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Essay

How energy flow shapes cell evolution

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How mitochondria shaped the evolution of eukaryotic complexity has been controversial for decades. The discovery of the Asgard archaea, which harbor close phylogenetic ties to the eukaryotes, supports the idea that a critical endosymbiosis between an archaeal host and a bacterial endosymbiont transformed the selective constraints present at the origin of eukaryotes. Cultured Asgard archaea are typically prokaryotic in both size and internal morphology, albeit featuring extensive protrusions. The acquisition of the mitochondrial predecessor by an archaeal host cell fundamentally altered the topology of genes in relation to bioenergetic membranes. Mitochondria internalised not only the bioenergetic membranes but also the genetic machinery needed for local control of oxidative phosphorylation. Gene loss from mitochondria enabled expansion of the nuclear genome, giving rise to an extreme genomic asymmetry that is ancestral to all extant eukaryotes. This genomic restructuring gave eukaryotes thousands of fold more energy availability per gene. In principle, that difference can support more and larger genes, far more non-coding DNA, greater regulatory complexity, and thousands of fold more protein synthesis per gene. These changes released eukaryotes from the bioenergetic constraints on prokaryotes, facilitating the evolution of morphological complexity.

To say that cells need a continuous flow of energy to stay alive is so banal that it's easy to overlook the implications for evolution. Yet those implications shaped the four billion-year long trajectory of cell evolution, and may explain why prokaryotes (bacteria and archaea) remain relatively simple in their morphology, if not in their genetics or biochemistry, whereas eukaryotes explored the realm of morphological complexity, despite being more limited metabolically [1]. Taking a bioenergetic view of evolution can also explain the apparently singular origin of all complex (eukaryotic) life on Earth. This complexity is primarily at the level of cellular morphology — no known prokaryote compares with an amoeba or a ciliate in morphological complexity. Considering energy flow in relation to genes helps to explain why.

Charging the membrane

The reason that bioenergetics has the power to shape cell evolution so profoundly derives from the requirement for membranes, as first conceived by Peter Mitchell in the 1960s [2]. Far from being simply 'chemistry in a bag', cells drive both carbon and energy metabolism (specifically CO_2 fixation and ATP synthesis) through the use of electrical membrane potential. As a rule of thumb, all cells use this potential to drive the fundamental processes of living [3]. The use of membrane potential to drive growth is as deeply conserved across the tree of life as the genetic code itself [3]. Membrane bioenergetics link energy flow to two aspects of cell structure: topology (that is, which membranes are charged) and an apparent requirement for polyploid genomes stationed next to membranes to control their electrical potential.

Membrane potential is produced by pumping protons (or other ions such as sodium) across a membrane. For bacteria and archaea, the membrane in question is the plasma membrane, which separates the cell from its environment. The resulting differences in proton concentration (pH) and electrical charge — with positively charged protons accumulating outside the cell (for example, in the periplasmic space) — is called the proton motive force. Overall, this force equates to 150–200 mV [2]. That might sound relatively trivial, but since the membrane is only ~5 nm thick, the field strength is 30 million volts per metre, equivalent to a bolt of lightning. Failure to control such intense electrical potential is linked with severe penalties including controlled cell death in both prokaryotes and eukaryotes [4].

