

Some like it hot: A hypothesis for establishment of the proto-mitochondrial endosymbiont during eukaryogenesis

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BACKGROUND

Available evidence suggests that two prokaryotes, an archaeon and a bacterium, collaborated [1-4] in the eventual formation of nucleated cells with arguably [5] increased complexity of form and function. However, the mechanisms by which bacteria and archaea cooperated in the formation of eukaryotes, and the selective pressures that promoted this partnership, remain a mystery [6-10].

Mitochondria are eukaryotic organelles thought to be derived from respiring, α -proteobacterial endosymbionts capable of generating ATP by oxidative phosphorylation [11]. The earliest eukaryote likely harbored mitochondria, since all characterized eukaryotic lineages show evidence of containing [12], or having once contained [13], these organelles. Consequently, it has been argued that mitochondria, and particularly the ATP that can be generated by these compartments, allowed for evolution toward an expanded number of proteins, an increase in overt specialization achievable by eukaryotic cells, and the eventual formation of complex multicellular organisms [14,15]. However, the relationship between mitochondrial ATP generation and its potency in allowing genome expansion has been a matter of debate [16,17]. Moreover, how and why an endosymbiont not yet converted to an organelle might purposefully provide ATP to its host is not clear [18,19].

Here, I propose that the initial driving force for integration of the proto-mitochondrial endosymbiont within the proto-eukaryotic host may not have been provision of ATP to its archaeal partner, but rather that heat generated by the endosymbiont allowed the archaeal host to endure lower temperatures at the outset of eukaryogenesis. I discuss how this arrangement may have led to the increased apparent complexity that is characteristic of eukaryotes.

ANCESTRAL ARCHAEA ARE HYPERTHERMOPHILIC

While eukaryotes are not found at temperatures higher than $\sim 60^{\circ}\text{C}$ [20,21], prokaryotic cells can proliferate at temperatures even exceeding 120°C [22]. Although some bacteria are hyperthermophiles [23], most enumerated hyperthermophilic prokaryotes that proliferate above 80°C are archaea, and the ancestral state of archaea is almost certainly hyperthermophily [24-26]. Only later were archaea able to populate

environments of lower temperature, with some archaea currently proliferating in habitats close to the freezing point of water [27]. Notably, there may be a trend toward more compact genomes as the optimal proliferation temperature of archaeal species increases [28,29]. In addition, a comprehensive analysis suggests that the protein evolution rate of hyperthermophilic archaea is reduced in comparison to archaea living at lower temperatures [24]. These findings suggest that high environmental temperature may be a general barrier to genome expansion and variation, thereby restricting phenotypic diversity.

One mechanism by which archaea may have adapted to lower temperatures is through abundant lateral gene transfer (LGT) from mesophilic bacteria already residing at lower temperatures [30-33]. Such gene transfers may have promoted improved protein folding or enzyme activity as organisms moved to colder locations. For example, many ancestral hyperthermophilic archaea lack specific chaperones, such as Hsp70 proteins, that were later acquired during relocation into cooler settings [29,34], suggesting that such chaperones may have initially promoted polypeptide folding or stability at reduced temperature. Furthermore, experimental evidence suggests that transfer of chaperone genes from a bacteria residing at low temperature can promote proliferation of a more thermophilic organism under cooler conditions [35]. Beyond the assistance provided by LGT in improving proteostasis, metabolic enzymes selected to perform within hyperthermophiles may not retain sufficient catalytic activity when moved to lower temperature [36,37], prompting the need for orthologous replacement by genes from other organisms.

Some ancestrally hyperthermophilic archaea were clearly able to establish themselves at lower temperature environments [27,33] and also commonly transit colder climates in order to seed new locations at their preferred temperature [38]. However, should the piecemeal lateral transfer or slow alteration of genetic information be the only path to the endurance of reduced temperature? What if an archaeal cell could efficiently generate its own heat, allowing the maintenance of elevated temperature even when encountering colder habitats?

MITOCHONDRIA GENERATE HEAT

In prokaryotes and prokaryote-derived organelles, energy from electrons can be converted to a proton gradient across a membrane by use of a proteinaceous electron transport chain (ETC). The resulting proton gradient can be coupled to the performance of work, such as flagellar movement or mechanochemical ATP synthesis [39-42]. The proton gradient can also be used to drive the entry of extracellular metabolites into a prokaryotic cell or eukaryotic organelle [43].

