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When bacteria meet mitochondria: The strange case of the tick symbiont *Midichloria mitochondrii* †

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7 **When bacteria meet mitochondria: the strange case of the tick symbiont**
8 ***Midichloria mitochondrii***

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44 **Keywords**

45 intracellular bacteria, symbiosis, mitochondria, *Midichloria mitochondrii*, *Ixodes ricinus*

49 **Abstract**

50

51 Mitochondria are key eukaryotic organelles, that perform several essential functions. Not
52 surprisingly, many intracellular bacteria directly or indirectly target mitochondria, interfering
53 with innate immunity, energy production or apoptosis, to make the host cell a more
54 hospitable niche for bacterial replication. The alphaproteobacterium *Midichloria mitochondrii*
55 has taken this behavior to another level by physically colonizing mitochondria, as shown by
56 transmission electron micrographs of bacteria residing in the mitochondrial intermembrane
57 space. This unique localization provokes a number of questions around the mechanisms
58 allowing and reasons driving intramitochondrial tropism. We suggest possible scenarios that
59 could lead to this peculiar localization and hypothesize potential costs and benefits of
60 mitochondrial colonization for the bacterium and its host.

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63

64 **Introduction**

65

66 Symbiosis, the tight interaction of organisms of different species, is ubiquitous in nature,
67 across all branches of the tree of life (Brucker and Bordenstein, 2012). Intracellular symbioses
68 are among the most fascinating, as they clearly require constant and complex interaction
69 between the symbiont and its host (Wernegreen, 2012). All symbiotic relationships can have
70 important impact on both partners, but intracellular symbioses can play key roles in the
71 evolution of hosts and symbionts (Archibald, 2015; Brucker and Bordenstein, 2012). The
72 archetype of intracellular symbiosis is the evolution of mitochondria from a bacterial ancestor,
73 an event that was instrumental in the evolution of eukaryotic cells and multicellularity (Gray
74 et al., 2001; Roger et al., 2017).

75

76 Despite their prevalence and foundational role in shaping eukaryotic evolution, our
77 knowledge of intracellular symbiosis between bacteria and eukaryotic cells remains sparse.
78 Recent studies have started to unveil the molecular basis of some specific cases of symbiotic
79 bacteria-cell interactions (e.g (Douglas, 2014)), but advances are limited by the paucity of
80 genetically amenable systems and culturable symbionts. Arthropods are among the most
81 studied symbiotic hosts, due to their extreme species-richness, broad distribution and
82 importance as pathogen vectors, but also due to their strong propensity to harbour symbionts
83 (Gil and Latorre, 2019). Many intracellular mutualists of arthropods play fundamental
84 nutritional roles, supplementing the diets of hosts that feed on unbalanced substrates such as
85 phloem sap or blood (Moran et al., 2008). Ticks are obligate hematophagous arthropods and
86 appear to be a true “receptacle” for a wide variety of both pathogenic and non-pathogenic
87 bacteria (Duron et al., 2017; Kernif et al., 2016). A portion of these tick-borne bacteria are
88 maternally inherited, obligate intracellular bacteria and are thought to be nutritional
89 symbionts (Bonnet et al., 2017). Here, we will focus on *Candidatus* *Midichloria mitochondrii*
90 (hereafter *M. mitochondrii*), a maternally inherited symbiont that can colonize the oocyte
91 mitochondria of the tick *Ixodes ricinus*, one of the most widespread ticks in Europe.

92

93 **Mitochondria, an emerging target for intracellular bacteria**

94

95 Mitochondria originated when an ancestral proteobacterium entered the ancestor of
96 eukaryotic cells, a unique and crucial event for the evolution of eukaryotic life. Determining
97 the nature of the bacterial lineage giving rise to the ancestor of mitochondria is a highly
98 debated topic, and among other hypotheses (Martijn et al., 2018), one indicates intracellular
99 alphaproteobacteria of the *Rickettsiales* order as possible candidates, based on analyses
100 indicating them as the closest extant relatives of the organelle (Andersson et al., 1998; Ferla
101 et al., 2013; Fitzpatrick et al., 2006; Sasser et al., 2011; Wang and Wu, 2015; Williams et al.,
102 2007). Mitochondria are essential eukaryotic organelles responsible for vital functions
103 including energy production, biogenesis of iron-sulphur clusters, porphyrin and lipids,
104 anaplerotic reactions, amino acid metabolism and programmed cell death (intrinsic apoptosis
105 (Green et al., 2014).

