Letters

The intracellular cyanobacteria of *Paulinella chromatophora*: endosymbionts or organelles?

Andrzej Bodył[1], Paweł Mackiewicz[2] and John W. Stiller[3]

[1] University of Wrocław, Zoological Institute, Department of Biodiversity and Evolutionary Taxonomy, ul. Przybyszewskiego 63/77, 51-148 Wrocław, Poland
[2] University of Wrocław, Faculty of Biotechnology, Department of Genomics, ul. Przybyszewskiego 63/77, 51-148 Wrocław, Poland
[3] Department of Biology, East Carolina University, Howell Science N108, Greenville, NC 27858, USA

Endosymbiotic relationships are common across the tree of life and have had profound impacts on cellular evolution and diversity. Recent molecular investigations of the amoeba *Paulinella chromatophora* have raised a timely and important question: should obligatory intracellular cyanobacteria in *Paulinella* be considered new organelles, or do plastids and mitochondria hold a unique stature in the history of endosymbiotic events? We argue that drawing a sharp distinction between these two organelles and all other endosymbionts is not supported by accumulating data, neither is it a productive framework for investigating organelle evolution.

How difficult is it to acquire an organelle?

In their 1985 landmark paper on organelle evolution, Cavalier-Smith and Lee [1] argued that establishing protein translocation machinery, complete with targeting sequences, must have been an exceedingly difficult and, therefore, unique event. This idea underlies most current hypotheses of organelle evolution and is echoed in recent discussions regarding *Paulinella* endosymbionts. Yoon *et al.* [2] suggested they be considered organelles, based on how thoroughly their metabolism and cell division is integrated with the *Paulinella* host cell and the likelihood that they import at least some proteins. By contrast, Theissen and Martin [3] argued for maintaining a clear distinction between stable endosymbionts and organelles; that is, only mitochondria and plastids pass a strict test of having well-developed protein import mechanisms. Growing evidence, however, indicates that the problem of developing protein import has been overemphasized.

First, irrespective of what is found in other stable endosymbioses, the problem of establishing protein import was clearly overcome in at least two separate situations during the integration of mitochondria and primary plastids. Moreover, host-to-plastid transport was subsequently recreated on multiple occasions in establishing secondary and tertiary plastids [4]. Second, both plastid and mitochondrial translocons turned out to be examples of evolutionary tinkering with pre-existing components in the hosts and endosymbionts, rather than being of *de novo* origin [5,6]. Third, potential targeting signals capable of directing protein import already existed in the bacterial ancestors of these organelles [7], or could have evolved from signal peptides [6,8]. In fact, structural and functional similarities between mitochondrial and plastid transit peptides, in addition to common interactions with cytosolic factors [9], indicate that the initial establishment of the mitochondrion could have pre-adapted eukaryotic cells for additional endosymbiotic bacterial associations.

Finally, a new model for the evolution of protein import into mitochondria, which explains how even complex import systems could have evolved on multiple occasions, has been proposed recently [6]. This model hypothesizes three main stages during the evolution of organelle import machineries: (i) initially, host-derived metabolic carriers, which are devoid of presequences, are translocated into the periplasmic space through pre-existing channels in the outer bacterial membrane; (ii) metabolic carriers with presequences are imported using established channels, such as pores for branched amino acids, embedded in both bacterial membranes; and (iii) these pathways are exploited and elaborated to begin to import matrix proteins using presequences. When interpreted within this kind of framework, emerging empirical data indicate that at least some modern endosymbionts are already well into such a transitional process.

Peculiar endosymbionts or new organelles?

Many insects harbor intracellular bacteria that produce and excrete essential amino acids into the host cytosol [10,11]. Although, in some cases, 'dead-end' bacterial cells with incomplete genomes are replaced by new endosymbionts [10], this is not the case for the bacteriocyte *Carsonella ruddii*. The genome of *Carsonella* is reduced to a mere 160 kb (within the range for organelles), has lost all genes for many essential functions, and no other bacterial endosymbionts are present to offer potential compensation [11]. Thus, *Carsonella* seems to be at the end of an endosymbiotic continuum in sap-feeding psyllid insects and represents the recent acquisition of a new type of organelle for biosynthesis of amino acids.

Comparable endosymbioses are found in many plants and protists [12]. For example, in the diatom *Rhopalodia gibba*, a highly reduced relative of the cyanobacterium *Cyanothece* was identified that seems to be far along the path of becoming a nitrogen-fixing organelle [13]. Another diatom, *Pinnularia nobilis*, harbors Gram-negative bacterial endosymbionts residing between envelope membranes of its secondary rhodophyte-derived plastid [14]. Their tight association with *Pinnularia* plastids is suggestive of new organelles, perhaps performing dark

Corresponding author: Bodył, A. (bodyl@biol.uni.wroc.pl).
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reactions in photosynthesis; it might even be that these bacteria were engulfed and integrated simultaneously with the rhodophyte during the secondary plastid endosymbiosis [14].

