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Lipid metabolism integration in Paulinellidae

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Abstract:

In this issue, Sato et al. (2020) have performed the first comprehensive analysis of glycerolipids in *Paulinella micropora*, a unicellular organism containing a photosynthetic organelle called the chromatophore, acquired by a primary endosymbiosis about 100 million years ago. Based on lipidomic analyses and metabolic labeling experiments, the provision of fatty acids and/or fatty acid precursors to the host cell, synthesized photosynthetically and rapidly by the chromatophore, is likely one of the early steps in the integration of this recent organelle. The capacity to produce membranes independently of any supply of organic carbon may be one of the critical benefits the heterotrophic host may get from its photosynthetic guest, and this may have been a fitness advantage for this slow growing species. Further work using *Paulinella* as a model may help understand the early steps of membrane lipid integration in the primary chloroplast, deriving from a more ancient endosymbiosis event, more than 1 billion years ago.

Main text:

Endosymbiosis events are key milestones in the evolution of eukaryotic cells, from the acquisition of the mitochondrion during eukaryogenesis (Imachi et al. 2020) to the emergence of the most sophisticated cellular architectures, following primary, secondary or serial endosymbioses. To our knowledge, the acquisition of photosynthesis by eukaryotes has always derived from the engulfment of a photosynthetic cell, prokaryotic or eukaryotic, leading to the emergence of primary or secondary photosynthetic organelles, respectively (for review, (Fussy and Obornik 2018; Marechal 2018)). The engulfment of a *symbiotic cell* inside a *host cell* is necessary to comprehend how novel subcellular compartments can appeared, but is not sufficient to understand the transition to a fully integrated organelle transmitted through generations.

One of the most important challenges for evolutionary science in the 21st century, is therefore to identify those ancestral partners at the origin of membrane bound compartments, and to reconstruct the transitions from free-living cells to obligate endosymbiotic organelles. This ambitious objective, considered sometimes unattainable (Koskela and Annila 2012), relies on multidisciplinary approaches, combining comparative and functional genomics, phylogenetics, molecular and biochemical analyses and cell biology evidence to propose reasonable scenarios ultimately testable in appropriate experimental systems. For organelles such as the *mitochondrion* or the *primary chloroplast*, ancestral endosymbionts are not clearly established yet (Ball et al. 2013; Cenci et al. 2017; Huang and Gogarten 2007, 2008; Marechal 2018; Sato and Awai 2017; Sato and Takano 2017). Models of eukaryogenesis include both mitochondrial-early and mitochondria-late scenarios (Poole and Gribaldo 2014). In the most recent model, mitochondria may have emerged early, deriving at least in part from the association of a facultatively aerobic organotrophic bacterium with an Archae host, belonging to the 'Asgard Archae' superphylum (Imachi et al. 2020). Acquisition of mitochondria may have occurred from 1.9 to 1 billion years ago (Marechal 2018). Concerning primary chloroplasts, molecular, biochemical and ultrastructural evidence support that this photosynthetic organelle derives from a single endosymbiotic event occurring 1.5 to 1 billion years ago (Jensen and Leister 2014). Although an ancestral photosynthetic cyanobacteria, seemingly unrelated to present phyla of cyanobacteria, is hypothesized to be at the origin of the primary chloroplast, major components, including plastid synthesizing enzymes, are of non-cyanobacterial origin (Sato and Awai 2017; Sato and Takano 2017). Contribution of other 'partners' have been suggested, including pathogenic bacteria such as *Chlamydiales*, via additional lateral gene transfers, but cellular relicts of these additional contributors have been lost and are no more visible in the ultrastructure of the organelle (Huang and Gogarten, 2007;2008;Ball et al., 2013;Cenci et al., 2017).

Having clues on the identity of endosymbiotic cells is not sufficient to understand how novel compartments have remained from cell division to cell division. Over evolutionary time, interdependency reaches such a level that the endosymbiont is no more able to live by its own, becoming a so-called *semi-autonomous organelle*, when still containing a functional genome, or a *completely integrated organelle*, when all necessary genes have been transferred to the host nucleus and the organellar DNA is eventually lost. The transfer of genes from the endosymbiont to the host genome, and the reduction of the endosymbiont genome are well-known markers of this integration. Less is known on the driving forces 'rewiring' the endosymbiont and host metabolic networks, optimizing cooperative pathways, developing systems transferring precursors from one partner to the other, eventually reducing redundant pathways that have become futile. In the case of cyanobacteria integration, the two-membrane envelope limiting the organelle, thought to derive from the two limiting membranes of the cyanobacteria ancestor, needs to be scrutinized as it is the interface where transporters facilitating novel exchanges of metabolites will be embedded, and because this membrane system needs to be transmitted from cell division to cell division.

