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Shigeki Mayama · Asuka Kuriyama

Diversity of mineral cell coverings and their formation processes: a review focused on the siliceous cell coverings

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Abstract Mineral cell coverings are found in various protists. Some macroalgae accumulate calcium carbonate in the intercellular space, and some unicellular organisms use calcium carbonate or silica for the construction of loricas, scales, and frustules. Diatoms are representatives of those utilizing silica for the material of the cell covering called a frustule. The development of the frustule is initiated in a silica-deposition vesicle (SDV), which occurs just beneath the plasma membrane and, subsequently, the silicified cell covering expands its area, following the expansion of the SDV from valve face to valve mantle. Sequential valve development with whole valves is reviewed in several diatoms placed in different phylogenetic positions. Every diatom commences its valve formation from its pattern center and then develops by means of individual procedures. The results indicate that the valve development reflects the phylogeny of diatoms. In addition, recent progress in silica biomineralization is briefly reviewed, and the phylogeny of ability concerning siliceous cell covering formation is inferred.

Key words Cell covering formation · Diatom · Mineral cell covering · Phylogeny · Silica biomineralization · Valve ontogeny

Introduction

Among protists, there are many kinds of organisms with mineral cell coverings. Along a rocky beach we can easily find calcareous red algae such as Corallinales, in which calcium carbonate accumulates in the intercellular space (Miyata et al. 1980; Okazaki et al. 1982). Similar calcareous walls are also known in green and brown algae, e.g., *Halimeda* (Borowitzka and Larkum 1977), *Acetabularia* (Okazaki and Katsumi 1984) and *Padina* (Okazaki et al. 1985). These seaweeds accumulate calcium carbonate outside of the organic cell wall, namely, in the intercellular space. In contrast to the macroalgae, some micro algae bear the mineral cell covering on or beneath the plasmalemma. The materials of the mineral covering are usually calcium carbonate, silica, manganese, or iron. The surface of the haptophyte cell may be covered with calcified scales or coccoliths, which are originally formed in the Golgi apparatus and then secreted from the cell (Okazaki and Furuya 1985). The cell coverings of some euglenoid algae, such as *Trachelomonas*, are impregnated with iron and/or manganese salts (Bold and Wynne 1978). Among the protists, however, the most frequently employed material of cell covering is silica.

S. Mayama (✉) · A. Kuriyama
Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo, 184-8501 Japan
Tel. +81-42-3297524; Fax +81-42-3297737
e-mail: mayama@u-gakugei.ac.jp

Diversity of siliceous cell coverings

Siliceous cell coverings are widely distributed in the Protista and show various types of morphology depending on the taxonomic group. Among the algae, they are found specifically in several groups of Heterokontophyta (Van den Hoek et al. 1995). Diatoms (Bacillariophyceae), which are representative of organisms with a siliceous cell covering, have a box-like covering called a frustule composed of two valves and many bands (copulae and pleurae). There are various shapes of the frustule ornamented by the sophisticated patterns of fine valve structure. The surface of the synurophycean cells is covered with delicately sculptured siliceous scales, on which long spine-like projections are often borne. Species of Parmales show a cell covering consisting of siliceous plates, which resemble the scales occurring on the surface of diatom auxospores (Mann and Marchant 1989), but the class to which this order belongs is still unknown because of a lack of successful cultivation. The cells of Dictyochophyceae, except species of the so-called Pedinellophyceae, have a basket-like siliceous skeleton with spinose projections. The skeleton is tubular and placed

outside of the plasmalemma, although the main part of the skeleton is contained inside an extensive viscous envelope. The cells of Chrysophyceae and Raphidophyceae are naked, but some species are known to form a siliceous cyst. In addition, the cyst formed by some species of Xanthophyceae is impregnated with silica. However, other classes of the Heterokontophyta, i.e., Phaeophyceae, Eustigmatophyceae, Pelagophyceae, Pedinellophyceae, and Bolidophyceae, do not have any siliceous cell covering, though the Bolidophyceae is demonstrated to be a sister group to the diatoms in the phylogenetic tree based on the small subunit coding region of ribosomal DNA (SSU rDNA; Guillou et al. 1999). Colorless heterokontophyta or heterotrophic stramenopiles (Patterson 1989), which branched off earlier than the autotrophic stramenopiles (Leipe et al. 1994; Van der Auwera et al. 1995), also do not have any siliceous cell covering.

