

# A Review of Mitoribosome Structure and Function does not Support the Serial Endosymbiotic Theory

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## Abstract

The theory of serial endosymbiosis states that eukaryotic cells, of which plants and animals are composed, evolved from the symbiosis of smaller aerobic and autotrophic prokaryotic cells (bacteria) living within larger prokaryotic cells. Unlike plant and animal cells, prokaryotic cells contain no membrane bound organelles or an organized nucleus. Based on this theory, organelles such as the mitochondria in modern eukaryotic cells would be the evolutionary descendents of the aerobic prokaryotic cells engulfed by the larger prokaryotes.

Mitochondria have a few minor characteristics that are similar to bacteria, such as their size, lack of introns in the mitochondrial genome, and a bi-layer cell covering that have been used to support the theory of serial endosymbiosis. However, there are also significant differences that make the transition of a bacterium into a eukaryotic mitochondrion impossible. Mitochondria and bacteria both have ribosomes, made of protein and ribonucleic acid (RNA), to catalyze the synthesis of proteins. When the theory of serial endosymbiosis was first proposed, it was assumed that ribosomes occurred in only two forms; a smaller 70S variety found in prokaryotes, and a larger 80S ribosome found in the cytosol of plant and animal cells (eukaryotes). According to the theory of serial endosymbiosis, the ribosomes present in mammalian mitochondria were expected to resemble the prokaryote 70S ribosome. However, the structure of mammalian mitochondrial ribosomes and their RNA and amino acid sequences indicate that mammalian mitochondrial ribosomes are completely different from prokaryotic ribosomes.

The evolution of mammalian ribosomes from prokaryotes requires major mutation and selection events to change a prokaryote-like ribosome into the mammalian mitochondrial ribosomes observed today. However, computer simulations with yeast and human genomes have shown that natural selection is unable to create new beneficial structures from random mutational events. Experiments introducing minor changes in the RNA and protein sequences of ribosomes have also demonstrated that these changes are deleterious and lead to decreased fitness.

It is apparent from the knowledge gained about mitochondria ribosome structure and function since the proposal of the Serial Endosymbiosis Theory that prokaryotes are not the ancestors of eukaryote or mammalian mitochondria.

**Keywords:** mitochondria, endosymbiosis, ribosome, eukaryote, prokaryote, ribonuclei acid (RNA) protein

## Introduction

Many people assume that “primitive” organisms are made of cells with fewer metabolic pathways, less organization, and smaller genomes than more “advanced” organisms. According to evolutionary theory, “primitive” one-celled organisms called prokaryotes (“first genome” or “before nucleus”) were the first cells to appear on Earth. The addition of metabolic pathways and larger genomes would eventually lead to the evolution of prokaryotes into more advanced cell-types and multicellular organisms.

“Advanced” organisms, called eukaryotes (“modern genome or nucleus”), are made of cells with more metabolic pathways, more organization, and larger genomes. Many eukaryote metabolic pathways and genomes are localized in compartments within cells to facilitate the increase in metabolic reactions necessary to maintain more complex systems. Compartmentalization, present in the

form of membrane bound organelles, is one of the distinguishing characteristics that separate eukaryotes from prokaryotes. Typical prokaryotes, like bacteria, are single-celled organisms lacking compartmentalization for many metabolic reactions. Plants (including algae), fungi, and animals have cells with many organelles including a nucleus, mitochondria, and chloroplasts (plants) that sequester reactions enabling more diverse cell-types and functions.

Because eukaryotes generally have more metabolic pathways and larger genomes, it is assumed that eukaryote cells evolved from the less complex prokaryotes. The leading hypothesis explaining the evolution of eukaryote cells from prokaryote cells is the Endosymbiotic Theory or Serial Endosymbiotic Theory (SET) popularized by Lynn Margulis (Margulis 1970). SET proposes that eukaryote cells evolved from a symbiotic community of smaller prokaryotes living within larger prokaryote cells. A simple scenario

for SET suggests a series of events beginning with the prokaryote plasma membrane folding inward, eventually evolving into the endomembranous system characteristic of the eukaryote endoplasmic reticulum and Golgi apparatus. Smaller aerobic prokaryote cells were engulfed by larger prokaryotes with the smaller prokaryotes eventually evolving into mitochondria. Chloroplasts originated when symbiosis between small autotrophic bacteria and larger prokaryotes resulted in the incorporation of the smaller autotrophic prokaryote into the biological system of the larger prokaryote (Campbell and Reece 2005).

