Trends in Genetics

CellPress
REVIEWS

Review

Mitochondrial DNA Transcription and Its Regulation: An Evolutionary Perspective

Gilad Barshad,[1] Shani Marom,[1] Tal Cohen,[1] and Dan Mishmar[1,*]

The bacterial heritage of mitochondria, as well as its independent genome [mitochondrial DNA (mtDNA)] and polycistronic transcripts, led to the view that mitochondrial transcriptional regulation relies on an evolutionarily conserved, prokaryotic-like system that is separated from the rest of the cell. Indeed, mtDNA transcription was previously thought to be governed by a few dedicated direct regulators, namely, the mitochondrial RNA polymerase (POLRMT), two transcription factors (TFAM and TF2BM), one transcription elongation (TEFM), and one known transcription termination factor (mTERF1). Recent findings have, however, revealed that known nuclear gene expression regulators are also involved in mtDNA transcription and have identified novel transcriptional features consistent with adaptation of the mitochondria to the regulatory environment of the precursor of the eukaryotic cell. Finally, whereas mammals follow the human mtDNA transcription pattern, other organisms notably diverge in terms of mtDNA transcriptional regulation. Hence, mtDNA transcriptional regulation is likely more evolutionary diverse than once thought.

Highlights

Unlike the traditionally held view, mtDNA transcription is regulated not only by dedicated factors but also by known regulators of nuclear transcription.

This suggests that although mtDNA transcription regulatory system is separated in space from the nucleus, the prokaryotic progenitor of mitochondria adapted to its host regulatory environment so as to enable coregulation.

mtDNA transcriptional regulatory elements are not confined to noncoding regions and may also reside within genes. Hence, extensive mtDNA rearrangements, such as insertions, deletions, and inversions that occurred during the course of evolution, would have altered orientation of such elements and may affect transcription.

Although mtDNA transcriptional regulation in mammals resembles that of humans, it can diverge considerably elsewhere.

The Mitochondrial Genome: Known versus Novelty in Transcriptional Regulation

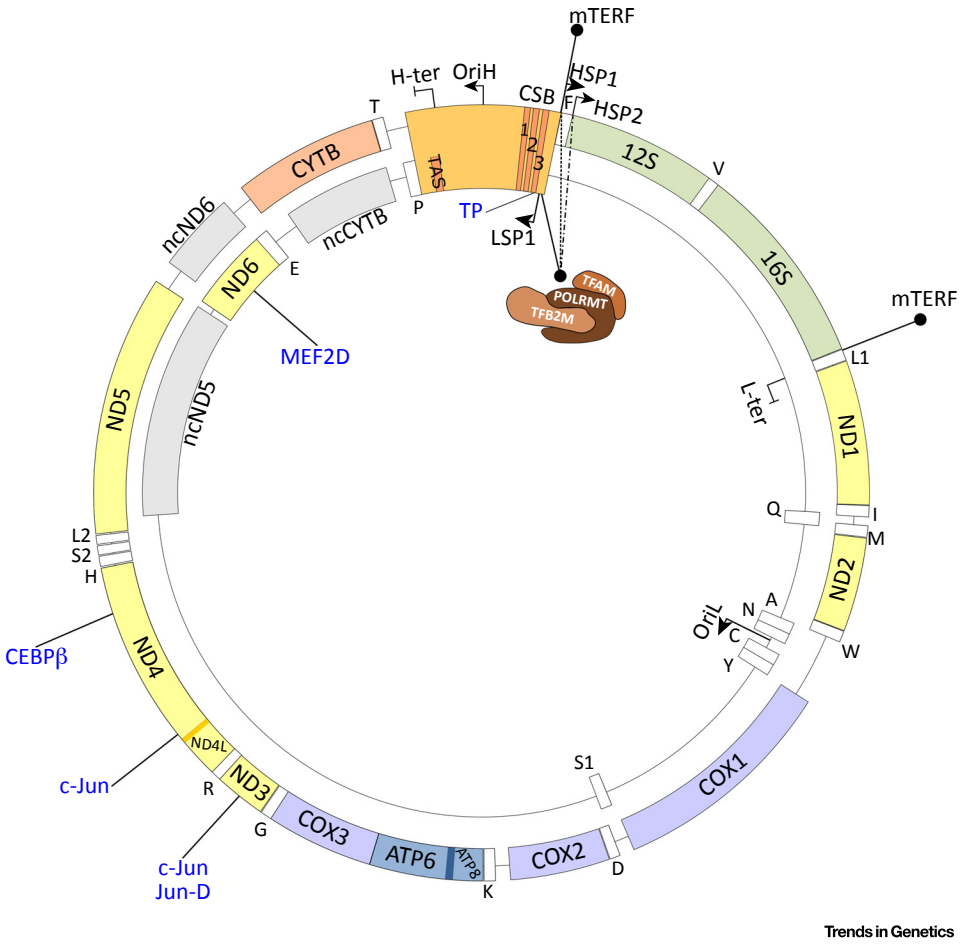
The very existence of an independent genome in the mitochondria (mtDNA) was first reported in the chick embryo [1]. Only a decade later, it was shown that human mtDNA is transcribed in a strand-specific, bacterial-like polycistronic manner [2]. Sequencing of the first draft of the human mitochondrial genome [3] in the early 1980s indicated that it is mostly coding (>90%), with each strand harboring different amounts of genetic information. The heavy strand encodes 12 protein subunits of the oxidative phosphorylation system (NADH ubiquinone oxidoreductase subunits ND1–ND5, ND4L, cytochrome oxidase bc1 subunit CytB, cytochrome oxidase subunits CO1–CO3, and ATP synthase subunits ATP6 and ATP8), two ribosomal RNA genes (12S and 16S), and 14 tRNAs. The light strand encodes for a single mRNA (NADH ubiquinone oxidoreductase subunits ND6) and eight tRNAs (Figure 1). Notably, recent analysis has revisited the genetic content of the mitochondrial genome and identified previously overlooked noncoding transcripts (Box 1; Figure 1).

Sequencing of the human mtDNA also identified the main noncoding region of the mammalian mtDNA: the displacement loop (**D-loop**) (see Glossary) (Figure 1). This enabled subsequent detailed identification of the mtDNA heavy- and light-strand promoters (HSP1 and HSP2 and LSP, respectively) within the D-loop in human [4,5] and mouse mtDNAs [6,7] (see Box 2 for discussion on the ongoing debate of HSP2 functionality) (Figure 1). These findings marked the beginning of our understanding of mtDNA transcriptional regulation at the molecular level. After the identification of the LSP and HSPs, considerable effort was invested in identifying strand-specific mtDNA transcription termination-associated sequences TAS1 and TAS2 in

[1]Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva 8410501, Israel

*Correspondence:
dmishmar@bgu.ac.il (D. Mishmar).

