Research has overturned endosymbiosis: the unbridgeable gap between prokaryotes and eukaryotes remains

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Cells are divided into two major groups: prokaryotes (i.e. without organelles) and eukaryotes (i.e. with organelles). Evolution postulates that prokaryotes evolved into eukaryotes. An enormous gap exists between these two cell types that could not have been bridged by transitional forms. The most popular effort to explain this gap is the endosymbiosis theory of Lynn Margulis. The proponents' theory proposes some proto-eukaryotic cells engulfed prokaryotes, and eventually the engulfed proteobacteria evolved into organelles in the primitive eukaryotes. The many major problems with this theory are reviewed, leading to the conclusion that it is widely accepted only because it is the most plausible evolutionary hypothesis and not because of empirical evidence. In fact, as documented in this paper, considerable evidence exists against the endosymbiosis theory.

Understanding the "evolution of eukaryotic cellular complexity is one of the grand challenges of modern biology."[1] Unlike prokaryotes, eukaryotic cells are highly compartmentalized and contain many different membrane-bound organelles that are absent from bacteria or archaea (non-bacterial single-celled prokaryotes). Along with many genetic and molecular differences, complex compartmentalized organelles are not found in prokaryotes. Evolutionists generally attempt to explain how this happened by endosymbiosis, claiming that an ancient archaean engulfed a proteobacterium that eventually gave rise to the first organelle, a mitochondrion.

Endosymbiosis is a phenomenon where one creature lives inside another in a mutually beneficial relationship, such as is observed in the case of certain bacteria that live inside termites, for example. In this paper, 'endosymbiosis' will refer not to such observed phenomena, but to the widely accepted hypothesis by the late Professor Lynn Margulis (figure 1) to explain the origin of mitochondria, which is the focus of this paper (sometimes referred to as 'primary endosymbiosis'). It is invoked in an attempt to bridge the gap existing between cells lacking compartmentalized organelles (prokaryotes) and those with them (eukaryotes). Of course, the differences are much more than just mitochondria. Some of the other organelles a eukaryote has in contrast to prokaryotes are a nucleus, nucleolus, rough and smooth endoplasmic reticulum, Golgi apparatus, centrioles, peroxisomes, and lysosomes.[2]

At some point in the distant past, this endosymbiotic theory states, a prokaryotic bacterium engulfed a theoretical proteobacterium which remained inside of the host in a symbiotic relationship. That means that hundreds of genes were somehow modified to completely new functions. Additionally, the theory suggests that thousands of genes from the proteobacterium were transferred into the protoeukaryote cell's nucleus as many other genes were discarded.

The prokaryotic-eukaryotic DNA chasm

Prokaryotes contain a single circular DNA molecule that occupies the nucleoid region and often also small rings of double-stranded extrachromosomal DNA called plasmids. Prokaryote DNA is "profoundly different from eukaryotes" that contain "two to four separate and independently transmitted nuclear, chloroplastic, microtubular, and mitochondrial DNA genomes".[3] The prokaryotic–eukaryotic contrast is so great that it constitutes "'the greatest single evolutionary discontinuity' of life" known, and the "origin of eukaryotes has remained one of the most enigmatic, controversial and challenging questions in evolution."[4] Only two naturalistic theories of organelle evolution remain; all others have been effectively refuted.[5]

The first theory is the autogenous 'self-generated' hypothesis, which postulates that organelles evolved gradually from some precursor organelle by natural selection of mutations. This view lacks evidence for all existing organelles, and consequently has largely been rejected and replaced with some form of endosymbiosis.

The second theory is endosymbiosis, also called serial endosymbiosis theory (SET), symbiogenesis, or the *xenogenous hypothesis*. The enveloped bacteria (figure 2)

Figure 1. Late evolutionist professor Lynn Margulis (1938–2011), advocate of the faulty endosymbiotic hypothesis of the origin of eukaryotes

subsequently evolved inside their hosts to gain specialized functions, some becoming mitochondria (figure 3) that eventually took over the role of providing energy by 'charging' ADP to ATP. This occurs by way of the ATP synthase enzyme, a molecular 'machine'in both eu- and prokaryotes.[6] Prokaryotes are single-celled microscopic organisms that have far lower energy requirements than eukaryotes.

The ATP synthesis machinery in a prokaryote is embedded in its cell membrane, which would have been the case for the engulfing bacterium, which as at this stage in the theory still a prokaryote. In contrast, eukaryotes use mitochondria to charge ADP, so when the ancient archaean supposedly engulfed a proteobacterium which would eventually become a mitochondrion, it still had to meet its energy needs while evolving from a prokaryote to a eukaryote. Furthermore, only a "small fraction of the proteins required for the propagation and function of mitochondria are coded by their genomes, while nuclear genes code the vast majority."[7] The heart of the endosymbiosis explanation for the chasm between prokaryotes and eukaryotes is mitochondrial DNA.

