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

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ABSTRACT

Eukaryotic cells are generally characterized by a considerable increase in subcellular compartmentalization in comparison to prokaryotes. Eukaryotes can also form multicellular organisms consisting of highly specialized cell types. Most evidence suggests that the earliest eukaryotes consisted of mitochondria derived from an α-proteobacterial ancestor enclosed within an archaeon-derived host cell. However, what specific benefits the archaeal host and the proto-mitochondrial endosymbiont each obtained from this endosymbiotic relationship remains unclear. In this work, we argue that endosymbiont-generated heat may have initially permitted an archaeal host living at very high temperatures to colonize a cooler environment, and we describe how subsequent events could have prompted the increased apparent complexity of eukaryotic cells.

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 ~60°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 are likely to have adapted to reduced temperature 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 hyperthermophillic archaea were clearly able to establish themselves at lower temperature environments [27, 33] and also commonly transit colder climes in order to seed new locations at their preferred temperatures [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 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℃ 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℃ 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 collection of heat-generating membranes within the cytosol then allowed the host to maintain the cell's internal temperature at a value higher than ambient temperature and to colonize a novel, cooler environment (Fig. 1). 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, and it has been suggested that the electron transport chains of endosymbionts and parasites may have increased latitude to lose energy as heat [66]. In addition, protons pumped to the bacterial periplasm either by ETC activity or by use of host and endosymbiont ATP to operate the ATP synthase in reverse [67, 68] 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 [69] and in the face of evidence suggesting that intracellular bacteria closely related to mitochondria may be unwilling to share ATP with host cells [70].

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, 71, 72] 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 (Fig. 2). 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 [73], and so movement to a cooler environment would potentially allow extraction of additional energy by ETC activity that could support both efficient ATP generation and a basal level of heat output.

Second, it has been argued that single cells are typically in temperature equilibrium with their environment [48, 74]. However, formation of extensive multicellular clusters with a reduced surface-area-to-volume ratio, if large enough [48], could promote the retention of endosymbiont-generated heat. Furthermore, specific shaping of such a biomass, or localization to a niche limiting heat transfer, may have limited heat loss from cells to the environment. In fact, large multicellular aggregates and biofilms, consisting of both archaea and bacteria, are commonly found [75], but no experimental data, to our knowledge, has carefully investigated the relationship between heat retention and the size and shape of a biomass. If such aggregates promoted heat retention by the proto-eukaryote, large-scale LGT between members of the resulting conglomerate may have contributed to an enriched 'gene menu' available for addition to the early eukaryote (Fig.

3). 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, 76, 77], a considerable amount of gene transfer to the nucleus from other bacterial sources clearly occurred during eukaryogenesis [78-80].

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 (Fig. 4), thereby maintaining such a lineage of proto-eukaryotes. Moreover, since multiple endosymbiont genotypes might be encompassed by the same cell, such genotypic heteroplasmy, with some endosymbiont electron chains better coupled to ATP synthesis than others, might allow further tailoring of heat production by selective pressure. 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 [81].

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, 82], and other reactions important for lipid metabolism or amino acid production can also be compartmentalized at these organelles [83-85]. 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.

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FIGURE LEGENDS

Figure 1. Internalization of heat generating bacteria could permit archaeal colonization of cooler environments. **A:** Ancestral archaeal cells eventually forming the proto-eukaryotic host (grey) would initially be limited to proliferation at higher temperatures, while the respiring proto-mitochondrial endosymbiont (orange) would be resident at lower temperatures. **B:** After encountering one another, the endosymbiont would be enclosed within the proto-eukaryotic cell. **C:** After sufficient endosymbiont load has been achieved, and the heat generated by electron transport and proton leak reaches a sufficient value, the proto-eukaryote may colonize a lower temperature environment.

Figure 2. A subsequent switch to higher ATP generation capacity could promote cellular complexity. **A:** After initially promoting heat generation and permitting movement of the proto-eukaryote to a cooler location, subsequent genetic changes obviate the need for maximal endosymbiont heat generation. **B:** Tighter coupling of electron transport to ATP synthesis can then evolve, leading to increased ATP abundance. **C:** Higher ATP availability resulting from mitochondrial activity may have increased the possibility of subcellular compartmentalization and, in particular, may have promoted the ability to phagocytose other cells (prey prokaryotes in blue).