 https://doi.org/10.1016/j.tig.2018.05.009



Trends in Genetics

Figure 1. Map of Coding, Noncoding, and Regulatory Elements in Human mtDNA. Genes encoded by the human mitochondrial DNA (mtDNA) are annotated and divided according to their mtDNA strand location: heavy strand (outer circle) or light strand (inner circle). The protein-coding genes are colored according to their oxidative phosphorylation protein complex assignment. Regions where open reading frame overlap (i.e., between ND4 and ND4L, and ATP6 and ATP8) are indicated in darker colors. rRNA genes are colored in light green. tRNA genes, indicated by empty rectangles, are marked according to the single-letter amino acid code. Noncoding transcripts are designated in gray. The main noncoding mtDNA region, the D-loop (orange segment), is depicted to encompass both mtDNA strands. Known regulatory elements and protein binding sites are indicated in black, whereas newer non-canonical elements are indicated in blue. CSB, conserved sequence blocks; TAS, termination-associated sequence; TP, newly discovered transcription pausing site [33]. Arrows indicate the orientation of the origin of replication per strand (OriH, OriL), as well as the promoters (HSP1, HSP2, and LSP). The transcription initiation complex (POLRMT, TFAM, and TFB2M) is indicated as brown clouds.

deciphering the functions served by the conserved sequence blocks CSB1–CSB3 [8], such as suggesting the roles played by the CSBs (in cell culture) [9], and specifically that of CSB2 (*in vitro*) [10], in the switch between mtDNA transcription and replication, and identifying the functional importance of the inter-promoter region [11] (Figure 1). In parallel, efforts focused on isolating the core factors involved in mtDNA transcription initiation and elongation. Such efforts led to the identification of mitochondrial RNA polymerase (POLRMT) [12–14], mitochondrial transcription factor A (TFAM) [15], and the mitochondrial transcription factor TFB2M and its paralog TFB1M [16]. It was further noted that only TFB2M unwinds the mtDNA as part of the transcription initiation complex, whereas TFB1M, which retained its ancestral

Glossary

Bilaterians: the animal kingdom can be divided according to those whose body plan presents longitudinal symmetry (i.e., bilaterians) and those lacking such a plan (non-bilaterians). The body plan of bilaterians comprises a head and tail, as well as a back and a belly. As such, they also present left and right sides. Non-bilaterians include, among others, organisms with radial symmetry, such as sea urchins and anemones.

D-loop: the displacement loop (D-loop) comprises the largest mtDNA noncoding region in mammals (~1100 nucleotides) and harbors most of the known mtDNA replication and transcription regulatory elements.

GRO-seq, PRO-seq: global run-on transcription with deep sequencing (GRO-seq), and its improved version, entitled precision run-on transcription (PRO-seq) are deep sequencing techniques designed to capture and sequence genome-wide nascent RNA transcripts in cells and tissue samples. The techniques are based on labeling nascent transcripts (usually by biotin), followed by capture, cDNA preparation, sequencing library preparation, and sequencing by the common deep sequencing platforms.

Indels: an acronym for insertion and deletion mutations.

Box 1. The Increased Complexity of the mtDNA Genetic Information

It has been thought for many years that the mtDNA of all studied vertebrates is mostly coding (>90%), harboring a total of 37 genes that are divided between the heavy- and light-strand transcripts: the heavy-strand transcript harbors 12 protein subunits of the oxidative phosphorylation system (NADH ubiquinone oxidoreductase subunits ND1–ND5, ND4L, cytochrome oxidase bc1 subunit CytB, cytochrome oxidase subunits 1–3, and ATP synthase subunits 6 and 8), two ribosomal RNA genes (12S and 16S), and 14 tRNAs; the light strand encodes for a single mRNA (NaDH ubiquinone oxidoreductase subunits ND6) and eight tRNAs. Although this was thought to constitute the ultimate gene content, the identification of a short 24-oligopeptide entitled 'Humanin', an open reading frame within the 16S rRNA [99], which perhaps possess neuroprotective properties, questioned this final gene content [100]. Thorough revisiting of the human mitochondrial transcriptome via analysis of RNA-seq from several cell lines revealed a previously un-noticed collection of small RNAs, with yet unknown function [31,101]. Subsequent analysis of human mtDNA revealed three long noncoding RNAs (lncRNA) that are transcribed from the opposite strand of the ND5, CytB, and ND6 genes, respectively, with the ND5 lncRNA found both at the 3′ end of the ND6 mRNA, as well as an independent processed transcript [102]. These mtDNA-encoded lncRNAs were suggested to form duplexes with their corresponding mRNAs to either stabilize or increase mRNA degradation rate; as the levels of such lncRNAs differ between tested tissue samples, Rackham *et al.* [102] speculated that the three lncRNAs may play a role in regulating the differential expression levels of ND5, CytB, and ND6 mRNAs. Taken together, the confirmed presence of these RNA species raises the possibility that similar to the nuclear genome, the mtDNA noncoding RNAs play a regulatory role over the canonical mtDNA-encoded genes.

methyltransferase activity on the 12S rRNA, does not contribute to mtDNA transcription [17] (Box 2). In addition, the mitochondrial transcription machinery was shown to involve an elongation factor (TEFM) that increases transcription processivity and modulates the switch between transcription and replication [18,19], a single-stranded DNA-binding protein 1 [20,21], and the family of mitochondrial transcription termination factors (mTERF1–mTERF4) [22,23]. Notably, only mTERF1 clearly terminates mtDNA transcription in the light strand [24] yet promotes HSP1 transcription [25], whereas mTERF2–mTERF4 display other prominent activities in addition to their putative involvement in mtDNA transcription, such as modulating mtDNA gene translation, and involvement in ribosome stability (reviewed in ref. [26]). All the above-mentioned proteins are essential for cell life, and their importance in mtDNA transcription and replication has recently been thoroughly reviewed [26,27] (Box 2). Nevertheless, most research on mtDNA transcription has been performed *in vitro* and in model organisms, thus by-and-large avoiding the impact of changes in mitochondrial genome organization and associated regulatory elements during the course of evolution [28]. As a result, such studies overlook potential additional regulatory elements (see discussion below). To address these caveats, the growing

Box 2. mtDNA Transcription: The Core Elements

Transcription of mitochondrial DNA (mtDNA)-encoded genes initiates from three strand-specific promoters located in the main mtDNA noncoding area (D-loop). Specifically, transcripts driven by the light-strand promoter (LSP) and the heavy-strand promoter 2 (HSP2) are genome sized polycistronic transcripts, whereas HSP1 drives transcription of the 16S and 12S ribosomal RNA $tRNA^{Phe}$ and $tRNA^{Val}$ genes [103,104]. Although there is ongoing debate as to the functionality of HSP2 [4,5,25,105,106], recent studies have provided evidence for the activity and importance of this promoter [33,103,107]. LSP transcripts may either synthesize most of the mtDNA light strand, or terminate at the conserved sequence block II (CSBII) [108], to generate a replication primer for the mtDNA heavy strand [9]. Transcription initiated by HSP1 preferentially terminate after the rRNA genes via MTERF1 binding within the $tRNA^{Leu(UUR)}$ gene [109,110].