From endosymbiosis to multicellular organisms

It has been long recognized that "The principle of endosymbiosis was suggested more than a century ago but was generally considered as 'entertaining fantasy'."[8] This view largely held until the work of Lynn Margulis, who developed the endosymbiosis idea in great detail.[9] Through her work and influence, endosymbiosis has moved from an obscure, poorly accepted idea to the most popular theory of organelle origins today.[10]

The common endosymbiosis scenario postulates that free-living eukaryotic cells eventually joined in communities now called multicellular organisms.[11] Problems were encountered soon after the endosymbiosis theory was proposed. For example, further research found that the mitochondria of fungi, plants, and animals were so different that endosymbiosis must have occurred independently many times, which only multiplies the highly improbable odds of the endosymbiotic scenario occurring even once.[12]

Margulis predicted that mitochondrial DNA will be different from nuclear DNA, but instead will consist of admixtures of sequences from eubacterial and archaeal genes.[13] In 1963, mitochondria were found to possess DNA independent of nuclear DNA.[14] Another important factor supporting endosymbiosis was the 1960s discovery of plastid DNA (cpDNA) in the chloroplast of plants called the plastome, which supporters argued made the endosymbiosis mechanism for the origin of organelles more plausible.[15]

The organelles called mitochondria are commonly "believed to have arisen only once in evolutionary history, but despite their common ancestry, mitochondrial DNAs vary extensively throughout eukaryotes in genome architecture and gene content."[16] Researchers have found that the largest mtDNA is in a freshwater protozoan, *Reclinomonas americana,* which has 69,034 nucleotides and 97 genes that encode 67 proteins, which includes at least 18 proteins not previously known to be encoded in mitochondria.[17] Endosymbiosis must, therefore, postulate that the engulfed bacteria lost 96–99% of their proteins, from ~1,600 down to below 67, depending on the specific engulfed protobacterium. For humans, only 37 genes are essential for cellular respiration.

Furthermore, comparisons of eukaryotic proteins in eukaryotic organelles has found they are not simply admixtures of sequences from archaea and eubacteria as endosymbiosis predicts, but are often unique, inconsistent with the idea that eukaryotic genomes are a combination of eubacterial and archaeal genes.[18]

All other examples examined also show major differences between mitochondrial and bacterial genomes, as expected given the protein differences described by Kirkland in a previous paragraph. Even for the best-known example (*R. americana* mtDNA), enormous differences exist between its 69-kbp (97 genes) and both the 580-kbp genome (470 genes) of *Mycoplasma genitalium* and the 1,830-kbp (1,743 genes) *Haemophilus influenzae* genome. Furthermore,

> "Comparison of the *Mycoplasma* and *Haemophilus* genomes suggested that their different gene contents reflect 'profound differences … between these two organisms' … . In this context, the *Reclinomonas* mitochondrial genome may be viewed as an extreme example of eubacterial genome reduction, such that

> the only genes remaining are related to mitochondrial gene expression (transcription, RNA processing and translation) and biogenesis of the protein complexes required for electron transport and coupled oxidative phosphorylation (including components implicated in mitochondrial protein transport and biosynthesis).”[19]

In short, these few examples illustrate the chasm existing between bacterial DNA and mtDNA which is only one of many problems with endosymbiosis.

Similarities and differences between mitochondria and bacteria

The endosymbiotic theory relies heavily on homology between organelles and bacteria. For example, each mitochondrion has a circular genome like bacteria, but much smaller and lacking histone proteins. The mtDNA is usually located in the mitochondrion’s matrix, although it is sometimes attached to the inner mitochondrial membrane.

Mitochondria also closely resemble purple-aerobic bacteria in size and shape. They both use oxygen in ATP production using the Krebs cycle. Certain antibiotics that kill bacteria also inhibit mitochondrial functions. These general similarities alone do not demonstrate endosymbiosis because, as documented below, many significant, often major and critical, differences exist.

One factor arguing for endosymbiosis is membrane composition. The outer membrane of both chloroplasts and mitochondria has both structural and chemical similarities to the prokaryotic cell membrane. Later research, though, determined that mitochondrial membranes are only superficially similar to prokaryotic cell membranes. One difference is that the proteobacterium alleged to have entered the protoeukaryote would have had a single membrane, whereas modern mitochondria have a double (inner and outer) membrane. The double membrane is not optional, but critical for its function to charge ADP. The inner membrane contains numerous plate-like folds called *cristae* that possess membranous sacks containing enzymes. Cristae can be either exclusively lamellar or exclusively tubular, but some mitochondria contain both types. Another difference is that the mitochondrial inner membrane has a different chemical composition from that of prokaryotes but is the same as in eukaryotes—contrary to the endosymbiosis theory’s prediction.

