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Mitochondrial diseases: Translation matters

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ABSTRACT

Mitochondrial diseases comprise a heterogeneous group of disorders characterized by compromised energy production. Since the early days of mitochondrial medical genetics, it has been known that these can be caused by defects in mitochondrial protein synthesis. However, only in recent years have we begun to develop a broader picture of the array of proteins required for mitochondrial translation. With this new knowledge has come the realization that there are many more neurological and other, diseases attributable to impaired mitochondrial translation than previously thought. Perturbation of any part of this intricate machinery, from the primary sequence of transfer or ribosomal RNAs, to the proteolytic processing of ribosomal proteins, can cause mitochondrial dysfunction and disease. In this review we discuss the current understanding of the mechanisms and factors involved in mammalian mitochondrial translation, and the diverse pathologies resulting when it malfunctions. This article is part of a Special Issue entitled 'Mitochondrial function and dysfunction in neurodegeneration'.

Introduction

Mitochondria are essential double-membrane organelles, whose primary function is energy production through the oxidative phosphorylation (OXPHOS) process. Additionally, mitochondria serve as the crossroads for many important biochemical pathways and perform crucial roles in regulating iron and calcium homeostasis, nitrogen metabolism, and apoptosis. OXPHOS catalyzes the oxidation of fuel molecules and the concomitant energy transduction into ATP via five complexes located in the inner mitochondrial membrane (IMM). Briefly, a series of redox reactions result in the reduction of oxygen to water while complexes I, III and IV of the respiratory chain pump protons across the IMM into the intermembrane space (IMS) generating a 'proton gradient' across the IMM. This proton gradient provides the driving force for the synthesis of ATP from ADP and inorganic phosphate (P_i) by complex V (or ATP synthase). Notably, the formation of the OXPHOS system is under the control of two separate genetic systems, the nuclear and the mitochondrial genomes. As a result, genetic defects of either mitochondrial or nuclear DNA can compromise ATP production and potentially cause human pathology at any age, with any symptoms, and by any mode of inheritance. In this review we will focus on protein synthesis in human mitochondria and on the role it plays in mitochondrial disorders, after recapitulating the processes required before mitochondrial protein synthesis can take place.

The mitochondrial genome

Human mitochondrial DNA (mtDNA) is a circular, double-stranded molecule with a striking economy of sequence organization, compressing 37 genes into 16.6 kilobase pairs of DNA. Unsurprisingly, there are no introns and the genes are arranged end to end with little or no intergenic regions. However, there is one sizeable non-coding region (NCR) that contains a number of important regulatory elements of replication and transcription (Fig. 1A). Mitochondrial DNA is devoted to the synthesis of 13 subunits of respiratory complexes I, III, IV, and V. In addition to the 13 proteins of the OXPHOS system, mtDNA encodes the 22 transfer RNAs and two ribosomal RNAs necessary for their translation within the organelle. The two strands of human mtDNA have different nucleotide compositions and are designated heavy (H) and light (L), according to their buoyant density on cesium chloride gradients. The H-strand contains most of the coding material, including 12 of the 13 protein-coding genes, both rRNAs, and 14 of the 22 tRNAs, whereas the DNA L-strand encodes a single protein and eight tRNAs.

The fact that mtDNA is a compartmentalized extrachromosomal element contributes to its unique genetic features. 1) A typical cell contains hundreds or thousands of mtDNA molecules. 2) Although in normal circumstances almost all copies share the same sequence (homoplasmy), some individuals harbor two (or more) mitochondrial genotypes (heteroplasmy). 3) Because there are many copies of mtDNA in a cell, a deleterious sequence variant can be tolerated until, or unless, the abundance of the defective mtDNAs exceeds a threshold, at which point mitochondrial and cellular dysfunction manifests. 4) Mitochondria are not partitioned equally at cell division, and so the proportion of mutant mtDNAs may shift in daughter cells,

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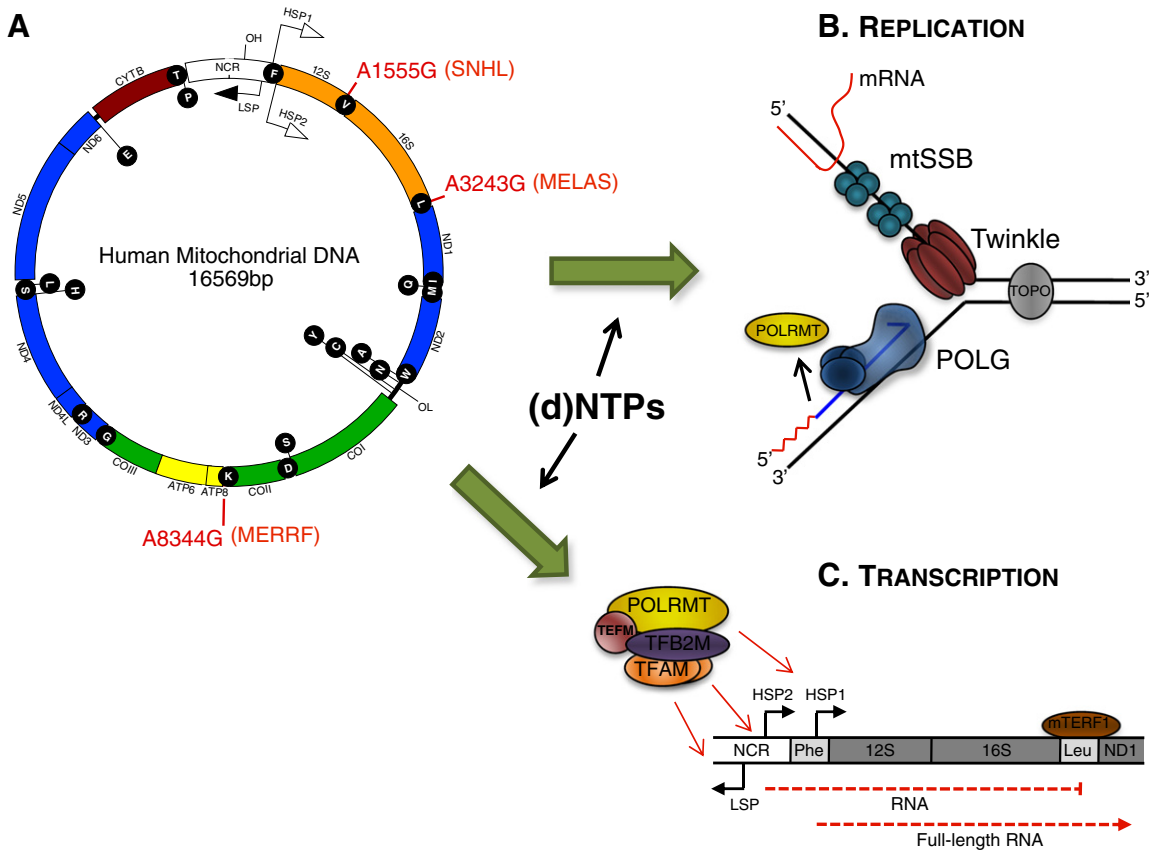

