Experimental Physiology

Special Review Series – Biogenesis and Physiological Adaptation of Mitochondria

Replication and transcription of mammalian mitochondrial DNA

Patricio Fernández-Silva *, José A. Enriquez and Julio Montoya

Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, Miguel Servet 177. 50013 Zaragoza, Spain

Mitochondria are subcellular organelles, devoted mainly to energy production in the form of ATP, that contain their own genetic system. Mitochondrial DNA codifies a small, but essential number of polypeptides of the oxidative phosphorylation system. The mammalian mitochondrial genome is an example of extreme economy showing a compact gene organization. The coding sequences for two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and 13 polypeptides are contiguous and without introns. The tRNAs are regularly interspersed between the rRNA and protein-coding genes, playing a crucial role in RNA maturation from the polycistronic transcripts. A single major non-coding region, called the D-loop region, contains the main regulatory sequences for transcription and replication initiation. This genetic organization has its precise correspondence in the mode of expression and distinctive structural features of the RNAs. The basic mechanisms of mitochondrial DNA transcription and replication and the main cis-acting elements playing a role in both processes have been determined. Many trans-acting factors involved in mitochondrial gene expression, including the RNA and DNA polymerases, have been cloned or identified. However, the regulatory mechanisms participating in mitochondrial gene expression are still poorly understood. The interest to complete this knowledge is increased by the involvement of mitochondria in human diseases, in basic processes such as heat production, Ca^{2+} homeostasis and apoptosis, and by their potential role in ageing and carcinogenesis. *Experimental Physiology* (2003) **88.1**, 41–56.

Contents Page

Mitochondria are eukaryotic organelles involved in many metabolic pathways, but their principal function is the generation of most of the cellular ATP through the oxidative phosphorylation system (OXPHOS) (Attardi & Schatz, 1988).

From the point of view of their biogenesis, mitochondria are unique organelles since they require the contribution of two physically separated genomes. The majority of mitochondrial proteins are encoded in the nucleus and synthesized in the cytoplasm, usually as precursors that must be imported and processed inside the organelle (see article by Stojanovski *et al.* in this issue). The mitochondrial genome contributes only a small set of polypeptides that are essential for the OXPHOS system functionality, as revealed by the appearance of human diseases linked to defects in their synthesis (Wallace, 1992).

Concerning its evolutionary origin, the most established and accepted hypothesis is that mitochondria derive from α-proteobacteria that entered the eukaryotic cell ancestor as an endosymbiont around two thousand million years ago (Lang *et al.* 1999). During the course of evolution most of the genes from the endosymbiont were transferred to

Publication of *The Physiological Society*

* Corresponding author: pfsilva@posta.unizar.es

the nuclear chromosomes. The existence of a genetic cytoplasmic element, different from the nucleus, was first proposed in the late 1940s after the description of yeast mutants showing respiratory defects transmissible through a cytoplasmic factor (Ephrussi *et al.* 1949). Mitochondrial DNA (mtDNA) was discovered in the 1960s (Nass & Nass, 1963), and since then a detailed picture of its structure, genetic content and mode of expression has been obtained.

The complete nucleotide sequence of mtDNA from many animal species is known (Wolstenholme, 1992; an updated compilation of such sequences can be found at: http://megasun.bch.umontreal.ca/gobase/), the human mitochondrial genome being the most well analysed. Actually, the first human genomic entity to be completely sequenced was mtDNA in the early 1980s (Anderson *et al.* 1981). Although the size, gene organization and expression mode is very different in different phyla, the basic gene content and function of mtDNA is extremely well conserved throughout evolution comprising a small set of protein subunits of the OXPHOS complexes and the rRNAs and tRNAs needed for their translation (Attardi & Schatz, 1988).

The mitochondrial genome has some characteristics that are different to those of the nuclear genome. These include the use of a divergent genetic code, transmission by maternal inheritance, a higher rate of mutation, the phenomenon of polyploidy and a specific organization and expression mode. Although most of the knowledge about the structure and the basic mechanisms of mtDNA replication and transcription was produced about two decades ago (Clayton, 1982; Attardi, 1985), the dissection of the machinery involved in these processes, their regulation and coordination with nuclear gene expression has progressed more slowly and much has still to be learned.

The discovery of an increasing number of human diseases caused by mutations in mtDNA (Zeviani & Antozzi, 1997; Wallace *et al.* 1999; see also article by von Kleist-Retzow *et al.* in this issue), the possible role of this genome in ageing (Wallace, 1997; see also article by Hofhaus *et al.* in this issue) and in the development of carcinogenesis (Penta *et al.* 2001) and the involvement of mitochondria in the process of apoptosis (Kroemer & Reed, 2000; see also article by Gulbins *et al.* in this issue) has fuelled an explosion in the research on mitochondria and mtDNA in recent years.

This review will focus on the main characteristics of the mechanisms and factors involved in the replication and transcription of mtDNA, in mammals and particularly in humans, with some hints on the regulation of both processes. Excellent reviews covering other aspects of these topics can be found in the literature (Attardi & Schatz, 1988; Wallace, 1992; Shadel & Clayton, 1997; Taanman, 1999; Lecrenier & Foury, 2000; Moraes, 2001).

Peculiarities of the mitochondrial genetic system

The studies in the field of mitochondrial genetics have unravelled a series of peculiarities and differences compared to the nuclear genome.

(1) Cells are polyploid with respect to mtDNA: most mammalian cells contain hundreds of mitochondria and, in turn, each mitochondrion contains several (2–10) copies of mtDNA (Shuster *et al.* 1988; Wiesner *et al.* 1992*b*). In a given individual, all mtDNA copies are thought to be identical, a condition known as homoplasmy, but mutations can arise, be maintained or amplified to different levels and coexist with wild-type mtDNA, giving rise to the condition of heteroplasmy. At cell division mitochondria and their genomes are randomly distributed to daughter cells and hence, starting from a given heteroplasmic situation, different levels of heteroplasmy and even homoplasmy can arise in different cell lineages. As a consequence of this it is common to find a 'threshold effect' in mtDNA-linked human diseases; the mutation has to reach a certain percentage, usually higher than 60–80 %, in order to manifest pathological effects (Lightowlers *et al.* 1997; Zeviani & Antozzi, 1997).

(2) The mitochondrial genome is maternally inherited; the few mitochondria from the sperm cell that could enter the oocyte during fertilization are actively eliminated by a ubiquitin-dependent mechanism (Sutovsky *et al.* 1999, 2000). During oogenesis, mtDNA suffers a bottleneck phenomenon by which only a small subset of mtDNA molecules are amplified and transmitted to the offspring (Marchington *et al.* 1998). This phenomenon explains the rapid shift to homoplasmy of transmitted mutations in one or a few generations (Zeviani & Antozzi, 1997). Recent data show that in some cases, related to human diseases, paternal mtDNA can escape the mechanism of active elimination and be transmitted to the muscle tissue of the offspring at relevant levels (Schwartz & Vissing, 2002).

(3) The evolution rate of mtDNA is much faster than that of the nuclear genome (Brown *et al.* 1979). Several reasons are invoked to explain this fact: mtDNA is less protected by proteins, it is physically associated with the mitochondrial inner membrane where damaging reactive oxygen species (ROS) are generated, and it appears to have less-efficient repair mechanisms than the nucleus (Pinz & Bogenhagen, 1998). This high mutation rate and the maternal inheritance pattern have made mtDNA sequence analysis an interesting tool in human population genetics and evolutionary studies (Stoneking, 1994). On the other hand, somatic accumulation of mtDNA mutations has been proposed to play a role in human ageing (Wallace, 1997; Michikawa *et al.* 1999; see also article by Hofhaus *et al.* in this issue).

(4) Mitochondrial genes are translated using a genetic code with some differences from the universal genetic code. Thus, in mammals, UGA specifies tryptophan instead of a termination codon, AUA, AUC and AUU are used as

initiation codons and AGA and AGG are termination codons instead of encoding arginine. In addition, a simplified codon–anticodon pairing system allows translation to proceed with only 22 tRNAs (Attardi & Schatz, 1988).

Structure, gene content and organization of mammalian mtDNA

(a) Structure. The structure, genetic content and organization of mtDNA are strongly conserved among mammals, hence human mtDNA can be considered as a model. Human mtDNA is a double-stranded, closed-circular molecule of approximately 16.6 kb, which corresponds to a molecular weight of *ca* 10 million Da (Anderson *et al.* 1981) and in most cells it represents only about 0.5–1 % of the total DNA content. The two strands of mtDNA can be distinguished because of their different G+T content and can be separated by density in denaturing gradients, giving a heavy or H-strand and a light or L-strand. The normal mtDNA state is thought to be as a super coiled structure and, as mentioned above, it is poorly associated with proteins, at least in comparison with nuclear DNA (Richter *et al.* 1988). Early reports suggested an association of human mtDNA with the mitochondrial inner membrane through a 'protein knob' (Albring *et al.* 1977). Actually, in yeast, mtDNA is organized as supramolecular structures called nucleoids, which contain several (3–4) mtDNA molecules and about 20 different polypeptides (Miyakawa *et al.* 1987; Kaufman *et al.* 2000) and are associated with mitochondrial membranes (Hobbs *et al.* 2001). To explain mtDNA segregation behaviour in mammals, the existence of nucleoids has been proposed (Jacobs *et al.* 2000), but it has not been confirmed yet and is currently under investigation.

A high proportion of mtDNA molecules in a metabolically active cell contain a triple-stranded structure called the displacement loop (D-loop), in which a nascent H-strand DNA segment of 500–700 nt remains annealed to the parental L-strand. The D-loop region is about 1 kb long in humans (Fig. 1) and it is the major non-coding segment, containing the main regulatory elements for mtDNA transcription and replication. This is also the region that is most variable in sequence and size among different species, although it contains some conserved elements with possible regulatory functions (see below) (Attardi & Schatz, 1988; Shadel & Clayton, 1997).

