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The Serial Endosymbiosis Theory: Cellular Origins and Intelligent Design Theory

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Since [Lynn Margulis gave the serial endosymbiosis theory (SET) its modern expression, it] has received nearly universal acceptance as an explanation for the origin of mitochondria and chloroplasts.

Even though the origin of cells remains largely unresolved, the serial endosymbiont theory is widely accepted as the means by which two organelles, mitochondria and chloroplasts, came to be. The serial endosymbiont theory hypothesizes that mitochondria and chloroplasts were derived from ancient bacteria that were engulfed by an ancient, nucleated cell and took up residence in the cytoplasm of the nucleated cell, until over time these internalized cells became organelles. Several lines of evidence support the serial endosymbiont theory and associations between several species of insects and various microbes also provide convincing examples of intermediates in the process by which a microorganism becomes an organelle. The pervasiveness of endosymbiosis in nature suggests that organisms have a tendency to form mutually beneficial relationships. This tendency to form such relationships reflects the goodness that God imparted to creation and is somewhat antithetical to traditional Neo-Darwinism. Alternatively, the data suggest that more purposeful forces or principles might guide the formation and subsequent maturation of such relationships.

The origin of cells is a subject of intense debate within biology, but most creationists and intelligent design theorists find the formation of any cell from nonliving molecules simply impossible.¹ Nevertheless there is substantial agreement, at least among the majority of mainstream scientists, on the origin of two subcellular structures within some cells.

According to contemporary evolutionary thinking, two compartments inside cells, mitochondria and chloroplasts, are descended from bacteria that were engulfed by ancient cells and took up residence inside their hosts. Normally the predatory cell would digest the bacteria as food, but for some reason the invaders were not digested this time. The two cells began a mutually beneficial relationship in which the hitchhiking bacterium gave chemical energy to the host and the

host protected the tiny interloper. Over time, this relationship grew into one of mutual dependence until the bacterial invader became recognizable only as an organelle, or miniature organ, within a host cell that depended heavily upon the activity of the newly-minted organelle for its survival.

The idea outlined above is called the serial endosymbiosis theory (SET). A. F. W. Schimper first proposed this idea in 1883,² but Lynn Margulis gave it its modern expression.³ Since then, the endosymbiont theory has received nearly universal acceptance as an explanation for the origin of mitochondria and chloroplasts. Even though the evidence used to support the endosymbiont theory is deep and broad, this theory proposes that mutually beneficial associations between organisms is a major driving force behind the formation of new species.⁴ Such an evolutionary mechanism is somewhat non-Darwinian, and even represents a challenge to modern neo-Darwinian thought.⁵

It is the goal of this article to present the data used to support the endosymbiont theory, especially the flood of new sequence data. However, the data that corroborates the endosymbiont theory also show that

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mitochondria and chloroplasts contain features that are not easily explained by contemporary neo-Darwinism. In fact, some aspects of the origins of these organelles might be better described by an appeal to a less orthodox explanation that requires purposeful, but not necessarily supernatural forces at work. Furthermore, SET supports a tendency for organisms to form interdependent and mutually beneficial relationships, which is not predicted by Darwin's theory of evolution via natural selection. Thus, even though creation contains cruel and harsh elements, it also features organisms working together rather than against each other.⁶ In this way, creation displays how people should work together in humility and mutual dependence, acknowledging our differential giftedness.⁷

Endosymbiosis and Creation

In *The Origin of Species*, Darwin issued this challenge to his readers:

Natural selection cannot possibly produce any modification in any one species exclusively for the good of another species; though throughout nature one species incessantly takes advantage of, and profits by, the structure of another ... If it could be proved that any part of the structure of any species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection.⁸

The selfish character of natural selection seems to contradict the way cooperative associations between very different organisms can mutually benefit each other. Certainly natural selection can account for the establishment of some mutualistic relationships,⁹ but the extent to which we observe endosymbiotic relationships in nature may cause one to ask if some other principle is at work. Normally bacterial cells are food for single-celled, nucleated organisms. Why would an organism form a metabolic bond with an organism it normally views as food?

Despite the pain and suffering in our world, it still declares the glory of God the Creator.¹⁰ It displays the power of God, which in the words of theologian John Rankin, is "the power to give."¹¹ This power to give is part of our being made in the image of God, since we can procreate or give life to another and give of ourselves to others. We show the ability to work cooperatively with mutual interdependence, which, in theory, is most clearly demonstrated in the church. Likewise nonhuman creation, or "nature," is endowed with the power to give, but it also displays the power to take and to destroy human life. Nevertheless, these same organisms still show the power to associate and form mutually beneficial relationships and this is, in an important way, a reflection of the glory of their Creator.

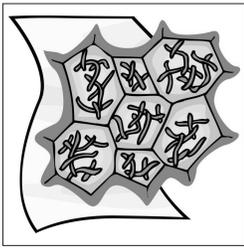
If evolutionary theory has taught theology anything, it is that death is usually necessary to make life possible.¹²

This principle even applied in Eden, where, even if vegetarian diets were the rule, Adam and Eve still needed to eat plants, which required the death of part of the plant. This principle seems to work spiritually as well, since the skin of a dead animal was required to cover the naked bodies of Adam and Eve after they sinned.¹³ Likewise the death of Jesus provides the free option of eternal life for all who embrace his call.¹⁴ Since organisms are able to work together for their own mutual benefit without necessarily killing one another, endosymbiosis seems to be an alternative to the principle of life for one organism arising from the death from another.

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Despite the explanatory power of natural selection and the successes it has had in explaining animal and human behavior, there are elements of human and nonhuman behavior that presently are inexplicable by natural selection alone.¹⁵ There are clear examples of human self-sacrifice that fly in the face of selectionist explanations, since, as sociobiologist Michael Ghiselin claims, if natural selection were true and sufficient as an explanation for human behavior, then there should be no genuinely disinterested behavior.¹⁶ Symbiosis is a major force that drives biodiversity, both presently and in the past.¹⁷ Could it be that the tendency for organisms to associate for each other's mutual benefit is another reflection of design in our world? Testing such a hypothesis would be difficult, but is worth pursuing.

Nature is a reflection of the Creator, even if it sometimes seems a cruel and unforgiving world. The ability of our world to make beauty and goodness from death and suffering is itself an illustration of the grace of God. We can do no less than embrace the good and fight the evil as stewards of the Gospel. We can also do no less to search for his ways within the world, even when they are hidden from plain view.



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Background

All living things are composed of cells and all cells come from pre-existing cells. This simple but elegant statement constitutes the cell theory. It is the culmination of the work of Robert Hooke (1635–1703), who first described microscopic cell remnants from a slice of cork in his popular 1665 book *Micrographia*; Antony van Leeuwenhoek (1632–1723), who described the first observations of living microorganisms using a simple microscope; Matthias Schleiden (1804–1881), who showed that different plant structures were made of cells in 1838; Theodor Schwann, (1810–1882) who extended Schleiden's observations to animals and embryos in 1839; and Rudolph Virchow (1821–1902), who demonstrated in 1858 that all cells come from previously existing cells. The cell theory remains a foundational concept of contemporary cell biology.

Modern organisms are composed of two distinct cell types. The prokaryotic cell type is a relatively simple cell that lacks internal compartments and contains a chromosome devoid of extensive secondary structure. Prokaryotes are well represented today by bacteria. The eukaryotic cell type contains an array of internal, membrane-bound compartments dedicated to specific functions. Because of their specialization, these compartments are called organelles.¹⁸ Eukaryotic cells compose all vertebrate and invertebrate animals, land plants, algae, fungi, and protozoans.

One particular compartment in eukaryotic cells, the nucleus, houses the cell's genetic information. Cells store genetic information in the form of a molecule called deoxyribonucleic acid or DNA, which is assembled into compact, linear macromolecular structures called chromosomes (Figure 1A). The entire complement of genes contained within the nuclei of the cells of an organism is called the genome and a branch of genetics called genomics entails the study of the entire genome of an organism. With the advent of high-throughput automated sequencing, we can determine the sequence of the entire genome of organisms. Today we have the completed sequence for the genomes of over 160 microorganisms and almost twenty-five multicellular organisms, ranging from fungi to humans.¹⁹

Accessing the genetic information stored in DNA requires the synthesis of an informational intermediate molecule called ribonucleic acid (RNA). The DNA molecule serves as a template or pattern for the synthesis of RNA molecules, and RNA synthesis requires a large protein complex called RNA polymerase (Figure 1B). Some RNA molecules called messenger RNAs (mRNAs) are used to make proteins, but RNAs can also perform other tasks.

To make proteins, mRNAs are transported from the nucleus and come into contact with a structure called a ribosome. Ribosomes are the protein-synthesizing machines of the cell and are an assembly of proteins and special RNA molecules called ribosomal RNAs (rRNAs). The ribosomes of eukaryotic cells are distinct from those of bacteria. Herein lies the reason why we can treat diseases with certain antibiotics like erythromycin, tetracycline, and streptomycin that inhibit protein synthesis in bacteria but not in people—ribosomes from bacteria are susceptible to these antibiotics, but such drugs do not affect our own ribosomes.²⁰

Ribosomes cannot make protein by themselves. Instead, they must have an mRNA to direct the synthesis of the protein, and without the mRNA, the ribosome is impotent to work. The sequence of the mRNA is a copy of one of the strands of the DNA molecule, and the ribosome uses this sequence to construct the protein. To make the protein, the ribosome needs the building blocks of proteins called amino acids. Small RNA molecules called transfer RNAs or tRNAs ferry the amino acids to the ribosome. Each specific tRNA carries a particular amino acid and the tRNA-amino acid conjugate comes to the ribosome when the ribosome has engaged a particular three-base sequence or codon in the mRNA. If the three-base codon corresponds to the sequence to which the tRNA can bind, then the tRNA delivers its amino acid payload to the ribosome and the ribosome attaches it to the growing protein (Figure 1C).

