The evidence for the endosymbiotic theory

Abstract

Modern eukaryotic cells were originally believed to have arisen directly from a single prokaryotic ancestor through serial mutation and the process of genetic drift. However, much evidence has lead scientists to believe that eukaryotes are the result of a merger between a prokaryotic cell, a relative of modern Î±-protebacteria, that became incorporated by a host archaeon. This is referred to as the endosymbiotic theory. Over the span of millions of years, symbiont DNA was transferred to the nucleus to give rise to the eukaryotic genome and the prokaryote gave rise to mitochondria. As such the two organisms effectively become a single organism, each unable to survive in the others' absence. The endosymbiotic theory is widely regarded due to the many shared biochemical and morphological characteristics of mitochondria with bacteria, including DNA organization and similarities protein synthesising machinery and membrane composition. In reviewing these characteristics I have come to conclude that an endosymbiotic event is the most plausible explanation for the development of mitochondria within the eukaryotic cell, despite the uncertainties regarding the nature of the prokaryotes' inclusion in the host cell.

Introduction

The term " endosymbiosis" refers to the event in which one organism takes up permanent residence within another, such that the two develop a mutually beneficial relationship1. The endosymbiotic theory was developed to explain the evolutionary discontinuity between the appearance of prokaryotes and eukaryotes, and the great many differences exhibited by the two taxa, described in Table. 1. Since mitochondria share so many biochemical and structural characteristics with Bacteria, an evolutionary relationship was proposed, in order to explain the transition from prokaryote to eukaryote2. Contrary to the traditional view that a series of chance mutations were responsible for the evolution of eukaryotic cells, the endosymbiotic theory states that mitochondria arose by the incorporation of a free living aerobic prokaryote, relating to an Î±-proteobacterium, into an anaerobic proto-eukaryote3. This is proposed to have occurred approximately 1. 5 billion years ago, between the first fossil record of aerobic prokaryotes and eukaryotes (Table 1), after oxygen had begun to accumulate in Earth’s atmosphere as a result of the emergence of photosynthetic organisms4 5. Rather than being digested, the prokaryote remained as a symbiont, helping in the production of ATP within the host by the process of oxidative phosphorylation, enabling it to survive the increasing oxygen concentrations, thereby giving it a selective advantage over anaerobic cells. Interdependence between the aerobic bacterium and the host cell developed and, the bacterium evolved into the mitochondrion. Photosynthetic eukaryotes originated in a similar manner by a secondary symbiosis between these organisms and photoautotrophic bacteria related to cyanobacteria.

Wallin proposed in 1923 that mitochondria originated from an endosymbiotic event when he observed that they closely resembled bacteria in size and complexity7. These and related observations led Lynn Margulis to publish Symbiosis in Cell Evolution in 1981, in which she argued that modern eukaryotes were originally formed from a fusion of separate species. Margulis also proposed, more controversially, that motile prokaryotic species such as Spirochaeta were incorporated and resulted in the evolution of the structures that provide cellular motion. Since this hypothesis is generally not accepted, it is not discussed in further detail here.

1. Over the next thirty years, further similarities between mitochondria and bacteria were found that supported Margulis' work. Table 1 described some of the similarities observed between prokaryotic cells and mitochondria when compared to eukaryotic cells. Included are the overall physiological similarities of mitochondria and prokaryotes, such as the similarity in size compared to eukaryotes, similarities between the protein-synthesising machinery such as the mitochondrial ribosomes and tRNA molecules, and the presence of a separate mitochondrial genome. 3 8

Table. 1 Summary of the similarities between prokaryotes and eukaryotes, and eukaryotic organelles.

Adapted from: Indiana University-Purdue University Department of Biology (2004) Class Notes: The Endosymbiotic Theory Available: http://www. biology. iupui. edu/biocourses/n100/2k4endosymb. html [Accessed 16/04/10]

Prokaryotes

Eukaryotes

Mitochondria of

Eukaryotic cells

DNA

1 single, circular chromosome

Multiple linear chromosomes

compartmentalized in a nucleus

1 single, circular

chromosome

Replication

Binary Fission involving Fts proteins

Mitosis

Process akin to binary Fission involving dynamin proteins

Ribosomes

30S and 50S Subunits

40S and 60S Subunits

30S and 50S Subunits

Electron Transport Chain

Found in the plasma membrane around cell

Found only in the cell’s mitochondria

Found in the plasma membrane around mitochondrion

Size (approximate)

~1-10 Î¼m

~50 – 500 Î¼m

~1-10 Î¼m

First appearance in fossil record

Anaerobic bacteria:

~3. 8 Billion years ago

Aerobic bacteria:

~2. 5 Billion years ago

~1. 5 billion years ago

~1. 5 billion years ago

What the endosymbiotic hypothesis does not make clear is the order of events regarding the formation of the nucleus and the acquisition of the prokaryotic cell containing the precursor mitochondrial genome. Two hypotheses have been put forward for the formation of the eukaryotic cell, illustrated in Fig. 2.