The formation process of the siliceous cell coverings

The studies of the formation of siliceous cell covering have proceeded more vigorously in diatoms than in other organisms, although some studies were carried out in *Mallomonas* (Synurophyceae). The siliceous scales of *Mallomonas* are formed in specialized vesicles called silica deposition vesicles (SDV), which are closely appressed to the outer membrane of the chloroplast endoplasmic reticulum, and then are secreted to the outside of the plasmalemma (Wujek and Kristiansen 1978; McGrory and Leadbeater 1981; Mignot and Brugerolle 1982; Beech et al. 1990). The cytoskeletal elements are involved in this process (Mignot and Brugerolle 1982; Brugerolle and Bricheux 1984). High molecular mass glycoproteins are associated with the adhesion of the individual components making up the scale case (Ludwig et al. 1996).

In diatoms, the valve formed subsequent to cytokinesis commences in the SDV, which is small at the beginning and lies just beneath the plasmalemma (Reimann 1964; Dawson 1973; Chiappino and Volcani 1977; Pickett-Heaps et al. 1979; Schmid and Schulz 1979). The microtubule center is observed to be associated with the initiation of the SDV in pennate diatoms (Pickett-Heaps et al. 1979; Pickett-Heaps et al. 1990), although it is not essential in all species. Experiments with anti-microtubule drugs have induced teratology around the raphe area in some pennate diatoms, depending on the drug concentration (Schmid 1980). The SDV gradually expands towards the valve mantle and further silica accumulation takes place. In various pennate diatoms, microfilaments are associated with the outgrowing edge of the SDV (Edgar and Pickett-Heaps 1984). Treatment with the drug cytochalasin D has suggested that an actin-dependent system may be involved in the lateral movement of the forming valve in *Hantzschia* (Cohn et al. 1989). In a centric diatom, *Coscinodiscus*, it is demonstrated that the interaction among the plasmalemma, endoplasmic reticulum, Golgi vesicle, fibrous material, and a special vesicle denoted as the areola vesicle, is involved in valve formation (Schmid 1986). Recently, Sumper (2002) described a nanopatterning model, in which repeated phase separation events by the fragmentation of polyamine-containing droplets during valve formation were assumed to produce a hierarchy of self-similar patterns in the sieve plates of *Coscinodiscus*. However, there is still a long way to go before it is fully understood how elaborate valves are controlled in the morphogenesis of the diatoms.

Valve ontogeny in diatoms

Usually ontogeny is a subject for multicellular organisms, but it could also be applied to some unicellular protists. The coccoliths of haptophytes show sequential formation of their components (Young et al. 1992), and the diatoms bear new valves through accurately defined formation processes.

The valve of each diatom has its own pattern of aligned areolae, which are pores penetrating the valve and are occluded by a delicate sieve plate with finer perforations or slits at one side of the opening. Between the regularly aligned areolae is a solid, siliceous rib called an interstria or virga (Cox and Ross 1981). Although each virga is sometime bifurcated distally, they are connected proximally to a ring, or a strip called an annulus or a sternum, respectively; in particular, the sternum combined with the raphe slit, which extends between both poles, is called the raphe sternum. Because each virga looks to grow from the annulus in the centric diatoms or the sternum in the pennate, these areas are called pattern centers (Mann 1984). In fact, sequential valve formation commences from a pattern center in many of the diatoms examined.

Both centric diatoms *Ditylum* and *Chaetoceros* form a siliceous annulus at first and then extend radial ribs centrifugally followed by their bifurcations (Li and Volcani 1985a, b; see also the diagrammatic representation in Fig. 1a). Vertical differentiation of the forming valve follows the horizontal differentiation and, subsequently, the valve is completed by the development of secondary horizontal differentiation in *Thalassiosira* and *Coscinodiscus* (Schmid and Schulz 1979; Schmid and Volcani 1983).

A pennate araphid diatom, *Fragilaria*, has a narrow linear sternum in the mature valve. However, although a single chain composed of several rings, which seem to be homologous to the annulus, appears associated with laterally developing virgae in the earlier stage, it fuses to make a linear sternum in the later stage; ultimately the delimitation of areolae by the frets arising between virgae completes the mature valve (Mayama, in preparation).

