Silicon nanotechnologies of pigmented heterokonts

Mikhail A. Grachev,* Vadim V. Annenkov, and Yelena V. Likhoshway

Summary

Many pigmented heterokonts are able to synthesize elements of their cell walls (the frustules) of dense biogenic silica. These include diatom algae, which occupy a significant place in the biosphere. The siliceous frustules of diatoms have species-specific patterns of surface structures between 10 and a few hundred nanometers. The present review considers possible mechanisms of uptake of silicic acid from the aquatic environment, its transport across the plasmalemma, and intracellular transport and deposition of silica inside the specialized Silica Deposition Vesicle (SDV) where elements of the new frustule are formed. It is proposed that a complex of silicic acid with positively charged proteins silaffins and polypropylamines remains a homogeneous solution during the intracellular transport to SDV, where biogenic silica precipitates. The high density of the deposited biogenic silica may be due to removal of water from the SDV by aquaporins followed by syneresis—a process during which pore water is expelled from the network of the contracting gel. The pattern of aquaporins in the silicalemma, the membrane embracing the SDV, can determine the pattern of species-specific siliceous nanostructures. *BioEssays* 30:328–337, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

Pigmented heterokonts are the most-successful group of algae in geological history. A majority of pigmented heterokonts is able to build their cell walls of amorphous silica. It was proposed that pigmented heterokonts appeared due to double symbiosis and to the simultaneous appearance of the ability to build cell walls of silica.[1] They include diatom algae, which appeared no earlier than 240 My BP,[2] and chrysophycean algae, whose fossils were found in sediments that were deposited about 600 My BP.[3] The most-ubiquitous representatives of this group of organisms are diatom algae. These eukaryotic unicellular, sometimes colonial organisms have conquered all sunlit aquatic and moist environments. Since the invention of the microscope,the unbelievable beauty of their forms has enthralled observers.[4–8]

All diatoms have the same basic plan of a box (hypotheca) with an overlapping cover (epitheca) separated by a series of siliceous rings—girdle bands. The sizes of diatoms vary between 2 and 2000 μm. Every element of the exoskeleton is decorated by a species-specific, genome-encoded pattern of nanostructures, such as pores and areolae, ridges, hollow processes and spines[7] (Fig. 1A–H). The linear sizes of these siliceous nano-structures vary between 10 and a few hundred nanometers; they consist of a few million to a few billion residues of silicic acid. Diatoms and other silicifying heterokonts are claimed to have invented ''silicon nanotechnologies''.

Oceanic diatoms yield about 25.8×10^9 tons of primary organic carbon per year, up to 20% of the total primary production of the Earth.[9] Chrysophycean algae are less abundant. They use silica for the synthesis of siliceous scales and statospores, which are also decorated with species-specific patterns of nano-structures (Fig. 1I–L). It is believed that scales of chrysophycean algae are evolutionary precursors of diatom valves.[4,7]

During the last decade, diatoms and other pigmented heterokonts have been extensively studied using molecular biology techniques. The molecular clock technique has allowed a convincing evolutionary tree to be proposed.[1] In 2004, the first diatom genome project was completed,[10] using a marine species *Thalassiosira pseudonana* Hasle & Heimdal. Its nuclear genome encodes 11,242 proteins.

The impressive success of molecular approaches has still not explained the central enigma of cell biology—how live cells transform the information encoded by genomes into a design of three-dimensional cell walls. Diatoms expose the enigma most dramatically because their siliceous cell walls are hard and strong,[11,12] unlike those of other organisms. Many generations of devoted and talented biologists have studied

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Abbreviations: SIT, Silicon Transporter; STV, Silicon Transport Vesicle; SDV, Silica Deposition Vesicle.

