Current Biology
Magazine

CellPress

Quick guide

The Pyrenoid

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What is the pyrenoid? The pyrenoid is a non-membrane-bound proteinaceous organelle that mediates approximately one-third of global CO_2 fixation. It is found in the chloroplasts of nearly all algae and a group of non-vascular plants. There, it enhances the rate of CO_2 fixation into organic carbon by supplying the CO_2-fixing enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) with a high concentration of its substrate, CO_2. The evolutionary history of the pyrenoid is presently unclear, and it is possible that the pyrenoid evolved independently in multiple lineages as a result of convergent evolution.

How did it get its name? The pyrenoid was first described in 1803 by the Swiss pastor Jean Pierre Étienne Vaucher, but was only named in 1882 by Friedrich Schmitz. Its name comes from the Greek word 'pyrene', which means 'fruit stone', a reference to its appearance as viewed through a light microscope. In the first 50 years of the 20th century, the pyrenoid was used as a taxonomic marker, though it is now known to be a weak one.

What is the overall architecture of the pyrenoid? All characterized pyrenoids contain a dense matrix where most of the cell's Rubisco is localized (Figure 1A). In most species, the pyrenoid matrix is traversed by membrane structures that extend from the photosynthetic thylakoid membranes. In a few species, thylakoid membranes penetrate into the pyrenoid matrix only if an environmental stress is imposed. These traversing membranes can form diverse architectures, including interconnected star-shaped tubule networks or parallel sheets. In the unicellular green alga *Chlamydomonas reinhardtii*, the traversing membranes enter the pyrenoid matrix as tubules and then form a reticulated network in the center of the matrix. In many species, the Rubisco matrix is surrounded by a sheath made of starch, a polymer of glucose.

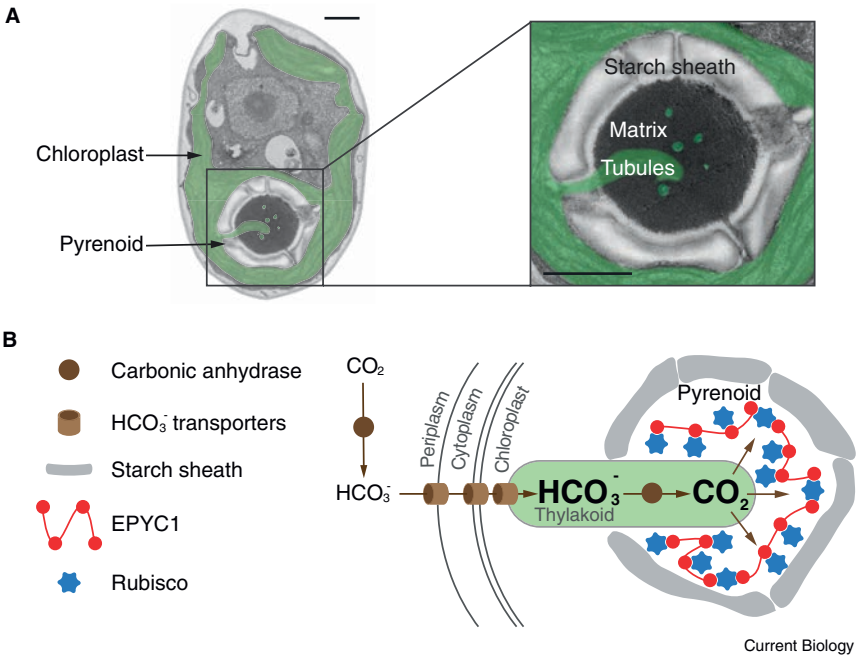

Figure 1. Ultrastructure and proposed mechanism of the pyrenoid.
(A) Transmission electron microscopy image of a *Chlamydomonas reinhardtii* cell grown in air (image courtesy of Moritz T. Meyer). The pyrenoid is found inside the chloroplast, and contains three sub-compartments: a spheroidal matrix, membrane tubules that traverse the matrix, and a starch sheath that surrounds the matrix. Scale bars: 1 μm. (B) Simplified model of a pyrenoid-based CO_2-concentrating mechanism. CO_2 outside the cell is converted to HCO_3^- (bicarbonate), which is transported across several membranes into the lumen of the thylakoid membranes. The thylakoid membranes enter the pyrenoid where they form the tubules; in these tubules HCO_3^- is converted back to concentrated CO_2, which enhances the activity of the CO_2-fixing enzyme Rubisco in the matrix. Rubisco is held together in the matrix by the linker protein EPYC1.