mtDNA transcription depends entirely on nuclear DNA-encoded factors. There are three core mtDNA transcription components in humans: (A) mitochondrial RNA polymerase (POLRMT), which has structural similarity to bacteriophage T7 RNA polymerase (RNAP); (B) mitochondrial transcription factor B2 (TFB2M); and (C) mitochondrial transcription factor A (TFAM). (B) TFB2M and TFB1M are ancient mammalian duplicates of the yeast mitochondrial transcription factor Mtf1p [111] and are homologous to bacterial rRNA dimethyltransferase. Of these, TFB2M became primarily a mitochondrial transcription initiation factor, and TFB1M retained the methyl transferase activity [16]. (C) TFAM preferentially binds the mtDNA promoters. Similar to other members of the high mobility group (HMG) proteins, TFAM can bind, unwind, and bend DNA with little sequence specificity. mtDNA transcription is initiated by TFAM binding to a site upstream of the transcription start site. TFAM, in turn, recruits POLRMT to the promoters, followed by recruitment of TFB2M to fully assemble the transcription initiation complex [104,112]. Finally, the mitochondrial transcription elongation factor (TEFM) joins, which increases transcription processivity [113].

use of deep sequencing techniques has enabled highly quantitative measurements of mtDNA transcript levels [29–31] and nascent mtDNA-encoded RNA transcripts (**GRO-seq, PRO-seq**) [32,33] regardless of the availability of genomic reference sequences. Such efforts laid the groundwork for functional assessment of mtDNA transcription *in vivo* both in model and non-model organisms, thus opening the door to future physiologically relevant studies of mtDNA transcription in multiple organisms so as to follow the process over the course of evolution.

mtDNA Transcription over the Course of Vertebrate Evolution

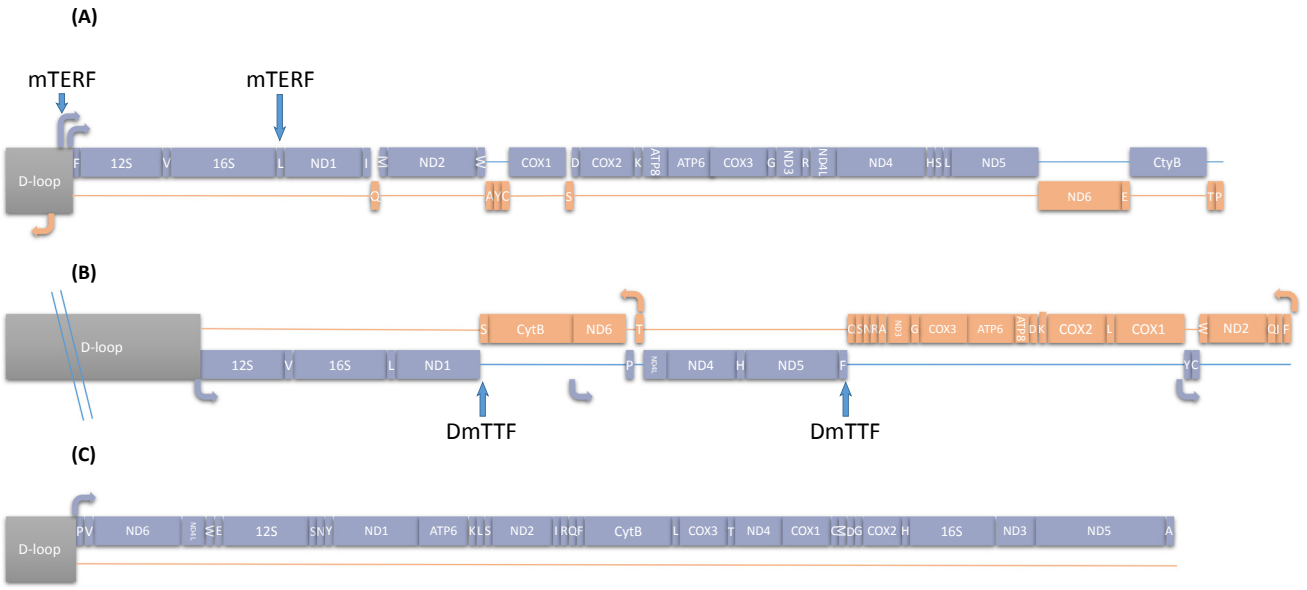
Human mtDNA transcriptional regulation, and to a similar extent that of mouse mtDNA, has been studied extensively [26]. However, can one assume that the human mode of mtDNA transcription applies to all mammals, vertebrates, and metazoans? In mammals, mtDNA gene content has been retained for the past ~200 million years, with changes occurring mainly in the noncoding sequences, including simple repeat **indels** [34,35], repeat copy number variations [8,36–38], and rare deletions [39,40]. Although findings from *in vitro* efforts hinted at potential impacts of such changes on mtDNA transcriptional regulation [41], no *in vivo* effects were demonstrated [39,40]. This may suggest that vertebrate mtDNA transcriptional regulation likely involves more DNA elements than first estimated (see further discussion below). Indeed, these may not necessarily be confined to noncoding mtDNA sequences [42,43].

Nevertheless, some noncoding mtDNA sequence changes that occurred over the course of vertebrate evolution affected mtDNA transcription. First, unlike the separate heavy and light strand promoters used in mammals, *Xenopus laevis* and *Gallus gallus* mtDNA transcription is instead governed by a single bidirectional promoter [44–46]. Second, it was discovered that the mtDNA of amphioxi (a group of fish-like marine chordates) nearly lacks a defined noncoding control region [47], suggesting that regulatory elements of mtDNA transcription are likely present throughout the mtDNA and not confined to noncoding regions. Thus, although vertebrate mtDNA gene content has remained relatively constant, transcriptional regulation must have diverged, albeit to an unknown extent. Such assessment awaits systematic investigation of mtDNA transcription in representatives of major phylogenetic metazoan clades.

The Variability of mtDNA Organization among Metazoans, and the Potential Impact of Such Changes on mtDNA Transcriptional Regulation

Advances in deep sequencing technologies, which do not rely on pre-existing reference sequences, have allowed access to mtDNA sequences from a variety of non-model organisms [28]. In considering all metazoans, including non-**bilaterians**, variability becomes apparent in terms of mtDNA gene order, gene content, and even genome structure (i.e., circular versus linear or fragmented circles). For example, recent analysis revealed split mtDNA in the nematode *Globodera ellingtonae*, where two co-segregating mtDNA molecules that together contain 12 protein-, 22 tRNA-, and two rRNA-coding genes are found [48]. These co-segregating mtDNA molecules differ in copy number, consistent with differential regulation, despite sharing the same 6.5-kb noncoding region. Although most nematode species possess a single circular mitochondrial genome, two species harbor a set of co-segregating five-split mtDNA molecules, which may differ in copy number. Much more fragmented mtDNA has been reported in human lice (~20 mini-chromosomes), although with relatively conserved gene content among the body louse, *Pediculus humanus*; the head louse, *Pediculus capitis*; and the pubic louse, *Pthirus pubis* [49]. Fragmented mtDNA was reported in the flagellate *Diplonema papillatum*, with some of these fragments containing truncating genes that are re-joined via trans-splicing following transcription. Finally, the mtDNA of trypanosomatid protozoa, the kinetoplast, harbors thousands of mini- and maxi-mtDNA circles that, unlike the mitochondrial genome in most