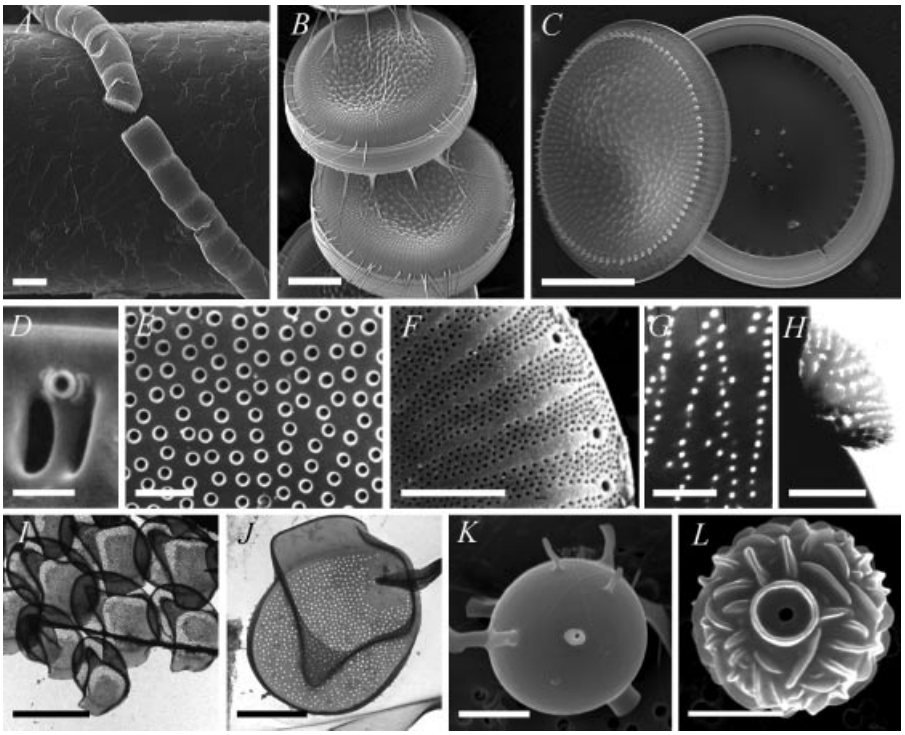


Figure 1. Structures of siliceous elements of frustules of diatoms (**A–H**) and chrysophycean algae (**I–L**): **A:** A colony of *Stephanodiscus meyeri* Genkal & Popovsk. on a human hair; **B:** cells of one of the biggest diatoms—*Cyclotella baicalensis* Skv.—connected together in colonies by mucilage strands; **C:** epitheca and hypotheca of *Cyclotella minuta* (Skv.) Antipova decorated by species-specific patterns of nano-structures on the inner and on the outer surfaces; **D:** a tube of a marginal fultoportula with two parallel supports on the inner surface of a frustule of *C. minuta*; **E:**—regular openings (diameter 260–290 nm) in a wall of *Stephanodiscus grandis* Churs.; **F:**—openings Ø 160–180 nm and 30–80 nm) in the marginal zone of *Cyclotella tuncaensis* Likhoshway; **G:**—smallest pores (Ø 40–50 nm) in girdle bands of *Aulacoseira baicalensis* (K. Meyer) Sim.; **H:** a fine siliceous cone (velum) sitting below a pore (areola) in the siliceous frustule of *A. baicalensis*; **I,J:** scales of *Mallomonas vanigera* Asmund (photos of O. I. Belyh); **K:** statospore with tubes Ø 240–470 nm of an unknown chrysophycean species from Lake Baikal; **L:** statospore with ridges of *Spiniferomonas trioralis* (Takahashi) Preisig & Hibberd (**K,L** photos A. D. Firsova). **A–F, K–L** SEM; **G–J** TEM. Scale bars: **A–C** 10 μm; **D** 1 μm; **E,F,J** 2 μm; **G,H** 0.5 μm; **I,K,L** 5 μm.

diatoms and created a complicated taxonomic system mostly based on the design of three-dimensional valves, but, to our knowledge, nobody has yet proposed a self-consistent mechanism for their synthesis. Stages of macro- and micro-morphogenesis of siliceous frustules of diatoms and the factors that are involved in these processes have been considered in detailed recent reviews,[6,13] the present review considers new possible mechanisms of morphogenesis.

Uptake of silicic acid from the environment

Silicifying pigmented heterokonts face a few difficult chemical problems. Firstly, they need to distinguish molecules of silicic acid from molecules of water. In the ocean, 56 million molecules of water are present per one molecule of silicic acid. Secondly, having captured silicic acid, cells have to direct it to appropriate destination points. Finally, they have to transform silicic acid into almost dry, dense and hard biogenic silica, actually quartz glass,[4] decorated with all the above-mentioned nanostructures.

Scales and chrysophycean cysts as well as hypothecae, epithecae and girdle bands of diatoms are synthesized in specialized subcellular particles, silica deposition vesicles (SDVs), are always bounded by a special lipid membrane, the silicalemma.[14,15] General problems of the cytology of diatoms are covered by several excellent reviews.[4,6,16]

An important discovery in the field of molecular biology of diatoms has been the detection of a novel class of membrane proteins, Silicon Transporters (SITs). They were first found in 1997 in a marine diatom *Cylindrotheca fusiformis* Reimann and Lewin.[17] Genes of a family of five similar proteins, candidates for the roles of SITs, were sequenced.[18] The predicted amino acid sequences suggested that the genes belonged to trans-membrane transporters. Transfer of one of these genes into the oocytes of *Xenopus laevis* induced uptake of silicic acid. Another *sit* gene was found in 2002 in a