Margulis proposed “that eukaryotes formed as a result of a gradual multi-endosymbiotic union with prokaryotes. In contrast, others, like de Duve and Stanier, proposed that phagotrophy, which requires a dynamic cytoskeleton, an endomembrane system, and the loss of the prokaryotic rigid cell wall, evolved prior to endosymbiosis.” In contrast to this proposal phylogenetics based on mtDNA later concluded mitochondria could have evolved only once from an α-proteobacterium.[20] The endosymbiosis view of organelle evolution is widely accepted not because of empirical evidence, but because no other theory is even remotely plausible.[21] For this reason, Battley describes endosymbiosis as “tentative at best”.[22]

Because no physical evidence exists for most steps of the transition from prokaryote cell to eukaryote cell, armchair reasoning (i.e. mitochondria and chloroplasts have small plasmid DNA that superficially resembles prokaryotic DNA) is exploited as support. In fact, organellar DNA is *more similar* to eukaryotic nuclear genes. A well-known example of some organelle genes resembling eukaryotic nuclear genes is the presence of introns, which are rarely present in prokaryote genes.[23]

Endosymbiosis does not solve the organelle origins problem

Although the endosymbiotic origin of mitochondria and chloroplasts is now textbook orthodoxy,[24] proposals that most “other cellular compartments are the result of symbiosis. … are not so widely accepted”.[25] For most other organelles, endosymbiosis is recognized by many researchers as an implausible explanation for their origin. (It is also an inadequate explanation for the mitochondrion and the chloroplast, though the chloroplast evolution theory will be addressed in a separate paper).

Endosymbiosis an inadequate explanation for mitochondria

A major scientific problem with the endosymbiosis hypothesis is that as soon as the theory first appeared it was (and still remains) untestable.[26] The problem is endosymbiosis “proposes no real mechanism and most textbooks show the simplistic picture of a cell that swallows another cell that becomes a mitochondrion.”[27] Actually, it is now far less plausible than when first proposed because a great deal more is known today about organelles (e.g. mitochondria) and bacteria.

Among the other basic problems with the theory are: What prevented the host cell from digesting the invading organism? and: Where did the many other structures required for a eukaryotic cell to survive come from (figure 4)? For example, microtubules are not explained by the theory, even though they are needed for cell division and motility in eukaryotic cells. De Duve notes that nothing is known about the evolution of the cell cytoskeleton system, which requires many new innovations to function. Similar lines of evidence cited to support the theory that spirochete bacteria gave rise to flagella are problematic. Tubulin, the primary component of microtubules in eukaryotic cells, has not been found in any prokaryote. For these reasons, most evolutionary biologists reject the idea that flagella, tubulin and most other cellular structures originated by endosymbiosis. At best, endosymbiosis explains the origin of one or two organelles. But for

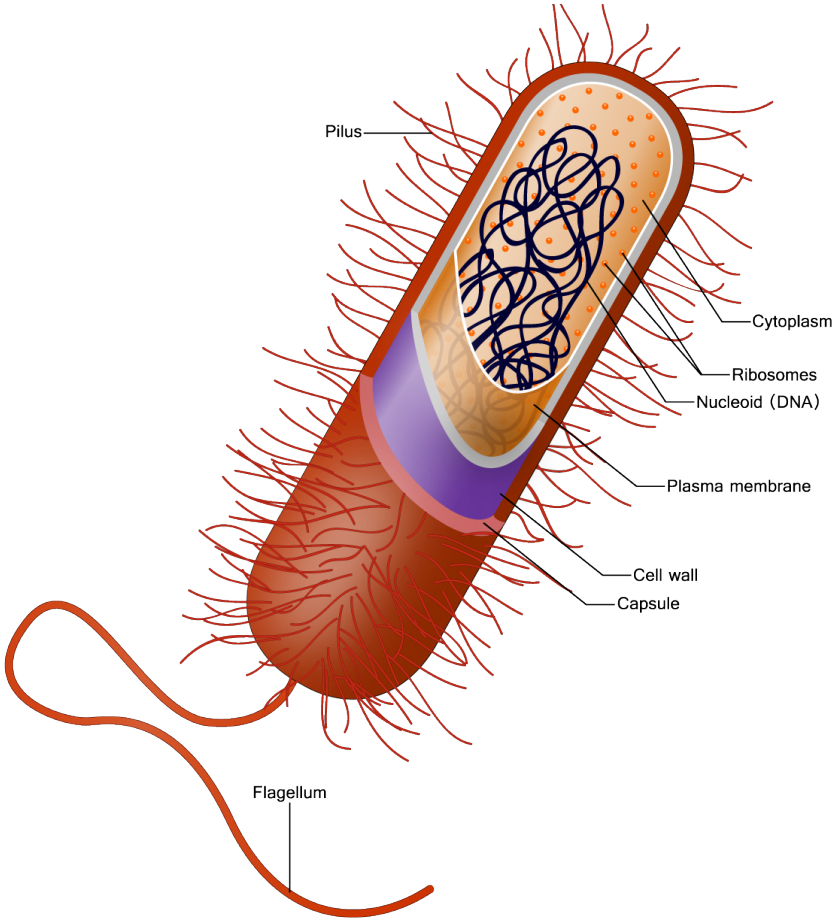

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Figure 2. Simplified representation of a bacterial cell and its interior

a eukaryotic cell to function, a whole new set of structures is required, all of which must evolve concurrently for functional integrity.