Ribosomes also receive assistance from some accessory proteins during the process of protein synthesis. One group of accessory proteins called initiation factors help the ribosome begin protein synthesis. A second

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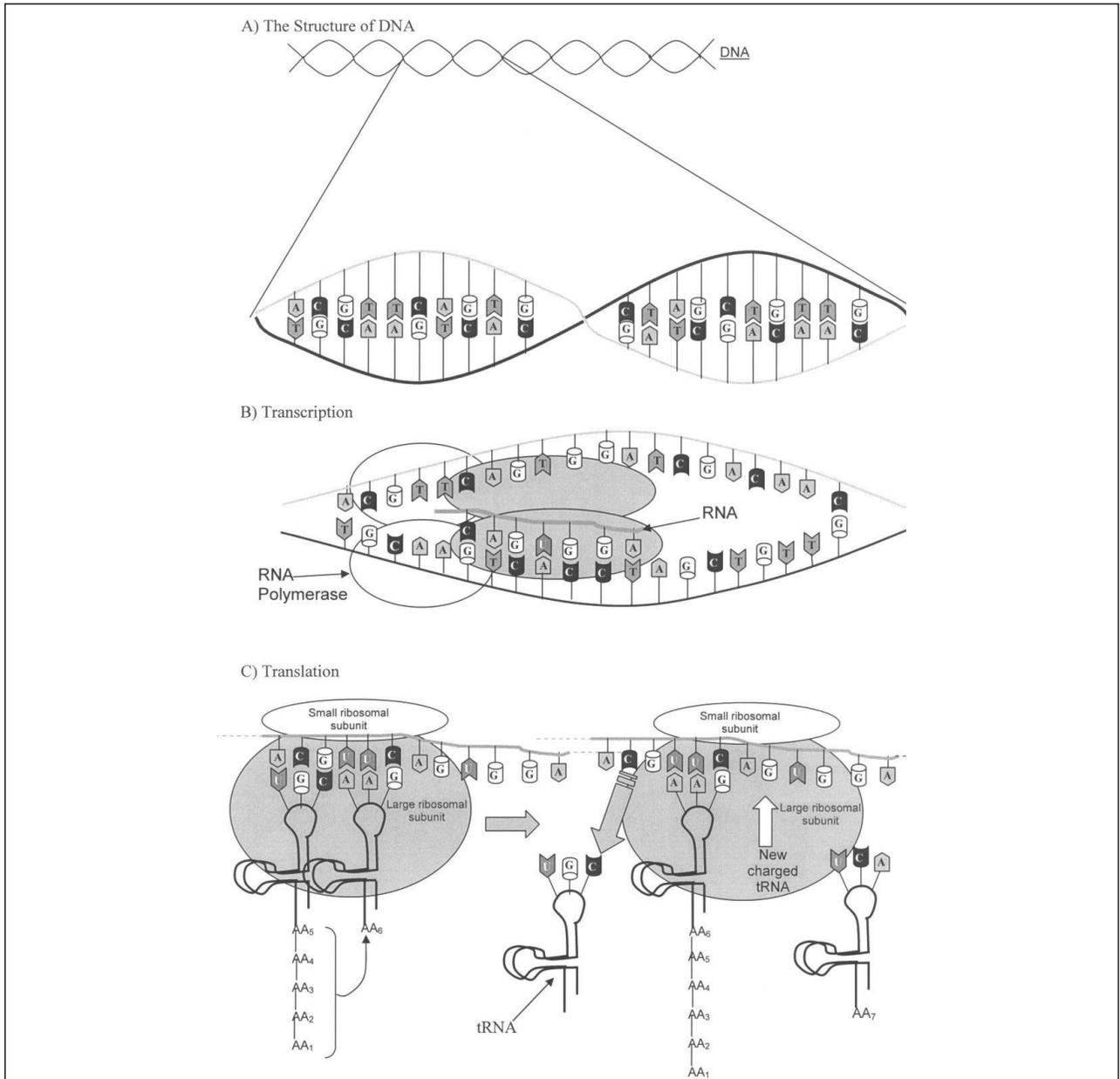
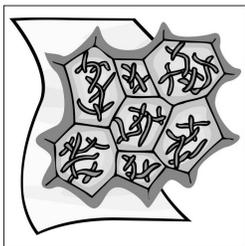


Figure 1. The Flow of Genetic Information. These three figures illustrate the basic elements of molecular biology and how genetic information is stored and accessed by the cell. **(A) A DNA or deoxyribonucleic acid molecule.** DNA is a polynucleotide molecule, which is to say that it is composed of a repeating chain of nucleotides. Nucleotides consist of three chemical entities; a phosphate, sugar, and nitrogenous base. The bases of DNA also show extremely specific rules of interaction; the base adenine always pairs with a thymine and cytosine always pairs with guanine on the opposing strand. Exceptions to these rules occur at the ends of some linear chromosomes. **(B) Transcription or the synthesis of RNA from DNA.** DNA is used as the pattern for RNA synthesis. The enzyme RNA polymerase synthesizes RNA from DNA and the enzyme must unwind the double helix before it can synthesize RNA. RNA polymerase uses the bases of DNA to synthesize an RNA molecule that is matched to one of the strands of the DNA molecule. RNA polymerase accesses the DNA at specific sequences called promoter sequences, which act as entry points for RNA polymerase. RNA is typically a single-stranded molecule. **(C) Translation or the synthesis of protein from an RNA molecule by a ribosome.** Most RNA molecules are messenger RNA molecules, which are used as a pattern for protein synthesis, although some RNAs play structural or regulatory roles. The protein synthesis machines of the cell are ribosomes, which are composed of two subunits, a small and large subunit. The ribosome “reads” the RNA three bases at a time and carrier molecules called tRNAs bring amino acids, the building blocks of proteins, to the ribosome, and the ribosome links these amino acids together to make a protein. Each messenger RNA has a distinct sequence, and from this sequence the ribosome makes a protein with a specific amino acid sequence. How does the ribosome know which amino acid should be added next? The tRNAs that carry the amino acids have a loop at the front of the molecule with a three-base sequence (anticodon). This front-loaded three-base sequence must match the three-base sequence (codon) of the messenger RNA at the ribosome. If it does not match, then the tRNA cannot bind to the ribosome. If it does, then the tRNA binds and its amino acid is added to the growing protein chain. Specific tRNAs with specific three-base sequences in their front loop carry specific amino acids, which means that only the amino acid coded for by the mRNA gets added to the growing protein. The ribosome also gets help during protein synthesis from a cloud of proteins that help start (initiation factors), maintain (elongation factors) and terminate (termination factors) translation. Also specific enzymes called aminoacyl-tRNA synthetases link the amino acids to their specific tRNAs.



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group called elongation factors help the ribosome execute the actual synthesis of proteins, and a third group known as termination factors help end the process of protein synthesis. Finally proteins called aminoacyl-tRNA synthetases attach the amino acids to the tRNAs for use in protein synthesis. All of these accessory proteins play crucial roles in carrying out and regulating protein synthesis. After proper translation of the mRNA, the protein is potentially ready to perform its function.

The Endosymbiont Theory and Its Evidence

Mitochondria and chloroplasts are two organelles in modern eukaryotic cells that are thought to have originated from bacteria that entered a proto-eukaryotic host cell and became part of it. Mitochondria are found

inside almost all eukaryotic cells, and they appear as small sacs surrounded by two membranes. Mitochondria are the powerhouses of the cell, since they make the bulk of the chemical energy required by the cell for its life-sustaining processes (Figure 2A). Chloroplasts, on the other hand, are only found in plants and algae. Green plants contain the pigment chlorophyll, which they use for the process of photosynthesis, and the green, tubular organelles called chloroplasts house chlorophyll and the photosynthetic machinery (Figure 2B). Without chloroplasts plants are unable to carry out photosynthesis and lose their green coloration.

If mitochondria and chloroplasts in modern cells descended from bacteria that came into larger cells and stayed, then these organelles should show similarities to bacteria. Dyer and Obar outline six specific criteria that should be met if chloroplasts and mitochondria descend from bacteria. First, the proteins and enzymes from mitochondria and chloroplasts should be more similar to those from bacteria than any other eukaryote. Second, we would expect these organelles to have retained their own genome and these genomes, including the genes they encode and their mechanisms of gene expression should be more like those of bacteria than eukaryotes. Third, the inheritance patterns of mitochondria and chloroplasts should be separate and distinct from the inheritance pattern of the nuclear genome. Fourth, the RNAs used by each organelle—the rRNAs, tRNAs, and mRNAs—should resemble those from bacteria more than they do those from eukaryotes. In the case of rRNAs, which are the essential structural and catalytic elements of ribosomes, the ribosomes of mitochondria and chloroplasts and their accessory proteins should also more closely resemble those from bacteria in size, structure, and function than those of eukaryotic cells. Fifth, we should be able to find a living bacterium that genetically resembles each organelle. Finally, we should be able to find evidence of organisms that have secondarily lost these organelles.²¹

Proteins are composed of chains of amino acids. By comparing the amino acid sequence of one protein to another, we can quantitatively determine the similarities between two proteins. With the aid of computers, we can compare the amino acid sequence

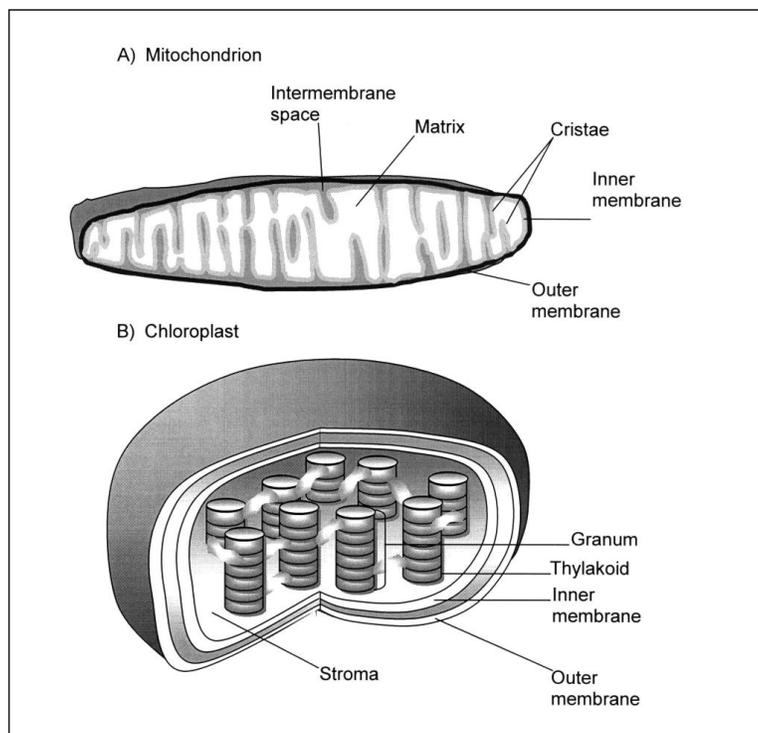


Figure 2. The internal organization of mitochondria and chloroplasts. (A) The internal structure of a mitochondrion. The central core region, or matrix, is surrounded by two membranes, a selectively permeable inner membrane and a relatively permeable outer membrane. The matrix contains the enzymes of the Krebs cycle and the mitochondrial genome. The components of the electron transport chain that are used for oxidative phosphorylation are embedded in the inner membrane, and this membrane is highly infolded in creases called cristae to increase its surface area. **(B) The internal structure of a chloroplast.** Like the mitochondrion, chloroplasts are surrounded by two membranes, but the inner membrane is not folded. The space surrounded by the inner membrane, the stroma, contains enclosed sacs called thylakoid membranes that are organized into stacks called grana. The stroma contains the enzymes of the Calvin cycle and the chloroplast genome. The thylakoid membranes contain the photosynthetic machinery and the electron transport chains that allow photophosphorylation.

similarities between groups of proteins and such comparisons can tell us a great deal about evolutionary relationships between organisms.²² If we use this approach to compare the amino acid sequences of proteins from mitochondria, with a variety of other extant organisms, the greatest similarities are found with proteins from a specific group of bacteria called the α -proteobacteria, particularly the *Rickettsia* subdivision of the α -proteobacteria.²³ Similar comparisons with proteins from chloroplasts show that the most similar proteins are found in a photosynthetic group of bacteria called the cyanobacteria.²⁴ Thus, the amino acid sequences of mitochondrial and chloroplast proteins are most similar to those from specific groups of bacteria.