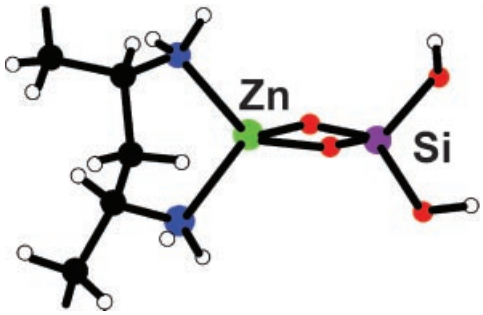
freshwater diatom *Synedra acus* Kütz.[19] Subsequently, fragments of *sit* genes and complete *sit* genes were amplified and sequenced starting with genomic DNA of many other diatoms.[20–24] In 2006, *sit* genes were first found in chrysophycean algae *Synura peternesii* Korshikov and *Ochromonas ovalis* Doflein.[25] In 2007, using immuno-electron microscopy with antibodies against synthetic peptides constructed according to the predicted amino acid sequence of *S. acus* SIT clusters of antigens of SIT were localized below openings in the siliceous frustule. SIT antigens were also found scattered around the cytoplasm.[26]

Intramembrane hydrophobic segments of predicted SITs are punctuated by hydrophilic sequences.[21] In spite of the evident general similarity of the predicted sequences and of the respective hydropathy profiles, the known overlapping parts of SITs do not have any universal continuous identical motifs longer than one amino acid residue. On the one hand, the molecule of silicic acid does not carry a significant charge. It does not contain any moieties that have an affinity to organic phases. On the other hand, silicic acid is irreversibly bound by positively charged residues of lysine and arginine. This limits the selection of motifs that could provide its reversible binding in the active center. The possibilities of direct structure–functional studies of SITs are limited because the proteins are not yet isolated in a pure form, and there is no experimental system that could allow modification of SIT and then study the consequences of this modification on the transport of silicic acid. Hence, it is only possible to speculate on the basis of comparison of SITs belonging to different species. In an early paper,[18] it was proposed that a cysteine residue in SIT takes part in transport of silicic acid, because uptake of silicic acid by live diatoms was inhibited by SH reagents such as copper and N-ethylmaleimide. However, comparative sequence analysis finally did not reveal any absolutely conserved cysteines. Sequencing revealed a conserved motif GXQ found four times, twice in the trans-membrane segments, and twice in extra-membrane loops.[22] These motifs were proposed for the role of binding and transport of silicic acid through the pore. The idea deserves thorough consideration.

It was proposed that SITs may bind silicic acid via an atom of zinc[21,23] since it was found in the 1980s that diatoms do not assimilate silicic acid in the absence of this metal.[27] It was noticed that SITs from *C. fusiformis* and a SIT from *S. acus* all contain a motif CMLD.[19] It was evident that this motif can potentially bind a zinc ion using side chains of C, M and D as ligands. Studies of a synthetic peptide NCMLDY confirmed this possibility—the rate of reaction of the cysteine residue of this peptide with an alkylating reagent strongly decreased in the presence of Zn^{2+}.[28] Later on, conserved CMLD or closely similar CMID motifs were found in a majority of SITs, most remarkably, in SITs of not only diatom, but also chrysophycean algae.[25] However, extensive sequencing has shown that there are exceptions.[10,22,24] Of the 125 known SITs (GenBank), the extra-membrane loop between trans-membrane segments TM 4 and TM 5 contains CMLD in 32, CMID in 55, SMID in 19, QMID in 6, HMID in 2, MMLD in 2, MMID in 5, and AMID in 4 proteins. The MXD motif (X = L, I) is strictly conserved. Methionine (M) and aspartic acid (D) residues can, in principle, act as ligands for Zn^{2+}, therefore, the proposed speculation on the role of this site in binding zinc[21,23] is still not invalid.

Zinc is an obligatory component of more than 300 diverse enzymes.[29] Zinc ions in enzymes stabilize enzyme–substrate complexes and act as so-called Lewis acids, promoting nucleophylic attack of those (e.g. carbon, phosphorus, silicon) atoms of substrates that are bound with them via oxygen atoms. Remarkably, zinc was found in a silicase of silicifying sponges,[30] and this protein was proposed for the role of an agent that binds silicic acid and performs its condensation (and decondensation of silica). If Zn^{2+} was bound by SITs, it would promote binding of silicic acid.

Silicic acid is adsorbed from its dilute (0.17 mM) solutions by a cross-linked polyvinylamine in the presence of Zn^{2+}.[31] The concentration of silicic acid in the supernatant above the polymer decreased twofold, as revealed by a color reaction with molibdate.[32] On the contrary, it did not fall in the absence of Zn^{2+}. The concentration of silicic acid in the starting solution was similar to that found in many fresh waters.[9] Ions of other transition metals, such as Cu^{2+}, Mn^{2+} and Ni^{2+}, did not induce binding of silicic acid by polyvinylamine. Quantum-mechanical calculations showed that silicic acid can indeed form a complex with Zn^{2+}- polyvinylamine.