106 Most of our knowledge on mitochondria stems from model organisms, essentially
107 yeast or mammalian cells, but most key mitochondrial functions and molecular machineries
108 appear largely conserved (Pernas and Scorrano, 2016; Westermann, 2010). Due to their
109 central role as metabolic and signalling hubs, mitochondria are a prime target for intracellular
110 bacteria, which can obtain nutrients from them or manipulate intrinsic apoptosis. Indeed,
111 several intracellular bacteria, associated with a range of eukaryotic hosts, can affect
112 mitochondria (reviewed in (Spier et al., 2019). The bacterial effects on mitochondria include
113 morphological and functional changes, as exemplified by the facultative intracellular
114 bacterium *Listeria monocytogenes*, which induces fragmentation of the mitochondrial
115 network while leading to a collapse of the mitochondrial membrane potential (Stavru et al.,
116 2011). Several bacteria target mitochondria through secreted proteins, that act directly on the
117 organelles. For example, *Helicobacter pylori* secretes the toxin VacA, which is inserted in the
118 mitochondrial inner membrane (Foo et al., 2010), while the *Listeria* toxin listeriolysin O inserts
119 into the plasma membrane, causing a calcium influx and thus indirectly leading to
120 mitochondrial fission (Stavru et al., 2011). Some bacteria even interact physically with
121 mitochondria, establishing close contacts. Stable contacts have been detected between
122 mitochondria and the avian pathogen *Chlamydia psittaci* , increasing concomitantly to
123 bacterial replication. This led the authors to suggest that mitochondria might provide ATP for
124 bacterial replication via the *C. psittaci* – encoded ATP-ADP transporter (Matsumoto et al.,
125 1991). In contrast, the human pathogen *Legionella pneumophila* was shown to establish
126 dynamic contacts with mitochondria that were lost at late timepoints of infection. In addition,
127 these contacts were also observed upon infection with avirulent *L. pneumophila* (deficient in
128 the type four secretion system), leading the authors to propose that transient mitochondrial
129 association with bacteria-containing phagosomes might represent a general, virulence-
130 independent host response to infection (Escoll et al., 2017). The recently described
131 alphaproteobacterium *Midichloria mitochondrii* (Sasser et al., 2006) provides one of the
132 most extreme examples of a physical interaction between an intracellular bacterium and
133 mitochondria.

134 135 ***Midichloria mitochondrii***

136
137 *Midichloria* is a genus of obligate intracellular bacteria belonging to the order Rickettsiales, a
138 group of alphaproteobacteria composed by intracellular bacteria (with one exception (Castelli
139 et al., 2019), which includes human pathogens (e.g many Rickettsia species (Parola et al.,

140 2013; Weinert et al., 2009), mutualists (Hosokawa et al., 2010; Landmann, 2019; Taylor et al.,
141 2005), reproductive parasites (Werren et al., 2008), and a number of less-studied lineages
142 with unknown interactions with their host (Castelli et al., 2016). *Midichloria* have been found
143 in several tick species, including *Ixodes ricinus* (Beninati et al., 2004), *Rhipicephalus bursa* (Epis
144 et al., 2008), *Amblyomma maculatum* (Budachetri et al., 2018), among others (Cafiso et al.,
145 2016; Epis et al., 2008). These bacteria are among the most abundant members of the
146 microbiome in multiple tick species (Di Lecce et al., 2018; Duron et al., 2017; Hernández-
147 Jarguín et al., 2018), suggesting a potential mutualistic relationship (Lo et al., 2006) and
148 mechanisms to avoid immune recognition by the host.

149 Among *Midichloria*, the most studied is *Candidatus* *Midichloria mitochondrii*
150 (hereafter *M. mitochondrii*). *M. mitochondrii* is present at high prevalence and abundance in
151 females and immature *I. ricinus* ticks (Lo et al., 2006; Sassera et al., 2008). In this species, most
152 characteristics of the host-symbiont relationship are those common to nutritional
153 mutualisms: high prevalence, widespread distribution, transovarial transmission, low
154 symbiont genetic diversity, and symbiont genome reduction while retaining B vitamin
155 synthesis genes (Al-Khafaji et al., 2019; Lo et al., 2006; Sassera et al., 2011). *Ixodes ricinus* is a
156 competent vector for a range of viral, parasitic and bacterial diseases of high medical impact
157 (Lejal et al., 2019; Moutailler et al., 2016; Reis et al., 2011). Diseases transmitted by *I. ricinus*
158 include the highly virulent tick-borne encephalitis caused by the homonymous virus,
159 rickettsioses, and Lyme disease caused by multiple *Borrelia* species (Boulanger et al., 2019).