Mitochondria and chloroplasts also contain their own genomes, and these genomes are every bit as important to the cell as that of the nucleus.²⁵ In fact, the DNA chromosomes found in the majority of chloroplasts and mitochondria consist of circular DNA molecules, much like the chromosomes of most bacteria,²⁶ although the size and gene content of mitochondrial and chloroplast genomes vary tremendously (Table 1).²⁷

In many cases, the genes encoded by the DNA chromosomes of mitochondria and chloroplasts are arranged in the same order as those found in bacteria.²⁸ Several molecular similarities exist between the genomes of chloroplasts and those of cyanobacteria, since the gene clusters from chloroplast genomes resemble those from cyanobacteria in both organization and structure. In chloroplast genomes, many genes contain promoters that greatly resemble bacterial promoters. When RNA polymerase begins making an RNA copy of the DNA, it always begins at a specific

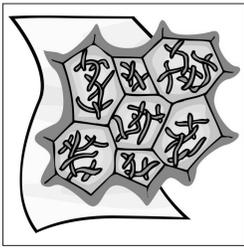
DNA site called the promoter. Promoters are specific DNA sequences that tell the RNA polymerase when and where to begin making RNA. These promoter-like sequences have also been demonstrated to play an essential role in the expression of chloroplast genes.²⁹ Some chloroplast genes also contain Shine-Dalgarno sequences, which are peculiar to bacteria and found at the front ends of messenger RNAs. Shine-Dalgarno sequences bind to the termini of 16S rRNAs and help ribosomes fasten to the mRNA so that it can use the RNA molecule to direct its protein synthesis.³⁰

Not only do mitochondria and chloroplasts possess their own genomes, but the inheritance patterns of these genomes are distinct from that of the nuclear genome. In most species, the inheritance of mitochondrial and chloroplast genomes is marked by uniparental inheritance, which is to say that the offspring of a mated individual possesses the mitochondrial or chloroplast genome of one parent, typically the mother.³¹ To illustrate this, classic experiments with frogs from the genus *Xenopus* showed that interspecific matings produced progeny with the mitochondrial DNA of the mother (Figure 3).³² Similar results are commonly observed in other vertebrates.³³ Similarly for chloroplast genomes, matings between various strains of the single-celled green alga *Chlamydomonas reinhardtii* have demonstrated uniparental inheritance of many chloroplast-encoded traits.³⁴ These modes of inheritance for organelle-based stand in stark contrast to the Mendelian inheritance patterns observed with genes from nuclear genomes.

All the RNAs found in mitochondria and chloroplasts, be they mRNAs,³⁵ rRNA,³⁶ or tRNAs are much more similar to those found in specific groups of bacteria than any

Organism	Size (base pairs)	Number of genes encoded	Mitochondrial or chloroplast genome
<i>Homo sapiens</i> (Human beings) ³⁸	16,569	37	Mitochondrial
<i>Saccharomyces cerevisiae</i> (Baker's yeast) ³⁹	85,779	35	Mitochondrial
<i>Marchantia polymorpha</i> (The common liverwort—a moss-like plant) ⁴⁰	186,608	75	Mitochondrial
<i>Marchantia polymorpha</i> ⁴¹	121,025	128	Chloroplast
<i>Arabidopsis thaliana</i> (A flowering plant) ⁴²	366,924	57	Mitochondrial
<i>Arabidopsis thaliana</i> ⁴³	154,478	136	Chloroplast
<i>Porphyra purpurea</i> (Red alga) ⁴⁴	~191,000	255	Chloroplast
<i>Zea mays</i> (Domestic corn) ⁴⁵	140,387	104	Chloroplast

Table 1. The Size and Number of Genes Encoded by Mitochondrial and Chloroplast Genomes From Distinct Organisms.



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Another prediction of the endosymbiont theory is that we should be able to find organisms in the process of forming an interdependent relationship with an indwelling microorganism in which the microbe has yet to completely lose its cellular identity. Such an association would constitute an intermediate to the formation of a cellular organelle.

eukaryote.³⁷ In fact the arrangement of the rRNA genes in chloroplast genomes bear exquisite similarities to those of cyanobacteria.⁴⁶ Mitochondria and chloroplasts also contain their own ribosomes and their ribosomes are much more similar to those from bacteria than the cytoplasmic ribosomes in eukaryotic cells.⁴⁷ For example, ribosomal subunits, the ribosomal accessory factors that assist ribosomal function and aminoacyl-tRNA synthetases from bacteria and chloroplasts are completely interchangeable. Such interchangeability also applies to mitochondrial ribosomes, but to a lesser extent.⁴⁸ Ribosomes from mitochondria and chloroplasts are also susceptible to the same antibiotics that typically inhibit bacterial ribosomes but never affect eukaryotic ribosomes.⁴⁹

With respect to a living representative, the protein and RNA sequences of mitochondria show the greatest similarities to those found in the microbiological agent of epidemic typhus, *Rickettsia prowazekii*.⁵⁰ Furthermore, genomes from mitochondria of the jakobid protist *Reclinomonas americana* resemble a miniaturized version of a bacterial genome and show striking genetic affinities to the genome of *Rickettsia prowazekii*.⁵¹ Thus in the case of mitochondria, we not only have a microorganism that most closely resembles modern mitochondria, but a eukaryote whose mitochondrial genome looks like a miniature version of the genome of *Rickettsia prowazekii*. For chloroplasts, repeated comparisons of chloroplast proteins and genomes from green plants with those of extant bacteria have demonstrated that the cyanobacteria are the most similar to chloroplasts, even though it is difficult to

determine the exact organism that is the most similar to modern chloroplasts.⁵²

Finally, there are copious examples of organisms that contain no mitochondria but have retained copies of mitochondrial genes in their nuclei,⁵³ and some that possess mitochondrial remnants.⁵⁴ For chloroplasts, there are non-photosynthetic, parasitic flowering plants, like *Epifagus virginiana*, whose chloroplast genomes have lost the photosynthetic genes,⁵⁵ and heterotrophic euglenoids like *Astasia longa* and parasitic protozoa that contain plastids with genomes that are clearly derived from chloroplasts.⁵⁶ Thus there are plenty of examples of secondary loss of these organelles. Hence mitochondria and chloroplasts contain proteins, genomes, RNAs, and ribosomes whose most similar counterparts are from bacteria. There are also extant organisms that resemble these organelles more than any other living thing and many examples of secondary loss of these organelles. These data corroborate the endosymbiont theory.

Intermediates between Cytoplasmic Microorganisms and Organelles

Another prediction of the endosymbiont theory is that we should be able to find organisms in the process of forming an interdependent relationship with an indwelling microorganism in which the microbe has yet to completely lose its cellular identity. Such an association would constitute an intermediate to the formation of a cellular organelle. Insects form extensive associations with bacteria and fungi,⁵⁷ and by carefully exam-

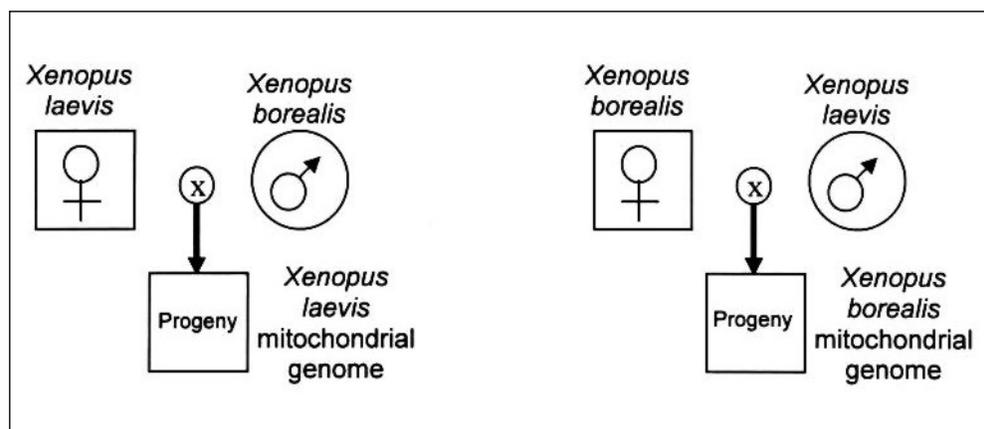


Figure 3. Maternal inheritance of *Xenopus* mitochondrial DNA. Reciprocal crosses between two species produce F₁ hybrids and each hybrid retains only the mitochondrial DNA from its mother.

ining several insect species and their indentured microbial servants, scientists have found what many think are such intermediates.

Small insects called aphids pierce plant tissues with syringe-like mouthparts and withdraw sap from the vascular elements of the plant. Consequently aphids can cause extensive damage to plants and pass plant viruses between plants, and are justly designated as plant pests. As a diet, plant sap is very rich in sugars but rather poor in amino acids, the building blocks of proteins. Therefore the aphid has a diet that is good for energy but poor for making proteins, since the insect cannot synthesize all of the amino acids it needs to stay alive. Instead the insect has a bacterium called *Buchnera* that lives inside the cells of its body that makes the amino acids it needs to live and reproduce.⁵⁸

Buchnera are small, round bacterial cells, which live inside specialized cells that compose a bilobed structure within the body cavity of the aphid called the bacteriome (Figure 4A). The bacteriome is composed of 60 to 90 large cells called bacteriocytes or mycetocytes (Figure 4B), and within each bacteriocyte live thousands of *Buchnera* (Figures 4B, 4C). These bacteria are vital to the growth and propagation of the aphids. In fact the hitchhiking *Buchnera* cells are passed from the aphid mother to her progeny.⁵⁹ If aphids are treated with antibiotics that kill off the bacterial cells, the insects show a rapid reduction in growth and eventually become sterile. Antibiotic-treated aphids without their bacterial symbionts can only grow if they are supplemented with amino acids.⁶⁰

Buchnera species that reside in different types of aphids are much more similar to each other than they are to any other organism. This strongly suggests that the ancestor of all modern aphids formed a symbiotic relationship with the ancestor of all modern *Buchnera* species that was then passed on to all the descendants of this aphid progenitor. Secondly, the microorganisms most closely related to *Buchnera* are members of the γ -proteobacteria group, which includes such familiar organisms as *Escherichia coli* (*E. coli*).⁶¹

A particular *Buchnera* strain called APS (formally referred to as *Buchnera* sp. APS) inhabits the body of the pea aphid, *Acyrtosiphon pisum*. The sequenced genome of *Buchnera* sp. strain APS is approximately four times smaller than that of *E. coli* K-12, and lacks genes for the biosynthesis of particular bacterial cell-surface components, regulatory systems that control gene expression during changes in environmental conditions and host defense systems that protect bacterial cells from viral infections. The gene order of *Buchnera* sp. APS is so similar to that of *E. coli* that the *Buchnera* genome looks like a diminutive version of the *E. coli* genome. The genome of *Buchnera* sp. APS also includes the genes necessary for the biosynthesis of all ten amino acids that are essential to the

aphid host, but lacks the genes for the biosynthesis of all amino acids that are nonessential to the aphid.⁶² These data show the complementarity of the symbiosis between *Buchnera* and the aphids—the endosymbiont provides the aphid with the materials that it cannot make or acquire from its diet and the host provides the endosymbiont with those materials that it cannot synthesize. Thus, aphids and their *Buchnera* symbionts fit each other like a hand and a glove. Nevertheless, *Buchnera* show definite affinities with the γ -proteobacteria and probably descended from them. Thus, *Buchnera* represents, in the minds of many biologists, an organism that is on its way to becoming an organelle, just like mitochondria or chloroplasts, and is intermediate between those organisms that have become internal compartments in cells, like mitochondria, and those that have yet to completely lose their cellular identity.

