How protein-Protein interactions contribute to pyrenoid formation in Chlamydomonas

Ananya Mukherjee
Louisiana State University

James V. Moroney
Louisiana State University

Follow this and additional works at: https://digitalcommons.lsu.edu/biosci_pubs

Recommended Citation

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.
The chloroplast pyrenoid, an important component of the CO₂ concentrating mechanism of algae, is a structure composed primarily of Rubisco. In Chlamydomonas, Rubisco in the pyrenoid is held together by the linker protein EPYC1. Atkinson et al. (2019) determined the regions of the Rubisco small subunit and EPYC1 that are important for the protein-protein interaction, thus making progress towards reconstruction of a pyrenoid in higher plants. Why is a protein soluble in one organism while its homologue in another species becomes part of a liquid-like cell structure? That is the question being addressed by Atkinson et al. (2019) in this issue of the Journal of Experimental Botany. It is even more striking when the protein is ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the most abundant soluble enzyme in plants and algae. In terrestrial plants, Rubisco behaves as a soluble protein found throughout the chloroplast stroma of leaf mesophyll cells. However, in most algae, Rubisco is found in a structure within the chloroplast called the pyrenoid.

The physiological consequences of this packaging of Rubisco are profound. In general, algae with pyrenoids have a much higher affinity for inorganic carbon (\( C_i = CO_2 + HCO_3^- + CO_3^{2-} \)) than terrestrial C₃ plants. These algae are able to raise the CO₂ concentration for Rubisco through the CO₂ concentrating mechanism (CCM). Our current thinking is that the packaging of Rubisco is a requirement for the CCM (Mackinder, 2018; Moroney and Ynalvez, 2007; Spalding, 2008). However, since CO₂ can readily cross cell membranes (Tolleter et al., 2017), how can a single-celled organism possibly concentrate CO₂? Current CCM models have cells accumulating HCO₃⁻, an ion that does not readily cross membranes, instead of CO₂ directly. After taking up HCO₃⁻, a key step in this process is the conversion of the accumulated HCO₃⁻ to CO₂ by the action of the enzyme carbonic anhydrase (CA). This raises the CO₂ concentration at Rubisco, which is located physically close to the CA. Then the Rubisco has a chance to fix the substrate CO₂ before the CO₂ diffuses away (Box 1A). If the pyrenoid is not correctly formed, the CO₂ will inevitably leak out of the cell (Box 1B). Another proposed reason for the pyrenoid organization is that it facilitates the recapture of some of the CO₂ as it leaks past Rubisco. In Chlamydomonas, the pyrenoid is surrounded by a starch sheath and the heteromeric protein LCIB/LCIC. The LCIB/C complex has been proposed to act as a CA, converting leaking CO₂ to the less permeant HCO₃⁻. Thus, CO₂ generated by the CCM must pass through Rubisco and then a CA layer before it has a chance to leave the chloroplast (Box 1A).

In Chlamydomonas, the protein sequence of the Rubisco small subunit (SSU) and the protein EPYC1 (essential pyrenoid component 1) are important to the formation of the pyrenoid (Mackinder et al., 2016; Meyer et al., 2012). The first evidence came from the work of Meyer et al. (2012), who showed that Chlamydomonas cells expressing the Arabidopsis SSU instead of the Chlamydomonas SSU failed to form a pyrenoid and failed to grow in ambient CO₂. However, it was interesting that the Rubisco in these cells, consisting of the Chlamydomonas large subunit (LSU) and Arabidopsis SSU, was still enzymatically active. Meyer et al. (2012) also identified regions in the Rubisco SSU necessary for pyrenoid formation. Replacing specific Chlamydomonas SSU α-helices with the corresponding sequence from plant Rubisco SSU resulted in cells without pyrenoids and with defective CCMs. In 2016, Mackinder et al. found that EPYC1 was also required for pyrenoid formation. EPYC1 is a linker protein which binds to Rubisco in Chlamydomonas and facilitates the liquid-like formation (Küken et al., 2018; Rosenzweig et al., 2017). It is not present in terrestrial plants. Loss of EPYC1 results in Chlamydomonas cells being unable to form a normal pyrenoid and develop a functional CCM (Mackinder et al., 2016).