(b) Gene content and organization. Mammalian mtDNA contains 37 genes corresponding to the RNA components of the mitochondrial translation apparatus (two ribosomal RNAs called 12S and 16S rRNAs and 22 tRNAs) as well as mRNAs for 13 polypeptides that are subunits of four OXPHOS complexes (Fig. 1). Seven of those polypeptides, ND1 to ND6 plus ND4L are subunits of complex I (NADH-dehydrogenase-ubiquinone reductase); one, cytochrome *b*, is part of complex III (ubiquinol-cytochrome *c* reductase); three, CO I, CO II and CO III, are the catalytic subunits of complex IV (cytochrome *c* oxidase), and ATPase 6 and 8 are subunits of complex V (ATP synthetase). These genes are asymmetrically distributed in mtDNA. The heavy or H-strand encodes most of the information: the two rRNAs, 14 tRNAs and the mRNAs for 12 out of the 13 polypeptides. The L-strand encodes the remaining eight tRNAs and only one mRNA, corresponding to ND6 subunit (Attardi & Schatz, 1988). These mtDNA-encoded protein subunits appear to be essential for the correct assembly or activity of the corresponding complex, as has been established in many cases (Holt *et al.* 1990; Hofhaus & Attardi, 1995; Bai & Attardi, 1998; Kadenbach *et al.* 1998).

One of the main characteristics of animal mtDNA is its compact gene organization, with all the coding sequences contiguous to each other or separated by a few bases and without introns (Anderson *et al.* 1981; Montoya *et al.* 1981) (Fig. 1). Some of the protein genes even overlap (ATPase 6 and 8 (46 nucleotides in humans), and ND4 and ND4L subunits (7 nucleotides in humans)) and in several cases part of the termination codons are not encoded in mtDNA, but are generated by the post-transcriptional polyadenylation of the corresponding mRNAs (Ojala *et al.* 1981). Another interesting feature of mitochondrial gene organization is that tRNA genes are regularly distributed among the other genes (Fig. 1), playing an important role in RNA processing (see below).

There are only two non-coding regions in mtDNA and they contain most of the known regulatory functions. The main one is the above-mentioned D-loop region, situated between the genes for $tRNA^{Phe}$ and $tRNA^{Pro}$, which contains the origin of replication for the H-strand (O_H) and the promoters for H- and L-strand transcription (Fig. 1). The D-loop structure from different animals also contains conserved sequences known as CSBs (conserved sequence blocks), and TASs (termination-associated sequences) and tRNA-like secondary structures (Shadel & Clayton, 1997), which probably play a role in mtDNA replication. The second non-coding region, which is much shorter, is a ~30 nucleotide-long segment which contains the origin of replication for the L-strand (O_L) and is located inside a tRNA cluster at about two-thirds of the mtDNA length from the O_H (Fig. 1).

As an example of the differences in gene organization among phyla, it can be mentioned that some yeast mitochondrial genomes (*Saccharomyces*) are about five times larger than human mtDNA, in spite of a similar gene content, and are composed mostly of linear molecules. On the other hand, several yeast mtDNA genes contain introns and the tRNAs are grouped in clusters (Attardi & Schatz, 1988).

mtDNA transcription and RNA processing

The particular features of the gene structure and organization of mammalian mtDNA are also reflected in its expression mode. Initial studies on mtDNA transcription were carried out about two decades ago in cultured human and mouse cells both *in vivo* and *in vitro* and have provided

most of the available evidence on this process. These studies have allowed characterization of mitochondrial RNAs in terms of identity, structure and metabolic properties (Montoya *et al.* 1982, 1983; Walberg & Clayton, 1983; Chang & Clayton, 1984; Yoza & Bogenhagen, 1984), and the dissection of the *cis*-acting elements required for mitochondrial transcription (Chang & Clayton, 1986*a*,*b*; Fisher *et al.* 1987). More recent studies using mammalian tissues have focused preferentially on the regulation of RNA synthesis in different physiological situations (reviewed by Enriquez *et al.* 1999*b*). Different strategies, from forward and reverse genetics to computer inter-species homology searching, are increasing our knowledge about the *trans*-acting factors involved in mitochondrial transcription (Fisher & Clayton, 1988; Fernández-Silva *et al.* 1997; Tiranti *et al.* 1997; Spelbrink *et al.* 2001; Falkenberg *et al.* 2002).

(a) Transcription products. From the *in vivo* studies with human and mouse cells it was deduced that both mtDNA strands are completely and symmetrically transcribed (Fig. 1). mtDNA transcription products were identified and characterized initially in human HeLa cells and include the two rRNAs (12S and 16S) and an rRNA precursor containing both rRNAs, the 22 tRNAs and 18 polyadenylated RNAs (Montoya *et al.* 1983). Most mature poly(A)-RNAs correspond to a single gene, while two of them, the mRNA for ND4 and ND4L subunits and the mRNA for ATPase 6 and ATPase 8 subunits contain two overlapping reading frames. The three largest RNA species (RNAs 1, 2 and 3) as well as the smallest poly(A)-RNA (RNA 18 or 7S) along with eight tRNAs are the transcription products of the L-strand, the remainder being transcribed from the H-strand (Attardi & Schatz, 1988) (Fig. 1).

Some of the structural features of mtRNAs are peculiar. Thus, the rRNAs are smaller than cytoplasmic or bacterial rRNAs, they are methylated and contain a short 3′ poly(A) tail of 1–10 residues (Dubin *et al.* 1982). Mitochondrial tRNAs are also smaller (59–75 nt) than their cytoplasmic or prokaryotic counterparts and have some structural differences. Most mitochondrial tRNAs lack the so-called constant nucleotide positions and the size of the 'DHU' loop is very variable or even absent in the case of $tRNA^{Ser}_{AGY}$ (Arcari & Brownlee, 1980; Bruijn *et al.* 1980). With this one exception, all mitochondrial tRNAs can fold adopting the typical cloverleaf structure, but their stabilization requires less tertiary interactions than cytoplasmic tRNAs. The only general tRNA features conserved are the structure of the anticodon region and the presence of a -CCA end that is not mtDNA encoded but post-transcriptionally added (Arcari & Brownlee, 1980). In several cases, tRNA sequences have additional functions such as in transcription termination or L-strand replication initiation. In addition, mitochondrial tRNAs play an important role in the processing of the polycistrons to yield the mature RNAs (see below). The mRNAs start directly at the initiation codon or have an extremely short untranslated 5′-end (1–3 nt) and contain a poly(A) tail of about 55 residues immediately after the stop codon, lacking the typical features of other mRNAs such as 5′ and 3′ untranslated regions, 3′ polyadenylation signals or the 5′-end 'cap' structure (Montoya *et al.* 1981).

As discussed below, mtDNA replication depends also on L-strand transcription which produces short transcripts required as primers for DNA synthesis.

(b) Transcription mechanism and *cis*-acting elements. Mapping of nascent transcripts and analysis of the transcript labelling kinetics *in vivo* have revealed that RNA synthesis in human mitochondria starts at three different initiation points located in the D-loop region, one for the L-strand (L) and two for the H-strand (H_1 and H_2) (Montoya *et al.* 1982, 1983; Bogenhagen *et al.* 1984).

The H-strand is transcribed by two overlapping units. One of them starts at the initiation site H_1, located 19 nt upstream of the $tRNA^{Phe}$ gene and ends at the 16S rRNA 3′-end. This transcription unit operates much more frequently than the second one and is responsible for the synthesis of the two ribosomal RNAs, $tRNA^{Phe}$ and $tRNA^{Val}$. The activity of this unit is linked to a transcription termination event taking place immediately downstream from 16S rRNA, inside the gene for $tRNA^{Leu}$ (Montoya *et al.* 1983; Kruse *et al.* 1989). The second transcription unit, operating with a frequency about 20 times lower, starts at the initiation site H_2, close to the 12S rRNA 5′-end and originates a polycistronic molecule covering almost the whole H-strand. The mRNAs for the 12 H-strand encoded polypeptides and 12 tRNAs derive from the processing of this polycistron. This transcription model explains how a differential regulation of rRNA *versus* mRNA transcription can be operated through the initiation of H-strand transcription at the two alternative sites (Montoya *et al.* 1983). Analysis of the effects of different ATP concentrations and intercalating drugs such as ethidium bromide on *in organello* transcription and the results of footprinting experiments have also provided support for this model of two H-strand transcription units (Gaines & Attardi, 1984; Gaines *et al.* 1987; Micol *et al.* 1997).

The L-strand gives rise to a single polycistron starting at the 5′-end of 7S RNA, about 150 bp away from the H_1 initiation point, from which the eight tRNAs and the ND6 mRNA are derived. The precise location and mechanism for H_2 and L-strand transcription termination is unknown (Attardi & Schatz, 1988).

In vitro run-off transcription assays using deletion, site-directed mutagenesis and linker substitution analysis of cloned mtDNA fragments as templates have allowed the determination of the promoter requirements for initiation of transcription at H_1 and L start points (Clayton, 1992).

The two major mammalian mitochondrial promoters containing the H_1 and L initiation points, called HSP_1 and LSP, respectively, are functionally independent and have a bipartite structure. First, they contain a promoter element with a consensus sequence motif of 15 bp which surrounds the initiation points and is essential for transcription. A

second element, located immediately upstream of the initiation point (−12 to −39 bp), is required for optimal transcription and can be considered as an enhancer. This regulatory element includes the binding sites for a transcription factor, mtTFA, and a distance of 10 bp between both regions is critical (Chang & Clayton, 1986*a*; Fisher *et al.* 1987; Fisher & Clayton, 1988). The second H-strand transcription initiation point, H_2, has a promoter with only a limited similarity to the 15 bp consensus sequence, and apparently does not have the upstream mtTFA binding element (Fig. 2).