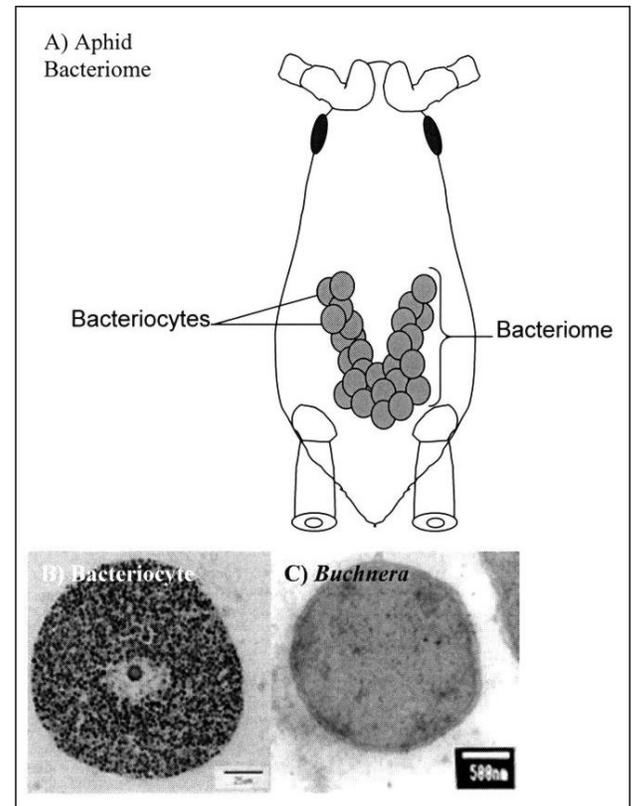
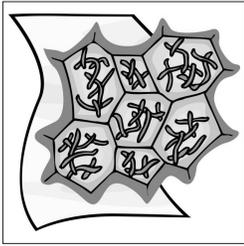


Figure 4. Aphid bacteriome and individual bacteriocytes and *Buchnera* cells. (A) Drawing of Aphid body with bacteriome positioned in the abdomen of the insect. The bacteriome is ventrally located, underneath the insect ovaries. It is also in contact with the insect hemolymph, the fluid that serves as the insect blood. The amino acids synthesized by the *Buchnera* are released into the hemolymph and carried to various parts of the body and the sugars acquired by feeding arrive to the bacteria via the same means. **(B) Electron micrograph of an individual bacteriocyte.** Each tiny dot in the cytoplasm of this cell is a *Buchnera* cell. The large dot in the center is the bacteriocyte nucleus. **(C) Electron micrograph of an individual *Buchnera* cell from the cytoplasm of a bacteriocyte.** Figure (A) was redrawn from M. B. Ponsen, "Alimentary Tract," Figure 2A in *Aphids: Their Biology, Natural Enemies, and Control*, ed. A. K. Minks and P. Harrewijn (Amsterdam: Elsevier, 1987), 79-97. Figures (B) and (C) were acquired from <http://buchnera.gsc.riken.go.jp/intro.html> and used with permission.



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While the prevailing model of neo-Darwinian evolution has tremendous explanatory power, it is difficult to determine how a force like natural selection might drive the transfer of genes from the mitochondrion to the nucleus in a hierarchical manner and at disparate rates.

It also should be emphasized that relationships between aphids and *Buchnera* are not unusual among the insects. Aphids are related to whiteflies and mealybugs, organisms that also feed on plant sap and harbor bacterial endosymbionts.⁶³ Cockroaches, tsetse flies, and carpenter ants also contain endosymbionts that are different from *Buchnera* and were probably acquired during independent events.⁶⁴ In fact, symbiotic associations are found in many organisms in a variety of ecosystems.⁶⁵ Therefore, endosymbiosis appears to be a robust association between eukaryotes and bacteria that occurred in the past and continues today. Endosymbiosis allows organisms to exploit new food sources and lifestyles, and it creates situations that are mutually beneficial to both organisms.

Organelle Origins and Intelligent Design

Based on the present available data, an endosymbiotic origin for mitochondria and chloroplasts seems to be a reasonable conclusion despite the unanswered questions that remain.⁶⁶ Chloroplasts from a variety of photosynthetic organisms show very similar features and have kept many of their bacterial features. It is difficult to convincingly explain these bacterial features in a non-historical manner. Despite this, it seems somewhat uncertain why some mitochondrial genomes are so different from their bacterial ancestors while chloroplast genomes have retained so many bacterial features. It could be that mitochondria were established much earlier in the eukaryotic lineage while chloroplasts are relative newcomers to eukaryotic cells. Even if this possibility is granted, it still does not explain the extensive remodeling of mitochondrial genomes versus chloroplast genomes, even though the larger size of chloroplast genomes might have more to do with their function.

Nevertheless, the remodeling of mitochondrial genomes seems to follow certain principles. First of all, molecular biologists have predicted that over time all the genes in organelles should experience transfer to the nucleus and deletion from the mitochondrial genome. The reason for this is a principle called Muller's ratchet, whereby deleterious mutations accumulate much more rapidly in

asexually propagated genomes than in sexually propagated ones, where recombination is possible. Therefore, the asexually propagated mitochondrial genome is much more subject to gene decay than the nuclear genome, and natural selection should favor the transfer of essential mitochondrial genes to the nuclear genome, where recombination can protect it from gene decay.⁶⁷

Perhaps a more pressing problem is the difficulty that one might have conceiving how genes from an enclosed compartment like the mitochondrion can migrate to another closed compartment of the cell, like the nucleus. Nevertheless several lines of evidence strongly argue that such transfers do occur. First, genomic sequencing projects have definitively demonstrated several cases where unequivocal copies of portions of the mitochondrial genome are inserted into the nuclear genomes of *Arabidopsis*, felines and humans.⁶⁸ Secondly, the transfer of marked chloroplast genes to the nucleus has actually been observed in transgenic tobacco plants, and at a rate that is comparable to the spontaneous mutation rate of nuclear DNA.⁶⁹ Given the frequency of gene transfer from chloroplasts to nuclei, it seems likely that the rates of gene transfer between mitochondria and nuclei are similar, especially since studies in yeast have observed a similar rate.⁷⁰ Balancing this tendency for nuclear transfer is the need for the maintenance of genomes in organelles so that they can detoxify dangerous reactive oxygen species that are side effects of their energy production mechanisms.⁷¹

Investigations into the transfer of genes from mitochondrial genomes to the nuclear genome have revealed surprisingly that this relocation seems to occur in some kind of hierarchical fashion.⁷² If we examine the mitochondrial ribosomal protein genes and determine if they are encoded by the mitochondrial or nuclear genome, we observe a loose hierarchy of transfer of genes to the nucleus. For example, the genes that encode the mitochondrial ribosomal proteins are designated *rps* for ribosomal protein small subunit, and numbered. The *rps1* gene typically undergoes nuclear transfer before all the other *rps* genes. The transfer of the *rps10* gene from the mitochondrial genome to the nuclear genome usually follows after *rps1*

was transferred, and *rps11* goes to the nucleus after *rps10*, and so on. There are exceptions to this order, but the overall trend seems to argue for a hierarchy of gene transfer from the mitochondrion to the nucleus (Table 2⁷³). Likewise the components of respiratory chain complex I also show an order to their nuclear transfer.⁷⁴ In both cases, the order of transfer does not correlate with the size of the gene or its genomic location (Table 2).

The observed order of transfer is also not an artifact of biological history, since mitochondrial genomes that encode a limited number of genes retain similar sets of genes, regardless of their phylogenetic placement. The transfer of genes to the nucleus differs within distinct evolutionary lineages and can also vary tremendously within

particular lineages. For example, two prasinophyte green algae, *Nephroselmis olivacea* and *Pedinomonas minor* possess mitochondrial genomes that radically differ in size, gene content, and order.⁷⁵ In the green plants, gene content comparisons of the mitochondrial genomes of four different organisms provide ample examples of differences in gene transfer within this evolutionary lineage (Table 3). Similar discrepancies are seen in these and other evolutionary lineages. However many genes are transferred to the nucleus; they are typically transferred in the order suggested in Table 2.

While the prevailing model of neo-Darwinian evolution has tremendous explanatory power, it is difficult to determine how a force like natural selection might drive

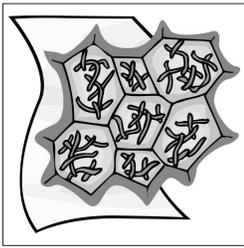
Table 2. Small Subunit Ribosomal Protein Genes Encoded by Mitochondrial Genomes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>rps1</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>rps10</i>	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>rps11</i>	+	+	+	+	+	-	-	-	-	+	-	-	-	-
<i>rps2</i>	+	+	+	+	+	+	-	-	-	-	-	-	-	-
<i>rps7</i>	+	+	+	+	+	+	-	-	+	-	-	-	-	-
<i>rps8</i>	+	+	+	+	+	+	+	-	-	-	-	-	-	-
<i>rps4</i>	+	+	+	+	+	+	+	-	+	-	-	-	-	-
<i>rps19</i>	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>rps13</i>	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>rps14</i>	+	+	+	+	+	+	+	+	-	-	-	-	-	-
<i>rps12</i>	+	+	+	+	+	+	+	+	+	+	-	-	-	-
<i>rps3</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	-

The "+" signifies that the mitochondrial genome of the designated organism encodes the indicated ribosomal protein and "-" signifies that the mitochondrial genome of the organism does not encode the indicated ribosomal protein.