Could a need to control this intense membrane potential have constrained

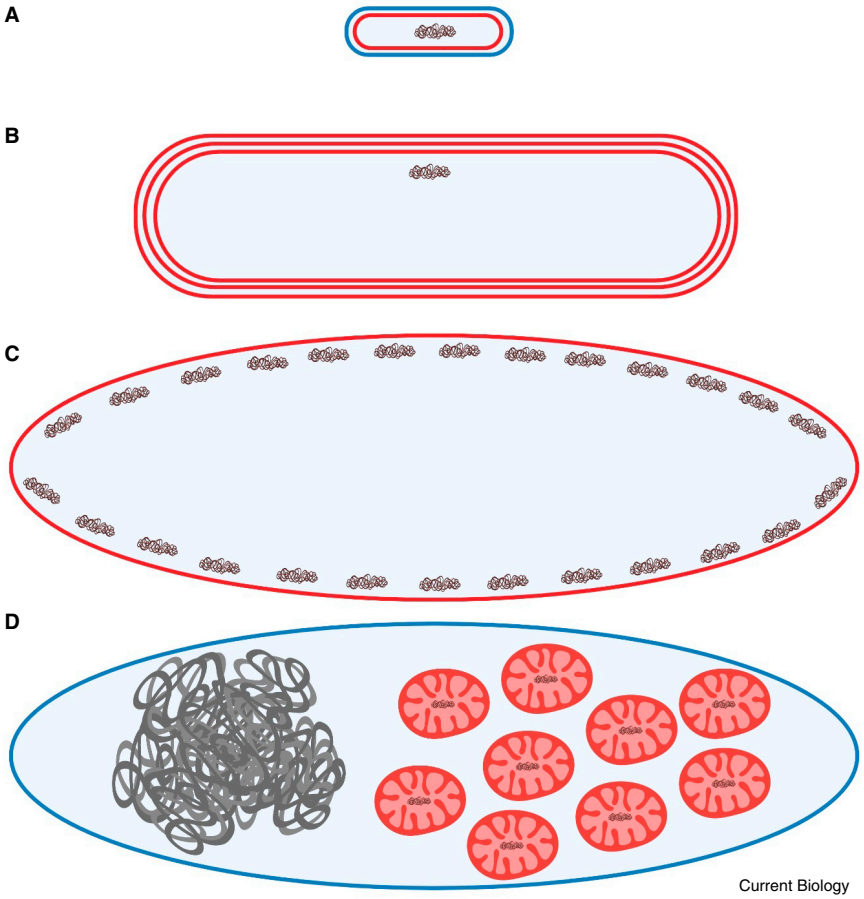


Figure 1. Topology of genes and bioenergetic membranes.
(A) A typical bacterium equivalent to *E. coli* with an electrically charged (bioenergetic) plasma membrane shown in red, and an uncharged outer membrane in blue. (B) A large bacterium with a haploid genome and multiple internal bioenergetic membranes. Such cells do not exist, for the postulated reason that a single genome cannot control energy flow across an extensive area of bioenergetic membranes. (C) A giant bacterium with extreme polyploidy, equivalent to *Epulopiscium*, with thousands of copies of its complete genome positioned close to an extensive bioenergetic plasma membrane; the internal volume is metabolically nearly inert. (D) A proto-eukaryotic cell in which a large host-cell genome is supported energetically by many mitochondria, with extreme polyploidy of their pared-down 'bioenergetic' genomes. The total DNA content is equivalent to giant bacteria, but its distribution exhibits a genomic asymmetry.

the evolution of bacteria and archaea? There are good reasons to think so. Consider the tree of life: there is at least as much genetic variation among bacteria and archaea as in eukaryotes, as judged by the number of genes in metagenomes or the variation between groups [5]. In other words, bacteria and archaea have explored genetic sequence space just as thoroughly as eukaryotes, yet did not evolve comparable morphological complexity. Although some eukaryote-like traits have evolved in prokaryotes, including even a form of phagocytosis-like cell engulfment in planctomycete bacteria [6], these traits are limited when compared with eukaryotic excesses. For example, phagocytic planctomycetes are usually less than 5 μm in diameter [6] and thousands of fold smaller than common eukaryotic amoebae in their cell volume. This universal limitation suggests that bacteria and archaea are not constrained by information alone: they made a start up the ramp towards eukaryotic complexity, but then stopped short. What else could limit prokaryotic evolution? The most likely answer is some kind of restrictive bottleneck, such as a constraint in cell structure. Previous proposals have included the 'catastrophic loss of the cell wall' or the attachment of bacterial chromosomes to the cell membrane [7]. But prokaryotes lacking cell walls [8] and those with free chromosomes and multiple origins of replication [9] show little tendency to evolve eukaryotic complexity, so those ideas are not borne out.

