



From microbiology to cell biology: when an intracellular bacterium becomes part of its host cell

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Mitochondria and chloroplasts are now called organelles, but they used to be bacteria. As they transitioned from endosymbionts to organelles, they became more and more integrated into the biochemistry and cell biology of their hosts. Work over the last 15 years has shown that other symbioses show striking similarities to mitochondria and chloroplasts. In particular, many sap-feeding insects house intracellular bacteria that have genomes that overlap mitochondria and chloroplasts in terms of size and coding capacity. The massive levels of gene loss in some of these bacteria suggest that they, too, are becoming highly integrated with their host cells. Understanding these bacteria will require inspiration from eukaryotic cell biology, because a traditional microbiological framework is insufficient for understanding how they work.

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We microbiologists can be a bit defensive about our organisms. We know that microbes are the most genetically and metabolically diverse organisms on Earth [1], that they drive nearly all biogeochemical cycles [2], and that all macroorganisms require the presence of microorganisms for optimal health and survival [3] (but that the opposite is not true). We know all of this, but despite our best efforts to remind our colleagues of these important facts at every possible opportunity, we still suspect that our macroorganism-focused friends really do not care.

Perhaps, sometimes, this defensiveness can breed zealotry. If a microbial zealot thinks of the eukaryotic cell at all, their focus is usually restricted to the mitochondrion and chloroplast. These structures are clearly the only interesting parts of eukaryotic biology, because they

are the only organelles of bacterial origin [4–7]. Although some microbiologists might allow that the morphological diversity of eukaryotes is interesting, to a microbial purist these beautiful forms are really just elaborate ways of moving around mitochondria and displaying chloroplasts.

No matter one's enthusiasm for the microbial world, the idea that the origin of the mitochondrion was important in the evolution of the eukaryotic cell is not controversial. If true eukaryotes existed before the mitochondrion, none apparently survived; all extant eukaryotes have mitochondria or degenerate mitochondria-like organelles called hydrogenosomes and mitosomes [5,8]. Arguments in the field of eukaryotic cell evolution now concern the timing and effect of the mitochondrial acquisition [9^{**},10–13]. Did a cell that looked at least a bit like a modern eukaryote become the mitochondrial host, perhaps engulfing it by endocytosis, or did the mitochondrial acquisition happen early, by other mechanisms, and drive the evolution of the eukaryotic cell? These are very difficult questions to answer, because no intermediate forms exist — the last universal eukaryotic ancestor had most of the canonical features seen in eukaryotes today [14^{*}].

However, recent results seem to be converging on a partial answer to these questions. Phylogenetic [15,16] and genomic [17^{*}] results both point to organisms from the TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota, Korarchaeota) superphylum of Archaea as our closest prokaryotic cousins [18,19^{**}]. TACK metagenomes encode pathways previously thought to be limited to eukaryotes, including endosomal sorting complexes required for transport (ESCRT) pathways, homologs of actin and tubulin, and the ubiquitin pathway [10,17^{*}]. Together these results suggest that eukaryotic organisms are not sister to Archaea as they are generally shown in textbooks [20], but rather they emerged *from* them. Although these issues are far from resolved [13], several lines of evidence point to the idea that the mitochondrion might have been acquired by a cell with some eukaryotic features, perhaps somewhat late in the evolution of the eukaryotic cell [12,21].

What makes all of these inferences difficult is the fact that the mitochondrial and chloroplast acquisitions each occurred only once, approximately 1.8 billion [22] and 1.5 billion [23] years ago, respectively. Drawing firm conclusions about the timing and nature of the organisms involved in these acquisitions will therefore probably

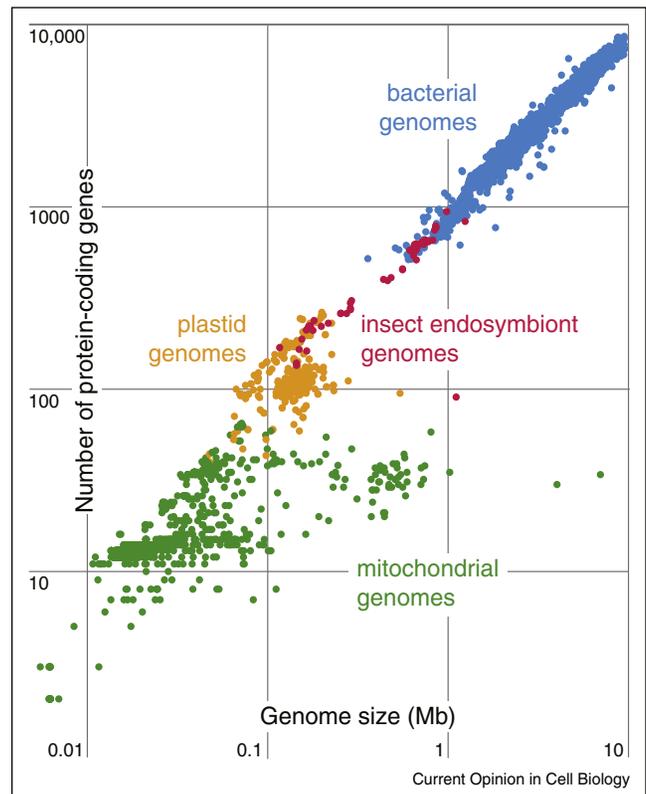
always be difficult [14*]. Of course it makes sense to try, because the only way to really understand the evolution of mitochondria and chloroplasts is to study the evolution of mitochondria and chloroplasts. But other, more modern endosymbioses are available that might tell us something about how organelles came to be, and why they look the way they do. While these newer symbioses will never recapitulate the evolution of the mitochondria and chloroplasts — nothing could — they can reveal the general outcomes that occur as a result of intimate, long-term endosymbiosis, and thus *may* have happened during their evolution [24**]. Or they may not, but it seems worthwhile to try this approach, too.