The  $\alpha$ -proteobacteria, such as *Paracoccus*, *Rickettsia*, and *Bartonella species*, are assumed to be similar to the ancestral prokaryotes that gave rise to mitochondria based on ribosomal RNA sequence comparisons (Andersson et al. 1998; Kurland and Andersson 2000; Margulis 1970; Suzuki et al. 2001b; Yang et al. 1985). These comparisons are also based on the assumption that a prokaryote ancestor of mitochondria transferred many of its genes to the host genome resulting in mitochondria with small genomes while the evolved eukaryote nucleus possessed nearly all the genes for mitochondria structure and function. This hypothesis is not without problems though.

The human and bovine mitochondrial genomes have just 37 genes, including 13 protein coding genes, 22 tRNA genes, and two rRNA genes. This configuration of genes is also present in all other known mitochondrial genomes of vertebrates. Not only do vertebrates have the same genes, the order of genes is identical in all species with the exception of a few species that have the order of just a couple of transfer RNA genes reversed (Macey et al. 1997; Mindell, Sorenson, and Dimcheff 1998). The content and order of genes is a strong indication of stasis with no transfer of mitochondrial genes to the nucleus in vertebrate species. In an evolutionary scenario this would represent approximately 400 million years of stasis. The genes for vertebrate mitochondria ribosome proteins are located in the nucleus as are several hundred other genes including those for oxidative phosphorylation and mitochondria architecture. In addition, mitochondria ribosome genes are scattered among the chromosomes of sequenced genomes such as humans and *Bos taurus*, and have introns characteristic of eukaryote genomes. *Escherichia coli*, a typical prokaryote, has many ribosomal protein genes clustered on the chromosome, and of course being prokaryote, these genes lack introns characteristic of the mitoribosome genes in humans and bovines.

*Rickettsia prowazekii*, a typical  $\alpha$ -proteobacterium suggested as a type of mitochondrial ancestor, has 834 known protein-coding genes (Andersson et al. 1998), which is more than 20 times the size of the vertebrate

mitochondrial genome and more than 12 times the size of the mitochondrial genome in the protozoan *Reclinomonas americana* (67 genes), which possesses the largest known eukaryote mitochondrial genome. Certainly, if SET is valid, all gene transfers had to occur before the radiation of vertebrates and likely before the radiation of one-celled eukaryotes.

*Rickettsia prowazekii* is commonly given as a representative of the type of prokaryote that could have been an ancestor of the eukaryote mitochondria. *Rickettsia prowazekii* has one of the smallest genomes and several genes are organized similar to the mitochondrial genome in *Reclinomonas americana*, believed to represent one of the first eukaryote cell types (Andersson et al. 1998). *Reclinomonas americana* mitochondria have the most protein coding genes (67) of any known eukaryote mitochondrial DNA including 49 orthologous protein-coding genes that are found in sequenced mitochondrial genomes of other eukaryotes (Gray, Burger, and Lang 1999; Gray et al. 1998).

Other sources of support for SET include the observation of many endosymbiotic organisms extant today, the similar structure of mitochondria and chloroplasts to bacteria (Margulis 1993; Zablensky et al. 1975), sequence comparisons between DNA in eukaryote organelles and prokaryotes (Yang et al. 1985), and cytochrome c comparisons (Dickerson 1980).

There are many examples of endosymbiosis observed between extant organisms. Lichens are made of both fungi and algal cells, invertebrates frequently carry different types of algae, and bioluminescent bacteria can be found in the cells of fish, cephalopods, and mollusks to name a few of the many examples given by Margulis (Margulis 1993). Serial endosymbiosis should not be confused with the endosymbiosis observed between many different kinds of organisms. SET proposes that symbiotic relationships between organisms observed in nature reflects a similar starting process for the evolution of eukaryote cells.

