Biology 3102 – Microbial Eukaryotes
Supplementary Course Material – Special Topic
Fall 2020

MINERALISED STRUCTURES

Scales, Skeletons and so on
In the Part #2, chapter 1 of the lecture notes we considered the cytoskeleton of eukaryotic cells, which is comprised of intracellular components made of protein. We will now discuss other important components that impart basic morphology and function on the cell – scales, skeletons and so on, especially those made from *inorganic* material.

There are two mineral constituents that predominate in the mineral skeletons and scales produced by microbial eukaryotes – silica and calcium carbonate.

Silica structures
Silica is used to form a variety of different structures in a broad range of microbial eukaryote groups. The more important of these have been briefly introduced in the lectures in Part 1 of the course. Here is a quick recap of some important eukaryotic microbes that produce silica structures: Some **Chrysophyceans**, especially the **Synurales** (photosynthetic stramenopiles) are covered in numerous silica scales that are attached to the cell membrane. **Diatoms** (also photosynthetic stramenopiles) are completely enclosed in a silica 'frustule'; Much of the total volume of silica in the frustule is in just two plates called the 'valves'. Most **Polycystine Radiolaria** have a large, usually single-piece silica skeleton. **Acanthoecid choanoflagellates** produce an openwork lorica constructed from numerous fine rods called 'costae'. The main group of **Filose testate amoebae,** namely **Euglyphida** (Rhizaria), produce a 'test' of overlapping silica scales. In addition, silica is often a component of the walls of cysts of a range of taxa, and has been detected as a minor component of the cell walls of various algae.

Assembly of silica structures (especially in diatoms)
Although most silica structures are located extracellularly once completed, all of the various silica components made by microbial eukaryotes are constructed intracellularly inside endomembrane compartments. These are called 'Silicon Deposition Vesicles', or SDVs. The membrane of the SDV is often referred to as the 'silicalemma'. The SDV

controls the local environment in which the silica structure is formed, and may help in imposing form on the silica structures developing within it.

The raw ingredient for the mineral component of frustules, scales etc is dissolved silica in the marine or freshwater, mostly in the form $Si(OH)_4$ (orthosilicic acid). The ultimate source of most of this silica is weathering of terrestrial rock, and dissolved silicon concentrations tend to be somewhat higher in freshwater than in the marine environment. Irrespective, the concentration in both marine and freshwater is not very high (<200 micromolar), and is extremely low in much of the photic zone of the ocean (generally <10 micromolar; sometimes <1 micromolar). This is well below solubility saturation for silica near neutral pH (~2 millimolar). As a consequence, cells usually have to actively acquire and greatly concentrate dissolved silicon from the environment in order to deposit it.

Most of our understanding of silicon accumulation and deposition in microbial eukaryotes comes from work on marine diatoms. At environmental silicon concentrations, diatoms import $Si(OH)_4$ though active transport (i.e. it will ultimately cost the cell energy to do this): Specific silicon transporter proteins called 'SITs' are embedded in the cell membrane and co-transport $Si(OH)_4$ and sodium ions (SITs are symporters that exploit the Na^+ gradient across the cell membrane). The cell thereby builds up a high apparent intracellular concentration of Si (well above theoretical saturation), allowing a high concentration of silicon to be achieved in turn in the SDV, most likely via assisted passive transport across the silicalemma. Silica is deposited within the SDV in a hydrated polymer form, without a strict crystal structure [i.e. amorphous; see images in the lecture slides]. The formation of silica structures in the SDV is helped by a slightly acidic pH, which reduces the solubility of $Si(OH)_4$, and slows spontaneous reorganisation after deposition. The form in which silica is deposited is also governed by the presence of proteins called silaffins and silacidins, which catalyse the precipitation of polymeric silica (assisted other large organic molecules - polyamines). The silica in diatom frustules is generally deposited in the form of numerous tiny spheres that are bonded together.

Genes encoding SITs that are closely related to those of diatoms have been found in some silica-producing chrysophyceans. Homologous SIT genes have also been detected in loricate choanoflagellates, albeit their sequences are quite divergent from those of diatoms. It is possible, though not convincingly proven, that the ability to deposit silica was acquired by loricate choanoflagellates through an event of lateral (horizontal) gene transfer, with the gene donor being a photosynthetic stramenopile.

