Mitochondrion 25 (2015) 67–75

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Review

Regulation of mitochondrial biogenesis through TFAM–mitochondrial DNA interactions
Useful insights from aging and calorie restriction studies

Anna Picca, Angela Maria Serena Lezza *

Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Via Orabona, 4, 70125, Bari, Italy

ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 24 September 2015
Accepted 1 October 2015
Available online 3 October 2015

Keywords:
Mitochondrial biogenesis
MtDNA
TFAM
TFAM–mtDNA interactions
Aging
Calorie restriction

ABSTRACT

Mitochondrial biogenesis is regulated to adapt mitochondrial population to cell energy demands. Mitochondrial transcription factor A (TFAM) performs several functions for mtDNA and interactions between TFAM and mtDNA participate to regulation of mitochondrial biogenesis. Such interactions are modulated through different mechanisms: regulation of TFAM expression and turnover, modulation of TFAM binding activity to mtDNA through post-translational modifications and differential affinity of TFAM, occurrence of TFAM sliding on mtDNA filaments and of cooperative binding among TFAM molecules, modulation of protein–protein interactions. The tissue-specific regulation of mitochondrial biogenesis in aging and calorie restriction (CR) highlights the relevance of modulation of TFAM–mtDNA interactions.

Contents

1. Mitochondrial biogenesis and interactions between mitochondrial transcription factor A and mitochondrial DNA

The very complex process of mitochondrial biogenesis depends on the coordinated expression of nuclear and mitochondrial DNA (mtDNA) and, as a result, it adapts mitochondrial population to cell energy demands. In fact, in response to different physiological and environmental conditions, cell metabolic functions require variable energy amounts, largely provided by mitochondrial oxidative metabolism. Such energy metabolism is usually efficient also due to the constant modulation of mitochondrial transcription. In particular, the transcriptional coactivators of the peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1) family are fundamental regulators of mitochondrial biogenesis and function (Handschin and Spiegelman, 2006; Finck and Kelly, 2006; Scarpulla, 2008; Mirebeau-Prunier et al., 2010; Philp et al., 2011). PGC-1α and PGC-1β are two members of this family which, although present in various cell types, are highly expressed in tissues as heart, brain, skeletal muscle and kidney, largely dependent on oxidative metabolism for their energy support; another family member, PGC-related coactivator (PRC), is ubiquitously present (Handschin and Spiegelman, 2006; Puigserver et al., 1998; Lin et al., 2002). The starting event of one pathway

* Corresponding author.
E-mail address: angelamariaserena.lezza@uniba.it (A.M.S. Lezza).

http://dx.doi.org/10.1016/j.mito.2015.10.001
1567-7249/

leading to mitochondrial biogenesis is the activation, by the PGC-1 family proteins, of transcription factors, as the nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2) and the estrogen related receptor alpha (ERRα) (C.A. Virbasius et al., 1993; J.V. Virbasius et al., 1993; Schreiber et al., 2003), that regulate the expression of mitochondrial proteins encoded by nuclear DNA (Anderson and Prolla, 2009). As a consequence of the activation of such transcription factors, an increase in the expression of many mitochondrial proteins, including those named mitochondrial transcription factor A (TFAM) and mitochondrial transcription factors B1 and B2 (TFB1M and TFB2M, respectively) (Wu et al., 1999; Rebelo et al., 2011) occurs. TFAM is a protein, member of the high-mobility-group (HMG) (Parisi and Clayton, 1991), able to bind, unwind and bend DNA without sequence specificity (Fisher et al., 1992). TFAM is involved in many functions: mtDNA transcription (Fisher and Clayton, 1988), mtDNA maintenance (Ekstrand et al., 2004) and replication and likely also mtDNA repair (Canugovi et al., 2010). Therefore, through regulation of TFAM levels, the PGC-1 coactivators can control the expression of mtDNA-encoded proteins (Scarpulla, 2008). Mammalian mtDNA is a circular double-stranded DNA, ~16.5 kb-long, coding for 37 genes: 13 subunits of four mitochondrial respiratory complexes, 2 rRNAs (12S rRNA and 16S rRNA), and 22 tRNAs (Attardi and Schatz, 1988). MtDNA presents two noncoding regions (NCR) that regulate mtDNA transcription and replication. The major NCR, namely the D-loop, encompasses in its about 900 bp of length the transcription promoter of both heavy and light strands (HSP1 and HSP2, LSP) and the origin of replication of the heavy strand (OriH). The association of multiple proteins with the D-loop region has been widely described, thus indicating this region as the major site of transcriptional regulation (Scarpulla, 2008). The minor NCR of 30 bp of length is located between the tRNACys and tRNAAsn coding site and encompasses the origin of replication of the L strand (OriL) (Fig. 1).

Rat circular mtDNA molecule consists of a heavy-strand (outer circle) and a light-strand (inner circle) that code for the 12S and 16S rRNA and 22 tRNA genes (single letter codes indicated under the corresponding tRNA genes) and subunits of four oxidative phosphorylation complexes: NADH dehydrogenase subunits (ND1–6 and ND4L), cytochrome b (Cyt b), cytochrome c oxidase subunits (COI–III), and ATP synthase subunits (A6, A8). At the top of the molecule is the stable, three-stranded structure of the displacement loop (D-loop),

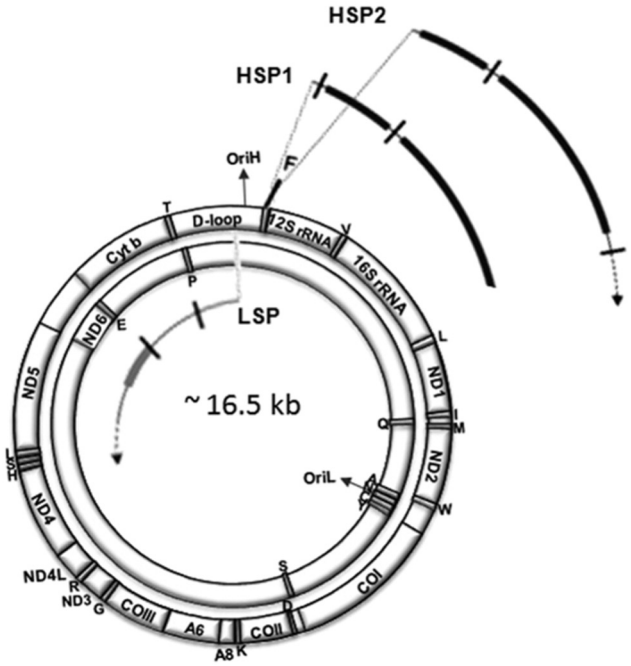

Fig. 1. Genetic map of rat mtDNA.

found in many mtDNA molecules *in vivo*. The D-loop region is the major noncoding portion of the molecule and harbors the L strand promoter (LSP), one of the H strand promoters (HSP1), and the origin of H strand synthesis (OriH). The second H strand promoter (HSP2) is immediately upstream of the 12S rRNA gene, in tRNAPhe (F). The L strand origin (OriL) is located in the "WANCY" cluster of tRNAs. Genes transcribed from the H strand promoters (the H strand is the template) or the LSP (the L strand is the template) are indicated, respectively, outside or inside the mtDNA molecule. Semicircular arcs connected to the mtDNA molecule by dashed lines represent mitochondrial transcripts, originating from HSP1, HSP2 and LSP.

