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## Ribosomal DNA intracellular journey and the new upcoming ribosomal organelle

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### Abstract

Human mitochondrial DNA (mtDNA) is 16,569 base pairs (bp) in length, coding for 37 genes. During the course of evolution, nearly all the genes expressing ribosomal proteins and ribosomal RNAs (rRNA) genes have been transferred from the mitochondria to the nucleus. However, mitochondrial DNA contains two ribosomal RNAs genes (12S and 16S), which have not yet transferred to the nucleus. These two, should, soon or later, end up in the nucleus. What does the future likely hold for these genes? In the nucleus everything has already been prepared, since all of the ribosomal protein genes are now chromosomally encoded, and nucleolar organizers, the nucleolus, and 45 rDNA are developed waiting for 12S rRNA and 16S rRNA genes to join them, thus enabling the formation of the third DNA-containing organelle, the ribosomal compartment. What could be the reason for this major event in the existence of life on Earth as it is currently understood? The advent of the ribosomal organelle will likely have an enormous impact on reproductive characteristics and on intellectual (thoughts, abstract and complex ideas, imagination, dreams, spirit) activity of the brain. Could this be the answer to the question: Are humans still evolving?

**Keywords:** Nucleolus; mitochondrion; gene transfer; ribosomal organelle; evolution

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### 1. Introduction

Attenuation of the mitochondrial genome entailed the transfer of genes from organelle to the nucleus. This event is an active, prevalent and ongoing process (Henze and Martin, 2001). When having a mitochondrial gene present in the nucleus but absent in mitochondria is beneficial self-fertilization dramatically increases both the rate and probability of gene transfer. However, in the absence of such a benefit, when mitochondrial mutation rates exceed those of the nucleus, self-fertilization decreases the rate and probability of gene transfer (Brandvain and Wade, 2009) Gene transfer is a process of fundamental importance to the establishment and evolution of organelles in eukaryotic cells. In the original ancient mitochondrial genome there were approximately 100 ribosomal genes, coding for ribosomal proteins and for ribosomal RNA. During evolution, all of them except two (12S rRNA and 16S rRNA) moved into the nucleus. If the ~98 ribosomal genes moved during evolution from mitochondria to the nucleus, is logical to assume the remaining two will

also do so. In amphibian oocytes, for instance, ribosomal genes have the capacity to form extra-chromosomal rDNA that replicates independently in the extra-chromosomal nucleoli (Blankenship, 2001). Because of the inherent compact structure of the somatic nucleolus of mammalian cells, it is difficult to individualize ribosomal genes from the bulk of the genome.

45S rDNA sites on the chromosomes are referred to as secondary constriction regions. Constriction sites and chromosomal fragility may be indicative of stalled replication. Chromosome gaps and breaks in plants were exclusively associated with the 45S rDNA and resembled fragile sites in human chromosomes (Gall, 1968).

Actively growing mammalian cells contain 5-10 million ribosomes, which is a sufficient reason, regarding energy consumption, for compartmentalization of the nucleolus. If the ribosomal organelle has to also include ribosomal protein genes (RPG), this may be possible by functional chromosomal translocation and inter-chromosomal recombination between chromosome pairs containing RPG and acrocentric chromosomes 13, 14, 15, 21, and 22.

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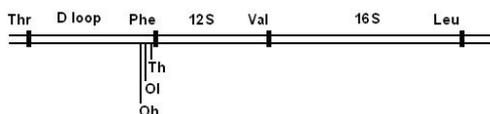
## 2. Organization of the human mitochondrial rDNA

Figure 1 shows the region of the mtDNA containing the 12S rDNA and 16S rDNA attached to the control region with regulatory functions. D loop is a stretch of ~1100 bp in length, marked by a triple stranded structure encompassing a duplicated short region of the heavy strand, the so-called 7S DNA. In the D loop, there is an origin of synthesis and transcription of heavy strand and an origin of transcription of light strand of the mtDNA. This region, the D loop, would be an ideal place for genetic recombination to become engaged in transfer of these 12S and 16S rDNA, as whole pieces, into the nucleolus.

