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Review

Mitochondrial DNA transcription and diseases: Past, present and future

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Abstract

The transcription of mitochondrial DNA has been studied for 30 years. However, many of the earlier observations are still unsolved. In this review we will recall the basis of mitochondrial DNA transcription, established more than twenty years ago, will include some of the recent progress in the understanding of this process and will suggest hypotheses for some of the unexplained topics. Moreover, we will show some examples of mitochondrial pathology due to altered transcription and RNA metabolism.

Keywords: Mitochondrial DNA; Mitochondrial transcription; Mitochondrial diseases; Transcriptional machinery

1. Introduction

Human mitochondrial DNA (mtDNA) is a super-coiled double-stranded closed circular molecule of approximately 16.5 kilobases (kb) [1,2]. There are several copies per mitochondrion and many hundred mitochondria per cell.

This molecule encodes 37 genes, 2 ribosomal RNAs (12 S and 16 S rRNAs), 22 transfer RNAs (tRNAs) and 13 polypeptides, all of them components of the oxidative phosphorylation (OXPHOS) system: seven (ND1–ND6 and ND4L) of the 46 complex I (NADH:ubiquinone oxidoreductase) polypeptides; one (apocytochrome b) of the 11 complex III (ubiquinol:cytochrome *c* oxidoreductase) subunits; three (COI–COIII) of the 13 complex IV (cytochrome *c* oxidase) polypeptides and two (ATP6 and ATP8) of the 16 complex V (ATP synthase) subunits.

According to their G+T bases composition, which confers to them a different density, the strands were denominated heavy (H) and light (L). One polypeptide (ND6) and eight tRNA genes are encoded in the L-strand while the rest are encoded in the H-strand.

The genetic organization of the mtDNA is extremely compact: polypeptide, tRNA and rRNA genes are smaller than the cytosolic and prokaryotic counterparts [3], there are overlapping genes, the 3′-end CCA of the tRNAs and even some termination codons are not mitochondrial encoded; the smaller size of the rRNAs has required the recruitment of new ribosomal proteins to substitute the lost functions of the removed rRNA segments [4], there are no intronic sequences and almost no non-coding nucleotides between genes.

A non-coding region (control region) of approximately 1.1 kb, located between the $tRNA^{Phe}$ and $tRNA^{Pro}$, contains the origin of replication for the heavy strand (O_H), the transcription promoters (LSP and HSP, light and heavy strand promoters, respectively) and the regulatory elements for the mtDNA expression [multiple TFAM binding sites, 3 conserved sequence blocks (CSB) and a termination associated sequence (TAS)]. However, the function for most of the control region is unknown and it is unexpected that in such an otherwise compact genome there are regions without any apparent function. In fact, the central domain of the control region, with as yet unknown role, is more evolutionary conserved than the known regulatory elements [5,6] and it might be involved in the replication or expression of the mitochondrial genetic information.

2. Mitochondrial RNA synthesis and processing

RNA is synthesized in human mitochondria by means of three transcription units that start at three different initiation points, one for the L-strand (L) and two for the H-strand (H_1 and H_2), that transcribe three polycistronic molecules [7–10] (Fig.

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1). Thus, the H-strand is transcribed by two overlapping units. One of them, more frequently used, starts at the initiation site H_1, located 16 nt upstream of the $tRNA^{Phe}$ gene (position 561) and ends at the 3′-end of the 16S rRNA. This transcription unit is responsible for the synthesis of the two rRNAs, $tRNA^{Phe}$ and $tRNA^{Val}$. The transcriptional activity of this unit is linked to a termination event that take place immediately downstream from 16 S rRNA, inside the gene for $tRNA^{Leu}$ [8,11]. The second transcription unit, less frequently used than the previous one, starts at the initiation site H_2, 2 nt upstream of the 5′-end of the 12 S rRNA gene (position 646) [8,10] and produces a polycistronic molecule covering almost the whole H-strand. The mRNAs for 12 polypeptides and 14 tRNAs derive from the processing of this polycistron. The L-strand gives rise to a single polycistron starting at the 5′-end of the RNA 18 (position 407), 154 bp away from the H_1 initiation point, from which the eight tRNAs and the ND6 mRNA are derived. Moreover, the H-strand replication requires short RNA primers originated by the processing of the L-strand promoter transcripts. Consequently, replication of mammalian mtDNA is linked and dependent on mitochondrial transcription, although a new mechanism of replication has recently been described [12,13]. The precise location and mechanism for H_2 and L-strand transcription termination is unknown [14], although the finding of H-strand ESTs with polyadenylation sites located immediately upstream of the H_1 site suggests that this point might be the termination site for the second transcription unit of the H strand [15], as previously suggested [16].

The polycistronic primary transcripts synthesized from the three initiation sites are processed, according to the "tRNA punctuation" model, to give rise to the mature rRNAs, mRNAs and tRNAs after the precise endonucleolytic cleavage on both sides of the tRNA molecules [8,17]. According to this model, the tRNA sequences on the nascent RNA chains would acquire the cloverleaf structure and act as the signals for the processing enzymes. The processing of the polycistronic molecules requires precise endonucleolytic cleavages at the 5′- and 3′-end of the tRNAs, the addition of the CCA to the 3′-end of the tRNAs, a polyadenylation activity for mRNAs and rRNAs, and, finally, the post-transcriptional modification of a subset of tRNA and rRNA nucleotides.

The 5′-endonucleolytic cleavage occurs first [18] and is performed by a mitochondrial RNase P [19,20]. The enzyme responsible for the 3′-end cleavage activity is a tRNAase Z [21]. The addition of the CCA to the 3′-end of the tRNAs is performed by an ATP(CTP):tRNA nucleotidyltransferase. This

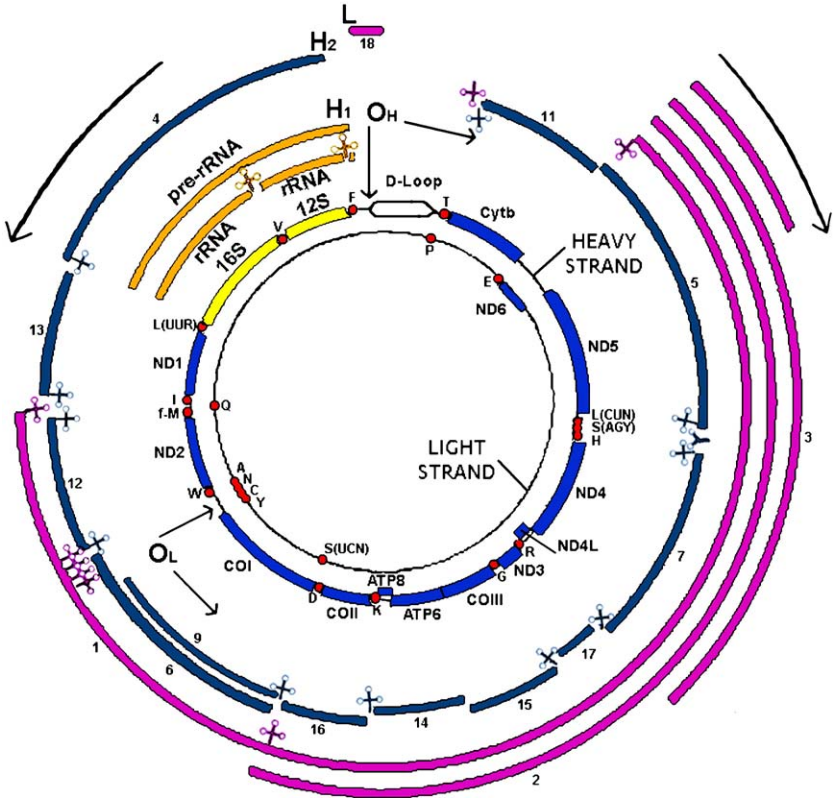

Fig. 1. Genetic and transcription map of human mtDNA. The two internal circles represent both mtDNA strands with the encoded genes in yellow (rRNAs), red dots (tRNAs) and blue (protein coding genes). External circles represent the RNAs transcribed from the heavy strand (in orange or in blue for the RNAs derived from the H_1 or H_2 transcription units) and light strand (in pink). ND1 to ND6 are subunits 1–6 of NADH dehydrogenase (complex I); cyt b, cytochrome *b* subunit of complex III; CO I, CO II and CO III, are subunits of cytochrome *c* oxidase (complex IV) and ATP6 and ATP8, subunits of ATP synthase (complex V). tRNA genes are indicated by the one letter code of the corresponding amino acid. O_H and O_L represent replication origins for the H- and L-strand, respectively, according to the classical model of replication. H_1, H_2 and L indicate initiation points for the three transcription units of the H- and L-strand, respectively. Arrows at the O_H and O_L, and in the outside part of the figure, indicate the direction of replication and transcription of both strands.