1.1. TFAM and mitochondrial transcription

According to the favored model for the mammalian transcription initiation complex, the basic machinery for mtDNA transcription includes three or two proteins ubiquitously present: mitochondrial RNA polymerase (POLRMT), TFB2M and TFAM (Parisi and Clayton, 1991; Falkenberg et al., 2002; Litonin et al., 2010). *In vitro* studies, showing that a relevant mitochondrial transcription could be initiated from HSP1 and LSP promoters in the absence of TFAM, suggested that, at least *in vitro*, TFAM may be dispensable for a basal mitochondrial transcription (Shutt et al., 2010). However, subsequent results demonstrated that, both in reconstituted "in vitro" transcription systems and in mitochondrial extracts, TFAM is an absolutely necessary core component of the complex required for initiation of human mitochondrial transcription (Shi et al., 2012). These contradictory data were reconciled suggesting that conditions able to induce breathing of the DNA template, as low salt reaction media or negative supercoiling, could promote TFAM-independent transcription (Shi et al., 2012). Anyhow, there is general consent that these three components are all crucial for the basic functioning of the transcription system (Bestwick and Shadel, 2013). The domain essential for TFAM-dependent transcriptional activation *in vitro* has been identified by mutational analysis and is the carboxyl-terminal (C-term) tail of the protein (Dairaghi et al., 1995a). *In vitro* results have demonstrated that transcription from both LSP and HSP1 is enhanced by TFAM presence, although the LSP promoter is activated by an amount of TFAM smaller than that effective for the HSP1 promoter (Shutt et al., 2010). Furthermore, since LSP primes mtDNA replication (Bonawitz et al., 2006) from OriH (Chang and Clayton, 1985; Chang et al., 1985), this creates an intimate relationship between transcription and replication of mtDNA. In particular, the ratio TFAM:mtDNA has been indicated as the means by which TFAM differentially regulates mitochondrial replication versus transcription (Shutt et al., 2011) and human TFAM should be one of the primary factors controlling the copy number of mtDNA in cells. The relevance of the ratio TFAM:mtDNA or, more in general, of TFAM/mtDNA interactions for the regulation of mitochondrial biogenesis has been demonstrated by means of different experimental approaches. Such regulation involves the balance between transcription and replication of mtDNA. The initial report dealt with the permanent overexpression of human TFAM in transgenic mice, leading to an increased mtDNA copy number without a similar stimulation of mitochondrial transcription and of the subsequent respiratory chain capacity and mitochondrial mass (Ekstrand et al., 2004). By the other side, TFAM transient overexpression in HeLa as well as in HEK cultured cells led to conflicting results. The increased concentration of intramitochondrial TFAM was sufficient to stimulate mitochondrial transcription, but not replication of full-length mtDNA molecules (Maniura-Weber et al., 2004). The use of a recombinant human mitochondrial transcription system supported the speculation that different amounts of bound TFAM could induce different transcription and replication potentials of single mtDNA molecules. Thus, low TFAM:mtDNA ratios should imply the preferential activation of LSP and favor mtDNA replication above mtDNA transcription (Shutt et al., 2011). Recently, the combination of single-molecule manipulation and fluorescence microscopy has allowed demonstrating that small changes

in TFAM concentrations have a large impact on mtDNA transcription and replication. According to the proposed model an increased ratio of TFAM to DNA inhibits DNA melting and thus blocks active transcription and replication of mtDNA (Shutt et al., 2011). TFAM levels are regulated *in vivo* by a number of various mechanisms that will be described in the following paragraphs.

1.2. TFAM and mtDNA replication

The replication of the mitochondrial genome in human cells is performed by an enzymatic machinery including at least five different proteins: the catalytic subunit of DNA polymerase γ (POLγA) and its processivity factor (POLγB); the helicase TWINKLE; the mitochondrial single-stranded DNA-binding protein (mtSSB); and the mitochondrial RNA polymerase (POLRMT). The detailed process of mtDNA replication still needs to be clarified. An alternate strand-displacement and strand-coupled models have been proposed and they are presently debated since biochemical data support both of them (Holt et al., 2000; Yang et al., 2002; Yasukawa et al., 2005; Brown et al., 2005). The recent identification of POLRMT as the primase necessary for initiation of DNA synthesis from OriL further highlights the intimate relationship between transcription and replication of mtDNA. In particular, analysis of replication by the strand-displacement mechanism has unveiled that synthesis of the lagging-strand DNA is preceded by OriL activation and primer synthesis by POLRMT that require the passage of the leading-strand DNA replication machinery. In fact, POLRMT binds to the stem-loop structure of the exposed OriL and initiates the RNA primer synthesis. POLRMT is substituted, after about 25 nts, by POLγ and DNA synthesis begins (Fusté et al., 2010). Although TFAM is not generally included in the basic replication machinery of mtDNA, its presence and activity are crucial for the direct regulation of mtDNA copy number (Ekstrand et al., 2004) as well as for other functions of the mitochondrial genome.

1.3. The histone-like role of TFAM

TFAM is considered the histone-like protein of mtDNA and the issue of TFAM:mtDNA ratio is a very relevant, still debated one, due to the very different values measured in cultured cells versus tissues. The laboratories of Kang (Takamatsu et al., 2002) and Larsson (Kukat et al., 2011) reported a very high number of bound TFAM molecules namely about 1000 proteins/mtDNA genome or higher in HeLa cells and other mammalian cell lines. This was suggestive of a very dense TFAM binding that could represent a potential block for the access of other proteins binding DNA as factors for transcription, replication and repair, although the number of TFAM molecules necessary to saturate the mtDNA genome has not yet been determined. A single copy of TFAM seems to occupy 22 bp of the LSP according to the first crystal structures of TFAM bound to a specific mtDNA sequence (Ngo et al., 2011; Rubio-Cosials et al., 2011). This would make the reported stoichiometry of 1000 or more TFAM molecules per mtDNA excessive, unless TFAM binds with an altered mode along the mtDNA molecule. A much lower ratio of 50 molecules of TFAM/mtDNA was reported in HeLa cells by Cotney et al., (2007), but the basis for this 20-fold discrepancy still needs to be resolved. Proteins associated with mtDNA constitute nucleoprotein particles called nucleoids (Bogenhagen et al., 2008). The major protein component of the nucleoids is TFAM that is essential in mtDNA packaging (Bonawitz et al., 2006; Fisher et al., 1989). Furthermore, presumably, the strong affinity of TFAM for non-specific DNA (NS) sequences (Fisher et al., 1989) should allow the factor also to perform an architectural (Kanki et al., 2004) or scaffolding function (Fisher and Clayton, 1988). Interestingly, according to Shutt et al., different amounts of bound TFAM could distinguish nucleoids among them and characterize their specific molecular functions, with protein amounts ranging from low in replicating mtDNA molecules, through moderate in genomes subjected to gene expression, to high amounts in quiescent nucleoids (Shutt et al., 2011) (Fig. 2).

Hypothetical situations elicited by different amounts of TFAM associated with mtDNA molecules/nucleoids. In the transcription complex are indicated: POLRMT, bound at the LSP and HSP promoter sites, TFBM and TFAM. Significant amounts of TFAM bound to mtDNA lead to relevant activation of both promoters and consequent transcription (Left). A very little amount or no TFAM bound to mtDNA probably leads to the absence of basal levels of transcription from both promoters and to residual mtDNA replication (Right).

Such suggestion is in good agreement with the idea that TFAM might greatly determine nucleoid volume through the formation of DNA loops, which reduce contour length, and through supercoiling at higher TFAM amounts, which modulates genome accessibility (Campbell et al., 2012). Several other proteins, including: RNA helicases, mitochondrial ribosomal proteins, chaperones and quality control proteases (Lon and ClpP) and lipid metabolic enzymes, are associated with nucleoids and all together make the right environment for key steps in mitochondrial biogenesis. Although no definitive conclusion has yet been reached about the number of mtDNA molecules included in single nucleoids or clusters of nucleoids in cultured cells (Bogenhagen, 2012), a very recent report demonstrated, by using multiple experimental approaches, that "in vitro" reconstituted nucleoids, nucleoids inside isolated mitochondria and nucleoids from mouse embryonic fibroblasts typically contain a single copy of mtDNA (Kukat et al., 2015).