Figure 3. The need to avoid heat loss may encourage lateral gene transfer from bacteria to the proto-eukaryote. **A:** Single cells carrying heat-generating endosymbionts may rapidly equilibrate their temperature with the environment. **B:** However, archaea often form mixed aggregates that include bacteria (colored ovals), and archaea-containing biofilms can be of significant size (not reflected here). By decreasing the surface-area-to-volume ratio, a greater amount of endosymbiont-generated heat might be retained (reflected by red cytoplasm). **C:** The formation of large conglomerates of cells, along with encouraging heat retention, might boost lateral gene transfer to the early eukaryotic cell.

Figure 4. Cell-to-cell variability in the number of heat-producing endosymbionts may allow a population to be resilient to environmental temperature changes. **A:** As an example, a population of cells adapted to narrow temperature range by a fixed genotype (blue rectangle) may not be able to easily endure or colonize environments of significantly different temperature. **B:** However, inherent variability in endosymbiont load among a

population of proto-eukaryotes might allow at least some members of an otherwise genotypically identical population to subsist or thrive at more widely divergent temperatures.

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B)

C)

higher
temperature
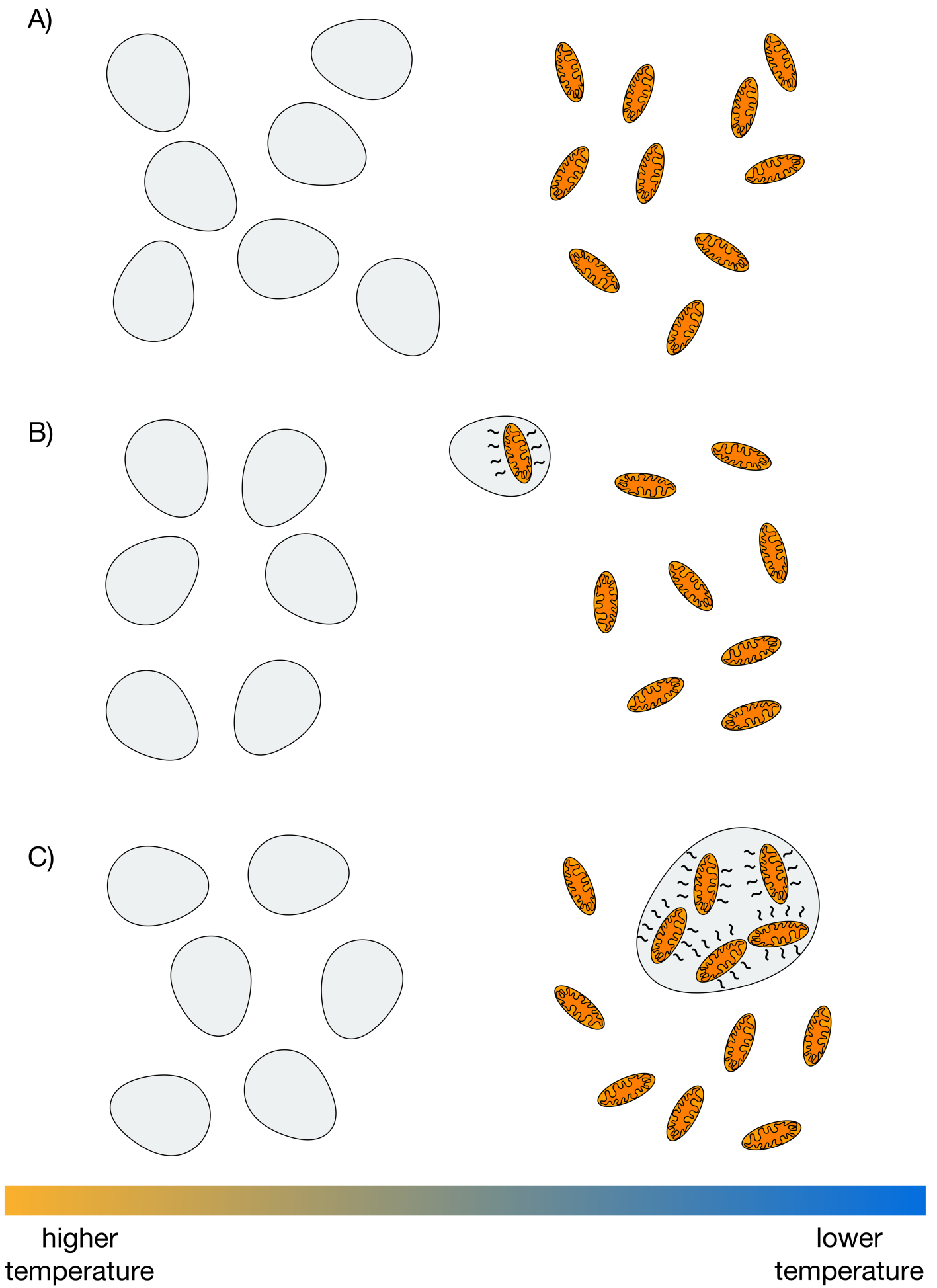
lower
temperature

Figure 1
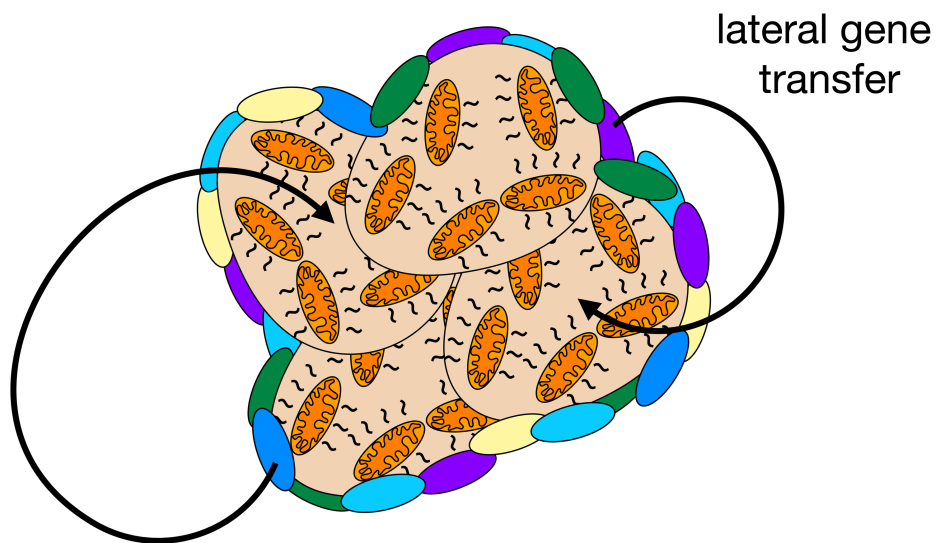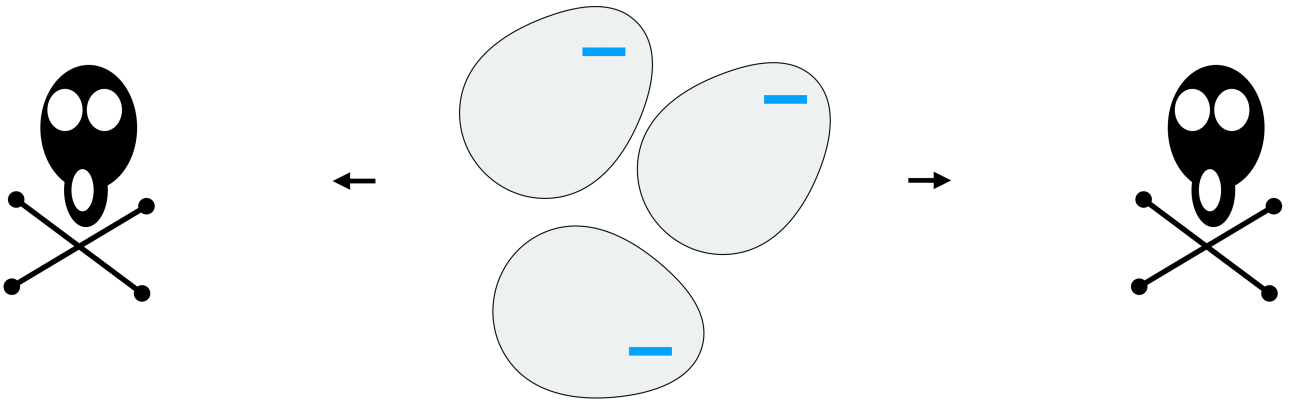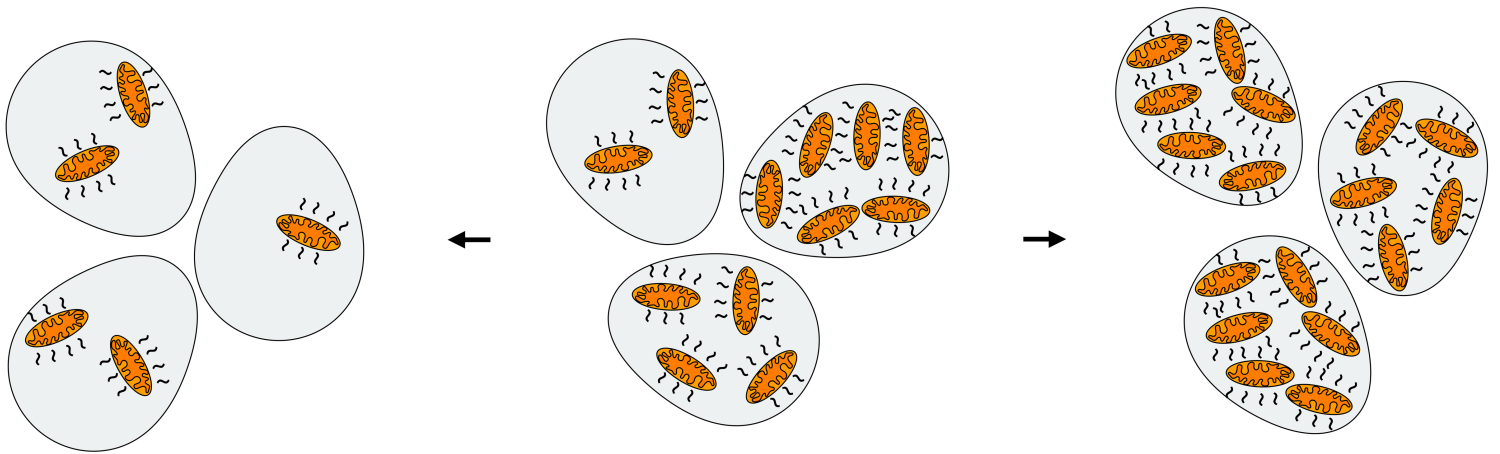
A)

