scientists and managers (*9*, *10*), and these can potentially be balanced with human demands. For example, in drought-prone rivers, flows can be designed to favor native over non-native fish species while simultaneously meeting human water needs (*11*).

The Sabo *et al.* paper reinforces the growing understanding that water managers can, and must, balance socioeconomic and ecological needs for regulated rivers. Incorporating flexibility into the design of new dams will enable proactive maintenance of downstream ecosystem services, even as future hydrology and social demands for water change (*6*). Such flexibility will be critical for adaptive management; it should be mandated for all new dam constructions and selectively required for existing dams.

With the likely expansion of the designer flow philosophy, careful evaluation of limitations and uncertainties is needed. For example, Sabo *et al.*'s work shows that production of key species in the Tonle Sap fishery is likely to be enhanced under a designed flow regime; however, it remains unclear whether such engineered flows support broader biodiversity that may promote sustainable fish populations.

Ultimately, managing rivers for multiple, sustainable benefits requires integrating scientific, social, and policy perspectives into operational decision frameworks (*12*). In rivers, optimizing among divergent stakeholder interests remains a challenge because flow-dependent benefits are distributed in complicated and often unspecified ways (*13*). For example, the water-dependent cultural values of local or indigenous peoples have historically been largely excluded from water management decisions and are therefore often deemphasized in decisions about building and operating water infrastructure (*14*). In the Mekong River, cultural values and economic livelihoods align around the native fisheries. Given this, Sabo *et al.*'s work is an exemplar of how ecological science can inform equitable distribution of river-dependent benefits toward achieving social-ecological sustainability in complex and contested river ecosystems. ■

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BIOCHEMISTRY

Putting the RuBisCO pieces together

Scientists find a way to build the major plant enzyme RuBisCO in bacteria

By **Todd O. Yeates** *and* **Nicole M. Wheatley**

Among the thousands of different enzymes that have evolved in nature, ribulose-1,5-bisphosphate carboxylase-oxygenase (known as RuBisCO) holds a special place. It is the enzyme in plants, algae, and many photosynthetic bacteria that ultimately takes energy derived from the Sun and uses it to convert or "fix" atmospheric CO_2 into organic forms of carbon that constitute the basis for life (*1*). Globally, RuBisCO fixes enormous quantities (gigatons) of carbon annually. To carry out such a massive chemical conversion requires a huge amount of the enzyme, especially because RuBisCO performs reactions quite slowly. Accordingly, RuBisCO is believed to be the most abundant enzyme on the planet (*1*, *2*). RuBisCO is unusual in other ways as well. Each RuBisCO enzyme in the cell requires a tremendous amount of help, from chaperones, to fold into the correct three-dimensional shape required for its function. The large number of chaperones is important for two reasons. One is that there are numerous questions about the identities of these chaperones and how they operate on a structural level. Another is that it has been difficult to produce correctly folded RuBisCO in laboratory experiments, which has also limited the ability to engineer alternate forms of the enzyme. On page 1272 of this issue, Aigner *et al.* (*3*) fill in some of the most important remaining pieces of the RuBisCO folding and assembly puzzle.

> ***"Can plant RuBisCO be engineered more easily now to make an improved enzyme for agricultural use?"***

The amino acid sequence of a protein encodes the final three-dimensional structure into which it folds. But to fold correctly, some proteins require help from chaperones (*4*, *5*). Intriguingly, most cellular proteins that need help folding get by with the assistance of one or a few generic chaperones that fold many different proteins, whereas RuBisCO requires numerous chaperones, including some that appear dedicated exclusively to that special task (*6*–*10*). The task is made more complex by the need to assemble 16 total protein subunits together (L_8S_8, eight large and eight small subunits) in one RuBisCO enzyme.

