Opinion

Chlamydia, cyanobiont, or host: who was on top in the ménage à trois?

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The endosymbiont hypothesis proposes that photosynthate from the cyanobiont was exported to the cytosol of the eukaryote host and polymerized from ADP-glucose into glycogen. *Chlamydia*-like pathogens are the second major source of foreign genes in Archaeplastida, suggesting that these obligate intracellular pathogens had a significant role during the establishment of endosymbiosis, likely through facilitating the metabolic integration between the endosymbiont and the eukaryotic host. In this opinion article, we propose that a hexose phosphate transporter of chlamydial origin was the first transporter responsible for exporting photosynthate out of the cyanobiont. This connection pre-dates the recruitment of the host-derived carbon translocators on the plastid inner membranes of green and red algae, land plants, and photosynthetic organisms of higher order endosymbiotic origin.

Tripartite symbiosis may explain the uniqueness of plastid endosymbiosis

The plant kingdom (Archaeplastida) comprises photoautotrophic eukaryotes bearing a plastid of primary endosymbiotic origin. Plastid endosymbiosis occurred some 1.6 billion years ago and involved the capture and retention of a cyanobacterium by a unicellular heterotrophic eukaryotic host, resulting in the ancestor of the plastid-containing modern alga [1–3]. Once the cyanobacterial endosymbiont was established, the three lineages of Archaeplastida diverged: the Glaucophyta (glaucophytes), the Rhodophyta (red algae), and the Chloroplastida (green algae and plants), which are now accepted as monophyletic lineages. However, this ancient event is not unique because the thecate amoeba *Paulinella chromatophora* has also been shown to have acquired its photosynthetic organelles in a similar manner to that of the Archaeplastida, although involving a different cyanobacterial endosymbiont [4–6].

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Plastid endosymbiosis led to the ability to perform oxygenic photosynthesis being transferred to eukaryotes. This in turn enabled the plastid-bearing organisms to colonize novel marine and terrestrial environments, thus maximizing primary production on Earth and paving the way for animal life to flourish. The capture of the endosymbiont, probably through a phagocytosis-related event, was followed by a first wave of gene losses that concerned those cyanobacterial functions that were no longer needed in a sheltered host-cell environment. These gene losses were followed by the transfer of most of the remaining 'essential' genes to the host nucleus, leading to the degenerate extant plastid genomes [7,8]. This process, which normally occurs on a large evolutionary timescale, can be recapitulated in the laboratory, suggesting that it is mechanistically relatively straightforward [9–11]. The transfer of the bulk of the cyanobiont genome to the nucleus implies the redirection of the gene products back to the compartment of provenance. It is assumed that the core protein import apparatus evolved by recruitment of pre-existing host and cyanobacterial components before the three Archaeplastida lineages diverged [12,13]. The appearance of this core apparatus, together with the evolution of an increasing diversity of transporters intensifying and diversifying the connectivity between the evolving plastids, define the hallmarks of processes known as the metabolic integration of the protoplastid. The three Archaeplastida lineages followed different paths of metabolic integration, thereby defining their biochemical specificities, which were optimally suited to the diverse environments that they initially colonized [13–18].

However, the establishment of a reliable metabolic and optimal connection between host and cyanobiont pre-dated all of these events and, thus, must have been of crucial importance for the successful enslavement of the endosymbiont at the onset of plastid endosymbiosis. Indeed, natural selection of plastid endosymbiosis relied on the export of photosynthate from the symbiont to its host. For a detailed discussion of metabolic integration and metabolite transport across the plastid envelope, see for example, [19].

