresh normal medium; Fig. 9. Lysed sibling cells after prolonged treatment.

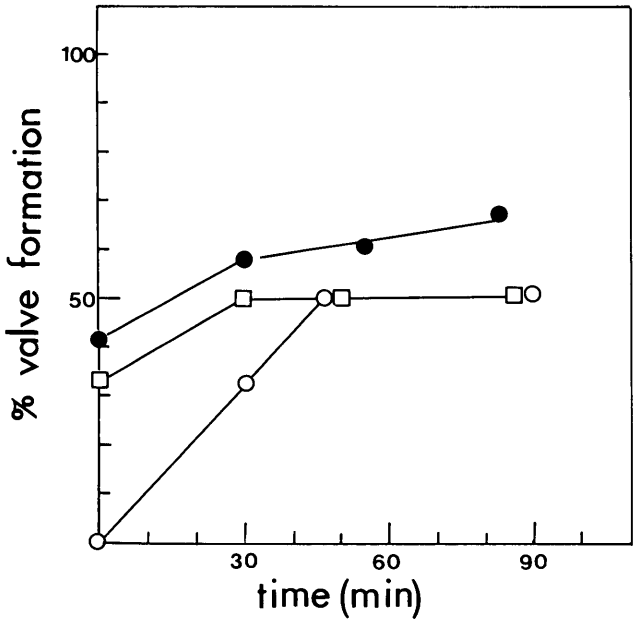


Fig. 10. Valve formation of post-telophase cells (○) and of cells with small embryonic valves (□, ●) incubated in 0.5μg mL^{-1} swansonine.

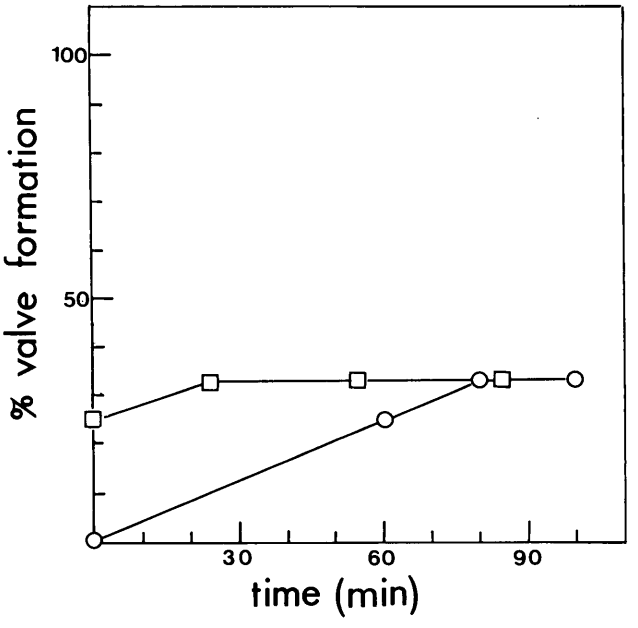


Fig. 11. Valve formation of post-telophase cells (○) and of cells with small embryonic valves (□) incubated in 1.0 μg mL^{-1} swansonine.

with fresh normal medium within 30 min, embryonic valves of the treated cells resumed expansion to their final size at a normal speed. Once the incubation time exceeded 30 min, however, the inhibitor-treated cells could not recover and lysed in 2.5 h.

