Title

Asymmetric cell-cell adhesion can trigger mitochondriogenesis: A hypothesis

Author

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Abstract

Without exception, modern hypotheses explaining the origin of eukaryotes assume evolution from two (or more) distinct prokaryotes. Almost all prokaryotic cell membranes possess a chemiosmosis system, whereas eukaryotic cell membranes have lost that ability. Nevertheless, no hypothesis describes how chemiosmosis might have been lost from one symbiont. This work proposes a novel hypothesis for the origin of eukaryotes, invoking the adhesion of two chemiosmosis-bearing membranes to promote eukaryogenesis. The intermembrane space between the adhered cell membranes accumulates protons, and the enhanced proton gradient across the membranes accelerates ATP synthesis in both symbionts. Next, the smaller symbiont is engulfed by the larger symbiont to expand the intermembrane space, and then the engulfed symbiont starts to supply a large quantity of ATP to the surrounding host to improve the evolutionary fitness of the whole symbiotic union. Finally, the host cell membrane acquires pluripotent membrane excitation in exchange for its own chemiosmosis system.

Text

The origin of eukaryotes is one of the greatest enigmas in evolutionary biology [1-6]. In 1905, the Russian botanist Constantin Mereschkowsky articulated the idea of symbiogenesis, proposing that two distinct organisms fused and resulted in a new species [7,8]. Several decades later, a seminal article by Lynn Sagan (later Margulis) in 1967 [9] introduced a lively debate about the origin of eukaryotes, and Margulis later proposed that many intracellular structures unique to eukaryotes

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have distinct endosymbiotic origins, known as the 'serial endosymbiosis theory' [10].

From then until the early 1990s, the standard hypotheses assumed, often tacitly, a strict aerobic ancestor of mitochondria [10-13]. These models claimed that the anaerobic host gained an energetic benefit from the aerobic symbiont. However, because the host could never obtain such a benefit unless it acquired a mechanism to exploit ATP synthesized by the symbiont simultaneously with symbiosis, these models were not at all realistic.

In 1998, William Martin and Miklós Müller proposed the hydrogen hypothesis, which explained the selective advantage of the development of symbiosis [14]. They proposed that the first symbiosis had been an anaerobic syntrophy mediated by hydrogen. According to the hydrogen hypothesis, an obligate anaerobic autotrophic microorganism and a facultative anaerobic heterotrophic microorganism approached each other for mutual benefit by anaerobic syntrophy, and over the course of time, the former engulfed the latter; finally, the engulfed microorganism evolved into an energy-transducing organelle such as a mitochondrion or hydrogenosome.

Although it is highly plausible that the first symbiotic relationship would have been anaerobic syntrophy, there is no necessity for one of the symbionts to be a facultative anaerobic heterotroph; instead, being an obligate anaerobic heterotroph is sufficient to establish this anaerobic syntrophy. Considering that the environment where the first syntrophy occurred is absolutely anaerobic, there is no selective advantage for the symbiont to conserve aerobic respiration. Additionally, even the hydrogen hypothesis does not explicitly explain what kind of selective pressure promotes the acquisition of an ATP translocator after engulfment of the symbiont. Acquisition of an ATP translocator is one of the most crucial steps in the process of evolving energy-transducing organelles [15,16]. Meanwhile, the standard hypotheses promoting the anaerobic encounter have not rationally explained why aerobic respiration would have been preserved and how the symbionts acquired ATP translocators.

Almost all of the modern hypotheses assume that an obligate anaerobic host eventually acquired aerobic respiration through endosymbiosis [4,6]. Since oxygen is highly toxic to obligate anaerobic

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organisms, the first symbiosis must take place in a strictly anaerobic environment. All of the models supposing that the first symbiosis occurs in an anaerobic environment and does not require aerobic respiration by the symbiont implicitly assume that the symbiont has aerobic respiration by chance and that the aerobic respiration simply happened to be conserved until the chance to utilize it again. Since mitochondria have their own genomes, it is possible to hypothesize their ancestors using molecular phylogenetic analyses. In recent decades, much evidence suggesting that mitochondria originated from an endosymbiosis between an archaeal host [17,18] and a prokaryote closely related to α-proteobacteria as a symbiont [19-21] has been widely accepted. In 2015, the novel archaeal phylum Lokiarchaeota was identified using a metagenomic analysis of deep marine sediment; Lokiarchaeota is attracting attention as a promising candidate for the archaeal host of the first eukaryote [22]. More recently, the Asgard superphylum, which contains Lokiarchaeota and three closely related archaeal groups, was proposed [23]. Although comparative genomic evidence has indicated that Lokiarchaeon is hydrogen-dependent and strictly anaerobic [24], it is unclear whether the ancestors of Lokiarchaeon were also strictly anaerobic. It is worth emphasizing that Lokiarchaeota and other Asgard archaea probably possess complex and energy-demanding cellular structures, and the energy productivity of hydrogen-dependent anaerobic respiration is generally low.