The monophyletic nature of the Archaeplastida has enabled the reconstruction of ancient metabolic networks from the detailed knowledge of the nature and composition of the pathways in the three Archaeplastida lineages [20–23]. This strategy led to the proposal in [21] that, at the onset of endosymbiosis, storage polysaccharides were

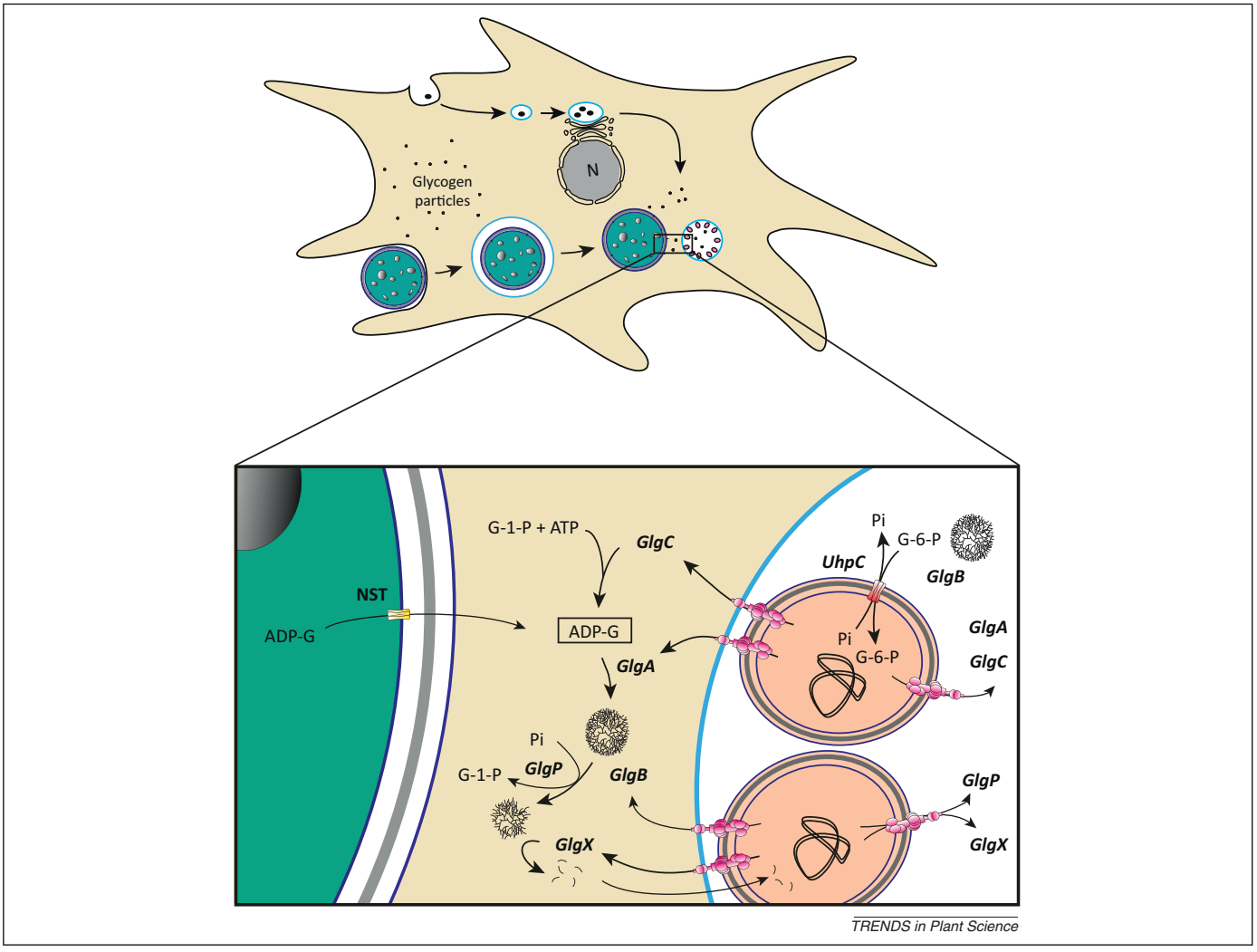

Figure 1. Ménage à trois with the host on top. In this proposed scheme, endosymbiosis was achieved through phagocytosis of a cyanobacterium (inclusion membrane shown in blue, with white cyanobacterial starch granules) in an ancestral amoeba that already contained an ancestral chlamydial symbiont (in pink) as initially proposed in [22]. The cyanobiont immediately escaped the phagocytosis vacuole and entered the cytosol with its outer and inner cell membranes exposed. A section of the cyanobiont and the inclusion vesicle that contained the chlamydial pathogens is enlarged. The inclusion vesicle is derived from endocytosis of the chlamydia elementary body at the surface of the amoeba. The escape to the cytosol of the cyanobiont was accompanied by the recruitment of the host-derived nucleotide-sugar transporter (NST; in yellow) to the inner membrane (see main text). Thus, ADP-glucose (ADP-G) was exported to the cytosol (in beige) but is neither produced nor used by eukaryotes. Therefore, to be metabolized, the only possible fate for the cytosolic ADP-G is to be polymerized through a bacterial glycogen synthase into the host glycogen pools. The presence of a chlamydial effector glycogen synthase establishes a tripartite symbiosis. It is thought that Chlamydiales secrete most of their glycogen metabolism enzymes as virulence effectors through the type-three secretion system (TTS). *GLGC* encodes the chlamydial ADP-glucose pyrophosphorylase, which synthesizes ADP-G from glucose 1-P (G-1-P) and ATP. *GLGA* encodes the chlamydial glycogen synthase that transfers Glc from ADP-G to the glycogen outer chains. *GLGB* encodes the chlamydial glycogen-branching enzyme that introduces the α-1,6 branches into the glycogen particle (shown in black). The outer chains of glycogen are recessed by glycogen phosphorylase encoded by the chlamydial *GLGP* gene. The GlgP enzyme releases G-1-P from the ends of the glycogen outer chains in the presence of orthophosphate and stops at a distance of four Glc residues from the branch, Debranching