This simple chemical system may mimic the mechanism by which SITs captures silicic acid from its highly diluted solutions in aquatic environments.

Silicic acid bound by SIT is discharged into the cell. Formally, SIT has to transport silicic acid against a steep concentration gradient. Transport of this kind would need an expenditure of energy. Earlier studies of the biochemistry of silicon transport led to a suggestion that the silicon transporter is in fact a symporter performing uphill transport of silicic acid at the expense of downhill transport of Na^{+}[33] whose concentration in the ocean is higher than that in live cells. However, the concentration of sodium in freshwater bodies is

much smaller than that in live cells. Contrary to the symporter hypothesis, freshwater diatoms evidently cannot use sodium as a source of energy for the transport of silicic acid. It is possible that marine and freshwater diatoms have different mechanisms for the supply of energy for silicic acid transport. Contrary to this suggestion, sequences of SITs of marine and freshwater diatoms do not fall into clades according to demands for salinity.[24]

It seems likely that SITs perform a catalytic function—transformation of silicic acid into an oligosilicate. A possible route is shown in Fig. 2. The key issue is the action of Zn^{2+} as a Lewis acid facilitating the attack of the Si atom of the first molecule of silicic acid by second molecule of silicic acid. The particular route shown in Fig. 2 stipulates the formation of cyclic trisilicate, a meta-stable six-member cycle, a long-known postulated intermediate in chemical polycondensation of silicic acid.[32] However, to arrest reverse reactions, it is necessary to transform trisilicate into a more stable compound, i.e., to have an acceptor of trisilicate at the outlet of SIT inside the cell.

It should be mentioned that, under certain conditions, silicic acid can be captured by diatom cells by a mechanism that does not involve work of SITs as transporters—it is (macro)-pinocytosis, a mechanism according to which silicic acid is internalized by an invaginating part of the plasmalemma.[34] An argument in favor of this mechanism is the fact that salinity of the growth medium affects the pattern of nanostructures formed inside the SDV—a phenomenon that is hard to explain if salts from the growth medium do not join the content of the SDV together with silicic acid. There are also other data that suggest that silica can be assimilated by diatoms without the participation of SITs—it is the so-called surge uptake of silicic acid,[35] which does not obey the Michaelis-Menten kinetics and hence does not need any enzyme-like transporter.

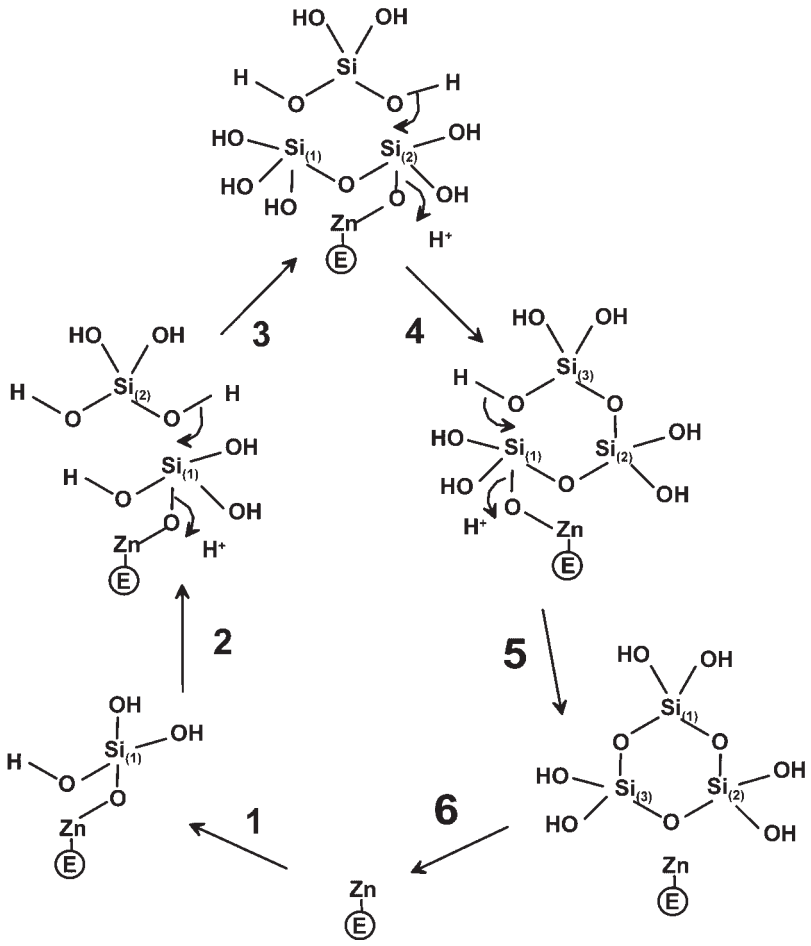


Figure 2. A scheme of catalytic condensation of silicic acid yielding cyclic trisilicate.