Eunotia is a pennate genus with a sternum and a characteristically short raphe, which branches away from the sternum, and is traditionally considered the most primitive of biraphid diatoms. Mann (1984), who inferred the scheme of raphe evolution, also placed the eunotioid stage somewhere between the araphid with labiate processes and the naviculoid with a mature raphe system. Our phylogenetic analyses also indicate that *Eunotia* is the first branched lineage in the raphid diatoms (Fig. 2). Our knowledge about the forma-

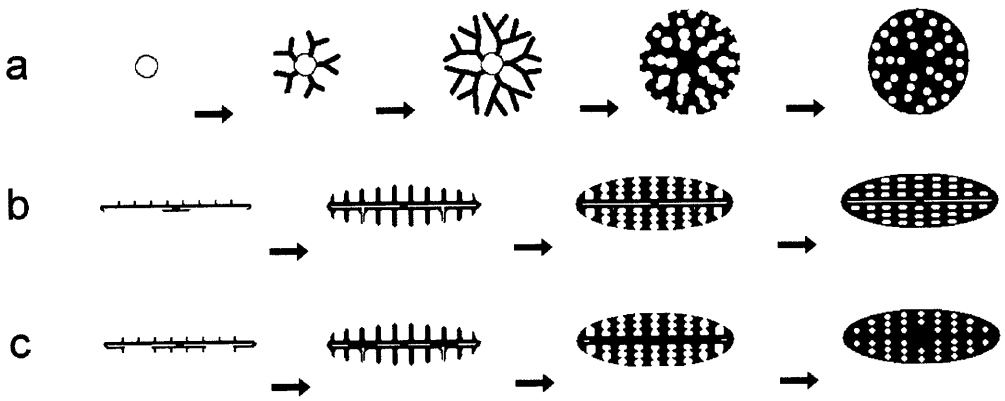

Fig. 1a–c. Diagrammatic representation of sequential valve development in diatoms. **a** Centric diatom: from annulus to radial furcated ribs to areolae formation. **b** Biraphid diatom or raphid valve of monoraphid diatom: from formation of primary side of raphe sternum through raphe formation to areolae formation. **c** Araphid valve of monoraphid diatom: from early formation of raphe slit, followed by its filling in, to areolae formation