Legend:

- (1) *Reclinomonas americana*, a jakobid protozoan.
- (2) *Marchantia polymorpha*, a moss-like plant called a liverwort.
- (3) *Nephroselmis olivacea*, a motile, single-celled green alga.
- (4) *Phytophthora infestans*, a stramenopile, a group that includes the oocytes or water molds and algae with two different flagella.
- (5) *Acanthamoeba castellanii*, a single-celled amoeba.
- (6) *Thraustochytrium aureum*, a stramenopile.
- (7) *Monosiga brevicollis*, a choanoflagellate (a protozoan that looks like a small piece of sponge tissue).
- (8) *Tetrahymena pyriformis*, a ciliated, single-celled organism.
- (9) *Arabidopsis thaliana*, a flowering plant.
- (10) *Porphyra purpurea*, a rhodophyte (red alga).
- (11) *Allomyces macrogynus*, a chytrid, an aquatic fungus with motile gametes.
- (12) *Schizosaccharomyces pombe*, a single-celled fungus (yeast).
- (13) *Plasmodium falciparum*, an apicomplexan (parasitic single celled organism that causes malaria).
- (14) *Homo sapiens*, human beings.



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The field of mitochondrial evolution might be a place where ID advocates can make some predictions and test them. If the evolution of mitochondria is driven largely by natural selection acting on mutations, then the random changes in mitochondrial genomes should be either inconsequential and carried on, selected for and inherited in the majority of cases, or selected against and not inherited in a certain percentage of the cases.

the transfer of genes from the mitochondrion to the nucleus in a hierarchical manner and at disparate rates. This suggests that another mechanism drives the hierarchical transfer of these genes to the nucleus.

Perhaps the new model of Intelligent Design (ID) could test its tenets in this case. ID is a somewhat recent proposal which posits that particular aspects of living organisms and the universe as well, are best

explained by intelligent causes.⁷⁶ Despite the strongly theistic overtones of such a proposal, ID advocates tend to disavow any attempt to identify the designer. Instead, ID proponents wish to consider certain aspects of living organisms as having been purposefully made rather than fashioned by wholly impersonal forces. Many scientists have strongly objected to this proposal because of its perceived introduction of supernatural explanations into science.⁷⁷ However, a

Table 3. The Partial List of Protein-Coding and tRNA Gene Content of Mitochondrial Genomes of Various Plants

Mitochondrial genes	<i>Marchantia polymorpha</i> (Liverwort) ⁷⁸	<i>Arabidopsis thaliana</i> ⁷⁹	<i>Oryza sativa</i> (Rice) ⁸⁰	<i>Beta vulgaris</i> (Sugar Beet) ⁸¹
<i>rps1</i>	+	–	+	–
<i>rps10</i>	+	–	–	–
<i>rps11</i>	+	–	–	–
<i>rps2</i>	+	–	+	–
<i>rps7</i>	+	+	+	+
<i>rps8</i>	+	–	–	–
<i>rps4</i>	+	+	+	+
<i>rps19</i>	+	–	+	–
<i>rps13</i>	+	–	+	+
<i>rps14</i>	+	–	–	–
<i>rps12</i>	+	+	+	+
<i>rps3</i>	+	+	+	+
<i>rpl2</i>	+	+	+	–
<i>rpl5</i>	+	+	+	+
<i>rpl6</i>	+	–	–	–
<i>rpl16</i>	+	+	+	–
<i>ccmB</i> ⁸²	+	+	+	+
<i>ccmC</i>	+	+	+	–
<i>ccmF_N</i>	+	–	+	+
<i>ccmF_{N1}</i>	–	+	–	–
<i>ccmF_{N2}</i>	–	+	–	–
<i>nad7</i>	–	+	+	+
Glycine tRNA	+	+	–	+
Phenylalanine tRNA	+	–	+	+

commitment to ID does not necessarily commit one to miraculous creation, and purposeful forces that are wholly natural in their scope and activity could account for the origin of various intelligently designed structures or phenomena.⁸³ ID has not won many converts in the journals to date, but this is not all that surprising, since ID advocates have mainly reinterpreted already existing data and have yet to formulate a list of predictions that can evince future experimental success at this time.⁸⁴

Can ID help explain the hierarchical transfer of rps or nad genes from mitochondrial to the nuclear genome? Perhaps it can ...

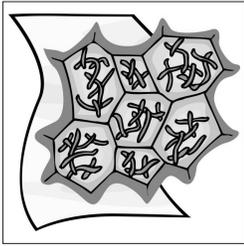
The field of mitochondrial evolution might be a place where ID advocates can make some predictions and test them. If the evolution of mitochondria is driven largely by natural selection acting on mutations, then the random changes in mitochondrial genomes should be either inconsequential and carried on, selected for and inherited in the majority of cases, or selected against and not inherited in a certain percentage of the cases. Changes in mitochondrial genomes should be steady with rare "quantum" events (large deletion, insertion, or inversion) that greatly change the structure of the mitochondrial genome. If, however, some kind of purposeful principle guides the sculpting of mitochondrial genomes, then we might expect a step-like series of changes in the structure of the mitochondrial genome until the genome becomes a kind of "optimal size" or "optimal structure." We should keep in mind that natural selection and ID need not be mutually exclusive, since the two could just as easily work side-by-side.⁸⁵ The difficulty is determining the contribution of natural selection as opposed to a contribution from some sort of as yet unidentified underlying principle that might guide mitochondrial genomic evolution.

Can ID help explain the hierarchical transfer of *rps* or *nad* genes from mitochondrial to the nuclear genome? Perhaps it can if we consider that ribosomes work as integrated wholes with "several well-matched, interacting parts that contribute to the basic function."⁸⁶ Given Muller's ratchet, we might predict that the genes most crucial to basic ribosomal function should experience the earliest transfer to the nucleus in order to protect them from gene decay, and those genes less constrained by amino acid specificity should experience later transfer to the nucleus.

The *rps1* gene typically is the first to experience transfer to the nucleus and ribosomal protein S1 has RNA unwinding activity,⁸⁷ is important for the binding of mRNA to the ribosome, influences the affinity of ribosomes for different mRNA initiation sequences, and is required for the translation of most or all natural mRNAs in bacteria.⁸⁸ Thus S1 ranks quite high in importance to the ribosome. The second gene to go to the nucleus is usually *rps10*, and this protein is not only an important ribosomal protein, but is also an inhibitor of transcription termination.⁸⁹ The third and fifth proteins to go to the nucleus are *rps11* and *rps7* and these proteins work together in the ribosome to control translational fidelity.⁹⁰ The fourth *rps* gene to go to the nucleus is *rps2*, and S2 assists in the incorporation of S1 into the 30S ribosomal subunit.⁹¹ S8 is encoded by the sixth gene to experience transfer to the nucleus, *rps8*, and S8 plays a key role in assembling the small ribosomal subunit. S8 binds independently of other ribosomal proteins to the central domain of 16S rRNA during 30S subunit assembly and with proteins S6, S11, S15 and S18 forms the side projection of the 30S subunit.⁹²

The seventh ribosomal protein gene, *rps4*, encodes S4, a protein that plays key roles in 30S subunit assembly and translational fidelity.⁹³ The next two *rps* genes transferred to the nucleus, *rps19* and *rps13* encode proteins that interact.⁹⁴ S19 constitutes part of the so-called "A" site of the ribosome,⁹⁵ and both proteins bind the 16S rRNA. The tenth and twelfth *rps* genes to go to the nucleus are *rps14* and *rps3*. *In vitro* studies have shown that these two proteins are required for ribosomal assembly, but not absolutely required for translation.⁹⁶ Therefore, these two ribosomal proteins are not as important as the others and there is less need to move them to the nucleus. The eleventh *rps* gene to experience transfer to the nucleus, *rps12*, encodes the famous S12 protein, which is the protein that undergoes alteration when bacterial cells become resistant to the antibiotic streptomycin.⁹⁷ However, mutations in *rps12* can actually increase translational accuracy.⁹⁸ Therefore, despite its importance in translation, the need for the cell to preserve *rps12* from gene decay is lower than other *rps* genes. Thus it appears that ribosomal protein genes are transferred to the nucleus in a hierarchy conditioned by the importance each gene to the function of the ribosome. This hierarchy is not irrevocable, but merely exists as a trend; since all ribosomal proteins are functionally important to the ribosome at some point in its activity. Thus the loose order of transfer is predicted.

ID theory also could potentially answer why chloroplast genomes are so homogenous relative to mitochondrial genomes and so bacterial in structure. Why should chloroplast genomes, which are so far removed from their cyanobacterial ancestors, keep their bacterial features? Could it be that the bacterial nature of chloroplast genomes is required for their function? This is a hypothesis that is testable and several experiments designed to



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evaluate it might provide ready answers. For example, we might expect that mutations that affect chloroplast function are lesions in some sort of bacterial-like regulation system. Furthermore, we might expect that complementation of chloroplast-specific mutations should work better with a bacterial gene rather than with a eukaryotic gene. Such experiments are readily workable in the single-celled alga *Chlamydomonas*, where a chloroplast-specific genetic system and a chloroplast transformation system are available.⁹⁹ Clearly these are questions for further research and it is possible that less orthodox ways of thinking about the origins of these organelles might be useful for further investigation and consideration.

Conclusion

Two organelles from contemporary eukaryotic cells, mitochondria and chloroplasts, probably descend from ancient bacterial cells that were engulfed by other, larger ancient nonbacterial cells and formed symbiotic relationships with their captors. The contemporary biological world contains many examples in which endosymbiotic relationships are in the process of forming and the creation of interdependent relationships could be one of the primary forces driving species diversification. Furthermore, the tendency of organisms to form mutually-dependent relationships is at odds with a pure, neo-Darwinian view of nature, and is probably part of the original goodness God builds into creation as he makes it. Because the formation of mitochondria and chloroplasts was probably due to purposeful rather than wholly purposeless processes, investigations into the evolutionary and genetic behavior of these organelles is potentially better aided by ID theory rather than bald neo-Darwinism. ✠

Notes

¹Walter L. Bradley and Charles B. Thaxton, "Information and the Origin of Life," in *The Creation Hypothesis*, ed. J. P. Moreland (Downer's Grove, IL: Intervarsity Press, 1994), 173–210. See also Stephen C. Meyer, "DNA and the Origin of Information," in *Mere Creation*, ed. W. A. Dembski (Downer's Grove, IL: Intervarsity Press, 1998), 113–47; David Rosevear, "The Myth of Chemical Evolution," *Impact* 313 (1999); and Stephen C. Meyer, "Word Games: DNA, Design and Intelligence," in *Signs of Intelligence*, ed. W. A. Dembski and J. M. Kushiner (Grand Rapids, MI: Brazos Press, 2001), 102–17.

²F. J. R. Taylor, "An Overview of the Status of Evolutionary Cell Symbiosis Theories," *Annals of the New York Academy of Sciences* 503 (1987): 1–16.

³Lynn Margulis, *Origin of Eukaryotic Cells* (New Haven, CT: Yale University Press, 1970). The serial endosymbiosis theory of Margulis additionally proposes a bacterial origin for cilia and flagella, the propulsion structures of many eukaryotic cells. This part of SET is only supported by very slim evidence and has received little support from other researchers.