SET gained further support as a portion of eukaryote mitochondrion structure became better understood revealing many similarities with bacteria outlined in another book by Margulis (Margulis 1993). These similarities include a similar size for mitochondria and many bacteria (<10 $\mu$ m), a two-layered cell or organelle covering, and a circular plasmid-like strand of DNA lacking introns. The theory became more popular when it was discovered that horizontal or lateral gene transfer might occur between mitochondria and the nucleus, and does occur between chloroplasts and the nucleus of individual plant cells (Adams, Ong, and Palmer 2001; Chacinska and Rehling 2004; Covello and Gray

1992). This provided a putative explanation for how many genes encoding eukaryote mitochondrial and chloroplast proteins are located in the nucleus instead of their respective organelles and how the genomes of these organelles became considerably smaller than their ancestral prokaryote counterparts (Adams and Palmer 2003).

In spite of the documented similarities existing between eubacteria and eukaryote mitochondria and chloroplasts, many problems remain unsolved in reconciling SET with the current knowledge of the biological systems of eukaryote and prokaryote organisms. With the increasing knowledge gained from sequencing many representative genomes in all domains of life and several thousand organelle genomes, the differences in structure and DNA sequence of organelle genomes and prokaryotes as SET ancestor organisms has strained the validity of SET. Several prominent researchers have noted the problems of endosymbiosis as a model for the origin of eukaryote cells. For example, Kurland and Andersson, stated that it is not possible to identify the ancestral host of the endosymbiont for SET and that the current endosymbiotic theory (in 2000) needed to be modified (Kurland and Andersson 2000). Gabaldon and Huynen also noted that there is no extant eukaryote whose amitochondriate state can be considered ancestral (Gabaldon and Huynen 2004) and acknowledged the implications for SET if this situation remained unchanged. After examining the properties of mammalian mitochondria ribosomes, O'Brien observed that the unusual properties of these ribosomes raise questions about their relationship to other kinds of ribosomes (O'Brien 2002). Obviously none of these researchers are in the process of abandoning evolutionary thought, but all have made honest evaluations concerning the unresolved situation with the evolutionary origin of eukaryote cells.

Not only has the identification of a representative ancestor remained unknown, but as more information has been collected on the structure of mitochondria in particular, the plausibility of evolutionary SET has become less tenable and any possibility of SET in a creation context is impossible. At the center of this controversy is the structure and function of the eukaryote mitochondria ribosomes and their lack of similarity to a putative prokaryote ribosome ancestor. It should be acknowledged that in an evolutionary scenario more than 2 billion years has passed which would allow for an incredible amount of divergence to take place in the resulting eukaryote organisms and the prokaryote ancestors (Adams and Palmer 2003; Gabaldon and Huynen 2004; Smits et al. 2007). However, for SET to be plausible there must be observable evidence connecting the descendants

(mitochondria) to an ancestor (prokaryote) and a feasible mechanism to convert the existing structures from the ancestral state.

### **Ribosomes and SET**

Within mitochondria are ribosomes that were assumed to be similar to bacterial ribosomes in size and structure. The structure and composition of mitochondrial ribosomes (mitoribosomes) compared to prokaryote ribosomes is the focus of this paper since it would take a book to cover all of the implications of SET based on the remaining evidence presented by Margulis and others. The importance of ribosomes to SET is easily understood by simply acknowledging that all biochemical pathways are affected by the structure, function and activity of ribosomes. This is true of all prokaryote, eukaryote, and organelle systems. Changes in ribosome structure and function affect all cell functions dependent on the synthesis of proteins.

Before the elucidation of ribosome structure, it was assumed that ribosomes came in two sizes, a smaller 70S ribosome found in prokaryotes and a larger 80S ribosome found in the cytosol of eukaryotes. It was expected that, based on SET, eukaryote mitochondrial ribosomes in general would be the smaller 70S ribosomes consistent with the notion that mitochondria descended from an aerobic prokaryote (O'Brien 2003). Recent advances in molecular biology techniques have identified the structural and functional components of ribosomes for many prokaryotes and eukaryotes, and the information from these studies is inconsistent specifically with an SET origin for eukaryote mitochondria ribosomes and for the origin of eukaryote cells from a prokaryote ancestor in general. There is a wide range of ribosome sizes depending on the organism and whether the ribosome is located in the cytosol, mitochondria, or chloroplasts of a eukaryote. It was also assumed specifically that vertebrates, including mammals, would have mitochondria ribosomes similar to the 70S ribosome in a prokaryote ancestor rather than their 80S cytosolic counterparts (Mears et al. 2006).

