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Genetic recombination and the origin of mitochondrion

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Division of ancestral prokaryotic genome into two circular double-stranded DNA molecules is a basis for future separate evolution of nuclear and mitochondrion compartments. Universal double sheet of lipid molecules by invagination, at the level of membrane-hairpin attachment, formed two-layered envelope completely surrounding those two DNAs. Presumed ancestral prokaryote in this case is an Archaebacteria, which would lead to formation of six main groups of organisms: archaebacteria (Archaea), eubacteria, Protista, Fungi, Plantae and Animalia.

KEY WORDS: *Recombination, Genetic; Mitochondria; Archaea; Evolution*

INTRODUCTION

Genome evolves by acquiring new sequences, by the recombination of existing ones and by mutation. Recombination occurs between corresponding sequences of DNA. One of typical examples is the reciprocal recombination between two copies of a sequence that is repeated in the same orientation, i.e. direct repeats (DR). Two double-strand circular DNA molecules, each retaining a single copy of the DR, are the result of this rearrangement. The requirements of the reciprocal recombination are two recombination sites and multiple copies of the enzyme from the site-specific nickase-ligase family. This reaction can be divided into two stages: formation of a synaptic complex containing two intertwined DR sites and enzyme molecules, and the breakage and reunion of DNA.

The second stage starts with two double-strand break resulting in four 3'-OH and four 5' -P free ends. The 3'-OH ends than attack the phosphate bonds at the corresponding position in the other strand of the duplex. This creates a hairpin structure at the one end in which the 3' end of one strand is covalently linked to the 5' end of the other strand. Strand breakage and reunion are accomplished by two successive ester exchanges in which bond energy is conserved. It resembles to the topoisomerase-, resolvase-, gyrase-, transposase-, invertase-like reaction, recombination of immunoglobulin genes with RAG1 and RAG2 proteins... The ancestral organism, in this case an Archaea, from the very beginning already had about 4000 genes, including those for energy production and conversion: glycolysis, pyruvate dehydrogenase complex, citric acid cycle, respiratory-chain ATP production; genes necessary for different types of recombination, for cell envelop biogenesis and others. Topological characteristics of DNA are the central aspect of all its functional activities - recombination, replication, and transcription. One of this is a double-helical region formed by base pairing between adjacent (inverted) complementary sequences called hairpin structure. During the performance of some of its functions, prokaryotic DNA molecule is typically attached to the plasma membrane, and hairpin structure is one of the candidates for this event. It is possible that in a very ancient prokaryotic cell, the plasma membrane with its attached DNA, could have invaginated and eventually formed a two-layered envelope of membrane completely surrounding DNA. This envelope is presumed to have pinched off (or spindled on) from the plasma membrane producing a DNA compartment surrounded by a double membrane.

SCENARIO

At an early stage of biogenesis there was a precursor autotrophic surface metabolist followed by semicellular organisms (1). Transition from surface organisms to cellular form of life suggests a step-by-step evolution of the nucleotide synthesis and base pairing leading to gene genesis. From this point, future steps of biogenesis are based on genome evolution as a reflection of cell-environment and its own properties. Segregation of ancestral archaebacterial genome (Table 1) into two compartments (Figure

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1) is a result of this stepwise evolution. It led to the increase of evolutionary stability of growing nuclear genome and was a basis for selective advantage, as well as for mitochondrial specialization. This scenario includes several steps: DNA-membrane attachment, DNA double-strand break, DNA ligation, and rolling over (or spindle off) of the attached membrane surrounding those two molecules. Activation of div sequences (Figure 1) is probably required for its association with cell membrane. The properties of the membrane fraction to bind DNA suggest that it includes components necessary to resolve this process. Which of these events cause the others is not obvious, but attachment of archaebacterial DNA to the membrane at the level or in the vicinity of div sequence, could provide a mechanism for both DNA segregation and invagination of the covalently closed circular (CCC) molecule (Figure 1) by two-layered lipid membrane. After step c) (Figure 1) there are several possibilities: 1. Invagination and enveloping of the mitochondrial genome, but not the nuclear; 2. Invagination and enveloping of both genomes; 3. Invagination and enveloping of nuclear but not mitochondrial genome; 4. Releasing of both genomes; 5. Archaebacterial undivided universal genome; 6. Archaea with invagination and enveloping of whole genome leading to amitochondriated eukaryotes (Protists).