During operation of the ETC, some energy is inevitably dissipated as heat during each electron transfer [44]. Moreover, once protons are pumped across the mitochondrial inner membrane (IM) by the ETC, they can leak back across the IM in a heat-producing futile cycle [45]. Indeed, approximately a quarter of protons pumped by the ETC in several mammalian tissues examined are not coupled to performance of useful work, and the magnitude of proton leak across the mitochondrial IM can range to even higher levels, depending upon tissue type [46,47]. While there is debate regarding the reliability of subcellular temperature measurements [48-51], studies reliant upon divergent approaches to investigating subcellular temperature suggest that differences in temperature between mitochondria and the cytosol can be quite substantial [52-55]. Indeed, fully functional mitochondria in cultured human cells appear to be maintained at temperatures nearly 10°C higher than the cellular environment, even in the absence of chemically-induced proton leak [54].

Moreover, cells can purposely augment thermogenesis by the expression of specific proteins promoting mitochondrial heat production. For example, cells can express uncoupling proteins to further increase proton leak across the IM, as illustrated by thermogenesis by brown fat in mammals [56]. Or, a cell might express alternative oxidases to allow greater flux of electrons through the ETC without maximal capture of energy through proton pumping, resulting in the conversion of residual energy to heat [57]. This approach is used for thermogenesis by some flowering plants [58] and can help maintain plant tissues at up to 35°C above ambient temperature [59]. Uncoupling proteins, like all proteins of the mitochondrial carrier family, are likely an eukaryotic invention [60,61]. Alternative oxidases, however, are also encoded by prokaryotes [62], including by several α -proteobacteria [63,64].

HEAT GENERATION PROVIDES AN IMMEDIATE SELECTIVE ADVANTAGE FOR MAINTAINING THE PROTO-MITOCHONDRION DURING EUKARYOGENESIS

I suggest a scenario in which a respiring proto-mitochondrial endosymbiont was encountered and enclosed by an archaeal host typically resident at high temperatures. The internal collection of heat-generating membranes then allowed the host to maintain the cell's internal temperature and to colonize a novel, cooler environment. Enclosure may have occurred either via phagocytosis of the endosymbiont by the host or, alternatively, by invasion of the host by the endosymbiont. The proto-mitochondrion was able to persist inside its host by utilization of host-provided metabolites, a situation not unlike many current host-endosymbiont relationships existing at present day [65]. Heat would be generated by dissipation of energy during passage of electrons through the ETC. In addition, protons pumped to the bacterial periplasm either by ETC activity or by use of host and endosymbiont ATP to run the ATP synthase in reverse [66,67] could leak through the bacterial IM, thereby intensifying heat production.

Importantly, this proposed scenario allows an immediate cooperative advantage for both host and endosymbiont. The host cell would receive heat required to endure or colonize a lower-temperature niche, and the endosymbiont would obtain sufficient metabolites from the host to provide heat energy to its host and to support its own maintenance. In contrast, views of initial proto-mitochondrion establishment during eukaryogenesis based on an exigent need for endosymbiont ATP production have been viewed with skepticism. First, one must propose that the host cell was incapable of fulfilling its ATP needs under selection and that the endosymbiont generated more ATP than it required before encountering the proto-eukaryotic host [19]. Second, one must assert that this endosymbiont was initially prepared and willing to export its ATP to the host, in spite of an initial lack of ATP/ADP antiporter currently used to exchange cytosolic ADP for ATP [68] and in the face of evidence suggesting that intracellular bacteria closely related to mitochondria may be unwilling to share ATP with host cells [69].

A MOVE TOWARD COMPLEXITY AT LOWER TEMPERATURES

As this proposed partnership allowed movement of host and its resident endosymbionts to cooler climates, the apparent barriers to genome size and diversity

presented by life at high temperatures [24,28,29,70,71] would have been circumvented. Moreover, the arrangement I propose may have set the stage for further progress toward the increased cellular complexity specifically characteristic of eukaryotic cells.

