Journal of Experimental Botany, Vol. 70, No. 19 pp. 5033–5039, 2019
doi:10.1093/jxb/erz299

eXtra Botany

Insight

How protein - protein interactions contribute to pyrenoid formation in Chlamydomonas

Ananya Mukherjee and James V. Moroney*

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA
*Correspondence: btmoro@lsu.edu

The chloroplast pyrenoid, an important component of the CO_2 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.

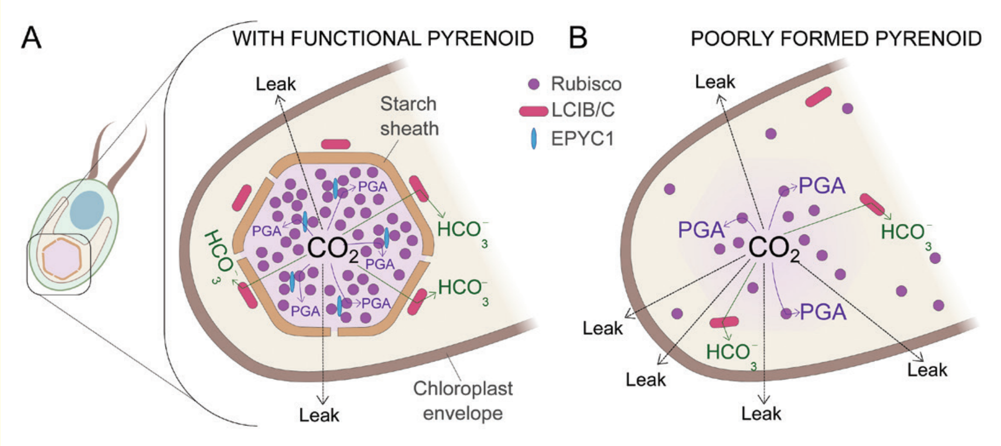
The physiological consequences of this packaging of Rubisco are profound. In general, algae with pyrenoids have a much higher affinity for inorganic carbon ($C_i = CO_2 + HCO_3^- + CO_3^{-2}$) than terrestrial C_3 plants. These algae are able to raise the CO_2 concentration for Rubisco through the CO_2 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 CO_2 can readily cross cell membranes (Tolleter *et al.*, 2017), how can a single-celled organism possibly concentrate CO_2? Current CCM models have cells accumulating HCO_3^-, an ion that does not readily cross membranes, instead of CO_2 directly. After taking up HCO_3^-, a key step in this process is the conversion of the accumulated HCO_3^- to CO_2 by the action of the enzyme carbonic anhydrase (CA). This raises the CO_2 concentration at Rubisco, which is located physically close to the CA. Then the Rubisco has a chance to fix the substrate CO_2 before the CO_2 diffuses away (Box 1A). If the pyrenoid is not correctly formed, the CO_2 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 CO_2 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 CO_2 to the less permeant HCO_3^-. Thus, CO_2 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 CO_2. 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 ($1A_{At}$). They then systematically replaced

Box 1. How the pyrenoid reduces CO_2 leakage in *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 HCO_3^- uptake and its subsequent conversion to 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 CO_2 concentrates in the pyrenoid. CO_2 that leaks past Rubisco is sometimes recaptured and converted to 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 HCO_3^- once converted to CO_2 easily leaks out and is not recaptured.



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 α-helices from the Chlamydomonas SSU into the Arabidopsis SSU was essential for interaction, while adding in the β sheets or the βA-βB 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 *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 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 *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 C_3 plant, as EPYC1 doesn't appear to need additional proteins to bind to Rubisco *in vitro*. However, *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 C_3 plants into a pyrenoid-like structure. Rubisco organized in this fashion should be more efficient at fixing CO_2 if a functional CCM is introduced into C_3 plants. Rubisco in a pyrenoid-like structure should better capture CO_2 generated by a CCM thus preventing CO_2 leakage (Box 1A).

Keywords: carbon dioxide concentrating mechanism, carbon dioxide fixation, Chlamydomonas, EPYC1, pyrenoid, Rubisco, Yeast 2 hybrid

---

References

Atkinson N, Velanis CN, Wunder T, Clarke DJ, Mueller-Cajar O, McCormick AJ 2019. The pyrenoidal linker protein EPYC1 phase separates with hybrid Arabidopsis-Chlamydomonas Rubisco through interactions with the algal Rubisco small subunit. Journal of Experimental Botany **70**, 5283–5269.

Furbank RT, Caemmerer S, Price GD. 2013. CO_2-concentrating mechanisms in crop plants to increase yield. Applying photosynthesis research to improvement of food crops. ACIAR Proceedings **140**, 130–137.

Küken A, Sommer F, Yaneva-Roder L, Mackinder LC, Höhne M, Geimer S, Jonikas MC, Schroda M, Stitt M, Nikoloski Z. 2018. Effects of microcompartmentation on flux distribution and metabolic pools in *Chlamydomonas reinhardtii* chloroplasts. eLife **7**, e37960.

Ma Y, Pollock SV, Xiao Y, Cunnusamy K, Moroney JV. 2011. Identification of a novel gene, CIA6, required for normal pyrenoid formation in *Chlamydomonas reinhardtii*. Plant Physiology **156**, 884–896.

Mackinder LCM, Chen C, Leib RD, *et al*. 2017. A spatial interactome reveals the protein organization of the algal CO2-concentrating mechanism. Cell **171**, 133–147.

Mackinder LC, Meyer MT, Mettler-Altmann T, *et al*. 2016. A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle. Proceedings of the National Academy of Sciences, USA **113**, 5958–5963.

Mackinder LCM. 2018. The Chlamydomonas CO_2 -concentrating mechanism and its potential for engineering photosynthesis in plants.New Phytologist **217**, 54–61.

McGrath JM, Long SP. 2014. Can the cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis. Plant Physiology **164**, 2247–2261.

Meyer MT, Genkov T, Skepper JN, Jouhet J, Mitchell MC, Spreitzer RJ, Griffiths H. 2012. Rubisco small-subunit α-helices control pyrenoid formation in Chlamydomonas. Proceedings of the National Academy of Sciences **109**, 19474–19479.

Moroney JV, Ynalvez RA. 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*. Eukaryotic Cell **6**, 1251–1259.

Rosenzweig ES, Xu B, Kuhn Cuellar L, *et al*. 2017. The Eukaryotic CO_2-concentrating organelle is liquid-like and exhibits dynamic reorganization. Cell **171**, 148–162.e19.

Spalding MH. 2008. Microalgal carbon-dioxide-concentrating mechanisms: Chlamydomonas inorganic carbon transporters. Journal of Experimental Botany **59**, 1463–1473.

Tolleter D, Chochois V, Poiré R, Price GD, Badger MR. 2017. Measuring CO_2 and HCO_3^- permeabilities of isolated chloroplasts using a MIMS-^{18}O approach. Journal of Experimental Botany **68**, 3915–3924.

Turkina MV, Blanco-Rivero A, Vainonen JP, Vener AV, Villarejo A. 2006. CO_2 limitation induces specific redox-dependent protein phosphorylation in *Chlamydomonas reinhardtii*. Proteomics **6**, 2693–2704.

Wunder T, Le Hung SC, Mueller-Cajar O. 2018. Reconstitution of the liquid liquid phase separation underlying the microalgal Rubisco supercharger. Biophysical Journal **114**, 61a.

Zhu XG, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. Annual Review of Plant Biology **61**, 235–261.