Glycerolipids make the bulk of biological membranes. They consist of a glycerol backbone, esterified by two fatty acids (FA) and harboring a polar head group. In a non-photosynthetic eukaryote, FA are synthesized by a multi-enzymatic fatty acid synthase of type 1 (FAS1) in the cytosol (**Figure 1A**). The FAS1-generated FA are then used, schematically, at the level of the endoplasmic reticulum to generate glycerolipids, such as phosphatidylcholine (**Figure 1A**). Cyanobacteria rely on a dissociated fatty acid synthase of type 2 (FAS2) for the assembly of their membrane glycerolipids (**Figure 1B**). Cyanobacteria membranes are enriched in specific glycerolipids including two major galactolipids (MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol), a sulfolipid (SQDG, sulfoquinovosyldiacylglycerol) and a phospholipid (PG, phosphatidylglycerol). In addition, a glucolipid (MGlDG, monoglucosyldiacylglycerol) is also present, being the precursor of MGDG by epimerization of its polar head group. MGDG, DGDG, SQDG and PG are conserved in cyanobacteria and primary plastids, whereas MGlDG is not. In spite of this conservation of lipid classes, numerous chloroplast proteins involved in their biosynthesis are of non-cyanobacterial origin (Boudiere et al. 2014; Petroutsos et al. 2014; Sato and Awai 2017; Sato and Takano 2017). Reconstructing the early steps of lipid metabolism rewiring is therefore extremely difficult in such an integrated organelle as the chloroplast.

The study of *Paulinella chromatophora* has allowed the discovery of an independent primary endosymbiosis involving the integration of a cyanobacterium into a Rhizarian amoeba, 100 to 60 million years ago, leading to the emergence of a photosynthetic organelle resembling primary plastids, termed the *chromatophore* (Marin et al. 2005; Nowack et al. 2008; Singer et al. 2017). Being more recent, the emergence of the chromatophore is therefore a unique opportunity to study early stages in the transition from a free-living α-cyanobacteria (here related to *Synechococcus* and *Prochlorococcus*) into an obligate endosymbiotic organelle. It must be stated that the chromatophore *is not* a chloroplast, and there is no reasonable argument to assess that both organelles should follow the same evolutionary sequences. Nevertheless, converging trends seem to exist, including the transfer of endosymbiont genes to the host nuclear genome, where addressing sequences were added allowing nuclear-encoded proteins to be imported 'back' to the photosynthetic organelle (Archibald 2017; Singer et al. 2017).

In this issue, Sato et al. (2020) have performed the first comprehensive analysis of glycerolipids in *Paulinella micropora*. The authors have confirmed the conservation of cyanobacteria lipids, i.e. MGDG, DGDG, SQDG, PG, as well as MGlDG, which is absent from primary plastid. This lipidomic analysis is consistent with the detection of cyanobacteria lipid synthesis genes in the chromatophore

genome (Sato and Awai 2017). Pulse-chase experiments with radiolabeled substrates show that MGlDG is the precursor of MGDG in *P. micropora*, like in cyanobacteria. The authors also found that the nuclear genome of *P. micropora* encoded two forms of FAS1, whereas the chromatophore genome contained homologues of FAS2 genes. Both FAS1 and FAS2 systems seem therefore to coexist (**Figure 1C**). Based on the realistic hypothesis that FAS2 is located in the stroma of the chromatophore, this machinery is expected to provide FA for the biosynthesis of plastid lipids, characterized as enriched in palmitic and hexadecenoic acids, having 16-carbon chain lengths and being saturated (16:0) or monounsaturated (16:1), respectively. Glycerolipids expected to be synthesized at the ER were enriched in longer-chained FA, with more unsaturations, such as 18:1, 20:4, 20:5 and 22:6. Based on metabolic labelling kinetics of photosynthetically fixed carbon, authors hypothesize that FAS2-generated FA may be exported and used for host-cell glycerolipid synthesis at the ER (**Figure 1C**, arrow 4). Alternatively, an acyl-CoA precursor (Sato et al. 2020) or possibly shorter-chained FA, may be exported from the chromatophore and feed cytosolic FA biosynthesis by FAS1 (**Figure 1C**, arrow 4').

