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A peptide adhesive molded by magnesium glues Rubisco's subunits together

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Rebekka M. Wachter[1]

From the School of Molecular Sciences and Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, Arizona 85287

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Rubisco enzymes play central roles in carbon fixation, with potential importance in biotechnology, but have eluded a full description of their multistep assembly and function. A new article describes the fascinating discovery that some archaeal Rubiscos contain a built-in assembly domain inserted into an otherwise canonical Rubisco fold, providing a tremendous expansion of our understanding of the diversity of naturally occurring Rubiscos.

Rubisco forms I and II are found in photosynthetic organisms and are responsible for almost all carbon fixation on earth, converting CO_2 and the sugar ribulose 1,5-bisphosphate (RuBP)[2] into 3-phosphoglycerate as part of the Calvin-Benson-Bassham (CBB) cycle (1). Form I Rubiscos (L8S8) partner the catalytic large subunits (LSU) with small subunits (SSU) to ensure optimal activity and are known to undergo a complex folding and assembly process. Formation of the holoenzyme requires highly specific chaperones such as RbcX, Raf1, and Raf2 to coordinate proper oligomerization to the hexadecameric state (2, 3). However, the mechanistic details of assembly and catalysis remain only partially understood, limiting biotechnological applications of these intriguing enzymes. Form II and Form III Rubiscos lack SSU. Form III Rubiscos, while using conserved active site features to catalyze the same carboxylation reaction as Form I and Form II (4), are expressed in anaerobic archaea that do not perform photosynthesis (5). Instead, these enzymes play a role in AMP metabolism, utilizing a phosphorylase and an isomerase in combination with Rubisco to generate adenine and 3-phosphoglycerate (5). In this process, the adenine base of AMP is salvaged, and the ribose moiety is fed into the central carbon metabolism. However, Form III enzymes can function as substitutes for endogenous Rubiscos in photosynthetic bacteria (4), meaning that they offer alternative assemblies to explore Rubisco structure and function. Gunn *et al.* (6) take advantage of this opportunity in their investigation of an archaeal Rubisco with an unusual insertion that defines a new self-association pathway and a new subtype of Rubisco sequences.

The methylotrophic methanogen *Methanococcoides burtonii* is an extremophilic archaeon that thrives in cold water and is capable of growing on methylamines and methanol. Based on sequence homology, the *M. burtonii* Rubisco had been classified as belonging to Form II, but it contains an unusual 29-residue sequence insertion into the TIM barrel fold of the LSU that deviates from this form. Gunn *et al.* (6) use X-ray crystallography to show that this sequence adopts a helical structure flanked by two irregularly structured but well-ordered peptide segments. The domain, which the authors term the "Rubisco assembly domain" (RAD), forms a knob-like extension to the surface of each LSU. In the ring-like L10 assemblies that are generated by the association of five dimeric (L2) subunits, each RAD domain makes molecular contact with the adjacent LSU (Fig. 1). Notably, the RAD domain uses the interfacial coordination of a magnesium ion to paste adjacent dimers together.

Broadly, the apparent function of the RAD domain in mediating assembly is reminiscent of the small subunit (SSU) found in Form I (L8S8) Rubiscos. The RAD consists of only 29 residues, compared with the 110–160 residues of the SSU that adopt the structure of a four-stranded anti-parallel β-sheet with two flanking helices (1). In the Form I hexadecameric assembly, each SSU contacts a substantial surface area of two adjacent LSU. In contrast, the RAD may be more appropriately classified as a subdomain, as it is unlikely to form an independently folding unit. Interestingly, *M. burtonii* Rubisco achieves full functionality only upon substrate-assisted assembly to ring-like complexes (7). It is tempting to speculate that, prior to assembly, part of the RAD helix may be disordered, presumably because it would lack magnesium ion coordination. Upon binding of an appropriate carbohydrate such as RuBP to the active sites, conformational adjustments may be transmitted over a distance of 44 Å to the dimer-dimer contact surfaces, thereby triggering assembly from the L2 to the L10 form. In this scenario, part of the RAD domain would undergo a disorder-order transition that is induced by RuBP binding, thereby facilitating magnesium ion coordination (Fig. 1).

With this structural information in hand, Gunn *et al.* (6) return to the classification of the *M. burtonii* Rubisco: their assessment of its sequence homology suggests it is intermediate between Forms II and III. However, based on both structure and function, Gunn *et al.* (6) choose to group this protein with Form III Rubiscos, defining a new subclass named IIIB.

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[1] To whom correspondence should be addressed. Tel.: 480-965-8188; Fax: 480-965-2747; E-mail: rwachter@asu.edu.

[2] The abbreviations used are: RuBP, ribulose 1,5-bisphosphate; LSU, large subunit(s); SSU, small subunit(s); RAD, Rubisco assembly domain.

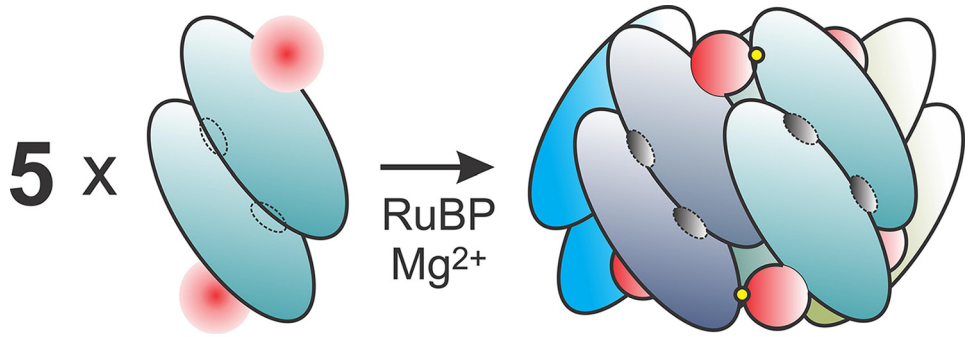

Figure 1. Cartoon of the *M. burtonii* Rubisco assembly mechanism. Dimeric units of the Rubisco LSU are colored in shades of *blue*. RADs are colored *red*, and magnesium ions are colored *yellow*. In the L2 form (*left*), the structure of the RAD domain is unknown and may be partially unstructured. In the fully assembled L10 form (*right*), the RAD domain adopts a distinct tertiary fold and links the dimeric units to their neighbors via interfacial magnesium ion coordination. The occluded active sites (*dashed lines*) are now occupied by RuBP.

The built-in assembly domain of the archaeal *M. burtonii* Rubisco provides a possible route to bypass the need for assembly factors, thereby providing opportunities for Rubisco engineering without the necessity to co-engineer a large array of auxiliary proteins. The co-evolution of assembly factors such as RbcX, Raf1, and Raf2 provides for interaction specificity with cognate large or small subunits of Rubisco. For this reason, the functional expression of Rubisco holoprotein in heterologous organisms remains quite challenging (8). Will we be able to learn from the structural and dynamical features of the RAD domain to design novel Rubisco proteins that undergo facile assembly in transgenic organisms? If so, the effects of improved Rubisco kinetics on photosynthetic performance and biomass accumulation could be evaluated more rapidly.

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