Mitochondrial evolution: Gene shuffling, endosymbiosis, and signaling

Parth K. Raval*, William F. Martin, Sven B. Gould

Genes for cardiolipin and ceramide synthesis occur in some alphaproteobacterial genomes. They shed light on mitochondrial origin and signaling in the first eukaryotic cells.

Mitochondria are curious entities. They supply energy for the cell and house numerous biosynthetic pathways. They used to be free-living bacteria but now are double membrane–bounded compartments within the cytosol of eukaryotic cells, where they perform a variety of functions. Mitochondria took up residence as endosymbionts, cells living within another cell, some 1.5 billion years ago (*1*). They have preserved many traits inherited from their free-living ancestors, the alphaproteobacteria, a highly diverse and versatile group of prokaryotes. But in the process of transforming from bacteria into mitochondria, they relinquished almost all of their genes to the nucleus, thereby contributing to the evolution of new functions that emerged during the course of eukaryote evolution.

The list of essential eukaryotic cell functions that involve mitochondria is long and still growing. It includes energy metabolism, redox balance, apoptosis (programmed cell death), iron-sulfur cluster assembly, the cell cycle, lipid synthesis, membrane flux, intracellular signaling, autophagy (degradation of intracellular proteins and organelles), and more (*2*–*5*). These essential functions of mitochondria are conserved across all eukaryotic groups, tracing the backbone of eukaryotic cell biology to the mitochondria of the last eukaryotic common ancestor (LECA) (*5*). Many molecular components of eukaryote-specific structures and processes stem from the genome of the mitochondrial symbiont. Knowing more about the nature of the bacterium that gave rise to mitochondria can thus help us understand how eukaryote cellular complexity came to be, and this is where Geiger *et al.* (*6*) weigh in with their investigation of genes for mitochondrial traits among alphaproteobacteria.

Geiger and colleagues (*6*) surveyed bacterial genomes for dozens of traits that link mitochondria to their free-living bacterial cousins. They sought genes for mitochondrial biosyntheses and for mitochondrion-specific operon structures typical of mitochondrial DNA (mtDNA). These traits are present in various free-living alphaproteobacteria, but not in all lineages, and they occur at different frequencies across different lineages. Geiger *et al.* (*6*) reasoned that those lineages with the highest frequencies of mitochondrion-specific traits would help identify the nature of the ancestor from which powerhouses of eukaryotic cells arose. An important message of their findings is that mitochondrial traits are not restricted to one particular alphaproteobacterial lineage, rather they are distributed, with some lineages having more and some having less. To use an analogy, it is as if someone had "shuffled the cards" of genes for mitochondrial traits among free-living bacteria, including alphaproteobacterial, in the roughly two billion years since LECA lived.

"Shuffling the cards" is exactly what lateral gene transfer (LGT) does (Fig. 1). LGT shuffles genes across genomes and species boundaries over evolutionary time, constantly generating new combinations and collections of genes in prokaryotic chromosomes. The set of genes we see in a given bacterial genome today will not be the same ones we would see a billion years from now because LGT will constantly bring in new genes at the expense of old ones. Even two strains of the same modern bacterial species can differ by 30% of their genes. Because LGT in prokaryotes generates gene flux over time, no single alphaproteobacterial lineage has the same collection of genes that the ancestor of mitochondria had 1.5 billion years ago.

This kind of fluidity in prokaryotic genomes is not a new insight, but it does complicate matters for phylogenetics experts trying to identify bacterial lineages that lived at the time of LECA. If we want to reconstruct the genome of the free-living mitochondrial ancestor, we have to look for its genes as they have been dispersed through geological time across modern bacterial genomes because the genome of the last common mitochondrial ancestor no longer exists, although its genes were deposited in the chromosomes of LECA through gene transfer from the mitochondrion's genome to the nuclear genome of its host. This is why the genes that Geiger *et al.* (*6*) studied for aerobic traits, anaerobic traits, and lipid metabolism, though acquired from one and the same mitochondrion, do not trace to one alphaproteobacterial lineage today (*7*).