Chemiosmosis is particularly universal

The last universal common ancestor (LUCA) definitely possessed a chemiosmosis mechanism [25-27]. The chemiosmotic proton circuit is still widely distributed; almost all obligate anaerobes, including methanobacteria, as well as aerobic organisms, synthesize ATP via chemiosmosis. Prokaryotes synthesize ATP using very diverse metabolic pathways. There are remarkably varied combinations of redox couples (pair of electron donor and acceptor) [28], whereas ATP is exclusively synthesized by ATP synthase in the chemiosmotic energy-transducing circuit that uses the electrochemical gradient across the cell membrane generated by redox reactions. Even obligatory fermentative

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bacteria retain ATP synthase, and no species that has completely lost ATP synthase has been found among obligate fermenters [29-31].

Among all of the 6,003 complete genomes of eubacteria (GenBank, 6 Dec 2016), only thirty genomes have completely lost ATP synthase components. All of them are symbionts with reduced genomes of less than 1 Mb (average 0.44 Mb) and fewer than one thousand genes (average 404 genes), such as *Phytoplasma* [32], *Buchnera* [33], and *Tremblaya* [34].

Among all 248 complete genomes of archaea (GenBank, 6 Dec 2016), there are only two genomes lacking ATP synthase. Both belong to symbionts with extremely small genomes that have lost many biosynthetic pathways (*Woesearchaeota*, 0.8 MB [35]; ectosymbiotic *Nanoarchaeota*, 0.6 MB [36]).

Chemiosmosis-bearing microorganisms create a proton gradient across the cell membrane through a proton drainage mechanism (Fig. 1a). ATP synthase produces ATP by using proton motive force (PMF), which is derived from a chemical gradient and a voltage gradient across the cell membrane. Almost all eubacteria and archaea have distinct F_1F_o and A_1A_o ATP synthases [37,38]. Eukaryotic mitochondria synthesize ATP by using the F_1F_o ATP synthase based on the PMF across the inner mitochondrial membrane.

The diffusion of protons pumped out from the mitochondrial matrix is partially blocked by the outer mitochondrial membrane and is finally strictly regulated by the cell membrane [39] (Fig. 1c). The difference in pH between cytoplasm and mitochondrial intermembrane space is measured as 0.2 – 0.7 [40,41]. In contrast, in the case of prokaryotes, the outside of the chemiosmosis-bearing cell membrane is the external environment, which is far larger than the cytoplasm or the mitochondrial intermembrane space, and the environmental pH is not always stable. Protons pumped out by prokaryotes quickly diffuse into the external environment, never accumulate around the cells, and do not increase the PMF. In this respect, the prokaryotic energy-transducing membrane is at a disadvantage compared to the inner mitochondrial membrane. It is known that protons accumulate in the periplasmic space between the inner and the outer membranes and that PMF increases in some microorganisms with cell walls [42].

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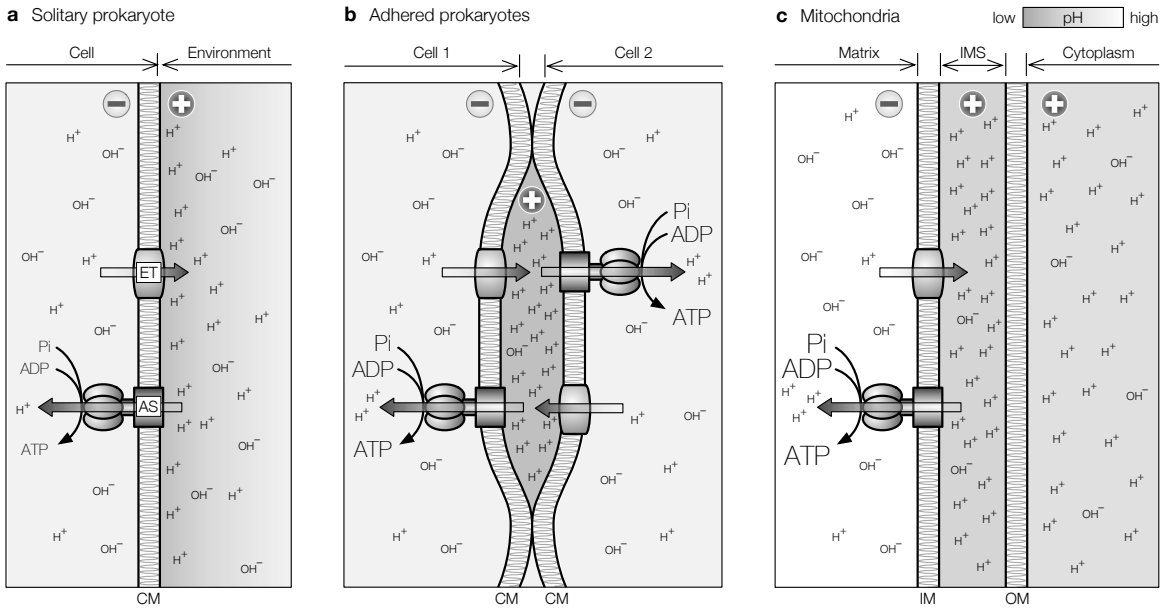
105 In the world before eukaryogenesis, a prokaryote that had acquired traits overcoming this
106 disadvantage might have been able to improve its evolutionary fitness.
107