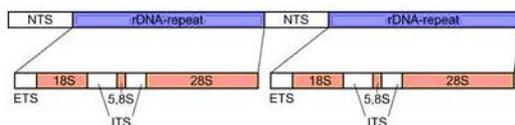
## 3. Infrastructure of the ribosomal acceptor

In higher eukaryotes, nuclear ribosomal DNA (rDNA) genes are usually organized in two multigene families. A major family encodes for 28S, 5.8S, and 18S rRNA (Fig. 2), and a minor family contains only 5S rRNA genes. The 5S rDNA consists of a conserved transcribing region of 120 bp, with a variable intergenic spacer (non-transcribed spacer-NTS). 5S rDNA is located elsewhere in the genome in most eukaryotes.

In the nucleus, the rDNA region of the chromosome is visualized as a nucleolus which forms expanded chromosomal loops with rDNA. These regions are also called nucleolar organizers, as they give rise to the nucleolus. The nucleolus has a tripartite organization that reflects spatial separation of the steps of ribosomal biogenesis. Initiation of the process starts in the fibrillar centers (FC), where 45S rRNA is transcribed by RNA polymerase I from rDNA.



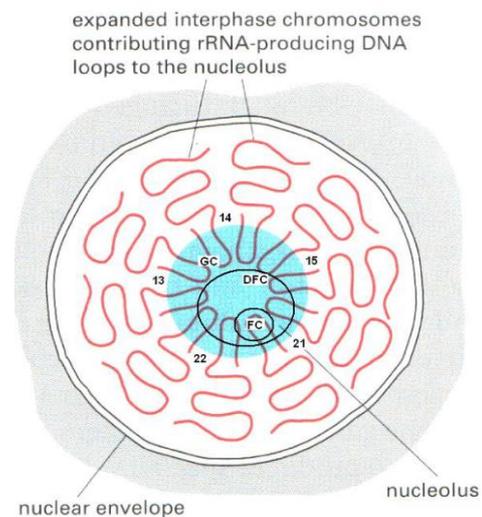
**Fig 1.** Organization of the human mitochondrial rDNA. 12S and 16S ribosomal genes. Thr, Phe, Val, Leu represent tRNA genes. Th–transcription origin of the heavy strand, Oh–origin of replication of heavy strand, Ol–origin of transcription of light strand



**Fig. 2.** Eukaryotic nuclear rDNA consists of a tandem repeat of a unit segment, an operon, composed of NTS, ETS, 18S, ITS1, 5.8S, ITS2, 28S frames. NTS–non-transcribed spacers, ETS–external transcribed spacers, ITS–internal transcribed spacers

Pre-rRNA processing, as well as rRNA assembly with ribosomal proteins and the 5S rRNA, occur in

the dense fibrillar component (DFC) and granular component (GC) of the nucleolus (Huang et al., 2008) (Fig. 3).



**Fig. 3.** Human nucleolus with chromatin loops containing rRNA genes on five pairs of interphase chromosomes (13, 14, 15, 21, and 22), and its tripartite organization with FC–fibrillar center, DFC–dense fibrillar component, and GC–granular component

Products of this process, the 40S and 60S ribosome subunits are exported, through the nuclear pores, to the cytoplasm. The internal density of the nucleolus should represent the future ribosomal matrix, much like the mitochondrial matrix. The nucleolus is assembled around several hundred copies of the repeated 45S rRNA gene clustered on the short arms of chromosomes 13, 14, 15, 21 and 22 (Hetman and Pietrzak, 2012; Henderson et al., 1973). Except for the ribosomal genes, no other active genes have been reported to reside within nucleoli. It would be reasonably expected that these two remaining genes end up in the nucleolus. Regarding the proteins, proteomic research in the human nucleolus has enabled the identification of ~350 proteins (Worton et al., 1998). Interestingly, a piece of evidence for protein synthesis even inside the nucleus has been reported (Andersen et al., 2002). These facts speak in favour of the nucleolus being well equipped to become a new cellular organelle.