The acquisition of mitochondria in eukaryotes was unquestionably a revolution in cell structure [10]. Mitochondria derive from heterotrophic bacteria via an endosymbiosis that occurred perhaps two billion years ago [11]. Topologically, mitochondria internalise the bioenergetic membranes, freeing up the plasma membrane for other tasks, including phagocytosis [12]. But mitochondria are much more than internal bioenergetic membranes, which are also found in many bacteria. Critically, they are semi-autonomous, locally controlled genetic units, with their own specialised genes and protein-synthesis machinery (Figure 1). Mitochondrial biogenesis requires replication of mitochondrial genes, the biosynthetic and respiratory machinery, and the membranes themselves. There is no known prokaryotic equivalent to such self-contained power-packs, as pointed out long ago by Stanier and van Niel in their 'Concept of a bacterium' [13]. For eukaryotes, more power requires more power-packs, each one with its own genetic machinery. As such, eukaryotes exhibit extreme polyploidy of the mtDNA, with each genome in close apposition with bioenergetic membranes.

Some have argued that mitochondrial genomes are mere vestiges of a bacterial genome, the bulk of which was relocated to the nucleus, where it is supposedly better protected from reactive oxygen species (ROS) or copying errors, and recombined by sex every generation [14]. But evolution speaks strongly against this position. After perhaps two billion years of coevolution within eukaryotic cells, all mitochondria capable of oxidative phosphorylation have retained a small genome encoding core respiratory membrane proteins, along with the translational machinery needed for local protein synthesis [15–17]. In contrast, mitochondria that lost the machinery for oxidative phosphorylation (such as mitosomes and hydrogenosomes) typically lost their vestigial genomes too [15–17].

Why respiring mitochondria need genes is still debated. The 'co-location for redox regulation' hypothesis postulates that genes need to be placed next to bioenergetic membranes for redox regulation [17]. By being retained locally, these genes enable swift responses to changes in substrate availability, ATP levels, oxygen tension, ROS flux and membrane potential, making mitochondria 'smart-organelles' [17]. Putting aside the details, the conservative position from evolution is simply that genes are needed next to highly charged bioenergetic membranes, or respiration goes wrong. The penalties must be severe, as this has apparently never happened.

The nature of the host cell that first acquired the mitochondrial predecessor has come into sharper focus in recent years. From phylogenetic analyses, it seems to have been an archaeon, probably related to the Asgard archaea [18]. Although they have normal archaeal genome sizes, the Asgard archaea harbour some strikingly eukaryotic-like genes, which hint at the presence of a relatively dynamic cytoskeleton and membrane remodelling [18]. Some of these archaea have been recently cultured and found to form extensive protrusions involved in heterotrophic feeding, such as amino-acid fermentations [19], but their internal morphology is archetypally prokaryotic in complexity and bears little resemblance to eukaryotes. Various bacteria and archaea are known that form processes including nanowires or cables for electron transfer, so Asgards are by no means unique in their morphological complexity [20]. Nor is their metabolism suggestive of great complexity: a metabolic reconstruction of the last Asgard common ancestor suggests that it may have been limited to hydrogen-dependent anaerobic metabolism using the acetyl CoA pathway [21] — not far up any ramp towards eukaryotic complexity.

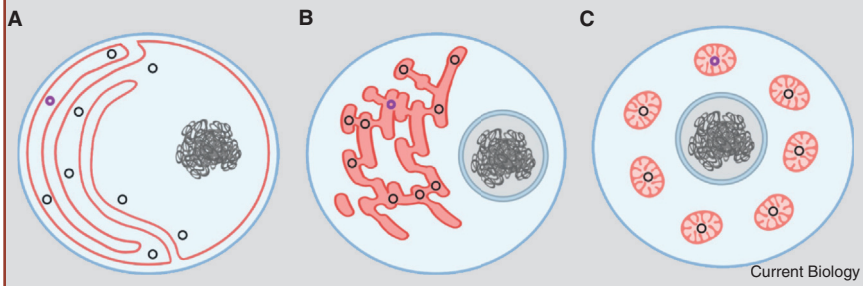
Multi-bacterial power without the overhead

The acquisition of endosymbiotic bacteria by an archaeal host cell led to a step-change in evolution, ultimately increasing eukaryotic 'energy per gene' by several orders of magnitude compared with bacteria [10]. The term 'energy per gene' has often been misconstrued to relate to the number of genes [22] or the costs of expressing a gene [23]. The term was actually intended to refer to the energy *availability* for gene expression, which is to say a cell's ability to pay for protein synthesis [10,24–26]. Protein synthesis accounts for 70–80% of the ATP budget of microbes: it is far more expensive than RNA or DNA synthesis, which together account for a relatively small fraction of the ATP budget [10]. An increase in energy per gene therefore equates to more energy available for gene expression, and does not imply a large increase in gene number.