160
161 Although it is largely unknown whether *M. mitochondrii* plays a role in pathogen
162 transmission, or is itself productively transmitted through blood feeding (Bazzocchi et al.,
163 2013; Cafiso et al., 2019; Mariconti et al., 2012a; Serra et al., 2019), one study found a positive
164 correlation between levels of the *Midichloria* symbiont of a different tick species (*Amblyomma*
165 *maculatum*) and the presence of the pathogen *Rickettsia parkeri* (Budachetri et al., 2018).
166 However, what makes the interaction between *M. mitochondrii* and its host unique is the
167 astonishing observation that *M. mitochondrii* is not only present in the cytoplasm, but a
168 portion of its population colonises the intermembrane space of mitochondria (Fig 1) and
169 appears to lead to mitochondrial matrix condensation (Sacchi et al., 2004). Such peculiar
170 localization, never reported in any other animal cell, raises major questions: (1) how and why
171 does *M. mitochondrii* get into mitochondria and how do mitochondria respond? (2) What is
172 the role of *M. mitochondrii* in the cell, and does this benefit the host? (3) why does invasion of
173 mitochondria not lead to cell death? In the following paragraphs, we will address these
174 questions by summarizing the available data and putting forward hypotheses and
175 experimental approaches to test them.

176
177

178 **Mitochondria as an unexpected ecological niche**

179
180 The origin and evolution of the intramitochondrial tropism (IMT) displayed by *M.*
181 *mitochondrii* are currently unknown. Another *Midichloria* symbiont was reported to be
182 capable of IMT, in *R. bursa* ticks (Epis et al., 2008). Although *Midichloria* are the only bacteria
183 capable of intramitochondrial tropism in a metazoan organism, other bacteria have been
184 reported as intramitochondrial or very closely associated to mitochondria in protists.
185 Examples for IMT/mitochondrial association are found in bacteria that colonize the protists
186 *Halteria geleiana* (Yamataka and Hayashi, 1970), *Urotricha ovata* Kahl (de Puytorac and Grain,

187 1972) and more recently two *Diplomonas* species, whose bacterial endosymbionts were the
188 founding members of the new genus “Candidatus Cytomitobacter” (Tashyreva et al., 2018).
189 The most parsimonious explanation to multiple phylogenetically unrelated bacteria invading
190 or interacting very closely with mitochondria is the parallel independent evolution of these
191 interactions, while a potential alternative would be an event of horizontal transfer of genes
192 that mediate IMT. Future studies will uncover whether these bacteria employ common
193 strategies to colonize mitochondria. In any case, the localization of bacteria in the
194 mitochondrial IMS raises the question of the physiological reasons and advantages this
195 localization might have. Here, we put forward hypotheses, focusing on the *M. mitochondrii* -
196 *I. ricinus* system and drawn from our knowledge of mitochondrial IMS biology in model
197 systems. In addition, our own unpublished transcriptome data of *M. mitochondrii* and/or *I.*
198 *ricinus* also support to a certain degree the existence of signaling and metabolic pathways, as
199 well as of homologs of the proteins that are mentioned in the following paragraphs.

200

201 *The IMS as a protected space: avoiding cytosolic host defence mechanisms and modulating*
202 *apoptosis*

203 One possible explanation for the localization of *M. mitochondrii* in the IMS is that it potentially
204 confers the ability to actively suppress dangerous pathways originating from mitochondria. In
205 addition, IMT can offer protection by escaping cytosolic defence machineries based on innate
206 immune recognition or xenophagy. It might also give the bacterium the capacity to modulate
207 the host mitochondrion so as to avoid apoptosis induction (Tiwari et al., 2015), e.g by
208 suppressing cytochrome c release and preventing cristae remodeling (Polčić et al., 2017) . This
209 could allow the suppression of mitophagy and Ca²⁺ signaling, which eventually might result in
210 Ca²⁺ overload in mitochondria, followed by permeability transition and cell death (Orrenius et
211 al., 2015). As, in contrast to other tissues, mitochondria are usually quite dormant in oocytes
212 (Tiwari et al., 2015), they could represent a protected space without much reactive oxygen
213 species production taking place. In addition, intramitochondrial *M. mitochondrii* might also
214 modulate the immune response by preventing the release of mitochondrial DNA that is known
215 to induce the innate immune response (Nakahira et al., 2011).