2. Modulation of TFAM–mtDNA interactions

The overall modulation of TFAM–mtDNA interactions results from various concurrent mechanisms including: a) regulation of TFAM expression and turnover; b) modulation of TFAM binding activity to mtDNA through post-translational modifications of TFAM and differential affinity of TFAM for mtDNA sequences; c) occurrence of TFAM sliding on mtDNA filaments and cooperative binding among TFAM molecules; d) modulation of protein–protein interactions as PGC-1α-TFAM, SIRT1-TFAM and p53-TFAM. According to many evidences, among such mechanisms the regulation of TFAM expression has a relevant role since TFAM is essential for mtDNA maintenance. This is highlighted by disruption of the Tfam gene in mouse, which causes loss of mtDNA (Larsson et al., 1998), and by the low amount of human TFAM, expressed in mouse heart depleted of the endogenous TFAM, which is sufficient to maintain respiratory chain function (Freyer et al., 2010). By the other side, overexpression of TFAM leads to increased mtDNA copy number (Ekstrand et al., 2004; Kanki et al., 2004) and in cultured mammalian cells this seems to protect against oxidative stress and related pathologies, such as cardiopathies or neurodegenerative diseases (Rubio-Cosials et al., 2011; Ikeuchi et al., 2005; Noack et al., 2006; Hayashi et al., 2008). Although TFAM is needed for mtDNA maintenance through compaction and organization of the molecule in the nucleoid, excessively high amounts of the factor might lead to the formation of complex structures and suppress accessibility of mtDNA to replication and transcription machineries. Effectively, it has been reported that overexpression of TFAM increases the amount of supercoiled mtDNA and originates replication intermediates similar to those observed during replication stalling caused by dideoxy cytosine treatment (Pohjoismäki et al., 2006; Kaufman et al., 2007). Therefore, according to experimental evidence, TFAM amount can directly regulate mtDNA copy number, but the latter can also reciprocally affect TFAM amount. In fact, autosomal recessive diseases causing a decreased mtDNA copy number, because of hindered mtDNA replication, lead to decreased TFAM amount (Joseph et al., 2004). Treatment of cultured cells with the intercalating dye ethidium bromide induces loss of mtDNA and a simultaneous reduction of TFAM protein (Maniura-Weber et al., 2004; Peralta et al., 2012). As for TFAM turnover, it involves the mitochondrial Lon protease (Matsushima et al., 2010), a quality control enzyme that

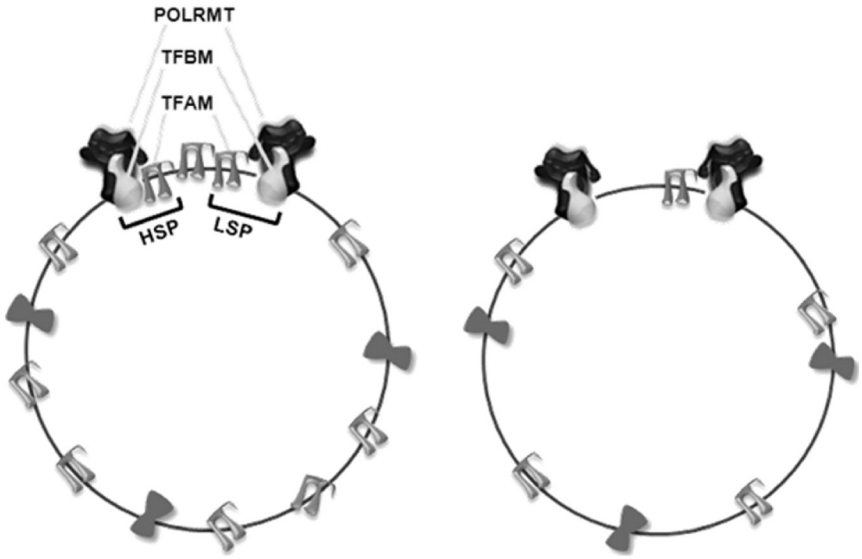

Fig. 2. Interactions TFAM–tDNA.

eliminates oxidized and misfolded proteins (Bender et al., 2011) and that also binds to specific regions of mtDNA (Liu et al., 2004; Lu et al., 2007). TFAM is degraded by Lon, but what triggers such Lon-mediated turnover might be the presence of oxidized TFAM or unbound TFAM (Matsushima et al., 2010); alternatively, since TFAM binds oxidized DNA more efficiently than unmodified DNA, Lon could remove TFAM from damaged DNA (Canugovi et al., 2010). Depletion of Lon leads to an increase in TFAM levels and mtDNA copy number, whereas overexpression of Lon leads to reduction of TFAM and a decrease in mtDNA copy number (Matsushima et al., 2010). However, an increasing number of reports suggest that the overall TFAM activity cannot be regulated only by changes in protein expression and turnover, but a more fine-tuned regulation is needed as indicated in HeLa cells where, through chromatin immunoprecipitation (Ohgaki et al., 2007) and sequencing experiments (Wang et al., 2013), a large non-specific binding across the mitochondrial genome was detected, together with a putative specific binding site upstream of the origin of light strand replication. Interestingly, other reports support the existence of a modulation of TFAM binding activity to mtDNA through post-translational modifications of TFAM and differential affinity of TFAM for mtDNA sequences. In particular, phosphorylation of TFAM by cAMP-dependent protein kinase has been shown, "in vitro" and in mitochondria isolated from HEK293 cells, to affect binding of the protein to mtDNA as well as its sensitivity to Lon-mediated proteolysis. The relationship between phosphorylation and degradation has been suggested to influence TFAM functions in mtDNA maintenance and expression (Lu et al., 2013). More recently, phosphorylation of TFAM by ERK "in vitro" and in cells has also been reported and indicated as another mechanism active in modulating TFAM through regulation of its binding activity (Wang et al., 2014). Dinardo et al., described acetylation of TFAM (Dinardo et al., 2003) and later its regulation by aging and calorie restriction has also been suggested (Hebert et al., 2013) as another pathway to modulate protein roles. Moreover, a site for potential ubiquitination has been identified in TFAM (Shi et al., 2011) and protein ubiquitination has been reported in the retina from an animal model of diabetes, where it should affect mitochondrial import and transcription in the organelle (Santos et al., 2014). A fine-tuning of TFAM binding activity is obtained also through modulation of specific and non-specific (NS) binding to DNA. Mouse TFAM–DNA affinity and specificity as well as the DNA binding activity of TFAM were analyzed by Surface Plasmon Resonance (SPR). This approach, by measuring changes in mass associated with DNA immobilized in a flow cell, allows performing a dynamic assay that is widely preferable to the electrophoretic mobility shift assay (EMSA). As a result, a sequence containing both mouse LSP and HSP1 relative to NS sequences (i.e. a COXI DNA sequence) was clearly preferred by mouse TFAM at low protein concentrations (estimated K_D 1.6×10^{-10} M and 1.6×10^{-9} M for each template, respectively) (Campbell et al., 2012; Kaufman et al., 2007). In a different experiment, TFAM demonstrated a preferential affinity with the LSP-Half site (K_D 1.7×10^{-9} M) versus the HSP-Half site (K_D 2.1×10^{-8} M). This quantitative determination of TFAM–DNA interaction specificity is in good agreement with earlier qualitative findings (Fisher et al., 1989, 1992; Dairaghi et al., 1995a) indicating that the difference between higher affinity (LSP) and lower affinity (NS) binding is 2–4-fold. These results suggest that TFAM distorts DNA near transcription start sites to prepare the template for transcription activation. Genomic compaction is achieved by TFAM binding and bending of NS–DNA and by the cooperative binding of TFAM to mtDNA (Campbell et al., 2012; Kaufman et al., 2007). Several evidences suggest a positive cooperativity of TFAM binding to mtDNA: presumably, the presence of one TFAM molecule bound induces an alteration at the local DNA structure that favors the proximal binding of the following molecule. Therefore, the distortion of DNA could mediate the "spread" of TFAM molecules across large stretches of DNA and then facilitate such cooperativity (Campbell et al., 2012). By the other side, since single TFAM molecules diffuse widely over DNA (sliding) and, by collisions, form patches on DNA in a cooperative manner, TFAM nonspecific DNA-binding dynamics might affect mtDNA compaction. Moreover, TFAM induces DNA compaction also by changing the flexibility of the DNA through its local denaturation (melting). Thus, specific regulation of transcription by TFAM requires both sliding of TFAM and DNA melting (Farge et al., 2012). While considering the potential role of TFAM in genome compaction and promoter selection, however, the factor capability to bind as a dimer, to induce DNA looping and to be driven in its binding to mtDNA by a relevant cooperativity have to be taken into account in the formulation of models. Last, but not least, modulation of TFAM–mtDNA interactions is also likely achieved through modulation of protein–protein interactions as the described PGC-1α-TFAM (Aquilano et al., 2010; Safdar et al., 2011), SIRT1-TFAM (Aquilano et al., 2010) and p53-TFAM (Saleem and Hood, 2013). In the first cited report PGC-1α was found in cross-linked nucleoids from murine liver suggesting its possible interaction with TFAM to modulate transactivation of mtDNA and mitochondrial biogenesis (Aquilano et al., 2010). The amount of PGC-1α-TFAM complex at the D-loop of mtDNA has been reported to increase in mouse skeletal muscle after an acute bout of endurance exercise. This had fostered the indication