organisms, are bound by histone-like proteins and are transcriptionally regulated by a set of unique proteins [50,51]. These differences in mtDNA organization raise several questions in terms of transcriptional regulation: Has the strand-specific polycistronic pattern of transcription been retained over the course of evolution, even in linear mtDNAs, such as found in medusozoan cnidarians [52]? How does coregulation of mtDNA genes occur in organisms with fragmented mitochondrial genomes [53]? If the mtDNA is transcribed as whole-genome polycistrons, why would gene order differences affect transcription? Investigating transcriptional regulation in such organisms is not only necessary for answering these questions but also will add novel and important insight into the molecular basis of mtDNA transcription. As a first step, *in vivo* patterns of mtDNA transcription were recently assessed in mammals and non-mammalian metazoans using precision run-on transcription (PRO-seq) experiments [33]. The results revealed that although all tested mammals (chimp, rhesus macaque, mouse, and rat) presented a human-like pattern of mtDNA transcription based on initiation of both the heavy and light strands within the D-loop, *Caenorhabditis elegans* mtDNA contains only one heavy-strand transcription initiation site (and no light-strand initiation site), while *Drosophila melanogaster* includes more than five different transcription initiation sites dispersed throughout the mtDNA (Figure 2). Such differential initiation patterns precisely follow gene location and orientation per mtDNA strand, which (as mentioned above) changed over the course of evolution. As such, it would appear that in addition to the known traditional mtDNA regulatory elements, previously overlooked transcriptional regulatory elements are present in the mtDNA (as further discussed below). Accordingly, it is possible that mtDNA rearrangements, such as inversions, deletions, and insertions, not only interfered with mtDNA gene sequences and gene order but also likely altered the orientation and importance of regulatory elements throughout the mitochondrial genome.



Trends in Genetics

Figure 2. mtDNA Transcription Initiation and Termination Maps Differ According to Strand Gene Content and Order. A consensus mammalian mtDNA map including transcription and termination sites as found in *Homo sapiens*, *Pan troglodytes*, *Macaca fascicularis*, *Mus musculus*, *Ratus ratus* (16569–16299 bp) (A) *Drosophila melanogaster* (19517 bp) (B), and *Caenorhabditis elegans* (13794 bp) (C). Transcription initiation sites are indicated by curved arrows, based on recently published PRO-seq experimental results [33]. Gene names and one-letter code tRNAs are indicated. The heavy strand is depicted in gray-blue, and the light strand is drawn in orange. Binding sites for *Drosophila* [98] and human [25] transcription termination factors are indicated.

The differential patterns of mtDNA transcriptional initiation, pausing, and termination revealed by the above-mentioned PRO-seq experiments underline the need to study patterns of mtDNA transcription in organisms across evolution. This could be tested at unprecedented resolution as functional genomics tools become more readily available for analyzing mtDNA transcriptional patterns of non-model organisms lacking a reference genome sequence.

mtDNA Transcriptional Regulation Is Not Solely Governed by Dedicated Factors: Evidence for Direct mtDNA Transcriptional Control by Regulators of Nuclear Gene Expression

The mtDNA transcription machinery retained some ancient prokaryotic properties, such as the generation of polycistronic mtDNA transcripts and the phage-like structure of POLRMT (Box 2). Despite such similarities, it is not unconceivable that the past 2 billion years of endosymbiosis were accompanied by adaptation of mitochondria to the general regulatory system of the cell. Accordingly, in addition to the core elements and factors that regulate mtDNA transcription, certain factors that regulate transcription in the nucleus are also imported into mitochondria, where they bind the mtDNA and directly regulate its transcription [54,55]. Evidence for such regulation first came with the observation of *in vitro* thyroid hormone binding to the D-loop and 12S rRNA gene [56–58], as well as mitochondrial localization of the hormone *in vivo* [59], followed by detection of D-loop-binding of the cAMP response element-binding protein (CREB) [60–62], which promotes mtDNA gene expression [63], and later, identification of mtDNA binding and *in vivo* transcriptional regulation by the myocyte enhancer factor 2D (MEF2D) [42]. A recent screen of multiple chromatin immuno-precipitation experiments coupled to deep sequencing (ChIP-seq) from the ENCODE consortium revealed *in vivo* mtDNA binding of c-Jun, JunD, and CEBP beta [43,64], as well as their *in vivo* mitochondrial localization in human cells [43]. This latter finding was consistent with the mitochondrial localization of AP-1 transcription factors (including c-Jun) in mouse brain mitochondria following *in vivo* treatment with the ionotropic glutamate receptor agonist kainate [65]. *In silico* screens suggested that the mtDNA harbors glucocorticoid receptor (GR)-binding elements [66]. These predictions were partially corroborated by mitochondrial localization of both GR and estrogen receptor (ER) beta [67,68], and by their effects on mtDNA gene expression [67,69], although *in vivo* mtDNA binding by both GR and ER has yet to be demonstrated. More recently, *in vivo* mtDNA binding within the D-loop was shown for the nuclear DNA chromatin modulator MOF acetyl transferase in mouse [70], the signal transducer and activator of transcription 3 (STAT3) in mouse keratinocytes [71], and the osteogenic cell line-specific transcription modulator NFATc1 in human mesenchymal stem cells after 28 days of differentiation induction [72]. The latter is consistent with differences in mtDNA transcripts between tissues [73]. Members of the nuclear factor-κB (NF-κB) family of transcription factors [p50/NF-κB1, p65 (RelA) and IκBα] that regulate cellular responses to stress and infection were also localized to mitochondria. One member of this family, p65 (RelA), reduced the levels of mtDNA-encoded CO3 and CytB mRNAs, possibly by binding to the D-loop in human cells in the absence of p53 [74]. *In vivo* mtDNA binding was reported for the mitochondrial Lon protease in G-quadruplex forming sequences [75], although the functional importance of this observation to mtDNA transcription remains unclear. Although such binding and the effect on mtDNA transcripts were reported, most of these studies assumed binding of the studied transcription factors to sequence elements within the D-loop, which includes most known regulatory elements of mtDNA transcription (Figure 1). D-loop binding was also reported for the DNA methyltransferase Dnmt1 in bovine retinal endothelial cells grown in high glucose, which was associated with reduced mtDNA transcripts levels [76]. Although this suggests a role for epigenetic modifications in mtDNA gene expression regulation, the importance of this phenomenon is still a matter of debate [77]. Nevertheless, D-loop binding was not the case for c-Jun, JunD, CEBP beta [43], and MEF2D [42], which bind within mtDNA genes. This suggests

that transcriptional regulatory elements within the compact human mtDNA are not confined to noncoding sequences. Hence, gene coding sequences could be written in two languages: the protein/RNA coding language and the regulatory language. As exons of certain genes in the nuclear genome can act as transcriptional regulatory elements of other unrelated genes [78], a similar logic may also apply to the mtDNA.