Fate of silicic acid inside the cell

Frustules of diatom algae contain an admixture of positively charged biopolymers[36–39] such as silaffins—small proteins post-translationally modified with oligomers of propylamine, as well as free polymers of propylamine. By analogy with ubiquitous polyamines putrescine and spermidine and their analogs,[40] these positively charged polymers could accumulate in acidic post-Golgian vesicles which capture oligosilicate and become Silicon Transport Vesicles (STVs).[41] Chemical studies have shown that oligomers of silicic acid form strong complexes with diverse polyamines.[32,42–45] Transport of silicic acid into polyamine-loaded vesicles would not need any special source of energy.

Diatoms can accumulate significant pools of "free silicic acid".[20,46] Reportedly, the concentrations of this "free silicic acid" within diatom cells can be as high as 20 g SiO_2/L,[20] much higher than the limit of solubility of amorphous silica in water (ca. 0.12 g SiO_2/L).[32] If such a high concentration of silicic acid were really present around the cytoplasm, the cell could not survive. The explanation lies in the method of determining "free silicic acid". A sample of diatoms is extracted in a large volume of hot water or strong acid. The extracted cells are removed by centrifugation, and a color reaction of the supernatant with molibdate is performed. The siliceous walls of the diatoms mostly remain intact. The amount of molibdate-reactive silicic acid found in the extract is assumed to be equal to that formerly present in live diatom cells, and give the unrealistically high intracellular concentrations of "free silicic acid".[46] It is evident that treatment with hot water or acid must change the form of silicic acid, compared to that present in the live cell.

Studies of live diatom cells with solid-state NMR revealed the presence of silicon atoms of three types.[47] The first type are internal atoms of silica of the core of the diatom valve—Si atoms binding four –O–Si moieties. This type is the major one, more than 70% of total Si. The second type are Si atoms binding three –O–Si and one –OH group. These atoms (about 20% of total Si) occur at the surface of polysilicate. The third type (2–5%) are Si atoms binding two –O–Si moieties and two hydroxyls. They occur in linear and cyclic oligo- and polysilicates. No free silicic acid $Si(OH)_4$ was found. These facts suggest that oligomeric forms of silicic acid, rather than free silicic acid, are present in live diatom cells. However, solid-state NMR data have to be taken with some precaution, because they were obtained with freeze-dried cells. Drying can result in polycondensation of free silicic acid. Similar results were obtained in another study.[48]

Magic angle spinning NMR and Fourier transform infrared spectroscopy analyses[49] performed on living diatoms revealed, in addition to some free silicic acid, a poorly condensed silica network interacting with template biomolecules. The data on the pool of condensable derivatives of silicic acid found in diatom cells do not rule out the possibility that this raw material for frustule synthesis is present inside the SDV.

Optical microscopy reveals "clouds of vesicles surrounding the Golgian apparatus which persistently move towards the SDV and fuse with the silicalemma in the course of the synthesis of the valves of diatom cells".[4] It has been suggested that these are silicon transport vesicles (STVs), which deliver silica to the SDV. The viewpoint was subsequently put under doubt because it seemed to contradict with data of transmission electron microscopy—the postulated STVs did not contain a sufficient amount of electron-dense silica.[13] However, STVs can be filled with a water-soluble complex of oligosilicates and polyamines and hence can carry silica and be electron-transparent.

Deposition of silica

It was reported that the frustules of a freshwater diatom *Cyclotella cryptica* Reimann, Lewin & Guillard have a density of ca. 1.46 g/cm^3,[50] but the method of treatment probably did not removes all organic matter. The density of the frustules of freshwater *Synedra acus* treated with hot concentrated nitric acid is 1.96 g/cm^3.[51] According to other data, the density of the silica of marine diatom frustules is 2.07 g/cm^3[52]—almost the density of melted (amorphous) quartz, which is 2.18 g/cm^3.[53]

How does excess water leave the synthesized frustule? Transport of water through membranes of live cells is mediated by special proteins—aquaporins.[54,55] These small proteins easily pass molecules of water, but arrest co-transport of protons. Aquaporins occur as aggregates consisting of four identical 24 kDa subunits. The productivity of aquaporins is extremely high—a pore of a single aquaporin can pass up to $3 \cdot 10^9$ molecules of water per second.[55] Water is passed inside or outside the cell, depending on the osmotic pressure. Motifs of aquaporins were found in GenBank containing the predicted proteome of the diatom *T. pseudonana*.[10,56] It is highly probable that removal of water from the SDVs of pigmented heterokonts in the course of the synthesis of siliceous elements is mediated by proteins of this kind. Remarkably, it was found[34] that silica of the frustule is denser when cells are grown in a less saline medium. This fact is in line with the proposal for the involvement of aquaporins in the removal of water from the SDV, because more water can be removed from a less-saline STV by osmotic pressure.

