

Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

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

Authors: Ball SG, Greub G

Journal: Microbes and infection

Year: 2015 Nov-Dec

Volume: 17

Issue: 11-12

Pages: 723-6

DOI: [10.1016/j.micinf.2015.09.007](https://doi.org/10.1016/j.micinf.2015.09.007)

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

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

4
5 **Steven G. Ball^{a,1} and Gilbert Greub^b**

6 ^a Univ.Lille, CNRS, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle,
7 F 59 000 Lille, France

8 ^b Center for Research on Intracellular Bacteria (CRIB), Institute of Microbiology, University
9 Hospital Center and University of Lausanne, 1011 Lausanne, Switzerland

10

11 ¹To whom correspondence should be addressed. Steven G. Ball , UMR8576 CNRS-USTL,
12 Bâtiment C9, Université des Sciences et Technologies de Lille, Cité Scientifique, 59655
13 Villeneuve d'Ascq Cedex, France. Phone : 33 3 20436543, Fax : 33 3 20436555, e-mail :
14 steven.ball@univ-lille1.fr

15

16

17

18

19 **Abstract**

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

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

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

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

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

72 One of the major weaknesses of the phylogenomic approaches summarized above
73 resides in problems inherent to very ancient phylogenetic signals taken as evidence for LGTs
74 (lateral gene transfers). The problems consist of signal erosion (hence of low bootstrap values
75 and of tree topology problems) and most of the time cannot be solved by phylogenetic tools
76 [14]. One attempt has been recently made to use alternative models of gene evolution to
77 master single phylogenies and recover more “accurate” phylogenetic trees [15]. However
78 these attempts generally successful for multigene phylogenies have, not surprisingly, failed to
79 resolve the single gene histories where signal erosion is evidenced. This has led those that

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

95 **The ménage à trois (MAT) hypothesis**

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

105 serious candidate for defining the original flux that established plastid endosymbiosis. All but
106 two components of this flux could be built from the likely preexisting enzymes of host and
107 cyanobiont glycogen metabolism [17, 18]. This would have been required if, as we believe,
108 the symbiotic flux defined the onset of endosymbiosis. Nevertheless the two key proteins that
109 established the first biochemical connection between the two unrelated enzyme networks did
110 not define obvious preexisting components. These key proteins defined a nucleotide-sugar
111 translocator (NST) on the cyanobiont inner membrane extracting the bacterial specific
112 metabolite ADP-Glc to the cytosol and a bacterial glucan synthase able to polymerize this
113 substrate, unrecognized by the host, in the cytosol's glycogen pools [16, 17]. Weber and
114 Bhattacharya had previously proven that all plastidial carbon translocators from extant red
115 and green algae, which export carbon from plastids to the cytosol, display a common
116 phylogenetic origin [20]. Furthermore they showed that this unique ancestral protein was
117 sister to host encoded NSTs of the endomembrane system [20]. Later Colleoni et al. (2010)
118 [21] demonstrated that these NSTs defined very efficient ADP-Glc translocators in liposome
119 transport assays. Hence a convincing case supports the recruitment of a host derived
120 endomembrane NST to achieve carbon export from the cyanobacterium. This leaves the ADP-
121 glucose utilizing glucan synthase as the only "foreign" protein, whose presence in the cytosol
122 at the time of plastid endosymbiosis, must be explained. The gene encoding this enzyme can
123 be considered at the center of the host-cyanobiont biochemical symbiosis [18]. Phylogenetic
124 analysis suggests that the gene was transferred from Chlamydiales to the Archaeplastida
125 ancestor following plastid endosymbiosis [3,6,8]. However at the very onset of the event,
126 there was no reason for a eukaryotic phagotroph to encode a bacterial glycogen synthase as it
127 uses a substrate that it does not produce. Nevertheless this paradox could be solved if one
128 hypothesizes the presence of a chlamydial intracellular pathogen encoding such an enzyme
129 and actively secreting the protein in the host cytosol as a virulence effector. This hypothesis