Indirect mtDNA Transcription Regulators

Regulation of mtDNA transcription occurs not only directly by binding of transcriptional regulators to the mtDNA but also indirectly. This could be achieved either via response to changes in the redox balance and ATP concentration [79,80] or by 'regulating the regulators' in the nucleus, as elaborated below. The nuclear respiratory factors NRF-1 and NRF-2 have a variety of impacts on mitochondrial activities [81]. Specifically, both NRF-1 and NRF-2 directly bind promoters of the mitochondrial transcription factors TFB2M and TFAM, as well as the mitochondrial dimethyl transferase TFB1M [82]. Silencing of the nuclear transcription factor Ying-Yang 1 (YY1) reduced mtRNA expression levels [83]. This is consistent with enrichment of YY1-binding motifs in upstream regulatory elements of oxidative phosphorylation genes [84]. The peroxisome proliferator-activated receptor gamma coactivator-1 (PGC1) family (PGC1-α, PGC1-β, and PRC) are considered major nuclear regulators of mitochondrial transcript levels. These factors regulate the activity of a variety of transcription factors, including (among others) NRF-1 [85–88], NRF-2 [89], and YY1 [83], while PRC regulates CREB [85,86]. Interestingly, although PGC1α does not present known DNA-binding domains and forms only protein–protein interactions, it complexes with TFAM at the mouse mtDNA D-loop [90]. Finally, the tumor suppressor protein p53 interacts with TFAM and affects mtDNA copy numbers, resulting in decreased mtDNA transcript levels [91,92]. Both the direct and indirect regulations of mtDNA transcription, in addition to both anterograde and retrograde signaling [93] (i.e., signal flow from and to the mitochondria, respectively), suggest that the long-time presence of mitochondria within eukaryotic cells was likely accompanied by the adaptation of mtDNA transcriptional regulation to that of the host.

First Clues for the Existence of Regulated Higher-Order mtDNA Organization

Given the above-mentioned information, one may wonder to what extent has mtDNA transcriptional regulation adapted to the host cell regulatory system. In the nuclear genome, higher-order organization of the chromatin plays a major role in the regulation of transcription, replication, and genome packaging. Unlike the nuclear genome, the mtDNA lacks histones, instead being packaged within a protein–DNA structure named after a similar prokaryotic

Box 3. The Mitochondrial Nucleoid

Similar to the chromosomes in the nucleus, the mtDNA is packed into a DNA-protein complex entitled the nucleoid, after the bacterial DNA-protein organization. Similar to the bacterial nucleoid, mitochondrial nucleoids are dynamic complexes that are associated with the mitochondrial membrane [114] and are tethered to the cytoskeleton with a transmembrane structure that allows them to 'migrate' within the mitochondrial network. The mitochondrial nucleoids are roughly spherical [95] and harbor one to ten mtDNA molecules [115,116]. They are organized in a layered structure: First, the core region contains the TFAM, mitochondrial single-stranded DNA binding protein in addition to replication- and transcription-related proteins such as the mtDNA polymerase (POLG/POLG2), POLRMT, TFB2M, mTERF, and the mtDNA helicase Twinkle. The peripheral region of the nucleoid contains chaperones, RNA processing and translation-related proteins, as well as temporarily recruited proteins [94,114]. Finally, the translation machineries inside and outside the mitochondria are found in physical proximity to the nucleoids, allowing efficient assembly of the OXPHOS complexes [114,116].

Each nucleoid can divide or move within the mitochondrial network as a single unit, or aggregate and create nucleoid clusters. Clusters of nucleoids will mostly contain nucleoids with newly synthesized mtDNA molecules, while nucleoids that are not replicating will remain outside of these clusters. Similarly to their bacterial counterparts, nucleoids with newly replicated mtDNA localize at the mitochondrial tips, next to the mitochondrial fission mediators Drp1 and Mff, where mitochondrial fission occurs [116–119].

structure, the nucleoid [94] (Box 3). A major component of the mitochondrial nucleoid is the transcription factor TFAM, which was proposed to have no binding sequence preferences and with elevated cellular concentrations being associated with increased condensation of the mtDNA [95]. However, others have suggested that TFAM shows some degree of differential binding preference for mtDNA sites and mtDNA strands that promote loop formation, DNA bending [96], and differential involvement of TFAM in the regulation of the three mtDNA promoters [11]. DNA loops were previously proposed to have been generated by mTERF, the mtDNA transcription termination factor [25]. Hence, it is plausible that mtDNA organization is more structured than once thought [97]. The recently reported involvement of the known chromatin modulator MOF acetyl transferase in mammalian mtDNA transcriptional regulation [70] tempts us to speculate that there might be regulatory crosstalk between the mtDNA and the nuclear genome not only at the level of transcriptional regulation but also possibly in modulation of the higher-order organization of the two genomes.

Concluding Remarks and Future Perspectives

Here, we have taken an evolutionary and genomics perspective to discuss mtDNA transcriptional regulation. Growing focus on investigating mitochondrial function in genomics deep sequencing techniques enabled the identification of direct mtDNA regulatory factors beyond the core mtDNA transcriptional regulators, as well as the identification of previously overlooked candidate regulatory elements throughout the mitochondrial genome, even within protein-coding genes. Some of the newly identified mtDNA gene transcription regulatory factors also play a role in regulating nuclear gene expression, thus emphasizing the regulatory crosstalk between the mitochondrial and nuclear genomes. This also stresses the importance of future characterization of the full repertoire of factors modulating such crosstalk and adaptation of the mitochondrion to its host regulatory system (see Outstanding Questions). Genomic techniques do not require prior knowledge of the reference genome sequence of the studied organism, thus enabling future investigation of mtDNA transcription in non-model organisms. With this in mind, the evolutionary diversity seen in mtDNA organization and sequence challenges the generality of the human and mouse mtDNA transcriptional regulation schemes, as well as the identities and locations of regulatory elements within mtDNA. This underlines the importance of future investigation of mtDNA gene expression regulation in organisms representing major clades across Eukarya.

Outstanding Questions

The human and mouse mtDNA transcriptional plans do not necessarily apply to non-mammals, especially organisms with rearranged mtDNAs. How does mtDNA transcription occur in organisms with altered mtDNA organization, or in those presenting fragmented genomes or linear mtDNAs?

What is the full repertoire of nuclear transcriptional regulators that are directly involved in mtDNA transcription? What is the full repertoire of factors that govern coregulation of nuclear and mitochondrial gene expression?

What is the molecular basis of the physiological response of the mtDNA transcriptional system?

What is the role of mtDNA-encoded noncoding RNA molecules in transcriptional regulation?

What is the nature of higher-order mtDNA structures? What are the controlling factors, besides TFAM?

Does higher-order mtDNA organization share similarity to bacterial intra-genome loops, or is it similar to the transcription-associated domains (TADs) seen in the nucleus?

Acknowledgments

This study was funded by the research grants from the Israel Science Foundation (372/17), the US-Israel Binational Science Foundation (2013060), and the US Army Life Sciences Division (LS-67993) awarded to D.M. G.B. was funded by a Darom scholarship. S.M. and T.C. were funded by Negev scholarships.