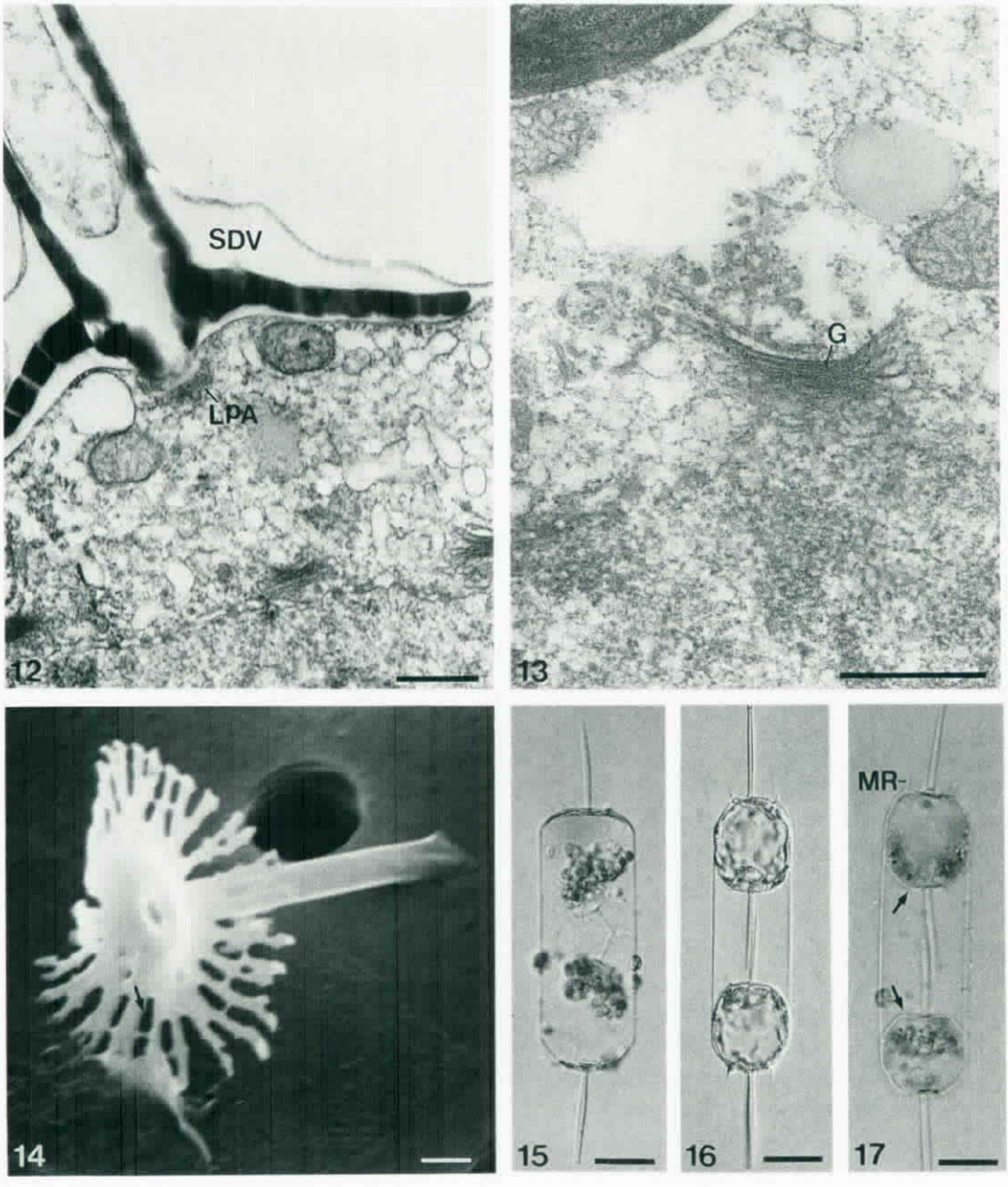
Thin sections of cells incubated in 0.5 μg mL^{-1} swansonine for 1 h, showed that the embryonic valves were still enclosed in the SDV (Fig. 12). The thickness of deposited silica was about one half of its final thickness and the characteristic hexagonal columns, a special morphological form of deposited silica recorded only from this diatom (Li and Volcani, 1985a), were barely visible. The labiate process apparatus, an organelle associated with labiate process formation, was still closely attached to the silicalemma (Fig. 12). The distribution of the Golgi apparatus in the treated cells was similiar to those in the normal developing cells; however, the cisternae were more flattened and many fewer small vesicles were present in the vicinity of the Golgi apparatus (Fig. 13). Scanning electron microscopy of the isolated developing valve of a treated cell showed that the rotae in the poroid areolae were not formed (Fig. 14).

Cytochalasin B Inhibits Silicification

Newly separated sibling cells, at all stages of valve formation, stopped expanding their new valves immediately in 1.5 μg mL^{-1} cytochalasin B and were usually lysed within 1.5 h (Fig. 15). Valve formation could recover in fresh normal medium, but the speed of valve formation was much slower, taking about 3-4 h, and the new valves were usually aberrant with no marginal ridges (Figs. 16, 17).

Discussion

In eukaryotic cells components of organelles that can not synthesize their own proteins must either be imported directly from the cytosol or transported through the endoplasmic reticulum and the Golgi apparatus by transport vesicles (Sabatini *et al.*, 1982; Kelly, 1985). It is generally agreed that the SDV in centric diatoms expands through the coalescence of small vesicles and hence the lipids required to make the silicalemma may well be carried by these vesicles. In the present study core glycosylation in the endoplasmic reticulum and terminal glycosylation in the Golgi apparatus were inhibited by 1-deoxynojirimycin and swansonine



Figs. 12–13. Sections of a cell treated with 0.5 μg mL^{-1} swansonine for 1 h, in TEM, showing that the half-formed new valve is still enclosed in the SDV (Fig. 12), and that cisternae of the Golgi apparatus (G) are thinner than normal (Fig. 13); LP labiate process; LPA labiate process apparatus. Scale bar = 0.5 μm.

Fig. 14. An isolated new value formed in 0.5 μg mL^{-1} swansonine, in SEM, showing the lack of rotae in the poroid areolae (arrow). Scale bar = 1 μm.