216

217 *The IMS: a microreactor?*

218 In addition to providing protection, the physical and chemical conditions of the IMS might
219 constitute a favourable environment for *M. mitochondrii*. For example, the proximity to the
220 mitochondrial respiratory chain might under certain conditions provide more suitable
221 conditions for bacterial growth. For example, it has been reported in mammalian cells that
222 inside mitochondria, close to the respiratory chain, the temperature can be significantly above
223 that of the cytosol (Chrétien et al., 2018). Whether this temperature difference is also found
224 in tick oocyte mitochondria remains an open question, and while the overall GC content of
225 the *M. mitochondrii* (37%) does not seem to be specifically tailored for high temperatures, this
226 needs to be confirmed by comparative analysis of its 16S rRNA GC content with that of non-
227 mitochondrial *Midichloriae* (Wang et al., 2006). The IMS might also serve as a hub to exchange
228 metabolites between host and bacterium. Indeed, ATP concentrations in the IMS are
229 comparatively high and might be used directly by the bacterium. The existence of a putative
230 ATP-ADP translocase in the bacterium can support this hypothesis, although *M. mitochondrii*
231 appears to have retained the ability to synthesize ATP (Sassera et al., 2011). In contrast,
232 several other obligate intracellular bacteria, which display highly reduced genomes, have lost

233 the ability to synthesize nucleotides and therefore rely on ATP import from the host (energy
234 parasitism, shown e.g. by Chlamydiae (Trentmann et al., 2007), *Lawsonia intracellularis*
235 (Schmitz-Esser et al., 2008) and *Rickettsia prowazekii* (Winkler and Daugherty, 1984). In
236 addition, the IMM and the IMS are important sites for cellular lipid metabolism, and the
237 bacterium might benefit from direct lipid supply. Moreover, positioning in the IMS might allow
238 the bacterium to influence AMP signaling (i.e. potentially to increase mitochondrial
239 biogenesis), that is in part driven by the IMS-localized adenylate kinase 2 enzyme (Dzeja and
240 Terzic, 2009).

241

242 *The IMS as shuttle for transfer to the next generation*

243 Transovarian transmission to the next generation is clearly the reason why many intracellular
244 symbionts reside in female reproductive organs, however in the specific case of *M.*
245 *mitochondrii* an additional factor contributing to its accumulation in oocytes could be the high
246 number of mitochondria found in oocytes (up to 100 times more than in an epithelial cell,
247 (Monnot et al., 2013). A last hypothesis on the reason for the IM localization is that
248 mitochondria in general might serve as shuttles that allow *M. mitochondrii* to efficiently pass
249 from one generation to the next, as the bacterium would then hijack the obligate maternal
250 inheritance of mitochondria to ensure its own transmission. After fertilization, mitochondria
251 initially do not have to divide but are distributed among cells of the early embryo. This might
252 therefore also form a way for the bacterium to efficiently spread to multiple tissues in the
253 next generation.

254

255

256 **Scenarios for mitochondrial colonization by *M. mitochondrii***

257

258 Electron micrographs (EM) support inference of various possible scenarios for the life cycle of
259 *M. mitochondrii*, and are complimented by similar work used to understand now well-
260 characterized intracellular bacteria, including pathogens like *Listeria monocytogenes* (Tilney
261 and Portnoy, 1989) or symbionts like *Wolbachia*, a member of the order *Rickettsiales* like *M.*
262 *mitochondrii* (Avakyan and Popov, 1984). *Wolbachia* accumulate inside a host-derived vacuole
263 (e.g. (Fischer et al., 2014)). It appears that cytosolic *M. mitochondrii* do not (see Fig. 1, (Sacchi
264 et al., 2004)). Although it is also possible that *M. mitochondrii* might temporarily reside in a
265 vacuole, which might allow the bacterium to escape into the cytosol or fuse with the
266 mitochondrial outer membrane, resulting in its observed intermembrane space (IMS)
267 localization (Fig 2). Alternatively, cytosolic bacteria could directly enter mitochondria, possibly
268 hijacking mitochondrial fusion. Unfortunately, EM precludes the analysis of dynamic events,
269 and therefore validation of either model critically depends on the ability to perform functional
270 tests, and therefore on the ability to manipulate *M. mitochondrii* in vitro, which is yet to be
271 developed.