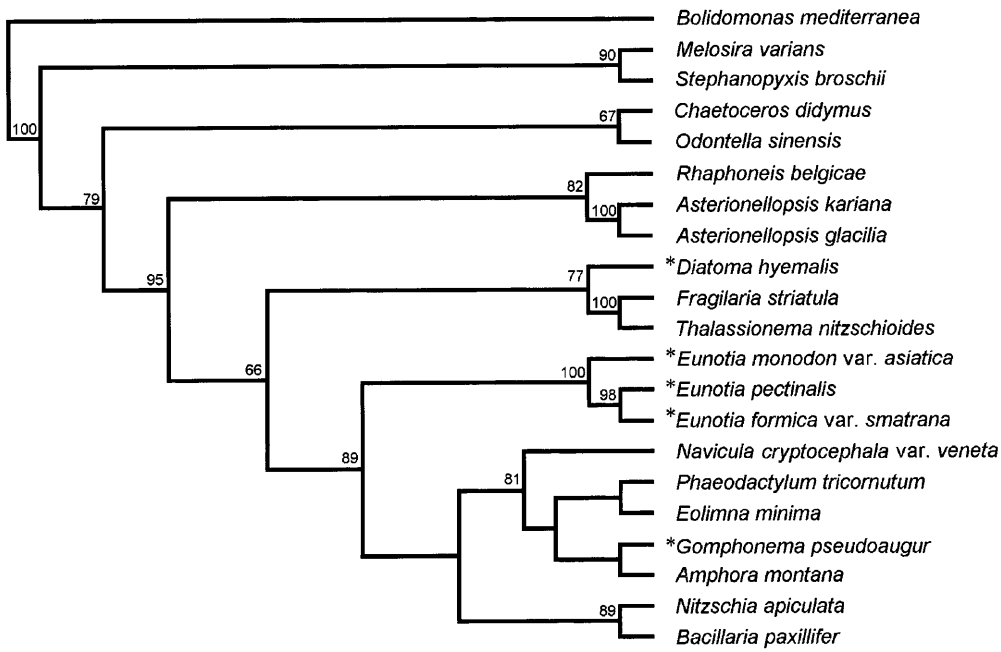

Fig. 2. Phylogeny of the diatoms from 18S rDNA sequence comparisons of 21 diatom taxa with *Bolidomonas mediterranea* as an outgroup. Sequence data are original here in species names with *asterisks*. The others are obtained from GenBank. Tree is inferred with the maximum parsimony computer program (DNAPARS, PHYLIP, Felsenstein 1993) using 1,554 aligned nucleotides. Only bootstrap values over 60% (1,000 replications) are given above the internal nodes. Note *Eunotia* is the first branching genus among raphid genera

tion of the eunotioid valve is still insufficient, but it has been elucidated that the forming valve with a sternum, which is similar to the araphid diatom in the stage shortly before maturation, is already developed prior to the formation of the raphe area. After completion of the primary side of the raphe rim by fusion of the developing virgae, the secondary side is formed by the fusion of two arms extending from both raphe endings (Fig. 3).

All biraphid diatoms except the eunotioid genera have a raphe sternum. Chiappino and Volcani (1977) demonstrated first the valve formation of the biraphid diatoms *Navicula pelliculosa*, though their material should have been identified as *Navicula atomus* (Mayama and Kobayasi 1988). In the earliest stage, the primary rib of the raphe sternum is deposited, followed by the reflexing of its ends at the poles and the centrifugal extension of the secondary arms from the central nodule (Fig. 1b). The reflexed primary rib and the secondary arms approach each other and fuse to complete the raphe system, as is seen in the eunotioid formation mentioned above. This stage is accompanied by the continued growth of the virgae. After the virgae reach the valve margin, the frets between the virgae develop to form the areolae. The formation processes of the virgae, areolae, and a central area are different in manner and order among biraphid genera (Cox 1999, 2001).

Monoraphid diatoms exhibit heterovalvy: one valve has the raphe (raphid valve), whereas the other is without (araphid valve). The developing pattern of the raphid valve is basically the same as that of biraphid diatoms (Fig. 1b), and yet the forming process of the araphid valve is not similar to that of araphid diatoms, but of the raphid diatoms (Fig. 1c). The araphid valve of *Achnanthidium saprophilum* (formerly *Achnanthes minutissima* var. *saprophila*) forms partial raphe branches in the early stage, but they are filled in later to complete the araphid valve (Mayama and Kobayasi 1989). The araphid valve of *Achnanthes coarctata* observed in cross section also shows a raphe slit, which is filled in later (Boyle et al. 1984). These facts indicate that

