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Review

Toward an understanding of the function of Chlamydiales in plastid endosymbiosis

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ABSTRACT

Plastid endosymbiosis defines a process through which a fully evolved cyanobacterial ancestor has transmitted to a eukaryotic phagotroph the hundreds of genes required to perform oxygenic photosynthesis, together with the membrane structures, and cellular compartment associated with this process. In this review, we will summarize the evidence pointing to an active role of Chlamydiales in metabolic integration of free living cyanobacteria, within the cytosol of the last common plant ancestor.

1. Introduction

Metabolic integration of established endosymbionts into novel organelles, such as the mitochondrion or plastids, defines events of the outmost rarity that have had far reaching consequences in the forging of the first eukaryotes and of all their photosynthetic derivatives. While the endosymbiont theory states that mitochondria and plastids derive from α-proteobacteria and cyanobacteria respectively, metabolic integration of these endosymbionts has evidently implied the massive participation of genes whose ancestry cannot be traced back to these two sole clades [1,2]. In particular, plastid endosymbiosis is known to correlate with a phylogenomic imprint from intracellular chlamydia pathogens specific and selective to all lineages derived from this unique event [3–10]. It has recently been shown that enzymes that are thought to have been responsible for photosynthetic carbon assimilation in the host cytosol, define metabolic effector proteins secreted by intracellular Chlamydiales pathogens ([10] highlighted by [11]; reviewed in [12]) the cytosol of their host. Hence the intracellular pathogens and incipient cyanobacterium were possibly tied together with their host in a tripartite symbiosis where the three partners coded essential components of a common photosynthetic carbon assimilation pathway [10]. This suggests that intracellular bacteria living as temperate pathogens or symbionts within eukaryotes may define major players down the path of metabolic integration of future organelles. Such intracellular bacteria are usually viewed as degenerate genomes that evolved from free living sister lineages by selective gene losses. (for review see [13]). However the intracellular lifestyle also implied the evolution of hundreds of protein effectors that ensures intracellular life either within phagocytosis derived vacuoles or more rarely in the cytosol. Because direct microinjection of free living bacteria in the eukaryotic cytosol, fails to yield any multiplication of the injected organisms unless they already define intracellular pathogens or symbionts [14], we believe that free-living cyanobacteria were driven into endosymbiosis thanks to helper intracellular symbionts. Recent work on the impact of chlamydia in plastid endosymbiosis has yielded an unexpectedly detailed molecular description of the early events that may have triggered plastid endosymbiosis, including the molecular nature of the symbiotic gene and the precise nature of the major carbon and ATP transporters involved. This speculative scenario is presently well sustained by a series of distinct phylogenetic and biochemical observations that, together, make a strong case for the implication of Chlamydiales in the initial steps of plastid endosymbiosis. In this review we will describe the evidence sustaining this hypothesis.

2. The chlamydial phylogenomic signal in the Archaeplastida genome

Chlamydiaceae, including genus Chlamydia and Chlamydophila are a family of obligate intracellular bacteria with a small size genome (<1 Mbp) that multiply in inclusion vesicles within the eukaryotic cytosol. Chlamydiae commonly infect animals, while related organisms from the order Chlamydiales, with a two to three-fold larger genomes, may infect a wider range of other eukaryotic phagotrophs. All Chlamydiales share a similar obligate intracellular life cycle (Fig. 1) consisting first of attachment of the infectious bacterium called the "elementary body" to an exposed membrane, followed by penetration through