108

109 **Figure 1 Closed intermembrane space improves the efficacy of chemiosmosis. a,** Chemiosmosis
110 of solitary prokaryote. **b**, Chemiosmosis around adhered cell membranes. **c**, Chemiosmosis of
111 mitochondria. ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, inorganic phosphate;
112 CM, cell membrane; IM, inner mitochondrial membrane; OM, outer mitochondrial membrane;
113 IMS, intermembrane space; AS, ATP synthase; ET, electron transport mechanism. Areas with high
114 and low pH are shaded dark and light grey, respectively. Circled '-' and '+' indicate relative
115 potential derived from an electrochemical gradient across a membrane.
116 (End of legend)
117

118 **Membranes adhesion generates more energy**
119 Since almost all prokaryotes have the chemiosmosis-bearing type of cell membrane, the two
120 microorganisms that became the ancestor of eukaryotes also almost undoubtedly had such energy-
121 transducing membranes.
122 Let us briefly explore a hypothetical symbiosis between chemiosmosis-bearing microorganisms

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(Fig. 2a). They would encounter each other in an environment that was comfortable for one or both of them. If two cells closely approach each other and their cell membranes form patches of adhesion, bubbly and semi-closed 'intermembrane spaces' will be generated. Such quasi-intermembrane spaces restrict the diffusion of protons, consequently increasing the PMF (Fig. 1b, Fig. 2b).

Derick Brown and colleagues have theoretically and experimentally shown that the energy efficiency of bacteria improves when they adhere to a solid surface, narrowing the extracellular space [43,44]. The highly permeable outer mitochondrial membrane still produces a pH difference between the cytoplasm and the mitochondrial intermembrane space. Therefore, even if the intermembrane adhesion bordering the quasi-intermembrane space is leaky, the proton equilibrium concentration should increase as the diffusion decelerates. As a result of PMF enhancement due to the formation of quasi-intermembrane space, the rate of ATP synthesis increases in both adherent cells. In addition, the pH of the quasi-intermembrane space should be more stable than that of the external environment, which can be perturbed. These will improve the evolutionary fitness enough to stabilize the formation of the quasi-intermembrane space, finally allowing the quasi-intermembrane space to develop into a true intermembrane space.

Each adhering microorganism gains a selective advantage, regardless of the type of redox couple used in their chemiosmotic mechanisms. It does not even matter if both microorganisms utilize aerobic respiration.

Since energy efficiency improves as the ratio of the area of the intermembrane space to the area of whole cell membrane increases, the intermembrane space will evolve to become wider and wider. Here, if the volumes of the two cells are not equal, the smaller cell (hereafter called the 'S cell') will be engulfed in the larger cell (hereafter called the 'L cell') with expansion of the intermembrane space (Fig. 2c). The S cell can incorporate the greater part of its cell membrane area into the intermembrane space by getting stuck in the surface of the L cell. In contrast, the outside of the L

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cell membrane, which is in contact with the external environment, cannot be incorporated into the intermembrane space, so the L cell cannot improve its energy productivity as much as the S cell. Therefore, unless the two cells are equal in size, the selective advantage obtained from the intermembrane space is inevitably asymmetric. The membrane adhesion itself is symmetrically mutual; thus, it does not allocate the roles of host and symbiont. To assign the roles, some fixed asymmetry is required. Not only the cell size but also other relationships such as syntrophy may cause the asymmetry, and these determinants may act additively, synergistically, or antagonistically.