One characteristic of most biogenic silica structures is that they have extremely elaborate morphologies. Diatom frustules, in particular, are often characterized as 'exquisitely sculpted', with the valves of diatoms showing various arrays of strengthening ribs, patterns of small pores (areolae), long extensions in some cases, and several other forms of elaborate patterning. The details of the process of forming these complex morphologies are not well understood at present. The girdle bands of diatoms are much simpler structures than the valves, but still have defined shapes, and substructure. Scheffel et al., (2011) identified ribbon-like structures made of protein that are tightly associated with the silica matrix of the girdle bands of a model diatom. The ribbon-like structures connect together into structures called 'microrings' that closely resemble the arrangement of the girdle bands of the cell. The structures include a new family of proteins called 'cingulins' that have similarities to silaffins (see above), and purified microrings will

catalyse the precipitation of silica on their surfaces *in vitro*. This suggests that the ribbons may act as some sort of template for each girdle band. Interestingly, however, no similar matrix was detected within the valves of the same model diatom.

One important part of the morphogenetic process is the fact that the silicalemma physically constrains silica deposition, and therefore, the shape of the SDV can mould the developing silica structure. The shape of the SDV depends on the size of the SDV (which varies over the course of development of the frustule) and on interactions with the cell cytoskeleton outside it. Even the position of cellular organelles within the cytoplasm that surrounds the SDV can play a role. In raphid pennate diatoms, for example, the first silica elements of the valve to be constructed are the margins of the raphe. These margins are laid down when the SDV is very long and thinly tubular; in at least some species one margin is laid down, from the centre outwards, and silicification 'grows' around the end of the raphe and continues down the other side to form the second margin. The SDV extends in association with cytoskeletal elements that include microtubules that originate from an MTOC that lies alongside the centre of the SDV. A striated fibre (i.e. a non-microtubular cytoskeletal structure) lies between the forming margins of the raphe, preventing the SDV from growing into this region and thereby stopping the raphe from being filled in. The actin cytoskeleton also plays a role in constraining SDV shape. In some pennate diatoms mitochondria lie close to the SDV, between developing lateral struts. The mitochondria may play a role in distorting the SDV to retard silica deposition between the struts.

One remarkable example of the role of the cytoskeleton in shaping the SDV, and hence the determining the shape of the frustule, is provided by the tremendously elongate spines (setae) of some centric diatoms (e.g. *Chaetoceros*). The setae are very fine tubular extensions of the valves (two per valve in *Chaetoceros*). During their development, the cell pushes out long, thin extensions. The SDV grows along with these extensions lying immediately under the cell membrane. A cylinder of actin filaments is present near the tip of the extension, lying immediately underneath the silicalemma. This cylinder determines precisely the inner diameter of the seta. As the seta grows, the actin ring moves to remain close to the tip (it may well move by actin treadmilling), thus continuously providing a 'mould' for the SDV.

Given the low concentration of silica in seawater, and its slightly alkaline pH, the silica in an exposed marine diatom frustule would be expected to gradually dissolve. This is problematic because diatom frustules are persistent through generations, and need to last a long time (in principle, indefinitely; in practice, several cell generations). However, the silica of the frustule is actually protected from direct contact with the water by a coating layer of organic material. This coating is rich in proteins and glycoproteins, but also contains lipid, and might be derived from the silicalemma.

Loricae of choanoflagellates

We will leave diatoms aside for a moment to consider acanthoecid choanoflagellates, which make a silica structure called the lorica. While diatoms make very large and complex individual silica structures, acanthoecid choanoflagellates make less bulky and structurally simpler assemblies built from long silica rods called 'costae'. However, these costae can be several times the linear dimensions of the cell itself.

How do acanthoecids go about producing extremely long structures intracellularly? It turns out that costae are not actually produced pre-formed within the cell. Instead, the

cell produces numerous shorter 'costal strips' within SDVs that are shorter than the diameter of the cell. Several costal strips become associated with a common, very delicate, organic material. They can adhere to each other at their ends to form full-length costae (vaguely analogous to the assembly of the poles in a hiking tent).