The provision of FA and/or FA precursors to the host, synthesized photosynthetically and rapidly by the chromatophore, are highlighted by Sato et al (2020) as one of the early steps in the integration of this recent organelle. The capacity to produce membranes independently of any supply of organic carbon may be one of the critical benefits the heterotrophic host gets from its photosynthetic guest, and this may have been a fitness advantage for this slow growing species. Question marks remain, such as the precise localization of glycerolipids within *P. micropora* cell, and the precise nature of the precursor(s) provided by the chromatophore to the ER for host membrane glycerolipid production. Further work using *Paulinella* as a model may help understand the early steps of membrane lipid rewiring following the acquisition of a photosynthetic organelle in other systems as well, including the primary chloroplast. Primary plastids show a much higher level of integration: FAS2-generated FA are first used in the plastid for the production of glycerolipids via a pathway termed 'prokaryotic'. Whereas FAS1 has been lost in the cytosol of Archaeplastida, plastid FA are exported to the cytosol and used in the ER to generate glycerolipid molecular species termed 'eukaryotic'. In Viridiplantae, such as Angiosperms, some precursors with 'eukaryotic' structures are then imported 'back' into the chloroplast to be used for plastid glycerolipid biosynthesis (**Figure 1D**, arrow 5). Plant chloroplast lipids are therefore synthesized by a cooperation of plastid-localized 'prokaryotic' and ER localized 'eukaryotic' pathways. Over evolution, redundant components of the 'prokaryotic' pathway have become futile (Marechal and Bastien 2014) and were lost independently in numerous clades of Angiosperms (Mongrand et al. 1998). Interestingly, the 'prokaryotic' pathway relies on non-cyanobacterial enzymes in primary plastids (Sato and Awai 2017), whereas it does rely on cyanobacterial proteins in the chromatophore. If appropriate tools were available to efficiently engineer *Paulinella* nuclear genome, one could test the effect of the introduction of some non-cyanobacterial genes producing galactolipids in the nuclear genome of green algae or plants and evaluate whether their presence could lead to the loss of cyanobacterial genes, in the same way this is hypothesized to have occurred in chloroplasts.

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Figure Legend

Figure 1. Integration of fatty acid and glycerolipid metabolism following the primary acquisition of a cyanobacteria by a eukaryotic host cell. Two independent primary endosymbiosis events have led to the emergence of either a chromatophore (chr) in Paullinelidae or a primary plastid (pl) in Archaeplastida. **A.** In mitochondriate eukaryotes, a fatty acid synthase of type 1 (FAS1) generates fatty acids (FA) in the cytosol (red arrow, 1). These FA are utilized as substrates in the endoplasmic reticulum (ER) for the production of glycerolipids. ER-generated glycerolipids serve as building blocks for the biogenesis of cell membranes (blue arrow, 2). These include the endomembrane system, comprising the ER, nuclear envelope (nuc), the Golgi apparatus, trans-Golgi network, vesicles, vacuoles and plasma membranes, all connected by direct membrane bridges or vesicular trafficking. ER-generated glycerolipids can also be transferred to the mitochondria (mit), via inter-organellar contact sites. **B.** In cyanobacteria, a dissociated fatty acid synthase of type 2 (FAS2) generates FA (yellow arrow, 3) utilized for the production of membrane glycerolipids. These include cyanobacteria-specific lipids such as galactolipids and the sulfolipid. **C.** In Paulinellidae, genomic evidence indicate that FAS2 and FAS1 systems coexist. Sato et al., 2020 have shown that FAS2-generated FA are likely used as substrates for the production of galactolipids and the sulfolipid, as well as glycerolipids usually synthesized in the ER, phosphatidylcholine and triacylglycerol. This suggests a transfer of chromatophore-generated FA to the ER (green arrow, 4) and/or precursors to cytosolic FAS1 (green arrow, 4'). **D.** In Archaeplastida, such as green algae and plants, the primary plastid, or chloroplast, is the site of FA synthesis for membrane glycerolipids. FAS2-generated FA are either used for plastid glycerolipid synthesis (yellow arrow, 2) or exported to the cytosol (green arrow, 4). Some ER-synthesized glycerolipids are imported back into the chloroplast (purple arrow, 5), where they serve as precursors for a part of plastid lipid synthesis. In the course of the evolution of Archaeplastida, the dependence of plastid on ER-synthesized glycerolipids increased, leading to a tight cooperation between these two organelles for FA and glycerolipid metabolism.