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endocytosis–phagocytosis, and then by modification of the endocytic vacuole to escape lysosomal digestion, and by active multiplication within this vacuole named the inclusion vesicle (for a review see [15]). This is followed by cell lysis or budding of the inclusion vesicles releasing novel infectious bacteria unable to replicate autonomously. In 1998, in the first genome description of a chlamydial intracellular pathogen infecting human cells (*Chlamydia trachomatis*), the authors surprisingly reported that a majority of the cases of LGT (lateral gene transfer), uniting Chlamydiae with eukaryotes, did not translate in the capture of genes from their animal target cells [3]. In fact, for some unknown reason, a majority of the 37 cases documented at that time united the pathogens with the green plants! This came as a total surprise since no extant plants are known to be susceptible to chlamydial infection, an observation which correlates with the requirement for exposed membranes in order to initiate infection. In their genome description, the authors proposed that the LGTs discovered in the *C. trachomatis* genome were ancient and dated back to the time when Chlamydial ancestors infected the amoebal ancestors of both the plant and animal lineage [3]. Because of their phagotrophic habit, such organisms were not covered by a continuous cell wall. This interpretation proved in part to be correct as the LGTs can indeed be traced back to over a billion years of evolution of these very ancient pathogens. However the assumed directionality of gene transfer, which was thought to consist of the capture of amoebal genes by the evolving pathogens, proved to be partly incorrect, as a majority (but by no means all) of these LGTs are now suspected to reflect the transfer of Chlamydial intracellular pathogen genes to the amoeba-like phagotroph that defines the common ancestor of plants, rather than the opposite. This common ancestor is the founder of the Archaeplastida, a group of eukaryotes containing the ancestor of all plastids (for review see [12]). Archaeplastida diversified into three major lineages: the glaucophytes, consisting of a small number of unicellular freshwater algae containing a peptidoglycan containing plastid called the muroplast; the Rhodophyceae, known as the red algae, a very diverse group of freshwater and marine unicellular and multicellular organisms, containing a rhodoplast, and the Chloroplastida, an equally diverse and complex set of marine and freshwater organisms harboring the chloroplast. Within the Chloroplastida, a particular lineage later established itself on land and gave birth to all "true plants". In 2007–2008, three distinct groups published that a specific chlamydial imprint could be evidenced in all Archaeplastida lineages [6–8]. The presence of at least a third of these Archaeplastida-specific LGTs in several of three (red algae, green algae and glaucophytes) genomes hinted that these LGTs happened in the common ancestor of the Archaeplastida. Because the common ancestor can be defined as the cell that resulted from plastid endosymbiosis, Peter Gogarten first proposed that the pathogens took an active role in metabolic integration of the protoplastid [6]. This hypothesis was also sustained by the two other studies [7,8] and by a more recent study that integrated the genomes of the major families of the order Chlamydiales [9]. It must be stressed that the phylogenomic signal, which is recovered by imposing a minimal bootstrap value of 70 in maximum likelihood phylogenies uniting Archaeplastida and chlamydial lineages at the exclusion of all other lineages, may not be powerful enough to distinguish issues of transfer directionality or to ascertain that the LGTs do relate to plastid endosymbiosis, especially when the LGTs are clade specific (that is when only one of the three Archaeplastida lineages is concerned). LGTs from bacteria are common in all eukaryotic lineages and we can predict a background of LGTs within the chlamydial phylogenomic signal which is most probably not related to plastid endosymbiosis. We presently estimate between 1/10 to at most 1/3 the number of LGTs within the chlamydial phylogenomic signal not related to plastid endosymbiosis [10]. This would leave us with a lower pessimistic and restrictive figure of 30 genes concerned with plastid endosymbiosis, and a maximum more optimistic figure of 50 genes. This figure is certainly dwarfed by the cyanobacterial phylogenomic signal which comes out one to two orders of magnitude stronger but it is nevertheless robust and only matched in plastid proteins by proteobacteria, as a whole, which define a far more prevalent and more diverse group of bacteria [1]. Phylogenomic approaches did not leave us any clues as to how and why this signal was generated at plastid endosymbiosis.

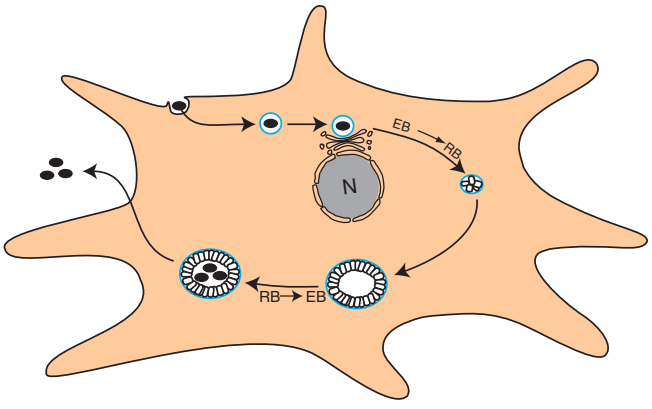

Fig. 1. Life cycle of chlamydiales pathogens. A small infectious chlamydia cell, called an elementary body (EB in solid black), interacts with the plasma membrane of an amoeba-like eukaryotic host. The endocytosis–phagocytosis vacuole is reprogrammed by the pathogen to become an inclusion vacuole thereby avoiding acidification and destruction. The EBs differentiate into actively multiplying reticulate bodies (RBs), which are thought to be attached to the inclusion vesicle through their TTS (type three secretion system, not displayed in this drawing). Some EBs detach from the inclusion vesicle, and redifferentiate into EBs. Progeny infectious EBs are released into the extracellular medium through lysis or fusion of the inclusion vesicle with the plasma membrane. EBs never divide in the extracellular medium.

3. Working out the plastid endosymbiosis symbiotic flux

Plastid endosymbiosis can be distinguished from mitochondrial endosymbiosis by a good knowledge of the setting and preexisting conditions. The nature of the host is universally accepted as being a standard heterotrophic and phagotrophic flagellate, while that of the future plastid was most certainly an ancestor of extant diazotrophic cyanobacteria. Because phagotrophy was observed in very early diverging prasinophyte green algae, we and many others reason that phagotrophy in the case of plastids defines a very obvious candidate mechanism for penetration of the plastid's ancestor into its eukaryotic host [16–18].

Such a good knowledge of the starting conditions certainly does not apply to mitochondrial endosymbiosis where the status of the host, and the entry mechanism remain obscure, further precluding inference of the very nature of the metabolic symbiosis that prompted this event [2]. Having accepted the phagotrophic and classical eukaryotic status of the host of plastid endosymbiosis, we are faced with a more restricted number of possibilities. Phagotrophy in all cases had to abort, and a symbiotic flux had to be installed between the two partners that give a selective advantage to this partnership. Because cyanobacteria are not reported to have the ability to live within eukaryotes, it is reasonable to assume that this unlikely partnership was selected because only cyanobacteria could provide the required metabolic traits. This of course leaves us with oxygenic photosynthesis, and to a lesser extent diazotrophy, as possible candidates for the installment of the symbiotic flux.

We have reviewed elsewhere the metabolic reasons explaining why maintenance of diazotrophy in a symbiont exporting photosynthetic carbon was metabolically impossible [19]. Briefly, ancestors of extant single-cell diazotrophic cyanobacteria, which we have hypothesized to define the plastid source [21], display a very tight circadian-clock regulation of cellular metabolism. Indeed Nitrogenase being equisitively