The work described by Atkinson et al. (2019) greatly extends these studies by investigating which regions of each protein are required for SSU-EPYC1 binding. Chlamydomonas has two genes encoding the SSUs, designated S1_Cr and S2_Cr. Atkinson et al. (2019) used quantitative yeast two-hybrid (Y2H) experiments to show that EPYC1 strongly interacts with both Chlamydomonas SSU homologs but not with the Arabidopsis SSU (1A₄ₐ). They then systematically replaced
parts of the Chlamydomonas SSU with the corresponding regions of the Arabidopsis SSU. They found that multiple parts of the Chlamydomonas SSU contributed to the SSU-EPYC1 interactions. Substituting in the two \( \alpha \)-helices from the Chlamydomonas SSU into the Arabidopsis SSU was essential for interaction, while adding in the \( \beta \) sheets or the \( \beta \mathrm{A}-\beta \mathrm{B} \) loop region greatly increased SSU-EPYC1 binding. Atkinson et al. (2019) repeated these Y2H experiments but with modifications of EPYC1. EPYC1 has four repeat regions with short terminus regions. They found that each of the repeated regions and the C terminus contributed to the binding of EPYC1 to the SSU. Thus, large portions of each protein were important to the strength of the protein-protein interaction. They also found that a mixture of EPYC1 and the Chlamydomonas SSU could phase separate and form liquid droplets \textit{in vitro}, indicating that a large number of components may not be needed to form a pyrenoid-like structure.

The question arises: can researchers reconstruct a pyrenoid in higher plants? Photosynthesis modelling suggests that introducing algal CCM bicarbonate transporters into \( \mathrm{C}_3 \) plants and packaging Rubisco could lead to a significant increase in photosynthetic efficiency (Furbank et al., 2013; McGrath and Long, 2014; Zhu et al., 2010). Atkinson et al. (2019) took a significant step towards building a pyrenoid by expressing EPYC1 in Arabidopsis wild type plants as well as Arabidopsis plants expressing the Chlamydomonas SSU. However, no Rubisco aggregation was seen in either; instead, an even distribution of Rubisco was seen throughout the chloroplast. It was encouraging that they were able to express EPYC1 in plants, although they postulate that the amount of EPYC1 present was still too low to expect liquid phase separation to occur, which is due to EPYC1-Rubisco interactions. An \textit{in vitro} assay developed by Wunder et al. (2018) showed that a critical EPYC1:Rubisco ratio is required before phase separation occurs; the EPYC1 producing plants in this paper may not have enough of the linker protein.

These results indicate that with a higher EPYC1: Rubisco expression, a packaging of Rubisco might be engineered in a \( \mathrm{C}_3 \) plant, as EPYC1 doesn’t appear to need additional proteins to bind to Rubisco \textit{in vitro}. However, \textit{in vivo}, other components might be required (Ma et al., 2011). Mackinder et al. (2017) showed that in Chlamydomonas, EPYC1 interacts with a protein kinase and 14-3-3 proteins, suggesting a role of phosphorylation in Rubisco-EPYC1 assembly. This finding fits with earlier studies showing that EPYC1 is a phosphoprotein (Turkina et al., 2006). The Atkinson et al. (2019) paper highlights the residues needed for EPYC1-Rubisco interaction in order to obtain a liquid-like pyrenoid. Thus, both EPYC1 and modified Rubiscos have been successfully expressed in heterologous systems of yeast and Arabidopsis by Atkinson et al., (2019) to show that the strength of the EPYC1-Rubisco interaction can be manipulated. This is a big step towards the end goal of organizing Rubisco in \( \mathrm{C}_3 \) plants into a pyrenoid-like structure. Rubisco organized in this fashion should be more efficient at fixing \( \mathrm{CO}_2 \) if a functional CCM is introduced into \( \mathrm{C}_3 \) plants. Rubisco in a pyrenoid-like structure should better capture \( \mathrm{CO}_2 \) generated by a CCM thus preventing \( \mathrm{CO}_2 \) leakage (Box 1A).

---

**Box 1. How the pyrenoid reduces \( \mathrm{CO}_2 \) leakage in \( \text{Chlamydomonas reinhardtii} \).**

A) In a functional pyrenoid, a starch sheath forms around the pyrenoid, which contains most of the Rubisco (indicated with purple). Rubisco interacts with EPYC1 (shown in blue) and that interaction aids in the formation of the pyrenoid. In this figure \( \mathrm{HCO}_3^- \) uptake and its subsequent conversion to \( \mathrm{CO}_2 \) with the help of carbonic anhydrase has not been shown for the sake of simplicity. The Rubisco product glycerate-3-phosphate (PGA), shown by purple arrows, forms when \( \mathrm{CO}_2 \) concentrates in the pyrenoid. \( \mathrm{CO}_2 \) that leaks past Rubisco is sometimes recaptured and converted to \( \mathrm{HCO}_3^- \) (indicated by green arrows) by LCIB/C (red), a stromal carbonic anhydrase, or exits the chloroplast entirely (dotted arrows). B) Absence of EPYC1 prevents the formation of a pyrenoid and accumulated \( \mathrm{HCO}_3^- \) once converted to \( \mathrm{CO}_2 \) easily leaks out and is not recaptured.
Keywords: carbon dioxide concentrating mechanism, carbon dioxide fixation, Chlamydomonas, EPYC1, pyrenoid, Rubisco, Yeast 2 hybrid

References

Furbank RT, Caemmerer S, Price GD. 2013. CO2-concentrating mechanisms in crop plants to increase yield. Applying photosynthesis research to improvement of food crops. ACIAR Proceedings 140, 130–137.