The *in vitro* transcription studies used in promoter analysis have failed to show any detectable H_2 initiation activity. In an alternative model derived from these studies, the existence of only one major transcription initiation point for each mtDNA strand is proposed. According to this model, transcription from H_1 would originate both mRNAs and

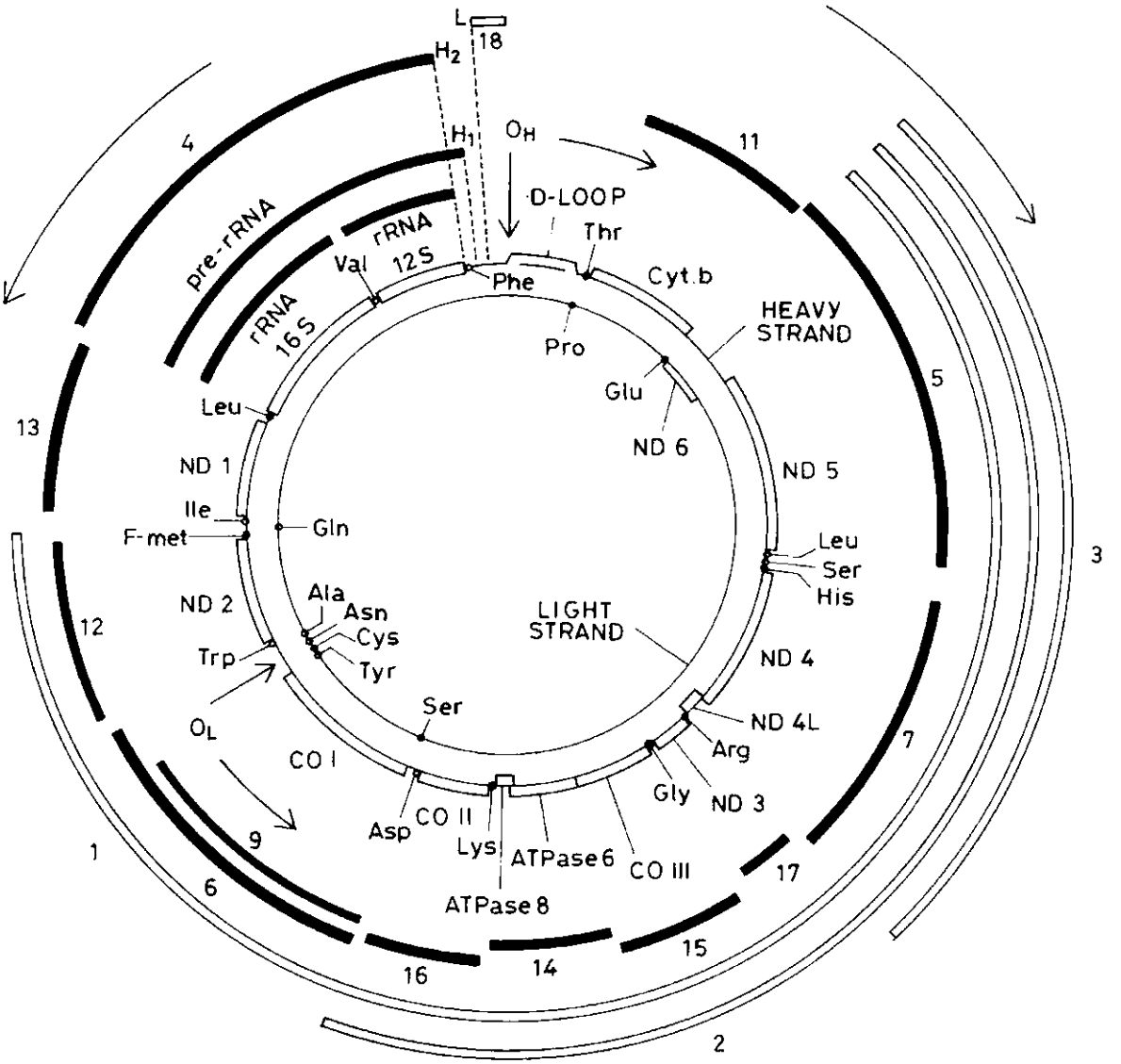

Figure 1

Genetic and transcription map of human mtDNA. The two internal circles represent both mtDNA strands with the encoded genes. External circles represent the RNAs transcribed from the heavy strand (filled lines) and light strand (open lines). 12S and 16S, ribosomal RNAs 12S and 16S; ND1 to ND6, subunits 1–6 of NADH dehydrogenase (complex I); cyt b, cytochrome *b* subunit of complex III; CO I, CO II and CO III, subunits of cytochrome *c* oxidase (complex IV); ATPase 6 and ATPase 8, subunits of ATP synthetase (complex V). tRNA genes are indicated by the three letter code of the corresponding amino acid. H_1, H_2 and L indicate transcription initiation points for the H- and L-strand, respectively. O_H and O_L represent replication origins for the H- and L-strand, respectively. Arrows indicate the transcription or replication direction.

rRNAs, and the regulation of the rRNA/mRNA ratio would be controlled only at the termination activity level (Clayton, 1992).

Evidence for transcription termination in the 16S rRNA/tRNA Leu^{UUR} boundary comes from the reduction of transcription velocity downstream of this region (Montoya *et al.* 1983), from the fact that the 3′-end of 16S rRNA molecules shows heterogeneity (Dubin *et al.* 1982; Etten *et al.* 1983), contrasting with the precise end of processed transcripts, and from numerous *in vitro* experiments showing specific transcription termination in that point (Kruse *et al.* 1989; Hess *et al.* 1991; Daga *et al.* 1993; Micol *et al.* 1996). For this transcription termination event to occur, both the presence of a tridecamer sequence in the tRNA Leu^{UUR} gene and a specific protein factor, mTERF, are necessary and sufficient (Christianson & Clayton, 1988). The termination-promoting activity of mTERF is bidirectional and, since no additional genes are encoded in this strand downstream of the mTERF binding site, it could also act on the L-strand transcription end (Shang & Clayton, 1994).

(c) RNA processing and maturation. The polycistronic primary transcripts originate the mature rRNAs, mRNAs and tRNAs after the precise endonucleolytic cleavage on both sides of the tRNA molecules (Ojala *et al.* 1981; Montoya *et al.* 1983). According to this model of 'tRNA punctuation', tRNA sequences located between each rRNA and mRNA would act as the signals for the processing enzymes after acquiring the cloverleaf structure on the nascent RNA chains. In the few cases where no tRNAs are flanking the mRNA termini, there are probably tRNA-like secondary structures which can be recognized by the processing machinery.

This processing requires at least four enzymatic activities: first, the tRNA 5′ and 3′ end endonucleolytic cleavages; second, a polyadenylation activity for rRNAs and mRNAs; and finally, the addition of the CCA to the tRNA 3′ end. According to Rossmanith and coworkers (1995) the 5′ endonucleolytic cleavage occurs first and is performed by a mitochondrial RNase P as had been previously suggested (Doersen *et al.* 1985). This kind of enzyme also exists in the nucleus and is usually a ribonucleoprotein with an essential RNA component (Puranam & Attardi, 2001).

The enzyme responsible for the 3′-end cleavage activity has not been identified. The addition of the CCA to the tRNA 3′-end is performed by an ATP(CTP):tRNA nucleotidyl-transferase which completes their maturation. Additional activities are needed for tRNA modification and are under investigation (Helm *et al.* 2000; Nagaike *et al.* 2001).

As mentioned before, both rRNAs and mRNAs are polyadenylated with tails of 1–10 and ~55 A residues, respectively. Polyadenylation is performed by a mitochondrial poly(A) polymerase and plays an important role in stabilizing the RNAs and in many cases it even contributes to the generation of the stop codons of some mRNAs (Ojala *et al.* 1981). There is evidence that the polyadenylation of mRNAs occurs during or immediately after cleavage (Amalric *et al.* 1978). It is possible that some, if not all, of these processing/maturation activities form a complex acting together and cotranscriptionally, as also seems to occur for RNApol II transcripts in the nuclear compartment (Bentley, 2002).

(d) Transcription machinery. The unravelling of the *trans*-acting factors involved in transcription has evolved more slowly than that of the *cis*-acting elements. mtDNA transcription requires an organelle-specific RNA polymerase (Tiranti *et al.* 1997) and at least three transcription factors: mtTFA (also called TFAM) (Fisher & Clayton, 1985, 1988) and either TFB1M or TFB2M for initiation (Falkenberg *et al.* 2002; McCulloch *et al.* 2002) and mTERF for termination of transcription (Daga *et al.* 1993; Fernández-Silva *et al.* 1997).

Human mtRNA polymerase (mtRNApol or POLRMT), a 120 kDa protein, which has recently been cloned, is homologous with phage polymerases and yeast mtRNA polymerases (Tiranti *et al.* 1997). In the absence of transcription factors, the mtRNApol is unable to recognize the mitochondrial promoters and shows little and only non-specific activity (Fisher & Clayton, 1985; Prieto-Martín *et al.* 2001).

mtTFA was, until very recently, the only known mitochondrial transcription initiation factor in mammals. When added to a fraction of partially purified mtRNApol, mtTFA was able to confer specific initiation capacity to the enzyme and increase its activity (Fisher & Clayton, 1985, 1988). This 25 kDa protein was cloned some time ago and belongs to the HMG (high mobility group) type of proteins (Parisi & Clayton, 1991). mtTFA, like other HMG family proteins, is able to wrap, bend and unwind DNA *in vitro* with a low degree of sequence specificity (Parisi & Clayton, 1991; Fisher *et al.* 1992). Binding sites for mtTFA are found upstream of the two more active transcription initiation points in the D-loop (H_1 and L), but both the binding and transcription stimulation activities are higher for the L promoter (LSP) than for the H_1 promoter (HSP_1) (Fisher *et al.* 1987; Fisher & Clayton, 1988; Ghivizzani *et al.* 1994). The current data suggest that mtTFA forms a complex with DNA through its two HMG-box domains, inducing a specific structural change in the promoter region of mtDNA that allows mtRNApol to initiate transcription (Shadel & Clayton, 1997).

In yeast, the specific transcription initiation factor, mtTF1 (Xu & Clayton, 1992), was different from mtTFA, and had a *Xenopus* homologue called mtTFB, pointing to the existence of additional transcription factors in vertebrates (Antoshechkin & Bogenhagen, 1995). Only this year, and based on computer homology searching, McCulloch and coworkers have identified a human protein potentially homologous to yeast mtTF1, which was able to stimulate transcription in a mitochondrial extract (McCulloch *et al.* 2002). They called this protein, which showed homology to RNA methyltransferases, TFBM. Following the same strategy, Falkenberg and collaborators have identified in humans and mice the same protein (termed now TFB1M)

along with a second related factor which they called TFB2M (Falkenberg *et al.* 2002). TFB2M shows at least two orders of magnitude more activity in stimulating specific transcription than TFB1M. Both factors seem to directly interact with mtRNApol forming a heterodimer, and one or the other, in addition to mtTFA, is required for the accurate initiation *in vitro* on both H_1 and L promoters, although they seem not to be required for transcription elongation (Falkenberg *et al.* 2002).