References

1. Nass, S. and Nass, M.M.K. (1963) Intramitochondrial fibers with DNA characteristics. *J. Cell Biol.* 19, 593–629
2. Aloni, Y. and Attardi, G. (1971) Symmetrical in vivo transcription of mitochondrial DNA in HeLa cells. *Proc. Natl. Acad. Sci. U. S. A.* 68, 1757–1761
3. Anderson, S. *et al.* (1981) Sequence and organization of the human mitochondrial genome. *Nature* 290, 457–465
4. Chang, D.D. and Clayton, D.A. (1984) Precise identification of individual promoter for transcription of each strand of human mitochondrial DNA. *Cell* 36, 635–643
5. Montoya, J. *et al.* (1982) Identification of initiation sites for heavy-strand and light-strand transcription in human mitochondrial DNA. *Proc. Natl. Acad. Sci. U. S. A.* 79, 7195–7199
6. Chang, D.D. and Clayton, D.A. (1986) Precise assignment of the heavy-strand promoter of mouse mitochondrial DNA: cognate start sites are not required for transcriptional initiation. *Mol. Cell. Biol.* 6, 3262–3267
7. Chang, D.D. and Clayton, D.A. (1986) Precise assignment of the light-strand promoter of mouse mitochondrial DNA: a functional promoter consists of multiple upstream domains. *Mol. Cell. Biol.* 6, 3253–3261
8. Sbisa, E. *et al.* (1997) Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. *Gene* 205, 125–140
9. Chang, D.D. and Clayton, D.A. (1985) Priming of human mitochondrial DNA replication occurs at the light-strand promoter. *Proc. Natl. Acad. Sci. U. S. A.* 82, 351–355
10. Pham, X.H. *et al.* (2006) Conserved sequence box II directs transcription termination and primer formation in mitochondria. *J. Biol. Chem.* 281, 24647–24652
11. Uchida, A. *et al.* (2017) Unexpected sequences and structures of mtDNA required for efficient transcription from the first heavy-strand promoter. *Elife* 6, e27283

12. Gaspari, M. *et al.* (2004) The mitochondrial RNA polymerase contributes critically to promoter specificity in mammalian cells. *EMBO J.* 23, 4606–4614
13. Reid, B.D. and Parsons, P. (1971) Partial purification of mitochondrial RNA polymerase from rat liver. *Proc. Natl. Acad. Sci. U. S. A.* 68, 2830–2834
14. Ringel, R. *et al.* (2011) Structure of human mitochondrial RNA polymerase. *Nature* 478, 269–273
15. Fisher, R.P. *et al.* (1987) Promoter selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements. *Cell* 50, 247–258
16. Falkenberg, M. *et al.* (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nat. Genet.* 31, 289–294
17. Metodiev, M.D. *et al.* (2009) Methylation of 12S rRNA is necessary for in vivo stability of the small subunit of the mammalian mitochondrial ribosome. *Cell Metab.* 9, 386–397
18. Minczuk, M. *et al.* (2011) TEFM (c17orf42) is necessary for transcription of human mtDNA. *Nucleic Acids Res.* 39, 4284–4299
19. Hillen, H.S. *et al.* (2017) Mechanism of transcription anti-termination in human mitochondria. *Cell* 171, 1082–1093
20. Kullar, P.J. *et al.* (2018) Heterozygous SSBP1 start loss mutation co-segregates with hearing loss and the m.1555A>G mtDNA variant in a large multigenerational family. *Brain* 141, 55–62
21. Tiranti, V. *et al.* (1995) Chromosomal localization of mitochondrial transcription factor A (TCF6), single-stranded DNA-binding protein (SSBP), and endonuclease G (ENDOG), three human housekeeping genes involved in mitochondrial biogenesis. *Genomics* 25, 559–564
22. Asin-Cayuela, J. *et al.* (2005) The human mitochondrial transcription termination factor (mTERF) is fully active in vitro in the non-phosphorylated form. *J. Biol. Chem.* 280, 25499–25505
23. Park, C.B. *et al.* (2007) MTERF3 is a negative regulator of mammalian mtDNA transcription. *Cell* 130, 273–285
24. Terzioglu, M. *et al.* (2013) MTERF1 binds mtDNA to prevent transcriptional interference at the light-strand promoter but is dispensable for rRNA gene transcription regulation. *Cell Metab.* 17, 618–626
25. Martin, M. *et al.* (2005) Termination factor-mediated DNA loop between termination and initiation sites drives mitochondrial rRNA synthesis. *Cell* 123, 1227–1240
26. Gustafsson, C.M. *et al.* (2016) Maintenance and expression of mammalian mitochondrial DNA. *Annu. Rev. Biochem.* 85, 133–160
27. Bestwick, M.L. and Shadel, G.S. (2013) Accessorizing the human mitochondrial transcription machinery. *Trends Biochem. Sci.* 38, 283–291
28. Lavrov, D.V. and Pett, W. (2016) Animal mitochondrial DNA as we do not know it: mt-genome organization and evolution in nonbilaterian lineages. *Genome Biol. Evol.* 8, 2896–2913
29. Cohen, T. *et al.* (2016) Ancient out-of-Africa mitochondrial DNA variants associate with distinct mitochondrial gene expression patterns. *PLoS Genet.* 12, e1006407
30. Kuznetsova, I. *et al.* (2017) Simultaneous processing and degradation of mitochondrial RNAs revealed by circularized RNA sequencing. *Nucleic Acids Res.* 45, 5487–5500
31. Mercer, T.R. *et al.* (2011) The human mitochondrial transcriptome. *Cell* 146, 645–658
32. Kwak, H. *et al.* (2013) Precise maps of RNA polymerase reveal how promoters direct initiation and pausing. *Science* 339, 950–953
33. Blumberg, A. *et al.* (2017) Initiation of mtDNA transcription is followed by pausing, and diverges across human cell types and during evolution. *Genome Res.* 27, 362–373
34. Bodenteich, A. *et al.* (1992) Dinucleotide repeat in the human mitochondrial D-loop. *Hum. Mol. Genet.* 1, 140
35. Szibor, R. *et al.* (1997) Mitochondrial D-loop 3′ (CA)n repeat polymorphism: optimization of analysis and population data. *Electrophoresis* 18, 2857–2860
36. Wilkinson, G.S. *et al.* (1997) Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. *Genetics* 146, 1035–1048
37. Nesbo, C.L. *et al.* (1998) Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). *Genetics* 148, 1907–1919
38. Zardoya, R. and Meyer, A. (1997) The complete DNA sequence of the mitochondrial genome of a 'living fossil,' the coelacanth (*Latimeria chalumnae*). *Genetics* 146, 995–1010
39. Bi, R. *et al.* (2010) The acquisition of an inheritable 50-bp deletion in the human mtDNA control region does not affect the mtDNA copy number in peripheral blood cells. *Hum. Mutat.* 31, 538–543
40. Behar, D.M. *et al.* (2008) A novel 154-bp deletion in the human mitochondrial DNA control region in healthy individuals. *Hum. Mutat.* 29, 1387–1391
41. Tan, B.G. *et al.* (2016) Length heterogeneity at conserved sequence block 2 in human mitochondrial DNA acts as a rheostat for RNA polymerase POLRMT activity. *Nucleic Acids Res.* 44, 7817–7829
42. She, H. *et al.* (2011) Direct regulation of complex I by mitochondrial MEF2D is disrupted in a mouse model of Parkinson disease and in human patients. *J. Clin. Invest.* 121, 930–940
43. Blumberg, A. *et al.* (2014) Transcription factors bind negatively-selected sites within human mtDNA genes. *Genome Biol. Evol.* 6, 2634–2646
44. L'Abbe, D. *et al.* (1991) The transcription of DNA in chicken mitochondria initiates from one major bidirectional promoter. *J. Biol. Chem.* 266, 10844–10850
45. Bogenhagen, D.F. and Romanelli, M.F. (1988) Template sequences required for transcription of *Xenopus laevis* mitochondrial DNA from two bidirectional promoters. *Mol. Cell. Biol.* 8, 2917–2924
46. Bogenhagen, D.F. *et al.* (1986) Identification of initiation sites for transcription of Xenopus laevis mitochondrial DNA. *J. Biol. Chem.* 261, 8488–8494
47. Boore, J.L. *et al.* (1999) Complete sequence, gene arrangement, and genetic code of mitochondrial DNA of the cephalochordate *Branchiostoma floridae* (*Amphioxus*). *Mol. Biol. Evol.* 16, 410–418
48. Phillips, W.S. *et al.* (2016) The mitochondrial genome of *Globodera ellingtonae* is composed of two circles with segregated gene content and differential copy numbers. *BMC Genomics* 17, 706
49. Shao, R. *et al.* (2012) Evolution of extensively fragmented mitochondrial genomes in the lice of humans. *Genome Biol. Evol.* 4, 1088–1101
50. Beck, K. *et al.* (2013) *Trypanosoma brucei* Tb927.2.6100 is an essential protein associated with kinetoplast DNA. *Eukaryot. Cell* 12, 970–978
51. Xu, C.W. *et al.* (1996) Nucleus-encoded histone H1-like proteins are associated with kinetoplast DNA in the trypanosomatid *Crithidia fasciculata*. *Mol. Cell. Biol.* 16, 564–576
52. Kayal, E. *et al.* (2012) Evolution of linear mitochondrial genomes in medusozoan cnidarians. *Genome Biol. Evol.* 4, 1–12
53. Valach, M. *et al.* (2017) Keeping it complicated: mitochondrial genome plasticity across diplonemids. *Sci. Rep.* 7, 14166
54. Leigh-Brown, S. *et al.* (2010) Nuclear transcription factors in mammalian mitochondria. *Genome Biol.* 11, 215
55. Szczepanek, K. *et al.* (2012) Multi-tasking: nuclear transcription factors with novel roles in the mitochondria. *Trends Cell. Biol.* 22, 429–437
56. Enríquez, J.A. *et al.* (1999) Direct regulation of mitochondrial RNA synthesis by thyroid hormone. *Mol. Cell. Biol.* 19, 657–670