Major ribosome differences and the mitoribosome

A major argument for the endosymbiotic theory of organelle origins was that the structure of mitochondrial ribosomes is "distinctly different" from that of eukaryotic ribosomes, and mitochondrial ribosomes resemble those of prokaryotes.[28] Contrary to the endosymbiotic theory, though, mammalian mitochondrial ribosomes, and the amino acid sequences that produce the ribosome, are completely different from the corresponding features in prokaryotes.[29] Mitochondrial ribosomes are in fact so different from bacterial ribosomes that they are designated as the *mitoribosome*.

When endosymbiosis was first proposed, it was assumed that ribosomes existed in only two forms, a smaller 70S variety used in prokaryotes, and a larger 80S ribosome used in eukaryotes. The S in 70S refers to the unit in which the sedimentation factor is expressed. The *sedimentation coefficient* measures basic morphological differences, a quantity related to the size of the particle which is equal to the terminal outward velocity of the particle when centrifuged in a standard fluid medium divided by the centrifugal force acting on it.[30] The ribosomes used in mammalian mitochondria were *expected* to resemble the prokaryotic 70S ribosome because they were similar in size. Instead, researchers found that "Mammalian mitochondrial ribosomes (55S) differ unexpectedly from bacterial (70S) and cytoplasmic ribosomes (80S), as well as other kinds of mitochondrial ribosomes."[31]

Mitochondria employ a system required to manufacture proteins that is also very different from bacteria. Mitochondrial ribosomes called mitoribosomes are described as 'undersized', mini-ribosomes having mini-RNA polymerase, and even mini-DNA.[32] Mitoribosomes differ from prokaryote ribosomes in RNA, protein content, and position/function of the ribosome parts, and are significantly different in DNA sequence (especially regions that do not contact the tRNA or growing polypeptide chain). Unique features of the mitochondrial ribosome include novel mRNAs that process mitochondrial mRNA and a novel guanosine triphosphate (GTP) binding site used during polypeptide elongation.

Other contrasts between bacterial ribosomes and mitoribosomes include many basic construction and assembly differences. Proteins comprise a larger portion of mitoribosomes than prokaryotic ribosomes. Some of these proteins are in novel positions and have functions different from the prokaryotic ribosome. One of many examples includes the 55S mitoribosome, which is held together by 15 intersubunit bridges and only six of these bridges are similar to those employed in prokaryotes.[33] Furthermore, 33 of the 81 proteins identified so far in human mitoribosomes have no homologues in prokaryotic ribosomes.[1,34]

These examples of the many differences between the prokaryotic and mitochondrial ribosome further illustrate the chasm between the two ribosomes. More examples could be documented and undoubtedly more will be discovered with further research.

The phagocytosis problem

Another issue is that phagocytosis supposedly brought a proteobacterium into prokaryotes by "phagocytosis" of

bacteria, but "The precise nature of the host cell that partnered with this endosymbiont is, however, very much an open question."[1] The problem is archaea—in fact all prokaryotes—"cannot perform phagocytosis, and there is not any reason to believe they ever had such abilities."[35] Consequently, "the means by which the endosymbiont entered its host is an enigma."[35] The reason why prokaryotes lack phagocytosis ability is because it is a complex process requiring

> "… a flexible cell wall, a dynamic internal cytoskeleton with motor proteins that interacts with a complex endomembrane system, lysosomes that bud from the Golgi complex and are targeted to food vacuoles, and particles enclosed in a phagocytotic cup that are based on the spatially controlled polymerization of actin. These characters are absent from prokaryotes."[36]

One theoretical solution to this problem suggests the invasive organism, "was a small (facultative) aerobic α-proteobacterium, which penetrated and replicated within the host periplasm, and later became the cell mitochondria."[37] This just-so story so far lacks empirical conformation.

Other ways mitochondria differ from bacteria

Mitochondria are important organelles because they contain the machinery and enzymes necessary to convert food into an energy-carrying molecule called *adenosine triphosphate* (ATP). The enzymatic process by which the mitochondria convert food to adenosine triphosphate (ATP) is called *oxidative phosphorylation*. The end process involves converting adenosine diphosphate (ADP) to the high-energy molecule ATP. Mitochondria produce 90% of the cell's energy, and their impairment causes several diseases that affect the central nervous system and, eventually, other systems.[38] The mitochondria produce this energy from fats, sugars, and protein; mitochondria are found in all human cells except enucleated red blood cells.

Called 'the powerhouses of the cell', we now know that the more-active cells, such as muscle, liver, and kidney tubule cells, contain large numbers of mitochondria. Conversely, the less-active cells, such as mucus-secreting cells, contain relatively few mitochondria. Other functions of the mitochondria are regulatory, including helping control the cytoplasmic calcium level.[39] They are also involved in specific kinds of lipid synthesis.[40]

The inner membranes contain a large set of enzymes that convert food to charge ADP by a series of reactions called the *Krebs cycle* or *citric acid cycle* that produces oxidative phosphorylation. The inner mitochondrial area, called the *matrix,* is filled with gel containing scores of different kinds of enzymes. The inner and outer membranes differ both in enzymatic activity and lipid composition. Some of the enzymes, such as ATPase, are permanently attached to the mitochondrial membrane.[41] In short, mitochondria are very different from bacteria.