Thus, the minimal machinery required for *in vitro* transcription initiation would include mtRNApol, mtTFA, and either TFB1M or TFB2M, which turns out to be more complex than was envisaged until very recently. The requirement for these different factors introduces more possible ways of mtDNA expression regulation, but their precise *in vivo* action on the different promoters and in replication still has to be clarified.

The existence of additional regulatory factors that could participate in the modulation of transcription in response to specific signals has been suggested (Tsiriyotis *et al.* 1997; Goglia *et al.* 1999). The evidence of such a factor is especially strong in the case of thyroid hormones, for which a mitochondrial receptor able to affect the transcription of mtDNA has been proposed (Wrutniak *et al.* 1995; Casas *et al.* 1999).

As already mentioned, transcription termination requires a 34 kDa protein, termed mTERF. This factor has three potential leucine zippers in its sequence, but binds DNA as a monomer and is able to bend DNA (Shang & Clayton, 1994; Fernández-Silva *et al.* 1997). Although it has been proposed to function as a physical barrier (Shang & Clayton, 1994), several observations argue against this assumption and suggest that some specific interaction with mtRNApol must exist. First, the efficiency of termination-promoting activity is very different with different RNA polymerases (Shang & Clayton, 1994); second, the *in vitro* expressed recombinant protein is able to bind DNA but does not promote transcription termination (Fernández-Silva *et al.* 1997); third, *in vitro* conditions which do not affect DNA binding by mTERF, such as intermediate concentrations of KCl, are able to abolish termination activity (Fernández-Silva *et al.* 1997); and finally, conditions which clearly modify the synthesis ratio mRNA/rRNA *in organello* do not affect the binding and occupancy of mTERF target

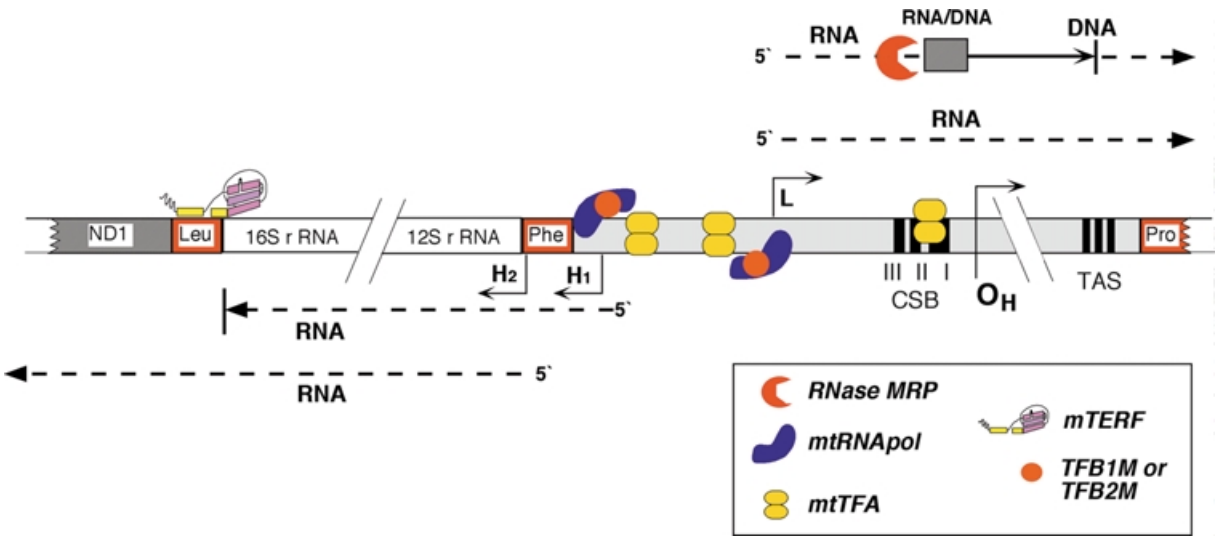

Figure 2

Schematic representation of the mammalian D-loop and transcription termination regions, showing the main elements and factors involved in transcription and in replication initiation. The non-coding regulatory D-loop region is shown in light grey, the rRNA coding region is shown in white, the ND1 coding region is shaded and the tRNA coding areas appear in red. The H_1 initiation point directs the transcripion of the rRNA region and its activity is linked to a termination event requiring the presence of the transcription termination factor, mTERF, bound to a tridecamer sequence inside the $tRNA^{Leu}$ gene. The H_2 initiation point directs the transcription of the whole H-strand and is around 20 times less active than H_1. L is the L-strand initiation point that gives rise to polycistronic transcripts for this strand and also produces RNA precursors for H-strand replication initiation. Transcription factor mtTFA binds to enhancer elements located upstream of the transcription initiation points H_1 and L, and to other phased regions of the D-loop, although only binding to the conserved sequence blocks (CSB) region is indicated. RNase MRP is a ribonucleoprotein, involved in the processing of L-strand primary transcripts, that generates primers for H-strand replication initiation. TAS, termination-associated sequences, around which nascent H-strand DNA molecules arrest, giving rise to the D-loop structure. Mitochondrial RNA polymerase (mtRNApol) forms a heterodimer either with transcription factors TFB1M or TFB2M with a 1:1 stoichiometry.

sequence as determined by footprinting analysis (Enriquez *et al.* 1999*a*).

mtDNA replication

mtDNA replication takes place in the mitochondrial matrix, independently from the cell cycle phase and from nuclear DNA (nDNA) replication (Bogenhagen & Clayton, 1977). Analysis of replicative intermediates of mammalian mtDNA has provided a generally accepted model, which describes replication as an asynchronous displacement mechanism involving two unidirectional, independent origins. The synthesis starts at O_H, which is located downstream of the LSP in the D-loop region, and proceeds along the parental L-strand to produce a daughter H-strand circle. When H-strand replication reaches O_L, the parental H-strand is displaced, the initiation site for L-strand synthesis is exposed and its replication starts and proceeds in the opposite direction producing a daughter L-strand (Fig. 1) (Shadel & Clayton, 1997).

(a) Replication mechanism and *cis*-acting elements. H-strand replication initiation requires short RNA primers originated by the processing of L-strand promoter transcripts. Consequently, replication of mammalian mtDNA is linked and dependent on mitochondrial transcription and, hence, on the *cis*-acting elements and *trans*-acting factors required for L-strand transcription (Clayton, 1992). In addition to these *cis*-acting elements, other D-loop sequences participate in the replication process, the so-called CSBs and TASs. CSBs are conserved sequences found in the D-loop region of all vertebrates examined and the experimental evidence supports the idea that they participate in the formation of a proper RNA primer for replication. Three of these sequences, CSB I, CSB II and CSB III are conserved among human, rat and mouse mtDNA and a major H-strand replication initiation point is almost always located near to CSB I (Walberg & Clayton, 1981). TASs are short (15 bp) sequences conserved in vertebrates and associated with the 3′-ends of arrested D-loop DNA strands (Doda *et al.* 1981) (Fig. 2).

The replication initiation process can be described in four steps: (1) mtRNA polymerase (associated with transcription factors) begins transcription from the L-strand promoter generating the primer precursor; (2) the newly synthesized RNA remains hybridized with a region of DNA upstream of O_H which contains the CSBs, forming a stable R-loop structure; (3) RNA processing activities cut the RNA primer precursor around or inside the R-loop structure generating the mature primer(s) for replication; (4) the mitochondrial DNA polymerase starts H-strand replication through the extension of an RNA primer (Lee & Clayton, 1996; Xu & Clayton, 1996; Shadel & Clayton, 1997) (Fig. 2).

After replication of the H-strand has started, two possible outcomes can occur. (1) H-strand synthesis events are arrested around the TAS sequences, creating the triplex D-loop structure (Clayton, 1982) (Fig. 2). This is the most common outcome. (2) In a minority of events the replication proceeds over the entire length of the genome. The function of the arrested nascent DNA strand and the mechanisms that decide if the elongation must proceed or not are unknown (Brown & Clayton, 2002).

In mammals, and in vertebrates in general, L-strand replication origin (O_L) is located far away from the H-strand origin (Fig. 1). The initiation for the replication of this strand occurs in a small non-coding region, as previously mentioned, that is thought to assume a stable stem–loop structure when it is exposed as a single strand. This initiation requires the action of a specific primase capable of generating short RNA primer molecules with 5′-ends mapping onto a T-rich portion of the loop in the stem-loop structure. Once initiated, L-strand replication proceeds over the entire length of the strand and ends after the H-strand (Wong & Clayton, 1985; Hixson *et al.* 1986).

This asymmetric replication of both strands allows the synthesis to be continuous. The whole process of replication has been estimated to take about 1 h, implying a slow polymerization rate of 270 nt min^{-1} (Graves *et al.* 1998). After the synthesis of the two strands is completed, the daughter mtDNA molecules must be separated, the RNA primers at both origins have to be removed and the corresponding gaps filled and ligated, and finally, the closed circular mtDNA must adopt its tertiary structure through the introduction of superhelical turns and interaction with different proteins (Lecrenier & Foury, 2000).

Recently, a second mechanism of mtDNA replication has been described as existing along with the one exposed above. This second mechanism, based on the observation of replicative intermediates in 2-D gels, corresponds to a coupled leading- and lagging-strand synthesis and would involve a reduced number of molecules, being particularly active in cells which have been partially depleted of their mtDNA (Holt *et al.* 2000). The relevance of this mechanism remains to be determined.