Nucleus formation preceded symbiosis of mitochondrion and chloroplast

Nucleus formed after symbiosis of mitochondrion and before chloroplast

Taken from Michael Madigan et al. Brock Biology of Microorganisms

(2008) 12th edition, Pearson Education Inc

The most widely regarded, summarised in Fig. 3, proposes that an ancestral prokaryote first developed a membrane around its DNA from infolding of the plasma, similar to the way in which the endomembranous system of the endoplasmic rectilium and Golgi apparatus is thought to have arisen3. This organism, dubbed the “ protoeukaryote” engulfed a small heterotrophic prokaryote, shown on Fig. 2 as the ‘ ancestor of mitochondrion’9.

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http://topicstock. pantip. com/wahkor/topicstock/2009/09/X8338687/X8338687-8. jpg

The second hypothesis, in contrast, considers that there could have been a nucleus was formed after the acquisition of the protomitochondrion, when a member of the Archaea acquired the bacterial ancestor of the mitochondrion through endocytosis11. This is known as the hydrogen hypothesis, proposed by Martin and Muller (1998), who claimed that the symbiotic relationship between the two cells was initially based on the host’s dependence on the hydrogen, evolved by the symbiont as a by-product of anaerobic respiration, as a source of energy. The nucleus was formed from the mitochondrial DNA from the symbiont and the free DNA residing in the nucleus12. Both models thus involve the transfer of a large portion of mitochondrial DNA to the host nucleus, resulting in the dependence of the symbiont upon the host.

An alternative hypothesis has recently been forwarded by Davidov and Jurkevitch, who propose that the ancestors of mitochondria were not endocytosed by Archaea but were predators that penetrated the host and devoured the host. The prey managed to survive and established a mutualistic relationship as in the previous hypotheses. (Davidov, Jurkevitch cited by Zimmer, 2009) This appears to be supported by the finding that certain species Rickettsia, obligate intracellular parasites, have more similar genomes to the than mitochondrion13 14.

However the eukaryotic cell arose, abundant evidence has accumulated that supports the endosymbiotic theory, and the evidence of similarities relating to different functions of bacteria and mitochondria are reviewed in this essay. There is also an increasing body of experimental evidence that suggests that endosymbiotic events occur in modern cells, and two such experiments and their implications on the endosymbiotic theory are reviewed here. Aside from these experiments, the evidence presented in this essay relates entirely to the emergence of mitochondria.

The Mitochondrial Genome

Mitochondria possess their own genomes that replicate that replicate independently from the nucleus, using DNA polymerases specific to the mitochondria. These processes, as well as the subsequent DNA transcription and protein synthesis take place in the matrix of mitochondria and occur throughout the cell cycle, which parallels the situation in bacteria but is not true of nuclear DNA2. The DNA of mitochondria is a single circular molecule of roughly the same size as the bacterial genome, and has a mean GC content ranging from 20-50%. This is close to the variation found in bacterial species and greater than observed for eukaryotes, reflecting phylogenetic relationships of mitochondria and bacteria2. Furthermore, like bacterial DNA, the DNA of mitochondria lacks both intervening sequences and the organisation into histones present in bacteria3. Comparative molecular sequencing of mitochondrial genes and the RNA they encode has revealed that the mitochondrial genome is more closely related to that of organisms such as the Î±-proteobacterium Rickettsia prowazekii than to the rest of the eukaryotic cell, indicating an extracellular origin10 15. Similar sequencing by Ito and Braithewaite has revealed that yeast mitochondrial DNA polymerase I is homologous in amino acid sequence the DNA polymerases, of E. coli and Streptococcus pneumoniae in both the 3′-5′ exonuclease and DNA polymerase domains. The similarity of the DNa replication machinery further implies that mitochondria evolved from prokaryotes.