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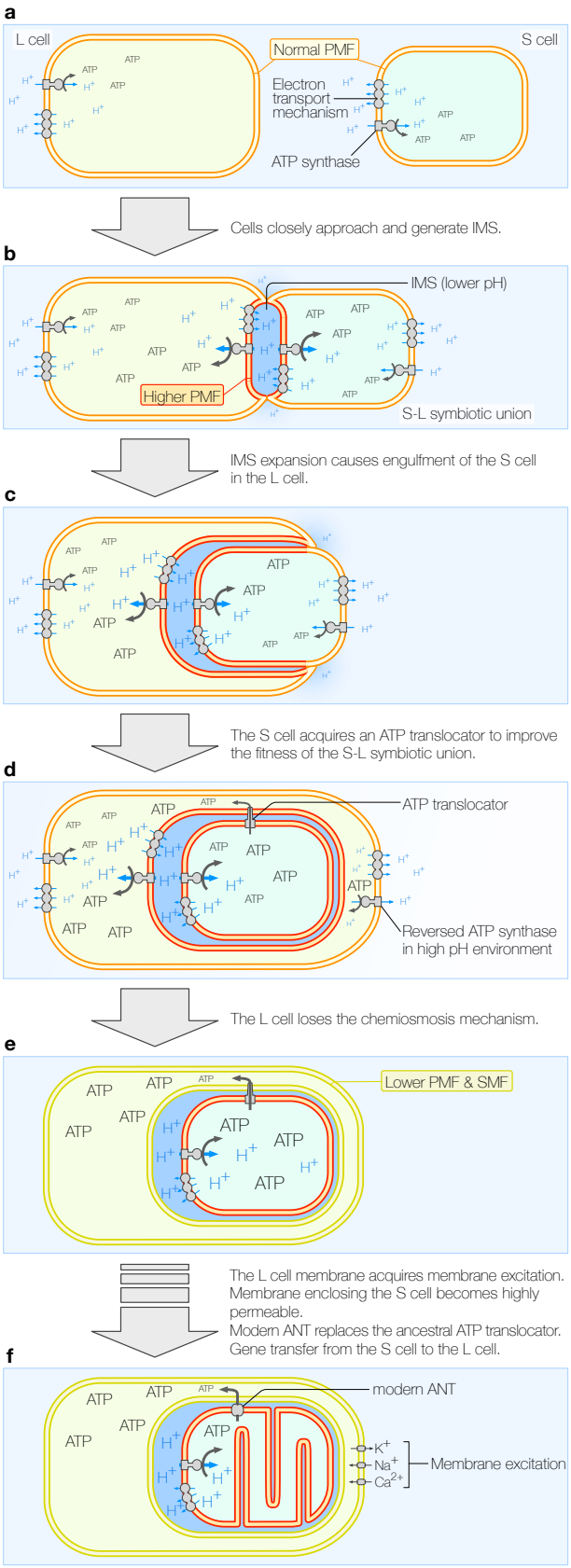

Figure 2 Hypothetical model to derive the ancestral state of eukaryotic energy transduction.

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(Legend of figure 2, continued)

a, Two chemiosmotic cells, future symbionts. **b**, Generation of intermembrane space by cell adhesion. **c**, Engulfment of one symbiont in another. **d**, Acquirement of ATP translocator. **e**, Loss of chemiosmotic mechanism from one symbiont. **f**, Eukaryote with mitochondria and membrane excitation. PMF, proton motive force; IMS intermembrane space; SMF, sodium motive force; ANT, adenine nucleotide translocator. The cytoplasm of the larger cell (L cell) and the smaller cell (S cell) are coloured in two slightly different shades of light green. Cell membranes are drawn as double lines, membranes with normal PMF are shaded orange, membranes with higher PMF are shaded red, and membranes with lower PMF are shaded dark yellow. The extracellular environments are coloured pale blue. Areas with lower pH values are shaded in a darker shade of blue, and areas with higher pH values are highlighted in a paler shade of blue. Blue gradients represent proton leakage from incompletely adhered membranes.

(End of legend)

Not exploitation but supply of ATP

When the S cell is almost completely engulfed in the L cell, the S-L symbiotic union encounters the external environment primarily via the membrane of the L cell. If the membrane of the L cell does not provide sufficient protection against natural selection, both the S-L symbiotic union and the S cell will be destroyed. The S cell has a high capacity for ATP production, even though it has little contact with the environment. In contrast, the L cell has limited energy productivity, whereas it must confront most of the selective pressures from the environment.

One of the ways to overcome this paradoxical situation is by the S cell supplying excess ATP to the L cell. Transfer of ATP from the S cell to the L cell contributes to improving the fitness of not only the L cell but also the S-L symbiotic union and the S cell itself. Therefore, it should be advantageous for the S cell to acquire an ATP translocator molecule. The ATP translocator improves the fitness of the whole S-L symbiotic union in which the S cell increases its ATP

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187 production rate as much as possible and supplies excess ATP to the L cell (Fig. 2d).
188 Conversely, even if the L cell acquires an ATP translocator to deprive the S cell of ATP, both
189 should gain in fitness. However, if the S cell is the proto-mitochondrion and the L cell is the proto-
190 cytoplasm, the L cell is likely to be an archaeon. To date, no ATP translocator encoded by archaea
191 has been identified, except for partial fragments from a marine sediment metagenome [45]. In
192 contrast, many eubacterial species, even α-proteobacteria, which are closely related to
193 mitochondria, contain an adenine nucleotide translocator (ANT) protein [16]. Therefore, it is
194 reasonable to assume that an S cell derived from eubacteria could have acquired an ATP
195 translocator to supply ATP to an L cell derived from archaea.
196 The present mitochondrial ANT is considered to have no phylogenetic relationship with the
197 prokaryotic ANT [16]. This can be explained by the ancestor molecule of the present mitochondrial
198 ANT having evolved later, from the eukaryotic transporter proteins, and replacing the ancestral
199 ATP translocator. I assume that the ATP translocator encoded by the S cell would not be a direct
200 ancestor of the current mitochondrial ANT.
201 Unlike the highly permeable mitochondrial outer membrane, the L cell membrane, which is itself an
202 energy-transducing membrane, must not have been permeable to ATP. Thus, it is necessary to
203 transport ATP across not only the S cell membrane but also the intermembrane space and the L cell
204 membrane. Actually, many eubacteria possess proteins that meet this requirement. Some
205 endospore-forming bacteria such as *Bacillus subtilis* possess some channel proteins that enable the
206 transport of metabolites from the mother cell to the forespore [46-48]. The forespore is encased within
207 a double membrane and the intermembrane space, and the channel complex bridges the mother cell
208 and the forespore across that distance. The channel proteins show similarity with the channel
209 proteins in type III secretion systems [46,47]. Type III secretion systems are widely distributed in
210 eubacteria, including α-proteobacteria, and they form the core of the flagellum and the
211 injectisome [49]. This type of channel protein may be a candidate for the first ATP transporter. At the
212 very least, it is plausible as a transporter of ATP across double membranes and is derived from