272 Once inside mitochondria, *M. mitochondrii* appears to manipulate the contacts that
273 normally keep the inner and outer membrane at a constant distance of about 20nm,
274 sometimes leading to substantial separation of the inner and outer membrane (Fig 1C and
275 (Sacchi et al., 2004)). 3D analyses and quantitative light microscopy will be required to address
276 whether *M. mitochondrii* modulates mitochondrial dynamics in addition to affecting
277 mitochondrial ultrastructure. Such approaches will also be instrumental to determine the
278 proportion of cytoplasmic versus mitochondrial *M. mitochondrii*. Strikingly, several examples

279 of mitochondria colonized by more than one bacterium have been reported, suggesting either
280 multiple rounds of invasion or bacterial division within the IMS. The presence of multiple
281 bacteria appears correlated with altered mitochondrial ultrastructure up to drastic
282 mitochondrial matrix condensation (Fig 1C and (Sacchi et al., 2004)) and it is reasonable to
283 expect these alterations to affect mitochondrial function. Surprisingly, these mitochondrial
284 changes do not correlate with an apparent induction of cell death. We hypothesize that the
285 bacterium actively counters apoptosis, similar to other obligate intracellular bacteria (Rudel
286 et al., 2010) .

287 While several tick species harbour bacteria belonging to the *Midichloria* genus,
288 currently the only complete genome available for this genus is that of *Midichloria*
289 *mitochondrii*, a putative symbiont of the hard tick *I. ricinus*. The reduced genome of *M.*
290 *mitochondrii* (1.2Mb) is indeed typical of rickettsial endosymbionts (Sassera et al., 2011).
291 Comparative genomics between *M. mitochondrii* and closely related species that do not
292 display IMT (e.g. *Midichloria* from *Ixodes holocyclus* (Beninati et al., 2009; Castelli et al., 2016)
293 will therefore be instrumental to uncover whether specific genes are associated with the
294 unique ability of the symbiont to invade mitochondria. However, the *M. mitochondrii* genome
295 already unveiled an intriguing and surprising finding: the presence of flagellar genes, including
296 components of the motor and export apparatus (Sassera et al., 2011). Even though the
297 flagellum of *M. mitochondrii* has never been visualized, hook and filament proteins have been
298 detected experimentally (Mariconti et al., 2012b). A typical PAMP (pathogen associated
299 molecular pattern), the flagellum can be recognized by Toll-like receptor family proteins,
300 which are highly conserved in eukaryotes (reviewed in (Leulier and Lemaitre, 2008).
301 Consequently, flagellar genes have been lost or are not expressed in several intracellular
302 bacteria or endosymbionts, including most members of the *Rickettsiales* (Toft and Fares,
303 2008). Given the immunogenicity and significant energetic cost associated with flagellar
304 synthesis (Toft and Fares, 2008), it is likely that *M. mitochondrii* flagella play an important role
305 in the bacterial life cycle. Such role may be difficult to imagine, if we only think about the
306 motility function of this structure, but flagella have recently been shown to have also other
307 functions, in particular bacterial adhesion and invasion (Haiko and Westerlund-Wikström,
308 2013) and symbiotic interactions (Shimoyama et al., 2009). Alternatively, the flagellum of *M.*
309 *mitochondrii* might actually represent a type three secretion system that could mediate
310 adhesion (e.g. to mitochondria) or contribute to host cell manipulation through the secretion
311 of bacterial proteins, similar to other intracellular bacteria (Ashida et al., 2007; Mueller et al.,
312 2014; Waterman and Holden, 2003; Zhang et al., 2012). The flagellum might thus be the key
313 to the interaction between the symbiont and the organelle.