that such complex is involved in the stimulation of mitochondrial biogenesis required to cope with the exercise-dependent, increased energy demands (Safdar et al., 2011). Furthermore, in the first quoted study SIRT1 and TFAM proteins were demonstrated to be associated with murine liver native nucleoids, leading to the indication of SIRT1 as a modulator of TFAM acetylation and binding activity (Aquilano et al., 2010). Finally, acute exercise has also been shown to stimulate translocation of p53 protein from nuclei to mitochondria and formation of p53-TFAM complexes at the mtDNA D-loop. Such complexes might lead to the increased mitochondrial transcription needed and verified after exercise (Saleem and Hood, 2013). Post-translational modifications of TFAM might likely influence the effects of the complexes, including the factor, on mtDNA functions. All these factors should mediate the crosstalk between nuclear and mitochondrial genomes, affecting the regulation of mitochondrial biogenesis in response to changing cell conditions.

3. TFAM–mtDNA interactions in aged and calorie-restricted animals

Even if TFAM–mtDNA interactions have been deeply studied *in vitro*, how TFAM regulates mtDNA copy number *in vivo* is still controversial. TFAM-mediated increase in mtDNA content has been explained by two different mechanisms. According to the first one, transcription-mediated priming of replication should be increased by a higher frequency of TFAM binding at LSP; the second one, instead, proposes that the genome-wide binding of TFAM stabilizes mtDNA content, maybe decreasing DNA turnover. These models are not alternate, but suggest that multiple mechanisms could regulate TFAM activity and contribute to copy number control (Campbell et al., 2012). Furthermore, modulation of TFAM expression and binding activity to mtDNA (Fisher et al., 1987) might be involved in another model proposed to explain TFAM-mediated copy number control and derived from experiments on tissue samples from aged, *ad libitum*-fed or calorie-restricted, rats (Picca et al., 2014). Although it is true that TFAM binds to any DNA sequence, it preferentially binds sites upstream of LSP and HSP in mammal mtDNA, as shown also by DNase I footprinting experiments (Fisher et al., 1992; Ohgaki et al., 2007). The wide distribution of TFAM across the whole mtDNA in HeLa cells has been recently demonstrated by chromatin immunoprecipitation analysis and this supports the abundant association of TFAM with mtDNA in cultured cells (Ohgaki et al., 2007; Wang et al., 2013). Therefore, we decided to evaluate the eventual alterations of the TFAM binding activity to specific regions of mtDNA in a physiological situation as aging of rat different tissues. Effectively, aging is a very complex phenomenon that implies the progressive structural and functional decline of tissues also through the age-dependent dysfunction of the mitochondrial respiratory complexes, reducing the amount of synthesized adenosine triphosphate (ATP) (Navarro and Boveris, 2004). According to the classic mitochondrial free radical theory of aging, mtDNA mutations originate an endogenous oxidative stress that progressively amplifies the mutational load leading to a *vicious cycle*. In the last few years, several findings have suggested that mtDNA mutations might be due more to replication errors and failure of the repair mechanisms rather than to damages by free radicals (Pinto and Moraes, 2015; Payne and Chinnery, 2015; Kauppila and Stewart, 2015; Szczepanowska and Trifunovic, 2015). In this view, the reported age-dependent accumulation of such mutations seems to occur by clonal expansion (Baines et al., 2014; Greaves et al., 2014). Furthermore, in current understanding, the involvement of mitochondrial dysfunction in aging goes further beyond the accumulation of mtDNA mutations, including alterations in other crucial mitochondrial functions as biogenesis, turnover, dynamics and protein quality control (Rugarli and Trifunovic, 2015). On the other hand, the role of ROS as signaling molecules has been reevaluated in several processes affecting aging as cellular senescence (Correia-Melo and Passos, 2015) and stem cell homeostasis (Ahlqvist et al., 2015). Finally, there is substantial evidence that mitochondrial oxidative stress, related to ROS and electron transport chain dysfunction, can extend lifespan regulating nuclear genes expression through retrograde signaling pathways (Ristow and Schmeisser, 2011; Schroeder et al., 2013). The involvement of mitochondria in aging is tissue-specific (Anderson and Weindruch, 2010) and is particularly relevant in tissues with a high dependence on oxidative metabolism such as the brain, heart and skeletal muscle (Wallace, 1992). However, other metabolically very active tissues, as liver, are also affected by the age-related mitochondrial dysfunction (Navarro and Boveris, 2004). Calorie restriction (CR) is so-far the only experimental approach able to delay or prevent the onset of several age-related alterations, also in mitochondria, in organisms ranging from yeast to man (Masoro, 2005; Guarente, 2008). Such treatment involves the administration of a nutrient dense diet that reduces calorie intake by 20–40% without malnutrition (Fontana, 2009) and exhibits a very marked tissue-specificity (Park and Prolla, 2005). Even if a precise pathway for

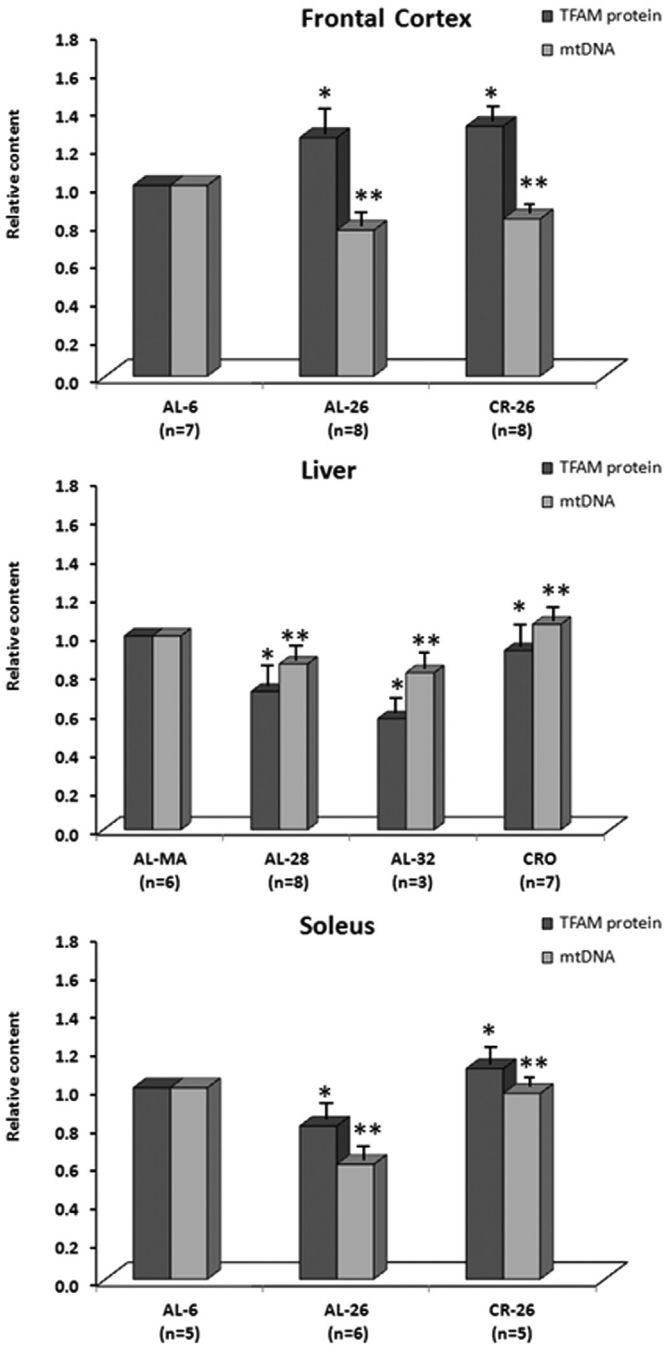

Fig. 3. Age- and CR-related changes of TFAM and mtDNA contents in three rat tissues.