(b) Replication machinery. The search for an enzymatic activity capable of processing L-strand transcripts at sites near O_H has led to the identification of the mitochondrial RNA-processing endoribonuclease or RNase MRP (Shadel & Clayton, 1997). This enzyme is a ribonucleoprotein present also in the nucleolus where it plays a role in the maturation of 5.8S rRNA. The capacity of this enzyme to process RNA primer precursors with a triple-stranded R-loop configuration at priming sites found *in vivo* has been demonstrated (Shadel & Clayton, 1997). Another enzyme potentially involved in RNA primer formation is the endonuclease G, although it has a wide spectrum of nucleolytic activities and its role in mitochondrial biogenesis is less clear (Cote & Ruiz-Carrillo, 1993).

mtDNA is polymerized by the action of a mitochondria-specific DNA polymerase, DNApol γ. This enzyme is composed in all vertebrates of two subunits: the large catalytic α subunit (125–140 kDa) (Gray & Wong, 1992; Graves *et al.* 1998), and the smaller β subunit (30–54 kDa) that appears to be involved in primer recognition and confers processivity to the former (Carrodeguas *et al.* 1999; Fan *et al.* 1999; Lim *et al.* 1999). DNApol γ also has a 3′-5′

exonuclease activity linked to the α subunit that would be responsible for the high fidelity of the enzyme (Longley *et al.* 2001).

Like nuclear DNA polymerases, DNApol γ requires many other factors to properly perform its function, but only a few have been characterized in detail. Thus, an ATP-dependent mitochondrial helicase responsible for unwinding DNA during replication has been described in sea urchin (Roberti *et al.* 1996) and bovine brain (Hehman & Hauswirth, 1992). Recently, a human protein with homology to the helicase domain of phage T7 primase/helicase has been identified and proposed to function as a ring helicase. Mutations in this mitochondrial factor, known as Twinkle, are linked to a subset of patients with autosomal dominant progressive external ophthalmoplegia (adPEO) (Spelbrink *et al.* 2001). Also, mitochondrially associated topoisomerases of types I and II have been described from different sources that would be responsible for the removal or introduction, respectively, of supercoils in mtDNA (Castora & Simpson, 1979; Castora *et al.* 1985; Kosovsky & Soslau, 1993). In that respect, a human mitochondrial topoisomerase I (TOPOImt) has been recently cloned, and shows a high degree of homology to the corresponding nuclear gene (Zhang *et al.* 2001).

Replicative intermediates of mtDNA contain long single-stranded DNA regions. A single-strand binding protein (mtSSB) exists in mitochondria to maintain the integrity of these regions and to increase the activity and fidelity of DNApol γ (Hoke *et al.* 1990; Farr *et al.* 1999). This small protein (16 kDa in humans) binds DNA as a homo-tetramer and is different from *E. coli* single-stranded DNA binding protein although it has a similar structure and DNA binding activity, and is essential for DNA maintenance in yeast and *Drosophila* (Van Dyck *et al.* 1992; Farr *et al.* 1999).

A DNA primase, different from nuclear primase, involved in L-strand replication initiation has been partially purified and seems to contain an RNA component essential for activity (Wong & Clayton, 1986). A mitochondrial DNA ligase, related to the nuclear ligase III has also been characterized in *Xenopus* at the protein level and recently cloned (Pinz & Bogenhagen, 1998; Perez-Jannotti *et al.* 2001).

As already indicated, mtTFA, in addition to being required for *in vitro* transcription initiation, seems to have a direct role in mtDNA maintenance. There seems to be a regularly spaced binding of mtTFA to the regulatory region of mtDNA, perhaps organizing the D-loop and allowing other specific factors involved in transcription and replication to interact with their target sequences (Ghivizzani *et al.* 1994). A direct correlation between mtDNA and mtTFA levels has been observed, although in some cases this could be explained as a secondary phenomenon due to the stability of the protein being dependent on DNA binding (Taanman, 1999). However, the heterozygous mtTFA knock-out mice show reduced mtDNA levels and homozygous knock-out embryos completely lack mtDNA and die (Larsson *et al.* 1998). In addition, the levels of this protein seem to exceed what would be expected for a transcription factor with regulatory functions and it has been proposed to act more in a histone-like fashion, covering the entire mtDNA molecule (Fisher *et al.* 1992; Ghivizzani *et al.* 1994). It was proposed in a recent report that the amount of mtTFA in human HeLa cells is about two orders of magnitude higher than previously estimated (Takamatsu *et al.* 2002), being abundant enough to entirely wrap mtDNA, as happens in yeast and possibly in *Xenopus* (Shen & Bogenhagen, 2001), and being in large excess with respect to transcription requirements. This report also suggests a role for mtTFA, along with mtSSB, in the turnover of the D-loop structure involved in mtDNA replication (Takamatsu *et al.* 2002). Recent data also suggest a possible role for human mtTFA and its yeast homologue ABF2 in recognition/repair of oxidative damage to DNA (Yoshida *et al.* 2002).

Regulation of mtDNA expression

The energetic demands can vary substantially between different cell types and in different physiological situations requiring an adaptation in mitochondrial biogenesis. Even inside the same cell, the energy requirements may be different depending on the existence of particular cell domains (e.g. in neurons or spermatozoa) (Overly *et al.* 1996). Mitochondrial biogenesis is a complex phenomenon requiring the participation and coordination of the nuclear and mitochondrial genomes and the molecular mechanisms involved in its control are still poorly understood. The OXPHOS system biogenesis alone involves more than one hundred genes, most of them encoded in the nucleus.

Different biogenetic programmes can be envisaged according to specific situations. Thus, mitochondrial proliferation (mass increase) would be the main purpose during situations of active cell growth and division. On the other hand, mitochondrial maturation or differentiation would be the aim during cell differentiation. In addition, a rapid mechanism for the adjustment of mitochondrial function in response to locally changing ATP demands and other extramitochondrial signals (mitochondrial local tuning) (Enriquez *et al.* 1999*b*) can also be considered. For each of these situations there are probably different regulatory mechanisms, and they can overlap, increasing the complexity of the process.

The first situation mentioned above, mitochondrial proliferation, requires the coordinated upregulation of nuclear and mitochondrial genes. The current data suggest that two types of nuclear factors, activators and coactivators, could be the main participants in the control of this kind of programme. The activators are transcription factors that include nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2), Sp-1, CREB and others. Coactivators are nuclear factors such as PGC-1 or PRC that interact with the DNA-bound transcription factors to regulate gene transcription in response to physiological signals of thermogenesis and cell proliferation (Wu *et al.* 1999; Andersson & Scarpulla, 2001; Carmona *et al.* 2002).

Recognition sites for NRF-1, NRF-2 and Sp-1 are common to many nuclear genes encoding respiratory subunits, to mitochondrial transcription and replication factors, as well as to certain haem biosynthetic enzymes and components of the protein import machinery (Scarpulla, 1997, 2002). By acting on the promoters of key structural OXPHOS genes and, simultaneously, on genes that control mtDNA gene expression, the coordination among both genomes would be achieved (Scarpulla, 1997; Enriquez *et al.* 1999*b*; see also article by Goffart & Wiesner in this issue). However, some situations of increased ATP demands show a different regulatory pattern, illustrating the complexity of the phenomenon. Thus, in the entry into proliferation of quiescent 3T3 fibroblasts induced by serum deprivation, an increase in the respiratory capacity can be achieved by the elevated expression of a key respiratory protein, namely cytochrome *c*, rather than through the coordinate increase in the synthesis of all the OXPHOS components (Herzig *et al.* 2000).

Mitochondrial differentiation implies changes in activity/composition of the organelles without an increase in number. In such a situation, described in rat liver soon after birth, post-transcriptional mechanisms such as RNA stability and translation efficiency of nuclear and mitochondrially encoded genes play an important role (Izquierdo *et al.* 1995; Preiss *et al.* 1995; Ostronoff *et al.* 1996).

Finally, there is evidence suggesting the existence of mechanisms for local regulation of mitochondrial biogenesis, in particular cell domains in neurons (Hevner & Wong-Riley, 1993; Nie & Wong-Riley, 1996) and of a direct response of the mitochondrial transcription apparatus to external signals in the absence of nuclear gene expression (Gaines *et al.* 1987; Enriquez *et al.* 1996, 1999*a*).

(a) Regulation of transcription. Some earlier observations suggested that mtDNA transcription was constitutive and not rate limiting for protein synthesis (England *et al.* 1978; Attardi & Schatz, 1988). However, changes in transcription activity and in the steady-state level of mtRNAs have been reported in a diverse series of situations in mammals, related to different stages of development (Cantatore *et al.* 1986; Loguercio-Polosa & Attardi, 1991), ageing (Gadaleta *et al.* 1990*b*; Fernández-Silva *et al.* 1991), change in cell energy demands (Williams, 1986; Hood *et al.* 1989; Hevner & Wong-Riley, 1993; Martin *et al.* 1993) or hormonal status (Mutvei *et al.* 1989; Gadaleta *et al.* 1990*a*; vanItallie, 1990; Wiesner *et al.* 1992*a*; Weber *et al.* 2002). The molecular mechanisms involved in mitochondrial transcription regulation, responsible for these changes, are poorly understood. mtTFA has been postulated as a key player in mtDNA transcription regulation and in coordination with nuclear gene expression (Garstka *et al.* 1994; Virbasius & Scarpulla, 1994; Montoya *et al.* 1997; Gordon *et al.* 2001). However, some observations seem to question a general role for this protein in the control of mtRNA levels. Thus, as mentioned above, mtTFA seems to be a very abundant protein (Takamatsu *et al.* 2002), and the reduction in its levels induced in heterozygous knock-out mice was accompanied in all tissues by a drop in mtDNA levels but not in mtRNA levels (Larsson *et al.* 1998).

By using isolated mitochondria as a model system, it has been demonstrated that some degree of autonomous regulation in the transcription of mtDNA can exist. Thus, in the absence of nuclear gene expression, the synthesis and maturation of mitochondrial mRNAs can be maintained during long periods of time (Enriquez *et al.* 1996). In addition, some external signals like ATP concentration or thyroid hormones are able to induce changes in mitochondrial transcription (Enriquez *et al.* 1996, 1999*a*). This kind of autonomous regulation would allow each individual organelle, set up with a maximum potential capacity by the nucleus, to rapidly control the actual level of expression of mtDNA-encoded genes, depending on the local energy demands and other external signals (Enriquez *et al.* 1999*b*).

