RAF1, suggesting that their roles are not entirely overlapping. Much insight is provided into the BSD2 assembly chaperone, which contains a DnaJ-like zinc-binding cysteinrich domain (8). A crystal structure of eight RuBisCO large subunits and eight BSD2 subunits appears to represent a stable late-stage intermediate in assembly; final displacement of the BSD2 subunits by RuBisCO small subunits completes the process. Intriguingly, the sites on the L\textsubscript{s} structure where BSD2 subunits bind and the sites where the small subunits finally sit are almost nonoverlapping; it appears that subtle conformational changes and steric effects couple the addition of small subunits to the departure of BSD2 subunits (see the figure).

Although Aigner et al. clarify much about the RuBisCO chaperone process (and how to implement it in bacteria for practical applications), some mysteries remain. The importance of RAF2 in assembling plant RuBisCO was confirmed, supporting earlier work in maize (9). But its mode of operation is still not clear. A recent structure of the cyanobacterial homolog of RAF2 (acRAF) showed it to be a de novo relic of a pterin dehydratase enzyme, which evolved a new function as a chaperone (10). Among the specialized RuBisCO assembly chaperones, RAF2 is now the only one for which no specialized RuBisCO assembly chaperones, given their role in RuBisCO assembly, consequently regulate RuBisCO degradation as well? RuBisCO researchers can now answer these and other questions about the molecular evolution and detailed mechanisms of chaperoning and assembly of RuBisCO, one of the most important enzymes on Earth.

The E. coli RuBisCO assembly assay provides a facile experimental system to tease apart the functions and evolution of RuBisCO chaperones. For instance, why has RuBisCO seemingly acquired more folding and assembly chaperones as it evolved? Can plant RuBisCO be engineered more easily now to make an improved enzyme for agricultural use? A recent finding suggests that some of the chaperones may have special regulatory roles beyond assembly, including degradation of RuBisCO under particular stress conditions, such as starvation, to provide amino acids (14, 15). RuBisCO is degraded to provide amino acids to cells during stress conditions and senescence (12). Could some of these chaperones, given their role in RuBisCO assembly, consequently regulate RuBisCO degradation as well? RuBisCO researchers can now answer these and other questions about the molecular evolution and detailed mechanisms of chaperoning and assembly of RuBisCO, one of the most important enzymes on Earth.

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BIOLICAL MATERIALS

Coherent nanoparticles in calcite

A toughening strategy known to metallurgists is also used by the brittlestar

By Dorothy M. Duffy

Living organisms use a wide range of minerals to perform a variety of functions, including familiar examples such as bones (for support), teeth (for mastication), and shells (for protection), as well as other less common functions, such as optical, magnetic, and gravity sensing. These biominerals are produced with elements that are present in the local environment under ambient conditions. The ability to mimic biological strategies to improve current materials and processing methods is a long-standing goal of material scientists. On page 1294 of this issue, Polishchuk et al. (1) characterized the properties of a biomineral in the skeleton of the brittlestar, Ophiocoma wendtii. An array of microlenses on their skeletons focus light onto an optical receptor, enabling them to detect shadows and hide from predators. Nanoprecipitates in these lenses also toughen the skeleton, an effect that is achieved in engineered metal alloys only through expensive heat treatments.

The lenses are made of single-crystal calcite, ~50 μm in size, arranged in a hexagonal pattern on their dorsal arm plates. The microstructure of these brittlestar lenses has been known since 2002 (2), but until now, little was known about the nanostructure. Polishchuk et al. found that the single-crystal calcite lenses contained arrays of calcite nanoparticles, ~5 nm in diameter, that have a higher magnesium content than the host crystal. Further investigation revealed that these nanoparticles were coherent with the host, meaning that the crystal lattice planes were continuous as they passed through the nanoparticles. Coherent nanoparticles, sometimes referred to as Guinier–Preston, or GP, zones (3, 4), are well known in metallurgy, where they are...
used to increase the strength of the metal. The presence of GP zones had not previously been detected in biominerals.