Organelle acquisitions and losses

The advanced level of integration of the *Paulinella* endosymbiont with its host [15] indicates the presence of well-developed metabolite exchange and, probably, import of at least some proteins such as membrane antiporters [2,16,17]. Therefore, we agree with Bhattacharya and Archibald [17] that the *Paulinella* endosymbiont should be called a plastid. More interesting than the question of nomenclature, however, is a consideration of how the *Paulinella* endosymbiosis fits into a broader phylogenetic context.

The presumed difficulty of establishing host-to-symbiont protein transport has strongly influenced constructions of evolutionary scenarios. It is widely assumed that organelles are far more difficult to acquire than to lose; as a result, many current hypotheses for plastid evolution, at both primary and secondary levels, postulate multiple losses (for examples, see Ref. [18]). By contrast, available empirical data demonstrate that endosymbionts are common and, once fully integrated into host-cell metabolism, are difficult or even impossible to lose [4,19,20]. Therefore, to resolve conflicts among phylogenetic datasets, it might be more reasonable to presume independent transformations of endosymbionts to organelles rather than numerous organelle losses [4].

Modern endosymbioses as models for organelle evolution

Over the past 20 years, knowledge of the diversity, genetics and biochemistry of plastids and mitochondria has broadened dramatically. Nevertheless, the processes by which they were integrated into eukaryotic cells remain hidden in the shadows of a billion or more years of evolutionary change. Molecular data from *Paulinella,* psyllid–*Carsonella* and other endosymbiotic associations at various stages of development can begin to illuminate the processes by which organelles are established and make possible empirical tests of currently hypothetical evolutionary scenarios. Drawing artificial distinctions between endosymbioses at different historical and developmental stages will not help to clarify the full implications of these exciting new investigations.

References

1 Cavalier-Smith, T. and Lee, J.J. (1985) Protozoa as hosts for endosymbioses and the conversion of symbionts into organelles. *J. Protozool.* 32, 376–379

2 Yoon, H.S. *et al.* (2006) Minimal plastid genome evolution in the *Paulinella* endosymbiont. *Curr. Biol.* 16, R670–R672

3 Theissen, U. and Martin, W. (2006) The difference between organelles and endosymbionts. *Curr. Biol.* 16, R1016–R1017

4 Bodył, A. and Moszczyński, K. (2006) Did the peridinin plastid evolve through tertiary endosymbiosis? A hypothesis. *Eur. J. Phycol.* 41, 435–448

5 Reumann, S. *et al.* (2005) Evolution of the general protein import pathway of plastids (review). *Mol. Membr. Biol.* 22, 73–86

6 Cavalier-Smith, T. (2006) Origin of mitochondria by intracellular enslavement of a photosynthetic purple bacterium. *Proc. Biol. Sci.* 273, 1943–1952

7 Lucattini, R. *et al.* (2004) Bacterial proteins predisposed for targeting to mitochondria. *Mol. Biol. Evol.* 21, 652–658

8 Liu, Y.Y. *et al.* (2000) A chloroplast envelope-transfer sequence functions as an export signal in *Escherichia coli*. *FEBS Lett.* 469, 61–66

9 Zhang, X.P. and Glaser, E. (2002) Interaction of plant mitochondrial and chloroplast signal peptides with the Hsp70 molecular chaperone. *Trends Plant Sci.* 7, 14–21

10 Perez-Brocal, V. *et al.* (2006) A small microbial genome: the end of a long symbiotic relationship? *Science* 314, 312–313

11 Nakabachi, A. *et al.* (2006) The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. *Science* 314, 267

12 Rai, A.N. *et al.*, eds (2002) *Cyanobacteria in Symbiosis*, Kluwer Academic Publishers

13 Prechtl, J. *et al.* (2004) Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. *Mol. Biol. Evol.* 21, 1477–1481

14 Schmid, A.M. (2003) Endobacteria in the diatom *Pinnularia*. I. 'Scattered ct-nucleoids' explained: DAPI-DNA-complexes stem from exoplastidial bacteria boring into the chloroplasts. *J. Phycol.* 39, 122–138

15 Kies, L. and Kremer, B.P. (1979) Function of cyanelles in the thecamoeba *Paulinella chromatophora*. *Naturwissenschaften* 66, 578–579

16 Weber, A.P. *et al.* (2006) Single, ancient origin of a plastid metabolite translocator family in Plantae from an endomembrane-derived ancestor. *Eukaryot. Cell* 5, 609–612

17 Bhattacharya, D. and Archibald, J. (2006) The difference between organelles and endosymbionts. Response to Theissen and Martin. *Curr. Biol.* 16, R1017–R1018

18 Nozaki, H. *et al.* (2003) The phylogenetic position of red algae revealed by multiple nuclear genes from mitochondria-containing eukaryotes and an alternative hypothesis on the origin of plastids. *J. Mol. Evol.* 56, 485–497

19 Waller, R.F. and McFadden, G.I. (2005) The apicoplast: a review of the derived plastid of apicomplexan parasites. *Curr. Issues Mol. Biol.* 7, 57–79

20 van der Giezen, M. *et al.* (2005) Mitochondrion-derived organelles in protists and fungi. *Int. Rev. Cytol.* 244, 175–225

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