### **Comparison of prokaryote and eukaryote ribosome structure**

All ribosomes are made of ribonucleic acid (RNA) and proteins. *Escherichia coli*, a typical prokaryote and model organism for ribosome structure and function, has 70S ribosomes typical of all prokaryotes (Cannone et al. 2002). Ribosomes and their RNA subunits are classified according to a sedimentation coefficient (S). Sedimentation coefficients are derived partly from the size of the molecules, protein and nucleic acid composition, and how dense or porous they are during centrifugation. Consequently,

some characteristics of a ribosome can be derived by knowing the composition of the ribosome and its sedimentation coefficient. Ribosomes with similar structure and composition will have similar sedimentation coefficients while larger ribosomes will generally have larger sedimentation coefficients than smaller molecules.

There are two subunits in the *E. coli* 70S ribosome. A 30S small subunit (ssu) composed of 1540 nucleotides of 16S ribosomal RNA (rRNA) and 21 proteins, and a 50S large subunit (lsu) composed of 23S and 5S rRNA (2800 nucleotides), and 31 proteins (Cannone et al. 2002; Garrett and Grisham 1999). The small (30S) and large (50S) subunits assemble to form a 70S ribosome during initiation of protein synthesis.

Mammalian cytosolic ribosomes are larger than their prokaryote counterparts having a 40S small subunit made of 18S rRNA (1900 nucleotides) and 33 proteins, and a large subunit made of 28S and 5S rRNA (4700 nucleotides), and 49 proteins (Garrett and Grisham 1999). Mammalian mitochondrial ribosomes (mitoribosomes) have a lower sedimentation coefficient (55S) than the ribosomes of prokaryotes, but are actually larger and heavier indicating they have an internal structure distinctly different from prokaryote ribosomes. Human mitoribosomes, typical of mammals, have a 28S small subunit made of 12S RNA (954 nucleotides) and 33 proteins, and a 39S large subunit made of 16S RNA (1558 nucleotides) and 48 proteins (Anderson et al. 1981; O'Brien 1971; Smits et al. 2007). The mammalian mitoribosomes have just 2/3 as much RNA as prokaryotes and 60% more protein than prokaryotes. Prokaryote ribosomes are 2/3 RNA and 1/3 protein by weight while the reverse is true of mammalian mitoribosomes, which are 2/3 protein and 1/3 RNA (Matthews et al. 1982).

Mammalian mitoribosomes have a larger mass than prokaryote 70S ribosomes mostly due to their larger proteins. The bovine (*Bos taurus*) mitoribosome, also typical of mammals, has a mass of  $2.64 \times 10^6$  Daltons (Da) determined from the mitochondrial ribosomal RNA and protein content (Matthews et al. 1982). This is larger than the mass ( $2.49 \times 10^6$  Da) of *E. coli* 70S ribosomes (Patel, Cunningham, and Hantgan 2001). The size of the bovine mitoribosome is also 24% larger than prokaryote 70S ribosomes when analyzed by electron microscopy (Patel, Cunningham, and Hantgan 2001).

### **Functional differences between prokaryote ribosomes and mitoribosomes**

The differences in protein and RNA content, and sedimentation coefficients between prokaryote ribosomes and eukaryote mitoribosomes are not trivial, and they are a clue that major structural differences exist between each kind of ribosome.

Because of the differences in RNA and protein content, it is assumed, according to SET, that eukaryote mitochondria ancestors lost some of their mitochondria ribosomal RNA and replaced it with novel proteins, bi-functional exapted proteins, or N- and C-terminal extensions of existing proteins (O'Brien 2002; Smits et al. 2007).

