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Blurred pictures from the crime scene: the growing case for a function of chlamydiales in plastid endosymbiosis

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Abstract

A number of recent papers have brought suggestive evidence for an active role of *Chlamydiales* in the establishment of the plastid. *Chlamydiales* define a very ancient group of obligate intracellular bacterial pathogens that multiply in vesicles within eukaryotic phagotrophic host cells such as animals, amoebae or other protists, possibly including the hypothetical phagotroph that internalized the cyanobacterial ancestor of the plastid over a billion years ago. We briefly survey the case for an active role of these ancient pathogens in plastid endosymbiosis. We argue that a good understanding of the *Chlamydiales* infection cycle and diversity may help to shed light on the process of metabolic integration of the evolving plastid.

Keywords: *Chlamydiales*, plastid, endosymbiosis, glycogen, ménage à trois hypothesis

The growing phylogenomic case for the involvement of Chlamydiales in plastid endosymbiosis

Chlamydiales define very ancient obligate intracellular pathogens of phagotrophic eukaryotes including animals and amoebae, already intracellular more than 1 billion years ago [1]. An unexpected phylogenomic signal uniting these pathogens to plants was discovered in the late nineties, when the first *Chlamydiales* genomes became accessible [2]. At that time, it was noticed that 35 out of a total of 894 genes within the human pathogen *Chlamydia trachomatis* genome shared a common ancestry with eukaryotes [2]. Surprisingly most of these were more closely related to plants than to animals, despite that the latter defined their natural hosts (an observation herein referred to as “the plant-chlamydia paradox”). This result was unexpected as plant chlamydial infections have yet to be reported. This absence of chlamydial plant pathogens can be understood by the presence of a thick and continuous cell wall, precluding as in fungi, the initial attachment of the chlamydial elementary bodies to an exposed membrane [1]. A number of subsequent studies have confirmed the initial report, and extended it to the diversity of the *Chlamydiales* order [3, 4, 5, 6, 7, 8, 9]. Furthermore, the subsequent genome sequencing of red algae and glaucophytes revealed the presence of genes that also shared a common ancestry with *Chlamydiales* and the green plants [4, 5, 6, 7, 8, 9] . Both red algae and glaucophytes define the eukaryotic photosynthetic sister lineages of the green algae and land plants. Together with the latter, they are called the Archaeplastida, as all of these lineages result from a single plastid endosymbiosis event that happened over a billion years ago [10, 11, 12]. The most recent analysis presently estimates at 24 the number of genes which are shared with Chlamydiales by at least two of the Archaeplastida lineages [9]. Quite significantly, the majority of these genes concern products which today are found within plastids or on plastidial membranes [5,6, 7, 8]. In addition to these 24 LGTs (lateral gene transfers), an equal number of lineage specific LGTs are also evidenced [5, 6, 7, 8, 9]. The

shared presence of a substantial portion of LGTs in several of the Archaeplastida lineages, strongly suggests that these gene transfers happened in their common ancestor. This observation dates these LGTs back to over a billion years, possibly back to the time of plastid endosymbiosis [12]. The ancient nature of these LGTs also solves the plant-chlamydia paradox, as most scientists agree on the phagotrophic nature of the ancient protist that internalized the future plastid [13]. Such an amoeba-like organism was indeed likely to have harbored exposed membranes in at least part of its life cycle, and was therefore likely to have been susceptible to infection by a chlamydial ancestor. However this does not explain the presence of a two-fold fold higher number of total candidate LGTs in Archaeplastida when compared to the animals, despite these being far better sampled in the databases [8]. Indeed, animals have been susceptible to chlamydial infection ever since they evolved from single cell phagotrophs, while Archaeplatida evolved autotrophy, and as a result lost phagotrophy early on. As a consequence, they gained diverse thick and continuous cell wall structures precluding entry of Chlamydiales. To explain their comparatively higher frequency of chlamydial LGTs, it was proposed first by Gogarten [4] and then by others [5,6] that the pathogens were initially persistent in the Archaeplastida ancestors and that this persistence suggested an active early and essential role in metabolic integration of the protoplastid.