First, after the early eukaryote had initially colonized environments of lower temperature with the help of proto-mitochondrial heat production, further genetic changes and acquisitions over an extended time period would have rendered unnecessary a priority on heat generation. Subsequently, better coupling of electron transport to ATP synthesis, coincident with the introduction of an ATP/ADP antiporter exchanging cytosolic ADP for ATP synthesized in the mitochondria, would have allowed greater ATP availability to the early eukaryotic cell. Higher ATP concentration may have promoted the ability to phagocytose other cells and to make efficient use of the nutrients acquired from prey toward cell division [9]. In addition, while a matter of debate [14-17], increased ATP availability may have led to augmented protein synthesis capacity and to a corresponding expansion in gene content. Supporting this idea, oxygen solubility increases with reduced temperature [72], and so movement to a cooler environment would potentially allow extraction of additional energy through ETC activity that could support both efficient ATP generation and a basal level of heat output.

Second, large multicellular aggregates and biofilms are commonly formed by prokaryotes [73], and the formation of extensive multicellular clusters with a reduced surface-area-to-volume ratio would have promoted the retention of endosymbiont-generated heat. Often, these aggregates can consist of mixed communities consisting of both archaea and bacteria. Consequently, large-scale LGT between members of the resulting conglomerate may have contributed to an enriched 'gene menu' available for addition to the early eukaryote. Indeed, while many bacteria-derived genes currently encoded by the nuclear genome of eukaryotes were transferred to this subcellular location from the proto-mitochondrial endosymbiont [5,74,75], a considerable amount of gene transfer to the nucleus from other bacterial sources clearly occurred during eukaryogenesis [76-78].

Finally, I note that production of heat by a proto-mitochondrial endosymbiont may have also provided flexibility to the eukaryotic ancestor population that would not have been available through adaptation to a cooler environment by fixation of mutations and

gene transfers. Since one might expect stochastic differences in the quantity of endosymbionts producing heat among a population of proto-eukaryotic cells, such a population of proto-eukaryotes might be robust against changes in environmental temperature. Upon encountering lower temperatures, those cells with more heat-producing endosymbionts would flourish, and conversely, upon meeting higher temperatures, those cells with a more limited endosymbiont load would prosper, thereby maintaining this lineage of proto-eukaryotes in a variable environment. Later, the cell might evolve mechanisms to directly take control of endosymbiont load in a bid to carefully balance heat requirements with the environmental temperature. Indeed, controlling the load of heat-producing endosymbionts might have been a driving force for the evolution of autophagy, since the process of autophagy, like the presence of mitochondria, appears to have been a feature of the last eukaryotic common ancestor [79].

CONCLUSION

While a well-recognized function of mitochondria is ATP production, mitochondria are also the location of several other conserved cellular processes. For example, iron-sulfur cluster generation appears to be a primary function of mitochondria [13,80], and other reactions important for lipid metabolism or amino acid production can also be compartmentalized at these organelles [81-83]. Moreover, as highlighted in this work, mitochondria can also be a source of heat production, and indeed the ability to convert energy from electrons into heat may have been the earliest basis for proto-mitochondrial endosymbiont integration with its archaeal host. While mitochondrial ATP generation undoubtedly played a significant role in the evolution of eukaryotes, a broader focus on the many functions of mitochondria lying outside of ATP production will be informative when considering the early evolution of the eukaryotic cell.

ACKNOWLEDGEMENTS

This work was supported by a European Research Council Starting Grant (637649-RevMito) to C.D.D. I thank Gülayşe İnce Dunn, Güleycan Lutfullahoğlu-Bal, Bengisu Seferoğlu, and Anı Akpınar for comments on this manuscript.