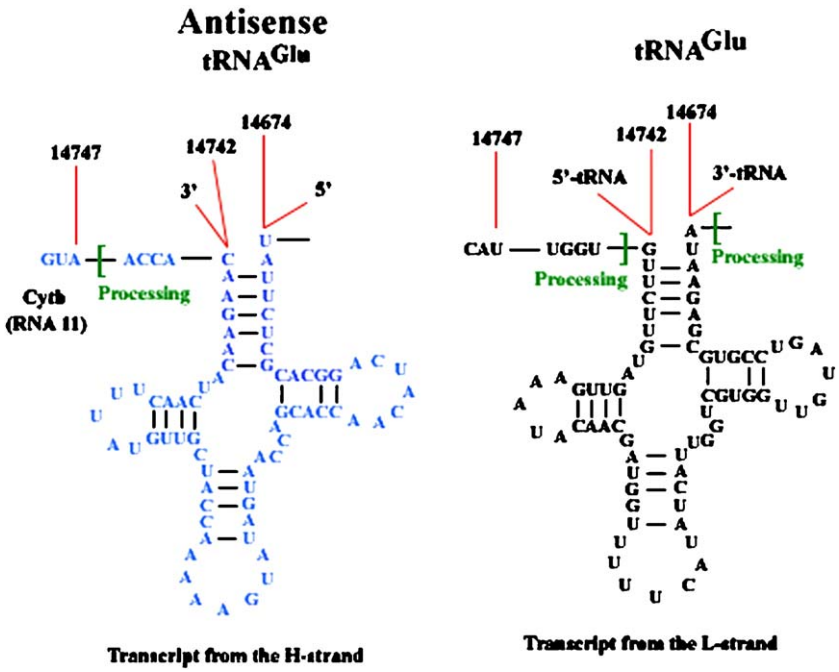

Fig. 2. Antisense-tRNA^{Glu} and tRNA^{Glu} sequences before processing. The first one is transcribed from the H-strand and the tRNA^{Glu} from the L-strand. Therefore, both will have complementary sequences and will be able to adopt cloverleaf structures, although the antisense sequence will not be a bone fide tRNA. The 3′-end of RNA 5 (mRNA for ND5), upstream of the antisense-tRNA^{Glu}, is not indicated in the figure since it has not been mapped.

CCA sequence acts as an antideterminant of the tRNase Z [22] avoiding its own removal from the tRNA. Additional activities are needed for tRNA modification [23,24].

As mentioned before, the mRNAs are polyadenylated with tails of ~55 A [25,26]. Interestingly, rRNAs transcribed from H_1 are not adenylated [27] or contain short tails (1–10 residues) of adenine at their 3′-end. However, the rRNAs transcribed from H_2 are polyadenylated [8] and they are considered end-products (possibly waste products), rather than precursors for ribosome formation [27]. Polyadenylation is performed by a mitochondrial poly(A)-polymerase [28], and in many cases it even produces the stop codons of some mRNAs [17]. The function of the 3′ poly(A) tails for RNA stability is still unknown. The addition of a 3′-end poly(A) extension in bacteria and organelles mediates rapid RNA decay [29]. However, in human mitochondria, a correlation between tail length and RNA stability has been observed [30]. Surprisingly, internal polyadenylation seems to be responsible for rapid RNA degradation in human mitochondria [15].

There are some cases in which there is not any tRNA separating the other RNAs of the polycistronic molecule. Such is the case of RNA 14 and 15. In this case, it is quite likely that another secondary structure can be recognized by the processing machinery. Mitochondrial mRNAs have considerable secondary structure in their 5′-terminal regions [31] and the first stem of the COIII mRNA contains an antideterminant CCA sequence in its 3′-region (see Fig. 9A in [31]). This fact might explain the processing only in the 5′-side of this secondary structure and then the production of the mature mRNA for COIII [32]. Also, RNA 5, 9 and 11 have an “antisense-tRNA” at either 3′- or 5′-end. Interestingly, RNAs 9 and 11 starts 9 and 4 nt away from their 5′ antisense-tRNAs while the RNAs located between true tRNAs are processed at the nucleotide closer to the tRNA [17,27,33]. Thus, it is possible that the antisense-tRNAs and the true tRNAs are not equally recognized by the processing machinery (Fig. 2). It is not known whether these antisense-tRNAs have any function or whether they are rapidly degraded. However, it has been shown that H-strand transcripts, both codogenic and non-codogenic, are more stable than L-transcripts [34].

The mitochondrial mRNAs start directly at the initiation codon or have an extremely short untranslated 5′-end (1–3 nt), lacking the typical features of other mRNAs such as 5′-end untranslated regions or the 5′-end ‘cap’ structure [33]. Mitochondrial mRNAs do not contain recognizable ribosome binding sites and it seems that they are translated from the first initiation codon at the 5′-end. In prokaryotic mRNAs the 5′-end leader sequence contains a stretch of nucleotides complementary to a region near the 3′-end of the rRNA. Eukaryotic mRNAs employ a 5′ terminal modification as a primary recognition signal. A particular protein mediates the binding of these mRNAs to the ribosome. Therefore, mammalian mitochondrial ribosomes must utilize different mechanisms to initiate protein synthesis. In this sense, mitochondrial mRNAs contain considerably secondary structure near their 5′ ends and initiation factors seem to be required for the proper recognition of these mRNAs as a prerequisite for initiation of protein synthesis [31].

Most mature poly(A)-RNAs correspond to a single gene but two of them, the mRNA for ND4 and ND4L subunits (RNA 7) and the mRNA for ATP6 and ATP8 subunits (RNA 14) contain two overlapping reading frames. The way by which the two

overlapping reading frames located in these RNAs are translated is still not completely understood. Two alternative mechanisms were proposed [35]. The first one involves translating each reading frame pair as a single polypeptide. This can happen if the ribosome slipped back one base during the translation of ATP8, which would result in premature termination at out-of-phase codons, followed by reinitiation at the ATP6 initiation codon. In the second one, the translation may require ribosome binding and initiation from a site in the first reading frame. The lack of a ribosome attachment site in the RNA 14, upstream of the ATP6 reading frame discard an independent entry site for ribosomes destined to translate the downstream reading frame [36]. However, other evidences support the ATP6 translation by initiation within the ATP8 gene [37].

3. Basic transcription machinery

MtDNA transcription requires an organelle-specific RNA polymerase (mtRPOL or POLRMT) [38] and at least three transcription factors: TFAM (or mtTFA) [39,40] and either TFB1M or TFB2M for initiation [41,42] and mTERF for termination of the transcription unit starting at H_1 [11,43,44] (Fig. 3).

The mtRPOL, encoded in the nuclear DNA (nDNA) (chromosome 19p13.3), is homologous to phage polymerases [38,45]. The cDNA for the human mtRPOL contains 3831 bp and codes for a protein of 1230 amino acids that, once it is internalized into mitochondria, eliminates 41 residues of the N-terminus giving rise to a mature protein of 1,189 amino acids. The amino-terminal region of the human mtRPOL contains two pentatricopeptide repeat motifs (PPR) that seem to be implicated in RNA processing in organelles [46]. In the absence of transcription factors, the h-mtRPOL is unable to recognize the mitochondrial promoters and shows little and only non-specific activity [39,47].