⁴Lynn Margulis, *Symbiotic Earth: A New View of Evolution* (New York: Basic Books, 1998), 5–12.

⁵Lynn Margulis and Dorion Sagan, *Acquiring Genomes: A Theory of the Origin of Species* (New York: Basic Books, 2002).

⁶Ps. 104:10–26.

⁷Rom. 12:4–13; 1 Cor. 12:4–31; Gal. 6:1–2; Eph. 4:11–13; Phil. 2:1–4.

⁸Charles Darwin, *The Origin Of Species* (London: Penguin Books, 1859), 228–9.

⁹Douglas J. Futuyma, *Evolutionary Biology* (Sunderland, MA: Sinauer Publishing, 1998), 551–4.

¹⁰Ps. 19:1–4.

¹¹John C. Rankin, *First the Gospel, Then Politics ...* (Hartford, CT: TEI Press, 1999), 20–9.

¹²John F. Haught, *God After Darwin* (Boulder, CO: Westview Press, 2000).

¹³Gen. 3:21.

¹⁴John 5:11.

¹⁵Jeffery P. Schloss, "Evolutionary Accounts of Altruism and the Problem of Goodness by Design," in *Mere Creation*, ed. W. A. Dembski (Downer's Grove: Intervarsity Press, 1998), 236–61.

¹⁶Michael T. Ghiselin, "Darwin and Evolutionary Psychology," *Science* 179 (1973): 964–8.

¹⁷Lynn Margulis, *Symbiotic Planet: A New View of Evolution*.

¹⁸An organelle, like the nucleus, houses the chromosomes and is responsible for gene expression and replication of the chromosomes prior to cell division. Another organelle called the mitochondrion generates the chemical energy for the cell, and in plant cells, an organelle called the chloroplast houses the photosynthetic machinery and uses energy captured from the sun to assimilate carbon dioxide into the plant.

¹⁹For a running total of sequenced genomes, see the Genomes Online Database at <http://wit.integratedgenomics.com/GOLD>.

²⁰For an accurate yet humorous and entertaining introduction to molecular biology and genetics, see Larry Gonick and Mark Wheelis, *The Cartoon Guide to Genetics* (New York: Harper Perennial, 1991).

²¹Betsy Dexter Dyer and Robert Alan Obar, *Tracing the History of Eukaryotic Cells: The Enigmatic Smile* (New York: Columbia University Press, 1994) 131–2. Dyer and Obar's points are reiterated here with some modifications. They state that we should not see living intermediates in the process of endosymbiogenesis, and this is incorrect, since we do see such intermediates. They do make the point that secondary loss is expected, which is reproduced here.

²²W. H. Li, *Molecular Evolution* (Sunderland, MA: Sinauer Associates, 1997).

²³D. Yang, et al., "Mitochondrial Origins," *Proceedings of the National Academy of Sciences USA* 82, no. 13 (1985): 4443–7; C. R. Woese, "Bacterial Evolution," *Microbiological Review* 51 (1987): 222–70;

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- M. W. Gray, "Rickettsia, Typhus and the Mitochondrial Connection," *Nature* 396, no. 6707 (1998): 109–10; M. W. Gray, G. Burger, and B. F. Lang, "Mitochondrial Evolution," *Science* 283, no. 5407 (1999): 1476–81; B. F. Lang, M. W. Gray, and G. Burger, "Mitochondrial Genome Evolution and the Origin of Eukaryotes," *Annual Review of Genetics* 33 (1999): 351–97. See also S. G. Andersson, et al., "The Genome Sequence of *Rickettsia prowazekii* and the Origin of Mitochondria," *Nature* 396, no. 6707 (1998): 133–40.
- ²⁴Michael W. Gray and William F. Doolittle, "Has the Endosymbiont Hypothesis Been Proven?" *Microbiological Reviews* 46, no. 1 (1982): 1–42. See also M. W. Gray, D. Sankoff, and R. J. Cedergren, "On the Evolutionary Descent of Organisms and Organelles: A Global Phylogeny Based on a Highly Conserved Structural Core in Small Subunit Ribosomal RNA," *Nucleic Acids Research* 12, no. 14 (1984): 5837–52; R. Cedergren, et al., "The Evolutionary Relationships Among Known Life Forms," *Journal of Molecular Evolution* 28, no. 1–2 (1988): 98–112; S. J. Giovannoni, et al., "Evolutionary Relationships Among Cyanobacteria and Green Chloroplasts," *Journal of Bacteriology* 170, no. 8 (1988): 3584–92; S. Turner, et al., "The Relationship of a Prochlorophyte *Prochlorothrix hollandica* to Green Chloroplasts," *Nature* 337, no. 6205 (1989): 380–2; C. F. Delwiche and J. D. Palmer, "The Origin of Plastids and Their Spread Via Secondary Endosymbiosis," in *Origins and Algae and Their Plastids*, ed. D. Bhattacharya (Vienna: Springer-Verlag, 1997), 53–86; and S. E. Douglas, "Plastid Evolution: Origins, Diversity, Trends," *Current opinion in Genetics and Development* 8 (1998): 655–61.
- ²⁵L. Margulis, *Symbiotic Earth: A New View of Evolution*, 22–8.
- ²⁶B. F. Lang, et al., "Mitochondrial Genome Evolution and the Origin of Eukaryotes."
- ²⁷Chloroplast genomes tend to be larger than mitochondrial genomes. Chloroplast genomes are also very bacterial-like and tend to be circular and lack extensive protein coatings like almost all bacterial genomes. See M. W. Gray and W. F. Doolittle, "Has the Endosymbiont Hypothesis Been Proven?"
- ²⁸M. W. Gray, et al., "Mitochondrial Evolution."
- ²⁹N. W. Gillham, "Chloroplasts and Mitochondria," in *Encyclopedia of Genetics*, ed. E. C. R. Reeve (Chicago: Fitzroy Dearborn Publishers, 2001) 721–35.
- ³⁰E. H. Harris, J. E. Boynton, and N. W. Gillham, "Chloroplast Ribosomes and Protein Synthesis," *Microbiological Reviews* 58, no. 4 (1994): 700–54. In at least one case, the Shine-Dalgarno sequences play a negative regulatory role. See W. Plader and M. Sugiura, "The Shine Dalgarno-like Sequence is a Negative Regulatory Element for Translation of Tobacco Chloroplast *rps2* mRNA: An Additional Mechanism for Translational Control in Chloroplasts," *Plant Journal* 34, no. 3 (2003): 377–82. In some cases, obliteration of them does not affect translation. See N. W. Gillham, "Chloroplasts and Mitochondria."
- ³¹Leland H. Hartwell, Leroy Hood, Michael L. Goldberg, Ann E. Reynolds, Lee M. Silver, and Ruth C. Veres, *Genetics: From Genes to Genomes* 2d ed. (New York: McGraw Hill Publishers, 2004), 535–43.
- ³²I. B. Dawid and A. W. Blackler, "Maternal and Cytoplasmic Inheritance of Mitochondrial DNA in *Xenopus*," *Developmental Biology* 29, no. 2 (1972): 152–61.
- ³³R. P. Wagner, "The Role of Maternal Effects in Animal Breeding. II. Mitochondria and Animal Inheritance," *Journal of Animal Science* 35, no. 6 (1972): 1280–7.
- ³⁴E. V. Armbrust, P. J. Ferris, and U. W. Goodenough, "A Mating Type-Linked Gene Cluster Expressed in *Chlamydomonas* Zygotes Participates in the Uniparental Inheritance of the Chloroplast Genome," *Cell* 74, no. 5 (1993): 801–11. Also see B. B. Sears and K. VanWinkle-Swift, "The Salvage/Turnover/Repair (STOR) Model for Uniparental Inheritance in *Chlamydomonas*: DNA as a Source of Sustenance," *Journal of Heredity* 85, no. 5 (1994): 366–76.
- ³⁵Michael W. Gray and William F. Doolittle, "Has the Endosymbiont Hypothesis Been Proven?"
- ³⁶For chloroplast rRNAs, see S. J. Giovannoni, et al., "Evolutionary Relationships among Cyanobacteria and Green Chloroplasts," *Journal of Bacteriology* 70, no. 8 (1988): 3584–92. For mitochondrial 5S rRNAs, see H. Kuntzel, M. Heidrich, and B. Piechulla, "Phylogenetic Tree Derived from Bacterial, Cytosol and Organelle 5S rRNA Sequences," *Nucleic Acids Research* 9, no. 6 (1981): 1451–61. For the 16S rRNAs of mitochondria, see M. W. Gray, D. Sankoff, and R. J. Cedergren, "On the Evolutionary Descent of Organisms and Organelles: A Global Phylogeny Based on a Highly Conserved Structural Core in Small Subunit Ribosomal RNA," *Nucleic Acids Research* 12, no. 14 (1984): 5837–52. Also see D. Yang, et al., "Mitochondrial Origins."
- ³⁷For tRNAs of both organelles, see M. Sprinzl, et al., "Compilation of tRNA Sequences and Sequences of tRNA Genes," *Nucleic Acids Research* 26, no. 1 (1998): 148–53.
- ³⁸S. Anderson, et al., "Sequence and Organization of the Human Mitochondrial Genome," *Nature* 290, no. 5806 (1981): 457–65; D. A. Clayton, "Replication and Transcription of Vertebrate Mitochondrial DNA," *Annual Review of Cell Biology* 7 (1991): 453–78.
- ³⁹F. Foury, et al., "The Complete Sequence of the Mitochondrial Genome of *Saccharomyces cerevisiae*," *Federation of European Biochemical Societies Letters* 440, no. 3 (1998): 325–31.
- ⁴⁰K. Oda, et al., "Gene Organization Deduced from the Complete Sequence of Liverwort *Marchantia polymorpha* Mitochondrial DNA: A Primitive Form of Plant Mitochondrial Genome," *Journal of Molecular Biology* 223, no. 1 (1992): 1–7.
- ⁴¹K. Ohyama, "Chloroplast and Mitochondrial Genomes from a Liverwort, *Marchantia polymorpha* – Gene Organization and Molecular Evolution," *Bioscience, Biotechnology and Biochemistry* 60, no. 1 (1996): 16–24. With exceptions, chloroplast genomes tend to be 120,000–160,000 base pairs in size. See M. Reith, "Molecular Biology of Rhodophyte and Chromophyte Plastids," *Annual Review of Plant Physiology and Plant Molecular Biology* 46 (1995): 549–75.
- ⁴²M. Unseld, et al., "The Mitochondrial Genome of *Arabidopsis thaliana* Contains 57 Genes in 366,924 Nucleotides," *Nature Genetics* 15, no. 1 (1997): 57–61.
- ⁴³S. Sato, et al., "Complete Structure of the Chloroplast Genome of *Arabidopsis thaliana*," *DNA Research* 6, no. 5 (1999): 283–90.
- ⁴⁴M. Reith and J. Munholland, "Complete Nucleotide Sequence of the *Porphyra purpurea* Chloroplast Genome," *Plant Molecular Biology Reporter* 13 (1995): 333–5. The chloroplast genome of *Porphyra purpurea* (a red alga) shows the highest reported gene content of all chloroplast genomes.
- ⁴⁵R. M. Maier, K. Neckermann, G. L. Igloi, and H. Kossel, "Complete Sequence of the Maize Chloroplast Genome: Gene Content, Hotspots of Divergence and Fine Tuning of Genetic Information by Transcript Editing," *Journal of Molecular Biology* 251, no. 5 (1995): 614–28.
- ⁴⁶E. H. Harris, J. E. Boynton, and N. W. Gillham, "Chloroplast Ribosomes and Protein Synthesis." Also see M. Sugiura, T. Hirose, and M. Sugita, "Evolution and Mechanism of Translation in Chloroplasts," *Annual Review of Genetics* 32 (1998): 437–59.
- ⁴⁷N. W. Gillham, *Organelle Heredity* (New York: Raven Press, 1978). Also see E. H. Harris, "Chloroplast Ribosomes and Protein Synthesis."
- ⁴⁸C. J. Schwartzbach and L. L. Spremulli, "Bovine Mitochondrial Protein Synthesis Elongation Factors. Identification and Initial Characterization of an Elongation Factor Tu-Elongation Factor Ts Complex," *Journal of Biological Chemistry* 264, no. 32 (1989): 19125–31. Also see H. K. Chung and L. L. Spremulli, "Purification and Characterization of Elongation Factor G from Bovine Liver Mitochondria," *Journal of Biological Chemistry* 265, no. 34 (1990): 21000–4; J. Ma and L. L. Spremulli, "Expression, Purification, and Mechanistic Studies of Bovine Mitochondrial Translational Initiation Factor 2," *Journal of Biological Chemistry* 271, no. 10 (1996): 5805–11.
- ⁴⁹For mitochondrial ribosomes, see H. de Vries, A. J. Arendzen, and A. M. Kroon, "The Interference of the Macrolide Antibiotics with Mitochondrial Protein Synthesis," *Biochimica et Biophysica Acta* 331, no. 2 (1973): 264–75. Also see N. D. Denslow and T. W. O'Brien, "Antibiotic Susceptibility of the Peptidyl Transferase Locus of Bovine Mitochondrial Ribosomes," *European Journal of Biochemistry* 91, no. 2 (1978): 441–8; R. J. LaPolla and A. M. Lambowitz, "Mitochondrial Ribosome Assembly in *Neurospora crassa*. Chloram-