57. Morrish, F. *et al.* (2006) Thyroid hormone receptor isoforms localize to cardiac mitochondrial matrix with potential for binding to receptor elements on mtDNA. *Mitochondrion* 6, 143–148
58. Casas, F. *et al.* (1999) A variant form of the nuclear triiodothyronine receptor c-ErbAalpha1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol. Cell. Biol.* 19, 7913–7924
59. Wrutniak, C. *et al.* (1995) A 43-kDa protein related to c-Erb A alpha 1 is located in the mitochondrial matrix of rat liver. *J. Biol. Chem.* 270, 16347–16354
60. Cammarota, M. *et al.* (1999) Cyclic AMP-responsive element binding protein in brain mitochondria. *J. Neurochem.* 72, 2272–2277
61. Lee, J. *et al.* (2005) Mitochondrial cyclic AMP response element-binding protein (CREB) mediates mitochondrial gene expression and neuronal survival. *J. Biol. Chem.* 280, 40398–40401
62. Ryu, H. *et al.* (2005) Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13915–13920
63. De Rasmo, D. *et al.* (2009) cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. *FEBS J.* 276, 4325–4333
64. Marinov, G.K. *et al.* (2014) Evidence for site-specific occupancy of the mitochondrial genome by nuclear transcription factors. *PLoS One* 9, e84713
65. Ogita, K. *et al.* (2002) Localization of activator protein-1 complex with DNA binding activity in mitochondria of murine brain after in vivo treatment with kainate. *J. Neurosci.* 22 (7), 2561–2570
66. Solakidi, S. and Sekeris, C.E. (2003) Oligonucleotide sequences similar to transcription factor consensi of nuclear genes are present in the human mitochondrial genome. *Anticancer Res.* 23, 1389–1393
67. Hsieh, Y.C. *et al.* (2006) Upregulation of mitochondrial respiratory complex IV by estrogen receptor-beta is critical for inhibiting mitochondrial apoptotic signaling and restoring cardiac functions following trauma-hemorrhage. *J. Mol. Cell. Cardiol.* 41, 511–521
68. Scheller, K. *et al.* (2000) Localization of glucocorticoid hormone receptors in mitochondria of human cells. *Eur. J. Cell Biol.* 79, 299–307
69. Psarra, A.M. and Sekeris, C.E. (2011) Glucocorticoids induce mitochondrial gene transcription in HepG2 cells: role of the mitochondrial glucocorticoid receptor. *Biochim. Biophys. Acta* 1813, 1814–1821
70. Chatterjee, A. *et al.* (2016) MOF acetyl transferase regulates transcription and respiration in mitochondria. *Cell* 167, 722–738
71. Macias, E. *et al.* (2014) Stat3 binds to mtDNA and regulates mitochondrial gene expression in keratinocytes. *J. Invest. Dermatol.* 134, 1971–1980
72. Lambertini, E. *et al.* (2015) Osteogenic differentiation of human MSCs: specific occupancy of the mitochondrial DNA by NFATc1 transcription factor. *Int. J. Biochem. Cell Biol.* 64, 212–219
73. Fernandez-Vizarra, E. *et al.* (2008) Mitochondrial gene expression is regulated at multiple levels and differentially in the heart and liver by thyroid hormones. *Curr. Genet.* 54, 13–22
74. Johnson, R.F. *et al.* (2011) p53-dependent regulation of mitochondrial energy production by the RelA subunit of NF-κB. *Cancer Res.* 71, 5588–5597
75. Lu, B. *et al.* (2007) Roles for the human ATP-dependent Lon protease in mitochondrial DNA maintenance. *J. Biol. Chem.* 282, 17363–17374
76. Mishra, M. and Kowluru, R.A. (2015) Epigenetic modification of mitochondrial DNA in the development of diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 56, 5133–5142
77. Mposhi, A. *et al.* (2017) Regulation of mitochondrial gene expression, the epigenetic enigma. *Front. Biosci. (Landmark Ed)* 22, 1099–1113
78. Birnbaum, R.Y. *et al.* (2012) Coding exons function as tissue-specific enhancers of nearby genes. *Genome Res.* 22, 1059–1068
79. Enriquez, J.A. *et al.* (1996) The synthesis of mRNA in isolated mitochondria can be maintained for several hours and is inhibited by high levels of ATP. *Eur. J. Biochem.* 237, 601–610
80. Amiott, E.A. and Jaehning, J.A. (2006) Mitochondrial transcription is regulated via an ATP "sensing" mechanism that couples RNA abundance to respiration. *Mol. Cell* 22, 329–338
81. Scarpulla, R.C. *et al.* (2012) Transcriptional integration of mitochondrial biogenesis. *Trends Endocrinol. Metab.* 23, 459–466
82. Gleyzer, N. *et al.* (2005) Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. *Mol. Cell. Biol.* 25, 1354–1366
83. Cunningham, J.T. *et al.* (2007) mTOR controls mitochondrial oxidative function through a YY1–PGC-1α transcriptional complex. *Nature* 450, 736–740
84. van Waveren, C. and Moraes, C.T. (2008) Transcriptional co-expression and co-regulation of genes coding for components of the oxidative phosphorylation system. *BMC Genomics* 9, 18
85. Vercauteren, K. *et al.* (2006) PGC-1-related coactivator: immediate early expression and characterization of a CREB/NRF-1 binding domain associated with cytochrome c promoter occupancy and respiratory growth. *Mol. Cell. Biol.* 26, 7409–7419
86. Vercauteren, K. *et al.* (2008) PGC-1-related coactivator complexes with HCF-1 and NRF-2β in mediating NRF-2 (GABP)-dependent respiratory gene expression. *J. Biol. Chem.* 283, 12102–12111
87. Andersson, U. and Scarpulla, R.C. (2001) Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Mol. Cell. Biol.* 21, 3738–3749
88. Shao, D. *et al.* (2010) PGC-1β-regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERRα. *Mitochondrion* 10, 516–527
89. Handschin, C. *et al.* (2007) PGC-1α regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy. *Genes Dev.* 21, 770–783
90. Safdar, A. *et al.* (2011) Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J. Biol. Chem.* 286, 10605–10617
91. Yoshida, Y. *et al.* (2003) P53 physically interacts with mitochondrial transcription factor A and differentially regulates binding to damaged DNA. *Cancer Res.* 63, 3729–3734
92. Lebedeva, M.A. *et al.* (2009) Loss of p53 causes mitochondrial DNA depletion and altered mitochondrial reactive oxygen species homeostasis. *Biochim. Biophys. Acta* 1787, 328–334
93. Chae, S. *et al.* (2013) A systems approach for decoding mitochondrial retrograde signaling pathways. *Sci. Signal.* 6, rs4
94. Lee, S.R. and Han, J. (2017) Mitochondrial nucleoid: shield and switch of the mitochondrial genome. *Oxid. Med. Cell. Longev.* 2017, 8060949
95. Kukat, C. *et al.* (2015) Cross-strand binding of TFAM to a single mtDNA molecule forms the mitochondrial nucleoid. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11288–11293
96. Kaufman, B.A. *et al.* (2007) The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. *Mol. Biol. Cell* 18, 3225–3236
97. Rebelo, A.P. *et al.* (2009) In vivo methylation of mtDNA reveals the dynamics of protein-mtDNA interactions. *Nucleic Acids Res.* 37, 6701–6715
98. Roberti, M. *et al.* (2006) The *Drosophila* termination factor DmTTF regulates in vivo mitochondrial transcription. *Nucleic Acids Res.* 34, 2109–2116
99. Lee, C. *et al.* (2013) Humanin: a harbinger of mitochondrial-derived peptides? *Trends Endocrinol. Metab.* 24, 222–228