314

315

316 **Possible implications of mitochondrial colonization by *M. mitochondrii***

317

318 Genomic information allows to formulate hypotheses concerning the impact of *M.*
319 *mitochondrii* on its host physiology, which await experimental testing. Ecological studies
320 detected high prevalence and low genetic variability of *M. mitochondrii* (Al-Khafaji et al., 2019;
321 Lo et al., 2006), suggesting it could be beneficial to *I. ricinus*, however this is not proven and its
322 specific impact on the host cell or host physiology are unclear as the mechanisms of
323 interaction with mitochondria. A current hypothesis is that the bacterium improves host

324 cellular respiration during blood-feeding, during which ixodid ticks are thought to enter a
325 hypoxic state. Indeed, the *M. mitochondrii* genome has been found to encode a complex IV
326 cbb3 cytochrome oxidase that allows oxidative phosphorylation at low oxygen tension
327 (Sassera et al., 2011) . This intriguing finding, together with the presence of a bacterially
328 encoded ATP transporter and an observed increase in the bacterial population in response to
329 low oxygen tension and high metabolic needs in the host has led to propose that *M.*
330 *mitochondrii* could assist the tick cellular respiration during this specific stage of its life cycle
331 (i.e., feeding; (Sassera et al., 2008)). In addition, *M. mitochondrii* might also provide *I. ricinus*
332 with metabolites or vitamins, as recently proven for another intracellular symbiont of the
333 *Francisella* genus, which provides B vitamins to its host, the African soft tick *Ornithodoros*
334 *moubata* (Duron et al., 2018). *I. ricinus* apparently lacks all enzymes for porphyrin (haem)
335 biosynthesis except for the last three enzymes (Perner et al., 2016). Curiously, the first enzyme
336 encoded in the *I. ricinus* genome is coproporphyrinogen III oxidase (CPOX). In higher
337 eukaryotes, this enzyme is localized in the IMS and it is tempting to speculate that *M.*
338 *mitochondrii* provides precursors for this enzyme. *M. mitochondrii* might also play a role in
339 the regulation of mitochondrial reactive oxygen species (ROS) production. Functionally, ROS
340 detoxification by antioxidants has been shown to play a role in maintaining fertility/fecundity
341 in *Drosophila* (Parkes et al., 1998) and mosquitoes (DeJong et al., 2007), and maternally
342 inherited arthropod symbionts such as *Wolbachia* have been shown to regulate ROS levels
343 (Zug and Hammerstein, 2015). In ticks, an example of ROS manipulation by bacteria is given
344 by the pathogen *A. phagocytophilum*, which has recently been shown to inhibit ROS
345 production and apoptosis to preserve its replicative niche (Alberdi et al., 2019).

346

347

348 ***Midichloria mitochondrii* in non-ovarian tissues**

349

350 Recently *M. mitochondrii* has been detected in other tick organs, including salivary glands
351 (Mariconti et al., 2012a), malpighian tubules, tracheae and guts (Olivieri et al., 2019). This
352 finding led to hypothesize different functions for the bacterial populations residing in different
353 organs. *M. mitochondrii* subpopulations could not just be supplying essential nutrients to the
354 host but also enhance the reproductive fitness, helping in anti-oxidative defence, energy
355 production, water balance and homeostasis. More 'selfish' reasons for the multiple
356 localization include ensuring both vertical transmission (*M. mitochondrii* in oocytes) and
357 horizontal transmission to the vertebrate host during the blood meal (*M. mitochondrii* in
358 salivary glands), as well as optimization of energy parasitism. While giving some answers,
359 these findings also open a novel question: is *M. mitochondrii* localized also in mitochondria in
360 tissues other than oocytes? The low bacterial load makes electron microscopy a frustrating
361 endeavor, and correlative approaches will be required to definitively answer this question.

362

363

364 **Outlook**

365

366 Mitochondria are highly conserved across almost all eukaryotes and are intensively studied
367 due to their role in aging, cancer and in a variety of pathologies (Nunnari and Suomalainen,
368 2012). Despite this, many aspects of mitochondrial biology are unknown and 30% of
369 mitochondrial proteins have an unknown function. Studying the crosstalk between *M.*
370 *mitochondrii* and mitochondria might provide novel insight into mitochondrial biology, much

371 like the study of *Listeria monocytogenes* has yielded invaluable insight into fundamental cell
372 biological processes of its eukaryotic host cells (Cossart, 2011).