TFAM was the first mitochondrial transcription factor identified. The 246 amino acids of the human pre-protein are encoded on chromosome 10q21 of the nDNA, that results in a mature factor of 202 residues after import and elimination of the 42 N-terminus amino acids. TFAM contains two HMG-boxes separated by a linker region and a basic C-terminal tail, each HMG-box has DNA-binding activity [40,48]. The C-terminal tail is required for promoter-specific transcription. Binding sites for TFAM are found upstream of the two more active transcription initiation points in the control region of the mtDNA (H_1 and L), but both the binding and transcription stimulation activities are higher for the L promoter (LSP) than for the H_1 promoter (HSP1) [40,49,50]. TFAM is able to wrap, bend and unwind DNA in vitro with a low degree of sequence specificity [48,51]. Since this protein has also non-specific DNA-binding activity, it was suggested that the main function of this factor is maintenance of mtDNA. This is underscored by the role it plays in transcription synthesizing RNA primers from the L-promotor in order to initiate replication [52,53]. However, according to the new model of mtDNA replication [12,13], this role would be less important, since replication would not need initiation of transcription from the L-promotor. On the other hand, TFAM knockout mice produced by Larsson and co-workers did not allowed discrimination between transcription regulation and mtDNA maintenance [54], however, increasing the intramitochondrial TFAM levels, either *in organello* or in vivo, is sufficient to stimulate mtDNA transcription and probably to initiate the synthesis of replication intermediates, but not sufficient for successful replication of full-length mtDNA copies [55,56].

There are two additional mitochondrial transcription factors: TFB1M and TFB2M [41,42]. The human TFB1M (h-mtTFB1) and TFB2M (h-mtTFB2) are encoded in seven and eight exons located on chromosomes 6q25.1–q25.3 and 1q44 of the nDNA, respectively. TFB2M shows at least two orders of magnitude more activity in stimulating specific transcription than TFB1M. Both factors seem to directly interact with the RNA polymerase forming a heterodimer, and, one or the other, in addition to TFAM, are required for the accurate initiation in vitro on both H_1 and L promoters, although they seem not to be required for transcription elongation [41]. Thus, the minimal machinery required for in vitro transcription initiation would include mtRPOL, TFAM, and either TFB1M or TFB2M. It has also been shown that TFB1M has an rRNA dimethylase activity [57]. Both, methylation and transcription activation activities may be independent [58].

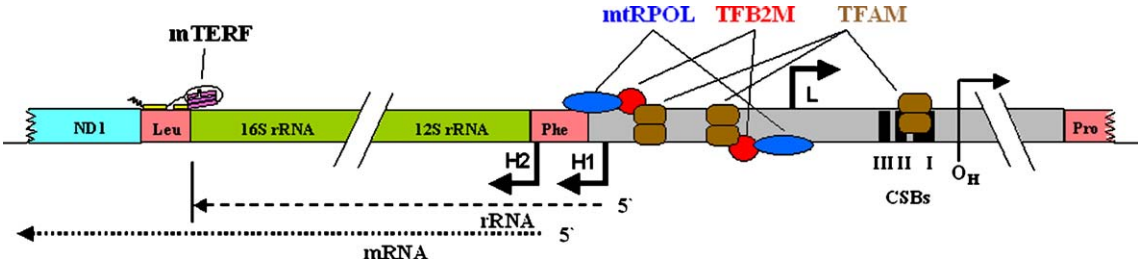

Fig. 3. Schematic representation of the human mitochondrial control and transcription termination regions, showing the main elements and factors involved in transcription. The non-coding regulatory region, rRNA, ND1 and tRNA sequences are shown in grey, green, blue and pink, respectively. Initiation at H_1 site transcribe the rDNA region ending at the 3′-end of the 16 S rRNA gene. This termination requires the presence of the transcription factor, mTERF, that binds to a sequence inside the $tRNA^{Leu}$ gene located just downstream of the rDNA area. Initiation at H_2 transcribes the whole H-strand. Transcription starting at L-strand promoter (L) originates a polycistronic RNA from which derives the RNA primer for initiation of H-strand replication. Transcription factor TFAM binds to enhancer elements located upstream of the transcription initiation points H_1 and L, and to other sequences such as the conserved sequence blocks (CSB). A heterodimer formed by mtRPOL and TFB2M would deliver the enzyme to the promoter region occupied by TFAM.

It is quite likely that the basic machinery for mtDNA transcription initiation requires, first, the bending and wrapping properties of TFAM and, then, the binding of TFB1M or TFB2M to RNA polymerase that would deliver the enzyme to the promoter region occupied by TFAM [59].

Transcription termination at the end of the transcription unit starting at H_1 and finishing at the 3′-end of 16S rRNA gene requires a protein, termed mTERF [11]. The human gene for this factor contains two exons and is located on chromosome 7q21–q22. The mature protein, produced once the pre-protein has been imported into mitochondria, has a molecular weight of approximately 39 kDa. This factor contains three potential leucine zippers and two separated basic binding domains in its sequence, binds DNA as a monomer and is able to bend DNA [44,60]. mTERF exists in mitochondria in two forms, an active monomer and an inactive homotrimer. A rearrangement in the interaction between leucine zippers would be responsible for the control of the activity of mTERF. Intramolecular interactions would maintain the tertiary structure of the active monomer, whereas the inactive form would depend on intermolecular interactions [61]. Recent work with rat mTERF has shown that this factor needs to be phosphorylated in order to be functional [62], although this fact is controversial, since the human factor seems to function independently of this modification [63].

4. Regulation of mitochondrial transcription

Variations in physiological situations and cell types require different energetic demands. Even more, inside the same cell, the energy requirements may be different depending on the existence of particular cell domains, and diverse biogenetic programs can be observed according to the specific situations [64]. Thus, during situations of active cell growth and division, mitochondrial proliferation is needed, while mitochondrial maturation or differentiation would take place during cell differentiation. In addition, it has been described that there is a rapid mechanism for the adjustment of mitochondrial function in response to local changes of ATP demands and other extramitochondrial signals [65]. The mechanisms that regulate mitochondrial activity during various situations may be different and very complex since they can also overlap [66].

Changes in transcription activity and in the steady-state level of the different mitochondrial RNA types have been reported in diverse series of situations in mammals, for example in different stages of development [67,68], ageing [69–71], change in cellular energy demands [72–75] or hormonal status [65,76–81]. The molecular mechanisms involved in the regulation of mitochondrial transcription, responsible for these changes, are still poorly understood.

Transcription regulation can be operated either at the level of initiation, termination or both. In particular, the transcription initiation rate at the two alternative H-strand promoters would determine the synthesis ratio of rRNAs versus H-strand mRNAs, which is supposed to be important for protein synthesis capacity [8,82]. The differential transcription of the H-strand is regulated not only through initiation at different sites, but also at the level of 3′-end termination of the rRNA transcription unit. The transcription termination factor mTERF plays a fundamental role in attenuation of this process [11]. As mentioned before the activity of this factor appears to be regulated by phosphorylation. While the DNA-binding activity of mTERF is unaffected by the phosphorylation state, only the phosphorylated form of the protein would be involved in termination [62]. The two alternative sites at which H-strand transcription can be initiated are physically related. While the activity of mTERF promotes the termination of transcripts synthesized from the first transcription unit, initiated at H_1, the second initiation site is refractive to the activity of mTERF. It is therefore likely that some communication exists between the sites of transcription initiation and termination. Accordingly, a new DNA target sequence for mTERF binding has been identified in the promoter region, albeit with a slightly lower affinity than that for the termination mTERF recognition site [62,83]. The fact that binding sites for mTERF are present both upstream

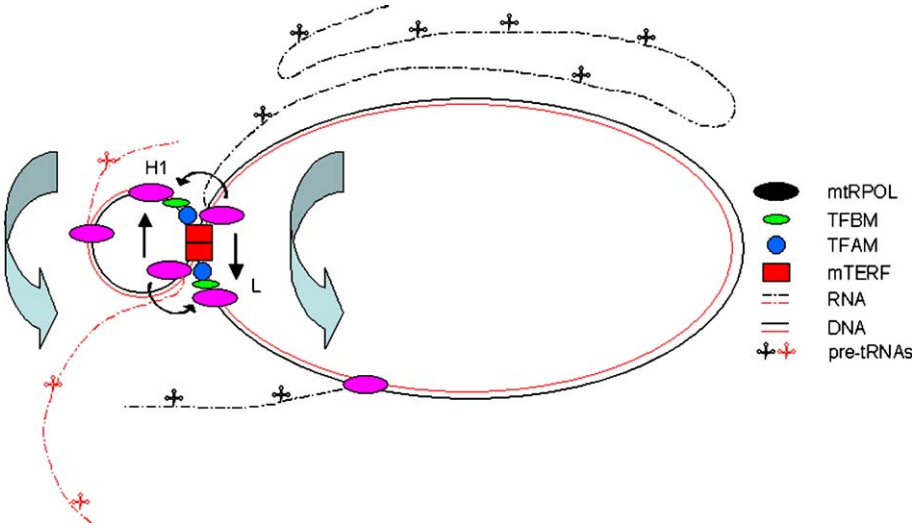

Fig. 4. Schematic representation of a proposed model for a double mitochondrial loop. According to this model, the rDNA and L-strand transcription would be regulated in parallel by allowing a recycling of the mtRPOL between the promoters.

and downstream from the rRNA transcription unit has led to the proposal that transcriptional termination and initiation are functional linked [84] (Fig. 4). In this model the mt-rRNA transcription unit would form a loop that facilitate the direct transfer of the mitochondrial RNA polymerase molecules that have terminated the transcription of nascent pre-rRNA chains to the transcription initiation site H_1. In this way, the transition to the pool of free mtRPOL is bypassed and the efficient recycling of this enzyme is ensued. Strong evidence for this model has recently been obtained indicating that the mechanism underlying this regulation of human mitochondrial rDNA transcription by mTERF, involving the simultaneous interaction of this factor with the initiation and termination sites and the resulting looping-out of the rDNA [10].

