Rubisco rescue

Rubisco catalyses the first step in photosynthetic carbon fixation, but it can be easily poisoned by side-products of its activity. Structural and functional analyses of a protein conserved across plants, algae and bacteria shows how one such blockage is both removed and recycled.

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The sunlight-driven conversion of carbon dioxide into organic matter fuels nearly all life on Earth. The enzyme Rubisco catalyses the fixation of carbon dioxide in photosynthesis, and thereby serves as the entry point for inorganic carbon into the biosphere. Rubisco limits biomass accumulation when sunlight is plentiful, and has therefore been heavily targeted in bioengineering efforts to boost crop yields. Central to the success of such strategies is a thorough understanding of the way in which this enzyme is assembled and activated. Writing in this issue of *Nature Plants*, Bracher and colleagues report the structure and function of a sugar phosphatase that contributes to the maintenance of carbon fixation through the highly selective breakdown of an endogenous inhibitor that would otherwise quickly overwhelm Rubisco.

Rubisco uses carbon dioxide to carboxylate the five-carbon compound ribulose bisphosphate (RuBP). The first step in this process involves the abstraction of a proton from Rubisco-bound RuBP to yield a highly reactive enediolate intermediate (Fig. 1). If all goes well, this intermediate accepts a molecule of carbon dioxide, setting in motion the synthesis of usable sugars. But it can also accept a molecule of oxygen, initiating the photorespiratory pathway that leads to the loss of fixed carbon. On occasion, the enediolate intermediate can accept a proton, generating xylulose bisphosphate (XuBP). Structurally highly similar to RuBP, XuBP inhibits Rubisco by binding to and ultimately blocking the enzyme’s active sites. XuBP is generated at particularly high rates in C3 plants, as they lack the ability to concentrate carbon dioxide at the site of fixation.

In general, the motor protein Rubisco activase (Rca) triggers the release of tight-binding inhibitors from Rubisco. Powered by ATP hydrolysis, Rca is thought to alter the conformational state of Rubisco to favour the opening of active sites. In recent years, interest in Rca has grown at a phenomenal rate due to the publication of its structure and function. Bracher and colleagues show that a second protein, XuBP phosphatase, degrades XuBP on release from Rubisco, yielding xylulose-5-phosphate (Xu5P), which can be recycled back to RuBP. Their findings suggest that Rca and XuBP phosphatase work together to restore carbon assimilation in the presence of XuBP.

**Figure 1** Saving Rubisco from itself. On binding to Rubisco, a proton is abstracted from RuBP generating a highly reactive enediolate intermediate that can accept a molecule of carbon dioxide, a molecule of oxygen or a proton. Acceptance of a proton leads to the generation of XuBP, an inhibitory compound that binds to the active sites of Rubisco and blocks activity. The motor protein Rca speeds up the release of XuBP from the active sites. Bracher and colleagues show that a second protein, XuBP phosphatase, degrades XuBP on release from Rubisco, yielding xylulose-5-phosphate (Xu5P), which can be recycled back to RuBP. Their findings suggest that Rca and XuBP phosphatase work together to restore carbon assimilation in the presence of XuBP. XuBP inhibits Rubisco when it binds to an inactive, decarbamylated form of the enzyme. Rca facilitates the release of inhibitory RuBP from Rubisco. On activation, the binding of RuBP leads to the formation of the enediolate intermediate. Rubisco is shown in pale yellow and inhibitors in red.
of crystallographic structures and the concomitant promise of structure-guided engineering. Rca is thought to play a pivotal role in the upkeep of Rubisco in light-adapted leaves and in the coordination of photosynthetic processes in response to changing light levels. Strikingly, this enzyme appears to dictate the rate of photosynthetic induction following the transition from low to high light conditions. Current protein engineering efforts are primarily aimed at increasing the thermal stability of Rca, and modulating its abundance and activity.

Although Rca oversees the release of XuBP from the active sites of Rubisco, the ultimate fate of this inhibitor has remained unclear. Bracher and colleagues shed light on this topic by reporting the structure and function of a highly selective sugar phosphatase that works in concert with Rca to prevent the build-up of inhibitory XuBP at Rubisco active sites. They analyse homologous phosphatases from the photosynthetic bacterium *Rhodobacter sphaeroides* and from the model plant *Arabidopsis thaliana*. Although they focus on these two species, the studied protein appears to be conserved across plants, algae and a number of photosynthetic bacteria. They show that the protein converts XuBP to the non-inhibitory compound xylulose-5-phosphate (Xu5P), an intermediate in the regeneration of RuBP by the Calvin cycle. Crystallographic and mutational analyses reveal that the cap domain of the enzyme confers selectivity for XuBP over RuBP. A particularly impressive feature of the study is the demonstration that XuBP phosphatase acts synergistically with Rca to restore the activity of XuBP-inhibited Rubisco — activity is restored only slightly in the presence of Rca, but fully in the presence of both Rca and XuBP phosphatase.

In many ways the regulation of XuBP levels by Rca and XuBP phosphatase mirrors that of carboxyarabinitol-1-phosphate (CA1P), a Rubisco inhibitor synthesized under dim light in the chloroplast of some plants. Like XuBP, CA1P is released from Rubisco by Rca, and dephosphorylated by its own phosphatase. However, whereas CA1P has a clear physiological function — the reduction of Rubisco activity at night — the inadvertent production of XuBP is more akin to a mechanistically unavoidable nuisance.

Rubisco’s oxygenating activity generates another inhibitor, pentodiulose bisphosphate (PDBP), which binds to the active sites of Rubisco and impedes function. This inhibitor may also be released by Rca, and appears to be metabolized by the CA1P phosphatase. High temperatures promote the oxygenation of RuBP by Rubisco and therefore increase the production of the PDBP misfire product. At the same time, heat is also expected to increase the production of XuBP because the drought conditions that often accompany heat waves cause stomatal closure, reducing the amount of carbon dioxide available for fixation. For reasons not completely understood, Rca appears unable to keep up with the rising demand for inhibitor removal at high temperatures. As such, the extent to which PDBP and XuBP attenuate the catalytic activity of Rubisco is likely to grow as the climate warms, although wedging Rubisco sites closed could provide adaptive advantages under some conditions.

The findings of Bracher and colleagues take us one step closer to uncovering the full suite of mechanisms that regulate biological carbon fixation. As nicely illustrated by the recent success in transferring a cyanobacterial Rubisco into a vascular plant, a thorough understanding of Rubisco regulation could pave the way to the rational engineering of crops for improved biomass accumulation. Such bioengineering efforts could prove key to meeting the food demands of a rapidly expanding global population in an increasingly uncertain climate.

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References