From a cell biology perspective, the most significant finding of Geiger *et al.* (*6*) concerns genes for the synthesis of two kinds of lipids that are typical for mitochondria: cardiolipin (CL) and ceramide. CL is a dimeric lipid, linking four fatty acid chains to three glycerol molecules (Fig. 1). CL typically comprises 15 to 20% of mitochondrial lipids; its dimeric nature gives it a conical shape. CL is synthesized in mitochondria where it is actively involved in respiration, energy production, ROS production, cristae morphology, mitochondrial fission and fusion, mitochondrial biogenesis, protein import, mitochondrial carriers, apoptosis, and mitophagy (*3*, *4*, *8*, *9*). Those are a lot of functions for a lipid, one might think, but CL is important. In eukaryotes,

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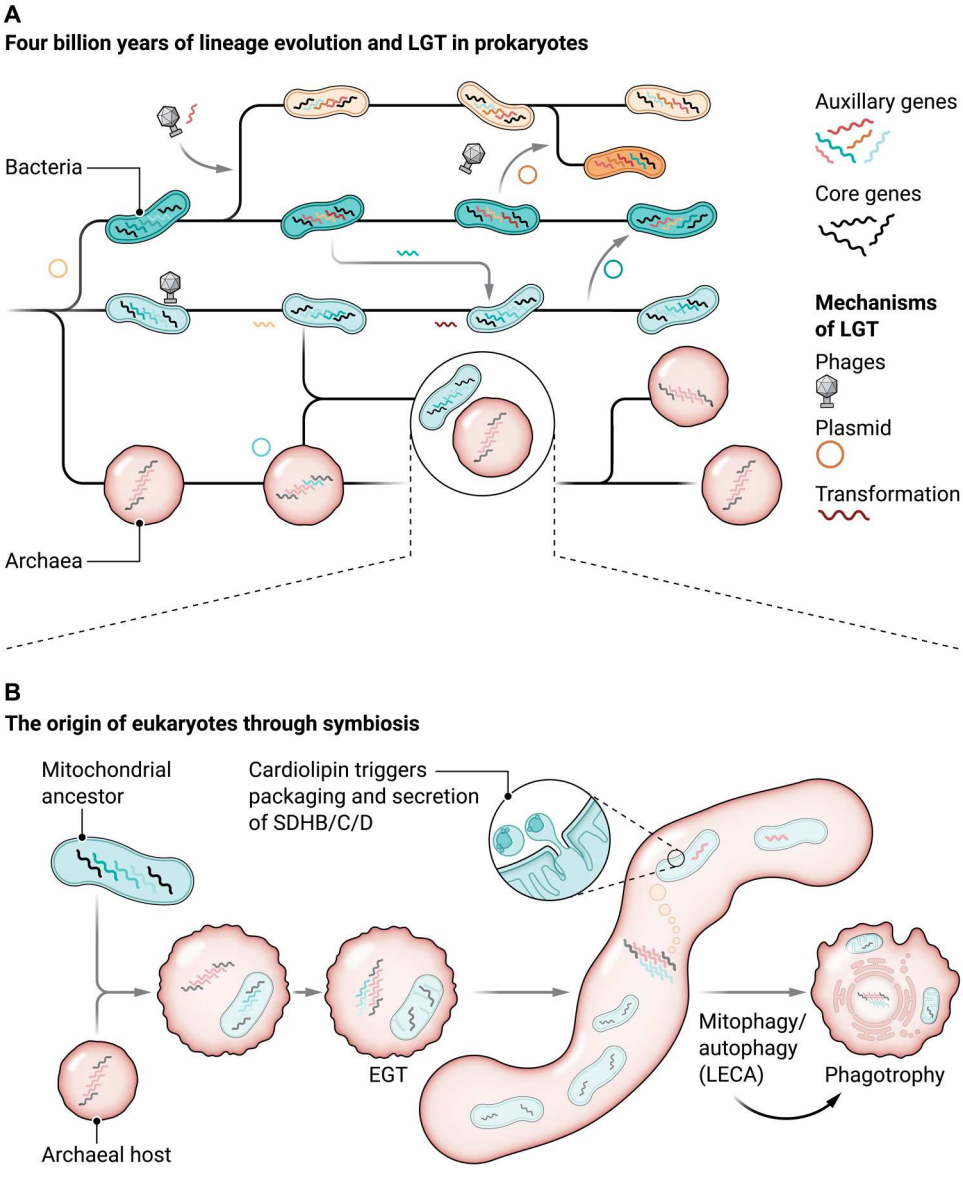

Fig. 1. Gene shuffling, endosymbiosis, and signaling. (**A**) Lateral gene transfer (LGT) continuously shuffles genes for physiology and metabolism (indicated by strands of different colors) in prokaryotes (bacteria and archaea). (**B**) Mitochondria (and the eukaryotic domain) are outcomes of a merger between (alphaproteo-)bacteria and archaea. Genes present in the alphaproteobacterial ancestor of mitochondria were also shuffled, via LGT, in its free-living relatives before, during, and after mitochondrial origin. Today, this ancestral combination of genes is dispersed across many modern bacteria. This includes genes to synthesize cardiolipin and ceramide that were present in the mitochondrial ancestor; after endosymbiosis, these genes were transferred to the host genome and were likely present in LECA. Mitochondrial signaling via these two lipids was likely established in LECA, triggering the degradation of malfunctioning mitochondria, a prerequisite for phagotrophy (the ability to engulf and digest prokaryotes as a food source). Credit: Austin Fisher/*Science Advances*.

there are two routes of CL synthesis that tend to be mutually exclusive, although a few eukaryotes possess both. Geiger *et al.* (*6*) identified alphaproteobacteria that encode the genes for both biosynthetic routes to CL synthesis, suggesting that the ancestral mitochondrion did as well, having donated its genes to LECA followed by differential loss during eukaryote lineages diversification.