The binding of mTERF to these two mtDNA sequences will create two different loops, one for the rDNA transcription unit and another one for the L-strand transcription unit (Fig. 4). The mTERF binding site to the promoter region is located between nucleotides 367 and 523, close to the initiation site for the L-strand [83]. Moreover, the termination-promoting activity of mTERF is bidirectional and, since no additional genes are encoded in this strand downstream of the mTERF binding site, this factor could also act on the L-strand transcription termination [60]. Interestingly, the rate of synthesis of rRNA is more than 15-fold higher relative to mRNA [7,8] and the L-strand is transcribed three times more frequently than the H-strand [85,86]. Thus, this difference of 5 times between the rRNA and the L-strand transcripts synthesis might be due to the fact that the L-strand transcription unit is 5 times larger. Moreover, there is a parallel regulation for the expression of both transcription units (H_1 and L) and different from the whole mtDNA transcription starting at H_2 [87]. Therefore, this mitochondrial loop could accelerate the transcription of both transcription units in a regulated way via repositioning the transcription machinery without diffusion from template DNA (Fig. 4).

Thus, a fairly complete picture is now available of mtDNA transcription in human cells. By contrast, very little is known about the regulation of these processes. For example, as we have seen, the H-strand expression responds to different stimuli than the rRNA and L-strand [87]. The mtDNA is a very compact and economic genome but this does not seem to be true for the transcription mechanisms since the L-strand is mostly non-codogenic. Which is the evolutionary reason to maintain transcription from both strands? ND6 is the only mitochondrial polypeptide encoded by the L-strand and seems to be necessary for complex I assembly [88]. Thus, a differential regulation of ND6 expression may be a mechanism to control complex I assembly and the entry of electrons to the respiratory chain. Alternatively, the polyadenylated RNA 1 transcript encompasses most of the L-strand and therefore it could act as an interfering RNA for H-strand transcripts. Then, a differential regulation of both H- and L-transcription units could avoid an interaction between them.

Screening the mitochondrial genome for binding sites for known nuclear transcription factors revealed the presence of some sequences with similarity to consensus sequences for twenty-one transcription factors, known to modulate nuclear genes [89]. Interestingly, the analysis of more than 2864 mitochondrial sequences and the information collected from mitomap.org shows a high conservation in 14 of them, thus supporting a potential role as regulatory elements. For example, similar sequences have been found for steroid and thyroid hormone binding nuclear receptors, which could act as hormone response elements (HREs). These hormones are major regulators of metabolic processes and their receptors have been detected in mitochondria [89–95]. Moreover, gel-retardation experiments showed that they were able to bind these HREs and that they can confer hormone-dependent activation of reporter genes in transfection experiments [94]. All these evidences suggest these hormone binding transcription factors could play a regulatory role in mitochondrial functions.

Surprisingly, one of the potential HREs for glucocorticoids is located between mitochondrial DNA positions 3228–3242 [94], overlapping with the sequence (3229–3256) protected by the mTERF factor [11]. Therefore, it is possible that glucocorticoids can regulate the rDNA loop formation [94].

Thyroid hormones are key players in energy metabolism. Hypothyroidism induces a decrease in the steady-state concentration of all mtRNAs, which is most pronounced for mRNAs, thus also changing the mRNA/rRNA ratio [76–78,80]. By using the *in organello* approach, a direct effect of thyroids hormones on the mRNA/rRNA ratio and on the protein–mtDNA interactions, probably through changes in H-strand promoter selection, was found. This effect was achieved with a low concentration of hormones and was saturable [80].

The processing of the polycistrons to yield the mature RNAs is another potential regulatory point, although available data suggest that this process is very rapid and not limiting. More important, it appears that changes in RNA stability determine, along with the transcriptional rate, the final steady-state level for each RNA species [96]. Because the mechanism of transcription for the mtDNA H-strand ensures the same rate of synthesis of each mRNA transcript, the differentiated steady-state levels of the individual transcripts must be achieved by the efficiency of their excision from the long precursor together with degradation rate [97].

5. Mitochondrial RNA metabolism and disease

Theoretically, mutations in mtDNA sequences or in nuclear genes involved in regulation of mitochondrial RNA metabolism might have phenotypic consequences. In this context, a high proportion of the somatic T414G transversion was found in the fibroblast mtDNA molecules of aged but was absent in younger individuals [98–100]. This transversion was also present in a high proportion of brains, but only in a small percentage of molecules in patients with Alzheimer disease (AD), whereas it was completely absent in all controls [101]. This mutation is located in the middle of the promoter core region for L-strand transcription, at a position adjacent to a segment with high affinity for TFAM.

Mutations in mtDNA elements related to the initiation of transcription are not the only ones possibly associated with particular phenotypes. The $tRNA^{Leu(UUR)}$ is a mutational hot spot. Eight pathological mutations have been described in the

$tRNA^{Leu(UUR)}$ gene region that is probably protected by mTERF binding [11], suggesting that other functions besides those related to the normal tRNA functions may be affected by these mutations. Thus, the A3243G transition located in the termination site of the H_1 transcription unit, substantially decreases mTERF binding [102,103] and results in severe impairment of transcription termination at the end of the 16 S rRNA gene [104].

As previously mentioned, tRNAs are considered processing signals and many tRNA mutations have been shown to affect RNA metabolism. For example, the MELAS A3243G mutation provokes a small but consistent increase in the steady-state levels of a new RNA (RNA 19), corresponding to 16S rRNA +$tRNA^{Leu(UUR)}$+ND1. This result suggests a defect on the $tRNA^{Leu(UUR)}$ processing [105]. Other pathologic $tRNA^{Leu(UUR)}$ mutations, such as 3256T, 3271C and 3302G, also increase the levels of RNA 19 [106–108]. Mutations in other mitochondrial tRNA genes also affect quantitatively the RNA processing efficiency. Thus, mutations at positions 7510G, 7511G and 7512G in the $tRNA^{Ser(UCN)}$ decrease this efficiency [109], and indeed it was shown that the 7512G mutations leads to the accumulation of a precursor transcript [110]. In addition, mutations at positions 7442Ci, 7443C, 7444T and 7445C and 7445G, adjacent to the 3′-end of the $tRNA^{Ser(UCN)}$ gene, have not only quantitative but also qualitative effects on the processing of this tRNA. The processing is produced by an aberrant cleavage one nucleotide in the 3′-direction from the normal processing site [109,111], however, this qualitative effect was not found in another study for 7445C [112].

Other mtDNA mutations affect RNA stability. A two residues microdeletion (μΔ9205) removing the ATP6 termination codon was found in a patient with mitochondrial disease. The polyadenylation profile of the RNA 14 revealed substantial abnormalities. Most of this mutated RNA terminated with short poly (A) extensions and the steady-state levels of the RNA 14 was markedly decreased. The enhanced turnover of RNA 14 was due to a translation-dependent deadenylation decay mechanism [113].