The function of all ribosomes is essentially identical and it should not be surprising that there are "conserved" (used here to denote similar RNA and amino acid sequence and structure) regions of ribosomes in the different domains of life. All ribosomes whether in a prokaryote, the eukaryote cytosol, the mitochondrial matrix, or the chloroplast stroma are the site of translation of the DNA code and protein synthesis. Initiation of protein synthesis, in prokaryotes, occurs when translation initiation sequences on messenger RNA (mRNA) bind complementary sequences along the rRNA of the small subunit. An initiation complex forms when initiation factors, the small subunit, mRNA, and the large subunit assemble. The peptidyl site (P-site), located on both small and large subunits, is initially occupied by a modified methionine on tRNA. (The modification is dependent on what kind of ribosome is involved). Elongation of the polypeptide chain begins as a cognate tRNA recognizes the correct codon on mRNA, occupies the aminoacyl site (A-site, comprised of rRNA from both subunits) and forms a peptide bond with the polypeptide chain attached to tRNA in the P-site. The uncharged peptidyl tRNA is released as the A-site tRNA is bound to the polypeptide chain and translocates to the vacant P-site with the assistance of an elongation factor and the expenditure of energy from the hydrolysis of guanosine triphosphate (GTP). These steps are repeated until the polypeptide is formed and translation terminates when the ribosome reaches a stop codon on mRNA (Garrett and Grisham 1999).

Because translation is basically the same process in all cell-types, mitochondria, and chloroplasts, it should not be surprising there is a degree of homology in the regions directly involved with the process of forming a nascent polypeptide (Mears et al. 2006). What is surprising is the degree of divergence between the rest of the ribosome in different cells and organelles. A brief comparison of the gross structure of prokaryote ribosomes and eukaryote mitochondria ribosomes does not confirm the "RNA replacement theory" of SET, but shows distinct kinds of ribosomes in prokaryotes and mitochondria.

Cryo-electron microscopy (cryo-em) of bovine (*Bos taurus*) mitoribosomes identified distinctly different structures for bovine mitoribosomes compared to prokaryote or eukaryote cytosolic ribosomes (Mears et al. 2002; Sharma et al. 2003). Cryo-em provided

evidence that the additional and enlarged proteins in mammalian mitochondria do not compensate for the missing RNA segments, but occupy different positions in the subunits of mitochondria (Sharma et al. 2003).

Sharma et al. gave many examples of the “divergent” structure of mitochondria and the following is a summary of some of the more important points of their data. The small and large ribosome subunits are joined by “bridges” during translation initiation and the structure and placement of these bridges are quite different in mammalian mitochondria compared to prokaryote ribosomes. Prokaryote 70S ribosomes have nine intersubunit bridges connecting the two ribosome subunits during translation (Yusupov et al. 2001; Wimberly et al. 2000). The 55S mitochondria is held together by 15 intersubunit bridges with only six of these bridges similar to those found in prokaryotes (Sharma et al. 2003). The nine intersubunit bridges in mitochondria, not found in prokaryotes, are dominated by protein-protein interactions while prokaryote ribosomes have RNA-RNA bridges (Sharma et al. 2003). While it is true that proteins are responsible for most of the bridge network in mitochondria instead of the RNA bridges in prokaryote ribosomes, it is crucial to recognize that the RNA bridges in prokaryote ribosomes are not replaced with protein bridges in mitochondria. The protein bridges in mitochondria are distinct from any of the bridges identified in prokaryotes and there are no prokaryote homologues for the nine “new” bridges in mitochondria.

Proteins do comprise a larger portion of the mitochondria than the prokaryote ribosome. Are these proteins actually compensating for the lesser amount of RNA in mitochondria or are they in novel positions with functions distinctly different from prokaryotes? The bridge structure discussed above shows that many of the additional proteins have different positions in the mitochondria than those in the prokaryote ribosome. This is true of many of the other proteins in the mitochondria as well. There are 33 proteins (out of 81 identified so far) in human mitochondria that do not have homologues in prokaryote ribosomes (Smits et al. 2007). One of the most distinctive differences in the mitochondria protein structure is the location of many of these proteins on the superficial portion of the ribosome (Sharma et al. 2003). In prokaryotes, proteins are located sporadically in patches on the outside of the ribosome (Carter et al. 2000; Sharma et al. 2003; Wimberly et al. 2000). Not only are the proteins in different positions, but cryo-em structures indicate that only ~20% of the RNA components missing in mitochondria (compared to prokaryote ribosomes) are replaced by mitochondria specific proteins (Mears

et al. 2006). Among the proteins that are homologous in ribosome position in both prokaryote ribosomes and mitochondria a general increase in size in the mitochondria is observed. This is due to the addition of N- and C-terminal extensions to assumed homologous proteins.