While nuclear DNA is contributed by both parents in sexual organisms, mtDNA is contributed almost entirely by the oocyte16. The symbiotic concept accounts for this non-mendelian mode of inheritance and indicates the evolutionary importance of maintaining uniparental heredity of nonchromosomal genes, due to the inherent redundancy that results from the fusion of gametes, This ensure that at least one copy of the organellar genome is maintained at each stage in the eukaryotic cell’s life cycle3.

DNA sequence analysis has also demonstrated the presence of mitochondrial DNA in the nucleus17. Biologists originally believed that the nuclear DNA of the eukaryote coded for mitochondria. However, Margulis reasoned that, if the endosymbiotic hypothesis represents the true course of events in the evolution of mitochondria, then upon entering a symbiotic partnership, the symbionts would lose all synthetic capabilities except the ability to replicate their own DNA. It has been shown that that the proteins that mediate function of the mitochondrion are encoded both in the mitochondrial and the nuclear genome and must be imported to the mitochondrion, shown in Fig. 3 which supports Margulis’ hypothesis. Margulis Although many mitochondrial genes have been found in the nucleus, nuclear genes direct the synthesis of only some of the proteins from which they are made, thus cells which lack mitochondria cannot generate them1. This appears to be the result of extensive gene transfer that took place after the endosymbiotic event which brought the two cells together. The transfer of essential genes to the host nucleus resulted in the progressive loss of independence after the host acquired the symbiont, resulting in an organelle unable to survive in oxic conditions outside the host3. O Daley proposes that the large transfer of mitochondrial DNA may have been the result of evolutionary pressure to accumulate DNA inside the nucleus, due to increased genetic variation 17 18. The High rate transfer of DNA between the nucleus and mitochondria demonstrates that the incorporation of the mitochondrial genome into the eukaryotic cell was vital in defining the eukaryotic genome18.

An external file that holds a picture, illustration, etc., usually as some form of binary object. The name of referred object is ch14f62. jpg.

Fig 3: Transfer of nuclear precursor proteins from the nucleus to the mitochondrion and subsequent production of mitochondrial protein. Red arrows indicate the site of action of a specific inhibitor of either nuclear or mitochondrial protein synthesis, discussed in more detail later in of this essay.

From Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts, and James D. Watson (1994) Molecular Biology of the Cell. 3rd edition, Garland Publishing Inc., New York.

Lipid membranes

Possibly the most convincing evidence of the extracellular origin of mitochondria is the similarity between the inner mitochondrial membrane and the membrane possessed by bacteria. Mitochondria are surrounded by two phospolipid membranes, and while the outer membrane resembles the eukaryotic membranes, including the membranes of other cellular organelles such as that of the nucleus and endoplasmic rectilium, the innermost membrane is chemically distinct to those found elsewhere in the eukaryotic cell6. Furthermore, Microscopic observation has enabled the comparison of cristae, invaginations of the inner mitochondrial membrane, shown in Fig. 4, to bacterial mesosomes, shown in Fig. 5. Both structures increase the surface area of their respective membranes and provide a site for the process of oxidative phosphorylation. Margulis has suggested that the similarity between mitochondrial cristae and the mesosomes possessed by many species of Bacteria (Fig. 5) also alludes to the extracellular origin of mitochondria3. Consistent with the endosymbiotic theory, it has been reasoned that the inner membrane once belonged to the bacterial symbiont, and that the outer membrane was a remnant of the phagocytic vacuole in which the symbiont was engulfed by the host cell, resulting in the development of cristae from mesosomes9.

Left:

Fig. 1: Structure of an idealised mitochondrion as compared to an electron micrograph. Illustrates the double membrane structure and the infoldings of the inner membrane known as cristae on which oxidative phosphorylation occurs.

Adapted from: Dr Jay Pitocchelli, Saint Anslem College (2001) Lecture Notes for Cell Biology. Available: http://www. anselm. edu/homepage/ jpitocch/genbio/mitochondrion. JPG [accessed 27/04/10]

Right:

Fig. 5 : Electron micrograph of thin section of T. dentrificans showing ‘ inverted Y’ form of simple mesosome-like body.

From: J W Greenawalt and T L Whiteside (Dec 1975). Mesosomes: membranous bacterial organelles. Bacteriological Review. 39(4): 405-463.