Besides mitochondrial mutations, trans-acting elements can also be related to mitochondrial diseases. The TFB1M and TFB2M initiation factors are closely related to rRNA methyltransferases [42]. Thus, these transcription factors can have other roles in mitochondrial gene expression. In fact, TFB1M expression in *E. coli* lacking the KsgA rRNA methyltransferase restores dimethylation of two adjacent residues in an stem–loop in the bacterial 16 S rRNA. Moreover, the resistance to the antibiotic kasugamycin is reversed by expression of TFB1M. The target adenine residues of this stem–loop are evolutionary conserved in the mitochondrial 12 S rRNA [57]. This mitochondrial stem–loop is close to the 3′-end of the 12 S rRNA and 28 nucleotides downstream of a pathogenic mtDNA mutation (A1555G) that predisposes individuals to deafness. Interestingly, there is evidence that the nuclear genetic background influences the phenotypic expression of this mutation. A polymorphism on chromosome 6 near the TFB1M gene, but located out of the coding region, was implicated as a nuclear modifying locus of the 1555G mutation in several pedigrees of mitochondrial deafness [114]. Several clues imply that the altered TFB1M expression affects the penetrance of the 1555G mutation by modifying the rRNA methylation. Thus, the methylation status of the analogous bacterial 16 S stem–loop determines sensitivity to aminoglycosides, the same antibiotics inducing deafness in patients with the mitochondrial mutation. The bacterial stem–loop structure is altered by the dimethylation of the conserved adenines what may suggests that the mitochondrial homologue structure could be also affected [115].

We have already seen how mitochondrial mutations in tRNA genes have been associated to different pathologic phenotypes. Recently, a candidate gene for prostate cancer susceptibility, ELAC2, has been identified. Several variants in this gene are significantly associated with the occurrence of this disease. Surprisingly, the prostate cancer susceptibility gene encodes the tRNase Z, the enzyme required for the 3′-end processing of the mitochondrial tRNAs [116].

The h-mtRPOL has an amino terminal extension with two pentatricopeptide (PPR) domains, a motif found in a family of proteins involved in RNA trafficking and metabolism. The leucine-rich PPR-motif containing (LRPPRC) protein, another member of this PPR family, has recently been associated with the French-Canadian type of the mitochondrial Leigh syndrome (LSFC) [117]. This protein is located in the mitochondria and appears to bind mt-mRNAs. It has been postulated that LRPPRC could function to facilitate the processing, trafficking and/or translation of human mtRNAs. The h-mtRPOL and LRPPRC may work together coupling post-transcriptional events directly to the transcription machinery, through the amino terminal domain of the mtRPOL.

As we have previously seen, the processing of nascent mitochondrial RNA chains seems to proceed concomitantly with the transcription process and polyadenylation would occur during or immediately after this cleavage [118]. Therefore, transcription, processing and maturation activities may form a ribonucleo-protein complex acting together. Then, the association between the LSFC disease and mutations in LRPPRC probably defines a novel pathogenic mechanism in humans involving defects in coupling during the mitochondrial gene expression pathway [119].

6. Future prospects

An enormous effort done in Attardi's and others laboratories unraveled the basis of mitochondrial transcription. However, some of the earlier findings remain still unexplained and many of them may be important in mitochondrial pathology. It is not rare to find patients with many evidences pointing to mtDNA disease without mtDNA "pathologic" mutations. As we have seen, tRNA [111] or non-coding mutations [112] can affect the RNA processing. For example, if the proposed secondary structure for RNA 15 is true, it would be possible that mutations in the first stem of this RNA could affect its processing. Interestingly, only two polymorphisms have been described in this stem in 2864 mitochondrial sequences and the mitomap database, one of them rebuilding a Watson–Crick base pair and the other one (nt 9212, a synonymous mutation) in a patient with Alzheimer disease. Moreover, synonymous changes affecting the stability of the mRNAs [120] or mitochondrial HREs could also have phenotypic effects by altering the transcription factor

binding, as already shown for nuclear HREs [121]. Therefore, we need to be more cautious when ruling out these changes as possible pathologic mutations [122].

Future work in order to know the finest details of the mitochondrial transcription process and machinery is necessary for studying other possible implications of RNA synthesis and processing with mitochondrial diseases and may allow to define therapeutic targets for their treatment.

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References

[1] S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. de-Bruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, H.P. Schreier, A.J.H. Smith, R. Stader, I.G. Young, Sequence and organization of the human mitochondrial genome, Nature 290 (1981) 427–465.

[2] R.M. Andrews, I. Kubacka, P.F. Chinnery, R.N. Lightowlers, D.M. Turnbull, N. Howell, Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (1999) 147.

[3] A. Schneider, D. Ebert, Covariation of mitochondrial genome size with gene lengths: evidence for gene length reduction during mitochondrial evolution, J. Mol. Evol. 59 (2004) 90–96.

[4] T.W. O'Brien, Evolution of a protein-rich mitochondrial ribosome: implications for human genetic disease, Gene 286 (2002) 73–79.

[5] E. Sbisa, F. Tanzariello, A. Reyes, G. Pesole, C. Saccone, Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications, Gene 205 (1997) 125–140.

[6] J.A. Drake, C. Bird, J. Nemesh, D.J. Thomas, C. Newton-Cheh, A. Reymond, L. Excoffier, H. Attar, S.E. Antonarakis, E.T. Dermitzakis, J.N. Hirschhorn, Conserved noncoding sequences are selectively constrained and not mutation cold spots, Nat. Genet. 38 (2006) 223–227.

[7] J. Montoya, T. Christianson, D. Levens, M. Rabinowitz, G. Attardi, Identification of initiation sites for heavy strand and light strand transcription in human mitochondrial DNA, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 7195–7199.

[8] J. Montoya, G.L. Gaines, G. Attardi, The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units, Cell 34 (1983) 151–159.

[9] D.F. Bogenhagen, E.F. Applegate, B.K. Yoza, Identification of a promoter for transcription of the heavy strand of human mtDNA: in vitro transcription and deletion mutagenesis, Cell 36 (1984) 1105–1113.

[10] M. Martin, J. Cho, A.J. Cesare, J.D. Griffith, G. Attardi, Termination factor-Mediated DNA loop between termination and initiation sites drives mitochondrial rRNA synthesis, Cell 123 (2005) 1227–1240.

[11] B. Kruse, N. Narasimhan, G. Attardi, Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination, Cell 58 (1989) 391–397.

[12] M. Bowmaker, M.Y. Yang, T. Yasukawa, A. Reyes, H.T. Jacobs, J.A. Huberman, I.J. Holt, Mammalian mitochondrial DNA replicates bidirectionally from an initiation zone, J. Biol. Chem. 278 (2003) 50961–50969.

[13] T. Yasukawa, M.Y. Yang, H.T. Jacobs, I.J. Holt, A bidirectional origin of replication maps to the major noncoding region of human mitochondrial DNA, Mol. Cell 18 (2005) 651–662.

[14] G. Attardi, G. Schatz, Biogenesis of mitochondria, Annu. Rev. Cell Biol. 4 (1988) 289–333.

[15] S. Slomovic, D. Laufer, D. Geiger, G. Schuster, Polyadenylation and degradation of human mitochondrial RNA: the prokaryotic past leaves its mark, Mol. Cell. Biol. 25 (2005) 6427–6435.

[16] E. Sbisà, M. Nardelli, F. Tanazariello, A. Tullo, C. Saccone, The complete and symmetric transcription of the main non coding region of rat mitochondrial genome: in vivo mapping of heavy and light transcripts, Curr. Genet. 17 (1990) 247–253.

[17] D. Ojala, J. Montoya, G. Attardi, tRNA punctuation model of RNA processing in human mitochondria, Nature 290 (1981) 470–474.

[18] W. Rossmanith, A. Tullo, T. Potuschak, R. Karwan, E. Sbisa, Human mitochondrial tRNA processing, J. Biol. Chem. 270 (1995) 12885–12891.

[19] C.J. Doersen, C. Guerrier-Takada, S. Altman, G. Attardi, Characterization of an RNase P activity from HeLa cell mitochondria: comparison with the cytosol RNase P activity, J. Biol. Chem. 260 (1985) 5942–5949.

[20] R.S. Puranam, G. Attardi, The RNase P associated with HeLa cell mitochondria contains an essential RNA component identical in sequence to that of the nuclear RNase P, Mol. Cell. Biol. 21 (2001) 548–561.

[21] L. Levinger, O. Jacobs, M. James, In vitro 3′-end endonucleolytic processing defect in a human mitochondrial tRNA(Ser(UCN)) precursor with the U7445C substitution, which causes non-syndromic deafness, Nucleic Acids Res. 29 (2001) 4334–4340.