It is not feasible to discuss all 48 homologous proteins individually for the purposes of this paper, but a discussion of the prokaryote small subunit protein S15 will provide a typical example of the significant differences between “homologous” prokaryote and mitochondria proteins and explain the importance for some degree of homology. Mammalian mitochondrial protein S15 is 2.4 times more massive than its homologous counterpart in *E. coli*. This size difference is attributable to the N- and C-terminal extensions on the mitochondria S15 protein (Suzuki et al. 2001a). The S15 proteins in both prokaryotes and mitochondria bind to the 16S RNA and 12S RNA, respectively, along the double-stranded helix 34 (*E. coli* numbering) (Suzuki et al. 2001a; Carter et al. 2000). S15 has four  $\alpha$ -helices enabling it to bind to the helix of double-stranded RNA in both prokaryote ribosomes and mitochondria in an area involved with the translocation of the growing polypeptide from the A-site to the P-site (Brink et al. 1994; Carter et al. 2000; Suzuki et al. 2001a). It should not be surprising then that the amino acid sequence of the S15  $\alpha$ -helices in both ribosome types share 36–38% homology among several prokaryotes (including *R. prowazekii* and *E. coli*) compared to mammalian mitochondria (Suzuki et al. 2001a). Prokaryotes have no homologous sequences to the N- and C-terminal extensions in mitochondria S15 protein and blast searches for paralogous DNA sequences have not been found in the human genome indicating that S15 N- and C-terminal extensions are novel, and did not originate from pre-existing sequences within the human genome (Suzuki et al. 2001a). This also appears to be true of the N- and C-terminal extensions for the other mitochondria proteins that are identified as “homologous” to prokaryote ribosomal proteins. This is important to note because the N- and C-terminal extensions would have to originate from newly created DNA sequences, not from existing DNA (such as pseudogenes, duplicated genes, or untranslated regions) that could be recruited to replace “lost” RNA.

Because of the different RNA and protein composition, and the distinctly different arrangement of RNA and protein in mitochondria compared to prokaryote ribosomes, it should not be surprising to find major differences in the specifics of the two translation systems. Mitochondria lack the ability to recognize Shine-Dalgarno sequences on mRNA, which are necessary for translation initiation in prokaryote

ribosomes, but obviously not for mitoribosomes (Sharma et al. 2003). Mammalian mitoribosomes also have an intrinsic GTP binding site in the protein of the small subunit, which is unique among all known translational systems (Denslow, Anders, and O'Brien 1991; O'Brien 2002) and quite different from the GTP binding site located on elongation factor G in prokaryotes (Bilgin et al. 1990; Carter et al. 2000). The unique features in mitoribosome structure and composition make it possible for mitoribosomes to process their unique mRNAs. Mitochondria mRNA lack modifications to the 5' and 3' ends found in cytosolic eukaryote mRNA or prokaryote mRNA, and have a start codon within three nucleotides of the 5' end, all unique features among mRNAs and translation systems (Anderson et al. 1982; Sharma et al. 2003).

Sequence comparisons between prokaryote and mitoribosome RNA genes (rDNA) or the rRNA sequences in the small or large subunits are difficult due to a lack of sequence homology. The sequence differences are so significant, that alignment programs available at the National Center for Biotechnology Information (NCBI) will not align these sequences partly due to the difference in length (for example, prokaryote *E.coli* ssu RNA is 1540 nucleotides, human mitoribosome ssu RNA is 954 nucleotides) and partly due to the different frequencies of rRNA nucleotides. An alignment can be forced manually using a program like ClustalX, but these alignments, which are highly subjective, show less than 40% homology between the two types of small subunit RNA with long stretches of gaps resulting in insignificant alignment scores.

A comparison of nucleotide frequencies reveals several striking deviations. Guanine is the most frequently occurring nucleotide (30%) in prokaryote ribosomal RNA (rRNA), but is less than 18% of the nucleotides in bovine mitochondria rRNA (Mears et al. 2006). Conversely adenine is found at only 25% of the sites in prokaryote rRNA, but 38% of bovine mitochondria rRNA sites (Mears et al. 2006). Although there are several stem loops with nucleotide pairing in the secondary structure of rRNA (Carter et al. 2000; Wimberly et al. 2000) it should be noted that much of the rRNA in ribosomes is not base-paired making the frequency of individual nucleotides far more important to the secondary structure and function in rRNA than the frequency of nucleotides in a double-stranded nucleic acid such as DNA.