- phenicol Inhibits the Maturation of Small Ribosomal Subunits," *Journal of Molecular Biology* 116, no. 2 (1977): 189-205; A. Dalhoff, "Pleiotropic Actions of Aminoglycosides," *Antibiotics and Chemotherapy* 39 (1987): 182-204. For chloroplast ribosomes, see N. W. Gillham, *Organelle Heredity*. Also see E. H. Harris, "Chloroplast Ribosomes and Protein Synthesis."
- ⁵⁰S. G. Andersson, et al., "The Genome Sequence of *Rickettsia prowazekii* and the Origin of Mitochondria."
- ⁵¹B. F. Lang, et al., "An Ancestral Mitochondrial DNA Resembling a Eubacterial Genome in Miniature," *Nature* 387, no. 6632 (1997): 493-7.
- ⁵²For a review, see Susan E. Douglas, "Plastid Evolution: Origins, Diversity, Trends." For genomic and protein phylogenies, see T. Rujan and W. Martin, "How Many Genes in *Arabidopsis* Come from Cyanobacteria? An Estimation from 386 protein phylogenies," *Trends in Genetics* 17, no. 3 (2001): 113-20. See also William Martin, et al., "Evolutionary Analysis of *Arabidopsis*, Cyanobacterial, and Chloroplast Genomes Reveals Plastid Phylogeny and Thousands of Cyanobacterial Genes in the Nucleus," *Proceedings of the National Academy of Sciences USA* 99, no. 19 (2002): 12246-51. Also see K. H. Chu, J. Oi, Z. G. Yu, and V. Anh, "Origin and Phylogeny of Chloroplasts revealed by a Simple Correlation Analysis of Complete Genomes," *Molecular Biology and Evolution* 21, no. 1 (2004): 200-6.
- ⁵³Just a few examples, in *Trichomonas*, see A. Germot, H. Philippe, and H. Le Guyader, "Presence of a Mitochondrial-Type 70-kDa Heat Shock Protein in *Trichomonas vaginalis* Suggests a Very Early Mitochondrial Endosymbiosis in Eukaryotes," *Proceedings of the National Academy of Sciences USA* 93, no. 25 (1996): 14614-7. In *Microsporidia*, see E. Peyretailade, et al., "Microsporidia, Amitochondrial Protists, Possess a 70-kDa Heat Shock Protein Gene of Mitochondrial Evolutionary Origin," *Molecular Biology and Evolution* 15, no. 6 (1998): 683-9. In *Giardia intestinalis* and *Entamoeba histolytica*, see N. Arisue, et al., "Mitochondrial-Type Hsp70 Genes of the Amitochondrial Protists, *Giardia intestinalis*, *Entamoeba histolytica* and two Microsporidians," *Parasitology International* 51, no. 1 (2002): 9-16.
- ⁵⁴Bryony A. P. Williams, Robert P. Hirt, John M. Lucocq, and T. Martin Embley, "A Mitochondrial Remnant in the Microsporidian *Trachipleistophora hominis*," *Nature* 418, no. 6900 (2002): 865-9.
- ⁵⁵K. H. Wolfe, C. W. Morden, and J. D. Palmer, "Function and Evolution of a Minimal Plastid Genome from a Nonphotosynthetic Parasitic Plant," *Proceedings of the National Academy of Sciences USA* 89, no. 22 (1992): 10648-52.
- ⁵⁶For *Astasia longa*, see G. Seimeister and W. Hachtel, "A Circular 73kd DNA From the Colourless Flagellate *Astasia longa* That Resembles the Chloroplast DNA of *Euglena*," *Current Biology* 15 (1989): 435-41. For malarial parasites and their plastids, see G. I. McFadden, "Mergers and Acquisitions: Malaria and the Great Chloroplast Heist," *Genome Biology* 1, no. 4 (2000): 1026.
- ⁵⁷A. E. Douglas, "Mycetocyte Symbiosis in Insects," *Biological Review of the Cambridge Philosophical Society* 64, no. 4 (1989): 409-34.
- ⁵⁸H. Ishikawa, "Biochemical and Molecular Aspects of Endosymbiosis in Insects," *International Review of Cytology* 116 (1989): 1-45.
- ⁵⁹Ibid.
- ⁶⁰P. Baumann, N. A. Moran, and L. Baumann, "The Evolution and Genetics of Aphid Endosymbionts," *BioScience* 47, no. 1 (1997): 12-20.
- ⁶¹P. Baumann, et al., "Genetics, Physiology, and Evolutionary Relationships of the Genus *Buchnera*: Intracellular Symbionts of Aphids," *Annual Review of Microbiology* 49 (1995): 55-94.
- ⁶²S. Shigenobu, et al., "Genome Sequence of the Endocellular Bacterial Symbiont of Aphids *Buchnera* sp. APS," *Nature* 407, no. 6800 (2000): 81-6.
- ⁶³P. Baumann, et al., "The Evolution and Genetics of Aphid Endosymbionts."
- ⁶⁴D. Schroder, et al., "Intracellular Endosymbiotic Bacteria of *Camponotus* Species (Carpenter Ants): Systematics, Evolution and Ultrastructural Characterization," *Molecular Microbiology* 21 (1996): 479-90; N. A. Moran and P. Baumann, "Phylogenetics of Cytoplasmically Inherited Microorganisms of Arthropods," *Trends in Ecology and Evolution* 9 (1993): 15-20; C. Bandi, et al., "The Establishment of Intracellular Symbiosis in an Ancestor of Cockroaches and Termites," *Proceedings of the Royal Society of London Series B* 259 (1995): 293-9; and S. Askoy, A. A. Pourhosseini, and A. Chow, "Mycetome Endosymbionts of Tsetse Fly Constitute a Distinct Lineage Related to *Enterobacteriaceae*," *Insect Molecular Biology* 4 (1995): 15-22.
- ⁶⁵Lynn Margulis, *Symbiotic Planet: A New View of Evolution*, 6-12.
- ⁶⁶For example, why do almost all mitochondria use a T7-like RNA polymerase, when the ancestral condition seems to be the four-subunit bacterial enzyme? See N. Cermakian, et al., "On the Evolution of the Single-Subunit RNA Polymerases," *Journal of Molecular Evolution* 45, no. 6 (1997): 671-81. Also see N. Cermakian, et al., "Sequences Homologous to Yeast Mitochondrial and Bacteriophage T3 and T7 RNA Polymerases are Widespread throughout the Eukaryotic Lineage," *Nucleic Acids Research* 24, no. 4 (1996): 648-54.
- ⁶⁷H. J. Muller, "The Relation of Recombination to Mutational Advance," *Mutation Research* 1 (1964): 2-9. See also C. G. Kurland, "Evolution of Mitochondrial Genomes and the Genetic Code," *BioEssays* 14 (1992): 709-14.
- ⁶⁸For *Arabidopsis*, see X. Lin, et al., "Sequence and Analysis of Chromosome 2 of the Plant *Arabidopsis thaliana*," *Nature* 402, no. 6763 (1999): 761-8. For felines, see J. V. Lopez, et al., "Numt: A Recent Transfer and Tandem Amplification of Mitochondrial DNA to the Nuclear Genome of the Domestic Cat," *Journal of Molecular Evolution* 39, no. 2 (1994): 174-90. For humans, see T. Tsuzuki, et al., "Presence of Mitochondrial-DNA-like Sequences in the Human Nuclear DNA," *Gene* 25, no. 2-3 (1983): 223-9. See also M. Fukuda, et al., "Mitochondrial DNA-like Sequences in the Human Nuclear Genome. Characterization and Implications in the Evolution of Mitochondrial DNA," *Journal of Molecular Biology* 186, no. 2 (1985): 257-66; H. Nomiya, et al., "Molecular Structures of Mitochondrial-DNA-like Sequences in Human Nuclear DNA," *Nucleic Acids Research* 13, no. 5 (1985): 1649-58; N. Kamimura, et al., "Three Separate Mitochondrial DNA Sequences are Contiguous in Human Genomic DNA," *Journal of Molecular Biology* 210, no. 4 (1989): 703-7; J. L. Blanchard and G. W. Schmidt, "Mitochondrial DNA Migration Events in Yeast and Humans: Integration by a Common End-Joining Mechanism and Alternative Perspectives on Nucleotide Substitution Patterns," *Molecular Biology and Evolution* 13, no. 6 (1996): 893; C. Herrnstadt, et al., "A Novel Mitochondrial DNA-Like Sequence in the Human Nuclear Genome," *Genomics* 60, no. 1 (1999): 67-77; and J. D. Yuan, et al., "Nuclear Pseudogenes of Mitochondrial DNA as a Variable Part of the Human Genome," *Cell Research* 9, no. 4 (1999): 281-90.
- ⁶⁹Chun Y. Huang, Michael A. Ayliffe, and Jeremy N. Timmis, "Direct Measurement of the Transfer Rate of Chloroplast DNA into the Nucleus," *Nature* 422 (2003): 72-6. Also see Pal Maliga, "Mobile Plastid Genes," *Nature* 422 (2003): 31-2.
- ⁷⁰P. E. Thorsness and T. D. Fox, "Escape of DNA from Mitochondria to the Nucleus in *Saccharomyces cerevisiae*," *Nature* 346 (1990): 376-9. In baker's yeast, mitochondrial DNA is used to heal double-stranded breaks in nuclear DNA. See M. Ricchetti, C. Fairhead, and B. Dujon, "Mitochondrial DNA Repairs Double-Stranded Breaks in Yeast Chromosomes," *Nature* 402 (1999): 96-100. See also X. Yu and A. Gabriel, "Patching Broken Chromosomes with Extracellular DNA," *Molecular Cell* 4, no. 5 (1999): 873-81. However this phenomenon has not been observed in other organisms. See A. Kirik, S. Salomon, and H. Puchta, "Species-Specific Double-Strand Break Repair and Genome Evolution in Plants," *EMBO Journal* 19, no. 20 (2000): 5562-6.
- ⁷¹H. L. Race, R. G. Herrmann, and W. Martin, "Why Have Organelles Retained Genomes?" *Trends in Genetics* 15, no. 9 (1999): 364-70.
- ⁷²M. W. Gray, et al., "Genome Structure and Gene Content in Protist Mitochondrial DNAs," *Nucleic Acids Research* 26, no. 4 (1998): 865-78. See also B. F. Lang, et al., "Mitochondrial Genome Evolution and the Origin of Eukaryotes."