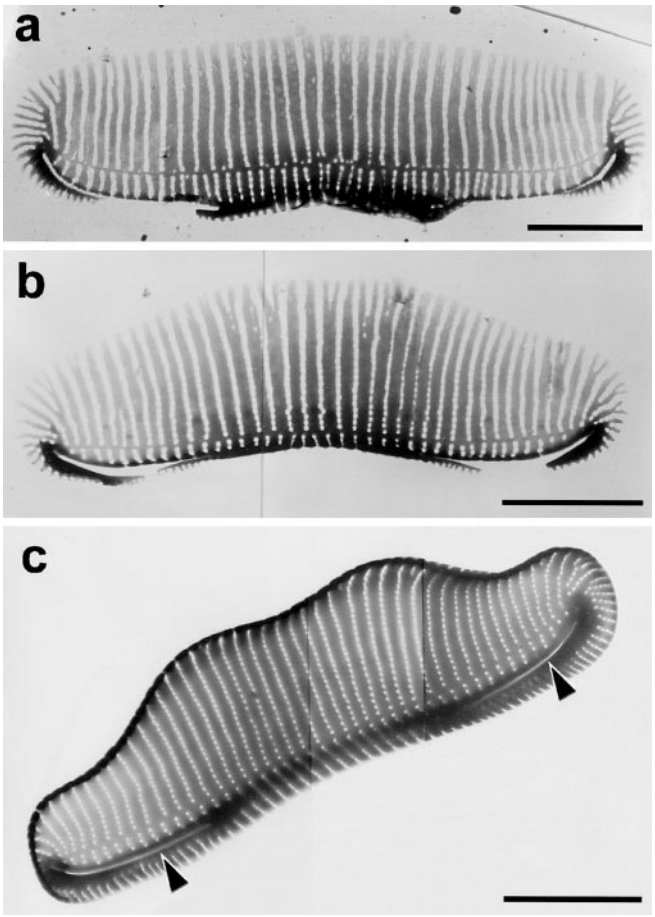

Fig. 3a–c. Sequential valve development of *Eunotia arcus* var. *bidens* in part. **a** Middle stage. Main part already formed shows features similar to araphid diatom. Primary side of the raphe area is not completed. **b** Middle/late stage. Primary side of the raphe area is completed by longitudinal fusion and arms are extending from raphe terminals to form secondary side. **c** Late stage. Completion of both sides of the raphe area and raphe slits (*arrow heads*), and delimitation of areolae by the frets arising between virgae. *Scale bars* = 5 µm

the araphid valve of the monoraphid diatoms is homologous to the raphid valve and also supports the speculation that monoraphid diatoms were derived from biraphid diatoms.

The order described above corresponds to the generally accepted order of diatom phylogeny in the bases of DNA sequence, morphology, and, in addition, fossil records if available. The ontogenetical study of diatoms indicates that the forming valves share some features with their ancestral groups in early developmental stages.

Progress in silica biomineralization research

According to a recent review by Scala and Bowler (2001), several proteins and genes for siliceous frustule formation of diatoms have been characterized. Both pleuralins (formerly HEPs) and silaffins are closely associated with the silica scaffold of the frustule and can be extracted with anhydrous hydrogen fluoride (Kröger et al. 1997, 1999, 2001; Kröger and Wetherbee 2000). Pleuralins are specifically localized around the overlapping zone of the epi- and hypocingulum, each of which is a set of pleurae (Kröger et al. 1997, 2001). Silaffins are polycationic proteins and classified into three peptides based on molecular mass. Silaffin-1A (4 kDa) and silaffin-1B (8 kDa) are proteolytically derived from a single gene denoted *SIL 1* (Kröger et al. 1999, 2001). Addition of silaffins to silicic acid solution can generate silica nanospheres within seconds in vitro (Kröger 1999). The size of the silica precipitant is dynamically controlled by the additional polyamines also extracted from diatom frustules (Kröger et al. 2000). Frustlins, calcium-binding glycoproteins, constitute the outer coat of the diatom wall, exhibit a ubiquitous distribution (Kröger et al. 1994, 1996, 1997), and appear to have a structural role in the casing of diatoms rather than a regulation of the silicification process (Van de Poll et al. 1999). The silicon transporter gene (*SIT*) family, identified from cDNAs isolated from *Cylindrotheca* (diatom), can specifically transport silica tetrahydroxide (Hildebrand et al. 1997, 1998). From the siliceous spicules of sponge, three similar subunits of proteins denoted Silicateins have been obtained (Shimizu et al. 1998).

Toward exploration of the origin of the siliceous cell covering

The algae bearing siliceous cell coverings are found in the Heterokontophyta, but not in other algal divisions. However, phylogenetic studies with SSU rDNA did not place them into monophyly (e.g., Saunders et al. 1997) because there are many other classes and orders without siliceous cell covering in the Heterokontophyta. Thus, algae groups with or without siliceous cell coverings are mosaically distributed in this division. Although the siliceous cell covering has not been found in the heterotrophic stramenopile, which is considered to be the ancestral host for the autotrophic heterokontophytes, there are other heterotrophic protists with siliceous cell coverings, i.e., filoseans, choanoflagellates, and polycystineans (one of the radiolarians). The filosean cells are covered with scaly tests, the choanoflagellates have spicular basket-like loricae, and the polycystinean cells have robustly skeletal shells. The molecular studies of SSU rDNA revealed that they belong to different lineages from the stramenopiles (Wainright et al. 1993; Bhattacharya et al. 1995; Zettler et al. 1997; Moon-van der Staay et al. 2001). Some other siliceous protists, such as a siliceous scale-bearing heliozoan (Kinoshita et al. 1995), have been also reported, but a large list by Bovee (1981) includes coverings of extraneous origin and also somewhat old and vague data.