REFERENCES

1. Margulis L. Origin of Eukaryotic Cells: Evidence and Research Implications for a Theory of the Origin and Evolution of Microbial, Plant, and Animal Cells on the Precambrian Earth. Yale University Press; 1970.
2. Schwartz RM, Dayhoff MO. Origins of prokaryotes, eukaryotes, mitochondria, and chloroplasts. *Science*. 1978;199: 395–403. doi:10.1126/science.202030
3. Doolittle WF. Revolutionary concepts in evolutionary cell biology. *Trends in Biochemical Sciences*. 1980;5: 146–149. doi:10.1016/0968-0004(80)90010-9
4. McInerney JO, O'Connell MJ, Pisani D. The hybrid nature of the Eukaryota and a consilient view of life on Earth. *Nat Rev Microbiol*. 2014;12: 449–455. doi:10.1038/nrmicro3271
5. Booth A, Doolittle WF. Eukaryogenesis, how special really? *Proc Natl Acad Sci U S A*. 2015;112: 10278–10285. doi:10.1073/pnas.1421376112
6. Poole AM, Gribaldo S. Eukaryotic origins: How and when was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology*. 2014;6: a015990–a015990. doi:10.1101/cshperspect.a015990
7. Martijn J, Ettema TJG. From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell. *Biochem Soc Trans*. 2013;41: 451–457. doi:10.1042/BST20120292
8. Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC. Molecular paleontology and complexity in the last eukaryotic common ancestor. *Crit Rev Biochem Mol Biol*. 2013;48: 373–396. doi:10.3109/10409238.2013.821444
9. Martin WF, Tielens AGM, Mentel M, Garg SG, Gould SB. The Physiology of Phagocytosis in the Context of Mitochondrial Origin. *Microbiol Mol Biol Rev*. 2017;81. doi:10.1128/MMBR.00008-17
10. López-García P, Eme L, Moreira D. Symbiosis in eukaryotic evolution. *J Theor Biol*. 2017. doi:10.1016/j.jtbi.2017.02.031
11. Gray MW. Mitochondrial evolution. *Cold Spring Harbor Perspectives in Biology*. 2012;4: a011403. doi:10.1101/cshperspect.a011403
12. van der Giezen M. Hydrogenosomes and Mitosomes: Conservation and Evolution of Functions. *Journal of Eukaryotic Microbiology*. 2009;56: 221–231. doi:10.1111/j.1550-7408.2009.00407.x
13. Karnkowska A, Vacek V, Zubáčová Z, Treitli SC, Petrželková R, Eme L, et al. A Eukaryote without a Mitochondrial Organelle. *Curr Biol*. 2016;26: 1274–1284. doi:10.1016/j.cub.2016.03.053
14. Lane N. Serial endosymbiosis or singular event at the origin of eukaryotes? *J Theor Biol*. 2017. doi:10.1016/j.jtbi.2017.04.031

15. Lane N, Martin W. The energetics of genome complexity. *Nature*. 2010;467: 929–934. doi:10.1038/nature09486
16. Lynch M, Marinov GK. Membranes, energetics, and evolution across the prokaryote-eukaryote divide. *eLife*. 2017;6: 621. doi:10.7554/eLife.20437
17. Lynch M, Marinov GK. The bioenergetic costs of a gene. *Proc Natl Acad Sci U S A*. 2015;112: 15690–15695. doi:10.1073/pnas.1514974112
18. Kurland CG, Andersson SGE. Origin and Evolution of the Mitochondrial Proteome. *Microbiol Mol Biol Rev*. 2000;64: 786–820. doi:10.1128/MMBR.64.4.786-820.2000
19. Martin W, Müller M. The hydrogen hypothesis for the first eukaryote. *Nature*. 1998;392: 37–41. doi:10.1038/32096
20. Brock TD. Life at high temperatures. Evolutionary, ecological, and biochemical significance of organisms living in hot springs is discussed. *Science*. 1967;158: 1012–1019. doi:10.1126/science.158.3804.1012
21. Forterre P. The common ancestor of archaea and eukarya was not an archaeon. *Archaea*. 2013;2013: 372396. doi:10.1155/2013/372396
22. Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J, et al. Cell proliferation at 122 degrees C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc Natl Acad Sci U S A*. 2008;105: 10949–10954. doi:10.1073/pnas.0712334105
23. Stetter KO. History of discovery of the first hyperthermophiles. *Extremophiles*. 2006;10: 357–362. doi:10.1007/s00792-006-0012-7
24. Groussin M, Gouy M. Adaptation to environmental temperature is a major determinant of molecular evolutionary rates in archaea. *Mol Biol Evol*. 2011;28: 2661–2674. doi:10.1093/molbev/msr098
25. Boussau B, Blanquart S, Neacsulea A, Lartillot N, Gouy M. Parallel adaptations to high temperatures in the Archaeal eon. *Nature*. 2008;456: 942–945. doi:10.1038/nature07393
26. Blank CE. Phylogenomic dating--the relative antiquity of archaeal metabolic and physiological traits. *Astrobiology*. 2009;9: 193–219. doi:10.1089/ast.2008.0248
27. Cavicchioli R. Cold-adapted archaea. *Nat Rev Microbiol*. 2006;4: 331–343. doi:10.1038/nrmicro1390
28. Sabath N, Ferrada E, Barve A, Wagner A. Growth temperature and genome size in bacteria are negatively correlated, suggesting genomic streamlining during thermal adaptation. *Genome Biol Evol*. 2013;5: 966–977. doi:10.1093/gbe/evt050
29. Laksanalamai P, Whitehead TA, Robb FT. Minimal protein-folding systems in hyperthermophilic archaea. *Nat Rev Microbiol*. 2004;2: 315–324. doi:10.1038/nrmicro866

30. Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, McInerney JO, et al. Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. *Proc Natl Acad Sci U S A*. 2012;109: 20537–20542. doi:10.1073/pnas.1209119109
31. Nelson-Sathi S, Sousa FL, Roettger M, Lozada-Chávez N, Thiergart T, Janssen A, et al. Origins of major archaeal clades correspond to gene acquisitions from bacteria. *Nature*. 2015;517: 77–80. doi:10.1038/nature13805
32. Deschamps P, Zivanovic Y, Moreira D, Rodríguez-Valera F, López-García P. Pangenome evidence for extensive interdomain horizontal transfer affecting lineage core and shell genes in uncultured planktonic thaumarchaeota and euryarchaeota. *Genome Biol Evol*. 2014;6: 1549–1563. doi:10.1093/gbe/evu127
33. López-García P, Zivanovic Y, Deschamps P, Moreira D. Bacterial gene import and mesophilic adaptation in archaea. *Nat Rev Microbiol*. 2015;13: 447–456. doi:10.1038/nrmicro3485
34. Petitjean C, Moreira D, López-García P, Brochier-Armanet C. Horizontal gene transfer of a chloroplast DnaJ-Fer protein to Thaumarchaeota and the evolutionary history of the DnaK chaperone system in Archaea. *BMC Evol Biol*. 2012;12: 226. doi:10.1186/1471-2148-12-226
35. Ferrer M, Chernikova TN, Yakimov MM, Golyshin PN, Timmis KN. Chaperonins govern growth of *Escherichia coli* at low temperatures. *Nat Biotechnol*. 2003;21: 1266–1267. doi:10.1038/nbt1103-1266
36. Sterner R, Liebl W. Thermophilic adaptation of proteins. *Crit Rev Biochem Mol Biol*. 2001;36: 39–106. doi:10.1080/20014091074174
37. Nguyen V, Wilson C, Hoemberger M, Stiller JB, Agafonov RV, Kutter S, et al. Evolutionary drivers of thermoadaptation in enzyme catalysis. *Science*. 2017;355: 289–294. doi:10.1126/science.aah3717
38. Wirth R. Colonization of Black Smokers by Hyperthermophilic Microorganisms. *Trends in Microbiology*. 2017;25: 92–99. doi:10.1016/j.tim.2016.11.002
39. Skulachev VP. Transmembrane electrochemical H⁺-potential as a convertible energy source for the living cell. *FEBS Lett*. 1977;74: 1–9. doi:10.1016/0014-5793(77)80739-4
40. Manson MD, Tedesco PM, Berg HC. Energetics of flagellar rotation in bacteria. *J Mol Biol*. 1980;138: 541–561. doi:10.1016/S0022-2836(80)80017-9
41. Nishihara Y, Kitao A. Gate-controlled proton diffusion and protonation-induced ratchet motion in the stator of the bacterial flagellar motor. *Proc Natl Acad Sci U S A*. 2015;112: 7737–7742. doi:10.1073/pnas.1502991112
42. Junge W, Nelson N. ATP synthase. *Annu Rev Biochem*. 2015;84: 631–657. doi:10.1146/annurev-biochem-060614-034124
43. West IC. Proton-Coupled Transport Mechanisms in Bacteria. *Biochem Soc Trans*. 1974;2: 800–803. doi:10.1042/bst0020800

