An unexpected sticking point for carboxysome assembly

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In order to improve photosynthetic efficiency, bacteria often enclose Rubisco and carbonic anhydrase into microcompartments called carboxysomes. Assembly of these complexes requires a protein called CcmM. It had previously been thought that CcmM mediated Rubisco assembly by displacing one of the Rubisco subunits, Ryan et al. show that despite having a three-dimensional structure that closely resembles the Rubisco small subunit, CcmM does not dislodge it, leading to a proposal for an alternative binding location. These results provide a new model for carboxysome assembly with implications for photosynthetic engineering.

Photosynthesis requires both light-dependent and light-independent reactions. The latter includes the actual step of carbon fixation, in which the enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) incorporates CO₂ into carbohydrate products. However, Rubisco can also catalyze the oxygenation of the ribulose biphosphate substrate, generating off-pathway products that require energy to recycle. To enhance the efficiency of photosynthesis, cells can increase the proportion of carbon dioxide relative to oxygen, which promotes the carboxylation reaction. Cyanobacteria achieve this by using a series of pumps to move HCO₃⁻ into the cytosol, and surrounding Rubisco and carbonic anhydrase in a protein-enclosed microcompartment called a carboxysome (1). The HCO₃⁻ that is pumped into the cytosol diffuses into the carboxysome where it is converted to CO₂ by carbonic anhydrase, increasing the effective CO₂ concentration at the Rubisco active site. These steps are of more than academic interest: Many studies are underway inserting bacterial Rubisco into plant chloroplasts that produce carboxysomes with encapsulated bacterial Rubisco (5). Understanding the assembly process will be required in order to incorporate plant Rubisco enzymes into a functional carboxysome.

CcmM is the protein that acts as a molecular glue to assemble Rubisco enzymes together inside the carboxysome. At one end of the protein, an N-terminal domain acts as a carboxic anhydrase, while at the other end there are 3–5 copies of an assembly domain separated by short linker regions. Cyanobacterial and plant Rubisco enzymes are hexadecameric, consisting of four dimers of large subunits, each of which contains an active site, and four small subunits at each end (Fig. 1a). The sequence of CcmM assembly domain resembles that of the Rubisco small subunit, leading to the suggestion that it may bind to the Rubisco large subunits by displacing the small subunits (6, 7) (Fig. 1a), but it was not known whether the three-dimensional structures also showed similarities.

A new study by Ryan et al. (8) answers this question, solving the crystal structure of a single CcmM assembly domain and confirming that it does indeed resemble that of the Rubisco small subunit (Fig. 1c). The structure shows that the CcmM assembly domain is missing two key motifs that are important for the binding of the Rubisco small subunit to the Rubisco large subunits, namely the N-terminal loop and hydrophobic β-bulge motifs. The absence of these motifs, together with differences in the nature of their surface properties, suggested to the authors that CcmM is likely to bind to Rubisco large subunits using a mechanism different from that of the small subunits and is unlikely to displace the small subunits.

To test whether the small subunits of Rubisco are indeed displaced upon binding of CcmM, Ryan et al. (8) used native MS and size exclusion chromatography methods to measure the size of the complex formed between isolated CcmM assembly domains and Rubisco. They showed that it was consistent with 1–5 CcmM assembly domains binding to each Rubisco hexadecamer, without the detection of any free Rubisco small subunits, strengthening the contention that CcmM can bind to Rubisco without displacing the small subunits (Fig. 1b). But, if CcmM does not displace the small subunits, does it bind? The authors suggest that the positive surface charge of CcmM, which is distinct from the more neutral/hydrophobic small subunit, might be complementary to a specific electropositive patch on the large subunits; it will be exciting to see if further studies confirm this putative location as the CcmM-binding site.

It could be argued that the structural studies and determination of the size of the protein complexes used only a single CcmM assembly domain and may not be representative of the
overall binding of the complete CcmM protein, which contains multiple assembly domains joined by short linker regions. To determine if this was indeed the case, Ryan et al. (8) used surface plasmon resonance binding assays to measure the binding affinity of CcmM constructs containing either a single assembly domain, two assembly domains joined by a linker, or all four assembly domains. These assays showed that all CcmM constructs bound Rubisco with a similar affinity, in the low micromolar range, confirming that a single CcmM binding domain binds in a fashion similar to the full-length protein and providing one of the first direct measurements of the affinity of Rubisco for one of its binding partners. In order to function properly, Rubisco interacts with a plethora of different binding partners, and we require detailed knowledge of these interactions if we are going to engineer photosynthesis to be more efficient. In addition to those involved in carboxysome formation, these include proteins involved in the Rubisco assembly process, Rubisco activase, which maintains Rubisco activity, and methyltransferases, which methylate specific lysine residues of Rubisco. Although the current study suggests a model in which CcmM interacts with Rubisco via a pocket between the dimers of large subunits, the exact nature of how CcmM binds is still unknown. Techniques such as cryo-EM are now allowing us to directly visualize many of the interactions with Rubisco for the first time (9, 10), providing an insight into how these proteins work together to optimize photosynthesis, and it is more than likely that we will soon be able to get a higher resolution structure of exactly how proteins are arranged in the carboxysome. The results presented here will certainly help in designing a CcmM glue that will stick to plant Rubisco, not just bacterial Rubisco. References

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**Editors’ Pick Highlight: A sticking point for carboxysomes**

**Figure 1.** Rubisco is a hexadecameric protein, with 8 large subunits (blue) forming the active core and 4 small subunits (green) at each end. CcmM consists of multiple assembly domains joined by flexible linker regions (magenta). While it had previously been proposed that CcmM assembled Rubisco in the carboxysome by displacing the Rubisco small subunits (a), Ryan et al. (8) propose a new model in which CcmM does not displace the small subunits, but instead binds at a different location on the large subunits (b), c, the structure of the CcmM assembly domain (magenta) closely resembles that of the Rubisco small subunit (green), but has a less extended N-terminal domain.

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**a) Previous model: CcmM binds to Rubisco by displacing the small subunits**

**b) New model: CcmM binds to Rubisco without displacing the small subunits**

**c) The CcmM assembly domain closely resembles the Rubisco small subunit**