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Experimental Sciences and Endosymbiosis Hypothesis: Solving a Paradox

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Abstract

Endosymbiosis Hypothesis is being included by most scientific books to elucidate the origin of the respiration organelle, mitochondria and the photosynthetic chloroplasts. In spite of some similarities between prokaryotes and cell organelles it still contradicts with major scientific subjects in Genetics, Molecular genetics and Experimental Biology. In this article, major criticism is directed toward gene transfer scenario and its relation with gene expression regulation, however, different aspects and subjects have been tackled and elucidated like; impact of differences in genetic code, prey- predator relationship, the reality of mitochondria and chloroplasts - double membrane envelope, the resemblance between organelles (mitochondria and chloroplasts) and bacteria in replication (binary fission) and chromosome structure, how Endosymbiosis scenario differs from natural gene transfer phenomena and the importance of signal peptide sequences in directing proteins toward organelles.

Key words: Endosymbiosis, evolution of mitochondria and chloroplasts, origin of cell organelles.

Introduction

Endosymbiosis Hypothesis presumed that the ancestral respiration organelle was a prokaryote (identified by Brooker, R. as a Purple bacteria) that was respiring free oxygen, and that the presumed ancestral organelles of photosynthesis was a prokaryote (identified by Brooker, R. as a bluish green bacteria or Cyanobacteria) that was making its own food through photosynthesis. The theory presumed that the ancestors of organelles have entered host cells (those host cells were living in zero-oxygen environment) as an internal parasite or as not digested prey.

The first scientist laid foundations of the Endosymbiosis hypothesis of organelles was the Russian C. Mereschkovky, then this hypothesis was developed by the American scientist Lynn Margulis. Endosymbiosis Hypothesis stated that: the mitochondria, the photosynthetic chloroplasts and the (9+2) basal bodies of flagella were themselves once free-living (prokaryotic) cells (Sagan, Lynn. (1967). There will be no comment in this paper on the basal bodies because it does not have DNA, and nobody had solid evidence that it had before.

Recently, Nakabachi, A. suggests that the psyllid Carsonella may have achieved organelle – like status (Nakabachi, A. et.al. 2006).

Most introduced evidence in favor of the hypothesis is the existence of similarities between bacteria and organelles like; chloroplasts ribosomal sequence (16S rRNA), method of reproduction, chromosome feature and other things. The traditional stepwise model of eukaryotes evolution was that the nucleus and microtubules evolved before the acquisition of mitochondria. However, Katz, L.A. introduced evidence that "Archezoa", the primitive eukaryotic group once harbored mitochondria (Katz, L. A. 1998). This finding has challenged the Endosymbiosis hypothesis scenario.

The Endosymbiosis hypothesis implies that a cooperative relationship had been established between the prokaryotic organisms and a host organism, where prokaryotic organisms abandon its unnecessary functions and became organelles inside cells. The cooperative relationship between them was, the prokaryotic organisms have to do aerobic respiration and photosynthesis (each according to its specialty) while the host organism provide food and protection (Campbell, N. A. et.al. 2012).

The Cyanobacteria fossil was found in rocks which belong to the beginning of Archean period (from 2.5- 3.8 billion years), so it is the oldest confirmed organism on earth. The proposed evolution of different kinds of bacteria that does not produce chlorophyll, and then evolution of all kinds of protists in the Archean period indicates it was evolved from Cyanobacteria. If this is true, then what was the fate of all genes that participated in synthesizing chlorophyll pigment and performing photosynthesis in Cyanobacteria? From the very beginning, is not it more fit for all prokaryotes and protists later (which the host organism belong to) to keep all the genes that were participating in photosynthetic process and be free from the assumed services rendered by the presumed prokaryote (who entered and then formed chloroplasts)? On the other hand, there is no mechanism enables a prokaryote or a protist to abandon a big number of its genes.

In 1985, Obar and Green proposed a stepwise model involving: (a) duplication of an organellar gene followed by the transfer of one copy to the nucleus (b) activation of the nuclear copy while keeping the orgnellar counterpart active (c) inactivation and subsequent loss of the organelle gene copy. This scenario sounds good if someone can test it experimentally. The only way to introduce a solid evidence for orgnellar gene transfer subject; is to specify a certain gene in certain cell organelle that is not found in nucleus, then to detect its existence in the nucleus of cell / cells of the lineage of that organism, and then to prove its loss from the cell organelle/ organelles of the lineage of that organism; while it still active in the nucleus. In this article we want to see if these steps are easy to follow.

