

This evolutionarily and functionally sharp distinction between organelles and endosymbionts — protein import, or not — was crisply articulated by Cavalier-Smith and Lee [9]. It has proven to be exquisitely robust.

Unless the *Paulinella* endosymbiont can be shown to possess a protein import apparatus, it is just another member in a long list of known cases of endosymbionts: the proteobacterial endosymbionts of insects such as *Buchnera*, *Wigglesworthia*, and *Wolbachia* [5,6,10], the methanogenic endosymbionts of anaerobic ciliates [11], the nitrogen-fixing symbionts in the diatom *Rhopalodia* [12], the chemosynthetic endosymbiont consortia of gutless tubeworms [13], the cyanobacterial endosymbionts of sponges [14], and endosymbionts that live within other prokaryotes [15] — to name just very few examples.

The rate-limiting step in the transition from endosymbionts to organelles would appear to be the origin of the protein import machinery itself [9]: the TIM and TOM complexes of mitochondria [7] and the TIC and TOC complexes of plastids [8].

The origin of those complexes allowed each organelle to specifically import proteins synthesized in the host's cytosol, thereby allowing the endosymbionts to relinquish their prokaryotic genes without relinquishing their prokaryotic biochemistry.

Calling the *Paulinella* endosymbiont a plastid or an organelle might make a story more exciting, but at the cost of scientific accuracy. Some proteobacterial endosymbionts of aphids have genomes smaller than those of some plastids [16]. Would anyone call those endosymbionts 'mitochondria'? Hardly.

For the same reasons, we should not call the *Paulinella* endosymbionts 'plastids' any more than we should say that sponges [14] have 'plastids'. There is a difference between endosymbionts and organelles.

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Response to Theissen and Martin

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Theissen and Martin [1] question the use of the term organelle — and, by extension, plastid — as applied to the photosynthetic inclusions of the filose amoeba *Paulinella chromatophora*. We suggest that the apparent degree of biochemical and cellular integration of host and 'endosymbiont' in this unicellular eukaryote distinguishes it from other examples of prokaryotic endosymbionts, warranting use of the term 'plastid'.

The question is as previously stated: "to what extent can the *P. chromatophora* endosymbiont be considered a bona fide organelle?" [2]. The answer depends on what future studies reveal about the biology of *Paulinella*. It also depends on one's definition of organelle. Theissen and Martin [1] argue that the difference between endosymbionts and organelles is protein import: all of the cytosolic proteins in an endosymbiont are encoded in its own genome, whereas most organellar proteins are encoded by nuclear DNA, translated in the host cytosol and targeted to the organelle using a protein import apparatus, as in mitochondria and plastids [3,4]. It will indeed be important to determine whether a rudimentary protein import apparatus is necessary in *Paulinella* and, if so, in which form it exists. Clearly it would look nothing like the TIC/TOC import apparatus that evolved once in canonical plastids [4].

Does this matter? How complex would such an import apparatus have to be to justify use of the terms 'organelle' and 'plastid'? For example, would the targeting of host- or endosymbiont-derived, nucleus-encoded proteins to the endosymbiont via the secretory pathway, as recently shown for carbonic anhydrase

in the *Arabidopsis* plastid [5], be considered enough to tilt the scale toward organelle? We believe it would.

For example, an irreversible, long-term metabolic and cell biological connection between host and photosynthetic 'endosymbiont' could develop entirely from host-derived systems (e.g., metabolite transporters integrated into the outer membrane of the endosymbiont, such as PfoTPT in *Plasmodium* [6]), in the absence of a protein import system (e.g., [7]). Over time, gene loss and endosymbiotic gene transfer could occur, with transferred genes potentially acquiring new functions in the host cell. This may already have occurred in *Paulinella* and very likely did so in the early stages of the evolution of canonical plastids [7]. At this stage of the association, is it 'endosymbiont' or 'organelle'?

Whereas Theissen and Martin [1] would say 'endosymbiont', we believe that the *Paulinella* endosymbiosis possesses landmark features that justify the use of 'plastid' as a term referring to a photosynthetic organelle of endosymbiotic origin: the most important of these is the fact that the obligate and permanent host-'endosymbiont' relationship occurs within a single-celled organism that has lost the ability to phagocytose prey and has become a photoautotroph. Other key features are the strict regulation of the number of photosynthetic bodies in *Paulinella* and the synchronization of their division and segregation [8–11] that appear to be controlled by host effectors. This may have been accomplished via endosymbiotic gene transfer followed by protein import, entirely through the action of host-derived gene products, or a combination of the two. In any case, as clearly stated by Archibald [2] and Yoon *et al.* [12], this needs to be proven. Regardless of whether the cyanobacteria-derived cytoplasmic bodies of *Paulinella* should be called 'endosymbionts', 'photosynthetic organelles', 'plastids' (our preference), or 'cyanelles' [2,11–14], the *Paulinella* nuclear genome will be important

for understanding the extent of organelle establishment in this organism.

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Males evolved from the dominant isogametic mating type

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In eukaryotes there are two main types of sexual reproduction: isogamous, with two similar-looking gametes, and oogamous, with distinct sperm and egg cells. Oogamous reproduction has apparently evolved from isogamous reproduction repeatedly in several eukaryotic lineages, most notably those leading to animals and flowering plants. But until now, there have been no molecular genetic data relating the sexes of oogamous organisms to the mating types of their isogamous ancestors. This may be because no extant isogamous organisms are known that are closely related to animals or land plants [1,2]. The oogamous multicellular green algae in the family Volvocaceae provide an ideal model for exploring such evolutionary relationships, because several mating-type-specific genes have been identified in the closely related isogamous, unicellular alga *Chlamydomonas reinhardtii* [3,4]. No mating-type-specific genes have been isolated previously from the Volvocaceae, however, possibly because sex-related genes evolve rapidly [5]. Here we report isolation of a male-specific gene from the oogamous volvocacean *Pleodorina starrii* (see Figure S1 in the Supplemental data available on-line with this issue) by PCR amplification using primers corresponding to the *minus-dominance (MID)* gene of *C. reinhardtii*. This *Pleodorina* gene, *PlestMID*, is only present in males, encodes a protein that is abundant in sperm nuclei, and is an orthologue of the *MID* gene of *C. reinhardtii* that causes cells to develop as 'mating type minus' (MT⁻) gametes [4]. Thus,