One of the major weaknesses of the phylogenomic approaches summarized above resides in problems inherent to very ancient phylogenetic signals taken as evidence for LGTs (lateral gene transfers). The problems consist of signal erosion (hence of low bootstrap values and of tree topology problems) and most of the time cannot be solved by phylogenetic tools [14]. One attempt has been recently made to use alternative models of gene evolution to master single phylogenies and recover more “accurate” phylogenetic trees [15]. However these attempts generally successful for multigene phylogenies have, not surprisingly, failed to resolve the single gene histories where signal erosion is evidenced. This has led those that

refuse to resolve uncertainties in tree building with biochemical arguments, to reject the proposed role of *Chlamydiales* in plastid establishment [16]. However we believe that the biochemical arguments made for critical single gene histories are sufficiently solid and sound to refuse this rejection [8,16]. In addition, the fact that *Chlamydiales* remain in all these phylogenies the closest taxa to the root of the Archaeplastida, despite the ever growing databases, gives us confidence that the uncertainties in most cases result from incorrect outgroup rooting of the important sections of the trees. One can easily obviate such problems by producing unrooted trees and propose sound rooting through biochemical arguments. The reader is referred to Domman et al. (2014) [15] and Ball et al. (2015) [16], for further documentation on the ongoing controversy. The picture that we get from these "problematic trees" is indeed partly blurred. In a fashion analogous to the analysis of a crime scene with a defective surveillance camera, the result remains a collection of blurred pictures, which yet still carry enough information to solve the crime. We believe that in this respect the most productive attitude is not to discard all of this useful information but to accept the trees at face value, but with caution, in order to build testable hypotheses (see conclusions)

The ménage à trois (MAT) hypothesis

Independently of the rising phylogenetic controversy, biochemical observations have accumulated that strengthens the case of the involvement of *Chlamydiales* in plastid endosymbiosis. A reconstruction of what could have been storage polysaccharide metabolism in the common ancestor of the Archaeplastida was recently proposed [17, 18]. This speculative reconstruction was initially made by comparing the extant biochemical pathways of storage polysaccharide metabolism in red and green algae [17, 18]. This speculative scenario was thereafter considerably strengthened by the subsequent observation of the extant glaucophyte pathways that displays all of its predicted basic properties [19]. The biochemical flux that was evidenced displays many unusual and attractive features that qualify it as a very

serious candidate for defining the original flux that established plastid endosymbiosis. All but two components of this flux could be built from the likely preexisting enzymes of host and cyanobiont glycogen metabolism [17, 18]. This would have been required if, as we believe, the symbiotic flux defined the onset of endosymbiosis. Nevertheless the two key proteins that established the first biochemical connection between the two unrelated enzyme networks did not define obvious preexisting components. These key proteins defined a nucleotide-sugar translocator (NST) on the cyanobiont inner membrane extracting the bacterial specific metabolite ADP-Glc to the cytosol and a bacterial glucan synthase able to polymerize this substrate, unrecognized by the host, in the cytosol's glycogen pools [16, 17]. Weber and Bhattacharya had previously proven that all plastidial carbon translocators from extant red and green algae, which export carbon from plastids to the cytosol, display a common phylogenetic origin [20]. Furthermore they showed that this unique ancestral protein was sister to host encoded NSTs of the endomembrane system [20]. Later Colleoni et al. (2010) [21] demonstrated that these NSTs defined very efficient ADP-Glc translocators in liposome transport assays. Hence a convincing case supports the recruitment of a host derived endomembrane NST to achieve carbon export from the cyanobacterium. This leaves the ADP-glucose utilizing glucan synthase as the only "foreign" protein, whose presence in the cytosol at the time of plastid endosymbiosis, must be explained. The gene encoding this enzyme can be considered at the center of the host-cyanobiont biochemical symbiosis [18]. Phylogenetic analysis suggests that the gene was transferred from Chlamydiales to the Archaeplastida ancestor following plastid endosymbiosis [3,6,8]. However at the very onset of the event, there was no reason for a eukaryotic phagotroph to encode a bacterial glycogen synthase as it uses a substrate that it does not produce. Nevertheless this paradox could be solved if one hypothesizes the presence of a chlamydial intracellular pathogen encoding such an enzyme and actively secreting the protein in the host cytosol as a virulence effector. This hypothesis

was verified by two distinct groups [8,22]. Glycogen metabolism enzymes in *Chlamydiales*, previously thought as house-keeping activities, are now accepted as important effectors that highjack the host carbon storage machinery. These results entail that the three genomes became united in a tripartite symbiosis through the shared coding of a common photosynthetic carbon assimilation pathway [8]. This is now defined as the MAT hypothesis (ménage à trois) [8,16]. As noted above, sequencing of the first glaucophyte genome confirmed that the predicted glycogen synthase was indeed found in the cytosol of *Cyanophora paradoxa* [19]. However proteomic analysis of the glaucophyte plastid (the muroplast) yielded only 12 candidate membrane bound transporters, 3 of which are suspected to define chlamydial LGTs [23]. Most importantly, the major transporter that exports photosynthetic carbon from the muroplast was proven biochemically to be UhpC, the chlamydial glucose-6-P/Pi exchanger, which defines the major carbon transporter in Chlamydiales [23,24]. To accommodate the presence of both the host derived NST and the chlamydial UhpC, it was proposed that the cyanobacterium and the pathogen entered the host simultaneously in the same inclusion vesicle [25]. This allowed conjugative transfer of the genes encoding the critical chlamydial transporters [26,27] and facilitated their correct localization at the onset of plastid endosymbiosis. It is known that extant *Chlamydiaceae* drive glycogen synthesis not only in the elementary bodies or the host cytosol but also predominantly in the inclusion vesicle [28]. Hence the same suite of chlamydial glycogen metabolism enzymes would ensure polysaccharide synthesis from photosynthetic carbon within the inclusion vesicle to the pathogen's major benefit. The host in this modified MAT model would only get the overflow of ADP-glucose through the inclusion vesicle NST of host origin. However the nature of the symbiotic link in the host cytosol would be the same as that described above. The modified MAT displays an immense advantage over the classical MAT hypothesis, as it offers a straightforward mechanism explaining how the free living cyanobacterium escaped the host