Lorica construction is a multigenerational process in many acanthoecid choanoflagellates. Prior to cell division, a cell that already has a lorica will manufacture all of the costal strips necessary for production of a new lorica, and exocytose them, but will keep them in bundles around the top of its existing lorica. After cell division, one daughter cell will inherit the parental lorica, while the other leaves with the bundles of costal strips. This second cell then uses its actin-supported microvilli and other cell movement to extend the bundles of costal strips into costae, and form a new lorica. This greatly reduces the time for which this second daughter cell is without a lorica.

Similar 'parental nuturing' occurs in some **euglyphid filose testate amoebae**. These rhizarian amoebae have tests made of overlapping silica scales. Before cell division the parental cell manufactures enough silica scales to make a second test. This test is assembled during cell division, with one daughter inheriting the parental test, and the other receiving the newly assembled test.

Calcium carbonate structures

Two groups of microbial eukaryotes dominate in terms of the use of calcium carbonate – coccolith-producing haptophytes (coccolithophores) and foraminifera (although it is worth noting that many macroalgal protists, especially some red algae and various macroalgal green algae, deposit large amounts of calcium carbonate in their extracellular matrices). These structures made by haptophytes and forams are on completely different size scales – Coccolithophores produce a series of individual scales that are a couple of micrometres across or less. Foraminiferan cells construct a multi-chambered test that is hundreds of micrometres wide, or even millimetres across.

Both coccolithophores and calcifying foraminifera are marine organisms. The concentration of calcium ions in seawater is fairly high (10 mM). In the ocean water, inorganic carbon is mostly in the form of bicarbonate (HCO_3^-). The overall reaction governing calcium carbonate precipitation is:

$$Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2$$

Unlike biogenic silica, biogenic calcium carbonate is laid down in regular crystalline forms: In the case of coccolithophorid haptophytes and most foraminifera this form is calcite. Calcite crystals have a rhombic structure, and the characteristic angles of calcite crystals (acute 78º; obtuse 102º) become apparent in close examination of certain biogenic calcium carbonate structures, including coccoliths [see pictures in the lectures].

While calcium ions are abundant in seawater, cells undergoing calcification within intracellular compartments (e.g. heterococcolith-producing haptophytes – see below) need

to import large quantities of calcium ions while not disrupting the extremely low calcium content of cytoplasm required for cell homeostasis. Coccolith-producing haptophytes appear to solve this problem by concentrating calcium within the endomembrane system, from whence it is trafficked to the coccolith vesicle (see below).

Coccoliths

Coccolithophorid haptophytes collectively actually produce two different kinds of coccoliths. The coccoliths most commonly depicted are called '**heterococcoliths**'. These usually have a spool-shaped structure (i.e. flattened disc-shapes, but with two flanges) and have complex morphologies [see supplementary notes #1 and the lecture slides]. The less commonly depicted type of coccoliths are the smaller and simpler '**holococcoliths**'. It was originally thought that heterococcoliths and holococcoliths were produced by different sorts of haptophytes, however, it has become clear that the two scale types can be produced by different life cycle stages of the same species – Heterococcoliths are produced by the diploid stage in the life cycle and holococcoliths by haploid cells. It is important to note that not all coccolithophore haploids produce holococcoliths; in several species, such as the famous and abundant *Emiliania huxleyi*, the haploid cells are non-calcifying. Here we will mainly discuss heterococcoliths, then return briefly to holococcoliths.

The production of **heterococcoliths** is intracellular. Initially a thin and relatively flat polysaccharide scale base is produced. This organic scale is synthesised within a Golgi-derived vesicle and is similar overall to the carbohydrate scales produced by non-coccolith-producing haptophytes. The scales contain large amounts of acidic polysaccharides (rather than cellulose, for example), which can bind to calcium ions (including to calcite). The endomembrane vesicle containing the organic scale, which will now be called the 'coccolith vesicle', is then associated with a 'reticular body' in most species. The reticular body is a very dynamic system of endomembrane tubules that appear to be responsible for feeding-in the inorganic substrates for calcification (especially calcium ions sequestered in the endomembrane system –see above), as well as additional calcium/calcite-binding coccolith-associated polysaccharides (and some protein; though the polysaccharides dominate the organic component of coccoliths, and have a better-defined role in coccolith formation – see below).