In reality, mitochondrial power probably enabled an expansion in eukaryotic genome size (from a maximum of 13 Mb in bacteria up to ~150,000 Mb in eukaryotes [27]), a rise in the number of protein-coding genes (4-fold on average) and most importantly, an increase in gene expression of hundreds to thousands-fold [10,25]. This transformative scaling up is linked with a mean increase in cell volume of around 15,000-fold [10]. Eukaryotes are composed of metabolically demanding machinery, mostly made up of proteins, so the high energy demands of gene expression plainly correlate with cell volume. Whereas an *Escherichia coli* cell has about 13,000 ribosomes, the ciliate *Tetrahymena thermophila* can have more than 100 million [28], an increase of about 8,000-fold. This grand expansion in cell volume, genome-size, number of protein-coding genes, and gene expression incurs soaring energetic costs, which are covered by the increase in eukaryotic energy per gene of 3–5 orders of magnitude [10,24–26] compared with prokaryotes.

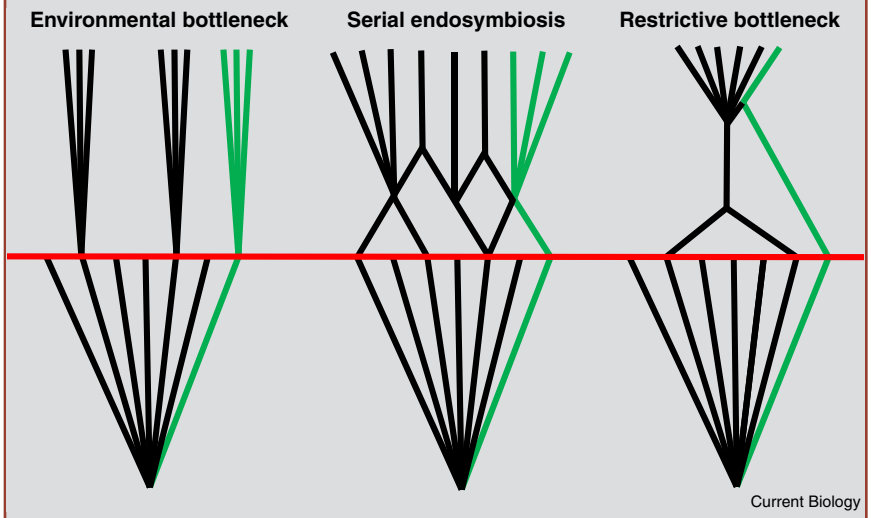
Mitochondria did not simply increase the area of internal bioenergetic membranes: the key to their advantage lies in the requirement for genomes to control respiration locally. As bacterial endosymbionts, mitochondria probably started out with 3,000–4,000 genes, which were ultimately whittled down to an average of a few dozen (ranging

Box 1. Why plasmids do not substitute for mitochondrial DNA.



Mitochondrial DNA can be similar in size and structure to bacterial plasmids (minicircles in the figure) [16]. In principle, it might seem possible to control respiration across an extensive area of bioenergetic membrane (red lines in figure) in giant bacteria through carefully positioned plasmids containing the same genes for oxidative phosphorylation as mitochondrial DNA. Yet this arrangement in (A) is never observed. Why not? There are various possibilities [24,25] but the degeneration of fused mitochondrial networks in which fission has been blocked [37] provides a clue. In large bacteria with invaginations of the plasma membrane (rather than discrete compartments) bioenergetic plasmids share a common continuous cytosol (A), making it hard to establish a correspondence between genotype and phenotype. When mitochondria fuse into laminating networks, multiple copies of mtDNA likewise share a common matrix space (B). This arrangement is likely to be beneficial in terms of the speed and efficiency of respiration, but if the inner mitochondrial membrane is continuous, then there is no direct correspondence between the genotype of any particular mtDNA and the phenotype of respiration. Fission regenerates discrete mitochondria with one or a few copies of mtDNA (C), in which a correspondence between genotype and phenotype can be established, facilitating the elimination of mtDNA mutations and opposing the degeneration of the system. A mutant plasmid or mtDNA is shown in purple in each case; only in (C) can the mutant be selected against on the basis of its specific phenotype.