There are multicellular organisms that deposit silica. Siliceous spicules of sponges may have some relationship to the lorica formation in choanoflagellates. Some land plants accumulate silica in tissues and vertebrates do so in bones, although they are not events in the cell covering. The organisms forming siliceous cell coverings are limited and

discontinuously distributed, regardless of autotrophy or heterotrophy. To interpret this situation, here we infer possibilities in the phylogeny of the ability to form a siliceous cell covering. Given that the types of covering are different, i.e., frustule, scale, plate, skeleton, and cyst, the ability to form the covering, which controls the whole shape, could have evolved independently within each taxon. As for the silica biomineralization in cell-covering formation, three scenarios are provided as follows.

1. All groups with a siliceous cell covering acquired the ability independently at different times. This could be possible in the heterotrophic protists, but seems rather unreasonable for the classes of Heterokontophyta in light of the principle of parsimonious evolution.

2. There was a common ancestor with the ability to form a siliceous cell covering. In the phylogenetic tree of SSU rDNA, Zettler et al. (1997) inferred the Polycystinea to be earlier organisms than the crown eukaryotes (Knoll 1992), whereas López-García et al. (2002) described it as a fast-evolving branch emerging in the apical region of the crown. In either phylogenetic tree, however, the first branching organism with siliceous cell covering is in the polycystinean lineage. If the ability of silica biomineralization in the cell covering was derived from the polycystinean lineage, a homologous gene concerning this event should be found in many lineages, as long as it has not been lost.

3. The heterokontophytes acquired the ability by horizontal gene transfer from the host within an endosymbiotic relationship. Though it is well known that many genes were transferred from the symbiont to the host nucleus during the organelle acquisition process, what we infer is the reverse of this gene-transfer process. Almost all the living polycystineans have symbiotic algae cells inside. They are considered to be Prasinophyceae, Haptophyceae, and Chrysophyceae, based on TEM observations (Anderson 1992). Recently, we found an endosymbiotic diatom, *Minutocellus polymorphus*, from a polycystinean *Sphaerozoum fusucum* (Mayama et al., in preparation). This endosymbiont was naked in the host, but cultivation of the isolate induced the proliferating cells to form siliceous frustules. It is known that the limited number of diatom species can live as endosymbionts in foraminiferan cells (Lee et al. 1992; Mayama et al. 2000). In this case, endosymbionts are also naked, and the isolates develop the frustules in culture conditions. All phylogenetic trees, including the Polycystinea, show earlier branching of this organism among the eukaryotes (Zettler et al. 1997; Moon-van der Staay et al. 2001; López-García et al. 2002) and, in fact, its earliest fossil record was from late Precambrian or Paleozoic (Anderson 1983). The mass extinction in the Permian–Triassic boundary (251±2 million years ago) has been explained by a long-term and worldwide marine anoxic event (Isozaki 1997). Many polycystineans also became extinct in this period. However, lodging symbionts, some polycystineans could surmount the severe times with the benefits from the algae. Because the origin of heterokontophyta inferred from the molecular clock-calculated data was at or shortly before the Permian–Triassic boundary (Medlin et al. 1997), probably the polycystineans could have the immature heterokontophyta as the symbiont. The anoxic environment continued nearly 20 million years across the Permian–Triassic boundary (Isozaki 1997), and we cannot exclude the possibility of the gene transfer occurring from the host to the naked endosymbiont during this period. In addition, it is noteworthy that the living polycystineans have endosymbiotic bacteroids or bacteria (Anderson and Matsuoka 1992; Matsuoka 1992), because their role as a vector may be expected in the ancient gene transfer event.

The most common element in the Earth's crust is silica, and it seems to be natural that diverse aquatic organisms would utilize it for the material of cell covering in their evolution. To date, we have had no tool for exploring the origin and lineage of the siliceous cell covering. However, recent findings in molecular studies concerning the biosilicamineralizaion encourage further development of this field. As the process of mineral cell covering formation appears to involve elaborate systems, collaboration between various research fields related to silica biomineralization is eagerly expected to lead to a better understanding of the evolution and phylogeny of the eukaryotic organisms.

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