B)

C)

Figure 2

A)

B)

C)

lateral gene
transfer
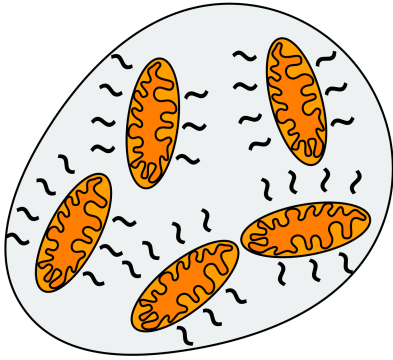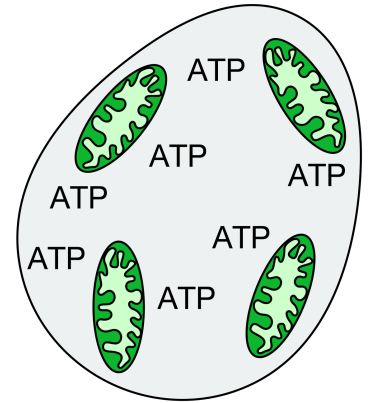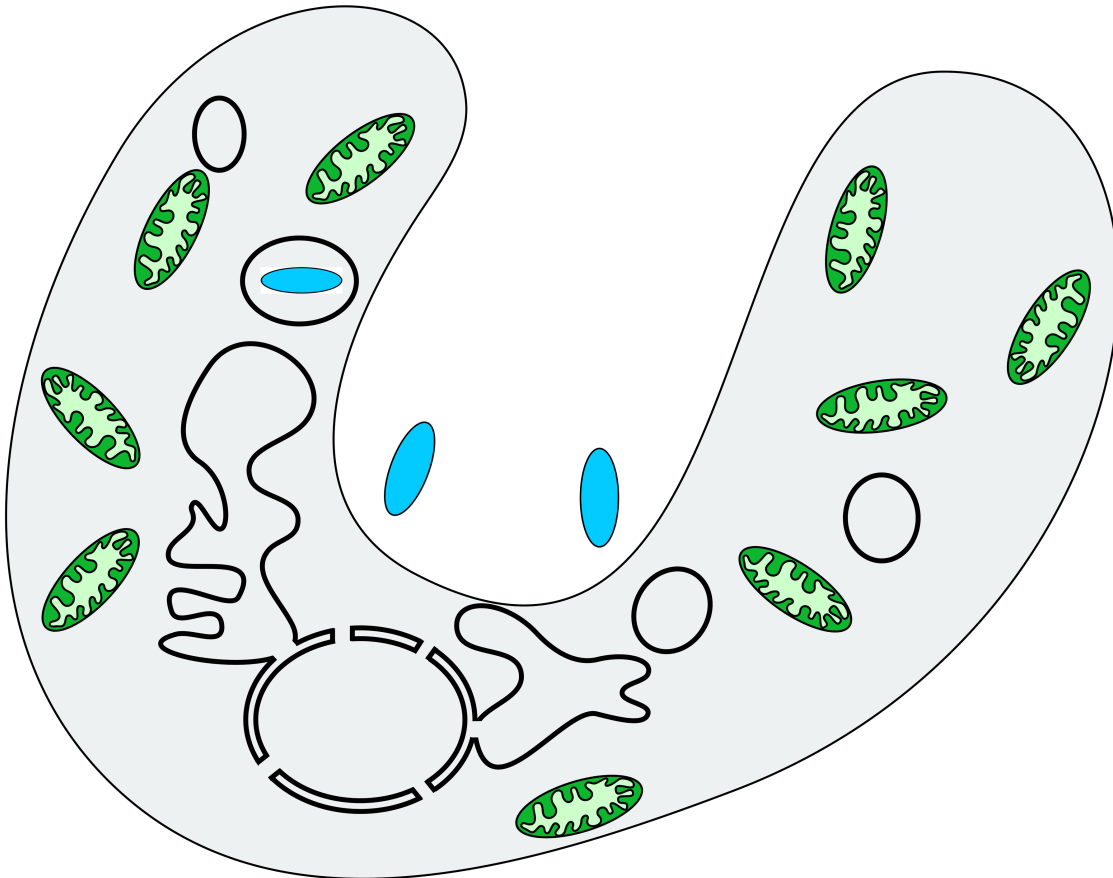