defense mechanism (autophagy, vacuole acidification) and became primed for intracellular life. It nevertheless requires a subsequent early escape of the cyanobiont from the inclusion in order to evolve the required common plastidial protein targeting machinery.

Conclusions

Hypotheses display true scientific value when they are sufficiently detailed as to lead to testable predictions. The MAT hypothesis typically offers this level of detail as it enables to build predictions amenable to experimental validation in several experimental systems. For instance the reconstruction of glycogen metabolism that was performed on the basis of the comparisons of the red and green algae extant biochemical networks was strengthened by studies performed in glaucophytes. The predicted chlamydial starch synthase was indeed found in the glaucophyte cytosol and used ADP-glucose as predicted (19). The MAT predicted that this enzyme must have defined an important chlamydial effector which was thereafter experimentally validated (8, 22). The “modified” MAT (25) predicts the existence of important host derived NSTs importing nucleotide sugars and present on the chlamydial inclusion vesicle membrane which can be further tested. The NST ancestor of extant plastidial carbon translocator was inferred in both MAT models to be an efficient ADP-Glc translocator which was proven on the suspected eukaryote ancestors by import experiments performed on yeast liposomes (21). Massive carbon export from the cyanobacterium predicts the latter must have suffered immediately from ATP depletion in darkness which is amenable to experimental testing in extant cyanobacteria thereby explaining the presence of the chlamydial ATP import protein on the inner plastid membrane. Both MAT models (8, 25) are indeed rich in predictions amenable to experimental testing. We agree that none of these experimental validations on their own are sufficient to “prove” the model but together they considerably strengthen the case as these findings must be considered as entirely coincidental if the MAT hypothesis is incorrect. The probability that this is untrue indeed diminishes with

each validated prediction. Finally perhaps an even more convincing approach would consist in repeating some aspects of the MAT in an experimental endosymbiosis system. We believe that the *Waddlia chondrophila* or *Estrella lausanensis – Dictyostelium discoideum* pathosystems offer a unique opportunity to establish endosymbiosis experimentally.This would in turn pave the way for important synthetic biology developments since such an experimental system could help establish free living bacteria together with their metabolic capabilities in the eukaryote cytosol.

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LITERATURE CITED

[1] Greub G, Raoult D. History of the ADP/ATP-translocase-encoding gene, a parasitism gene transferred from a *Chlamydiales* ancestor to plants 1 billion years ago. Appl Environ Microbiol. 2003;69:5530-5535

[2] Stephens RS, Kalman S, Lammel C., Fan J, Marathe R, Aravind L et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 2008;282:754-59.

[3] Brinkman FS., Blanchard JL, Cherkasov A, Av-Gay Y, Brunham RC, Fernandez RC et al. Evidence that plant-like genes in *Chlamydia* species reflect an ancestral relationship between Chlamydiaceae, cyanobacteria and the chloroplast. Genome Res. 2002;12:1-9.

[4] Huang J, Gogarten, P. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? Genome Biol. 2007;8:R99

[5] Becker B, Hoef-Emden K., Melkonian M. Chlamydial genes shed light on the evolution of photoautotrophic eukaryotes. BMC Evol. Biol. 2008 8:203.

[6] Moustafa A., Reyes-Prieto A., Bhattacharya D. Chlamydiae has contributed at least 55 genes to Plantae with predominantly plastid functions. PLoS ONE 2008;3:e2205.

[7] Collingro A., Tischler P, Weinmaier T, Penz, T, Heinz E, Brunham RC et al. Unity in variety: The pan-genome of the Chlamydiae. Mol. Biol. Evol. 2011;28:3253–70.

[8] Ball SG, Subtil A, Bhattacharya D, Moustafa A, Weber APM., Gehre L et al. Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? Plant Cell 2013;25:7-21.

214 [9] Deschamps P. Primary endosymbiosis: have cyanobacteria and Chlamydiae ever been
215 roommates? Acta Soc. Bot. Pol. Pol. 2014;83:291-302.