44. Murphy MP. Slip and leak in mitochondrial oxidative phosphorylation. *Biochim Biophys Acta*. 1989;977: 123–141. doi:10.1016/S0005-2728(89)80063-5
45. Brand MD, Chien LF, Ainscow EK, Rolfe DF, Porter RK. The causes and functions of mitochondrial proton leak. *Biochim Biophys Acta*. 1994;1187: 132–139. doi:10.1016/0005-2728(94)90099-X
46. Rolfe DF, Brand MD. Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. *Am J Physiol*. 1996;271: C1380–9.
47. Brand MD. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Experimental Gerontology*. 2000;35: 811–820. doi:10.1016/S0531-5565(00)00135-2
48. Baffou G, Rigneault H, Marguet D, Jullien L. A critique of methods for temperature imaging in single cells. *Nat Meth*. 2014;11: 899–901. doi:10.1038/nmeth.3073
49. Baffou G, Rigneault H, Marguet D, Jullien L. Reply to: "Validating subcellular thermal changes revealed by fluorescent thermosensors" and "The 10(5) gap issue between calculation and measurement in single-cell thermometry". *Nat Meth*. 2015;12: 803–803. doi:10.1038/nmeth.3552
50. Suzuki M, Zeeb V, Arai S, Oyama K, Ishiwata S. The 10(5) gap issue between calculation and measurement in single-cell thermometry. *Nat Meth*. 2015;12: 802–803. doi:10.1038/nmeth.3551
51. Kiyonaka S, Sakaguchi R, Hamachi I, Morii T, Yoshizaki T, Mori Y. Validating subcellular thermal changes revealed by fluorescent thermosensors. *Nat Meth*. 2015;12: 801–802. doi:10.1038/nmeth.3548
52. Nakano M, Arai Y, Kotera I, Okabe K, Kamei Y, Nagai T. Genetically encoded ratiometric fluorescent thermometer with wide range and rapid response. *PLoS ONE*. 2017;12: e0172344. doi:10.1371/journal.pone.0172344
53. Okabe K, Inada N, Gota C, Harada Y, Funatsu T, Uchiyama S. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. *Nature Communications*. 2012;3: 705. doi:10.1038/ncomms1714
54. Chretien D, Benit P, Ha HH, Keipert S, El-Khoury R. Mitochondria Are Physiologically Maintained At Close To 50°C. *bioRxiv*. 2017. doi:10.1101/133223
55. Sakaguchi R, Kiyonaka S, Mori Y. Fluorescent sensors reveal subcellular thermal changes. *Current Opinion in Biotechnology*. 2015;31: 57–64. doi:10.1016/j.copbio.2014.07.013
56. Busiello RA, Savarese S, Lombardi A. Mitochondrial uncoupling proteins and energy metabolism. *Front Physiol*. 2015;6: 36. doi:10.3389/fphys.2015.00036
57. Moore AL, Siedow JN. The regulation and nature of the cyanide-resistant alternative oxidase of plant mitochondria. *Biochim Biophys Acta*. 1991;1059: 121–140. doi:10.1016/S0005-2728(05)80197-5