Concerted evolution of the gene family is thought to be mediated by inter-chromosomal recombination between rDNA repeats, but such events would also result in conservation of the sequences distal to the rDNA on those five pairs of chromosomes. The rRNA genes are transcribed in a telomere-to-centromere direction, the 5' end of the cluster and adjacent non-rDNA sequences are conserved on the five pairs of chromosomes, and the 5' end of the cluster is positioned ~3.7 kb

upstream from the transcription initiation site of the first repeat unit. These data support a model of concerted evolution by inter-chromosomal recombination.

By elaborate processing of a single polycistronic 45S rRNA, the precursor would transcribe raising the three ribosomal RNAs—28S, 18S, 5.8S (Iborra et al., 2001). Maturation of precursors of the small and large ribosomal subunits (SSU, and LSU respectively) are also nucleolar (Maidak et al., 1997). LSU assembly in the nucleolus involves incorporation of the 5S rRNA which is transcribed from non-nucleolar genes by RNA polymerase-3 (Kressler et al., 2010). Stimulation of rDNA transcription is sufficient to increase the entire process of ribosomal biogenesis (White, 2008). On this basis, the organisation and process flow, finishing with the formation of the ribosomal compartment, may be deduced. Transfer of the 12S rDNA and 16S rDNA to the nucleus would be a sufficient signal for the formation of the putative new organelle. The transcription-dependent nucleolar compartmentalization of various proteins offers a number of potential stress factors that can be released on cell injury (Laferte et al., 2006). Conversely, nuclear export and inactivation of stress signaling messengers, such as p53 may require an *intact nucleolus* (Boulons et al., 2010).

12S rRNA is a component of the 40S small subunits of the ribosome. Like the large 16S rRNA, it has a structural role acting as a scaffold defining the position of the ribosomal protein. The 3' end contains the anti Shine-Dalgarno sequence which binds upstream from the AUG start codon on the mRNA. The 3' end of 16S RNA binds to S1 and S21 and is known to be involved in initiation of protein synthesis (Boyd et al., 2011). 12S rRNA interacts with 16S, aiding in the binding of two ribosomal subunits. 16S rRNA stabilizes correct codon-anticodon pairing in the A site of the ribosome. Mammalian mitochondrial 12S rRNA and 16S rRNA are much shorter than bacterial 16S rRNA and 23S rRNA.

Eukaryotic ribosomal genesis involves 80 ribosomal proteins, more than 200 non-ribosomal proteins and 75 small nucleolar RNAs. Unlike the rRNAs, each mammalian ribosomal protein typically is encoded by a single gene. Out of 80 ribosomal protein genes, 75 are mapped, and they are widely dispersed. Both sex chromosomes and at least 20 of the 22 chromosomes carry one or more ribosomal protein genes. Chromosome 19 contains twelve rp genes (Geige and Brennicke, 1999).

#### 4. Presentation of the hypothesis-Mechanisms for the transfer of the genes to the nucleus

There are several ways for genes to transfer from organelles to the nucleus. Genes can be transferred by RNA editing, by direct DNA transfer, by nuclear recombination involving DNA derived from the mitochondrion, by minicircles, etc. There is also a reverse process, the back transfer from nucleus to the organelles, for the same genes that had been earlier passed to the nucleus.

RNA editing usually involves the conversion of uracil into cytosine (U to C) in primary mRNA. A gene coding for RPS2 protein is present in the mitochondrial genome of several cereals, with seven editing sites in its mRNA, but it does not exist in dicotyledons (Vaitilingom et al., 1998; Stupar et al., 1998). Instead, in these species, the mt rps2 gene has been transferred into nucleus. The transfer mechanism of plant mitochondrial DNA to the nucleus proceeded through a cDNA intermediate. However, in several mitochondrial genomes there are no editing sites in the rRNAs and tRNAs, probably because of the conserved sequences of those genes. Therefore, potential transfer of these two genes, 12S rDNA and 16S rDNA through editing can probably be excluded.

Regarding direct DNA transfer, the best known example is *Arabidopsis* mtDNA. The transferred piece of this mtDNA is 620 kb long, and originated from internal duplications of large segments of this complete mtDNA (Stupar et al., 2001; Stupar et al., 2011; Stupar et al., 2012).