216 [10] Rodríguez-Ezpeleta N, Brinkmann H, Burey SC, Roure B, Burger G, Löffelhardt W et al.
217 Monophyly of primary photosynthetic eukaryotes: Green plants, red algae, and glaucophytes.
218 Curr. Biol. 2005;15:1325–30.

219 [11] Chan CX, Yang EC, Banerjee T, Yoon HS, Martone PT, Estevez JM et al. Red and green
220 algal monophyly and extensive gene sharing found in a rich repertoire of red algal genes.
221 Curr. Biol. 2011;21:328-33.

222 [12] Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. A molecular timeline for the
223 origin of photosynthetic eukaryotes. Mol. Biol. Evol. 2004;21:809–18.

224 [13] Bhattacharya D, Price DC, Yoon HS, Yang EC, Poulton NJ, Andersen RA et al. Single
225 cell genome analysis supports a link between phagotrophy and primary plastid
226 endosymbiosis. Sci Rep. 2012 2:356.

227 [14] Philippe H, Brinkmann H, Lavrov DV, Littlewood DT, Manuel M, Wörheide G et al.
228 Resolving difficult phylogenetic questions: why more sequences are not enough. Plos Biology
229 2011;9(3):e1000602.

230 [15] Domman D, Horn M, Embley M, Williams TA. Plastid establishment did not require a
231 chlamydial partner. Nat. Commun. 2015;6:6421.

232 [16] Ball SG, Colleoni C, Kadouche D, Ducatez M, Arias MC et al. Toward an understanding
233 of the function of Chlamydiales in plastid endosymbiosis. Biochim Biophys Acta
234 2015;847:495-504

235

236 [17] Deschamps P, Colleoni C, Nakamura Y, Suzuki E, Putaux JL, Buléon, A et al. Metabolic
237 symbiosis and the birth of the plant kingdom. Mol. Biol. Evol. 2008;25:536–48.

238 [18] Ball SG, Colleoni C, Cenci U, Raj JN, Tirtiaux C. The evolution of the glycogen and
239 starch pathway in eukaryotes gives molecular clues to understand the establishment of plastid
240 endosymbiosis. J. Exp. Bot. 2011;62:1775–1801.

241 [19] Price DC, Chan CX, Yoon HS, Yang EC, Qiu H, Weber APM. et al. *Cyanophora*
242 *paradoxa* genome elucidates origin of photosynthesis in algae and plants. Science
243 2012;335:843–47.

244 [20] Weber APM, Linka M, Bhattacharya D. Single, ancient origin of a plastid metabolite
245 translocator family in Plantae from an endomembrane-derived ancestor. Eukaryot. Cell
246 2006;5:609–12.

247 [21] Colleoni C, Linka M, Deschamps P, Handford MG, Dupree P, Weber APM et al.
248 Phylogenetic and biochemical evidence supports the recruitment of an ADP-glucose
249 translocator for the export of photosynthate during plastid endosymbiosis. Mol. Biol. Evol.
250 2010;27:691–701.

251 [22] Lu, C., Lei, L., Peng, B., Tang, L., Ding, H., Gong, S., et al. *Chlamydia trachomatis*
252 GlgA is secreted into host cell cytoplasm . PLoS One 2013;8(7):e68764.

253 [23] Facchinelli. F, Pribil M, Oster U, Ebert NJ, Bhattacharya D, Leister. D et al. Proteomic
254 analysis of the *Cyanophora paradoxa* muroplast provides clues on early events in plastid
255 endosymbiosis, Planta 2013;237: 637-51.

256 [24] Schwöppe C, Winkler HH, Neuhaus HE. Properties of the glucose-6-phosphate
257 transporter from *Chlamydia pneumoniae* (HPTcp) and the glucose-6-phosphate sensor from
258 *Escherichia coli* (UhpC). J. Bacteriol. 2002;184: 2108–15.

[25] Facchinelli F, Colleoni C, Ball SG and Weber AP. Chlamydia, cyanobiont, or host: who was on top in the ménage à trois? Trends Plant Sci. 2013;18: 673–79.

[26] Greub G, Collyn F, Guy L, Roten CA. A genomic island present along the bacterial chromosome of the Paréachlamydiaceae UWE25, an obligate amoebal endosymbiont encodes a potentially functional F-like conjugative DNA transfer system. BMC Microbiol. 2004;4:48.

[27] Bertelli C, Greub G. Lateral gene exchanges shape the genomes of amoeba-resisting microorganisms. Front Cell Infect. Microbiol. 2012;2:110.

[28] Nguyen BD, Valdivia RH.Virulence determinants in the obligate intracellular pathogen *Chlamydia trachomatis* revealed by forward genetic approaches. Proc Natl Acad Sci U S A 2012;109:1263–68.

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