It is likely that siliceous frustules of diatoms are molded within SDV of a dissolved complex of oligosilicates and polyamines delivered by STVs.

Transitions leading to spontaneous precipitation of polymers are long known under the name of syneresis. By definition, syneresis is a process "during which the network of a gel contracts and expels the pore liquid".[42] A classical example of syneresis is the production of cheese.[57–60] Syneresis also takes place during the coagulation of blood

and in many other important biological processes. Syneresis can be induced by increasing the concentration of the polymer, and begins after this concentration passes a certain critical value. The position of the critical point depends on physico-chemical conditions such as temperature, ionic strength, pH, etc. Even above the critical point, syneresis only occurs after a certain induction period.[42] Contraction of the gel network is due to irreversible aggregation of polymer chains.

It must be emphasized that the formation a gelatinous gel does not significantly reduce the micro-viscosity, and hence permeability for solvent. The coefficients of diffusion of small molecules in gelatinous gels remain almost the same as those in pure solvents. In contrast, amorphous precipitates, which are expelled due to syneresis of the gel are impermeable to solvents. Complete removal of mechanically captured pore solvent cannot take place unless the sediment is completely broken up to let droplets escape along cracks.[59,60]

We believe that molding of solid siliceous frustules of diatoms is performed by means of regulated syneresis, which takes place within the SDV and is mediated by aquaporins. The following facts support this suggestion. (1) Syneresis is irreversible; indeed, diatom algae frustules do not rapidly dissolve in water. (2) Polymers that precipitate via syneresis are always amorphous. Thorough studies of diatom valves with optical and X-ray diffraction techniques did not reveal any traces of crystallinity of their silica.[61–63] (3) Transmission electron microscopy of growing diatom frustules often reveals round electron-transparent "nano-bubbles" surrounded by electron-dense solid silica (Fig. 3), e.g. in the growing valves of *S. acus.* No bubbles of this kind are seen in mature frustules (see Fig. 15 in Ref. 64).

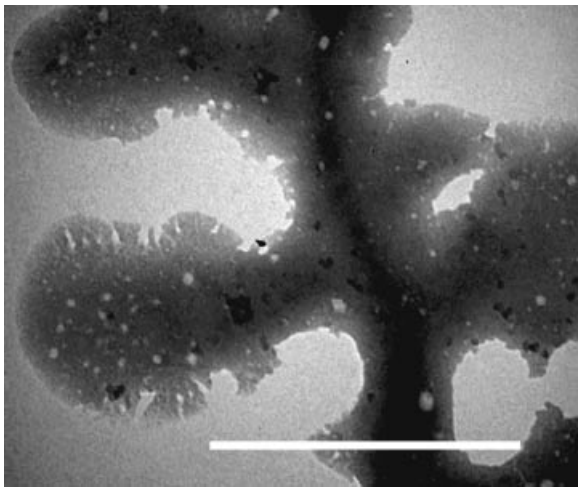


Figure 3. A growing valve of *S. acus*. Direct (no-staining) TEM. Photo of Ol. Kaluzhnaya. Scale bar 1 μm.

The high density of the biogenic silica of diatom frustules is evidently a consequence of the small thickness of its deposited elementary layers, which allows almost all the droplets of sequestered pore water to escape.

Layers of silica are revealed by transmission electron microcopy of growing valves.[65] A recent study[66] of thin sections by means of transmission electron microscopy has shown that frustules of *S. acus* synthesized at toxic concentrations of germanic acid consist of smooth layers of silica, instead of normal patterns of siliceous nanostructures. It was proposed that the admixture of germanic acid affects the process of coagulation of the silica gel. The layered structure suggests that removal of water is performed in batches. In our opinion, the data presented above suggest that siliceous elements form from the liquid content of the SDV, rather than from hard siliceous nanospheres. At the final stages of morphogenesis biogenic silica reaches the highest density, strength and rigidity.

So, what determines the species-specific patterns of nanostructures decorating diatom frustules and girdle bands and scales of chrysophyceans? This is the most intriguing question.

Models of morphogenesis

A few mathematical models have been proposed earlier to illustrate the possibilities of self-organization in the formation of the nanopatterns of diatom frustules. A convincing model was described by Parkinson et al.[67] The model is two-dimensional, SDV is flat. According to the model, nano-particles of silica arrive at the periphery of the SDV, and wander around the vesicle by means of molecular diffusion until they meet a previously deposited mass of silica and stick to it due to surface tension. This model easily gives a fractal structure that looks like a tree. Fractal features do appear in real diatom valves (see for example Fig. 1H). To give regular patterns the model requires another reasonable assumption. It was assumed that silica particles are delivered by usual trafficking along microtubules, and that microtubules (= sources of siliceous nanoparticles) anchored around the circular periphery of the SDV sit at approximately equal distances from each other. This supplement to the model produces regular nano-patterns that have much in common with frustules of real diatoms.