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proteins widely distributed in eubacteria.

Liberated membrane from chemiosmosis

If the amount of ATP supplied by the engulfed S cell greatly exceeds that produced by the L cell itself, the L cell does not necessarily need to conserve its own energy-transducing system. Under such conditions, accidental mutations in the components of the energy-transducing system of an L cell in an S-L symbiotic union do not significantly impair the fitness of the symbiotic union, which can survive as well as intact siblings (Fig. 2e). In such a chemiosmosis-deficient L cell, the membrane potential becomes less polarized, and not only the PMF but also the sodium motive force decreases; consequently, passive sodium transport into the cytoplasm decreases. As sodium influx decreases, the demand for active sodium transport out of the cell in order to maintain cytoplasmic sodium homeostasis also decreases, thereby lessening ATP consumption. Thus, the L cell can lose its own chemiosmosis system if it satisfies its energy demands with only ATP supplied from the engulfed S cell, thereby slightly improving energy efficiency and fitness as well.

I propose another reasonable explanation for how the L cell lost its own energy-transducing system. If an S-L symbiotic union, in which the S cell is completely encased in the L cell, migrated into a higher pH environment, the cytoplasmic pH value of the L cell could have been lower than the pH of the external environment. Even in this case, as long as the low pH of the intermembrane space was preserved, both the L cell and the S cell could have sustained energy production by chemiosmosis along the intermembrane space. However, in this situation, ATP synthase, which is located on the outside membrane and is in contact with the high pH environment, hydrolyses ATP to generate the PMF instead of dissipating it (Fig. 2d). Under these conditions, the evolutionarily optimal strategy for the S-L symbiotic union would be for the L cell to abandon ATP synthase, suppress the ATP waste, and depend entirely on the S cell for ATP.

Once the L cell loses its own chemiosmosis system, it can no longer dissolve the symbiosis with the S cell. If the L cell were to lose the encapsulated S cells, it would quickly fall into severe energy

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starvation. Therefore, the L cell should evolve a robust mechanism to retain the engulfed S cells. For example, if some essential genes of the S cell are transferred to the L cell genome, the L cell will be able to forcibly keep the S cell.

Initially, the evolutionary fitness gained by loss of L cell chemiosmosis may not be so large. However, this change provides an indispensable foundation for acquiring the prominent and diverse functions peculiar to eukaryotes in the long term.

Because the membrane potential has a striking impact on energy production efficiency as well as on chemical gradients of ions, preserving the membrane potential optimal for energy production inevitably takes the highest precedence as long as the membrane is capable of energy transduction. In contrast, an L cell that has lost its chemiosmosis system can choose other membrane potentials and can utilize them for other functions without any constraint of the PMF.

Not only is membrane excitation one of the unique characteristics of eukaryotes, it is also widespread among them. As is widely known, higher animals utilize membrane excitation for a broad range of intracellular functions, such as vesicle secretion and myocyte contraction, and intercellular communications, such as neurotransmission and heart contraction. In addition to multicellular organisms, many unicellular eukaryotes are also known to use membrane excitation. For example, ciliate protozoans such as *Paramecium*, which branched early from other eukaryotes, use membrane excitation to control swimming behaviour [50]. By acquiring novel functions based on membrane excitation, eukaryotes have improved their fitness and adapted to unexplored niches (Fig. 2f).

It would be too idealistic to think that the eukaryotic cell membrane accidentally lost chemiosmosis before or after symbiogenesis and consequently acquired significant convenience derived from the unconstrained membrane potential. It is much more plausible that the host (the L cell) had acquired the symbiont (the S cell), which took over energy production by supplying ATP, followed by the host rationally abandoning its chemiosmosis system and thereby obtaining the function of fast and

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265 flexible membrane excitation.