Calcium carbonate deposition then begins at specific points on the surface of the organic scale. In a typical heterococcolith there are several dozen individual calcium carbonate crystals, which are arranged in two different orientations. Crystal formation begins from a ring of nucleation sites around the rim of the organic scale, and as the crystals grow to one another they form a 'protococcolith ring' as an early stage of the development of the mineralized scale. The two crystal orientations alternate around this ring and interact as they grow (e.g. blocking growth in particular directions by adjacent crystals). The complex morphology of heterococcoliths seems to be governed at least in part by; (i) the initial conditions of nucleation (orientation of the crystals, spacing, etc), (ii) the constraints of being inside a vesicle, which initially surrounds the developing scale quite closely, and (iii) the presence of Coccolith-Associated Polysaccharides (CAPs) in the vesicle. Some CAPs bind preferentially to the acute step-edges of calcite, thereby 'blocking' them, while leaving the obtuse step-edges open to support further crystal growth; this preference favours elongation of the calcite along one particular crystallographic axis. By this

restriction of growth in particular directions, the calcite crystals within the forming coccolith take drastically different shapes to the standard non-biogenic calcite crystals. It is thought that a comparatively small number of specific polysaccharides denote the sites of nucleation on the base scale, and promote crystal growth there (and presumably determine the orientation at which each crystal will form). Although the CAPs tend to result in flattened crystals (an effect that can be demonstrated *in vitro*, incidentally), they apparently also guide more complex crystal growth in some cases (e.g. the production of long, thin elements). The interactions of these polysaccharides with calcite are pH-dependent (requiring a low pH), suggesting the possibility of the cell manipulating the vesicle pH over time to further fine-tune crystal growth.

Holococcoliths are also produced on an organic scale base. Unlike heterococcoliths they have only one basic crystal type (e.g. the crystals are oriented with a common axis, and have a simpler, more regular morphology). Interestingly, the calcium carbonate appears to be added **after** the scale is secreted, rather than within a vacuole within the cell (although the sites of calcification are within a space covered by an envelope of organic material). The individual calcite crystals attached to the scale tend to more closely resemble non-biogenic crystals (i.e. more evenly rhomboid rather than flattened).

Foraminiferan tests (shells)

While foraminifera have a single test, this is continually built on over the life of the cell by the addition of ever-larger chambers. Some planktonic foraminifera can produce a new chamber every day, although the speeds of growth shown by benthic foraminifera are typically much lower and they accumulate chambers more slowly (it is worth noting that benthic foraminifera can have life spans measured in months or even years).

Here we will be considering typical 'calcareous' foraminifera that construct their test entirely by deposition (there are other foraminifera, 'agglutinants', that accumulate mineral particles from the environment). When a foraminiferan constructs a new chamber it first secretes a thin organic 'theca' of the appropriate size around the main body of cytoplasm. The cell also migrates a thin veil of cytoplasm about the whole of the test – both the new chamber and the existing chambers – such that the whole test is effectively enclosed in a vacuole. Calcium carbonate is then laid down in two distinct phases. The first phase consists of calcite crystals that nucleate only in association with the newly-made organic theca. Then, a thicker secondary layer of calcite crystals is added over the whole test, including all the old chambers. The structure, and proportion of impurities in this second layer is different to that in the primary layer.

What are the functions of external mineral scales?