[22] L. Levinger, M. Morl, C. Florentz, Mitochondrial tRNA 3′ end metabolism and human disease, Nucleic Acids Res. 32 (2004) 5430–5441.

[23] M. Helm, H. Brule, D. Friede, R. Giege, D. Putz, C. Florentz, Search for characteristic structural features of mammalian mitochondrial tRNAs, RNA 6 (2000) 1356–1379.

[24] T. Nagaike, T. Suzuki, Y. Tomari, C. TakemotoHori, F. Negayama, K. Watanabe, T. Ueda, Identification and characterization of mammalian mitochondrial tRNA nucleotidyltransferases, J. Biol. Chem. 276 (2001) 40041–40049.

[25] M. Hirsch, S. Penman, Post-transcriptional addition of polyadenylic acid to mitochondrial RNA by a cordycepin-insensitive process, J. Mol. Biol. 83 (1974) 131–142.

[26] D. Ojala, G. Attardi, Identification and partial characterization of multiple discrete polyadenylic acid containing RNA components coded for by HeLa cell mitochondrial DNA, J. Mol. Biol. 88 (1974) 205–219.

[27] D.T. Dubin, J. Montoya, K.D. Timko, G. Attardi, Sequence analysis and precise mapping of the 3′-ends of HeLa cell mitochondrial ribosomal RNAs, J. Mol. Biol. 157 (1982) 1–19.

[28] R. Tomecki, A. Dmochowska, K. Gewartowski, A. Dziembowski, P.P. Stepien, Identification of a novel human nuclear-encoded mitochondrial poly(A) polymerase, Nucleic Acids Res. 32 (2004) 6001–6014.

[29] D. Gagliardi, P.P. Stepien, R.J. Temperley, R.N. Lightowlers, Z.M. Chrzanowska-Lightowlers, Messenger RNA stability in mitochondria: different means to an end, Trends Genet. 20 (2004) 260–267.

[30] T. Nagaike, T. Suzuki, T. Katoh, T. Ueda, Human mitochondrial mRNAs are stabilized with polyadenylation regulated by mitochondria-specific poly(A) polymerase and polynucleotide phosphorylase, J. Biol. Chem. 280 (2005) 19721–19727.

[31] N.D. Denslow, G.S. Michaels, J. Montoya, G. Attardi, T.W. O'Brien, Mechanism of mRNA binding to bovine mitochondrial ribosomes, J. Biol. Chem. 264 (1989) 8328–8338.

[32] G. Attardi, RNA synthesis and processing in mitochondria, in: D. Apirion (Ed.), Processing of RNA, CRC Press, Boca Raton, Florida, 1983, pp. 227–280.

[33] J. Montoya, D. Ojala, G. Attardi, Distinctive features of the 5′-terminal sequences of the human mitochondrial mRNAs, Nature 290 (1981) 465–470.

[34] A. Tullo, F. Tanzariello, A.M. Derchia, M. Nardelli, P.A. Papeo, E. Sbisa, C. Saccone, Transcription of rat mitochondrial NADH-dehydrogenase subunits presence of antisense and precursor RNA species, FEBS Lett. 354 (1994) 30–36.

[35] S. Anderson, M.H.L. de-Brujin, A.R. Coulson, I.C. Eperon, F. Sanger, I. Young, Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian genome, J. Mol. Biol. 156 (1982) 683–717.

[36] A. Chomyn, P. Mariottini, N. Gonzalez-Cadavid, G. Attardi, D.D. Strand, D. Trovato, M. Riley, R.F. Doolittle, Identification of the polypeptides encoded in the ATPase 6 gene and in the unassigned reading frames 1 and 3 of human mitochondrial DNA, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 5535–5539.
[37] I.M. Fearnley, J.E. Walker, Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase, EMBO J. 5 (1986) 2003–2008.
[38] V. Tiranti, A. Savoia, F. Forti, M.F. DApolito, M. Centra, M. Racchi, M. Zeviani, Identification of the gene encoding the human mitochondrial RNA polymerase (h-mtRPOL) by cyberscreening of the expressed sequence tags database, Hum. Mol. Genet. 6 (1997) 615–625.
[39] R.P. Fisher, D.A. Clayton, A transcription factor required for promoter recognition by human mitochondrial RNA polymerase, J. Biol. Chem. 260 (1985) 11330–11338.
[40] R.P. Fisher, D.A. Clayton, Purification and characterization of human mitochondrial transcription factor 1, Mol. Cell. Biol. 8 (1988) 3496–3509.
[41] M. Falkenberg, M. Gaspari, A. Rantanen, A. Trifunovic, N.G. Larsson, C.M. Gustafsson, Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA, Nat. Genet. 31 (2002) 289–294.
[42] V. McCulloch, B.L. Seidel-Rogol, G.S. Shadel, A human mitochondrial transcription factor is related to RNA adenine methyltransferases and binds s-adenosylmethionine, Mol. Cell. Biol. 22 (2002) 1116–1125.
[43] A. Daga, V. Micol, D. Hess, R. Aebersold, G. Attardi, Molecular characterization of the transcription termination factor from human mitochondria, J. Biol. Chem. 268 (1993) 8123–8130.
[44] P. Fernandez-Silva, F. Martinez-Azorin, V. Micol, G. Attardi, The human mitochondrial transcription termination factor (mTERF) is a multizipper protein but binds to DNA as a monomer, with evidence pointing to intramolecular leucine zipper interactions, EMBO J. 16 (1997) 1066–1079.
[45] T.E. Shutt, M.W. Gray, Bacteriophage origins of mitochondrial replication and transcription proteins, Trends Genet. 22 (2006) 90–95.
[46] S. Mili, S. Pinol-Roma, LRP130, a pentatricopeptide motif protein with a noncanonical RNA-binding domain, is bound in vivo to mitochondrial and nuclear RNAs, Mol. Cell. Biol. 23 (2003) 4972–4982.
[47] A. Prieto-Martín, J. Montoya, F. Martínez-Azorín, A study on the human mitochondrial RNA polymerase activity points to existence of a transcription factor B-like protein, FEBS Lett. 503 (2001) 51–55.
[48] M.A. Parisi, D.A. Clayton, Similarity of human mitochondrial transcription factor 1 to high mobility group proteins, Science 252 (1991) 965–969.
[49] R.P. Fisher, J.N. Topper, D.A. Clayton, Promoter Selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements, Cell 50 (1987) 247–258.
[50] S.C. Ghivizzani, C.S. Madsen, M.R. Nelen, C.V. Ammini, W.W. Hauswirth, In organello footprint analysis of human mitochondrial DNA: human mitochondrial transcription factor A interactions at the origin of replication, Mol. Cell. Biol. 14 (1994) 7717–7730.
[51] R.P. Fisher, T. Lisowsky, M.A. Parisi, D.A. Clayton, DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein, J. Biol. Chem. 267 (1992) 3358–3367.
[52] J. Montoya, A. PerezMartos, H.L. Garstka, R.J. Wiesner, Regulation of mitochondrial transcription by mitochondrial transcription factor A, Mol. Cell. Biochem. 174 (1997) 227–230.
[53] S. Gensler, K. Weber, W.E. Schmitt, A. PerezMartos, J.A. Enriquez, J. Montoya, R.J. Wiesner, Mechanism of mammalian mitochondrial DNA replication: import of mitochondrial transcription factor A into isolated mitochondria stimulates 7S DNA synthesis, Nucleic Acids Res. 29 (2001) 3657–3663.
[54] N.G. Larsson, J.M. Wang, H. Wilhelmsson, A. Oldfors, P. Rustin, M. Lewandoski, G.S. Barsh, D.A. Clayton, Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice, Nat. Genet. 18 (1998) 231–236.
[55] H.L. Garstka, W.E. Schmitt, J. Schultz, B. Sogl, B. Silakowski, A. Perez-Martos, J. Montoya, R.J. Wiesner, Import of mitochondrial transcription factor A (TFAM) into rat liver mitochondria stimulates transcription of mitochondrial DNA, Nucleic Acids Res. 31 (2003) 5039–5047.
[56] K. Maniura-Weber, S. Goffart, H.L. Garstka, J. Montoya, R.J. Wiesner, Transient overexpression of mitochondrial transcription factor A (TFAM) is sufficient to stimulate mitochondrial DNA transcription, but not sufficient to increase mtDNA copy number in cultured cells, Nucleic Acids Res. 32 (2004) 6015–6027.
[57] B.L. SeidelRogol, V. McCulloch, G.S. Shadel, Human mitochondrial transcription factor B1 methylates ribosomal RNA at a conserved stem–loop, Nat. Genet. 33 (2003) 23–24.
[58] V. McCulloch, G.S. Shadel, Human mitochondrial transcription factor B1 interacts with the C-terminal activation region of h-mtTFA and stimulates transcription independently of its RNA methyltransferase activity, Mol. Cell. Biol. 23 (2003) 5816–5824.
[59] E. Fernández-Vizarra, P. Fernandez-Silva, M.J. López-Pérez, J. Montoya, Advances in mammalian mitochondrial DNA transcription, in: F. Villarroya (Ed.), New Frontiers in Mitochondrial Biogenesis and Disease, Research Signpost, Kerala, India, 2005, pp. 1–19.
[60] J. Shang, D.A. Clayton, Human mitochondrial transcription termination exhibits RNA polymerase independence and biased bipolarity in vitro, J. Biol. Chem. 269 (1994) 29112–29120.
[61] J. Asin-Cayuela, M. Helm, G. Attardi, A monomer-to-trimer transition of the human mitochondrial transcription termination factor (mTERF) is associated with a loss of in vitro activity, J. Biol. Chem. 279 (2004) 15670–15677.
[62] A. Prieto-Martin, J. Montoya, F. Martinez-Azorin, Phosphorylation of rat mitochondrial transcription termination factor (mTERF) is required for transcription termination but not for binding to DNA, Nucleic Acids Res. 32 (2004) 2059–2068.
[63] J. Asin-Cayuela, T. Schwend, G. Farge, C.M. Gustafsson, The human mitochondrial transcription termination factor mTERF is fully active in vitro in the non-phosphorylated form, J. Biol. Chem. 280 (2005) 25499–25505.
[64] C.C. Overly, H.I. Rieff, P.J. Hollenbeck, Organelle motility and metabolism in axons vs. dendrites of cultured hippocampal neurons, J. Cell Sci. 109 (1996) 971–980.
[65] J.A. Enriquez, P. FernandezSilva, J. Montoya, Autonomous regulation in mammalian mitochondrial DNA transcription, Biol. Chem. 380 (1999) 737–747.
[66] P. Fernandez-Silva, J.A. Enriquez, J. Montoya, Replication and transcription of mammalian mitochondrial DNA, Exp. Physiol. 88 (2003) 41–56.
[67] P. Cantatore, P.L. Polosa, F. Fracasso, Z. Flagella, M.N. Gadaleta, Quantitation of mitochondrial RNA species during rat liver development: the concentration of cytochrome oxidase subunit I (CoI) mRNA increase at birth, Cell Differ. 19 (1986) 125–132.
[68] P. Loguercio-Polosa, G. Attardi, Distinctive pattern and transcriptional control of mitochondrial protein synthesis in rat brain synaptic endings, J. Biol. Chem. 266 (1991) 10011–10017.
[69] M.N. Gadaleta, V. Petruzella, M. Renis, F. Fracasso, P. Cantatore, Reduced transcription of mitochondrial DNA in the senescent rat. Tissue dependence and effect of L-carnitine, Eur. J. Biochem. 187 (1990) 501–506.
[70] P. Fernandez-Silva, V. Petruzzella, F. Fracasso, M.N. Gadaleta, P. Cantatore, Reduced synthesis of mtRNA in isolated mitochondria of senescent rat brain, Biochem. Biophys. Res. Commun. 176 (1991) 645–653.
[71] A.L. Andreu, M.A. Arbos, A. PerezMartos, M.J. LopezPerez, J. Asin, N. Lopez, J. Montoya, S. Schwartz, Reduced mitochondrial DNA transcription in senescent rat heart, Biochem. Biophys. Res. Commun. 252 (1998) 577–581.
[72] R.S. Williams, Mitochondrial gene expression in mammalian striated muscle. Evidence that variation in gene dosage is the major regulatory event, J. Biol. Chem. 261 (1986) 12390–12394.
[73] D.A. Hood, R. Zak, D. Pette, Chronic stimulation of rat skeletal muscle induces coordinate increases in mitochondrial and nuclear mRNAs of cytochrome-c-oxidase subunits, Eur. J. Biochem. 179 (1989) 275–280.
[74] R.F. Hevner, M.T. Wong-Riley, Mitochondrial and nuclear gene expression for cytochrome oxidase subunits are disproportionately regulated by functional activity in neurons, J. Neurosci. 13 (1993) 1805–1819.
[75] I. Martin, O. Vinas, T. Mampel, R. Iglesias, F. Villarroya, Effects of cold environment on mitochondrial genome expression in the rat-evidence for