Geiger *et al.* (*6*) also surveyed the distribution and phylogeny of genes for synthesis of another lipid too often overlooked in evolutionary issues, ceramide, a sphingolipid that uses serine instead of glycerol in the polar head group (Fig. 1). Sphingolipids are central to many signaling processes in eukaryotes. Their synthesis occurs in mitochondria and at mitochondrial-associated membranes (MAMs), the membrane contact sites between the endoplasmic reticulum and the outer mitochondrial membrane. The process starts at serine-palmitoyl transferase (SPT), an enzyme specific to the sphingolipid synthesis pathway. As for other mitochondrial traits sampled, the genes for ceramide biosynthesis are present in some alphaproteobacteria, being more frequent in some groups than in others, but not restricted to one single

lineage (*6*)—the workings of LGT. Accumulation of both ceramide and CL in mitochondria signals to the cell to induce mitophagy, under certain conditions even apoptosis (*8*,*9*).

The approach taken by Geiger *et al*. (*6*) examines microbial evolution as it occurs in nature: one gene at a time and every gene for itself. In prokaryotic genomes, genes and operons (clusters of co-regulated genes) for physiological traits can be continuously transferred and thus evolve, over time, independently from one another so as to generate new combinations of genes and biochemical capabilities across lineages. Each of these new combinations has the potential to be useful, hence selectable under specific environmental conditions. This patchwork mode of evolution contrasts with that observed for prokaryotic ribosomal proteins, which are universally present in all lineages and rarely the target of LGT (*7*). These contrasting evolutionary modes in prokaryotes—a lateral inheritance of physiological traits and vertical inheritance of ribosomes—generate conflicting pictures of mitochondrial evolution, but both pictures are valid. Prokaryotic distributions of genes for physiological traits (*6*) do not map cleanly onto phylogenies of ribosomal proteins because genes for traits undergo LGT. To get a fuller picture of mitochondrial origin, we need to take both physiological traits and phylogeny into account.

The most exciting aspect of the new work (*6*) exposes mitochondrial signaling in LECA. The genes for CL and ceramide synthesis are traced to alphaproteobacteria, the ancestors of mitochondria, and both lipids are not only involved in mitochondrial signaling but also involved in the induction of mitophagy (*4*). Mitophagy is a process in which defective mitochondria are targeted for degradation in the digestive tract of the cell: autophagosomes and lysosomes. Mitophagy can be induced by nutrient limitation and can weed out malfunctioning mitochondria but also provides means to use defective mitochondria as a food source under starvation conditions. It is curious that mitophagy is conserved across all eukaryotic groups (*8*), whereas phagotrophy—the process of eating bacteria for a living—is not (*10*). This is exemplified by the fungi, the vast and ancient eukaryotic group where mitophagy was discovered but phagotrophy never occurs.

The presence of mitophagy but the absence of phagotrophy in LECA makes sense. Any host cell housing intracellular bacteria absolutely requires means to dispose of dysfunctional endosymbionts (mitophagy). The ability to engulf bacteria (phagocytosis) is useless unless the cell already has the intracellular machinery (autophagy) to digest them. Today, CL and ceramide in mitochondrial membranes signal to the cytosol that the endosymbiotic organelle is ready for elimination via mitophagy. This could be the ancestral state and the initial selective pressure for the origin of vesicle flux, autophagosomes, and lysosomes in eukaryotic cells: endosymbiont cleanup.

The origin of many cell biological traits of nucleated cells can be understood as evolutionary responses to the challenges posed by the existence of permanent endosymbionts in an archaeal cytosol that was on its way to becoming eukaryotic (*5*). Once LECA had signaling, mitophagy, and lysosomal digestion in place for disposal of rogue mitochondria, then diversifying eukaryotic lineages could have coopted this machinery during the subsequent evolution of pinocytotic and phagotrophic lifestyles, where food particles are acquired from outside the cell and digested within. The functions and conserved antiquity of mitochondrial signaling in mitophagy, mediated by CL and ceramide, suggest that these pathways arose in response to the presence of mitochondria in LECA.

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