Box 2. The puzzling monophyletic origins of complex cells.



All eukaryotes share a long list of basal traits, from the structure of the nucleus to the deeply conserved endomembrane systems, to processes such as meiosis. The simplest explanation for this common ancestry is some form of population bottleneck, but different types of bottleneck make different predictions. The horizontal red bar in the figure depicts a bottleneck, with only prokaryotes below the bar and complex cells above. For an environmental bottleneck, such as a snowball Earth or oxygenation after the Great Oxidation Event (left), the prediction is that the best pre-adapted groups would radiate to give polyphyletic origins of complexity. For example, photosynthetic bacteria (green) should give rise to complex algae, while osmotrophic bacteria should give rise to fungi and so on. Cell-level complexity should then differ in these polyphyletic complex groups. The serial endosymbiosis theory (centre) makes a similar prediction — different endosymbioses in disparate environments should give rise to polyphyletic origins of complexity, with the example of photosynthesis shown again in green. What phylogenomics actually shows is closer to the restrictive bottleneck shown on the right, in which the bottleneck seems to relate to some constraint from cell structure rather than the environment. Here, an endosymbiosis between an archaeal host cell and bacterial endosymbiont gives rise to a monophyletic origin of eukaryotes, potentially through the restructuring of genomes in relation to bioenergetic membranes. The acquisition of a cyanobacterial symbiont, shown in green, only affects one eukaryotic group, the algae, which otherwise share all eukaryotic cell-level traits. This scheme is supported by phylogenomics, and also offers a possible explanation for the shared cell-level structure of eukaryotes — it arose through selection for coadaptation and conflict resolution between host cell and endosymbiont [35]. This perspective offers a rich vein of explanation, suggesting possible accounts for the origin of the nucleus [38] and endomembrane systems [39], as well as meiotic sex itself [24] and the evolution of two sexes [40].

between 3 and ~100) [15,16]. Although many genes migrated to the nucleus through endosymbiotic gene transfer, many others must simply have been lost, especially those encoding traits no longer needed in an endosymbiont, such as a cell wall or flagellum. The energy savings enabled by gene loss are colossal. Think of the eukaryotic cell as having multi-bacterial power. Each mitochondrion has overhead costs for making ATP. Any genes that are transferred to the nucleus and then expressed at the same level, to do the same job, incur an equal energetic cost; there are no savings there. But if a gene is simply lost, along with its function, then the endosymbiont would produce just as much ATP, but its gene-expression costs would be lower. So, eukaryotes have multi-bacterial power with lower overhead. If only 5% of the genes from each of 100 endosymbiont genomes were permanently lost, the energy savings (from not making those proteins) have been calculated at around 50 billion ATPs [25]. That could in principle pay for all kinds of new functions. For example, assuming a 24-hour life cycle, these energy savings could fuel the *de novo* synthesis of four micrometers of actin cytoskeleton every second! That enormous surplus of ATP surely enabled the physical expansion of eukaryotes and ultimately made possible extravagant forms of phagocytosis, photosynthesis and osmotrophy.

The idea that mitochondrial bioenergetics underpinned the evolution of burgeoning complexity in eukaryotes is appealing in its simplicity, but has been challenged. For example, it has been argued that there is no sharp division between prokaryotes and eukaryotes — that the costs of gene expression scale comparably across all microbes [23]. So, if the cell volume and protein content double, then the ATP and ribosome requirements would roughly double in both eukaryotes and prokaryotes [23]. That is true, but ignores the capacity of cells to provide the ATP required to meet those costs [26]. The capacity for ATP synthesis scales with the area of bioenergetic membranes. But crucially, expanding the membrane area by an average of 15,000-fold (as in eukaryotes) can only be achieved by increasing the total number of mitochondria, each one with its own necessary genome controlling respiration locally [17]. Scaling up requires extreme polyploidy of the mitochondrial genome [10]. Large amoeba might have as many as 300,000 copies of mtDNA [29,30].