Genes controlling mitochondria

As noted, the mitochondrion is a unique organelle because it contains its own DNA (mtDNA) in the form of plasmids. Mitochondrial DNA is used exclusively for the organelle's own functions, specifically to provide control, although not complete, over its own replication.

Human mtDNA has been completely sequenced. Its 16,569 base pairs code for 37 genes that include only 13 protein-coding genes, 22 tRNA genes, and two rRNA genes, all of which are essential.[42] Respiration systems cannot function unless all these proteins and tRNAs are present. These are only a *few* of the genes required by human mitochondria. Close to 90% of proteins imported from the cytoplasm are encoded in the nucleus, indicating a high level of integration that argues endosymbiosis is untenable.[43]

Because most genes controlling mitochondria are not located in the organelles themselves, but in the cell's nucleus,[44] a transfer of genes from the organelles to the host nucleus must be postulated by endosymbiosis supporters. This problem is not minor: "the migration of genes from endosymbionts to the nucleus is remarkable because it seems to have raised more difficulties than it solved."[45] Another problem is:

> "… in what form do the transferred genes physically make that intracellular journey—as RNA, as cDNA, as pieces of organelle DNA, or as whole organelle chromosomes? Current views focus upon cDNA as the vehicle, based upon some examples from plants. But other mechanisms, involving direct transfer of DNA from organelle chromosomes, could also account for the available data."[46]

The analogy is not unlike hypothesizing the moving of a small house into a larger house as a means of explaining the larger house's rooms when they can be explained more easily, even from an evolutionary standpoint, by hypothesizing their individual separate construction. This concern is significant in that the genes in mitochondria were a major original evidence for the endosymbiosis theory. From a Darwinist standpoint, the hypothesis which endosymbiosis replaced, the process of ingrowing membranes within the host cells forming all of the organelles, including the nucleus, appears more plausible and thus still has adherents. As the problems with endosymbiosis accumulate, the membrane-ingrowth hypothesis may again become popular.[47]

In short, genetics suggests this gene transfer *must* have occurred if endosymbiosis occurred. The real problems are: how pre-eukaryotic cells survived until the genes were transferred, how they survived before this transfer, and why and how they were transferred. Mitochondrial replication appears to require the nuclear control system because, as far as is known, it is universal. Nonetheless, why many genes important for mitochondrial function are located in the nucleus

and how they got there is the subject of much evolutionary speculation.[48]

One of the most unexpected discoveries has been the paucity of genes that would support endosymbiosis. One study found, in contrast to the theory's expectations, that comparisons with the organism, called the α-proteobacterial endosymbiont, widely believed to be the bacterial endosymbiont

> "… have identified a conserved core of proteins descended from the α-proteobacterial endosymbiont that gave rise to the mitochondrion and was the source of the mitochondrial genome in contemporary eukaryotes. A surprising result of phylogenetic analyses is the relatively small proportion (10–20%) of the mitochondrial proteome displaying a clear α-proteobacterial ancestry. A large fraction of mitochondrial proteins typically has detectable homologs [sequence similarity] only in other eukaryotes and is presumed to represent proteins that emerged specifically within eukaryotes."[49]

The authors concluded:

> "Understanding the origin and evolution of the mitochondrion remains a challenge, despite the flood of relevant biochemical, cell and molecular biological, and phylogenetic data and insights that have accumulated in the almost five decades since the modern resurrection of the long-standing endosymbiont hypothesis; the idea that this organelle is a tamed and highly reworked endosymbiotic bacterium. The abundance of information bearing on eukaryotic cell evolution (particularly and most recently sequence data) and differences over how the data are analyzed and interpreted have prompted a plethora of often-conflicting ideas about when and how, within an endosymbiotic context, the mitochondrion originated."[50]

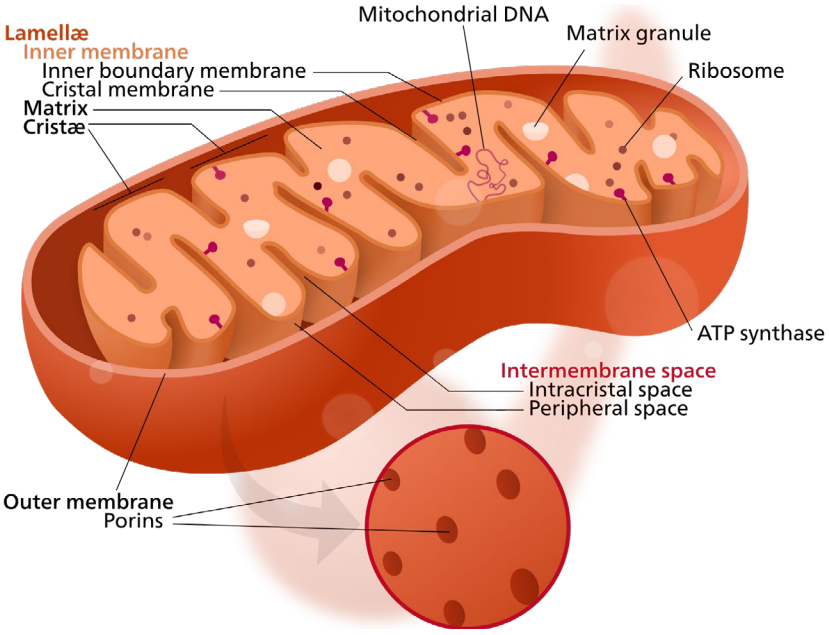

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Figure 3. Simplified diagram of a mitochondrion. Note the striking difference to the bacterial cell in figure 2.