The role of thyroid hormones (THs) requires particular attention since they are key players in energy metabolism and much effort has been devoted to the analysis of their effects on mitochondrial biogenesis. Hypothyroidism induces a decrease in the steady-state concentration of all mtRNAs, which is most pronounced for mRNAs, thus also changing the mRNA/rRNA ratio (Mutvei *et al.* 1989; Gadaleta *et al.* 1990*a*; vanItallie, 1990). By using the *in organello* approach, a direct effect of THs on the protein–mtDNA interactions and on the mRNA/rRNA ratio, probably through changes in H-strand promoter selection, was found. This effect was achieved with a low concentration of hormones and was saturable (Enriquez *et al.* 1999*a*). Furthermore, a truncated form of the C-ERB α-1 nuclear receptor has been described in rat liver mitochondria (the so-called p43) which is able to bind to the mtDNA thyroid-responsive elements (TRE) (Wrutniak *et al.* 1995), and to stimulate mitochondrial transcription *in organello* when overexpressed in whole cells (Casas *et al.* 1999). All this evidence points to the existence of a mitochondrial thyroid hormone receptor that would mediate this direct effect, allowing a rapid coordination with nucleus-mediated actions. The existence of such mitochondrially specific factors may extend to other hormones (Tsiriyotis *et al.* 1997; see also article by Scheller & Sekeris in this issue) and to intracellular messengers such as cAMP (Papa *et al.* 1999).

Transcription regulation can be operated either at the level of initiation, termination or both. In particular, the transcription initiation rate at the two alternative H-strand promoters would determine the synthesis ratio of rRNAs *versus* mRNAs, which is supposed to be determining for protein synthesis (Wiesner *et al.* 1994). The processing of the polycistrons to yield the mature RNAs is a second potential regulatory point, although available data suggest that this process is very rapid and not limiting. More important appear to be the possible changes in RNA stability, that will determine, along with the transcriptional rate, the final steady-state level for each RNA species (Chomyn & Attardi, 1992).

Recent advances, such as the cloning of the two new transcription factors TFB1M and TFB2M (Falkenberg *et al.* 2002), will facilitate the understanding of the basic molecular mechanisms involved in mitochondrial transcription regulation.

(b) Regulation of replication. In addition to the general regulatory mechanisms found in the expression of nuclear genes (transcription, RNA stability, translation and protein turnover), mtDNA gene expression can be regulated at the level of gene dosage. In a variety of cell lines and tissues there is a correlation between mtDNA copy number and total cell DNA, with the ratio being tissue specific (Shay *et al.* 1990). However, mtDNA levels can change in a given cell type, and gene dosage has been postulated as the main regulatory mechanism in some situations, such as muscle changes during endurance training and electrical stimulation (Williams, 1986). How does the cell sense its mtDNA level to adjust replication accordingly? Several observations have suggested the existence of an as yet unidentified limiting factor(s) that would determine the level of mtDNA. Thus, mammalian cells, like yeast cells, tend to maintain a constant mass of mtDNA, rather than a constant number of mitochondrial genomes (Tang *et al.* 2000). In addition, competition for replication and maintenance among different mtDNA genotypes co-existing in the same cells has been documented and supports the limiting factor hypothesis (Jenuth *et al.* 1997; Moraes *et al.* 1999). This limiting factor could be any of the proteins involved in replication (mtTFA for example), but it could also be the size of nucleoside pools as suggested by the observation that defects in nucleotide metabolism are associated with mtDNA instability in human diseases (Nishino *et al.* 1999; Kaukonen *et al.* 2000). The expression of several replication factors do not seem to change in a series of situations where mtDNA levels are modified (Davis *et al.* 1996; Schultz *et al.* 1998; Moraes *et al.* 1999). Only the accessory β subunit of DNApol γ shows changes that can be correlated with changes in mtDNA levels during *Drosophila* development (Lefai *et al.* 2000).

In view of the replication mechanism previously described, several points for its control can be envisaged. Since replication is dependent on RNA primer synthesis, transcription initiation could be the first point of control. However, it is not known if a specific initiation event is required for DNA replication, perhaps guided by a given factor (Shadel & Clayton, 1997). mtTFA could be involved in this process since this protein seems to be required, particularly for L-strand transcription initiation, and the heterozygous knock-out mice for this factor show reduced mtDNA levels (Larsson *et al.* 1998; Gensler *et al.* 2001). A second point of possible regulation is the cleavage of nascent RNAs for primer formation. Since mtTFA also binds at sites near RNase MRP cleavages (Fig. 2), it has a potential role in primer formation. Finally, some mechanism must exist to determine whether nascent D-loop strands terminate or continue to completely replicate mtDNA. Since these nascent DNA strands frequently terminate at the TAS, a template-dependent event is suggested that could be regulated. A 48 kDa protein binding to TAS has been described in cows that could be involved in this process (Madsen *et al.* 1993). Furthermore, a role in mtDNA copy number control has been recently assigned to this replication termination event (Brown & Clayton, 2002). Again, mtTFA and/or mtSSB could participate at this point of regulation (Takamatsu *et al.* 2002).

In addition to the aforementioned mechanisms for mtDNA gene expression control, other levels of regulation should be considered. In particular, changes in mitochondrial translation efficiency have been reported, associated with developmental stages or mitochondrial functionality (Loguercio-Polosa & Attardi, 1991; Ostronoff *et al.* 1996).

Future prospects

Despite great progress in this field in the past few decades, many aspects of mitochondrial biogenesis, and in particular of mtDNA gene expression regulation, remain to be fully elucidated. Of particular interest would be to define the role of mtDNA gene expression in determining the respiratory capacity of the cell: to what extent it is a regulated process, in which steps it is regulated and how it is coordinated with the nuclear gene expression. Some tissue-specific features in these regulatory mechanisms can be expected (Wiesner *et al.* 1992*a*; see also article by Kunz in this issue).

Other unanswered questions are the control of mtDNA copy number and the relevance of the recently described alternative mtDNA replication mechanism (Holt *et al.* 2000). It will also be interesting to determine the reach of the phenomenon of mtDNA paternal transmission (Schwartz & Vissing, 2002), with potential consequences for human diseases.

The role of mtTFA in transcription and replication, and of the two transcription factors TFB1M and TFB2M (Shoubridge, 2002), also needs further clarification. The participation of mtDNA expression in the signal transduction networks operating in the cell and the role of the proposed mitochondrial hormone receptors are other open questions (Papa *et al.* 1999; see also articles by Weitzel & Seitz; and Scheller & Sekeris, in this issue).

The recently initiated generation of mouse models for mtDNA-linked diseases will help to solve some of those open questions.

Albring M, Griffith J & Attardi G (1977). Association of a protein structure of probable membrane derivation with HeLa cell mitochondrial DNA near its origin of replication. *Proc Natl Acad Sci U S A* **74**, 1348–1352.

Amalric F, Merkel C, Geldfand R & Attardi G (1978). Fractionation of mitochondrial RNA from HeLa cells by high-resolution electrophoresis under strongly denaturing conditions. *J Mol Biol* **118**, 1–25.

Anderson S, Bankier AT, Barrell BG, de-Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier HP, Smith AJH, Stader R & Young IG (1981). Sequence and organization of the human mitochondrial genome. *Nature* **290**, 427–465.

Andersson U & Scarpulla RC (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.

Antoshechkin I & Bogenhagen DF (1995). Distinct roles for two purified factors in transcription of *Xenopus* mitochondrial DNA. *Mol Cell Biol* **15**, 7032–7042.

Arcari P & Brownlee GG (1980). The nucleotide sequence of a small (3S) seryl-tRNA (anticodon GCU) from beef heart mitochondria. *Nucleic Acids Res* **8**, 5207–5212.

Attardi G (1985). Animal mitochondrial DNA, an extreme example of genetic economy. *Int Rev Cytol* **93**, 683–711.

Attardi G & Schatz G (1988). Biogenesis of mitochondria. *Annu Rev Cell Biol* **4**, 289–333.

Bai YD & Attardi G (1998). The mtDNA-encoded ND6 subunit of mitochondrial NADH dehydrogenase is essential for the assembly of the membrane arm and the respiratory function of the enzyme. *EMBO J* **17**, 4848–4858.

Bentley D (2002). The mRNA assembly line, transcription and processing machines in the same factory. *Curr Opin Cell Biol* **14**, 336–342.

Bogenhagen DF, Applegate EF & Yoza BK (1984). Identification of a promoter for transcription of the heavy strand of human mtDNA, *in vitro* transcription and deletion mutagenesis. *Cell* **36**, 1105–1113.

Bogenhagen DF & Clayton DA (1977). Mouse L cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. *Cell* **11**, 719–727.

Brown TA & Clayton DA (2002). Release of replication termination controls mitochondrial DNA copy number after depletion with 2′,3′-dideoxycytidine. *Nucleic Acids Res* **30**, 2004–2010.

Brown WM, George JM & Wilson AC (1979). Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci U S A* **76**, 1967–1971.

Cantatore P, Polosa PL, Fracasso F, Flagella Z & Gadaleta MN (1986). Quantitation of mitochondrial RNA species during rat liver development, the concentration of cytochrome oxidase subunit I (CoI) mRNA increase at birth. *Cell Differ* **19**, 125–132.

Carmona MC, Iglesias R, Obregon MJ, Darlington GJ, Villarroya F & Giralt M (2002). Mitochondrial biogenesis and thyroid status maturation in brown fat require CCAAT/enhancer-binding protein alpha. *J Biol Chem* **277**, 21489–21498.

Carrodeguas JA, Kobayashi R, Lim SE, Copeland WC & Bogenhagen DF (1999). The accessory subunit of *Xenopus laevis* mitochondrial DNA polymerase gamma increases processivity of the catalytic subunit of human DNA polymerase gamma and is related to class II aminoacyl-tRNA synthetases. *Mol Cell Biol* **19**, 4039–4046.

Casas F, Rochard P, Rodier A, Cassarmalek I, Marchalvictorion S, Wiesner RJ, Cabello G & Wrutniak C (1999). A variant form of the nuclear triiodothyronine receptor c-ErbA alpha 1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol Cell Biol* **19**, 7913–7924.

Castora FJ, Lazarus GM & Kunes D (1985). The presence of two mitochondrial DNA topoisomerases in human acute leukemia cells. *Biochem Biophys Res Commun* **130**, 854–866.

Castora FJ & Simpson MV (1979). Search for a DNA gyrase in mammalian mitochondria. *J Biol Chem* **254**, 11193–11195.