CR has not been defined yet, among the likely multiple cellular mechanisms at the basis of CR efficacy, for a long time, the reduction of age-related oxidative stress obtained large credit (Guarente, 2008; Barja, 2007). However, the regulation of mitochondrial metabolism through activation of the PGC-1α-dependent cascade of mitochondrial biogenesis has also gained a wide consensus as one of the means by which CR counteracts the age-dependent dysfunction (Anderson and Prolla, 2009). Interestingly, more recently, the beneficial effects of CR on the aging process have been suggested to act through the attenuation of the age-related decline in mitochondrial function by means of a complex crosstalk among energy-sensing pathways (Ruetenik and Barrientos, 2015).

The tissue-specific effects of aging and CR on TFAM amount (Picca et al., 2013a,b) and mtDNA content (Picca et al., 2013a,b; Cassano et al., 2006) in three rat tissues (Picca et al., 2014) can be compared in Fig. 3.

TFAM and mtDNA relative contents in (A) frontal cortex, (B) liver, and (C) soleus skeletal muscle (Picca et al., 2013a,b, 2014). The histograms show the relative content of TFAM protein (dark bar) in aged, *ad libitum*-fed or calorie-restricted rats, compared to young rats, all normalized with respect to β-actin. *$p < 0.05$ versus the value of the young rats (fixed as 1); n, number of analyzed animals. The clear bars in the histograms show the relative content of mtDNA in aged, *ad libitum*-fed or calorie-restricted rats, compared to young rats. **$p < 0.05$ versus the value of the young rats (fixed as 1); n, number of analyzed animals.

As for TFAM amount, we found an age-related increase in the frontal cortex versus a decrease in the liver and soleus. The CR effect in the frontal cortex, consistent with what previously reported (Picca et al., 2013b), appeared very mild, leading to a TFAM increase not statistically different from that in the aged *ad libitum*-fed rats. On the contrary, CR completely prevented the age-related decrease in TFAM in the other post-mitotic tissue, the soleus, suggesting a relevant sensitivity to nutrient availability as well as in the liver. We analyzed in the same groups of samples the mtDNA content by Real Time-PCR and *in vivo* TFAM-binding activity to specific mtDNA regions with the mtDNA immunoprecipitation (mIP) technique, followed by the semi-quantitative (Picca et al., 2014) or quantitative determination (Picca et al., 2013a,b) of the mtDNA amount bound by TFAM at the assayed regions. We found a shared age-related decrease in mtDNA content and, again, a differential response in sensitivity to CR between the frontal cortex by one side and the soleus and liver by the other. The common age-related loss of mtDNA, however, might be explained by two different tissue-specific mechanisms. In fact, the age-related decrease in TFAM-binding in the frontal cortex might imply decreased mtDNA replication and/or increased mtDNA damage, not counteracted by the usual repair mechanisms (Picca et al., 2013b). On the contrary, the age-related increased TFAM-binding at both origins of replication in the liver and soleus might explain the mtDNA loss through a hindered mtDNA replication. The increased physical occupation by TFAM of the sequences usually involved in the primase activity performed by POLRMT might lead to the corresponding loss of mtDNA (Picca et al., 2013a; Cassano et al., 2006; Pesce et al., 2005; Barazzoni et al., 2000). Furthermore, the age-related increase in TFAM-binding at the replication origins described in rat soleus and liver (Picca et al., 2013a, 2014) might physically prevent also the access of factors/activities involved in the repair mechanisms to fix the age-related mtDNA modifications and/or mutations. It might occur, in aged soleus and liver, an effect, due to increased TFAM-binding, similar to that demonstrated by *in vitro* titration experiments, where the increase in TFAM concentrations, above a certain threshold, prevented rather than enhanced transcription at the HSP2 promoter region (Shutt et al., 2011; Dairaghi et al., 1995b; Lodeiro et al., 2012; Zollo et al., 2012). Furthermore, the absence of an age-related decrease in mitochondrial transcription in rat liver and soleus muscle (Barazzoni et al., 2000; Gadaleta et al., 1990) is consistent with an increase in TFAM-binding at the D-loop sub-region, not exceeding a functional threshold above which transcription efficiency might be affected. On the contrary, a suboptimal binding of TFAM at the D-loop region in the frontal cortex might explain also the brain-specific, age-related, reduced mitochondrial transcription previously described (Gadaleta et al., 1990). The full efficacy of CR in preventing the age-related mtDNA loss in both the soleus and liver might be linked to the preservation of TFAM-binding activities, especially at the origins of replication, similar to those of the younger animals. On the contrary, CR in frontal cortex did not significantly preserve mtDNA content and TFAM binding activity. The age- and CR-related modulation of TFAM-binding activity to the mtDNA regions encompassing the origins of replication, namely LSP for the H strand and OriL for the L strand, allow us to indicate such binding among the molecular triggers for the physiological control, exerted by TFAM, above mtDNA copy number (Fig. 3).

The effects on mitochondrial biogenesis deriving from such *in vivo* modulation of TFAM-binding to mtDNA are strongly consistent with findings from very recent *in vitro* experiments demonstrating that high TFAM:mtDNA ratios reduce the progression of replication and transcription complexes. At a low TFAM to DNA ratio (1 TFAM/60 bp),

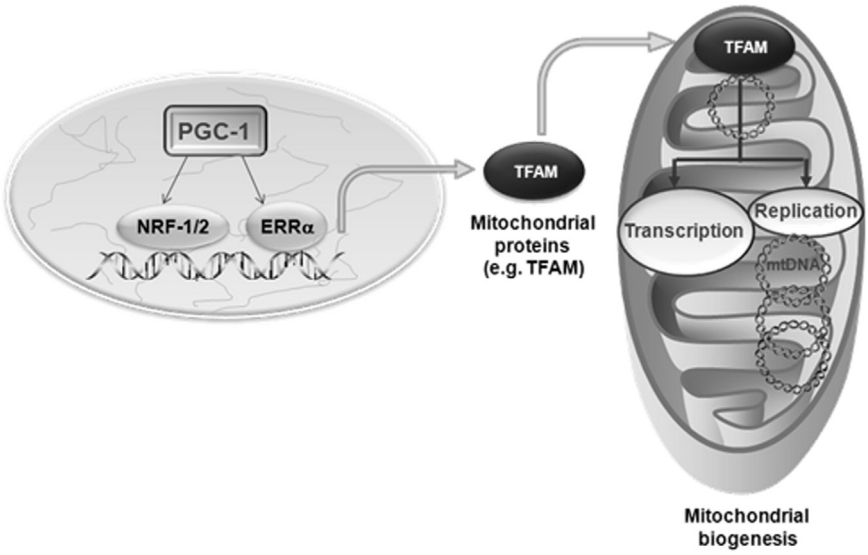

Fig. 4. Regulation of mitochondrial biogenesis.

a robust DNA synthesis has been observed, whereas a TFAM to DNA ratio increased to 1 TFAM/12 bp has led to a strong decrease in DNA synthesis (Farge et al., 2014).