In the first case there is an open possibility for evolution of future nuclear genome by acquiring new sequences and specialization of the mitochondrion. One of the organisms that arose after this (archaebacteria cyanobacteria-like precursor) is a good candidate for second dividing recombination event that leads to the chloroplast chromosome formation (Scheme 1). Scheme 1 shows overall view of phylogeny after two cycles of reciprocal genetic recombination of the universal ancestral Archaea genome. Table 1 shows possible gene/gene classes contents of the Archaea donor of the mitochondrial genome. This cell has genetic starting material to evolve to the eukaryotic lineage, which means it has genes necessary to perform all forms of energy production and genetic recombination.

Table 1. Gene/genes classes contents of the Archaea before genome division

Part: Nuclear (about 3.000 genes)	Mitochondrial (about 800 genes)
*Cell division	Pyruvate dehydrogenase complex
DNA replication, recombination, repair, modification	Glycosis (Embden-Meyerhoff pathway)
	PFO - ferredoxin oxidoreductase
Transcription	Hydrogenase
Translation	Respiratory chain enzymes
Cell envelope biogenesis	Citric acid cycle
**Membrane proteins	Aldehyd/alcohol dehydrogenase
Coenzyme metabolism	Acetil CoA synthase
Signal proteins	**Heat shock proteins
Carbon catabolism	Signal proteins
Heat shock proteins	t RNA genes
Protease	Peptidase
Transport and binding proteins	Protease
Nucleotide biosynthesis and metabolism	RNA synthesis
Amino acid biosynthesis and metabolism	Membrane proteins
	DNA replication

*By losing part of the genome responsible for cell division, mitochondrion has to find another way to divide