In other words, the model of Parkinson et al.[67] suggests that the structure of the siliceous frustule depends on the delivery of silica to the SDV by STVs along elements of the cytoskeleton, microtubules, which are organized in a species-specific way. The data discussed above do not fully support this model in that silica is delivered to the SDV as solid building blocks. However, it is very likely that the cytoskeleton determines the pattern of silica deposition. This possibility was also suggested in earlier papers (for review see Refs 4,13).

Is it possible that the pattern of aquaporins sitting in the silicalemma determines the species-specific pattern of siliceous elements?

Imagine an aquaporin sitting in the silicalemma of an SDV filled with a solution of a complex of oligosilicates with polyamines (Fig. 4).

Let the osmotic pressure allow water escape through the aquaporin pore. Evidently, the entrance of the pore will become a source of silica. After a while, the concentration of silica around the pore will increase and a ring of polymerized gelatinous silica gel can appear. This ring will be permeable to water, and removal of water will continue. The concentration of silica within the gel will increase. Finally, syneresis will occur, and a ring of anhydrous silica will appear. Pumping of water through the pore will cease. If the aquaporin shifts laterally due to two-dimensional diffusion in the silicalemma, a new silica ring will appear, and so on. One of the SEM images by A.-M.Tiffani (Fig. 5A) is a good illustration of this possibility. It is seen that the frustule of *Aulacodiscus oregonus* Harley & Bailey at an early stage of its development looks like a ''bubble pattern'' consisting of numerous rings of different diameters. Openings of some of these rings are filled with silica at subsequent stages of development. Other openings are transformed into the so-called alveolae.

Under appropriate conditions, a silica well, a silica disc or a silica nanosphere could form instead of a ring. This simple model gives numerous possibilities for genetic regulation combined with self-organization. In some respects, it is similar to the phase-separation model of diatom frustules morphogenesis.[38]

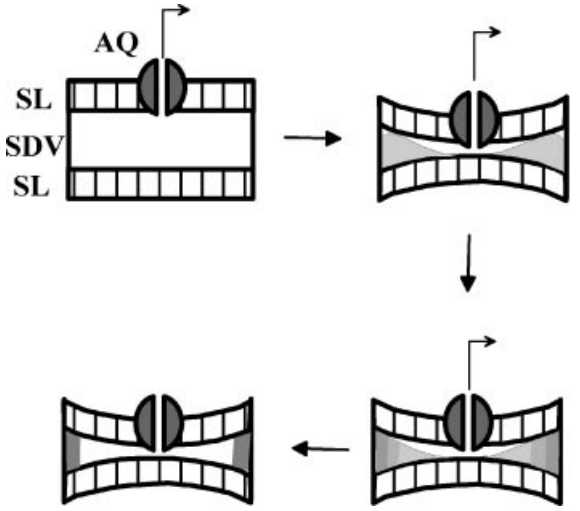


Figure 4. A scheme of the formation of a ring of quartz glass induced by an aquaporin. AQ, aquaporin, SL, silicalemma. An arrow above an aquaporin molecule efflux of water.

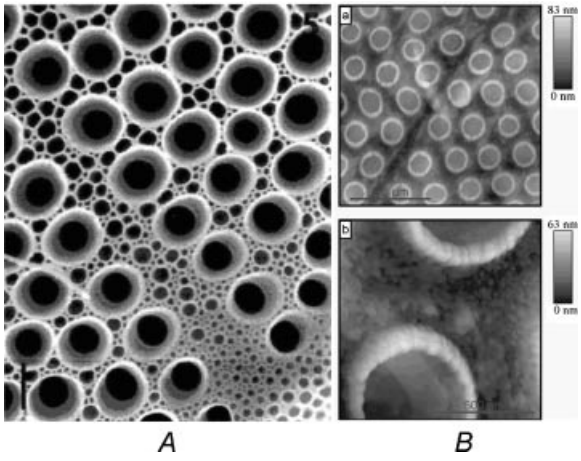


Figure 5. An image of frustule of *A. oregonus* at an early stage of its development (A, scale bar 1 μm), reprinted with kind permission of Springer Science and Business Media[8] and an image of the internal surface of a valve of *C. granii* obtained by means of atomic-force microscopy at two magnification, reprinted with permission from Noll F, Sumper M, Hampp N 2002 Nano Lett 2:91–95 copyright (2002) American Chemical Society.

Assuming that the height of the silica ring shown in Fig. 4 is 50 nm, that its diameter equals 500 nm, and that the concentration of dissolved silica within the SDV is 200 g/L (ten times higher than maximal reported for whole diatom cells), the height of a cylinder which would have to be ''desiccated'' by the aquaporin would be about 250 nm. Remarkably, a hexagonal pattern of 500 nm wide, 50 nm high craters covers the inner surface of the valve of *Coscinodiscus granii* Gough (Fig. 5B).[68] The possibility of molding of such an ornament starting with a 250 nm layer of dissolved silica does not seem unrealistic.