Observations of living cells have shown mitochondria to be dynamic organelles that change their shape, move from place to place inside the cytoplasm, and undergo both fusion with one another and fission (Karp, G., 2002). This shows that it is not easy to specify certain mitochondria and follow what would happen to it.

It is known that each animal and plant cells contain many organelles. The number of cell organelles varies widely among organisms and even between different tissues of the same organism. For example, in humans; erythrocytes (red blood cells) do not contain any mitochondria, whereas, liver cells and muscle cells may contain hundreds or even thousands. Moreover, each cell contains many mitochondria with multiple copies of mitochondrial DNA (mtDNA).

The presence of many organelles in each cell imposes a big obstacle in the way of gene transfer mechanism, so all scientists who have done experiments to prove reality of gene transfer subject have to answer serious questions in order to their results could be scientifically accepted.

Most researchers who tried to prove reality of gene transfer mechanism did not rely on original mice mitochondrial genes, but they used foreign genes. Usually scientists use particle bombardment (gene gun) method to transfer genes into organelles. In this method, usually many copies of the desired gene segments are places on the surface of the bombarded particles. Suppose a mouse skin tissue contains an average of 50 mitochondria in each cell. In gene transfer processes it is not easy to determine the destination of DNA segments. Upon applying the previous method on certain mouse skin cells, how researchers could distinguish between organelles that have received a foreign DNA segment and that have not. On the other hand, upon detecting a foreign gene in the nucleus; how researchers could determine if it comes from an organelle or it comes from the cytoplasm? As a matter of fact, bombarded DNA segments will not enter each organelle, some organelles may receive a DNA segment while others may not and some other DNA segments will reside temporarily in the cytoplasm.

In spite of contradictions of Endosymbiosis hypothesis with most experimental sciences, but it still dominates most scientific books, so, scientists have to introduce a clear and solid evidence whether Chloroplasts and Mitochondria were free- living prokaryotes or not. To look for such evidence scientists have to take into consideration the following:

I. Is the presumed prokaryotic entry into the host organism due to predation?

1. If the reason of the prokaryotic entry into the host organism was for predation, then digestive enzymes should have killed and digested the prokaryote and not make an endosymbiotic relation with it. If the predator digestive enzymes were not active enough or inefficient, then the presumed predator should be sick and suffering from hunger that caused by inability to digest preys. Science sense says, weak and energy- deficient organism cannot ingest or host the proposed prokaryote for even a short period of time.
2. Scientists assume that the host organism does not respire free Oxygen (O_2), I.e., it is an anaerobic bacteria that takes energy from oxidation of organic compounds) and they assume that respiration organelles were aerobic bacteria that was respiring free O_2. This assumption itself denies the possibility of meeting between these organisms. The atmosphere that has O_2 is considered toxic for anaerobic bacteria (Campbell, N. A. et.al. 2012), and vice versa. It does not make sense to find an anaerobic organism hanging out in a toxic environment. Let us assume that the scientifically unacceptable meeting has happened, then after engulfing of the aerobic prokaryote, it is expected that the prokaryote will die inside the host (predator) as a result of suffocation. The lack of O_2 may cause suffocation to the prokaryote within few minutes.
3. If protists did not contain mitochondria or chloroplasts at the Archean period (from 2.5- 3.8 billion years), this means that those organisms were free-living organisms, acquiring their energy from very efficient mechanisms other than mitochondria. The presumed prokaryote invasion would impose a big burden (extra energy generating mechanism) on the host, because the protist cannot digest food (preys), and consequently it cannot provide organic molecules to the invading organism to oxidize (burn) and generate energy (ATP). So, selection will be against protists that have got the proposed mitochondria. The host was happy with its very efficient energy generating mechanism, why to adopt an extra, new, fuel consuming mechanism (mitochondria)?
4. This case of engulfing a smaller prokaryote and the then digestive enzymes cannot digest the prokaryote resembles a state when a prokaryote is seized or fell in captivity. It is expected that the prokaryote will deal with this condition as a state of stress. It is known when a prokaryote faces a stress it goes into capsulation. It is expected that the capsule of the guest prokaryote will resist entry or exit of molecules. It is expected that the guest prokaryote will stay in the capsule until the days of the host life passes, so, it is a matter of few days or months and the whole Endosymbiosis scenario will be over. In normal cases, digestive enzymes secretion is fast and digestion starts before the prey get capsulated. The previous scenario shows that initiation of Endosymbiosis contradicts with the real life of prey and predator.