a tissue-specific increase in the liver, independent of changes in mitochondrial gene abundance, Biochem. J. 296 (1993) 231–234.
[76] A. Mutvei, S. Kuzela, B.D. Nelson, Control of mitochondrial transcription by thyroid hormone, Eur. J. Biochem. 180 (1989) 235–240.
[77] M.N. Gadaleta, V. Petruzzella, F. Fracasso, P. Fernández-Sílva, P. Cantatore, Acetyl-L-carnitine increases cytochrome oxidase subunit I mRNA content in hypothyroid rat liver, FEBS Lett. 277 (1990) 191–193.
[78] C.M. vanItallie, Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear-encoded subunit cytocrome c oxidase, Endocrinology 127 (1990) 55–62.
[79] R.J. Wiesner, T.T. Kurowski, R. Zak, Regulation by thyroid hormones of nuclear and mitochondrial genes encoding subunits of cytochrome-c oxidase in rat liver and skeletal muscle, Mol. Endocrinol. 6 (1992) 1458–1467.
[80] J.A. Enriquez, P. Fernandez-Silva, N. Garrido-Perez, M.J. Lopez-Perez, A. Perez-Martos, J. Montoya, Direct regulation of mitochondrial RNA synthesis by thyroid hormone, Mol. Cell. Biol. 19 (1999) 657–670.
[81] K. Weber, P. Bruck, Z. Mikes, J.H. Kupper, M. Klingenspor, R.J. Wiesner, Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle, Endocrinology 143 (2002) 177–184.
[82] R.J. Wiesner, V. Aschenbrenner, J.C. Ruegg, R. Zak, Coordination of nuclear and mitochondrial gene expression during the development of cardiac hypertrophy in rats, Am. J. Physiol. 267 (1994) C229–C235.
[83] A. Prieto-Martín, J. Montoya, F. Martinez-Azorin, New DNA-binding activity of rat mitochondrial transcription termination factor (mTERF), J. Biochem. (Tokyo) 136 (2004) 825–830.
[84] F. Martinez-Azorin, The mitochondrial ribomotor hypothesis, IUBMB Life 57 (2005) 27–30.
[85] G. Attardi, C. Dotersen, G. Gaines, J. Montoya, C. Guerreir-Takada, S. Altman, New insights into the mechanism of RNA processing in human mitochondria, in: E. Quagliarello, E.C. Slater, F. Palmieri, C. Saccone, A.M. Kron (Eds.), Achievements and Perspectives in Mitochondrial Research, Elsevier Sciences, 1985, pp. 145–163.
[86] G. Attardi, Animal mitochondrial DNA: an extreme example of genetic economy, Int. Rev. Cyt. 93 (1985) 683–711.
[87] G. Gaines, C. Rosi, G. Attardi, Markedly different ATP requirements for rRNA synthesis and mtDNA light strand transcription versus mRNA synthesis in isolated human mitochondria, J. Biol. Chem. 262 (1987) 1907–1915.
[88] Y.D. Bai, G. Attardi, The mtDNA-encoded ND6 subunit of mitochondrial NADH dehydrogenase is essential for the assembly of the membrane arm and the respiratory function of the enzyme, EMBO J. 17 (1998) 4848–4858.
[89] S. Solakidi, C.E. Sekeris, Oligonucleotide sequences similar to transcription factor consensi of nuclear genes are present in the human mitochondrial genome, Anticancer Res. 23 (2003) 1389–1393.
[90] C. Demonacos, R. Djordjevicmarkovic, N. Tsawdaroglou, C.E. Sekeris, The mitochondrion as a primary site of action of glucocorticoids: the interaction of the glucocorticoid receptor with mitochondrial DNA sequences showing partial similarity to the nuclear glucocorticoid responsive elements, J. Steroid Biochem. Mol. Biol. 55 (1995) 43–55.
[91] C. Wrutniak, I. Cassar-Malek, S. Marchal, A. Rascle, S. Heusser, J.M. Keller, J. Flechon, M. Dauca, J. Samarut, J. Ghysdael, G. Cabelo, A 43-kDa protein related to c-Erb A α1 is located in the mitochondrial matrix of rat liver, J. Biol. Chem. 270 (1995) 16347–16354.
[92] C. Wrutniak-Cabello, F. Casas, G. Cabello, Thyroid hormone action in mitochondria, J. Mol. Endocrinol. 26 (2001) 67–77.
[93] K. Scheller, P. Seibel, C.E. Sekeris, Glucocorticoid and thyroid hormone receptors in mitochondria of animal cells, International Review of Cytology, 2003, pp. 1–61.
[94] A.M. Psarra, S. Solakidi, C.E. Sekeris, The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells, Mol. Cell. Endocrinol. 246 (2006) 21–33.
[95] A. Pedram, M. Razandi, D.C. Wallace, E.R. Levin, Functional estrogen receptors in the mitochondria of breast cancer cells, Mol. Biol. Cell 17 (2006) 2125–2137.
[96] A. Chomyn, G. Attardi, Recent advances on mitochondrial biogenesis, in: L. Ernster (Ed.), Molecular Mechanisms in Biogenesis, Elsevier Science Publishers B. V., 1992, pp. 483–509.
[97] J. Piechota, R. Tomecki, K. Gewartowski, R. Szczesny, A. Dmochowska, M. Kudla, L. Dybczynska, P.P. Stepien, E. Bartnik, Differential stability of mitochondrial mRNA in HeLa cells, Acta Biochim. Pol. 53 (2006) 157–168.
[98] Y. Michikawa, F. Mazzucchelli, N. Bresolin, G. Scarlato, G. Attardi, Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication, Science 286 (1999) 774–779.
[99] R. Del Bo, M. Crimi, M. Sciacco, G. Malferrari, A. Bordoni, L. Napoli, A. Prelle, I. Biunno, M. Moggio, N. Bresolin, G. Scarlato, G. Pietro Comi, High mutational burden in the mtDNA control region from aged muscles: a single-fiber study, Neurobiol. Aging 24 (2003) 829–838.
[100] P.E. Coskun, E. Ruiz-Pesini, D.C. Wallace, Control region mtDNA variants: longevity, climatic adaptation, and a forensic conundrum, PNAS 100 (2003) 2174–2176.
[101] P.E. Coskun, M.F. Beal, D.C. Wallace, Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10726–10731.
[102] A. Chomyn, A. Martinuzzi, M. Yoneda, A. Daga, O. Hurko, D. Johns, S.T. Lai, I. Nonaka, C. Angelini, G. Attardi, MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcritps, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 4221–4225.
[103] S.C. Nam, C. Kang, DNA light-strand preferential recognition of human mitochondria transcription termination factor mTERF, J. Biochem. Mol. Biol. 38 (2005) 690–694.
[104] J.F. Hess, M.A. Parisi, J.L. Bennett, D.A. Clayton, Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies, Nature 351 (1991) 236–239.
[105] M.P. King, Y. Koga, M. Davidson, E.A. Schon, Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the transfer RNA(Leu)(UUR) mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes, Mol. Cell. Biol. 12 (1992) 480–490.
[106] L.A. Bindoff, N. Howell, J. Poulton, D.A. Mccullough, K.J. Morten, R.N. Lightowlers, D.M. Turnbull, K. Weber, Abnormal RNA processing associated with a novel transfer RNA mutation in mitochondrial DNA—A potential disease mechanism, J. Biol. Chem. 268 (1993) 19559–19564.
[107] Y. Koga, M. Davidson, E.A. Schon, M.P. King, Fine mapping of mitochondrial RNAs derived from the mtDNA region containing a point mutation associated with MELAS, Nucleic Acids Res. 21 (1993) 657–662.
[108] H.L. Hao, C.T. Moraes, Functional and molecular mitochondrial abnormalities associated with a C→T transition at position 3256 of the human mitochondrial genome—The effects of a pathogenic mitochondrial tRNA point mutation in organelle translation and RNA processing, J. Biol. Chem. 271 (1996) 2347–2352.
[109] H. Yan, N. Zareen, L. Levinger, Naturally occurring mutations in human mitochondrial pre-tRNASer(UCN) can affect the tRNase Z cleavage site, processing kinetics and substrate secondary structure, J. Biol. Chem. 281 (2006) 3926–3935.
[110] M. Mollers, K. Maniura-Weber, E. Kiseljakovic, M. Bust, A. Hayrapetyan, M. Jaksch, M. Helm, R.J. Wiesner, J.C. von Kleist-Retzow, A new mechanism for mtDNA pathogenesis: impairment of post-transcriptional maturation leads to severe depletion of mitochondrial tRNASer (UCN) caused by T7512C and G7497A point mutations, Nucleic Acids Res. 33 (2005) 5647–5658.
[111] M. Toompuu, L.L. Levinger, A. Nadal, J. Gomez, H.T. Jacobs, The 7472insC mtDNA mutation impairs 5′ and 3′ processing of tRNA(Ser (UCN)), Biochem. Biophys. Res. Commun. 322 (2004) 803–813.
[112] M.X. Guan, J.A. Enriquez, N. Fischel-Ghodsian, R.S. Puranam, C.P. Lin, M.A. Maw, G. Attardi, The deafness-associated mitochondrial DNA mutation at position 7445, which affects tRNASer(UCN) precursor processing, has long-range effects on NADH dehydrogenase subunit ND6 gene expression, Mol. Cell. Biol. 18 (1998) 5868–5879.
[113] R.J. Temperley, S.H. Seneca, K. Tonska, E. Bartnik, L.A. Bindoff, R.N. Lightowlers, Z.M. Chrzanowska-Lightowlers, Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria, Hum. Mol. Genet. 12 (2003) 2341–2348.