The overwhelming evidence for the unique mitoribosome structure and function compared to prokaryote ribosome structure and function is compelling evidence that mitoribosomes are distinctly different from prokaryote ribosomes and do not share a common prokaryote ancestor. The structure of ribosomes in organelles, prokaryotes, and the

eukaryote cytosol are so different that they are easily distinguishable from each other. This uniqueness even extends into the different domains of life where archaea and bacteria ribosomes are distinctly different, having no "gray areas" of confusion between the identity of each type of ribosome (Roberts et al. 2008).

### **Changing a Prokaryote Ribosome into a Mitoribosome**

It certainly could be argued that 2 billion years of evolution have created the vast differences between extant prokaryote ribosomes and their, now apparent, distant "relatives" the vertebrate mitoribosome. What mechanisms would promote or even permit the conversion of a prokaryote-type ribosome into the distinctly different mitoribosome? Was it an accumulation of nearly neutral mutations in the DNA coding for the ribosomal components and translation machinery proteins resulting in a transition to an entirely different ribosome? Or was it strong environmental pressures that encouraged selection of DNA, RNA, and protein alterations that facilitated the conversion of prokaryote-type ribosomes into mitoribosomes?

It is well-documented that mitoribosomes and prokaryote ribosomes are not under the classical definition of the neutral model of the theory of evolution (Hasegawa, Cao, and Yang 1998; Rand 2008). The simplest reason for this omission is the high nonsynonymous/synonymous substitution rates within species and between species, a condition not compatible with the neutral theory. Only a discussion of mutation and natural selection will be considered since there is much evidence that mitoribosomes, especially, are under strong selective forces.

### **Prokaryote antibiotic resistance; a model for the consequences of ribosome change**

The fast generation times and high mutation rates in prokaryotes permits limited genetic alteration enabling adaptations to changing microbial environmental conditions. These alterations are limited by the cost to the metabolic machinery of affected organisms and are eliminated from the population when environmental conditions return, favoring the pre-mutation phenotype (Criswell et al. 2006). Mutations in ribosomal RNA and proteins conferring antibiotic resistance have provided an excellent model for the limitations of genetic variation in many prokaryotes and an understanding of a few of these mechanisms will clarify the limitations of ribosome change in extant prokaryote translation systems. There are numerous papers documenting the fitness cost to prokaryotes and other microbes that accumulate mutations in ribosomal RNA

and proteins conferring resistance to specific antibiotics. (See Criswell et al. 2006 for many of these references.) However, more importantly is the effect these mutations have on ribosome function and the plausibility that mutation is a possible mechanism for the conversion of a prokaryote ribosome into a distinctly different mitoribosome.

Many *E. coli* mutations conferring antibiotic resistance (and a host of other bacteria) have been documented and the mechanisms of resistance well-studied. One antibiotic resistant mutation that is well understood is a DNA mutation that changes the base pairing in helix 34 of the ribosomal 16S RNA conferring resistance to the antibiotic spectinomycin (Brink et al. 1994). A C1192G mutation in helix 34 and its base paired partner G1064C provide low levels of resistance to spectinomycin, but more importantly, result in a five-fold decrease in growth rates (Brink et al. 1994). Chloramphenicol acetyl transferase (CAT) activity in *E. coli* carrying this double-mutant was only 20% of normal levels (non-mutant or wild-type) verifying the cost to the metabolic machinery of this mutation (Brink et al. 1994). The reduced levels of CAT activity resulted from the reduced ability of the *E. coli* to assemble 70S ribosomes from the small subunits carrying the double mutant (Brink et al. 1994). Mutants, not in the presence of spectinomycin would be (and are) quickly eliminated by their inability to compete with strains carrying the more effective RNA sequences and returning the population back to the more efficient metabolic mechanisms carried in their DNA. Although this is only one example of the consequences of altering the prokaryote ribosome it is typical of the mutations that are documented conferring resistance to antibiotics. Mutations cause major shifts in ribosome secondary structure and have pronounced consequences that are magnified throughout the cell as all metabolic activities are affected by the fidelity of protein synthesis performed by ribosomes (Allen and Noller 1989).