130 was verified by two distinct groups [8,22]. Glycogen metabolism enzymes in *Chlamydiales*,
131 previously thought as house-keeping activities, are now accepted as important effectors that
132 hijack the host carbon storage machinery. These results entail that the three genomes
133 became united in a tripartite symbiosis through the shared coding of a common photosynthetic
134 carbon assimilation pathway [8]. This is now defined as the MAT hypothesis (ménage à trois)
135 [8,16]. As noted above, sequencing of the first glaucophyte genome confirmed that the
136 predicted glycogen synthase was indeed found in the cytosol of *Cyanophora paradoxa* [19].
137 However proteomic analysis of the glaucophyte plastid (the muroplast) yielded only 12
138 candidate membrane bound transporters, 3 of which are suspected to define chlamydial LGTs
139 [23]. Most importantly, the major transporter that exports photosynthetic carbon from the
140 muroplast was proven biochemically to be UhpC, the chlamydial glucose-6-P/Pi exchanger,
141 which defines the major carbon transporter in *Chlamydiales* [23,24]. To accommodate the
142 presence of both the host derived NST and the chlamydial UhpC, it was proposed that the
143 cyanobacterium and the pathogen entered the host simultaneously in the same inclusion
144 vesicle [25]. This allowed conjugative transfer of the genes encoding the critical chlamydial
145 transporters [26,27] and facilitated their correct localization at the onset of plastid
146 endosymbiosis. It is known that extant *Chlamydiaceae* drive glycogen synthesis not only in
147 the elementary bodies or the host cytosol but also predominantly in the inclusion vesicle [28].
148 Hence the same suite of chlamydial glycogen metabolism enzymes would ensure
149 polysaccharide synthesis from photosynthetic carbon within the inclusion vesicle to the
150 pathogen's major benefit. The host in this modified MAT model would only get the overflow
151 of ADP-glucose through the inclusion vesicle NST of host origin. However the nature of the
152 symbiotic link in the host cytosol would be the same as that described above. The modified
153 MAT displays an immense advantage over the classical MAT hypothesis, as it offers a
154 straightforward mechanism explaining how the free living cyanobacterium escaped the host

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

158 **Conclusions**

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

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

187 **Acknowledgments**

188 SB was supported by the CNRS, the Université des Sciences et technologies de Lille, the
189 Région Nord Pas de Calais, the ANR grants (“starchevol” and “ménage à trois”), whereas G
190 Greub is supported by SNSF grants.

191

192

193 **LITERATURE CITED**

- 194 [1] Greub G, Raoult D. History of the ADP/ATP-translocase-encoding gene, a parasitism
195 gene transferred from a *Chlamydiales* ancestor to plants 1 billion years ago. *Appl Environ*
196 *Microbiol.* 2003;69:5530-5535
- 197 [2] Stephens RS, Kalman S, Lammel C., Fan J, Marathe R, Aravind L et al. Genome sequence
198 of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science*
199 2008;282:754-59.
- 200 [3] Brinkman FS., Blanchard JL, Cherkasov A, Av-Gay Y, Brunham RC, Fernandez RC et al.
201 Evidence that plant-like genes in *Chlamydia* species reflect an ancestral relationship between
202 Chlamydiaceae, cyanobacteria and the chloroplast. *Genome Res.* 2002;12:1-9.
- 203 [4] Huang J, Gogarten, P. Did an ancient chlamydial endosymbiosis facilitate the
204 establishment of primary plastids? *Genome Biol.* 2007;8:R99
- 205 [5] Becker B, Hoef-Emden K., Melkonian M. Chlamydial genes shed light on the evolution of
206 photoautotrophic eukaryotes. *BMC Evol. Biol.* 2008 8:203.
- 207 [6] Moustafa A., Reyes-Prieto A., Bhattacharya D. Chlamydiae has contributed at least 55
208 genes to Plantae with predominantly plastid functions. *PLoS ONE* 2008;3:e2205.
- 209 [7] Collingro A., Tischler P, Weinmaier T, Penz, T, Heinz E, Brunham RC et al. Unity in
210 variety: The pan-genome of the Chlamydiae. *Mol. Biol. Evol.* 2011;28:3253–70.
- 211 [8] Ball SG, Subtil A, Bhattacharya D, Moustafa A, Weber APM., Gehre L et al. Metabolic
212 effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? *Plant*
213 *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.

- 259 [25] Facchinelli F, Colleoni C, Ball SG and Weber AP. Chlamydia, cyanobiont, or host: who
260 was on top in the ménage à trois? Trends Plant Sci. 2013;18: 673–79.
- 261 [26] Greub G, Collyn F, Guy L, Roten CA. A genomic island present along the bacterial
262 chromosome of the Paréachlamydiaceae UWE25, an obligate amoebal endosymbiont encodes
263 a potentially functional F-like conjugative DNA transfer system. BMC Microbiol. 2004;4:48.
- 264 [27] Bertelli C, Greub G. Lateral gene exchanges shape the genomes of amoeba-resisting
265 microorganisms. Front Cell Infect. Microbiol. 2012;2:110.
- 266 [28] Nguyen BD, Valdivia RH. Virulence determinants in the obligate intracellular pathogen
267 *Chlamydia trachomatis* revealed by forward genetic approaches. Proc Natl Acad Sci U S A
268 2012;109:1263–68.

269

270

271 .

272

273

274

275

276

277