4. Current and future developments

As highlighted above, modulation of TFAM interactions with mtDNA is crucial to regulate mitochondrial biogenesis and adapt it to the always changing cell conditions. Such modulation can be obtained likely through multiple mechanisms among which alteration of the binding activity of TFAM to mtDNA and regulation of TFAM expression appear relevant (Fig. 4).

PGC-1 coactivators are master regulators of mitochondrial biogenesis, obtained through the coordinated expression of mitochondrial proteins with a double genetic origin. The PGC-1-mediated activation of transcription factors as NRF-1/2 and ERRα increase the expression of mitochondrial proteins encoded by nuclear DNA as TFAM. Upregulation of TFAM drives an increase in replication and expression of mtDNA.

In particular, due to the key role exerted on TFAM expression by the PGC-1 family of coactivators as well as by the mitochondrial quality control, acting through mitophagy, there should exist different molecular sensors linking the regulation of TFAM expression to the cell/environmental stimuli. Such link could be pursued through the regulation of the PGC-1 coactivators. Induction of mitochondrial biogenesis, obtained through the sequential activation of a number of transcription factors as NRF-1/2 and ERRα, leads to increased expression of nuclear DNA-encoded mitochondrial proteins. These include TFAM, whose upregulation raises both mtDNA replication and expression of mtDNA-encoded proteins. Due to the relevant role in mitochondrial biogenesis, which should be induced to counteract the age-related reduction of mitochondrial functionality, TFAM is a potential target for gene therapy (Keeney et al., 2009). TFAM ability to act as a cytoprotectant in cancer chemotherapy and to confer resistance to drugs such as cisplatin will also be thoroughly examined in the next future (Pastukh et al., 2007). Recent findings about the effects of aging, CR and other conditions on TFAM binding to mtDNA indicate that this kind of modulation can integrate with that resulting from TFAM expression and lead to a very complex regulation of mitochondrial biogenesis. Further study is, therefore, needed to shed light on the very intricate control of mitochondrial biogenesis, deeply affected by the changes in mitochondrial energy metabolism occurring during lifespan and pathological conditions (Scarpulla, 2008).

Conflict of interest

The authors have no actual or potential conflict of interest associated with this research.

Acknowledgments

This research was supported by grant to AMSL (Contributo di Ateneo Bari 2012). We thank Flavio Fracasso, MS, for assisting with the figures.

References

Ahlqvist, K.J., Suomalainen, A., Hämäläinen, R.H., 2015. Stem cells, mitochondria and aging. Biochim. Biophys. Acta 1847, 1380–1386.
Anderson, R., Prolla, T., 2009. PGC-1alpha in aging and anti-aging interventions. Biochim. Biophys. Acta 1790, 1059–1066.
Anderson, R.M., Weindruch, R., 2010. Metabolic reprogramming, caloric restriction and aging. Trends Endocrinol. Metab. 21, 134–141.
Aquilano, K., Vigilanza, P., Baldelli, S., Pagliei, B., Rotilio, G., Ciriolo, M.R., 2010. Peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. J. Biol. Chem. 285 (28), 21,590–21,599.
Attardi, G., Schatz, G., 1988. Biogenesis of mitochondria. Annu. Rev. Cell Biol. 4, 289–333.
Baines, H.L., Stewart, J.B., Stamp, C., Zupanic, A., Kirkwood, T.B.L., Larsson, N.-G., et al., 2014. Similar patterns of clonally expanded somatic mtDNA mutations in the colon of heterozygous mtDNA mutator mice and ageing humans. Mech. Ageing Dev. 139, 22–30.
Barazzoni, R., Short, K.R., Nair, K.S., 2000. Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart. J. Biol. Chem. 275, 3343–3347.
Barja, G., 2007. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res. 10, 215–224.
Bender, T., Lewrenz, I., Franken, S., Baitzel, C., Voos, W., 2011. Mitochondrial enzymes are protected from stress-induced aggregation by mitochondrial chaperones and the Pim1/LON protease. Mol. Biol. Cell 22, 541–554.
Bestwick, M.L., Shadel, G.S., 2013. Accessorizing the human mitochondrial transcription machinery. Trends Biochem. Sci. 38, 283–291.
Bogenhagen, D.F., 2012. Mitochondrial DNA nucleoid structure. Biochim. Biophys. Acta 1819, 914–920.
Bogenhagen, D.F., Rousseau, D., Burke, S., 2008. The layered structure of human mitochondrial DNA nucleoids. J. Biol. Chem. 283, 3665–3675.
Bonawitz, N.D., Clayton, D.A., Shadel, G.S., 2006. Initiation and beyond: multiple functions of the human mitochondrial transcription machinery. Mol. Cell 24, 813–825.
Brown, T.A., Cecconi, C., Tkachuk, A.N., Bustamante, C., Clayton, D.A., 2005. Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. Genes Dev. 19, 2466–2476.
Campbell, C.T., Kolesar, J.E., Kaufman, B.A., 2012. Mitochondrial transcription factor A regulates mitochondrial transcription initiation, DNA packaging, and genome copy number. Biochim. Biophys. Acta 1819, 921–929.
Canugovi, C., Maynard, S., Bayne, A.C., Sykora, P., Tian, J., Souza-Pinto, N.C., et al., 2010. The mitochondrial transcription factor A functions in mitochondrial base excision repair. DNA Repair (Amst) 9, 1080–1089.
Cassano, P., Sciancalepore, A.G., Lezza, A.M., Leeuwenburgh, C., Cantatore, P., Gadaleta, M.N., 2006. Tissue-specific effect of age and caloric restriction diet on mitochondrial DNA content. Rejuvenation Res. 9, 211–214.
Chang, D.D., Clayton, D.A., 1985. Priming of human mitochondrial DNA replication occurs at the light-strand promoter. Proc. Natl. Acad. Sci. U. S. A. 82 (2), 351–355.
Chang, D.D., Hauswirth, W.W., Clayton, D.A., 1985. Replication priming and transcription initiate from precisely the same site in mouse mitochondrial DNA. EMBO J. 4 (6), 1559–1567.
Correia-Melo, C., Passos, J.F., 2015. Mitochondria: are they causal players in cellular senescence? Biochim. Biophys. Acta 1847, 1373–1379.
Cotney, J., Wang, Z., Shadel, G.S., 2007. Relative abundance of the human mitochondrial transcription system and distinct roles for h-mtTFB1 and h-mtTFB2 in mitochondrial biogenesis and gene expression. Nucleic Acids Res. 35, 4042–4054.
Dairaghi, D.J., Shadel, G.S., Clayton, D.A., 1995a. Addition of a 29 residue carboxyl-terminal tail converts a simple HMG box-containing protein into a transcriptional activator. J. Mol. Biol. 249, 11–28.
Dairaghi, D.J., Shadel, G.S., Clayton, D.A., 1995b. Human mitochondrial transcription factor A and promoter spacing integrity are required for transcription initiation. Biochim. Biophys. Acta 1271, 127–134.
Dinardo, M.M., Musicco, C., Fracasso, F., Milella, F., Gadaleta, M.N., Gadaleta, G., et al., 2003. Acetylation and level of mitochondrial transcription factor A in several organs of young and old rats. Biochem. Biophys. Res. Commun. 301, 187–191.
Ekstrand, M.I., Falkenberg, M., Rantanen, A., Park, C.B., Gaspari, M., Hultenby, K., et al., 2004. Mitochondrial transcription factor A regulates mtDNA copy number in mammals. Hum. Mol. Genet. 13, 935–944.
Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N.-G., Gustafsson, C.M., 2002. Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat. Genet. 31, 289–294.
Farge, G., Laurens, N., Broekmans, O.D., van den Wildenberg, S.M., Dekker, L.C., Gaspari, M., et al., 2012. Protein sliding and DNA denaturation are essential for DNA organization by human mitochondrial transcription factor A. Nat. Commun. 3, 1013.
Farge, G., Mehmedovic, M., Baclayon, M., van den Wildenberg, S.M.J.L., Roos, V.H., Gustfsson, C.M., et al., 2014. In vitro-reconstituted nucleoids can block mitochondrial DNA replication and transcription. Cell Rep. 8, 1–9.
Finck, B.N., Kelly, D.P., 2006. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J. Clin. Investig. 116, 615–622.
Fisher, R.P., Clayton, D.A., 1988. Purification and characterization of human mitochondrial transcription factor 1. Mol. Cell. Biol. 8, 3496–3509.
Fisher, R.P., Topper, J.N., Clayton, D.A., 1987. Promoter selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements. Cell 50, 247–258.
Fisher, R.P., Parisi, M.A., Clayton, D.A., 1989. Flexible recognition of rapidly evolving promoter sequences by mitochondrial transcription factor 1. Genes Dev. 3, 2202–2217.
Fisher, R.P., Lisowsky, T., Parisi, M.A., Clayton, D.A., 1992. DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein. J. Biol. Chem. 267, 3358–3367.
Fontana, L., 2009. The scientific basis of caloric restriction leading to longer life. Curr. Opin. Gastroenterol. 25, 144–150.
Freyer, C., Park, C.B., Ekstrand, M.I., Shi, Y., Khvorostova, J., Wibom, R., et al., 2010. Maintenance of respiratory chain function in mouse hearts with severely impaired mtDNA transcription. Nucleic Acids Res. 38, 6577–6588.
Fusté, J.M., Wanrooij, S., Jemt, E., Granycome, C.E., Cluett, T.J., Shi, Y., et al., 2010. Mitochondrial RNA polymerase is needed for activation of the origin of light-strand DNA replication. Mol. Cell 37 (1), 67–78.