Competition between different kinds of ribosomes has also been observed in *E. coli* confirming that aberrant ribosomes are unlikely to be incorporated into the metabolic machinery when wild-type or cell-specific ribosomes are present. *E. coli*, transformed with a plasmid carrying *Salmonella enterica* specific spectinomycin resistant 16S ribosomal genes, was unable to acquire resistance to spectinomycin (O'Connor and Dahlberg 2002). Although the genes for *Salmonella* ribosomes are 97% identical in DNA sequence to *E. coli* ribosomes (Krawiec and Riley 1990), it is likely that the small subunits from the *Salmonella* 16S RNA gene were unable to compete with the *E. coli* ribosomal proteins and translation factors for ribosomal assembly (O'Connor and Dahlberg 2002). The inability of *E. coli* to assemble

ribosomes with the “foreign” 16S RNA component is evidence that major changes in the prokaryote genome are not incorporated into the biochemistry of the organism or its genetic material. This is an indication that structures capable of providing a temporary advantage to the cell require a host of simultaneous changes in the cell machinery to utilize aberrant components resulting from sudden multiple changes to designed components.

For SET to be feasible, it is necessary to identify the observable mechanisms that would convert prokaryote ribosomes into the characteristic mitoribosomes observed today in prokaryote or protista (*R. americana*) systems. With the small mitochondrial genome found in *R. americana* and the 400 million years of stasis (according to evolution) observed in the vertebrate mitochondrial genome there is no observable evidence that any part of SET took place after the divergence of vertebrates.

### Can selection change a ribosome?

The primary assumed mechanism for evolution is mutation and natural selection. The evidence above demonstrates that selection does occur among prokaryotes that acquire mutations in ribosomes resistant to antibiotics. Selection has also been documented in the population of mitoribosomes in the germ-line of mammals. But can natural selection really choose mutations that enable an organism to make significant permanent changes to its genome, biochemistry, and consequently phenotype that are beneficial to a proposed different environment?

A team of scientists when they worked at the Institute for Creation Research developed a computer simulation program testing the hypothesis that natural selection could use mutations to fix beneficial changes in the genome while eliminating deleterious mutations and consequently evolving an organism into a new form or kind (Sanford et al. 2008). The results of their simulations with human and yeast genomes demonstrated that selection could remove very few deleterious mutations while selecting for beneficial mutations. A summary of their population simulations revealed the following principles: 1) Deleterious mutations continue to accumulate over time. 2) Selection was unable to separate deleterious mutations from beneficial mutations. Both types of mutations are inherited together. 3) The net effect of mutation is always negative. 4) Because the overall effect is always negative, fitness always declines with time. This results in an increase in genetic entropy or loss of information leading to extinction and not new kinds of organisms or biological systems.

The results of the Sanford et al. work is confirmed by the experimental evidence of antibiotic resistant organisms that lose fitness as mutations accumulate.

The proposed “road” to a mitoribosome from a prokaryote ribosome ancestor contradicts the observable and experimental evidence indicating stasis among these important biological machines.

## Conclusion

Mitoribosomes are distinctly different from prokaryote ribosomes in structure and in the specifics of translation and protein synthesis. Mitoribosome structure is distinctly different from prokaryote ribosomes, evidenced by a larger mass and size but smaller sedimentation coefficient. Mitoribosomes differ from prokaryote ribosomes in RNA and protein content, position and function of ribosomal RNA and proteins, and are significantly different in sequence, especially in regions that do not contact the tRNA or the growing polypeptide chain. Mitoribosomes have many features unique to a mitochondrial translation system including novel mRNAs, modifications in mitoribosomes to process mitochondrial mRNA, and a novel GTP binding site during polypeptide elongation.

A feasible scenario to convert prokaryote ribosomes to mitoribosomes is not forthcoming. Mutations, in both prokaryote ribosomes and eukaryote organisms (humans), have demonstrated that mutation and natural selection are not capable of producing new biological systems or organisms. Mutations lead to reduced fitness and extinction in biological systems. A high nonsynonymous/synonymous substitution rate in the mitochondrial genomes within species and between species is evidence that the neutral theory of evolution is not applicable to these systems.

The lack of an observable, feasible amitochondriate ancestor and the lack of transitional ribosomes and translation systems have led many researchers to question the current Serial Endosymbiosis Theory and to suggest modifications to the theory. The comparison of ribosomes and translational systems in this paper provides evidence that the Serial Endosymbiosis Theory is not feasible given the current data available for review.

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