**Gene duplication occurred prior to division of ancient genome and make easy future gene transfer

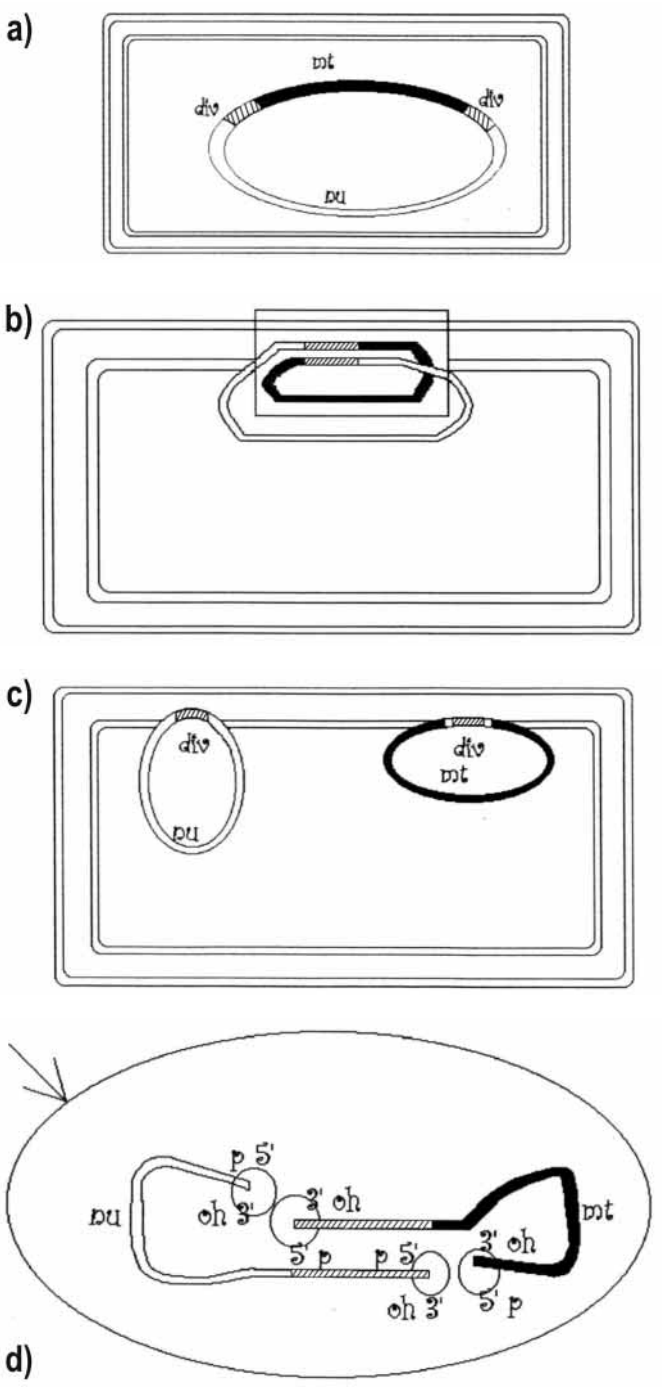
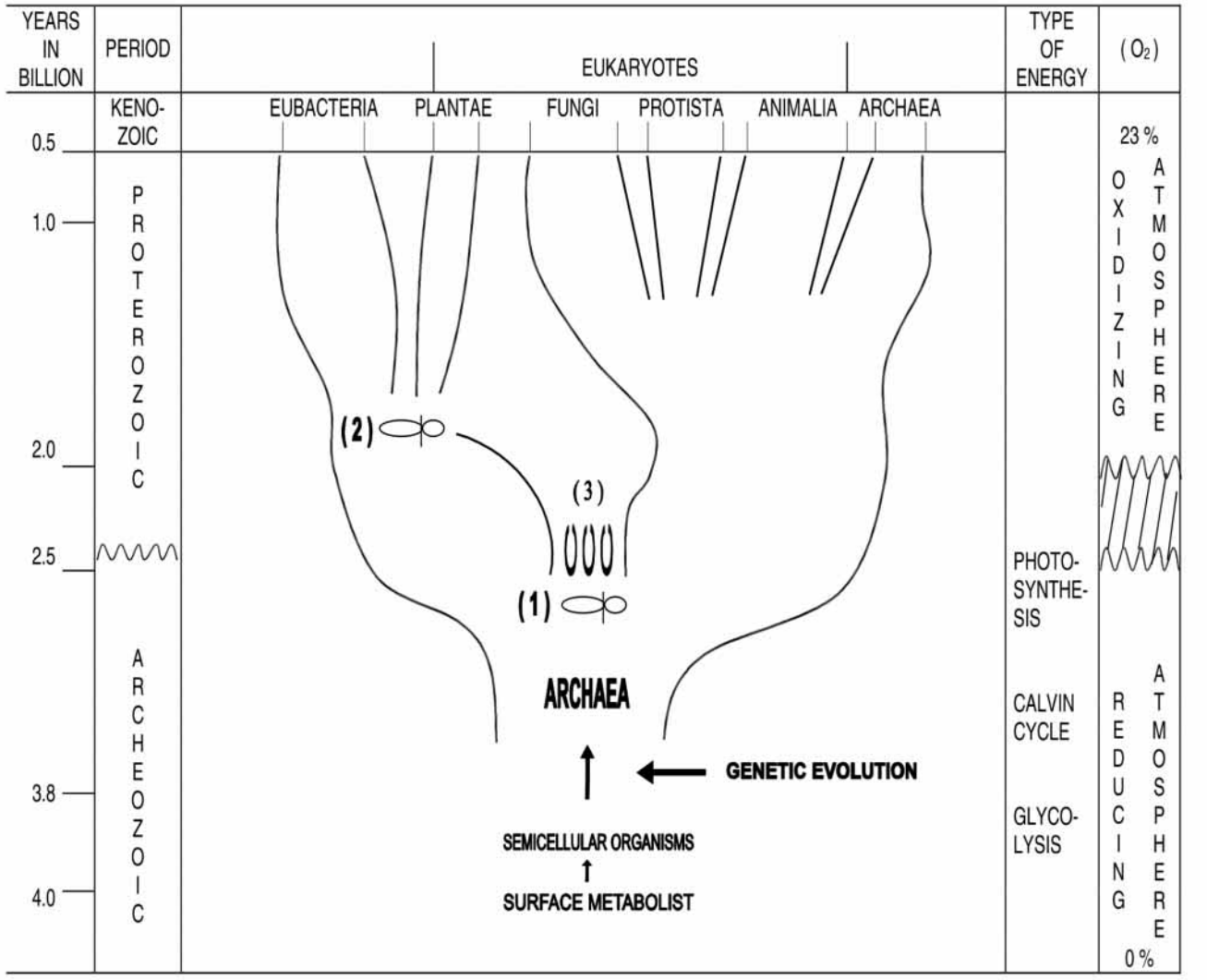


Figure 1. Reciprocal recombination between direct repeats excises the material between them, one product has a "nuclear genes" compartment and another has a "mitochondrial genes" compartment. Each product of recombination has one copy of the direct repeats. (a) Archaebacterial genome with two direct repeats called *div* (dividing) sequences (green boxes), between them is a nuclear (*nu*-clear region) and mitochondrial (*mt*-blue region) gene contents. (b) Pairing of direct repeats (like figure eight) and its attachment to the membrane is initiation signal for recombinase enzyme action (breakage/joining). (c) Recombination releases material between repeats as covalently closed circular (CCC) molecule attached to the membrane. (d) The view from below position (b) between inner membrane and inter-membrane space. Recombinase enzyme (circles) introduces double-strand break at the level/vicinity of *div* site leaving four 3' ends and four 5' ends; subsequent joining reaction by the same enzyme, covalently closed those two molecules. Arrow indicates outer membrane carpet.