Another interesting method for direct DNA transfer is found in Dinoflagellate chloroplasts. These have “mini-circles”, each coding a single gene (“one gene—one circle”) instead of the typical large multigenic circle. How the minicircles formed from a large circular piece of DNA is not clear, but minicircles may provide insight into finding a mechanism for gene transfer, because they can be easily packaged for the journey. Single gene minicircles have been found in the mitochondrial genome of mesozoans (McFadden, 1999).

Recombination is an inexhaustible source of genetic variation and new functions. Recombination involves the transfer of bulk DNA and recombination into the nuclear chromosome. For example, a piece of DNA from the rice mitochondrion containing a fragment of the rps19 coding region, a fragment of the rps3 coding region, and a segment of the group II intron in the rps3 gene have recombined into the 3' region of a nuclear gene for vacuolar ATPase (Kubo et al., 2001).

The transfer of Cox2 protein with novel gene activation steps into the mitochondria of legume species indicates that the presequence was cleaved in a three-step process which was independent of assembly. In this case, the Cox2 presequence of 136

amino acids (aa) consists of three regions: 20 aa for mitochondrial targeting, 104 aa for import of Cox2 protein into the mitochondria, and the last 12 aa for maturation of the imported protein (Daley et al., 2002). This indicates inexhaustible possibilities for gene transfer and cell ingenuity in solving their own needs.

### 5. Transfer of 12S DNA and 16S DNA into the nucleus

Nearby the nucleolus is the field of telomerase (reverse transcriptase) activity. Telomerase is a specialized example of reverse transcriptase, an enzyme that can synthesize a DNA sequence using an RNA template (3'...AACCCCAAC...5'). Telomerase acts at the level of telomeres, located so close to the nucleolus, that they are not, in fact, spatially separated. If this enzyme was involved in the whole process, what would the scenario be?

Several important steps can be delineated: I.–mRNA of the 12S and 16S DNA processing, with splicing off the tRNA for valine; II–entry of this mRNA into the nucleus; III–reverse transcription of 12S+16S ribosomal mRNA; IV–ds DNA synthesis; V–genetic recombination of new (12S+16S) DNA with rDNA in the nucleolus; VI–extra-chromosomal separation of full rDNA and circularization of the new operon genome; VI–membrane formation around the ribosomal genome (Fig. 4).

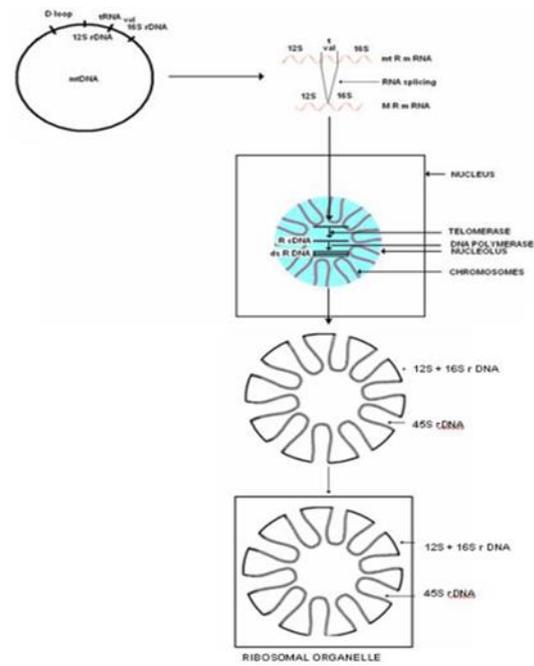
There are two ways for a newly formed organelle to exit the nucleus. One is through the nuclear membrane, and the other through nuclear pores. The best moment for this event would be at cell division, when proteins participating in this process, especially in the synthesis of membrane, are abundant. The human nuclear membrane is an uninterrupted single lipid bilayer that has an outer face and inner face. It is possible that the ribosomal genome could, at least initially, gain its unique membrane inside the nucleus, during cell division. The examples of planctomycetes (Martin, 2005) and *Ignicoccus*, in which the cytosol is surrounded by two complete and distinct membranes (Kupar et al., 2010), indicate that new membrane can arise *de novo* in evolution, at least in prokaryotes. The trigger for the whole cascade of events may be the entry of these two genes into the nucleus.