Gadaleta, M.N., Petruzzella, V., Renis, M., Fracasso, F., Cantatore, P., 1990. Reduced transcription of mitochondrial DNA in the senescent rat. Tissue dependence and effect of L-carnitine. Eur. J. Biochem. 187, 501–506.
Greaves, L.C., Nooteboom, M., Elson, J.L., Tuppen, H.A.L., Taylor, G.A., Commane, D.M., et al., 2014. Clonal expansion of early to mid-life mitochondrial DNA point mutations drives mitochondrial dysfunction during human ageing. PLoS Genet. 10, e1004620. http://dx.doi.org/10.1371/journal.pgen.1004620.
Guarente, L., 2008. Mitochondria — a nexus for aging, calorie restriction, and sirtuins? Cell 132, 171–176.
Handschin, C., Spiegelman, B.M., 2006. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr. Rev. 27, 728–735.
Hayashi, Y., Yoshida, M., Yamato, M., Ide, T., Wu, Z., Ochi-Shindou, M., et al., 2008. Reverse of age-dependent memory impairment and mitochondrial DNA damage in microglia by an overexpression of human mitochondrial transcription factor A in mice. J. Neurosci. 28, 8624–8634.
Hebert, A.S., Dittenhafer-Reed, K.E., Yu, W., Bailey, D.J., Selen, E.S., Boersma, M.D., et al., 2013. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. Mol. Cell 49, 186–199.
Holt, I.J., Lorimer, H.E., Jacobs, H.T., 2000. Coupled leading- and lagging strand synthesis of mammalian mitochondrial DNA. Cell 100, 515–524.
Ikeuchi, M., Matsusaka, H., Kang, D., Matsushima, S., Ide, T., Kubota, T., et al., 2005. Overexpression of mitochondrial transcription factor A ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 112, 683–690.
Joseph, A.M., Rungi, A.A., Robinson, B.H., Hood, D.A., 2004. Compensatory responses of protein import and transcription factor expression in mitochondrial DNA defects. Am. J. Physiol. Cell Physiol. 286 (4), C 867–C 875.
Kanki, T., Ohgaki, K., Gaspari, M., Gustafsson, C.M., Fukuoh, A., Sasaki, et al., 2004. Architectural role of mitochondrial transcription factor A in maintenance of human mitochondrial DNA. Mol. Cell. Biol. 24, 9823–9834.
Kaufman, B.A., Durisic, N., Mativetsky, J.M., Costantino, S., Hancock, M.A., Grutter, P., 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.
Kauppila, J.H.K., Stewart, J.B., 2015. Mitochondrial DNA: radically free of free-radical driven mutations. Biochim. Biophys. Acta 1847, 1354–1361.
Keeney, P.M., Quigley, C.K., Dunham, L.D., Papageorge, C.M., Iyer, S., Thomas, R.R., et al., 2009. Mitochondrial gene therapy augments mitochondrial physiology in a Parkinson's disease cell model. Hum. Gene Ther. 20, 897–907.
Kukat, C., Wurm, C.A., Spahr, H., Falkenberg, M., Larsson, N.-G., Jakobs, S., 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.
Kukat, C., Davies, K.M., Wurm, C.A., Spåhr, H., Bonekamp, N.A., Kühl, I., et al., 2015. Cross-strand binding of TFAM to a single mtDNA molecule forms the mitochondrial nucleoid. PNAS 112, 11288–11293.
Larsson, N.-G., Wang, J., Wilhelmsson, H., Oldfors, A., Rustin, P., Lewandoski, M., et al., 1998. Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat. Genet. 18, 231–236.
Lin, J., Puigserver, P., Donovan, J., Tarr, P., Spiegelman, B.M., 2002. Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J. Biol. Chem. 277, 1645–1648.
Litonin, D., Sologub, M., Shi, Y., Savkina, M., Anikin, M., Falkenberg, M., 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, 18129–18133.
Liu, T., Lu, B., Lee, I., Ondrovicová, G., Kutejová, E., Suzuki, C.K., 2004. DNA and RNA binding by the mitochondrial lon protease is regulated by nucleotide and protein substrate. J. Biol. Chem. 279, 13902–13910.
Lodeiro, M.F., Uchida, A., Bestwick, M., Moustafa, I.M., Arnold, J.J., Shadel, G.S., 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.
Lu, B., Yadav, S., Shah, P.G., Liu, T., Tian, B., Pukszta, S., et al., 2007. Roles for the human ATP-dependent Lon protease in mitochondrial DNA maintenance. J. Biol. Chem. 282, 17363–17374.
Lu, B., Lee, J., Nie, X., Li, M., Morozov, Y.I., Venkatesh, S., et al., 2013. Phosphorylation of human TFAM in mitochondria impairs DNA binding and promotes degradation by the AAA+ Lon protease. Mol. Cell 49, 121–132.
Maniura-Weber, K., Goffart, S., Garstka, H.L., Montoya, J., Wiesner, R.J., 2004. 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, 6015–6027.
Masoro, E.J., 2005. Overview of caloric restriction and ageing: an update. Mech. Ageing Dev. 126, 913–922.
Matsushima, Y., Goto, Y.-I., Kaguni, L.S., 2010. Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). Proc. Natl. Acad. Sci. U. S. A. 107, 18410–18415.
Mirebeau-Prunier, D., Le Pennec, S., Jacques, C., Gueguen, N., Poirier, J., Malthiery, Y., et al., 2010. Estrogen-related receptor alpha and PGC-1-related coactivator constitute a novel complex mediating the biogenesis of functional mitochondria. FEBS J. 277, 713–725.
Navarro, A., Boveris, A., 2004. Rat brain and liver mitochondria develop oxidative stress and lose enzymatic activities on aging. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287 (543), R1244–R1249.
Ngo, H.B., Kaiser, J.T., Chan, D.C., 2011. The mitochondrial transcription and packaging factor Tfam imposes a U-turn on mitochondrial DNA. Nat. Struct. Mol. Biol. 18, 1290–1296.
Noack, H., Bednarek, T., Heidler, J., Ladig, R., Holtz, J., Szibor, M., 2006. TFAM-dependent and independent dynamics of mtDNA levels in C2C12 myoblasts caused by redox stress. Biochim. Biophys. Acta 1760, 141–150.
Ohgaki, K., Kanki, T., Fukuoh, A., Kurisaki, H., Aoki, Y., Ikeuchi, M., et al., 2007. The C-terminal tail of mitochondrial transcription factor A markedly strengthens its general binding to DNA. J. Biol. Chem. 141, 201–211.
Parisi, M.A., Clayton, D.A., 1991. Similarity of human mitochondrial transcription factor 1 to high mobility group proteins. Science 252, 965–969.
Park, S.K., Prolla, T.A., 2005. Lessons learned from gene expression profile studies of aging and caloric restriction. Ageing Res. Rev. 4, 55–65.
Pastukh, V., Shokolenko, I., Wang, B., Wilson, G., Alexeyev, M., 2007. Human mitochondrial transcription factor A possesses multiple subcellular targeting signals. FEBS J. 274, 6488–6499.
Payne, B.A., Chinnery, P.F., 2015. Mitochondrial dysfunction in aging: much progress but many unresolved questions. Biochim. Biophys. Acta 1847, 1347–1353.
Peralta, S., Wang, X., Moraes, C.T., 2012. Mitochondrial transcription: lessons from mouse models. Biochim. Biophys. Acta 1819 (9–10), 961–969.
Pesce, V., Cormio, A., Fracasso, F., Lezza, A.M., Cantatore, P., Gadaleta, M.N., 2005. Age-related changes of mitochondrial DNA content and mitochondrial genotypic and phenotypic alterations in rat hind-limb skeletal muscles. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 60, 715–723.
Philp, A., Belew, M.Y., Evans, A., Pham, D., Silvia, I., Chen, A., et al., 2011. The PGC-1{alpha}-related co-activator (PRC) promotes mitochondrial and myogenic adaptations in C2C12 myotubes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301, 864–872.
Picca, A., Pesce, V., Fracasso, F., Joseph, A.-M., Leeuwenburgh, C., Lezza, A.M.S., 2013a. Aging and calorie restriction oppositely affect mitochondrial biogenesis through TFAM binding at both origins of mitochondrial DNA replication in rat liver. PLoS One 8 (9), e74644.
Picca, A., Fracasso, F., Pesce, V., Cantatore, P., Joseph, A.-M., Leeuwenburgh, C., et al., 2013b. Age- and calorie restriction-related changes in rat brain mitochondrial DNA and TFAM binding. AGE 35, 1607–1620.
Picca, A., Pesce, V., Fracasso, F., Joseph, A.-M., Leeuwenburgh, C., Lezza, A.M.S., 2014. A comparison among the tissue-specific effects of aging and calorie restriction on TFAM amount and TFAM-binding activity to mtDNA in rat. Biochim. Biophys. Acta 1840 (7), 2184–2191.
Pinto, M., Moraes, C.T., 2015. Mechanisms linking mtDNA damage and aging. Free Radic. Biol. Med. 85, 250–258.
Pohjoismäki, J.L.O., Wanrooij, S., Hyvärinen, A.K., Goffart, S., Holt, I.J., Spelbrink, J.N., et al., 2006. Alterations to the expression level of mitochondrial transcription factor A, TFAM, modify the mode of mitochondrial DNA replication in cultured human cells. Nucleic Acids Res. 34, 5815–5828.
Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M., Spiegelman, B.M., 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829–839.
Rebelo, A.P., Dillon, L.M., Moraes, C.T., 2011. Mitochondrial DNA transcription regulation and nucleoid organization. J. Inherit. Metab. Dis. 34, 941–951.
Ristow, M., Schmeisser, S., 2011. Extending life span by increasing oxidative stress. Free Radic. Biol. Med. 51, 327–336.
Rubio-Cosials, A., Sidow, J.F., Jiménez-Menéndez, N., Fernandez-Millan, P., Montoja, J., Jacobs, H.T., et al., 2011. Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. Nat. Struct. Mol. Biol. 18, 1281–1289.
Ruetenik, A., Barrientos, A., 2015. Dietary restriction, mitochondrial function and aging: from yeast to humans. Biochim. Biophys. Acta 1847, 1434–1447.
Rugarli, E., Trifunovic, A., 2015. Is mitochondrial free radical theory of aging getting old? Biochim. Biophys. Acta 1847, 1345–1346.
Safdar, A., Little, J.P., Stokl, A.J., Hettinga, B.P., Akhtar, M., Tarnopolsky, M.A., 2011. Exercise increases mitochondrial PGC-1α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. J. Biol. Chem. 286 (12), 10,605–10,617.
Saleem, A., Hood, D.A., 2013. Acute exercise induces tumour suppressor protein p53 translocation to the mitochondrial and promotes a p53-Tfam-mitochondrial DNA complex in skeletal muscle. J. Physiol. 591 (14), 3625–3636.
Santos, J.M., Mishra, M., Kowluru, R.A., 2014. Post-translational modification of mitochondrial transcription factor A in impaired mitochondria biogenesis: implications in diabetic retinopathy and metabolic memory phenomenon. Exp. Eye Res. 121, 168–177.
Scarpulla, R.C., 2008. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol. Rev. 88, 611–638.
Schreiber, S.N., Knutti, D., Brogli, K., Uhlmann, T., Kralli, A., 2003. The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor alpha (ERRalpha). J. Biol. Chem. 278, 9013–9018.
Schroeder, E.A., Raimundo, N., Shadel, G.S., 2013. Epigenetic silencing mediates mitochondria stress-induced longevity. Cell Metab. 17, 954–964.
Shi, Y., Chan, D.W., Jung, S.Y., Malovannaya, A., Wang, Y., Qin, J., 2011. A data set of human endogenous protein ubiquitination sites. Mol. Cell. Proteomics 10 (M110.002089).
Shi, Y., Dierckx, A., Wanrooij, P.H., Wanrooij, S., Larsson, N.-G., Wilhelmsson, L.M., et al., 2012. Mammalian transcription factor A is a core component of the mitochondrial transcription machinery. PNAS 109, 16510–16515.
Shutt, T.E., Lodeiro, M.F., Cotney, J., Cameron, C.E., Shadel, G.S., 2010. Core human mitochondrial transcription apparatus is a regulated two-component system in vitro. Proc. Natl. Acad. Sci. U. S. A. 107, 12133–12138.
Shutt, T.E., Bestwick, M., Shadel, G.S., 2011. The core human mitochondrial transcription initiation complex: it only takes two to tango. Transcription 2, 55–59.
Szczepanowska, K., Trifunovic, A., 2015. Different faces of mitochondrial DNA mutators. Biochim. Biophys. Acta 1847, 1362–1372.
Takamatsu, C., Umeda, S., Ohsato, T., Ohno, T., Abe, Y., Fukuoh, A., et al., 2002. Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. EMBO Rep. 3, 451–456.