of the glycogen core structure occurs through the chlamydial GlgX-debranching enzyme, which releases the remaining four Glc residues in the form of oligosaccharide in the cytosol (maltotetraose). Eukaryotes cannot degrade this maltotetraose, which can only be metabolized by the pathogens. During normal infection, the rationale for the pathogens is to induce the synthesis of glycogen in the cytosol at the beginning of the infection when cytosolic ATP and orthophosphate are high and low, respectively. At the end of the infection, when the ratio of ATP to orthophosphate has substantially decreased, the pathogens bypass the highly regulated glycogen phosphorylase of the host by secreting their own enzyme, thereby producing G-1-P and maltotetraose that are actively metabolized by the bacteria. The host glycogen metabolism machinery based on UDP-glucose is not shown. Upon establishing endosymbiosis with the cyanobiont, the host metabolizes photosynthates through catabolism of the cytosolic glycogen pools. The enlarged section shows the pink chlamydial reticulate bodies attached to the inclusion vesicle by the TTS (shown in red). It is thought that the Chlamydiae also secrete enzymes of glycogen metabolism within the inclusion vesicle (shown in white), leading to intravesicular glycogen metabolism.

synthesized in the cytosol of the host from a pre-existing host glycogen metabolism network (Figure 1). Carbon was proposed to flow from two directions: host carbohydrates were polymerized as usual from UDP-glucose in the host cytosol according to host regulatory networks; however, more importantly, photosynthate was proposed to be exported from the cyanobiont to the host cytosol in the form of the bacterial-specific glycosyl-nucleotide, ADP-glucose, which is devoted to glycogen synthesis in bacteria, through an ADP-glucose export protein [24]. This carbon was polymerized into host glycogen in the host cytosol because of the presence of an ADP-glucose-specific glucan (glycogen) synthase. Such a connection would have been optimal because only the carbon committed to storage through bacterial ADP-glucose pyrophosphorylase would be exported. In the cytosol, the only possible fate of this carbon unrecognized by eukaryotic metabolism would be polymerization into glycogen. The carbon stores would then be degraded through the host glycogen mobilization machinery solely controlled by host needs. In such a