Figs. 15–17. Sibling cells treated with 1.5 μg/mL cytochalasin B in differential interference contrast microscopy, showing lysed cells with incomplete new valves after prolonged treatment (Fig. 15); and valve formation recovery with transfer to fresh normal medium (Figs. 16, 17). Parts of the cytoplasmic contents of the sibling cells in Fig. 17, were removed by 2% SDS to show the new valves with no marginal ridges (arrows); MR, marginal ridge. Scale bar = 25 μm.

respectively, resulting in the termination of SDV expansion and of silicification. As an organelle, the SDV may have its protein components transport either from the cytosol or from fusing vesicles. The results clearly indicate that the protein components of the SDV are delivered by the small vesicles, and that these small vesicles are derived from the Golgi apparatus. Glycosylation of the silicalemma-related proteins might be a key point of their subsequent packing and budding processes. The present data provide no clues as to the site of biosynthesis of the mannose-containing polysaccharide that may be involved in silica deposition in the SDV of *D. brightwellii* (Li *et al.*, 1989).

In most cells the Golgi apparatus and the endoplasmic reticulum break up into smaller fragments and vesicles during mitosis (Lucocq and Warren, 1987). For a protein that has already reached the endoplasmic reticulum, further glycosylation may take about half an hour or up to several hours to reach its final destination (Palade, 1975; Kelly, 1985) and the secretory activities of the Golgi apparatus are greatly inhibited during mitotic metaphase (Warren, 1983). In centric diatoms, including *D. brightwellii,* SDV formation and silicification start before the completion of cytokinesis (Li and Volcani, 1985a, b, c), and in *Attheya decore* West the SDV is present even before the spindle has disappeared completely (Schnepf *et al.*, 1980). Fragmentation of the Golgi apparatus and endoplasmic reticulum during mitosis was also observed in *D. brightwellii.* It seems impossible that the endoplasmic reticulum and Golgi apparatus could have time, following their reassembly at the end of mitosis, to form the small vesicles in time to initiate the SDV. A more plausible model is that enough small vesicles have been formed prior to or during mitosis to build about half the SDV, and these vesicles are prevented from fusing with each other until the concentration of an unknown cytoplasmic messenger is altered.

In normal cell wall formation, components of the valve are always formed according to a determined sequence (Schmid and Volcani, 1983; Li and Volcani, 1985a, b). It is intriguing to know whether this sequence can be selectively bypassed or altered. Developing cells treated with microtubule inhibitors produce aberrant valves, which vary in form from one another species and usually lack certain valve components. The results show that parts of the sequence can be selectively omitted without affecting the progress of silicification (Li and Volcani, 1984). However, in the present study developing valves of the 1-deoxynojirimycin and swansonine treated cells do not thicken and their rotae are not formed, once SDV expansion is inhibited. The results imply that the biochemical environment of silica deposition in the SDV changes during silicification, possibly as a result of the addition of material from coalescing small vesicles. A previous study has also shown that the composition of the matrix in the SDV can change during valve formation since in the same valve component the presence of mannose varies with the degree of silicification (Li *et al.*, 1989).

The marked tendency of the small vesicles to accumulate at the growing edges of the SDV draws attention to the specific SDV-cytoskeleton-small vesicles interactions that must occur in diatoms. Microtubules usually play a major role in directing the secretory vesicles in regulated polarized cells (Kelly, 1985). However, in centric diatoms microtubules are only known to be associated with the formation of these wall components that involve the development and maintenance of cytoplasmic protrusions during silicification (Li and Volcani, 1984), thus a direct relationship between microtubules and the small vesicles is unlikely. The fact that the SDV expansion stops immediately in the cytochalasin B treated cells of *D. brightwellii* at all developing stages and that valve formation can be recovered after the removal of the drug, strongly suggest that microfilaments, instead of microtubules, are involved in transporting or organizing the small vesicles to form the SDV.

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矽藻矽沈積囊胞的起源

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本篇論文探討中心型矽藻 *Ditylum brightwellii* 矽沈積囊胞 (SDV) 的起源。我們使用穿透電子顯微鏡術及抑制劑研究其源自內質網及高氏體的可能性，並了解 Microfilaments 在 SDV 形成時所扮演的角色。由實驗證明，在細胞分裂之初，子細胞內即含有足以形成半個 SDV 的小囊胞。發育中的細胞經抑制劑處理，其分別影響內質網及高氏體內的蛋白醣化作用，SDV 即不能繼續擴張，其內的矽沈積也被中斷。顯示 SDV 的囊膜及醣化蛋白均源自內質網及高氏體，此外，Microfilaments 也可能參與了小囊胞組聚爲 SDV 的工作。