The microlenses of the brittlestar have a dual function. Not only do they focus light onto a receptor, but they are also part of the skeleton and, as such, they need to have robust mechanical properties. Like all minerals, calcite is very brittle, and it is susceptible to fracture. Organisms have developed a number of strategies to increase the toughness of biominerals. One of the most common, used in both bone (5) and seashells (6, 7), is the formation of complex hierarchical structures of hard and soft materials (8). The combination of hard and soft material is also used in synthetic composites, such as the carbon-fiber-reinforced polymers that have numerous high-strength, low-density applications.

Such complex structures would not be suitable for optical lenses, so these brittlestars required an alternative toughening strategy. The similarity of the magnesium-rich nanoparticles in the calcitic lenses to coherent nanoparticles in metals suggests that they may play a role in the enhancement of the mechanical properties. In metals, GP zones increase the tensile strength by inhibiting dislocation motion, but they also decrease ductility (increase brittleness) by the same mechanism.

Failure of brittle materials, such as calcite, occurs via a different mechanism than in metals, as dislocation mobility is limited. Brittle materials break catastrophically when a combination of the applied stress and the crack length reaches a critical value, known as the fracture toughness. The fracture toughness of a material is a measure of its resistance to fracture and is related to the energy required to extend existing cracks in a material. Fracture toughness can be increased by introducing interfaces to the material, as in bones, shells, and synthetic composites. It can also be increased by introducing a compressive stress, to counteract the applied tensile stress. Synthetic brittle materials that use such a strengthening mechanism include tempered glass and prestressed concrete.

The hypothesis put forward by Polishchuk et al. is that the magnesium-rich nanoparticles induce a compressive stress in the host calcite material, which increases the fracture toughness. The stress originates from the smaller lattice parameters of magnesium-rich calcite and the coherent nature of the lattice planes, which together create a tensile stress in the particles and a compensating compressive stress in the surrounding host crystal (see the figure). The hypothesis is supported by measurements of fracture toughness, which was found to be on the order of a factor of 2 higher than for single-crystal geological calcite.

Coherent nanoparticles in metals, such as aluminum, are formed by cooling a molten mixture of aluminum and copper to produce a supersaturated solid solution of copper atoms in aluminum (9). The solid solution is then heated, or annealed, and nanoparticles of a second phase, which have a high concentration of copper atoms, precipitate from the solid solution. The size of nanoparticles is controlled by the annealing temperature, as growth is limited by the rate at which the copper atoms diffuse through the crystal.

Biominerals must form under ambient conditions, and many biominerals are known to form from an amorphous precursor. As the solubility of magnesium in amorphous calcium carbonate is high, this precursor may be rich in magnesium. Magnesium is much less soluble in crystalline calcite than in amorphous calcium carbonate, so crystallization results in a supersaturated solid solution. Over time, the excess magnesium atoms diffuse to form coherent magnesium-rich precipitates. Such a formation mechanism of coherent nanoparticles in calcite is analogous to that of copper-rich nanoparticles in aluminum, as both evolve from supersaturated solid solutions.

Polishchuk et al. identified a toughening mechanism that does not disrupt the primary function of the biomineral—that is, the focusing of light. It is possible that prestressing by coherent nanoparticles occurs in other dual-function biominerals, where toughening with hierarchical structures is not an option, as it would disrupt the primary function of the biomineral. Future investigations should explore other dual-function biominerals to determine the extent to which nature employs such a strategy. Future research should also attempt to replicate the formation of coherent nanoparticles in minerals or ceramics by crystallization from an amorphous precursor, with the aim of developing an energy-efficient process for increasing the fracture toughness of ceramic materials.

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10.1126/science.aaq0111
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Science 358 (6368), 1254-1255.
DOI: 10.1126/science.aaq0111