### 6. Implication of the hypothesis-Medical implications

rRNA is very important in medicine and in evolutionary analysis. rRNA is a target of several antibiotics: erythromycin, chloramphenicol, sarcin, streptomycin. rRNA is the only one among all the genes which is present in the cells of all organisms.

rDNA, in particular 12S and 16S rDNA, are used in sequencing to identify an organism's taxonomic group, and estimate rates of species divergence.

Keeping in mind the role of mitochondria and nucleolus in the function of the cells, we can estimate the impact of this evolutionary change on human health.



**Fig. 4.** Exotic fashion of the elaborated mechanism of rDNA transfer from mitochondrial genome to the nucleolus. Starts with RNA splicing of the tRNA for valine. Mature rRNA enters the nucleus, and in the nucleolus with the aid of telomerase and DNA polymerase becomes ds rDNA. 45S rDNA breaks at the fragile site and allows rDNA to recombine

This change would lead to a balance between mitochondrial and ribosomal organelle functions, affecting the biochemistry of the whole cell. Loss of the ribosomal operon would allow the mitochondrion to more easily and efficiently perform its functions. Isolated, the ribosomal organelle would better regulate its own role. There would be an influence on the basic cellular functions from synthesis of Fe-S proteins to neuronal growth and long-term maintenance of mature neurons. Importantly, change in these five pairs of chromosomes would allow the nuclear genome to deal with even more luxurious functions, and it is envisaged this could well impact on the higher fields of brain activity. This would without doubt increase and improve biological and mental performance.

### 7. Instead of a conclusion

The three most important events in biological

evolution are development of the nucleus and mitochondrion, origin of the chloroplast, and future formation of the ribosomal organelle.

In the near future, through genetic recombination, a third, novel DNA-containing organelle will be made - the ribosomal compartment. Evolution is not random, spontaneous or instinctive, but rather, it has a specific purpose. The purpose of the whole of biological evolution (as we currently understand it in philosophical terms) is to develop a brain in order to know the absolute truth, to recognize ultimate intelligence. This means that the capacity of the human non-coding DNA sequences will increase. All 20,000-25,000 coding genes in the human genome have the same functions as in all other organisms and are used for non-intellectual activities. One of the best examples of universalization in biology is glycolysis, the enzymes of which are the same for all organisms. Another prominent example of universalization in biology is found in the cytochromes, cytochrome bc<sub>1</sub> complex in mitochondria and cytochrome b<sub>6</sub>f in chloroplasts. These two quinol oxidoreductase in respiration ("reverse photosynthesis") and in photosynthesis are closely related, and appear to share a common ancestor. In contrast though, we do not yet know a single biochemical pathway, nor genes, involved in the development or in the creation of thoughts. If thoughts are genetically controlled, i.e. if they are results of gene expression, there would be a periodicity in their appearance.

In the human genome there are about 25,000 genes with known or unknown functions. The functions of about 30% of the genes from completely sequenced genomes are not known. Is there some new process in the cell, the existence of which is not known? From the early onset of biological evolution, the first semi-cellular organisms did not possess any genetic material and those organisms were able to live for a long time in evolutionary terms, to their physical, not biological, death. What will happen when the new ribosomal organelle appears? Organisms in which these changes occur will live much longer than we can suppose, and presumably, all on account of higher intelligence.

This will certainly have an impact on reproductive activity. Reduced reproductive activity may allow the use of the remaining 80%-90% of the brain capacity that we currently do not use. The Y chromosome is already in the phase of losing its genetic importance. However, the greatest gains are likely to be in intellectual development. Finally, it is clear that other primates aside from man possess this same genetic organization of mitochondrial DNA. This begs the question: In which organisms

will this new change occur? (This is not a call for the extermination of primates).

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