266

267 **Conclusion**

268 My hypothetical model is not necessarily incompatible with established hypotheses such as the
269 hydrogen hypothesis; instead, it can complement several other models. Moreover, it also
270 emphasizes the possibility that both microorganisms in the symbiotic union could be aerobic.
271 This hypothesis can explain the origins of mitochondria and hydrogenosomes. Because
272 mitochondria and hydrogenosomes have a single origin, their common ancestor would have been a
273 facultative anaerobic prokaryote. If the aerobic respiration mechanism of the mitochondrion was
274 reasonably conserved under selective pressures rather than by chance, then it is unlikely that the
275 ancestral symbiotic union was harboured in a strictly anaerobic environment for enough time for
276 neutral genes to be lost by genetic drift. In this case, the possible route is one of the following. (1)
277 The first symbiosis occurred in an aerobic or a microaerobic environment and was followed by
278 some symbiotic unions that migrated into a strictly anaerobic environment and lost aerobic
279 respiration. Hydrogenosomes evolved from these symbiotic unions, while mitochondria evolved
280 from their siblings, which had remained in the aerobic environment. (2) The first symbiosis
281 occurred in a strictly anaerobic environment. Then, some symbiotic unions, which became free-
282 living, escaped into an aerobic environment before loss of the aerobic respiration function through
283 genetic drift, and mitochondria evolved from these symbiotic unions. Clearly, the former
284 explanation is simpler and less restrictive than the latter.
285 It is very likely that both of the organisms in the first symbiotic union had an energy-transducing
286 membrane, whether they used aerobic or anaerobic respiration. If that was the case, the cell
287 membrane of the host microorganism must have lost its chemiosmosis system sometime during the
288 evolution of eukaryotes.
289 Here, I have illustrated a highly plausible process of mitochondriogenesis in which adhesion of the
290 two energy-transducing membranes gives rise to a selective advantage by itself and in which, if

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there is a difference in the cell size, one membrane should lose its energy production ability after a series of evolutionary adjustments. Furthermore, the model demonstrates that the evolution of an ATP translocator can be promoted by the mutual benefit derived from ATP transport between symbiotic cells. The model also suggests that the host cell can free its membrane potential from the constraint of energy production by disrupting chemiosmosis, consequently acquiring membrane excitability, which is a highly effective and multipurpose trait that has never been acquired by prokaryotes.

Numerous testable predictions can be derived from this hypothesis, as various metagenome analysis technologies are rapidly being developed along with long-read and single-cell sequencing technologies [51]. If phylogenomic analyses reveal the aerobic ancestry of the host, the hypothesis proposed here becomes one of very few candidates that can explain the aerobic origin of eukaryotes. I predict that symbiotic-adhered prokaryotes, which can be detected as frequently accompanying genomes owing to their lower separability, should be found. Finally, I predict that proto-eukaryotic endosymbiotic unions, in which the host and the symbiont are an archaeon and an eubacterium, may be discovered using the forthcoming single-cell metagenome technologies. Furthermore, some endosymbiotic unions may be found, showing that the host cell membrane is deficient in chemiosmosis.

References

1 de Duve, C. Essay - The origin of eukaryotes: a reappraisal. *Nat. Rev. Genet.* **8**, 395-403, doi:10.1038/nrg2071 (2007).

2 Keeling, P. J. The Impact of History on Our Perception of Evolutionary Events: Endosymbiosis and the Origin of Eukaryotic Complexity. *Cold Spring Harbor Perspect. Biol.* **6**, doi:10.1101/cshperspect.a016196 (2014).

3 McInerney, J. O., O'Connell, M. J. & Pisani, D. The hybrid nature of the Eukaryota and a

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consilient view of life on Earth. *Nat. Rev. Microbiol.* **12**, 449-455, doi:10.1038/nrmicro3271 (2014).

4 Archibald, J. M. Endosymbiosis and Eukaryotic Cell Evolution. *Curr. Biol.* **25**, R911-R921, doi:10.1016/j.cub.2015.07.055 (2015).

5 Lopez-Garcia, P. & Moreira, D. Open Questions on the Origin of Eukaryotes. *Trends Ecol Evol* **30**, 697-708, doi:10.1016/j.tree.2015.09.005 (2015).

6 Martin, W. F., Garg, S. & Zimorski, V. Endosymbiotic theories for eukaryote origin. *Philos Trans R Soc Lond B Biol Sci* **370**, 20140330, doi:10.1098/rstb.2014.0330 (2015).

7 Mereschkowsky, C. Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biol. Centralbl.* **25**, 593-604 (1905).

8 Martin, W. & Kowallik, K. V. Annotated English translation of Mereschkowsky's 1905 paper 'Über Natur und Ursprung der Chromatophoren im Pflanzenreiche'. *Eur. J. Phycol.* **34**, 287-295, doi:10.1017/S0967026299002231 (1999).

9 Sagan, L. On the origin of mitosing cells. *J Theor Biol* **14**, 255-274, doi:10.1016/0022-5193(67)90079-3 (1967).

10 Margulis, L. *Symbiosis in cell evolution*. (W. H. Freeman, 1981).

11 de Duve, C. Evolution of the peroxisome. *Ann N Y Acad Sci* **168**, 369-381, doi:10.1111/j.1749-6632.1969.tb43124.x (1969).