- ⁷³Table modified from B. F. Lang, et al., "Mitochondrial Genome Evolution and the Origin of Eukaryotes."
- ⁷⁴Respiratory chain I composes part of the energy-producing machinery of the mitochondrion.
- ⁷⁵M. Turmel, et al., "The Complete Mitochondrial DNA Sequences of *Nephroselmis olivacea* and *Pedinomonas minor*. Two Radically Different Evolutionary Patterns within Green Algae," *Plant Cell* 11, no. 9 (1999): 1717-30.
- ⁷⁶William A. Dembski, *Intelligent Design* (Downer's Grove, IN: Intervarsity Press, 1999) 153-83. Also see Michael Behe, *Darwin's Black Box* (New York: Touchstone, 1996).
- ⁷⁷N. Shanks and K. H. Joplin, "Redundant Complexity: A Critical Analysis of Intelligent Design in Biochemistry," *Philosophy of Science* 66 (1999): 268-98; J. A. Coyne, "God in the Details: The Biochemical Challenge to Evolution," *Nature* 383 (1996): 227-8.
- ⁷⁸K. Oda, et al., "Gene Organization Deduced from the Complete Sequence of Liverwort *Marchantia polymorpha* Mitochondrial DNA: A Primitive Form of Plant Mitochondrial Genome."
- ⁷⁹Joachim Marienfeld, Michael Unseld, and Axel Brennicke, "The Mitochondrial Genome of *Arabidopsis* Is Composed of Both Native and Immigrant Information," *Trends in Plant Science* 4 (1999): 1360-85. Also see H. Handa, "The Complete Nucleotide Sequence and RNA Editing Content of the Mitochondrial Genome of Rapeseed (*Brassica napus* L.): Comparative Analysis of the Mitochondrial Genomes of Rapeseed and *Arabidopsis thaliana*," *Nucleic Acids Research* 31, no. 20 (2003): 5907-16.
- ⁸⁰Y. Notsu, et al., "The Complete Sequence of the Rice (*Oryza sativa* L.) Mitochondrial Genome: Frequent DNA Sequence Acquisition and Loss during the Evolution of Flowering Plants," *Molecular Genetics and Genomics* 268, no. 4 (2002): 434-45.
- ⁸¹T. Kubo, et al., "The Complete Nucleotide Sequence of the Mitochondrial Genome of Sugar Beet (*Beta vulgaris* L.) Reveals a Novel Gene for tRNA^{Cys}(GCA)," *Nucleic Acids Research* 28, no. 13 (2000): 2571-6.
- ⁸²This nomenclature for cytochrome *c* biogenesis genes follows that established by Sandrine Emmanuelle Faivre-Nitschke, et al., "Wheat Mitochondria *ccmB* encodes the Membrane Domain of a Putative ABC Transporter Involved in Cytochrome *c* Biogenesis," *Biochimica et Biophysica Acta* 1519 (2001): 199-208.
- ⁸³William A. Dembski, "Introduction: What Intelligent Design is Not," in *Signs of Intelligence*, ed. W. A. Dembski and J. M. Kushiner (Grand Rapids: Brazos Press, 2001), 7-23.
- ⁸⁴Some ID advocates have generated original research. See William Dembski, *The Design Inference* (Cambridge: Cambridge University Press, 1998). However, Dembski's work has been severely criticized. See Robert T. Pennock, *Tower of Babel* (Cambridge, MA: The MIT Press, 2000) and Mark Perakh, *Unintelligent Design* (Amherst, NY: Prometheus Books, 2004).
- ⁸⁵Michael J. Behe, *Darwin's Black Box*, 5-6.
- ⁸⁶*Ibid.*, 39.
- ⁸⁷W. Szer, J. M. Hermoso, and M. Boublik, "Destabilization of the Secondary Structure of RNA by Ribosomal Protein S1 from *Escherichia coli*," *Biochemical and Biophysical Research Communications* 70 (1976): 957-64.
- ⁸⁸J. A. Steitz, et al., "RNA-RNA and Protein-RNA Interactions during the Initiation of Protein Synthesis," in *Nucleic Acid-Protein Interactions*, ed. H. J. Vogel (New York: Academic Press Inc., 1977), 491-508. Also see M. A. Sorenson, J. Fricke, S. Pederson, "Ribosomal Protein S1 is Required for Translation of Most, If Not All, Natural mRNAs in *Escherichia coli* in vivo," *Journal of Molecular Biology* 280, no. 4 (1998): 561-9.
- ⁸⁹C. L. Squires and D. Zaporjets, "Proteins Shared by the Transcription and Translation Machines," *Annual Review Microbiology* 54 (2000): 775-98.
- ⁹⁰F. Robert and L. Brakier-Gingras, "A Functional Interaction between Ribosomal Proteins S7 and S11 within the Bacterial Ribosome," *Journal of Biological Chemistry* 278, no. 45 (2003): 44913-0.
- ⁹¹I. Moll, S. Grill, A. Grundling, and U. Blasi, "Effects of Ribosomal Proteins S1, S2 and the DeaD/CsdA DEAD-Box Helicase on Translation of Leaderless and Canonical mRNAs in *Escherichia coli*," *Molecular Microbiology* 44, no. 5 (2002): 1387-96.
- ⁹²W. A. Held, B. Ballou, A. Mizushima, and M. Nomura, "Assembly mapping of 30 S ribosomal proteins from *Escherichia coli*. Further Studies," *Journal of Biological Chemistry* 249, no. 10 (1974) 3103-11. See also V. Nowotny and K. H. Nierhaus, "Assembly of the 30S Subunit from *Escherichia coli* Ribosomes Occurs via Two Assembly Domains which are Initiated by S4 and S7," *Biochemistry*, 27, no. 18 (1988): 7051-5.
- ⁹³On S4 and 30S subunit assembly, see M. Nomura and W. A. Held, "Reconstitution of Ribosomes: Studies of Ribosome Structure, Function and Assembly," in *Ribosomes*, ed. M. Nomura, A. Tissieres, and P. Lengyel (New York: Cold Spring Harbor Press, 1988) 193-224. On S4 and translational fidelity, see D. I. Andersson, K. Bohman, L. A. Isaksson, and C. G. Kurland, "Translation Rates and Misreading Characteristics of *rpsD* Mutants in *Escherichia coli*," *Molecular and General Genetics* 187, no. 3 (1982): 467-72.
- ⁹⁴T. Pohl and B. Wittmann-Liebold, "Identification of a Cross-Link in the *Escherichia coli* Ribosomal Protein Pair S13-S19 at the Amino Acid Level," *Journal of Biological Chemistry* 263, no. 9 (1988): 4293-301; J. Schwarzbauer and G. R. Craven, "Evidence that *E. coli* Ribosomal Protein S13 has Two Separable Functional Domains Involved in 16S RNA Recognition and Protein S19 Binding," *Nucleic Acids Research* 13, no. 18 (1985): 6767-86.
- ⁹⁵F. L. Lin, L. Kahan, and J. Ofengand, "Crosslinking of Phenylalanyl-tRNA to the Ribosomal A Site Via a Photoaffinity Probe Attached to the 4-Thiouridine Residue is Exclusively to Ribosomal Protein S19," *Journal of Molecular Biology* 172, no. 1 (1984): 77-86; F. L. Lin, M. Boublik, and J. Ofengand, "Immunoelectron Microscopic Localization of the S19 site on the 30S Ribosomal Subunit Which is Crosslinked to a Site Bound Transfer RNA," *Journal of Molecular Biology* 172, no. 1 (1984): 41-55.
- ⁹⁶V. Ramakrishnan, V. Graziano, and M. S. Capel, "A Role for Proteins S3 and S14 in the 30S Ribosomal Subunit," *Journal of Biological Chemistry* 261, no. 32 (1986): 15049-52.
- ⁹⁷J. M. Musser, "Antimicrobial Agent Resistance in Mycobacteria: Molecular Genetic Insights," *Clinical Microbiological Reviews* 8, no. 4 (1995): 496-514.
- ⁹⁸T. Hosaka, et al., "The Novel Mutation K87E in Ribosomal Protein S12 Enhances Protein Synthesis Activity during the Late Growth Phase in *Escherichia coli*," *Molecular Genetics and Genomics* 271, no. 3 (2004): 317-24.
- ⁹⁹See K. L. Kindle, K. L. Richards, and D. B. Stern, "Engineering the Chloroplast Genome: Techniques and Capabilities for Chloroplast Transformation in *Chlamydomonas reinhardtii*," *Proceedings of the National Academy of Sciences USA* 88, no. 5 (1991): 1721-5. See also E. H. Harris, "Chlamydomonas as a Model Organism," *Annual Review of Plant Physiology and Plant Molecular Biology* 52 (2001): 363-406; M. M. el-Sheekh, "Stable Chloroplast Transformation in *Chlamydomonas reinhardtii* Using Microprojectile Bombardment," *Folia Microbiologica (Praha)* 45, no. 6 (2000): 496-504.

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