The inner mitochondria and bacterial membranes also share many biochemical features. Table 2 illustrates the observations made by Parsons, that the outer mitochondrial membrane is more similar in density and lipid composition to that of the endoplasmic reticulum of Serratia than of the inner mitochondrial membrane3 19. It has also been noted that Î²-barrel transmembrane proteins are exclusively found in the bacterial membranes and in the outer membrane of mitochondria, and that the amino acid sequences of these proteins show high similarity6. Additionally, LACTB, a protein that derives from bacterial penicillin-binding protein of peptidoglycan, has been found in the intermembrane space of eukaryotic mitochondria20. While mitochondria lack peptidoglycan, the presence of a vestigial peptidoglycan-forming protein provided further evidence that mitochondria are descended from bacteria.

Table 2: Comparison of structural features and lipid composition of inner and outer Mitochondrial membranes and ER of Serratia.

Adapted from D. F. Parsons (1867) Ultrastructural and molecular aspects of cell membranes. Proceeings of the Seventh Canadian Cancer Research Conference 7: 193-246.

The nature of the mitochondrial respiratory system raises another significant line of evidence supporting the endosymbiotic theory. The production of energy via the electron transport chain by mitochondria is associated only with the inner membrane, as in prokaryotes, and does not occur in the outer membrane, as evidenced by the difference in electron transport protein content in the mitochondrial membranes, shown in Table 29. Additionally, the membrane potential across the inner membrane that is necessary for the production of ATP is not found in the outer membrane of the mitochondrion or in eukaryotic membranes. Table 3 also demonstrates that that bacteria such as P. denitrificans and mitochondria share many respiratory features, such as the sensitivity of the oxidative chain to antimycin, which disrupts proton gradient formation across the membrane. Furthermore, the electron-transport chains of bacteria and mitochondria both contain a membrane-bound enzyme complex that accepts electrons solely from ubiquinone-10 quinine carrier3. Taken together, this evidence supports the theory that the outer membrane of mitochondria was formed from the vesicular membrane during an endosymbiotic event and the inner membrane formed from the prokaryote engulfed2.

Table 3: Mitochondrial features of Paracoccus denitrificans

Illustrates the similarities of the respiratory system of mitochondria to the systems of Paracoccus , that are also found in many other bacteria. These strikingly similar respiratory features indicate that microbes such as P.

denitrificans may be the ancestors of mitochondria. Many of these features are found in other bacteria. After Whateley (1977) Adapted from Lynn Margulis. Symbiosis in Cell Evolution (1981) W. H. Freeman & Co Ltd.

Respiratory Chain

Succinate and NADH dehydrogenases

Ubiquinone-10 is the sole quinine

Cytochromes a and a3 act as oxidase

Sensitive to low concentrations of antimycin

Oxidative Phosphorylation

Respiratory control is released by ADP or by uncouplers of oxidative phosphorylation

ATPase has tightly bound nucleotise exchangeable on energization

Membrane Phospholipids

Phosphotidylcholine is the main constituent

All fatty acids are straight-chain and monounsaturated

Mitochondrial division

The way in which mitochondria divide also provides evidence for their extracellular origin. It has been shown that these organelles multiply semi-autonomously, not through mitosis as eukaryotic cells are, but rather through a process similar to binary fission of pre-existing organelles21. Mitochondria been found to possess dynamin proteins, mechanochemical

GTPases, that are related to the FtsZ, filamentous temperature sensitive, proteins found in bacteria. Bacterial FtsZ2 proteins interact to form a divisome ring complex that from a division furrow from outside, a similar mechanism has been observed by the Dnm1 protein of mitochondria, where the proteins constrict the membrane from the outside, illustrated in Fig. 6. Furthermore, evidence of FtsZ proteins have been found in the mitochondria of the alga Mallomonas splendens that are proteins closely related to those of Î±-proteobacteria. The FtsZ protein is located in patches on the mitochondrial membrane, near the centre or at the ends of mitochondria, similar to the location of Dnm1. The similarities in the molecules involved in initiating bacterial and organelle division further emphasise the evolutionary ties that these organelles have to bacteria. 9 22 23

Fig. 6: A model of the mechanism of mitochondrial division: Dynamins are targeted to their site of action by other proteins. After modification by GTP, Dynamins from rings that tighten around the site of division and constrict to cut the mitochondrion into two. This mecahism parrales that of FtsZ, the tubulin protein of bacteria.