Mitochondria dependent on the nuclear genome

Another major problem with endosymbiosis is that the mtDNA genome is not independent but is functionally integrated with the nuclear genome. Over 90 proteins are required to produce mitochondrial ribosomes, and nearly all of them are supplied to the organelle from the host nucleus. (Of the references checked, none could give a specific number which, as of this writing, is unknown.) These genes are encoded by nuclear DNA; the resultant proteins are synthesized in the cell cytosol, and then individually transported into the organelle.

Human mitochondria "must import 99 percent of their proteins from the cytoplasm."[51] It would be far simpler (and therefore per the 'law of parsimony' a better explanation) to evolve mitochondria from scratch than to incorporate an independent organism which required: 1) the loss of most of its genes; 2) the evolution of many new ones, which involves; 3) the fact that most of the genes required to function must have originally evolved in the nucleus and, without these genes, the mitochondrion could not function. Furthermore, use of the two-gene system, nuclear and mitochondrial, requires the evolution of extremely complex import machinery involving complex surface receptors, binding relays, and a target signalling system.[52]

Another difficult problem is that some mitochondria use genetic codons that are different from *all* bacterial and eukaryotic codes. For example, the codon CUA normally codes for leucine, but in yeast mitochondria codes for threonine. In fact, multiple codes exist to code for threonine, and there are expanded codon recognition patterns for other amino acids.[53] For these reasons, some theorize that the mitochondrial code has been evolving in certain yeast mitochondria, because, as far as is known, all yeast mitochondria have the same code differences compared to bacteria.[54] As more genetic sequencing is completed on different mitochondria of other organisms, other coding differences will likely be found.

A mitochondrial code that was the same as used in bacteria, but different from that used in eukaryotes, if that were the case, might argue for endosymbiosis. But the difference that actually exists has forced some evolutionists to hypothesize

that the mitochondrial code is more 'primitive' and the bacterial code more evolved. In contrast to this view, the difference is better explained by the view that the mitochondrial code is designed for the specific needs of mitochondria, specifically to *prevent* exchange of mtDNA genes with nuclear genes.

Moves the problem elsewhere

Another major problem with the endosymbiosis theory is that it does not solve the problem of organelle evolution. Instead, it avoids the problem because it *starts* with the existence of a complex, functioning system that it cannot explain. For purposes of argument,

> "… suppose that the symbiosis Margulis envisions was in fact a common occurrence throughout the history of life. The important question for us biochemists is, can symbiosis explain the origin of complex biochemical systems?
>
> "Clearly it cannot. The essence of symbiosis is the joining of two separate cells, or two separate systems, *both of which are already functioning*. In the mitochondrion scenario, one preexisting viable cell entered a symbiotic relationship with another such cell. Neither Margulis nor anyone else has offered a detailed explanation of how the preexisting cells originated."[55]

Furthermore, the proponents of the symbiotic theory must "assume that the invading cells could already produce energy from foodstuffs; they [also] explicitly assume that the host cell already was able to maintain a stable internal environment that would benefit the symbiont."[56]

Margulis and Sagan proposed that the earliest eukaryotic cells were the protoctists: the amoebas, diatoms, giant kelps, and red seaweeds.[57] These eukaryotic creatures, though, are in most ways more similar to the 'higher' level eukaryotes than to prokaryotes. Even though the endosymbiosis theory does not fit the facts reviewed in this paper, it, nonetheless, is periodically recycled when alternative theories are shown to be wrong.[2]

Another indication of problems in endosymbiosis is the widespread disagreement by researchers about mechanisms underpinning the concept. For example, Margulis and Sagan note that certain bacteria have been renamed 'archaea' by Carl Woese, a terminology now widely accepted.[56]

> "This classification rejects endosymbiosis and is a 'denial of their bacterial nature' because it results in elevating 'the group "archaea" to parallel status with other bacteria and all eukaryotes'. The result is three fundamental groups called domains, or superkingdoms, which contradicts the endosymbiont theory."[58]

Another concern is that endosymbiosis involves relatively rapid evolution that contradicts Darwinian gradual evolution. In view of the fact that no viable gradualist explanation for the evolution of eukaryotes exists, evolutionists were motivated to blend Darwinian gradualist and non-gradualist positions. O'Malley writes that, as Maynard Smith argued, endosymbiosis is explained by the standard mutations, and macromutations.[59] Other researchers argue that other factors must be accounted for, making eukaryote evolution even more complex (consequently making it less probable). For example, Edgar adds that "the concurrent evolution of the L-ascorbic acid redox system should be considered a key factor leading to evolution of multicellular eukaryotes and remains involved in the maintenance of multicellularity and many other eukaryotic characteristics."[60]

Why organelle evolution is impossible

Behe argues that the gap existing between eukaryotic and prokaryotic cells cannot ever be bridged because of irreducible complexity. The complexity of even a simple machine can be reduced only so far—below this, the machine cannot function. The classic example is a standard mouse trap, which must have a minimum of five main parts to operate: a platform, a holding bar, a hammer, a catch, and a spring.