Fig. 1. The human mitochondrial genome and factors involved in mtDNA replication and transcription. A) A schematic diagram of human mitochondrial DNA encoding 13 protein, 2 rRNA, and 22 tRNA coding genes. The location of three of the most common mitochondrial mtDNA gene mutations with their associated clinical phenotypes are indicated. B) The core mtDNA replication machinery in mammals includes DNA polymerase POLG, Twinkle helicase, and mtSSB. Replication also involves RNA priming by POLRMT, mtDNA topology alteration by topoisomerases, and possibly RNA incorporation into the lagging strand during leading strand synthesis. C) Mammalian mtDNA transcription initiates from three promoters, one for the light strand (LSP) and two for the heavy strand (HSP1, HSP2). The core mtDNA transcription machinery includes RNA polymerase POLRMT and its accessory subunit TEFM, transcription factor TFB2M, and transcription activator TFAM. RNA synthesis beginning from HSP2 is terminated by mTERF1 within the $tRNA^{Leu(UUR)}$ gene while transcription started at HSP1 results in near genome-length polycistronic transcripts. The building blocks of nucleic acid are (deoxy)nucleotides ((d)NTPs).

affecting the phenotype accordingly. Although phenotypic selection will favor fully functional mitochondria, some mutant mtDNAs possess a replicative advantage and this may be key to their selection and persistence. 5) The mitochondria of spermatozoa degenerate after fertilization and so mtDNA is exclusively maternally inherited (Al Rawi et al., 2011). 6) There is a marked restriction in mitochondrial numbers during oogenesis and this 'bottleneck' can explain the striking generational shifts in the levels of different mtDNA variants, which were first described in cows (Olivo et al., 1983) and later in humans (Blok et al., 1997).

Mitochondrial DNA maintenance, replication and transcription

Mitochondrial DNA replication is an ongoing process in most cells and tissues that have been examined and, unlike nuclear DNA, mtDNA synthesis occurs independently of the cell cycle, and post-mitosis. Two modes of mtDNA replication have been proposed to operate in mammalian cells and tissues (Pohjoismäki and Goffart, 2011). In one mode, the two strands of DNA are synthesized concurrently as in nuclear DNA replication, whereas in the other there is a considerable delay between the initiation of synthesis of the two strands. The latter mode of replication exposes the lagging-strand template, which is either coated with protein or hybridized to RNA. The mtDNA replication apparatus is known to include a DNA polymerase (POLG), a DNA helicase (Twinkle) and mitochondrial-specific single-stranded DNA binding protein (mtSSB) (Fig. 1B). Other factors critical for mtDNA replication are RNase H1 and DNA ligase III, which are shared with the nucleus. Notwithstanding this knowledge, we are far from having a complete inventory of proteins required for mtDNA replication and maintenance; however it is of particular note that mitochondrial nucleoprotein complexes, or nucleoids, are closely associated with the machinery of mitochondrial protein synthesis (He et al., 2012b). Some of the nuclear-encoded factors that are important for mtDNA maintenance have a prolonged interaction with mtDNA, as epitomized by the major mtDNA packaging protein TFAM, whereas others do not interact directly with either mtDNA or mtDNA-binding proteins. The latter group includes enzymes involved in *de novo* synthesis of nucleotides or nucleotide salvage pathways, and some, such as thymidine phosphorylase and the accessory subunit of ribonucleotide reductase, RRM2B, even reside outside mitochondria (Spinazzola, 2011).

The mitochondrial genome relies on only three promoters to produce its 11 mRNAs, 22 tRNAs, and two rRNAs. Two are on the heavy strand (HSP1 and HSP2), whereas there is one lone light strand promoter (LSP). LSP and HSP1 are located in the NCR, while *in vitro* techniques have recently corroborated the earlier mapping of HSP2 to the $tRNA^{Phe}$ gene adjacent to the NCR (Fig. 1A) (Zollo et al., 2012). All three produce polycistronic transcripts, and the products of HSP1 and HSP2 partly overlap. The primary purpose of HSP1 is to produce rRNAs for mitochondrial ribosomes (mitoribosomes), and transcription beyond the rRNA genes is prevented by the mitochondrial transcription termination factor, mTERF1. HSP2 additionally