II. Is the double membrane existence is a solid evidence for the reality of the prokaryote invasion?

The existence of double membranes for mitochondria or chloroplasts does not mean that the out membrane was part of the host plasma membrane. As a matter of fact, when an amoeba engulfs a food source (or a prey) by its pseudopods it forms a food vacuole from constituents in the cytoplasm, and the amoeba membrane (called plasma lemma) returns to its original nature. If the amoeba membrane itself forms the food vacuoles, then the amoeba membrane will diminish and lost in few days.

Let us assume that the mitochondria or chloroplasts out membrane was part of the host plasma membrane, then after the first replication the out membrane will not be reconstructed in progeny, because the prokaryote does not have genes to build it. So, all progenies of the first prokaryote (mitochondria or chloroplasts) will have one membrane.

On the other hand, the double membrane design of mitochondria has an important function for ATP generation. The inner (much convoluted) one carries the respiratory electron transport chains and ATP synthase responsible for ATP generation. The mitochondrial Matrix, enclosed by the inner membrane, contains the enzymes of tricarboxylic acid cycle. Protons are assembled in the space between the two membranes to form H+ - gradient. The protein- enzyme complex act to use the energy resulted from the gradient to generate ATP molecules (Campbell, N. A. et.al. 2012, Horton, H. R.et. al. 2002). The presence of the outer membrane is essential for hydrogen ions assembly (in spite of repulsion of protons to each other) in order to form the gradient. So, the double membrane is essential for ATP generation function.

If the enzymes and other constituents of the mitochondria were under control of mutations and natural selection, then the host will die before this marvelous essential function is achieved (good mutations takes a long time). Moreover, the mitochondria and chloroplasts out membranes have specialized receptors for internal polypeptides that hold signal peptides (N- terminal leader sequence that target them to outer membrane of the organelle envelope), however, the host plasma membrane does not and should not have those receptors. i.e. usually the inner surface of membranes have internal receptors used for secretion of certain molecules outside cells, while the outer surface of membranes usually have receptors for external environmental signals. Upon

vacuole formation (swallowing a prokaryote), the host membrane inner surface will be the vacuole (organelle) outer surface, then how proteins will be received and introduced into the organelles?.

Although, nuclei of eukaryotic cells possess double membrane envelopes (the outer and the inner membranes meet at nuclear pores) we cannot say; cell nuclei were prokaryotes.

III. Is the presumed prokaryotic entry into the host organism due to parasitism?

Suppose the prokaryote entered the host cell as a parasite, so, it is going to take a long time for the prokaryote to adapt the new environment inside the host, and then to accept a cooperative relation, and then to start abandon certain genes. The expected result for such case is the death of the host organism (the life span is short) before such relation is established,

Additionally, suppose the genes that enable the prokaryote to live free were abandoned and necessary genes essential for the new function was conserved. As a matter of fact, there is no specialized mechanism for such activities. The important question is: who was discriminating necessary genes (and conserve them) from unnecessary genes and discard them. Moreover, who is going to determine the gene boundaries in order to cut at the correct edge?

There is no mechanism to orient or direct DNA segments that come from the prokaryote toward the nucleus, and that comes from the nucleus towards the prokaryotic genome. Up to my knowledge, science has not discovered any receptor to receive DNA segments and orient them towards the prokaryote plasma membrane (organelle), while science discovered hundreds of nucleases in the cytoplasm, which are able to destroy non enveloped or unmethylated double- stranded DNA segments.

Let us assume that all necessary tools and materials were available to transfer DNA segments from the prokaryote towards the nucleus and vice versa. Through this chaos of DNA transfer, waiting for good mutations to occur and produce a suitable mechanism is going to take a long time. So, before this very slow event is achieved, life of both organisms will end before they can divide or reproduce. Consequently, all metabolic pathways will stop including Endosymbiosis. It is known that bacterial life is measured in minutes and hours and the host life may be measured in days or months, while good mutations need years.

On the other hand, who has evidence that the prokaryote can make a successful parasitism process, because it is not equipped for parasitism. Then, who has evidence that the prokaryote was able to reproduce successfully in disharmony and after it had lost a large part of its genes. It is found that the human mitochondrial genome size equals 1% of a typical bacterial chromosome (Lewin, B. 2004). This assumes that around 99% of the prokaryotic genome was lost or transferred to the host nucleus. How this could happen without a suitable mechanism?