References:

Archibald, J.M. (2017) Evolution: Protein Import in a Nascent Photosynthetic Organelle. *Curr Biol* 27: R1004-R1006.

Ball, S.G., Subtil, A., Bhattacharya, D., Moustafa, A., Weber, A.P., Gehre, L., et al. (2013) Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? *Plant Cell* 25: 7-21.

Boudiere, L., Michaud, M., Petroutsos, D., Rebeille, F., Falconet, D., Bastien, O., et al. (2014) Glycerolipids in photosynthesis: composition, synthesis and trafficking. *Biochim Biophys Acta* 1837: 470-480.

Cenci, U., Bhattacharya, D., Weber, A.P., Colleoni, C., Subtil, A. and Ball, S.G. (2017) Biotic Host-Pathogen Interactions As Major Drivers of Plastid Endosymbiosis. *Trends Plant Sci* 22: 316-328.

Fussy, Z. and Obornik, M. (2018) Complex Endosymbioses I: From Primary to Complex Plastids, Multiple Independent Events. *Methods Mol Biol* 1829: 17-35.

Huang, J. and Gogarten, J.P. (2007) Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome biology* 8: R99.

Huang, J. and Gogarten, J.P. (2008) Concerted gene recruitment in early plant evolution. *Genome biology* 9: R109.

Imachi, H., Nobu, M.K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., et al. (2020) Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* 577: 519-525.

Jensen, P.E. and Leister, D. (2014) Chloroplast evolution, structure and functions. *F1000Prime Rep* 6: 40.

Koskela, M. and Annila, A. (2012) Looking for the Last Universal Common Ancestor (LUCA). *Genes (Basel)* 3: 81-87.

Marechal, E. (2018) Primary Endosymbiosis: Emergence of the Primary Chloroplast and the Chromatophore, Two Independent Events. *Methods Mol Biol* 1829: 3-16.

Marechal, E. and Bastien, O. (2014) Modeling of regulatory loops controlling galactolipid biosynthesis in the inner envelope membrane of chloroplasts. *Journal of theoretical biology* 361: 1-13.

Marin, B., Nowack, E.C. and Melkonian, M. (2005) A plastid in the making: evidence for a second primary endosymbiosis. *Protist* 156: 425-432.

Mongrand, S., Bessoule, J.J., Cabantous, F. and Cassagne, C. (1998) The C16:3/C18:3 fatty acid balance in photosynthetic tissues from 468 plant species. *Phytochemistry* 49: 1049-1064.

Nowack, E.C., Melkonian, M. and Glockner, G. (2008) Chromatophore genome sequence of Paulinella sheds light on acquisition of photosynthesis by eukaryotes. *Curr Biol* 18: 410-418.

Petroutsos, D., Amiar, S., Abida, H., Dolch, L.J., Bastien, O., Rebeille, F., et al. (2014) Evolution of galactoglycerolipid biosynthetic pathways--from cyanobacteria to primary plastids and from primary to secondary plastids. *Prog Lipid Res* 54: 68-85.

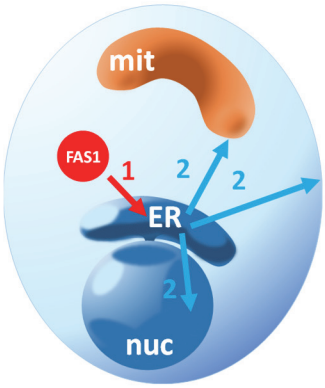
Poole, A.M. and Gribaldo, S. (2014) Eukaryotic origins: How and when was the mitochondrion acquired? *Cold Spring Harbor perspectives in biology* 6: a015990.