To illustrate the possibilities of self-organization of a pattern molded by means of aquaporin-induced syneresis, we present results of modeling of a pennate diatom (Fig. 6). The formalistics is much the same as that presented by Parkinson et al.,[67] but the SDV is oval rather than circular. It is seen that the pattern of silica deposition obtained with the model is similar to that observed at the early stages of morphogenesis of *S. acus*.

The most-mysterious example of a silicon nanotechnology used by pigmented heterokonts is the rapid (15 minutes) synthesis of the labiate process of a diatom *Ditylum brightwellii* (T. West) Grunow, a perfect 20 000 nm-long cylindrical siliceous tube having a diameter of 1000 nm and walls about 200 nm thick (see Fig 84 in Ref. 4). The synthesis of this tube begins with a complicated subcellular structure. The tube grows at its distal end with the deposition of silica proceeding at the cylinder edge. The growing tube remains wrapped by plasmalemma and silicalemma. The distance between the

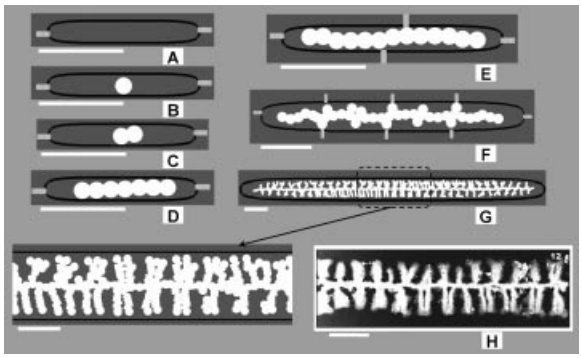


Figure 6. A model of the initial stages of the growth of a pennate diatom; formalism is similar to that described by Parkinson et al.[67] Black line, silicalemma; light bars, axes of microtubules delivering aquaporins; white circles, silica discs deposited due to syneresis. Scale bar 1000 nm. **A–E:** Formation of axial filament; **F:** branching due to arrival of new microtubules; **G:** an advanced stage of the model; **H:** silica of an early stage of development of *S. acus* as revealed by TEM.[64] Details of the model: rectangular grid, 12 000 × 660, square pixels, radius of the deposited silica disc 100 nm, 1 pixel = 3.33 nm. Form of the silicalemma—elliptic $(x/a)^4 + (y/b)^4 = 1$. At zero time, a = 220 pixels, b = 32 pixels. Minimal distance between microtubule axes is 420 pixels (1400 nm).

silicalemma and the surface of the siliceous tube is less than 100 nm. STVs have not been observed arriving at the distal edge. The syneresis model gives a simple explanation: STVs filled with the soluble complex of oligosilicates with polyamines arrive at the proximal end of the growing cylinder and, by means of fusion, discharge their content into the SDV. The solution flows towards the distal end. Silica is deposited at the distant edge due to syneresis induced by a circle formed of a given number of aquaporins. To form a circle having a diameter of 1000 nm, it would be necessary to join about 200 aquaporin tetramers. The task could be performed by a special circular organic filament (e.g. actin[4]).

It is known that, in mammals, aquaporin water channels are sequestered into intracellular vesicles and that these vesicles are moved by microtubules to distant destination points in order to perform passive water transport.[69] It is easy to imagine that aquaporins are delivered to silicalemmas of pigmented heterokonts in a similar way.

It is known that, on mixing with concentrated solutions of silicic acid, silaffins and polymers of propylamine produce opaque sediments built of siliceous nanospheres and ultra-thin films.[36–39,70] Taking different combinations of polyamines, it is possible to obtain various self-organized (e.g. hexagonal) nanopatterns of precipitated silica.[71,72] It is believed by many workers that species-specific patterns of silica are formed by diatoms due to a time-dependent change in the repertoire of these polycations, especially because silaffins undergo extensive post-translational modification such as phosphorylation and introduction of quaternary ammonium groups. However, it is hard to imagine how processes of this kind could give more specific, genome-encoded elements of design such as, for example, labiate processes. The major problem would be delivery of the right combinations of silaffins and polyamines at the right time and destination points within the SDV. However, it is easy to incorporate the polyamine-mediated mechanism into the regulated aquaporin-induced syneresis model.

The beauty and the sophisticated forms attract many workers to explain the silicon nanotechnologies of pigmented heterokonts. The publications are numerous but the key questions remain unanswered. How is silicic acid captured from the aquatic environment? How is its intracellular transport performed? How does silica precipitate within the silica deposition vesicle? How are the species-specific siliceous patterns formed? It is due time to propose and to verify new working hypotheses.

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