IV- Is it possible for a prokaryote to abandon a substantial part of its genetic material?

In order to defend gene transfer and gene abandon mechanisms of Endosymbiosis scenario, some refers to two mechanisms, one used by Red Blood Cells when it abandons its nuclei and the other used by Agrobacterium, when it infects a plant.

1. The eukaryotic Red Blood Cells are programmed to abandon its nuclei (through a programmed process called erythropoiesis), because it can perform its function without its nucleic DNA (genome). This is a different subject, because cyanobacteria and all prokaryotes do not have nuclei. If any prokaryote abandons its genetic material it will die within hours, while Red Blood Cells can live from 3- 4 months without its genome.

On the other hand, somatic cell hybrids discard whole chromosomes (not segments). In these hybrids, human cells fused with rodent (rat or mouse) cells. A peculiar characteristic of human – rodent hybrids is that they randomly discard the human chromosomes (Brooker, R.J. 1999). This case is a one way one time process, so, it is different from Endosymbiosis.

2. *Agrobacterium tumefaciens*; a bacterium that lives in the rhizosphere of plants and is responsible for crown gall disease. This disease infects broad- leaf plants. Scientists found that these bacteria contain small circular DNA called tumor- inducing plasmids (Ti- plasmid). Upon infection, a segment of plasmid DNA marked from both sides by left and right borders, known as T- DNA is transferred from the bacterium to the infected plant tissue. Around 13 genes participate directly or indirectly in cutting, transferring, packaging and introducing the single- stranded T- DNA into the plant nucleus (10).

The following points show that mutual transfer of genes between the prokaryote and the eukaryote nucleus is not possible; compared to natural DNA transfer process.

a. This transfer mechanism is designed to run from outside to inside. The *Agrobacterium* adheres to the cell wall, open an entrance and then sends it T-DNA into cell nucleus. If the *Agrobacterium* is an internal parasite it does not need or possess this mechanism. so, the comparison here is not justified.

b. The gene transfer mechanism between prokaryotes, known as conjugation is specific for fertility factor. The F factor DNA transfer occurs from one cell to another through a pilus (Griffiths, A.J. et.al. 2002). It is not random

and it's a one way one strand process. The same thing occurs with T- DNA of *Agrobacterium*, where it is inserted into the plant cell once. I.e., there is no continuous give and take mechanism as the Endosymbiosis hypothesis proposes.

c. It is found that genes located inside the T-DNA have eukaryotic features, rather than prokaryotic genes (Slater, A.et. al 2003). This refers to a specific gene transfer mechanism from prokaryotes to eukaryotes. Basically, prokaryotes should have gene promoters that have prokaryotic and not eukaryotic features. However, having several (not one, not two, not three genes) different genes located inside the T-DNA region having eukaryotic features mean those prokaryotic genes are programmed to work in eukaryotic cells. Such case is not encountered in any other prokaryotes.

V. why the organelles (mitochondria and chloroplasts) and bacteria have things in common?

1. The resemblance between the organelles (mitochondria and chloroplasts) and bacteria in replication (binary fission) and chromosome structure is a matter of regulatory necessity. Scientific researchers discovered that at certain stages of certain cells, a big number of mitochondria should operate to meet the cell demand for energy. For example, during oogenesis in human, increments in mitochondrial numbers parallel the increase in cytoplasmic volume. Premigratory primordial germ cells (PGCs) have less than 10 mitochondria, while 100 mitochondria are present in ovarian PGCs and 200 can be found in oogonia. Primordial follicle oocytes contain 10000 mitochondria (John, J. C. 2013).

So, to increase the number of mitochondria in developing cells; mitochondrial organelles should divide and multiply independently from the mother cell. Moreover, this division should be easy and fast. So, how should it be?

The fastest replication of an organism is when it has a circular chromosome because in contains one origin of replication compared to the linear chromosomes that have tens of origin of replications. The fastest replication can be achieved by binary fission as in bacteria. So, scientific logic suggests, that mitochondria should possess its own, circular DNA and its subsequent replication machinery, as that of bacteria. This collection should be present in mitochondria in order to divide freely and easily independent from the mother cell division. This is the reason behind the similarity between cell division in bacteria and that of organelles.