100. Tajima, H. *et al.* (2002) Evidence for in vivo production of Humanin peptide, a neuroprotective factor against Alzheimer's disease-related insults. *Neurosci. Lett.* 324, 227–231

101. Antonicka, H. *et al.* (2013) The mitochondrial RNA-binding protein GRSF1 localizes to RNA granules and is required for post-transcriptional mitochondrial gene expression. *Cell Metab.* 17, 386–398

102. Rackham, O. *et al.* (2011) Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. *RNA* 17, 2085–2093

103. Zollo, O. *et al.* (2012) Transcriptional requirements of the distal heavy-strand promoter of mtDNA. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6508–6512

104. Morozov, Y.I. *et al.* (2014) A novel intermediate in transcription initiation by human mitochondrial RNA polymerase. *Nucleic Acids Res.* 42, 3884–3893

105. Montoya, J. *et al.* (1983) The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units. *Cell* 34, 151–159

106. Litonin, D. *et al.* (2010) Human mitochondrial transcription revisited: only TFAM and TFB2M are required for transcription of the mitochondrial genes in vitro. *J. Biol. Chem.* 285 (24), 18129–18133

107. Lodeiro, M.F. *et al.* (2012) Transcription from the second heavy-strand promoter of human mtDNA is repressed by transcription factor A in vitro. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6513–6518

108. Wanrooij, P.H. *et al.* (2010) G-quadruplex structures in RNA stimulate mitochondrial transcription termination and primer formation. *Proc. Natl. Acad. Sci. U. S. A.* 107 (37), 16072–16077

109. Kruse, B. *et al.* (1989) Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination. *Cell* 58, 391–397

110. Fernandez-Silva, P. *et al.* (1997) 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, 1066–1079

111. Cotney, J. and Shadel, G.S. (2006) Evidence for an early gene duplication event in the evolution of the mitochondrial transcription factor B family and maintenance of rRNA methyltransferase activity in human mtTFB1 and mtTFB2. *J. Mol. Evol.* 63, 707–717

112. Hillen, H.S. *et al.* (2017) Structural basis of mitochondrial transcription initiation. *Cell* 171, 1072–1081

113. Posse, V. *et al.* (2015) TEFM is a potent stimulator of mitochondrial transcription elongation in vitro. *Nucleic Acids Res.* 43, 2615–2624

114. Bogenhagen, D.F. *et al.* (2008) The layered structure of human mitochondrial DNA nucleoids. *J. Biol. Chem.* 283, 3665–3675

115. Kukat, C. *et al.* (2011) Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. *Proc. Natl. Acad. Sci. U. S. A.* 108, 13534–13539

116. Iborra, F.J. *et al.* (2004) The functional organization of mitochondrial genomes in human cells. *BMC Biol.* 2, 9

117. Ban-Ishihara, R. *et al.* (2013) Dynamics of nucleoid structure regulated by mitochondrial fission contributes to cristae reformation and release of cytochrome c. *Proc. Natl. Acad. Sci. U. S. A.* 110 (29), 11863–11868

118. Lewis, S.C. *et al.* (2016) ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. *Science* 353, aaf5549

119. Kukat, C. and Larsson, N.G. (2013) mtDNA makes a U-turn for the mitochondrial nucleoid. *Trends Cell Biol.* 23, 457–463