How does the pyrenoid work? The thylakoid membranes that traverse the pyrenoid are thought to supply a high concentration of CO_2 to Rubisco in the matrix, increasing the rate of CO_2 fixation. CO_2 cannot be efficiently concentrated directly because it is a small uncharged molecule that diffuses easily through membranes. Therefore, instead of directly concentrating CO_2, cells first convert CO_2 to the intermediate HCO_3^- (bicarbonate), which is charged and therefore can be actively concentrated across membranes (Figure 1B).This concentration is mediated by the coordinated action of HCO_3^- transporters and carbonic anhydrases, which catalyze the conversion of CO_2 to HCO_3^-. A carbonic anhydrase in the periplasm of the algal cell facilitates conversion of CO_2 to HCO_3^-, which is then imported through the successive action of transporters located on the plasma membrane, the chloroplast envelope, and thylakoid membranes. Inside the thylakoid lumen, a carbonic anhydrase converts HCO_3^- back to CO_2, which then diffuses across the pyrenoid matrix to feed Rubisco. This last conversion step appears to be directly energized in the thylakoid lumen by the high proton concentration, which is produced from light energy by the photosynthetic electron transport chain. When a starch sheath is present around the matrix, it may serve as a barrier to minimize CO_2 leakage from the pyrenoid. Although a few organisms appear to have CO_2-concentrating mechanisms but no pyrenoid, the pyrenoid appears to provide a significant growth advantage by producing a point source where CO_2 is released, and by densely clustering Rubisco around this point source.

What is the molecular structure of pyrenoid? The molecular structure of the pyrenoid has been most extensively characterized in *Chlamydomonas*. Approximately 100 proteins were identified in the *Chlamydomonas* pyrenoid proteome, and the sub-pyrenoid localization and

protein–protein interactions of many of them have been identified. Current work is focused on determining how these proteins work together to form the overall pyrenoid structure and enable its function. The best-characterized sub-compartment of the pyrenoid is the matrix, which is primarily composed of Rubisco holoenzymes linked together by a disordered protein called 'essential pyrenoid component 1' (EPYC1). The matrix was long thought to be a crystalline solid; however, we now know that, at least in *Chlamydomonas*, the matrix behaves as a phase-separated liquid-like body, which mixes internally, divides by fission, and dissolves into the surrounding compartment under certain conditions. Purified Rubisco and EPYC1 are both sufficient and necessary to form phase-separated liquid droplets *in vitro*, suggesting that the matrix arises from co-condensation of these two components.

How is pyrenoid formation regulated? The pyrenoid in *Chlamydomonas* is not a static structure; it dissolves and condenses in response to environmental cues. The pyrenoid condenses in light conditions when CO_2 availability is low. It dissolves when CO_2 is abundant, during cell division, and at night for cells grown in a day–night cycle.

How is the pyrenoid inherited during cell division? During vegetative growth of *Chlamydomonas*, pyrenoids typically divide by fission; the pyrenoid appears to be bisected by the contracting cleavage furrow of the dividing chloroplast. However, a few minutes before this event, a substantial portion of the pyrenoid matrix dissolves into the surrounding chloroplast, and this material condenses back into the pyrenoid after chloroplast division is completed. In rare cases where pyrenoid fission fails and one of the daughter chloroplasts does not inherit a pyrenoid, it still inherits dissolved pyrenoid building blocks, which appear to condense *de novo* into a pyrenoid after chloroplast division is complete. This dissolution and condensation may therefore serve as a backup mechanism to ensure inheritance of a pyrenoid even when fission fails. Observations of other algal species suggest that their pyrenoids have similar properties.

Can the pyrenoid be engineered into other organisms? Modeling studies suggest that the transfer of a pyrenoid into C3 crops such as wheat and rice could improve water and nitrogen-use efficiencies, and increase yields by up to 60%. Pyrenoid engineering efforts are still at an early stage; but already, encouraging results have been obtained. First, nearly all algal proteins tested localize to the correct sub-cellular compartment in higher plants without any changes to their protein sequence, suggesting that transferring the pyrenoid will not require extensive protein-engineering work. Second, the first steps of reconstituting a pyrenoid matrix in higher plants are well underway. In order to form a pyrenoid matrix, it is thought that the small subunit of Rubisco found in higher plants needs to be exchanged for the *Chlamydomonas* homolog to enable its binding to the EPYC1 linker protein. Excitingly, this exchange of Rubisco small subunits is tolerated by the vascular plant *Arabidopsis*. These early advances may pave the way for crops with synthetic pyrenoids that produce more food with fewer resources to enable a more sustainable world.

What major questions remain unanswered? Despite recent progress, we are only beginning to understand how the pyrenoid works at a molecular level. What is the structural basis for pyrenoid matrix formation? How are the phase transitions of the matrix catalyzed and regulated? How are pyrenoid tubules shaped and what are their molecular functions? How is the starch sheath nucleated and shaped? How are proteins targeted to the pyrenoid? How are the pyrenoid's three sub-compartments held together? How is the pyrenoid positioned to its canonical location? What is the full set of proteins required for a minimal pyrenoid to operate? If pyrenoids evolved multiple times through convergent evolution, what common structural and functional principles do they share? These and other questions are sure to yield exciting discoveries over the coming years as this fascinating organelle emerges from obscurity.

Where can I find out more?

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