12 John, P. & Whatley, F. R. Paracoccus denitrificans and the evolutionary origin of the mitochondrion. *Nature* **254**, 495-498, doi:10.1038/254495a0 (1975).

13 Kurland, C. G. & Andersson, S. G. E. Origin and evolution of the mitochondrial proteome. *Microbiol. Mol. Biol. Rev.* **64**, 786-+, doi:10.1128/MMBR.64.4.786-820.2000 (2000).

14 Martin, W. & Müller, M. The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37-41, doi:10.1038/32096 (1998).

15 Gray, M. W. The pre-endosymbiont hypothesis: a new perspective on the origin and evolution of mitochondria. *Cold Spring Harb Perspect Biol* **6**,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2802v1 | CC BY 4.0 Open Access | rec: 13 Feb 2017, publ: 13 Feb 2017

343 doi:10.1101/cshperspect.a016097 (2014).

344 16 Amiri, H., Karlberg, O. & Andersson, S. G. Deep origin of plastid/parasite ATP/ADP
345 translocases. *J Mol Evol* **56**, 137-150, doi:10.1007/s00239-002-2387-0 (2003).

346 17 Van Valen, L. M. & Maiorana, V. C. The archaebacteria and eukaryotic origins. *Nature* **287**,
347 248-250, doi:10.1038/287248a0 (1980).

348 18 Doolittle, W. F. Revolutionary Concepts in Evolutionary Cell Biology. *Trends Biochem.Sci.*
349 **5**, 146-149, doi:10.1016/0968-0004(80)90010-9 (1980).

350 19 Andersson, S. G. *et al.* The genome sequence of *Rickettsia prowazekii* and the origin of
351 mitochondria. *Nature* **396**, 133-140, doi:10.1038/24094 (1998).

352 20 Williams, K. P., Sobral, B. W. & Dickerman, A. W. A robust species tree for the
353 alphaproteobacteria. *J Bacteriol* **189**, 4578-4586, doi:10.1128/JB.00269-07 (2007).

354 21 Wu, M. *et al.* Phylogenomics of the reproductive parasite *Wolbachia pipientis* *w*Mel: a
355 streamlined genome overrun by mobile genetic elements. *PLoS Biol* **2**, E69,
356 doi:10.1371/journal.pbio.0020069 (2004).

357 22 Spang, A. *et al.* Complex archaea that bridge the gap between prokaryotes and eukaryotes.
358 *Nature* **521**, 173-179, doi:10.1038/nature14447 (2015).

359 23 Zaremba-Niedzwiedzka, K. *et al.* Asgard archaea illuminate the origin of eukaryotic cellular
360 complexity. *Nature* **541**, 353-358, doi:10.1038/nature21031 (2017).

361 24 Sousa, F. L., Neukirchen, S., Allen, J. F., Lane, N. & Martin, W. F. Lokiarchaeon is
362 hydrogen dependent. *Nat Microbiol* **1**, 16034, doi:10.1038/nmicrobiol.2016.34 (2016).

363 25 Martin, W. & Russell, M. J. On the origins of cells: a hypothesis for the evolutionary
364 transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from
365 prokaryotes to nucleated cells. *Philos Trans R Soc Lond B Biol Sci* **358**, 59-83; discussion
366 83-55, doi:10.1098/rstb.2002.1183 (2003).

367 26 Koonin, E. V. Comparative genomics, minimal gene-sets and the last universal common
368 ancestor. *Nat Rev Microbiol* **1**, 127-136, doi:10.1038/nrmicro751 (2003).

27 Lane, N., Allen, J. F. & Martin, W. How did LUCA make a living? Chemiosmosis in the origin of life. *Bioessays* **32**, 271-280, doi:10.1002/bies.200900131 (2010).

28 Nealson, K. H. & Conrad, P. G. Life: past, present and future. *Philos Trans R Soc Lond B Biol Sci* **354**, 1923-1939, doi:10.1098/rstb.1999.0532 (1999).

29 Pedersen, M. B., Gaudu, P., Lechardeur, D., Petit, M. A. & Gruss, A. Aerobic respiration metabolism in lactic acid bacteria and uses in biotechnology. *Annu Rev Food Sci Technol* **3**, 37-58, doi:10.1146/annurev-food-022811-101255 (2012).

30 Das, A. & Ljungdahl, L. G. *Clostridium pasteurianum* F_1F_o ATP synthase: operon, composition, and some properties. *J Bacteriol* **185**, 5527-5535, doi:10.1128/JB.185.18.5527–5535.2003 (2003).

31 Kalnenieks, U., de Graaf, A. A., Bringer-Meyer, S. & Sahm, H. Oxidative phosphorylation in *Zymomonas mobilis*. *Archives of Microbiology* **160**, 74-79, doi:10.1007/bf00258148 (1993).