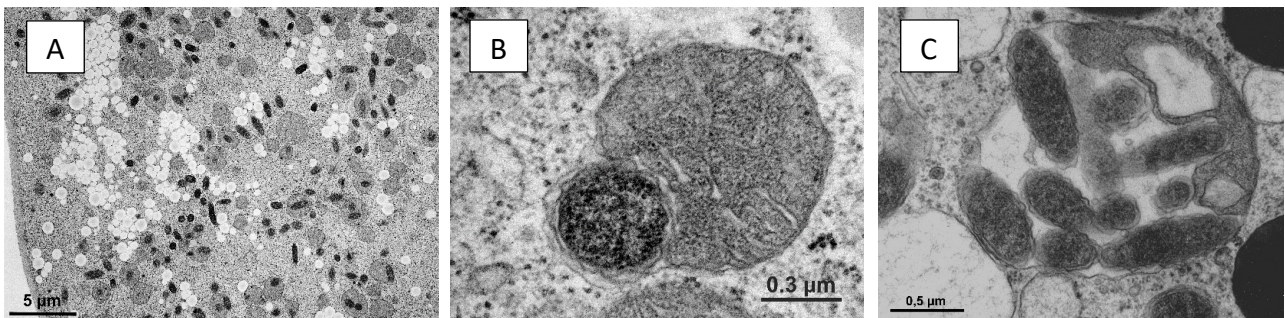
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387

390 **Figure 1**391 **Transmission electron micrograph of *I. ricinus* oocytes colonized by *M. mitochondrii*.**392 A Low magnification image displaying cytosolic and mitochondrial *M. mitochondrii*. B

393 Mitochondrial intermembrane space localization of a single bacterium. C Multiple bacteria

394 colonize an inflated intermembrane space, while the mitochondrial matrix is highly

395 condensed. Courtesy of Emanuela Clementi and Luciano Sacchi.

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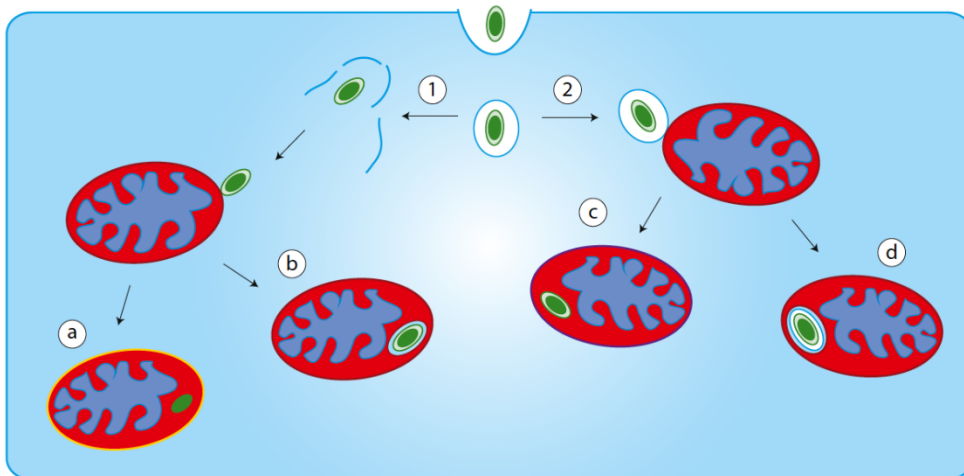
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412 **Figure 2**413 **Different scenarios for the life cycle of *M. mitochondrii*.** To date no electron micrographs have414 shown *M. mitochondrii* entering cells, this could occur upon colonization of other tissues or

415 during horizontal transmission. After cell invasion, the bacterium (green) either escapes from

416 the phagosome (1) or remains phagosome-bound (2). After escape, different scenarios could

417 result in colonization of the mitochondrial intermembrane space: (a) of the bacterium and its

418 outer membrane fuses with the mitochondrial outer membrane. (b) The mitochondrion

419 “phagocytoses” the cytosolic bacterium. (c) The phagosomal membrane enclosing the

420 bacterium fuses with the mitochondrial outer membrane. (d) The mitochondrion

421 “phagocytoses” the phagosome containing the bacterium, which would result in two host-

422 derived membranes in addition to the bacterial membranes.

423

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