What happens if bacteria are scaled up to eukaryotic volumes? Few such behemoths exist but some are known, such as *Epulopiscium* and *Thiomargarita*. These are larger than most eukaryotic cells, with an extensive surface area of bioenergetic membranes, albeit much of their internal volume is metabolically inert. If genome outposts are needed to control respiration, these giant cells should exhibit extreme polyploidy too. That is indeed the case. *Epulopiscium* has up to 200,000 copies of an identical 2.8 Mb genome — each ~150 times larger than a mitochondrial genome — placed roughly equidistantly along the plasma membrane [31]. *Thiomargarita* has some 15,000 copies, again placed right next to the plasma membrane [31]. When

the costs of extreme polyploidy are taken into consideration the difference between bacteria and eukaryotes is clear [10] (Figure 1). Consider the cost of expressing 100,000 mitochondrial versus 100,000 bacterial genomes. Giant bacteria must carry 260,000 Mb more polyploid DNA than comparably sized eukaryotes such as amoeba. Given a standard prokaryotic gene density of 1,000 genes per Mb, that's 26 million *more* genes that need to be expressed. No wonder giant bacteria are so rare.

The paucity of giant bacteria points to another interesting problem: empirical analysis of real cells can mislead. Since eukaryotic-sized bacteria that lack extreme polyploidy are simply not known, it is not possible to measure their metabolic rates or the costs of gene expression. Log-log plots showing the number of ATP synthases or ribosomes against cell volume invariably cluster all bacteria down at the base of the plot, whereas eukaryotes scale up over the next 3–5 orders of magnitude [23]. The rarity of giant bacteria necessarily limits the generalisability of any empirical analysis [32]. Biology needs to explain not only what is seen, but also what is not seen. The simple prediction from bioenergetics is that large bacteria will be more polyploid, with genomes placed right next to bioenergetic membranes, but surprisingly little is known about ploidy at present [32]. Large cyanobacteria have hundreds of copies of their complete genome [33] as does the large freshwater bacterium *Achromatium* [34]. These genomes are indeed placed right next to membranes between the calcite granules that make up the bulk of cell volume [34]. But the costs of polyploidy in scaling up gene expression means that eukaryotic-sized giant bacteria are extreme outliers.

The complex consequences of endosymbiosis

The difference between endosymbiotic bacteria and polyploid genomes is that bacteria can grow and divide, competing amongst themselves and losing genes over time [10,24,25]. Genomes aren't autonomous and can't copy themselves or compete in that way. Polyploid genomes therefore tend to remain similar in size over generations and can't specialise for bioenergetics. Endosymbiosis is arguably necessary to fashion small, specialised 'bioenergetic' genomes like mitochondrial genomes, with all the energetic advantages that gene loss confers [10]. Although eukaryotes are defined by their true nucleus, it is more helpful to think of them as being defined by genomic asymmetry: a massively expanded nuclear genome is supported energetically by hundreds or thousands of tiny mitochondrial genomes. These genomes are integral parts of discrete functional units (the mitochondria), which enables selection for discrete mitochondrial phenotypes associated with specific genotypes. That sets mitochondrial DNA apart from plasmids, which are not part of discrete functional units and therefore lack these tight phenotypic associations (Box 1). Breaking of genomic symmetry entails a functional cooperation — one promoted by endosymbiosis.

If endosymbiosis is necessary for the evolution of eukaryotic complexity, then the rare occurrence of endosymbioses between prokaryotes could explain the apparently singular origin of eukaryotes and perhaps many unique eukaryotic traits that did not evolve in prokaryotes, such as meiotic sex [24]. There is a striking paradox about the deep conservation of virtually all aspects of eukaryotic cell structure, from the nucleus itself to endomembrane systems and traits such as mitosis and meiosis. Given that plants, animals, fungi and protists have radically different lifestyles, it's unlikely that the evolution of shared eukaryotic traits reflects adaptation to any specific external environment (Box 2). But these conserved traits could reflect adaptation in response to a common internal feature — endosymbionts [25]. Both host cells and endosymbionts certainly had their own interests [35]. In exchange for unprecedented supplies of energy, this intimate and ultimately obligate relationship must have provoked stress in a 'naïve' archaeal host. Endosymbiosis did not abruptly transform the host cell into a hopeful monster, but it permanently altered the selective forces operating on proto-eukaryotic cells and the long-term evolutionary outcomes. For example, the expansion in genome size permitted by the acquisition of mitochondria may have necessitated the evolution of meiosis and sex from lateral gene transfer in prokaryotes, utilizing the same machinery for homologous recombination [24].