Furthermore, it is expected to find similarities between the ribosomes of organelles and that of cyanobacteria, because both have similar apparatus to perform similar function. 70S ribosomes are performing well in translation of mRNA molecules that are transcribed from circular condensed chromosome.

On the other side, gene arrangement and structure is different and puzzling. The genes of yeast and mammalian mitochondria code for virtually identical mitochondrial proteins. Vertebrate mitochondrial genomes are very small, with an extremely compact organization of continuous genes (have prokaryotic feature) whereas yeast mitochondrial genomes are larger and have some complex interrupted genes (have eukaryotic feature).

VI- Gene structure and gene expression system is not similar in prokaryotes and eukaryotes?

1. Eukaryotic genes differ from prokaryotic ones. Eukaryotic genes are long DNA sequences, it is interrupted, composed of a number of exons and introns. After processing of pri- mRNA inside the nucleus, intron DNA sequences are spliced and discarded. In general, prokaryotic gene structure does not contain introns that are why prokaryotic chromosomes are called condensed. Prokaryotic genes do not have introns and a lot of its mRNAs are polycistronic (Lewin, B. 2004). If cell organelles were free-living prokaryotes and Endosymbiosis scenario is real, then we have to find some mitochondrial or chloroplast genes of highly developed organisms are having a number of introns, and to find some polycistronic genes in eukaryote's nucleus.
2. Mechanism of transcription of genes in prokaryotes differs completely from mechanism of transcription in eukaryotes. The transcriptional enzymes, the transcription factors and binding sites on promoters differ between prokaryotes and eukaryotes. The binding sites of a typical promoter of prokaryotic genes is formed from hexamer bases at -35 and hexamer bases on -10 site, while, a binding site of a typical promoter of eukaryotic genes is formed from hexamer bases at -25, at the TATA box (Brooker, R.J. (1999, Lewin, B. 2004). In another word, if a complete gene transferred from guest organism, the prokaryote, into the nucleus of the host, then the transcription enzyme RNA Polymerase II will not recognize the binding sites and then it will not be transcribed and vice versa. So, what is the importance of a gene transfer if the gene will stay unrecognizable? I.e., any movement of gene from the prokaryote to the nucleus or from the nucleus to the prokaryote will be considered a loss of the genetic material.
3. It is known that most prokaryotic genes are arranged in clusters of related genes known as Operons. In the Operon system, each cluster of genes is controlled by one promoter. In this case cutting and transferring genes might end up with promoterless genes. This type of promoterless genes can be considered lost genes, so the proposed scenario would be destructive for genes.

4. Experimental science proved that DNA polymerase gamma is responsible for mitochondrial DNA replication. It is encoded in the nucleus, but it localizes in the mitochondria (Brooker, R.J. 1999, Lewin, B. 2004). This enzyme is unique and is different than its counterpart in bacteria. If the origin of mitochondria is a prokaryote, then it should use DNA polymerase I or III for its DNA replication as in *E. coli* (prokaryote).
5. Translation of messenger RNA (m RNA) in prokaryotes is done at 70S ribosomes while in eukaryotes is done at 80S ribosomes. The genetic codes for UGA sequence in mitochondrial genome for mammals, *S. cerevisiae, D. melanogaster, M. capricolum* and flowering plants means the amino acid tryptophan, while in nuclear DNA in all the previous organisms it means a stop codon. If gene transfer between the prokaryote chromosome and the eukaryotic nucleus occurred as Endosymbiosis hypothesis says, then big problems will result in translation of mRNAs of genes from each resource. Consequently, there will be a substantial destruction for the coding and translation system in first eukaryotes, and then it could be a dead end for all eukaryotes. As far as the coding and translation system is perfect in eukaryotes, then, saying the origin of organelles is free- living prokaryotes is just a speculation.

VII- Discrimination and then isolation of necessary genes from unnecessary genes in the guest prokaryote is not possible?

If prokaryotic genes had been transferred into the nucleus of a eukaryote (predator) to alleviate the contradiction between cytoplasmic genes and the nucleus genes is correct, then how this contradiction or conflict had been settled and how contradicting genes been determined? How the contradicting genes been cut off at the correct nucleotide sequence? Did DNA cutting was done by one restriction enzyme or more? And how DNA segments were transferred? Do DNA segments know where to go? If this is what really happened, then there must be a great organizer behind this job who knows exactly the reality of the problem, and knows where the gene starts and where it ends.

