CellPress

Current Biology
Magazine

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?

Atkinson, N., Feike, D., Mackinder, L.C., Meyer, M.T., Griffiths, H., Jonikas, M.C., Smith, A.M., and McCormick, A.J. (2016). Introducing an algal carbon-concentrating mechanism into higher plants: location and incorporation of key components. Plant Biotechnol. J. *14*, 1302–1315.

Freeman Rosenzweig, E.S., Xu, B., Cuellar, L.K., Martinez-Sanchez, A., Schaffer, M., Strauss, M., Cartwright, H.N., Ronceray, P., Plitzko, J.M., Forster, F., *et al*. (2017). The eukaryotic CO_2-concentrating organelle is liquid-like and exhibits dynamic reorganization. Cell *171*, 148–162.

Hennacy, J.H., and Jonikas, M.C. (2020). Prospects for engineering biophysical CO_2 concentrating mechanisms into land plants to enhance yields. Annu. Rev. Plant Biol. *71*, 10.1146.

Long, S.P., Marshall-Colon, A., and Zhu, X. (2015). Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell *161*, 56–66.

Mackinder, L.C.M., Meyer, M.T., Mettler-Altmann, T., Chen, V.K., Mitchell, M.C., Caspari, O., Freeman Rosenzweig, E.S., Pallesen, L., Reeves, G., Itakura, A., *et al.* (2016). A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle. Proc. Natl. Acad. Sci. USA *113*, 5958–5963.

Mackinder, L.C.M., Chen, C., Leib, R.D., Patena, W., Blum, S.R., Rodman, M., Ramundo, S., Adams, C.M., and Jonikas, M.C. (2017). A spatial interactome reveals the protein organization of the algal CO_2-concentrating mechanism. Cell *171*, 133–147.

Meyer, M.T., Whittaker, C., and Griffiths, H. (2017). The algal pyrenoid: key unanswered questions. J. Exp. Bot. *14*, 3739–3749.

Mukherjee, A., Lau, C.S., Walker, C.E., Rai, A., Prejean, C.I., Yates, G., Emrich-Mills, T., Lemoine, S.G., Vinyard, D.J., Mackinder, L.C.M., *et al.* (2019). Thylakoid localized bestrophin-like proteins are essential for the CO_2 concentrating mechanism of *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *116*, 16915–16920.

Schmitz, F.K.J. (1882). Die Chromatophoren der Algen. (Bonn: Max Cohen & Sohn).

Toyokawa, C., Yamano, T., and Fukuzawa, H. (2020). Pyrenoid starch sheath is required for LCIB localization and the CO_2-concentrating mechanism in green algae. Plant Physiol. https://doi.org/10.1104/pp.19.01587.

Wang, L., Yamano, T., Takane, S., Niikawa, Y., Toyokawa, C., Ozawa, S.I., Tokutsu, R., Takahashi, Y., Minagawa, J., Kanesaki, Y., *et al.* (2016). Chloroplast-mediated regulation of CO_2-concentrating mechanism by Ca^{2+}-binding protein CAS in the green alga *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *113*, 12586–12591.

Wang, Y., Stessman, D.J., and Spalding, M.H. (2015). The CO_2 concentrating mechanism and photosynthetic carbon assimilation in limiting CO_2: how *Chlamydomonas* works against the gradient. Plant J. *82*, 429–448.

Wunder, T., Cheng, S.L.H., Lai, S.-K., Li, H.-Y., and Mueller-Cajar, O. (2018). The phase separation underlying the pyrenoid-based microalgal Rubisco supercharger. Nat. Commun. *9*, 5076.

Zhan, Y., Marchand, C.H., Maes, A., Mauries, A., Sun, Y., Dhaliwal, J.S., Uniacke, J., Arragain, S., Jiang, H., Gold, N.D., *et al.* (2018). Pyrenoid functions revealed by proteomics in *Chlamydomonas reinhardtii*. PLoS One *13*, e0185039.

Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014, USA.
*E-mail: mjonikas@princeton.edu

Quick guide

Lens eyes in protists

Dan-E. Nilsson[1,*] and Justin Marshall[2]

Which protists have eyes? A group of dinoflagellates, the warnowiids, carry a unique structure that resembles a lens and a retina (Figure 1). The eye-like structure is called an ocelloid and is obviously surprising to see in a single-celled organism. The lens clearly refracts light and it is placed such that it will pass light to the retina-like structure. These facts, along with the shape of the whole structure, and its orientation during directional swimming strongly suggest that the ocelloid is a visual organ guiding behaviours in a unicellular organism. This says something about how constrained eye evolution is. Animal eyes are made of many different types of tissue that form the essential structures for catching pictorial information. In warnowiid dinoflagellates, almost identical structures have evolved as organelles within the single cell.

What are dinoflagellates? They are a diverse phylum of unicellular eukaryotes that are ecologically important components of phytoplankton. Some species cause red tides and others, such as *Noctiluca*, make water glow with bioluminescence at night. Many are armoured with cellulose plates that give the cells species-specific shapes, whereas other groups, such as the eye-bearing warnowiids, belong to the naked dinoflagellates. A distinctive feature of dinoflagellates is their flagellar arrangement with one ribbon-like transverse flagellum and one longitudinal flagellum of more conventional appearance. Many species contain chloroplasts and are autotrophic, whereas others are heterotrophic or mixotrophic.

In which ways are warnowiids special? Apart from containing the unique ocelloid, warnowiids possess various types of mechanical equipment of extreme complexity in their cells, such as pistons for propulsion and harpoon-like nematocysts for impaling other cells. The nematocysts, which are also found in a sister group to warnowiids, are at least superficially similar to the stinging nematocysts of jellyfish and other cnidarians. Warnowiids have been demonstrated to use nematocysts to attack and catch prey. Despite the heterotrophic lifestyle typical for warnowiids, they often contain photosynthetic chloroplasts. Surprisingly, these chloroplasts may not be their own, but rather stolen 'kleptochloroplasts' maintained from the prey they have caught and metabolised. They often engulf phototrophic species of dinoflagellates, and apparently sometimes hijack the chloroplasts and use them as power stations for as long as they last.

What is the ocelloid made of? Being a unicellular organism, warnowiids have to use organelles, not cells, to build their eyes. There is a cornea, a lens, a retinal body and a dark pigment screen that stops light from entering the retinal body from behind (Figure 1). It turns out that the cornea is formed by modified mitochondria and the retinal body is a highly modified chloroplast. Where the photoreceptors would have been located in an animal eye there are densely packed and strangely modified thylakoid membranes. It thus seems that warnowiids have turned their own chloroplast into a retina, and this might be the reason why some have to steal chloroplasts from other protists for photosynthesis.

Are ocelloids really eyes? The structure and position of the ocelloid strongly suggest that it serves the function of an eye. In some species, eye movements have been reported, as if they were looking for something in different directions. Yet, there is still no conclusive evidence that ocelloids are used for vision, and nothing is known about how the behaviour is controlled by visual information. It has been claimed that warnowiids cannot have vision because they have no brain. This is of course nonsense. The complex behaviour of many protists is based on processing of external information. But protists clearly have to use other means than neurons to process the information needed to perform behaviours. Light striking the eye-spot of the euglenoid flagellates is known to influence swimming direction and flagellar beats. The warnowiids have this system more refined.