Aigner *et al.* found that seven chaperones—chaperonin 60-subunit α1 (Cpn60α), Cpn60β, 20-kDa chaperonin (Cpn20), RuBisCO assembly factor 1 (RAF1), RAF2, ribulose bisphosphate carboxylase factor X (RbcX), and bundle-sheath defective–2 (BSD2)—help plant RuBisCO fold and assemble when the proteins are all expressed together in the bacterium *Escherichia coli* (see the figure). The Cpn60α-Cpn60β chaperone complex provided for general adenosine 5′-triphosphate–dependent protein folding of the large subunit of RuBisCO. The Cpn60 complex forms a large, barrel-shaped structure and is assisted by small capping subunits, Cpn10 or Cpn20. Misfolded proteins are drawn into the Cpn60 barrel interior where they can be refolded within a protected environment. Consistent with previous reports (*11*, *12*), the authors found that two slightly different versions of Cpn60—Cpn60α and Cpn60β—are required in combination for plant RuBisCO large-subunit folding, and they cannot be replaced by their homolog in *E. coli*, GroEL. This is in contrast to cyanobacterial chaperonins, which can be substituted by GroEL to fold cyanobacterial RuBisCO.

Although the general Cpn60-Cpn20 chaperone complex can fold RuBisCO large subunits, more specialized chaperones are needed to assemble RuBisCO into its final L_8S_8 form. Aigner *et al.* clarify the role of all four of the known plant RuBisCO-specific chaperones: RbcX, RAF1, RAF2, and BSD2. Previous studies showed that RbcX (*6*) and RAF1 (*7*) both bind and stabilize pairs of RuBisCO large subunits during the multistep assembly process, suggesting that they might be functionally redundant. But, Aigner *et al.* show that RbcX cannot effectively replace

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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 cysteine-rich 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_8 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 defunct 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 three-dimensional structural data are available when bound to RuBisCO subunits or peptide fragments, leaving open its mechanism of action. The roles of other endogenous *E. coli* chaperones, DnaK, DnaJ, and GrpE (*13*), were not evaluated in Aigner *et al*.'s work, leaving open the possibility of their additional contribution to plant RuBisCO assembly.

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 might 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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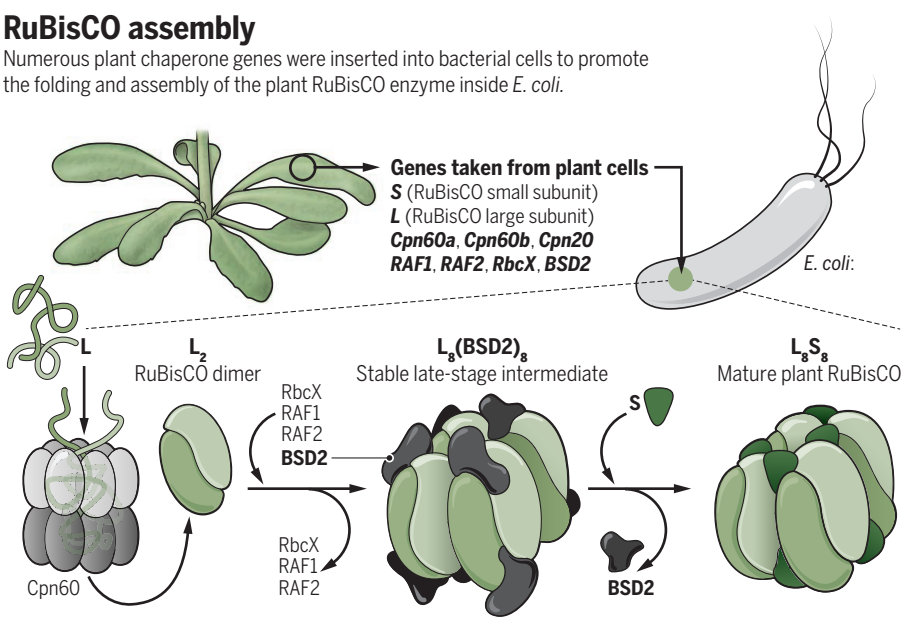
RuBisCO assembly

Numerous plant chaperone genes were inserted into bacterial cells to promote the folding and assembly of the plant RuBisCO enzyme inside *E. coli*.



BIOLOGICAL 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 nanoprecipitates, ~5 nm in diameter, that have a higher magnesium content than the host crystal. Further investigation revealed that these nanoprecipitates 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

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