The fact that all eukaryotes share a large number of traits that are essentially

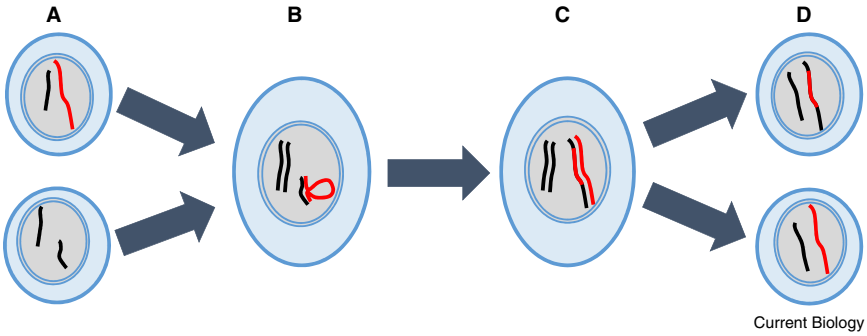


Figure 2. Only sex can load genes reciprocally.
The acquisition of mitochondria permitted larger genomes, while forcing host cells to adapt to a rapidly changing internal environment. Different adaptations to this internal environment can be pooled by meiotic sex, which is reciprocal and systematic across the whole genome. The early evolution of sex could therefore explain why all eukaryotic cells share the same traits, from the nucleus to endomembrane systems. (A) Two gametes each contain two chromosomes, one of which is homologous, while the other (in red) only has matching sequences in the end regions. (B) The chromosomes line up in the zygote before undergoing meiosis. The red chromosome pairs in the matching end regions, leaving a non-homologous loop. (C) Incorporation of missing DNA by standard homologous recombination, as in bacterial transformation via lateral gene transfer. In contrast to meiotic sex, however, bacterial lateral gene transfer is piecemeal and non-reciprocal, and so does not accumulate genes in all the cells of a population. (D) Regeneration of haploid gametes, which now all contain the missing DNA, illustrating how meiotic gene loading can in principle accumulate all eukaryotic traits in a sexually recombining population adapting to mitochondria. Although this model technically violates Mendel's law of segregation, it is a predictable intermediate between prokaryotic lateral gene transfer and true meiosis in modern eukaryotes.

absent from bacteria and archaea suggests that they arose in a sexually reproducing population [24,25]. Only sex (as opposed to cloning or lateral gene transfer) will accumulate traits within a population (Figure 2). This in turn implies that there must have been a tight population bottleneck at the origin of eukaryotes, as the only survivors were part of a single, sexually reproducing population; there are no known examples of cells that diverged before the evolution of the endomembrane system, the nucleus, mitosis, a dynamic cytoskeleton or vesicular trafficking [24,25]. Although it is possible that they were all outcompeted to extinction by more sophisticated eukaryotes, the existence of a large group of morphologically simple cells, the Archezoa — once thought to be evolutionary intermediates lacking mitochondria and some endomembrane systems [11] — shows that the niche is ecologically viable. The finding that mitochondria are an ancestral feature of all eukaryotes could explain the apparently singular origin of the complex eukaryotic cell: not only is endosymbiosis between two prokaryotes rare, but the potential for conflict in the fledgling symbionts was always more likely to end in extinction than complexity.

If the requirement for electrically charged membranes did shape the trajectory of cell evolution on Earth, as argued here, how did cells come to be constrained that way in the first place? Whereas the machinery for ATP synthesis is dauntingly complex, the iron–sulfur proteins involved in CO_2 fixation — such as the energy-converting hydrogenase — are far simpler and have plausible prebiotic precursors [3]. One hypothesis suggests that geologically sustained proton gradients in hydrothermal vents could modulate the reduction potentials of H_2 and CO_2, driving the formation of organic molecules [36]. Given that such vents seem to be ubiquitous on wet, rocky planets and moons, it's feasible that life across the universe could be constrained by electrical charges on membranes too. If so, then the peculiar trajectory of life on Earth might turn out to be predictably normal. In any case, the structure of energy flow in relation to genes and cell membranes is likely to have shaped cell evolution in pervasive and unexpected ways.

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