Insect endosymbionts are missing (genomic) links between bacteria and organelles

It is now widely appreciated that all animals form symbioses with bacteria [3,25]. Insects are especially interesting in this regard because they form many intracellular symbioses — that is, they allow bacteria to live inside their cells — that are not pathogenic from the host perspective [25–28]. These intracellular bacteria have broad effects on insect biology. They are sometimes reproductive manipulators [29], but often provide an array of protective functions for the host [30,31]. Most relevant here are the special cases where intracellular bacteria provide absolutely critical nutrients for sap-feeding insects [32–34]. Like all animals, sap-feeding insects cannot make 10 of the required 20 amino acids (the essential amino acids), and so must get them from their diet. This is a problem, because essential amino acids are not found at high levels in plant sap [35]. Sap-feeding insects solved this dilemma by establishing intracellular symbioses with bacteria and fungi that function as little essential amino acid producing factories. Symbiosis with these microbes thus allows exploitation of a new food source, but also then *requires* the presence of these microbes for the host's continued survival. It is important to realize that once you go down this 'rabbit hole,' it is very difficult to go back [36]. Just as eukaryotic cells are strictly dependent on their mitochondria, sap-feeding insects are strictly dependent on their nutritional bacterial endosymbionts.

Comparing genome size and coding capacity for bacteria, chloroplasts, and mitochondria reveals three interesting features (Figure 1). The first is that genome size and coding capacity form an unbroken continuum from free-living bacteria (the largest genomes, typically spanning 3–14 megabase pairs (Mb) in length) to host associated bacteria (typically the smaller genomes centered around 1 Mb), to the endosymbionts of insects (the smallest bacterial genomes, spanning 0.1–1 Mb), followed by the chloroplast and mitochondrial genomes. The second feature, related to the first, is that the genomes of insect endosymbionts are the smallest of all sequenced bacteria and overlap organelle genomes in terms of size and number of genes. The third is that the age of endosymbiosis is reflected in genome size.

Figure 1



Genomes from bacteria, insect endosymbionts, chloroplasts, and mitochondria form an unbroken continuum of size and coding density. The plot is truncated at 10 Mb and 10,000 genes.

The oldest endosymbionts, the mitochondria (~1.8 billion years old [22]), have the most reduced genomes encoding the fewest genes. These are followed by the genomes from chloroplasts (~1.5 billion years old [23]), and then the endosymbionts of sap-feeding insects (of various ages, but in the range of 30–300 million years old) [37]. Age recapitulates genome size in endosymbionts.

Of course, measuring genome size and counting numbers of protein-coding genes tell us little about how organisms function. It is instructive to dig a bit deeper and compare the genes retained and lost on organelle and endosymbiont genomes.

Genes retained on organelle and insect endosymbiont genomes

First, a disclaimer: The coding capacity of mitochondrial genome varies widely (Figure 1), making sweeping generalizations about gene content difficult. Some mitochondrial genomes encode 100 genes on a single distinctly bacterium-like chromosome, some retain just a few genes on small linear chromosomes, and some have been completely eliminated [38,39]. So it is only by ignoring this diversity that allows me to say the genes retained on

organelle and tiny endosymbiont genomes can be grouped into three broad categories [39–43]. The first relates to translation. Ribosomal RNAs, tRNAs, and ribosomal proteins are universally found on all organelle and endosymbiont genomes. The second are transcription-related genes. The larger mitochondrial, most chloroplast, and endosymbiont genomes encode some fraction of the core bacterial transcriptional machinery. Finally, in what could be considered the ‘signature gene set,’ genes related to the actual function of the organelle or endosymbiont are found. In chloroplasts, these genes are related to generating energy from sunlight [41]. In mitochondria, these genes are related to energy generation from electron transport [40]. In insect nutritional endosymbionts, these genes are related to essential amino acid and vitamin production [34,44]. Genes retained on these genomes thus reflect the core informational processes of translation (mostly), transcription (a bit), and replication (but hardly), as well as the function the endosymbiont or organelle provides to its host.