Sato, N. and Awai, K. (2017) "Prokaryotic Pathway" Is Not Prokaryotic: Noncyanobacterial Origin of the Chloroplast Lipid Biosynthetic Pathway Revealed by Comprehensive Phylogenomic Analysis. *Genome Biol Evol* 9: 3162-3178.

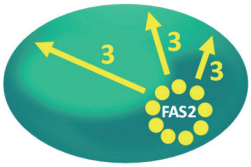
Sato, N. and Takano, H. (2017) Diverse origins of enzymes involved in the biosynthesis of chloroplast peptidoglycan. *J Plant Res* 130: 635-645.

Sato, N., Yoshitomi, T. and Mori-Moriyama, N. (2020) Characterization and biosynthesis of lipids in Paulinella micropora MYN1. Evidence for efficient integration of chromatophores into cellular lipid metabolism. *Plant Cell Physiol*.

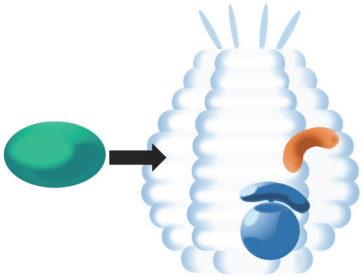
Singer, A., Poschmann, G., Muhlich, C., Valadez-Cano, C., Hansch, S., Huren, V., et al. (2017) Massive Protein Import into the Early-Evolutionary-Stage Photosynthetic Organelle of the Amoeba Paulinella chromatophora. *Curr Biol* 27: 2763-2773 e2765.



A. Mitochondriate Eukaryote



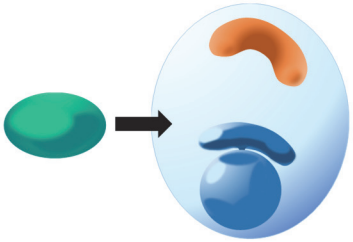
B. Cyanobacteria

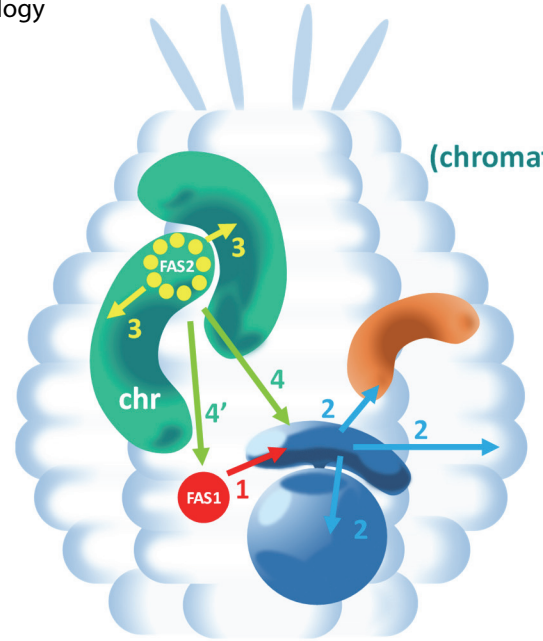


100 Mya?

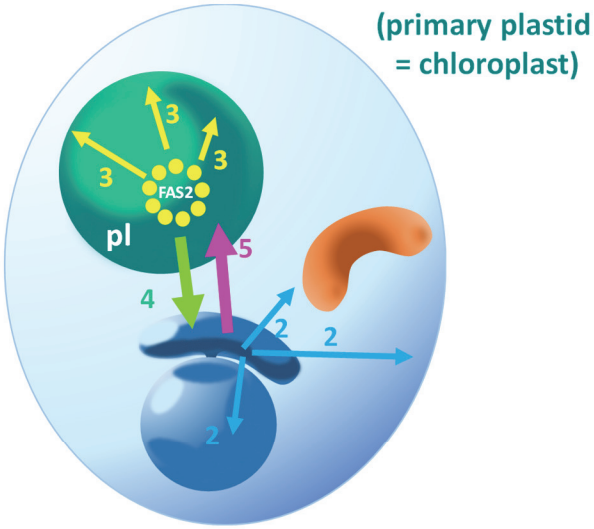
Independent Primary Endosymbiosis Events

1,500 Mya ?





C. Paulinellidae



D. Archaeplastida
(e.g. Green Algae, Plants)