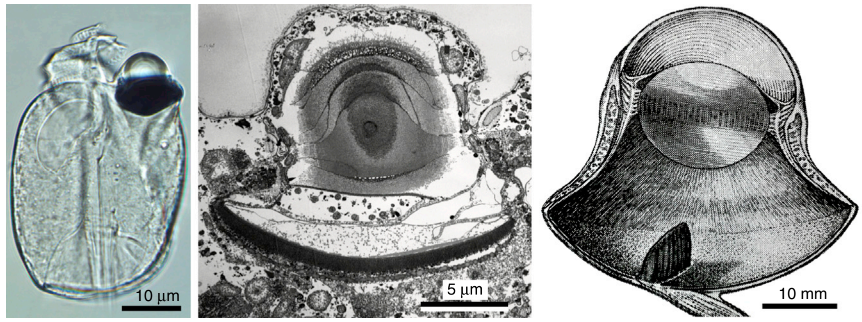

Figure 1. Dinoflagellate and owl eyes.
The warnowiid dinoflagellate *Erythropsidinium* with its forward-pointing ocelloid (left). An electron microscopic view of the ocelloid with its lens and retina-like body (middle). For comparison, the eye of an Eagle owl (*Bubo bubo*) which has a similar layout, although the diameter is 2000 times larger (right). Left and middle images courtesy of F. Gómez and T. Gojobori, respectively; right image modified from Walls (1942) with permission from Cranbrook Institute of Science.

How similar are the ocelloids to animal eyes? Compared to animal eyes, warnowiid ocelloids are small, with a lens diameter rarely more than 10 µm. Despite and indeed because of this size difference, it is striking how similar their 'eye' design is to that of several animals. This includes the two-thousand-times larger eyes of owls (Figure 1) and eyes of intermediate size in spiders, beetle larvae and deep-sea fish. The basic layouts of all these eyes are similar, a cornea and lens that focuses light onto a retina with restricted circumference. That is, the eye is not a full globe or sphere, the retina restricted to around a 90° radius. This sort of tube or bell-shaped eye has a number of advantages. It allows an increase in sensitivity due to the overall light gathering capability that the larger optics allow, but without the need to build a full-sized and spherical eye around those optics. This is a cheaper solution — the energetic budget of running and building large eyes is considerable — but it does have one obvious disadvantage in its restricted angle of view. This is solved differently among the animals with such eyes. Spiders have four pairs that point in different directions to cover all directions of view; owls have famously rotatable heads and the warnowiids twirl around pointing their 'eye' in many directions as they swim. Deep-sea fish often concentrate their visual effort and tube-eyes on looking up as that is where most of the light and information comes from.

Are warnowiids predators? If they are hunting other protists it would seem that predation is the right word. But strictly it is not predation because their typical 'prey' are photosynthetic species, in food webs equivalent to plants, which means that warnowiids are in fact herbivores. It just so happens that in their world, the grass swims and has to be hunted down. Studying warnowiids is like joining Alice in Wonderland in a strange world down the rabbit hole where everything gets curiouser and curiouser.

Where can I find out more?

Gavelis, G., Hayakawa, S., White III, R.A., Gojobori, T., Suttle, S.A., Keeling, P.J., and Leander, B.S. (2015). Eye-like ocelloids are built from different endosymbiotically acquired components. Nature *523*, 204–207.
Gavelis, G.S., Wakeman, K.C., Tillmann, U., Ripken, C., Mitarai, S., Herranz, M., Özbek, S., Holstein, T., Keeling, P.J., and Leander, B.S. (2017). Microbial arms race: ballistic "nematocysts" in dinoflagellates represent a new extreme in organelle complexity. Sci. Adv. *3*, e1602552.
Gómez, F. (2017). The function of the ocelloid and piston in the dinoflagellate Erythropsidinium (Gymnodiniales, Dinophyceae). J. Phycol. *53*, 629–641.
Gómez, F., Lopez-Garcia, P., and Moreira, D. (2009). Molecular phylogeny of the ocelloid-bearing dinoflagellates *Erythropsidinium* and *Warnowia* (Warnowiaceae, Dinophyceae). J. Eukaryot. Microbiol. *56*, 440–445.
Hayakawa, S., Takaku, Y., Hwang, J.S., Horiguchi, T., Suga, H., Gehring, W., Ikeo, K., and Gojobori, T. (2015). Function and evolutionary origin of unicellular camera-type eye structure. PLoS One *10*, e0118415.
Taylor, F.J.R. (1980). On dinoflagellate evolution. Biosystems *13*, 65–108.
Walls G.L. (1942). The Vertebrate Eye and its Adaptive Radiation. (Michigan: Cranbrook Press).

[1]Lund Vision Group, Department of Biology, Lund University, Sölvegatan 35, 22 362, Lund, Sweden. [2]Sensory Neurobiology Group, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland 4072, Australia.
*E-mail: dan-e.nilsson@biol.lu.se