Genes missing from organelle and endosymbiont genomes

If the genes present on organelle and endosymbiont genomes reveal what they do for their hosts, the genes that are *missing* hint at what the host must provide to the symbiont. Said another way: How do organelles and endosymbionts survive with such tiny genomes?

The broad outlines of how mitochondria and chloroplasts survive with genomes encoding tens or hundreds of genes are well known. The majority of gene products needed for organelle function are encoded by genes on the nuclear genome, which in many cases have been relocated there by horizontal gene transfer (HGT) during the establishment of the organelles [45,46]. Both mitochondria and chloroplasts have elaborate protein import systems, again encoded on the host genome, which transport these required proteins back into the organelles [47,48]. The targeting and taxonomic affiliation of these proteins can be complex, especially in the photosynthetic eukaryotes [49]. As one example, consider the aminoacyl-tRNA synthetases (aaRSs), the enzymes used to charge tRNAs with the appropriate amino acid. These are essential and ubiquitous enzymes, and most cellular organisms encode genes for twenty enzymes that charge the appropriate tRNA with one of 20 amino acids (or use some defined alternative pathways to perform the same function) [50]. Both mitochondria and chloroplasts have their own translation system requiring their own charged tRNAs, but their genomes encode no aaRSs. How does this work?

The simple expectation is that the nuclear genome of photosynthetic eukaryotes would encode 60 different aaRSs: 20 from the eukaryotic lineage, 20 that have been horizontally transferred from the ancestral mitochondrial genome, and 20 that have been horizontally transferred

from the ancestral chloroplast genome. But in *Arabidopsis thaliana*, only 45 aaRSs are found on the nuclear genome [51]. Twenty-four of these are of mitochondrial or chloroplast origin, and of these 24, 15 are targeted to both mitochondria and chloroplasts [51]. It is not just aaRSs that are targeted to multiple compartments in the cell — and it is not just *Arabidopsis* that shows these complex targeting patterns [52] — many important proteins related to tRNA biogenesis and genome replication, recombination, and repair are also dual targeted [49]. Eukaryotes are mosaic organisms, and have figured out how to build organelles from taxonomically diverse genes sets.

When the first insect endosymbiont genome was sequenced, the lack of genes required to produce components of the cell envelope such as lipopolysaccharides and phospholipids was surprising, and it suggested that these bacteria required extensive cooperation from the host [32]. Looking more broadly across the many tiny endosymbiont genomes now available reveals profound levels of gene loss in fundamental cellular processes [42–44]. The tiniest genomes such as those from *Nasuia*, *Tremblaya*, and *Hodgkinia* encode few or no genes involved in constructing a cell envelope, and are missing many aaRSs and genes involved in genome replication and repair [42–44]. These genomes therefore not only show parallels to organelle genomes in terms of size and genes retained, but also in genes lost.

From microbiology to eukaryotic cell biology

The similarities between insect endosymbionts and organelles are clear. Both are of bacterial origin and have experienced large levels of gene loss. Both encode their tiny gene sets on genomes that are often, but not always [53–56], small in size. Both have lost genes to autonomously construct cell envelopes, along with essential translation-related genes such as tRNAs and aaRSs. Both rely on bacterial genes that have been horizontally transferred from other bacteria to the host genomes [45,57,58*,59–61]. (There is now evidence that protein made from at least one of these horizontally transferred genes is transported back into insect endosymbiont cells [62*].) Both endosymbionts and organelles are absolutely required for normal host function and host survival. The question is: What do these parallels tell us about how endosymbionts actually work?

There are many more questions than answers, especially when considering these symbionts as I have for most of my career — that is, as a microbiological zealot. How do you make a bacterium without the ability to make a cell envelope, and without all of the required aaRSs? The cell biological and organelle literature at least provide a bit of focus. The endoplasmic reticulum (ER) contains most of the enzymes eukaryotic cells use to build membranes, and mitochondrial membrane systems are likely

constructed via special ER interaction sites [63,64*] (yet they somehow retain some bacterial lipid signatures [65]). What kind of interactions might endosymbionts have with the ER, and could this be how membranes are built in endosymbionts that lack the ability to do it themselves? If so, did they coopt the mitochondrial system, or did they find another way to do it? Mitochondria and chloroplasts retain the ability to translate some proteins but must import aaRSs and tRNAs of diverse taxonomic origins to do so. Some endosymbionts with tiny genomes such as *Hodgkinia* have incomplete sets of aaRSs and tRNAs, but shotgun proteomics shows that *Hodgkinia* protein synthesis still occurs [66]. Has *Hodgkinia* coopted the preexisting aaRS targeting system to become another cellular compartment that requires import of cytoplasmic or mitochondrial aaRS, or has it found another way to assure charged tRNAs are available for translation? These are important questions. They are also, alas, not microbiological questions. Answering them will require a jump into cell biology from a field dominated by microbe-loving scientists.

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