58. Wagner AM, Krab K, Wagner MJ, Moore AL. Regulation of thermogenesis in flowering Araceae: the role of the alternative oxidase. *Biochim Biophys Acta*. 2008;1777: 993–1000. doi:10.1016/j.bbabi.2008.04.001
59. Knutson RM. Heat production and temperature regulation in eastern skunk cabbage. *Science*. 1974;186: 746–747. doi:10.1126/science.186.4165.746
60. Kunji ERS. The role and structure of mitochondrial carriers. *FEBS Lett*. 2004;564: 239–244. doi:10.1016/S0014-5793(04)00242-X
61. Haferkamp I, Schmitz-Esser S. The plant mitochondrial carrier family: functional and evolutionary aspects. *Front Plant Sci*. 2012;3: 2. doi:10.3389/fpls.2012.00002
62. Pennisi R, Salvi D, Brandi V, Angelini R, Ascenzi P, Polticelli F. Molecular Evolution of Alternative Oxidase Proteins: A Phylogenetic and Structure Modeling Approach. *Journal of Molecular Evolution*. 2016;82: 207–218. doi:10.1007/s00239-016-9738-8
63. Roberts CW, Roberts F, Henriquez FL, Akiyoshi D, Samuel BU, Richards TA, et al. Evidence for mitochondrial-derived alternative oxidase in the apicomplexan parasite *Cryptosporidium parvum*: a potential anti-microbial agent target. *International Journal for Parasitology*. 2004;34: 297–308. doi:10.1016/j.ijpara.2003.11.002
64. Atteia A, van Lis R, van Hellemond JJ, Tielens AGM, Martin W, Henze K. Identification of prokaryotic homologues indicates an endosymbiotic origin for the alternative oxidases of mitochondria (AOX) and chloroplasts (PTOX). *Gene*. 2004;330: 143–148. doi:10.1016/j.gene.2004.01.015
65. Moya A, Peretó J, Gil R, Latorre A. Learning how to live together: genomic insights into prokaryote-animal symbioses. *Nat Rev Genet*. 2008;9: 218–229. doi:10.1038/nrg2319
66. Dimroth P, Cook GM. Bacterial Na⁺ - or H⁺ -coupled ATP synthases operating at low electrochemical potential. *Adv Microb Physiol*. 2004;49: 175–218. doi:10.1016/S0065-2911(04)49004-3
67. Campanella M, Parker N, Tan CH, Hall AM, Duchon MR. IF(1): setting the pace of the F(1)F(o)-ATP synthase. *Trends in Biochemical Sciences*. 2009;34: 343–350. doi:10.1016/j.tibs.2009.03.006
68. Karlberg O, Canback B, Kurland CG, Andersson SG. The dual origin of the yeast mitochondrial proteome. *Yeast*. 2000;17: 170–187. doi:10.1002/1097-0061(20000930)17:3<170::AID-YEA25>3.0.CO;2-V
69. Winkler HH, Neuhaus HE. Non-mitochondrial ATP transport. *Trends in Biochemical Sciences*. 1999;24: 64–68. doi:10.1016/S0968-0004(98)01334-6
70. Friedman R, Drake JW, Hughes AL. Genome-wide patterns of nucleotide substitution reveal stringent functional constraints on the protein sequences of thermophiles. *Genetics*. 2004;167: 1507–1512. doi:10.1534/genetics.104.026344
71. Drake JW. Avoiding dangerous missense: thermophiles display especially low mutation rates. Casadesús J, editor. *PLoS Genet*. 2009;5: e1000520. doi:10.1371/journal.pgen.1000520

72. Ming G, Zhenhao D. Prediction of oxygen solubility in pure water and brines up to high temperatures and pressures. *Geochimica et Cosmochimica Acta*. 2010. doi:10.1006/mvre.1998.2088
73. Fröls S. Archaeal biofilms: widespread and complex. *Biochem Soc Trans*. 2013;41: 393–398. doi:10.1042/BST20120304
74. Hewitt V, Alcock F, Lithgow T. Minor modifications and major adaptations: the evolution of molecular machines driving mitochondrial protein import. *Biochim Biophys Acta*. 2011;1808: 947–954. doi:10.1016/j.bbame.2010.07.019
75. Gray MW. Mosaic nature of the mitochondrial proteome: Implications for the origin and evolution of mitochondria. *Proc Natl Acad Sci U S A*. 2015;112: 10133–10138. doi:10.1073/pnas.1421379112
76. de Duve C. The origin of eukaryotes: a reappraisal. *Nat Rev Genet*. 2007;8: 395–403. doi:10.1038/nrg2071
77. Kurland CG, Collins LJ, Penny D. Genomics and the irreducible nature of eukaryote cells. *Science*. 2006;312: 1011–1014. doi:10.1126/science.1121674
78. Gabaldón T, Huynen MA. Reconstruction of the proto-mitochondrial metabolism. *Science*. 2003;301: 609–609. doi:10.1126/science.1085463
79. Yang J, Chai X-Q, Zhao X-X, Li X. Comparative genomics revealed the origin and evolution of autophagy pathway. *Jnl of Sytematics Evolution*. 2017;55: 71–82. doi:10.1111/jse.12212
80. Braymer JJ, Lill R. Iron-Sulfur Cluster Biogenesis and Trafficking in Mitochondria. *J Biol Chem*. 2017. doi:10.1074/jbc.R117.787101
81. Tatsuta T, Langer T. Intramitochondrial phospholipid trafficking. *Biochim Biophys Acta*. 2017;1862: 81–89. doi:10.1016/j.bbalip.2016.08.006
82. Ahn CS, Metallo CM. Mitochondria as biosynthetic factories for cancer proliferation. *Cancer Metab*. 2015;3: 1. doi:10.1186/s40170-015-0128-2
83. Makiuchi T, Nozaki T. Highly divergent mitochondrion-related organelles in anaerobic parasitic protozoa. *Biochimie*. 2014;100: 3–17. doi:10.1016/j.biochi.2013.11.018