32 Oshima, K. *et al.* Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. *Nat Genet* **36**, 27-29, doi:10.1038/ng1277 (2004).

33 Perez-Brocal, V. *et al.* A small microbial genome: the end of a long symbiotic relationship? *Science* **314**, 312-313, doi:10.1126/science.1130441 (2006).

34 McCutcheon, J. P. & von Dohlen, C. D. An interdependent metabolic patchwork in the nested symbiosis of mealybugs. *Curr Biol* **21**, 1366-1372, doi:10.1016/j.cub.2011.06.051 (2011).

35 Castelle, C. J. *et al.* Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Curr Biol* **25**, 690-701, doi:10.1016/j.cub.2015.01.014 (2015).

36 Wurch, L. *et al.* Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. *Nat Commun* **7**, 12115, doi:10.1038/ncomms12115 (2016).

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395 37 Mulkidjanian, A. Y., Makarova, K. S., Galperin, M. Y. & Koonin, E. V. Inventing the
396 dynamo machine: The evolution of the F-type and V-type ATPases. *Nat. Rev. Microbiol.* **5**,
397 892-899, doi:10.1038/nrmicro1767 (2007).
398 38 Gruber, G., Manimekalai, M. S., Mayer, F. & Muller, V. ATP synthases from archaea: the
399 beauty of a molecular motor. *Biochim Biophys Acta* **1837**, 940-952,
400 doi:10.1016/j.bbabio.2014.03.004 (2014).
401 39 Orij, R., Brul, S. & Smits, G. J. Intracellular pH is a tightly controlled signal in yeast.
402 *Biochim. Biophys. Acta-Gen. Subj.* **1810**, 933-944, doi:10.1016/j.bbagen.2011.03.011
403 (2011).
404 40 Cortese, J. D., Voglino, A. L. & Hackenbrock, C. R. The ionic strength of the
405 intermembrane space of intact mitochondria is not affected by the pH or volume of the
406 intermembrane space. *Biochim Biophys Acta* **1100**, 189-197 (1992).
407 41 Porcelli, A. M. *et al.* pH difference across the outer mitochondrial membrane measured with
408 a green fluorescent protein mutant. *Biochem Biophys Res Commun* **326**, 799-804,
409 doi:10.1016/j.bbrc.2004.11.105 (2005).
410 42 Yang, M. *et al.* Biocompatible click chemistry enabled compartment-specific pH
411 measurement inside E. coli. *Nat Commun* **5**, 4981, doi:10.1038/ncomms5981 (2014).
412 43 Hong, Y. & Brown, D. G. Alteration of bacterial surface electrostatic potential and pH upon
413 adhesion to a solid surface and impacts to cellular bioenergetics. *Biotechnol Bioeng* **105**,
414 965-972, doi:10.1002/bit.22606 (2010).
415 44 Albert, L. S. & Brown, D. G. Variation in bacterial ATP concentration during rapid changes
416 in extracellular pH and implications for the activity of attached bacteria. *Colloids Surf B*
417 *Biointerfaces* **132**, 111-116, doi:10.1016/j.colsurfb.2015.05.020 (2015).
418 45 Wawrik, B. *et al.* Methanogenic paraffin degradation proceeds via alkane addition to
419 fumarate by '*Smithella*' spp. mediated by a syntrophic coupling with hydrogenotrophic
420 methanogens. *Environ Microbiol* **18**, 2604-2619, doi:10.1111/1462-2920.13374 (2016).

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46 Meisner, J., Wang, X., Serrano, M., Henriques, A. O. & Moran, C. P. A channel connecting the mother cell and forespore during bacterial endospore formation. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 15100-15105, doi:10.1073/pnas.0806301105 (2008).

47 Camp, A. H. & Losick, R. A novel pathway of intercellular signalling in *Bacillus subtilis* involves a protein with similarity to a component of type III secretion channels. *Mol. Microbiol.* **69**, 402-417, doi:10.1111/j.1365-2958.2008.06289.x (2008).

48 Crawshaw, A. D., Serrano, M., Stanley, W. A., Henriques, A. O. & Salgado, P. S. A mother cell-to-forespore channel: current understanding and future challenges. *FEMS Microbiol Lett* **358**, 129-136, doi:10.1111/1574-6968.12554 (2014).

49 Diepold, A. & Armitage, J. P. Type III secretion systems: the bacterial flagellum and the injectisome. *Philos Trans R Soc Lond B Biol Sci* **370**, doi:10.1098/rstb.2015.0020 (2015).

50 Eckert, R. & Brehm, P. Ionic mechanisms of excitation in Paramecium. *Annu Rev Biophys Bioeng* **8**, 353-383, doi:10.1146/annurev.bb.08.060179.002033 (1979).

51 Gawad, C., Koh, W. & Quake, S. R. Single-cell genome sequencing: current state of the science. *Nat Rev Genet* **17**, 175-188, doi:10.1038/nrg.2015.16 (2016).

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