Adapted from: Suzanne Hoppins, Laura Lackner, and Jodi Nunnari (2007) The Machines that Divide and Fuse Mitochondria. Annual Review of Biochemistry Vol. 76: 751-780

Ribosomal Similarities

The protein-synthesising machinery of mitochondria shares more similarities with bacteria than that of the eukaryote cytoplasm. For example, the initiating amino acid in the transcripts of bacteria and mitochondria is Nformylmethionine, whereas protein synthesis in the cytosol of eukaryotic cells begins with methionine. The structure of the mitochondrial ribosomes also differs from those found in the eukaryotic cytoplasm, in that they are more similar in size and share the same subunit structure, described in Table. 1 3 9. The sequences of 16S ribosomal RNAs are closer to certain aerobic eubacteria than many other bacteria are, for example, Wolters and Erdmann have confirmed, by phylogenetic analyses, that the primary and secondary structure of 5S and 16S rRNA of angiosperm mitochondria share specific signatures with a particular type of purple bacteria, the rhodobacteria25. Conversely, mitochondria show no homologies in these traits with the eukaryotic cell cytoplasm3. These similarities appear to confirm the phylogenetic relationship of these organisms and the organelles.

Similarities between the ribosomes of bacteria and mitochondria are further evidenced by the action of a number of antibiotics that affect only bacterial, mitochondrion ribosomes protein-synthesising machinery. Fig. 3, above, demonstrates specific inhibitors of protein synthesis. These similarities the action of antibiotics between three types of ribosomes are illustrated in Table 4 3. For example, neomycin and streptomycin act by binding the 30S subunit of mitochondria and bacteria and inhibiting protein chain initiation, while chloramphenicol blocks the attachment of amino acid to tRNA9. None of these chemicals interfere with protein synthesis in the cytoplasm of the eukaryotes. Conversely, cyclohexamide and anisomycin affect only the protein synthetic machinery of eukaryotic cells, and have no inhibitory effects on mitochondria or on bacteria. Another example, rifampicin, inhibits the RNA polymerase of bacteria and mitochondria, but has no such effect on eukaryotic nuclear RNA polymerase. It is notable that each antibiotic, except for Puromycin, affects both mitochondrial and bacterial or cytoplasmic ribosomes, and this would appear to suggest a relationship between the protein synthesising machinery of mitochondria and that of prokaryotic bacteria from which they were thought to have originated3.

Experimental Evidence

Laboratory experiments have been conducted to confirm the establishment of endosymbioses in several organisms. Kwang Jeon of the University of Tennessee has demonstrated that, under laboratory conditions, it is possible to observe the establishment of a stable amoeba-bacteria symbiosis. After over 20 years of culture, a strain of Amoeba proteus became infected with a large number of bacteria. These became integrated as necessary cell components after initially being pathogenic to the host cells26. The amoeba’s dependence on the endosymbiotic bacteria was also demonstrated by removing the nucleus of an infected cell and placing it into another cell that had previously had its nucleus removed. Treatment with chloramphenicol also killed the majority of the endosymbiotic bacteria, which rendered the amoebae unable to survive. Thus, Jeon had proven that the host nucleus had become dependent on the symbionts 3, 27.

Finally, Okamoto and Inouye have shown that some organisms can take opportunistic advantage of a similar process to endosymbiosis, by observation of a heterotrophic protist that engulfed a unicellular green alga and used the products of its photosynthesis. Inside the host cell, the alga underwent morphological changes, including the loss of flagella and cytoskeleton. The heterotrophic host switched its source of nutrition and became an autotroph, and became capable of phototaxis, the ability to move towards light. (Kimball, 2007) The acquisition of the alga by the protist and subsequent changes in both cells are believed to represent the early stages of a secondary endosymbiosis in process, and the conclusions of both experiments illustrate the possibility of secondary endosymbiosis occurring in modern cells in a similar way in which the symbionts from which mitochondria are descended were acquired28.

Conclusion

Based upon the large body of available evidence contributed by scientists in the years since the endosymbiotic hypothesis was first proposed, including the conclusions of various experiments and the sequence data of nucleic acids and proteins, I have concluded that modern eukaryotic cells arose by a stable incorporation of prokaryotic endosymbionts. This dramatic change was then the driving force behind the evolution of new species and eventually more complex organisms4. However, the question of which order eukaryotes came to possess nuclei and respiratory organelles is still the subject of much debate, and the fact that some genes remain encoded in the mitochondria rather than being completely transferred to the nucleus has not been accounted for16. Despite these uncertainties, the endosymbiotic theory remains the most probable explanation for the similarities between mitochondria and Bacteria, and the large disparity between Bacteria and eukaryotes. The next steps in the development of this theory may require new methods of reconstructing events that occurred billions of years ago, in order to answer one of the greatest uncertainties in evolutionary biology, regarding the origin of the eukaryotes.