Chang DD & Clayton DA (1984). Precise identification of individual promoters for transcription of each strand of human mitochondrial DNA. *Cell* **36**, 635–643.

Chang DD & Clayton DA (1986*a*). Precise asignment of the light strand promoter of mouse mitochondrial DNA: a functional promoter consist of multiple upstream domains. *Mol Cell Biol* **6**, 3253–3261.

Chang DD & Clayton DA (1986*b*). Precise asignment of the heavy-strand promoter of mouse mitochondrial DNA: cognate start sites are not required for transcriptional initiation. *Mol Cell Biol* **6**, 3262–3267.

Chomyn A & Attardi G (1992). Recent advances on mitochondrial biogenesis. In *Molecular Mechanisms in Biogenesis*, ed. Ernster L, chap. 20, pp. 483–509. Elsevier Science Publishers B. V., Amsterdam.

Christianson TW & Clayton DA (1988). A tridecamer DNA sequence supports human mitochondrial RNA 3′-end formation *in vivo*. *Mol Cell Biol* **8**, 4502–4509.

Clayton DA (1982). Replication of animal mitochondrial DNA. *Cell* **28**, 693–705.

Clayton DA (1992). Transcription and replication of animal mitochondrial DNAs. *Int Rev Cytol* **141**, 217–232.

Cote J & Ruiz-Carrillo A (1993). Primers for mitochondrial DNA replication generated by endonuclease-G. *Science* **261**, 765–769.

Daga A, Micol V, Hess D, Aebersold R & Attardi G (1993). Molecular characterization of the transcription termination factor from human mitochondria. *J Biol Chem* **268**, 8123–8130.

Davis AF, Ropp PA, Clayton DA & Copeland WC (1996). Mitochondrial DNA polymerase gamma is expressed and translated in the absence of mitochondrial DNA maintenance and replication. *Nucleic Acids Res* **24**, 2753–2759.

de Bruijn MH, Schreier PH, Eperon IC & Barrell BG (1980). A mammalian mitochondrial serine transfer RNA lacking the 'dihydrouridine' loop and stem. *Nucleic Acids Res* **8**, 5212–5222.

Doda JN, Wright CT & Clayton DA (1981). Elongation of displacement-loop strands in human and mouse mitochondrial DNA is arrested near specific template sequences. *Proc Natl Acad Sci U S A* **78**, 6116–6120.

Doersen CJ, Guerrier-Takada C, Altman S & Attardi G (1985). Characterization of an RNase P activity from HeLa cell mitochondria. Comparison with the cytosol RNase P activity. *J Biol Chem* **260**, 5942–5949.

Dubin DT, Montoya J, Timko KD & Attardi G (1982). Sequence analysis and precise mapping of the 3′-ends of HeLa cell mitochondrial ribosomal RNAs. *J Mol Biol* **157**, 1–19.

England JM, Costantino P & Attardi G (1978). Mitochondrial RNA and protein synthesis in enucleated African green monkey cells. *J Mol Biol* **119**, 455–462.

Enriquez JA, Fernández-Silva P, Garrido-Perez N, Lopez-Perez MJ, Perez-Martos A & Montoya J (1999*a*). Direct regulation of mitochondrial RNA synthesis by thyroid hormone. *Mol Cell Biol* **19**, 657–670.

Enriquez JA, Fernández-Silva P & Montoya J (1999*b*). Autonomous regulation in mammalian mitochondrial DNA transcription. *Biol Chem* **380**, 737–747.

Enriquez JA, Fernández-Silva P, Perez-Martos A, Lopez-Perez MJ & Montoya J (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.

Ephrussi B, Hottinguer H & Tavlitzki J (1949). Action de l'acriflavine sur les levures. II. Etude génétique du mutant "petite colonie". *Ann Inst Pasteur* **76**, 351–367.

Etten RAV, Bird JW & Clayton DA (1983). Identification of the 3′-ends of the two mouse mitochondrial ribosomal RNAs. *J Biol Chem* **258**, 10104–10110.

Falkenberg M, Gaspari M, Rantanen A, Trifunovic A, Larsson NG & Gustafsson CM (2002). Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nat Genet* **31**, 289–294.

Fan L, Sanschagrin PC, Kaguni LS & Kuhn LA (1999). The accessory subunit of mtDNA polymerase shares structural homology with aminoacyl-tRNA synthetases. Implications for a dual role as a primer recognition factor and processivity clamp. *Proc Nat Acad Sci U S A* **96**, 9527–9532.

Farr CL, Wang Y & Kaguni LS (1999). Functional interactions of mitochondrial DNA polymerase and single-stranded DNA-binding protein. Template-primer DNA binding and initiation and elongation of DNA strand synthesis. *J Biol Chem* **274**, 14779–14785.

Fernández-Silva P, Martinez-Azorin F, Micol V & Attardi G (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.

Fernández-Silva P, Petruzzella V, Fracasso F, Gadaleta MN & Cantatore P (1991). Reduced synthesis of mt RNA in isolated mitochondria of senescent rat brain. *Biochem Biophys Res Commun* **176**, 645–653.

Fisher RP & Clayton DA (1985). A transcription factor required for promoter recognition by human mitochondrial RNA polymerase. *J Biol Chem* **260**, 11330–11338.

Fisher RP & Clayton, DA (1988). Purification and characterization of human mitochondrial transcription factor 1. *Mol Cell Biol* **8**, 3496–3509.

Fisher RP, Lisowsky T, Parisi MA & Clayton DA (1992). DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein. *J Biol Chem* **267**, 3358–3367.

Fisher RP, Topper JN & Clayton DA (1987). Promoter selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements. *Cell* **50**, 247–258.

Gadaleta MN, Petruzzella V, Fracasso F, Fernández-Silva P & Cantatore P (1990*a*). Acetyl-L-carnitine increases cytochrome oxidase subunit I mRNA content in hypothyroid rat liver. *FEBS Lett* **277**, 191–193.

Gadaleta MN, Petruzella V, Renis M, Fracasso F & Cantatore P (1990*b*). Reduced transcription of mitochondrial DNA in the senescent rat. Tissue dependence and effect of L-carnitine. *Eur J Biochem* **187**, 501–506.

Gaines G & Attardi G (1984). Highly efficient RNA-synthesizing system that uses isolated human mitochondria, new initiation events and *in vivo*-like processing patterns. *Mol Cell Biol* **4**, 1605–1617.

Gaines G, Rosi C & Attardi G (1987). Markedly different ATP requirements for rRNA synthesis and mtDNA light strand transcription versus mRNA synthesis in isolated human mitochondria. *J Biol Chem* **262**, 1907–1915.

Garstka HL, Facke M, Escribano JR & Wiesner RJ (1994). Stoichiometry of mitochondrial transcripts and regulation of gene expression by mitochondrial transcription factor A. *Biochem Biophys Res Commun* **200**, 619–626.

Gensler S, Weber K, Schmitt WE, Perez-Martos A, Enriquez JA, Montoya J & Wiesner RJ (2001). Mechanism of mammalian mitochondrial DNA replication, import of mitochondrial transcription factor A into isolated mitochondria stimulates 7S DNA synthesis. *Nucleic Acids Res* **29**, 3657–3663.

Ghivizzani SC, Madsen CS, Nelen MR, Ammini CV & Hauswirth WW (1994). In organello footprint analysis of human mitochondrial DNA, human mitochondrial transcription factor A interactions at the origin of replication. *Mol Cell Biol* **14**, 7717–7730.

Goglia F, Moreno M & Lanni A (1999). Action of thyroid hormones at the cellular level, the mitochondrial target. *FEBS Lett* **452**, 115–120.

Gordon JW, Rungi AA, Inagaki H & Hood DA (2001). Effects of contractile activity on mitochondrial transcription factor A expression in skeletal muscle. *J Appl Physiol* **90**, 389–396.

Graves SW, Johnson AA & Johnson KA (1998). Expression, purification, and initial kinetic characterization of the large subunit of the human mitochondrial DNA polymerase. *Biochemistry* **37**, 6050–6058.

Gray H & Wong TW (1992). Purification and identification of subunit structure of the human mitochondrial DNA polymerase. *J Biol Chem* **267**, 5835–5841.

Hehman GL & Hauswirth WW (1992). DNA helicase from mammalian mitochondria. *Proc Natl Acad Sci U S A* **89**, 8562–8566.

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

Herzig RP, Scacco S & Scarpulla RC (2000). Sequential serum-dependent activation of CREB and NRF-1 leads to enhanced mitochondrial respiration through the induction of cytochrome c. *J Biol Chem* **275**, 13134–13141.

Hess JF, Parisi MA, Bennett JL & Clayton DA (1991). Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* **351**, 236–239.

Hevner RF & Wong-Riley MT (1993). Mitochondrial and nuclear gene expression for cytochrome oxidase subunits are disproportionately regulated by functional activity in neurons. *J Neurosci* **13**, 1805–1819.

Hixson JE, Wong TW & Clayton DA (1986). Both the conserved stem-loop and divergent 5′-flanking sequences are required for initiation at the human mitochondrial origin of light-strand DNA replication. *J Biol Chem* **261**, 2384–2390.

Hobbs AE, Srinivasan M, McCaffery JM & Jensen RE (2001). Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. *J Cell Biol* **152**, 401–410.

Hofhaus G & Attardi G (1995). Efficient selection and characterization of mutants of a human cell line which are defective in mitochondrial DNA-encoded subunits of respiratory NADH dehydrogenase. *Mol Cell Biol* **15**, 964–974.

Hoke GD, Pavco PA, Ledwith BJ & Tuyle GCV (1990). Structural and functional studies of the rat mitochondrial single-stranded DNA binding protein P16. *Arch Biochem Biophys* **282**, 116–124.

Holt IJ, Harding AE, Petty RKH & Morgan-Hughes JA (1990). A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* **46**, 428–433.

Holt IJ, Lorimer HE & Jacobs HT (2000). Coupled leading- and lagging-strand synthesis of mammalian mitochondrial DNA. *Cell* **100**, 515–524.

Hood DA, Zak R & Pette D (1989). Chronic stimulation of rat skeletal muscle induces coordinate increases in mitochondrial and nuclear mRNAs of cytochrome-c-oxidase subunits. *Eur J Biochem* **179**, 275–280.