A mouse trap will not function until *every* one of its necessary parts is in place, each of which must be designed properly to articulate with the other parts. One contrary suggestion is to propose that some of these parts can be eliminated by various methods, such as nailing the trap to the floor. This approach does not eliminate a part but replaces it with another part; the floor is used as the base. Likewise, organelles will not function unless *every* required, properly designed and manufactured part exists, and *all* of them must be properly assembled to form an operating system.[25]

Organelles are very complex structures, consisting of multi-thousands of smaller complex parts, and the irreducible complexity concern is *also* very likely true of each individual part in each organelle. A cell cannot survive without ribosomes, each of which contains thousands or even tens of thousands of molecules, each one of which must be assembled to exacting specifications.[61] Thus, cellular life is impossible until all of its necessary parts are manufactured and properly assembled. Even though DNA is described as representing "massive intelligence … [it] has by itself neither a future nor a present. DNA without a cell to sustain and express it has no physiologic meaning."[62]

Few scientists have even endeavoured to speculate on the details of the transitional forms between the hypothetical pre-organelles, let alone present evidence for the multi-thousands of transitional forms required to create a reasonable scenario that could bridge the free-living cells and the cells with organelles used in multicellular organisms.

The cell's transport system is another example that illustrates why the concept of irreducible complexity makes organelle evolution impossible. After proteins are manufactured, they do not float around freely inside the cell, but must be transported by an appropriate mechanism to wherever they

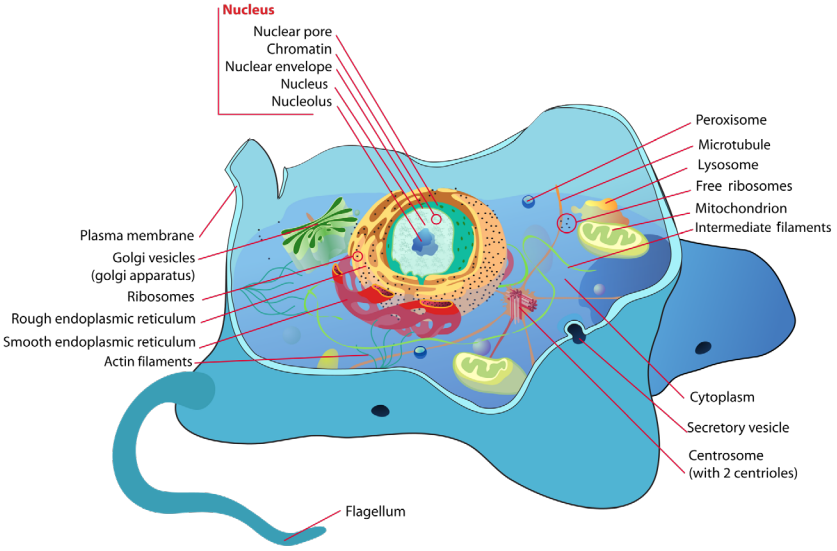

Figure 4. Endosymbiosis attempts to explain the evolutionary origin of only one organelle in animal cells. As of this date, the evolutionary origin of the 17 basic structures shown in the diagram above, including the mitochondrion, have no universally accepted explanation after 150 years of attempts. For most of the 17 structures, only tentative just-so stories exist. All of these structures are required in some form for a eukaryote cell to exist.

are needed. Two common mechanisms to transport proteins are *gated transport* and *vesicular transport*.

Gated transport requires construction of a door between the cytoplasm and the nuclear membrane and a chemical sensor (a protein that has the correct identification tag). When the protein package approaches the sensor, it opens the gate, allowing the protein to pass through. This control mechanism requires the protein to have the proper identification tag and a gate programmed to open in response. The gate itself also contains many parts, thus introducing another level of irreducible complexity. Each of these gated transport components is complex and consists of thousands of parts at the molecular level, *all* of which must exist for the gated transport system to function.

The vesicular transport system also uses a set of specially designed sensors. But instead of a gate, the proper identification tag causes the compartment membrane to bulge outward, pinching off and forming a vesicle that totally surrounds the protein. The transport vesicle then travels to a destination predetermined by its identification tag. If the vesicle tag and identification sensor match, another sensor recognizes the vesicle, and it merges with the compartment.