The most commonly proposed function of rigid scales and skeletons is protection against predation and/or parasitism. Larger eukaryotic microbes such as diatoms are often subject to grazing by animals, as well as predation by other microbial eukaryotes. Protection against predation by animals could take two forms – one would be resistance to mechanical crushing forces (reducing predation by weak-jawed predators). The other would be an increase in external dimensions of cell (reducing predation by predators with small mouths or feeding apparatuses). In the case of the silica frustules of diatoms, mechanical tests

show that these rigid structures are extremely strong and could resist crushing by some small planktonic animals that would otherwise be expected to be able to consume particles of that size. An increase in silicification of the frustule in response to the presence of active herbivores has been documented in lab experiments, suggesting an adaptive response, and thus consistent with a protective function. Studies of protists grazing on the model coccolithophorid *E. huxleyi* have shown significantly lower growth rates for the predators when they are fed calcifying strains than when fed on noncalcified strains, perhaps because the coccoliths reduce digestion efficiency. This again is consistent with, but does not prove, a grazing-protection function.

Protection against parasites is another plausible primary function for scales. Eukaryotic microbes are subject to attack from parasites ranging from viruses to other eukaryotes. In particular, viruses that infect eukaryotes generally require contact with the cell membrane to penetrate the host cell and initiate infection. Complete scale layers like those of coccolithophorids, or frustules, might be a reasonably effective protection against many parasites (note that the small pores in diatom frustules – areolae – are too small to admit most virus particles). It must be noted that many eukaryotic cells have layers of *organic* scales (see Notes from Part 1 of the course), which could also be protective in similar ways. Also, it is notable that the well-studied 'EhV' virus of *E. huxleyi* specifically attacks the coccolith-bearing diploid cells, and not the non-calcified haploid cells (albeit there are many differences between diploids and haploids in addition to presence/absence of coccoliths).

Another role suggested for mineral scales is in buoyancy regulation. Silica and calcite are much denser than seawater, and production and selective release of scales as 'ballast' could help planktonic cells to counterbalance changes in the buoyancy of the cell proper, or to migrate vertically (see notes on 'Locomotion'). Benthic foraminifera may use their often extremely heavy tests to avoid suspension in turbulent water, as well as to protect from mechanical abrasion.

One function that has been proposed specifically for calcium carbonate structures is the enhancement of photosynthesis through increasing the availability of carbon dioxide (CO_2), which is required for carbon fixation (CO_2 specifically is the substrate for the carbon fixation enzyme RubisCO). The rate of interconversion between HCO_3^- and CO_2 is very slow, and photosynthetic organisms run the risk of being limited by CO_2 availability; consequently algae employ various 'carbon concentrating mechanisms' to increase CO_2 availability within (ultimately) the plastid.

However, recall that the overall calcification reaction is:

$$Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2$$

Thus, precipitating $CaCO_3$ will also produce carbon dioxide within the cell, and might therefore act as a sort of carbon concentrating mechanism in coccolithophorid algae. It has been suggested that the algal symbionts of foraminifera might benefit from a similar process. The idea is controversial, however: laboratory studies have not shown a positive relationship between calcification and photosynthesis in model coccolithophorids, at least

under nutrient-replete conditions. There are similar experimental counter-indicators for a photosynthesis enhancement by calcification in symbiont-bearing forams.

Interestingly, a role in enhancing photosynthesis via increasing CO_2 availability has been proposed for the *silica* frustule of diatoms. It appears that the silica of the frustule has a buffering role, which increases the efficiency of extracellular carbonic anhydrase (the enzyme that catalyses the interconversion of HCO_3^- and CO_2).

Recently, it has been suggested that the presence of coccoliths, and the fine structuring of diatom frustules, might both influence the physical properties of the fluid layer around cells, and enhance nutrient uptake under real-world conditions (e.g. moderate turbulence). This possibility has not yet been extensively studied.

Finally, it is worth thinking about what producing mineralised scales etc. means for the cell in terms of investment of resources. There is a tendency to assume that mineral structures are costly items to produce, and that they would need to confer a substantial selective advantage to be worth the energy expended to produce them. However, it must be considered that organisms that do <u>not</u> use minerals for building structures like scales may produce similar structures anyway from expensive-to-produce organic material, mainly carbohydrates. These organic materials actually cost more energy to produce than would depositing an equivalent amount of silica, for example. In other words, if it is advantageous for a cell to be enclosed in a scale layer or similar (e.g. to reduce predation or virus exposure), it may well be cheaper to make this layer from silica or calcium carbonate, than to build it primarily from (say) cellulose.

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