Virbasius, C.A., Virbasius, J.V., Scarpulla, R.C., 1993a. NRF-1, an activator involved in nuclear-mitochondrial interactions, utilizes a new DNA-binding domain conserved in a family of developmental regulators. Genes Dev. 7, 2431–2445.

Virbasius, J.V., Virbasius, C.A., Scarpulla, R.C., 1993b. Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. Genes Dev. 7, 380–392.

Wallace, D.C., 1992. Mitochondrial genetics: a paradigm for aging and degenerative diseases? Science 256, 628–632.

Wang, Y.E., Marinov, G.K., Wold, B.J., Chan, D.C., 2013. Genome-wide analysis reveals coating of the mitochondrial genome by TFAM. PLoS One 8 (8), e74513.

Wang, K.Z., Zhu, J., Dagda, R.K., Uechi, G., Cherra III, S.J., Gusdon, A.M., et al., 2014. ERK-mediated phosphorylation of TFAM downregulates mitochondrial transcription: implications for Parkinson's disease. Mitochondrion 17, 132–140.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.

Yang, M.Y., Bowmaker, M., Reyes, A., Vergani, L., Angeli, P., Gringeri, E., et al., 2002. Biased incorporation of ribonucleotides on the mitochondrial L-strand accounts for apparent strand-asymmetric DNA replication. Cell 111, 495–505.

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

Zollo, O., Tiranti, V., Sondheimer, N., 2012. Transcriptional requirements of the distal heavy-strand promoter of mtDNA. Proc. Natl. Acad. Sci. U. S. A. 109, 6508–6512.