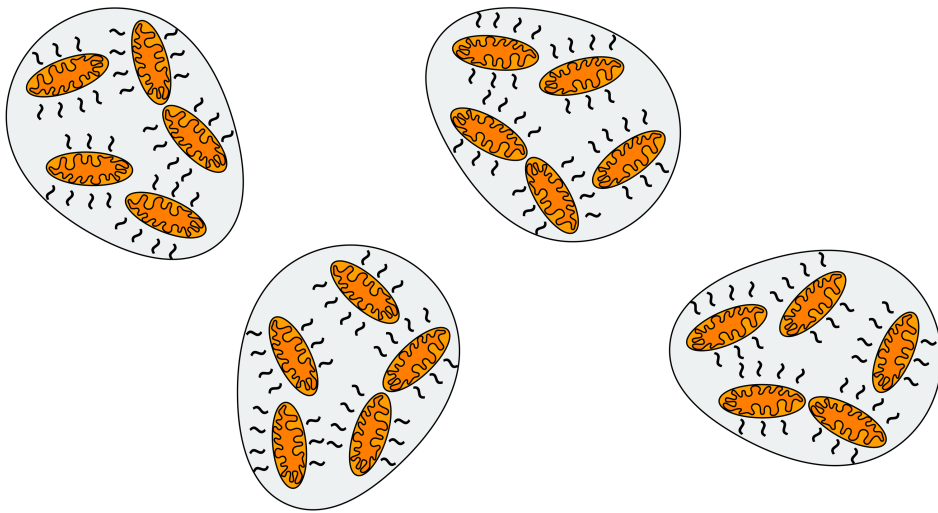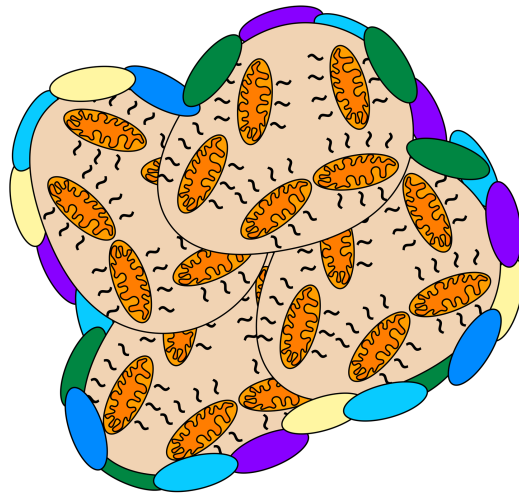
Figure 3

A)

B)

higher
temperature

lower
temperature

Figure 4