Then, the pinching-off process is reversed to allow the proteins to be carried inside the new compartment. The almost certainly irreducible complexity of the system must include two complex sensor systems, two identification tags as well as the vessel itself. At a level beyond this, each sensor identification tag and the carrier vessels are, at the molecular level, likewise constructed from thousands of parts, each of which is also likely an example of irreducible complexity. The vesicle must contain all of the structures that allow it to bud off from the original compartment and then to unite with another compartment.[25]

Other problems with endosymbiosis

The standard endosymbiosis theory has recently been under attack from several fronts, and some researchers are now arguing for a new theory to explain the evolution of organelles. Some of these scientists believe that a new theory "could solve some nagging problems with the prevailing theory" of endosymbiosis. The details of this new theory are still vague. They admit that, even if a new theory is elegantly argued though, there will likely be a lot of difficulties some new hypothesis doesn't account for. Evolutionists also conclude that we may have to admit that

> "… the mitochondrion was a lucky accident. First, the ancestral cell—probably an archaebacterium, recent genetic analyses suggest—acquired the ability to engulf and digest complex molecules. It began preying on its microbial companions. At some point, however, this predatory cell didn't fully digest its prey, and an even more successful cell resulted when an intended meal took up permanent residence and became the mitochondrion."[63]

Furthermore, scientists believed for decades that

> "… they had examples of the direct descendants of those primitive eukaryotes: certain protists that lack mitochondria. But recent analysis of the genes in those organisms suggests that they, too, once carried mitochondria but lost them later (*Science*, 12 September 1997, p. 1604). These findings hint that eukaryotes might somehow have acquired their mitochondria before they had evolved the ability to engulf and digest other cells."[63]

Another problem with endosymbiosis is the widespread disagreement by researchers about the mechanism. One summary concluded:

> "… that 'mitochondrion-early' models that postulate the acquisition of the protomitochondrion by an archaeal host, are more plausible than 'mitochondrion-late' models. However, since prokaryotes are unable to perform phagocytosis, such models failed to suggest a reasonable mechanism by which the endosymbiont got access into its host."[64]

One solution to all these problems is more time for it to evolve. As Professor Edgar explains, it took 0.5 billion years for prokaryotes (bacteria and archaea) to evolve "quite complex biochemistry and some eukaryote characteristics" but

> "… the transition from unicellular prokaryotes to multicellular, aerobic eukaryotes took a further 2.5 billion years to begin. The key factor or factors that eventually caused this long-delayed transition is a question that has been a focus of considerable research and a topic of discussion over many years."[60]

Conclusions

Two major groups of organisms exist: prokaryotes and eukaryotes. No intermediate organisms have ever been found between them, with part-developed organelles. With possible minor exceptions, what is found is either an absence of organelles, or fully functional and fully developed organelles.[65] "No missing links between eukaryotes and bacteria exist, either in the fossils or in life."[66] Furthermore, it is even difficult to postulate by compiling just-so stories how the links between prokaryotes and eukaryotes could exist.

Endosymbiosis postulates that mitochondria were once free-living bacteria, and that "early in evolution ancestral eukaryotic cells simply ate their future partners."[67] Both the gradual conversion and endosymbiosis theories require thousands of transitional forms, with each new one providing the cell with a competitive advantage over the unaltered cells.

The endosymbiosis idea is popular, not because of the empirical evidence, but because no other hypothesis is remotely plausible. The complete absence of fossil and other evidence is another problem. Thus, Professor Battley describes the endosymbiosis notion as "tentative at best".[22] A major problem with endosymbiosis is that it has always been untestable.[68] More research and knowledge has motivated one researcher who is at the forefront of this field to conclude in 1998 that the studies

> "… published over the past two or three years, much of them from genome-sequencing projects, have hinted that it is time for a new theory. In particular, it is turning out that eukaryotic nuclear genomes carry many genes of bacterial (sometimes α-proteobacterial) origin which have nothing to do with mitochondrial functions. Moreover, mitochondrion-free eukaryotes that we had come to think of as direct descendants of ancient proto-eukaryotes carry mitochondrial genes in their nuclear genomes."[69]

Endosymbiosis theory has come under attack from many other quarters, and no doubt these attacks will continue.[70] The former head of Clemson University's Genetic Lab called endosymbiosis a theory in crisis that, at best, explains very little of the evolution of eukaryotes from prokaryotes. The origin of the entire new cell system, the eukaryotes, remains to be explained. He concluded:

> "… [the] sequences of many eukaryotic genomes are now clearly showing that the gene repertoires needed for the mitochondria to function are *not* derived from bacteria but are remarkably unique to the type of creature in which they are found. While certain genetic similarities do exist, these correspondences are plausibly explained by the standard engineering concept of code re-use—common code to solve similar problems. The wealth of genomic data is now utterly destroying the idea of evolution on all fronts, even in the area of endosymbiosis, one of the secularists' favorite theories."[71]

There are major problems with endosymbiosis theory. Mitochondria differ from bacteria—genetically, structurally, functionally, and operationally. Furthermore, time has not been helpful in explaining these differences within the theory's parameters but has only increased the contrast between the two.

This review can only outline some of the major problems with the attempt to deal with the chasm between prokaryotes and eukaryotes via the endosymbiosis concept. To effectively critique this problematic idea would really require an entire paper for each of the problem areas concerned.

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