PERSPECTIVES: BIOMINERALIZATION

The Diatom Glasshouse

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Diatoms are unicellular microalgae with highly sculpted walls of silica. Because living cells must constantly interact with their environment, the diatom walls have myriad openings (such as pores and slits) that facilitate such exchanges. The intricate patterns and symmetries (see the figure) are species-specific and genetically determined (*1*). On page 584 of this issue, Kröger *et al.* (*2*) shed light on some of the organic molecules that are crucial for the formation of these diatom walls.

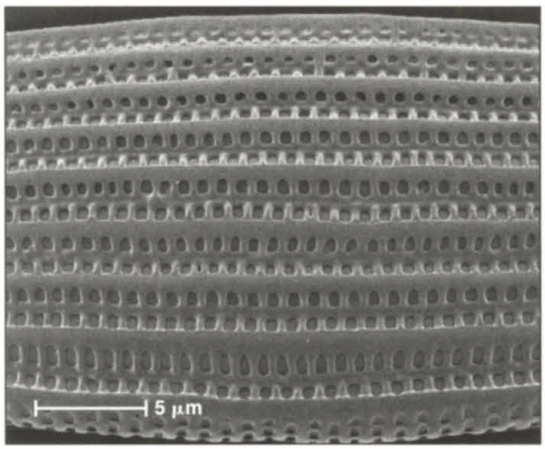
The high degree of complexity and hierarchical structure displayed by diatom silica walls is achieved under mild physiological conditions. The biological processes that generate patterned biosilica are therefore of interest to the emerging field of nanotechnology.

The success of diatoms in processing silica results from specific interactions at the organic-inorganic interface, between highly modified peptides called silaffins and silica. Kröger *et al.* first isolated silaffins from *Cylindrotheca fusiformis* (*3*) and subsequently from a range of diatoms (*4*). In vitro, silaffins catalyze the polymerization of silica spheres—tiny structures reminiscent of the nanoparticles known to constitute diatom biosilica (*5, 6*). Kröger *et al.* have now further defined the structure of the silaffins and discuss their pivotal role in the nanofabrication of diatom biosilica (*2*).

Diatom wall formation and silicification occur in the complex environment provided by specialized silica deposition vesicles (SDVs) located in the cell cytoplasm. Two primary mechanisms operate concurrently during silica wall biogenesis and mineralization.

Large-scale patterning and silicification are determined by the environment and constraints of the SDV. Cytoplasmic components imprint on the SDV to mold and shape the forming wall, followed by the precipitation of silica in the SDV. These macromorphogenic processes restrict SDV expansion to form distinct morphological features such as pores, slits, and chambers (*1*). Macromorphogenesis alone is, however, insufficient to explain the nanostructure of diatom walls (*6*). Micromorphogenic processes, which occur in the lumen of the SDV, depend on the activities of organic matrices at the inorganic interface.

The extraction of organic molecules embedded in diatom silica requires harsh conditions that often damage their structure and function. Initial extractions of mature diatom silica by Kröger *et al.* yielded wall-associated proteins that were not localized to the SDV during silicification (*7, 8*). However, extraction using anhydrous hydrofluoric acid yielded low molecular weight peptides, allowing the isolation and characterization of silaffins (*3*).



Intricate walls. Scanning electron micrograph of the silica wall of the marine benthic diatom *Amphora coffeaeformis*. Note the ornate structure, patterning, and porosity of the silica wall.

Silaffins nucleate silica spheres of uniform morphology when added to a solution of silicic acid, although the size, shape, rate of precipitation, and pH of formation differ from those in diatoms. Further refinement of the extraction procedures yielded modified silaffins that could direct silica polymerization via pendant polyamines grafted onto the protein backbone (*4, 9*). These modified silaffins dramatically alter the rates of silicate precipitation; the process is accelerated in a mildly acidic environment, a condition thought to characterize developing SDVs.

The presence of polyamines on the silaffins not only provides a possible template for nucleation, but might also control the silica colloid size within the SDV. The globular silica particles observed by electron and atomic force microscopy to constitute diatom silica (*5, 6*) may reflect the chain lengths of the polyamines that are used to direct silica deposition (*4*). The discovery of these molecules in a range of diatoms further demonstrates their role in the controlled polymerization of silica. Sumper (*10*) has proposed a possible mechanism through which these polymerization determinants could also contribute to the formation of silicified structure.

Kröger *et al.* (*2*) further refine the silica extraction procedures to yield silaffins in their native state, in which both the polyamine "tails" and phosphorylation are preserved. The native silaffins are capable of assembling into supramolecular complexes by the intermolecular interactions between the negatively charged phosphate groups and the polyamine moieties. The supramolecular silaffin assemblies therefore nucleate rapid silica formation, and the data suggest that the positioning of silaffin nucleation sites may have a major role in micromorphogenesis.

Characterizing additional organic molecules (such as polysaccharides) present in diatom biosilica now becomes a priority. The focus must be on the identification of organic templates within the SDV and the mechanisms of pattern generation. Assays for determining templating molecules will necessarily be complex. Silaffins nucleate and precipitate silica and have the capacity to generate organic-mineral nanostructures. In contrast, templating molecules have no apparent activity in the assays on their own; they affect the fine patterning of biosilica only in the presence of the nucleating molecules. Other molecules may have an inhibitory effect on silicification, even in the presence of the nucleating molecules.

It will be important to determine how the activities of one group of molecules modulate the activities of another. The answer to this question will be of value in developing the means to manipulate nanofabrication in materials science.

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