[114] Y. Bykhovskaya, E. Mengesha, D. Wang, H. Yang, X. Estivill, M. Shohat, N. Fischel-Ghodsian, Human mitochondrial transcription factor B1 as a modifier gene for hearing loss associated with the mitochondrial A1555G mutation, Mol. Genet. Metab. 82 (2004) 27–32.
[115] J.P. Rife, P.B. Moore, The structure of a methylated tetraloop in 16S ribosomal RNA, Structure 6 (1998) 747–756.
[116] H. Takaku, A. Minagawa, M. Takagi, M. Nashimoto, A candidate prostate cancer susceptibility gene encodes tRNA 3′ processing endoribonuclease, Nucleic Acids Res. 31 (2003) 2272–2278.
[117] V.K. Mootha, P. Lepage, K. Miller, J. Bunkenborg, M. Reich, M. Hjerrild, T. Delmonte, A. Villeneuve, R. Sladek, F. Xu, G.A. Mitchell, C. Morin, M. Mann, T.J. Hudson, B. Robinson, J.D. Rioux, E.S. Lander, Identification of a gene causing human cytochrome *c* oxidase deficiency by integrative genomics, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 605–610.
[118] F. Amalric, C. Merkel, R. Geldfand, G. Attardi, Fractionation of mitochondrial RNA from HeLa cells by high-resolution electrophoresis under strongly denaturing conditions, J. Mol. Biol. 118 (1978) 1–25.
[119] G.S. Shadel, A dual-function mitochondrial transcription factor tunes out deafness, Mol. Genet. Metab. 82 (2004) 1–3.
[120] J. Duan, M.S. Wainwright, J.M. Comeron, N. Saitou, A.R. Sanders, J. Gelernter, P.V. Gejman, Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor, Hum. Mol. Genet. 12 (2003) 205–216.
[121] J.M. Grad, J.L. Dai, S. Wu, K.L. Burnstein, Multiple androgen response elements and a Myc consensus site in the androgen receptor (AR) coding region are involved in androgen-mediated up-regulation of AR messenger RNA, Mol. Endocrinol. 13 (1999) 1896–1911.
[122] J.V. Chamary, J.L. Parmley, L.D. Hurst, Hearing silence: non-neutral evolution at synonymous sites in mammals, Nat. Rev., Genet. 7 (2006) 98–108.