On the opposite, if this job had happened randomly, through trial and error, then DNA cutting was done randomly. In this case, some one can imagine a gene and a half is going out and a gene and a quarter is getting in. this random process definitely will destroy most of the prokaryote and eukaryote genes.

If gene transfer between the prokaryote and the eukaryote nucleus is real, and had been accomplished in a random way by certain restriction enzymes, then we should find some left over genes remained in the organelles, like, genes related to growth or defense, however, few of left over genes will not affect the protist fitness. In human and yeast, almost all mitochondrial genes are responsible for production of rRNA, tRNA or electron transport proteins, however, no left over genes.

As a matter of fact, the scenario of random gene transfer between the organelle and the eukaryote nucleus is not scientific, and who claims contrary should review his experiment approach and results. As mentioned earlier, conjugation phenomenon is not random, where genetic material is transferred from one prokaryotic cell to another (not a nucleus) through a pilus(Griffiths, A.J. F. et. al. 2002, Lewin, B. 2004). In conjugation, one of the strands in the double - stranded fertility factor (F factor) DNA is cut at a location known as the origin of transfer. The cut DNA strand is then transferred in a linear manner. The other strand of the F factor DNA remains in the donor cell, which is contrary to Endosymbiosis scenario.

VIII- Miscellaneous subjects

1. Science has revealed that mitochondria are dealing with five different genetic codes (Lewin, B. 2004). Evolutionists interpretation concerning this subject is; the existence of five genetic codes mean that prokaryotes had invaded eukaryotes five times. To comment on this subject we might say, if one prokaryote had invaded a eukaryote cell once then there should be one common genetic code. But, if prokaryotes had invaded eukaryotes five times then we should see five different kinds of mitochondria, the difference comes from differences among organisms in addition to different transferred DNA segments.

   The existence of five different kinds of prokaryotes each adopting different genetic code, in relatively short period of time is against scientific sense, because this means, changing genetic codes is possible, easy and not harmful. This is untrue, because this will show wide differences in genetic codes among all organisms, but this is unreal.

   On the other hand, if the five different kinds of prokaryotes had invaded eukaryotes at relatively long time intervals, then we have to find different respiration mechanisms, each according to the mutation types, but this is unreal. Moreover, according to this subject we might find different kinds of respiration organelles in the same organism, which is not confirmed by science.
2. The liverwort moss appeared in the Ordovician era around 490 million years ago. The chloroplast genome DNA sequence of liverwort moss (its size 155 kb) and tobacco plant (its size 121 kb) have been completed. A comparison study between chloroplast genes showed that , in spite of differences in

length , but the arrangement of genes is similar and the total number of genes is almost identical (Lewin, B. 2004). This study shows clearly that the arrangement and total number of chloroplast genes between the liverwort moss, that evolutionists considered primitive and a tobacco plant, that is considered relatively recent are similar (Campbell, N. A. et.al. 2012). On the other hand, sequencing of the complete mitochondrial genome has been completed for more than 75 animal species. With few exceptions, a typical animal mitochondrial genome contains 13 protein- coding genes. Since no one has solid evidence a billion years ago if organelles were having much higher gene number, then logically it means that chloroplasts of specific plants and mitochondria of specific animals have a defined gene number that is needed for its function and suitable for its physiology. This defined gene number is an evidence that mitochondrial genome has not changed since a long time and never been a free-living prokaryote. i.e. such similarity and sequence confirms that there were no random cutting and transfer of genes.

3. Bacteria have one circular chromosome localized in a region called nucleoid, while there are several nucleoids for mitochondria and chloroplasts. Furthermore, sometimes there are more than one genome copy inside each nucleoid. For example, inside each mouse mitochondrial organelle in average 5- 6 copies of mitochondrial chromosomes, and inside each plant chloroplast organelle around 60 copies of chloroplast chromosomes(Brooker, R.J. 1999), and it might reach 100 copies(Slater, A.et. al 2003). Up to my knowledge, no one has encountered or documented such phenomenon in prokaryotes. This phenomenon indicates that organelles have specific functions that had nothing to do with prokaryotes.

Conclusion

The previous analysis and discussion of these evidences showed that Endosymbiosis Hypothesis is not standing on a solid scientific ground. Hence, there is a need for further investigations to solve the contradiction with experimental sciences in Genetics, Molecular genetics and Biology.

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