Shema 1. Hypothetical phylogenetic tree of the biogenesis based on the surface metabolism and subsequent recombinant division of the archaebacterial genome. (1) First division of archaebacterial genome; (2) Second division of cyanobacteria precursor-like genome; (3) After first recombination events - evolution of the cytoskeleton and endomembranes (having in mind that genes for cell envelope biogenesis already exist in Archaea).

DISCUSSION

Dawning and setting of the Archeozoic eon

Mitochondrion genesis and mitochondrial genome processing (genes transfer between *cp* - *mt* - *nu*) is one of the crucial point in the evolution, which begins with biochemical - energetic development of the membrane (at the time of the very early beginning of biogenesis cellular matrix did not exist) (1). Fundamental similarities in the metabolic pathways in cellular organisms provide a starting evidence for evolutionary conservatism operating over four billion years. If mitochondrial genome arises after separation from the nuclear one, then Archaebacteria is the oldest group of cellular form of life and worldwide ancestor for all living organisms.

After first RNA base pair forming [A - U (2)] further acquisition of G is inevitable (G - U base pair are found in critical and most conservative rRNA and tRNA sequences):

$$\begin{matrix} A - U \\ \\ U - G \end{matrix} \quad => \text{A-U-G.}$$

This leads to conclusion that AUG is the first triplet of nucleotide. By this way it is possible to construct progenome of the progenot, that means alignment of the gene classes in Archaea - genes for cell envelope - for energy production and conversion - for DNA replication, repair and recombination. It might be that first DNA sequence serves as both gene and promoter at the same time and RNA as a primer for replication and expression. Next 1.5 billion years of evolution of the progenome results in a universal Archaea genome with about 3000-4000 genes coding for all fundamental metabolic pathways common for all organisms.

Archaea possesses several characteristics that distinguish it from both eubacteria and eukaryotes and place it at a unique position, not as an intermediate but as a starting point. At that very

moment, before CCC progenome division, two factors play a leading part: capacity of the ancient genome and oxygen concentration. It seems likely that capacity of this genome depends upon gene number rather than nucleotide number.

Maximal capacity of genes number (about 4000) and oxygen concentration of about 1 percent of present-day levels fall at the same time, some 2.5 billion years ago, and it was an expected moment in time for ancestral genome division. The major part of the genome is sequestered in nucleus. To prevent integrity of the individual genes against interruption, nuclear DNA is segregated into chromosomes, plasmids, transposons, nucleolus, satellite DNA sequences, and episomes, because of its specialized functions. This all happens in the same manner - by means of genetic recombination.

To give proof of the reciprocal recombination engagement in the origin of mitochondrial genome, several things have to be done:

(1) To search for div sites in the mitochondrial and nuclear genome sequences.

(2) To search for the function of spacer sequences, unknown open reading frames (ORFs) specificity, in order to find new enzymes (such as new recombinase that catalyzes the introduction of P nucleotides at coding ends). To give functional vision of the "non-coding" regions by simulated base substitution to discriminate inactivated (silenced) or hidden genes of the archaean origin actively participated before 2.5 billion years.

(3) Cancer cell as a model for study reversible evolution of free living organisms. Cancerogenesis is a synonym for reversible evolution, cell descents on one's back, precisely at separation of nu from mt compartment. At this very moment disordered equilibrium between membrane basal metabolism (loss of contact inhibition and aberrant expression of transmembrane genes - future oncogenes) and accelerated cell division (by trigger silenced ancient genes - future oncogenes) have great influence over energy production (enormous glycolysis increase). Expression of gene cluster, gene transfer (*mt* - *nu*), and genetic recombination study of this transient phase can prove obvious succession of events in step-by-step evolution.

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REFERENCES

1. Wachtershauser G. Before Enzymes and Templates: Theory of Surface Metabolism. Microbiological Reviews. 1988:452-84.
2. Wachtershauser G. An all-purine precursor of nucleic acids. Proc Natl Acad Sci 1988;85:1134-5.