Izquierdo JM, Ricart J, Ostronoff LK, Egea G & Cuezva JM (1995). Changing patterns of transcriptional and post-transcriptional control of beta-F-1-ATPase gene expression during mitochondrial biogenesis in liver. *J Biol Chem* **270**, 10342–10350.

Jacobs HT, Lehtinen SK & Spelbrink JN (2000). No sex please, we're mitochondria, a hypothesis on the somatic unit of inheritance of mammalian mtDNA. *Bioessays* **22**, 564–572.

Jenuth JP, Peterson AC & Shoubridge EA (1997). Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nat Genet* **16**, 93–95.

Kadenbach B, Possekel S, Huttemann M & Arnold S (1998). Biochemical defects and genetic abnormalities in cytochrome c oxidase of patients with Leigh syndrome. *Biofactors* **7**, 273–276.

Kaufman BA, Newman SM, Hallberg RL, Slaughter CA, Perlman PS & Butow RA (2000). In organello formaldehyde crosslinking of proteins to mtDNA, identification of bifunctional proteins. *Proc Natl Acad Sci U S A* **97**, 7772–7777.

Kaukonen J, Juselius JK, Tiranti V, Kyttala A, Zeviani M, Comi GP, Keranen S, Peltonen L & Suomalainen A (2000). Role of adenine nucleotide translocator 1 in mtDNA maintenance. *Science* **289**, 782–785.

Kosovsky M & Soslau G (1993). Immunological identification of human platelet mitochondrial DNA topoisomerase-I. *Biochim Biophys Acta* **1164**, 101–107.

Kroemer G & Reed JC (2000). Mitochondrial control of cell death. *Nat Med* **6**, 513–519.

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

Lang BF, Gray MW & Burger G (1999). Mitochondrial genome evolution and the origin of eukaryotes. *Annu Rev Genet* **33**, 351–397.

Larsson NG, Wang JM, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS & Clayton DA (1998). Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat Genet* **18**, 231–236.

Lecrenier N & Foury F (2000). New features of mitochondrial DNA replication system in yeast and man. *Gene* **246**, 37–48.

Lee DY & Clayton D (1996). Properties of a primer RNA-DNA hybrid at the mouse mitochondrial DNA leading-strand origin of replication. *J Biol Chem* **271**, 24262–24269.

Lefai E, Fernández-Moreno MA, Alahari A, Kaguni LS & Garesse R (2000). Differential regulation of the catalytic and accessory subunit genes of *Drosophila* mitochondrial DNA polymerase. *J Biol Chem* **275**, 33123–33133.

Lightowlers RN, Chinnery PF, Turnbull DM & Howell N (1997). Mammalian mitochondrial genetics, heredity, heteroplasmy and disease. *Trends Genet* **13**, 450–455.

Lim SE, Longley MJ & Copeland WC (1999). The mitochondrial p55 accessory subunit of human DNA polymerase gamma enhances DNA binding, promotes processive DNA synthesis, and confers N-ethylmaleimide resistance. *J Biol Chem* **274**, 38197–38203.

Loguercio-Polosa P & Attardi G (1991). Distinctive pattern and transcriptional control of mitochondrial protein synthesis in rat brain synaptic endings. *J Biol Chem* **266**, 10011–10017.

Longley MJ, Nguyen D, Kunkel TA & Copeland WC (2001). The fidelity of human DNA polymerase gamma with and without exonucleolytic proofreading and the p55 accessory subunit. *J Biol Chem* **276**, 38555–38562.

McCulloch V, Seidel-Rogol BL & Shadel GS (2002). A human mitochondrial transcription factor is related to RNA adenine methyltransferases and binds s-adenosylmethionine. *Mol Cell Biol* **22**, 1116–1125.

Madsen CS Ghivizzani SC & Hauswirth WW (1993). Protein binding to a single termination-associated sequence in the mitochondrial DNA D-loop region. *Mol Cell Biol* **13**, 2162–2171.

Marchington DR, Macaulay V, Hartshorne GM, Barlow D & Poulton J (1998). Evidence from human oocytes for a genetic bottleneck in an mtDNA disease. *Am J Hum Genet* **63**, 769–775.

Martin I, Vinas O, Mampel T, Iglesias R & Villarroya F (1993). Effects of cold environment on mitochondrial genome expression in the rat – evidence for a tissue-specific increase in the liver, independent of changes in mitochondrial gene abundance. *Biochem J* **296**, 231–234.

Michikawa Y, Mazzucchelli F, Bresolin N, Scarlato G & Attardi G (1999). Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* **286**, 774–779.

Micol V, Fernández-Silva P & Attardi G (1996). Isolation and assay of mitochondrial transcription termination factor from human cells. *Methods Enzymol* **264**, 158–173.

Micol V, Fernández-Silva P & Attardi G (1997). Functional analysis of *in vivo* and in organello footprinting of HeLa cell mitochondrial DNA in relationship to ATP and ethidium bromide effects on transcription. *J Biol Chem* **272**, 18896–18904.

Miyakawa I, Sando N, Kawano S, Nakamura S & Kuroiwa T (1987). Isolation of morphologically intact mitochondrial nucleoids from the yeast, *Saccharomyces cerevisiae. J Cell Sci* **88**, 431–439.

Montoya J, Christianson T, Levens D, Rabinowitz M & Attardi G (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.

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

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

Montoya J, Perez-Martos A, Garstka HL & Wiesner RJ (1997). Regulation of mitochondrial transcription by mitochondrial transcription factor A. *Mol Cell Biochem* **174**, 227–230.

Moraes CT (2001). What regulates mitochondrial DNA copy number in animal cells? *Trends Genet* **17**, 199–205.

Moraes CT, Kenyon L & Hao HL (1999). Mechanisms of human mitochondrial DNA maintenance, the determining role of primary sequence and length over function. *Mol Biol Cell* **10**, 3345–3356.

Mutvei A, Kuzela S & Nelson BD (1989). Control of mitochondrial transcription by thyroid hormone. *Eur J Biochem* **180**, 235–240.

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

Nass MMK & Nass S (1963). Intramitochondrial fibers with DNA characteristics. *J Cell Biol* **19**, 593–629.

Nie F & Wong-Riley MT (1996). Mitochondrial- and nuclear-encoded subunits of cytochrome oxidase in neurons, differences in compartmental distribution, correlation with enzyme activity, and regulation by neuronal activity. *J Comp Neurol* **373**, 139–155.

Nishino I, Spinazzola A & Hirano M (1999). Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* **283**, 689–692.

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

Ostronoff LK, Izquierdo JM, Enriquez JA, Montoya J & Cuezva JM (1996). Transient activation of mitochondrial translation regulates the expression of the mitochondrial genome during mammalian mitochondrial differentiation. *Biochem J* **316**, 183–191.

Overly CC, Rieff HI & Hollenbeck PJ (1996). Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons. *J Cell Sci* **109**, 971–980.

Papa S, Sardanelli AM, Scacco S & Technikova-Dobrova Z (1999). cAMP-dependent protein kinase and phosphoproteins in mammalian mitochondria. An extension of the cAMP-mediated intracellular signal transduction. *FEBS Lett* **444**, 245–249.

Parisi MA & Clayton DA (1991). Similarity of human mitochondrial transcription factor 1 to high mobility group proteins. *Science* **252**, 965–969.

Penta JS, Johnson FM, Wachsman JT & Copeland C (2001). Mitochondrial DNA in human malignancy. *Mutat Res* **488**, 119–133.

Perez-Jannotti RM, Klein SM & Bogenhagen DF (2001). Two forms of mitochondrial DNA ligase III are produced in *Xenopus laevis* oocytes. *J Biol Chem* **276**, 48978–48987.

Pinz KG & Bogenhagen DF (1998). Efficient repair of abasic sites in DNA by mitochondrial enzymes. *Mol Cell Biol* **18**, 1257–1265.

Preiss T, Sang AE, Chrzanowska-Lightowlers ZM & Lightowlers RN (1995). The mRNA-binding protein COLBP is glutamate dehydrogenase. *FEBS Lett* **367**, 291–296.

Prieto-Martín A, Montoya J & Martínez-Azorín F (2001). A study on the human mitochondrial RNA polymerase activity points to existence of a transcription factor B-like protein. *FEBS Lett* **503**, 51–55.

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

Richter C, Park JW & Ames BN (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A* **85**, 6465–6467.

Roberti M, Musicco C, Polosa PL, Gadaleta MN & Cantatore P (1996). DNA-helicase activity from sea urchin mitochondria. *Biochem Biophys Res Commun* **219**, 134–139.

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

Scarpulla RC (1997). Nuclear control of respiratory chain expression in mammalian cells. *J Bioenerg Biomembr* **29**, 109–119.

Scarpulla RC (2002). Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim Biophys Acta* **1576**, 1–14.

Schultz RA, Swoap SJ, McDaniel LD, Zhang B, Koon EC, Garry DJ, Li K & Williams RS (1998). Differential expression of mitochondrial DNA replication factors in mammalian tissues. *J Biol Chem* **273**, 3447–3451.

Schwartz M & Vissing J (2002). Paternal inheritance of mitochondrial DNA. *N Engl J Med* **347**, 576–580.

Shadel GS & Clayton DA (1997). Mitochondrial DNA maintenance in vertebrates. *Annu Rev Biochem* **66**, 409–435.

Shang J & Clayton DA (1994). Human mitochondrial transcription termination exhibits RNA polymerase independence and biased bipolarity in vitro. J *Biol Chem* **269**, 29112–29120.

Shay JW, Pierce DJ & Werbin H (1990). Mitochondrial DNA copy number is proportional to total cell DNA under variety of growth conditions. *J Biol Chem* **264**, 14802–14807.

Shen EL & Bogenhagen DF (2001). Developmentally-regulated packaging of mitochondrial DNA by the HMG-box protein mtTFA during *Xenopus* oogenesis. *Nucleic Acids Res* **29**, 2822–2828.

Shoubridge EA (2002). The ABCs of mitochondrial transcription. *Nat Genet* **31**, 227–228.

Shuster RC, Rubenstein AJ & Wallace DC (1988). Mitochondrial DNA in anucleate human blood cells. *Biochem Biophys Res Commun* **155**, 1360–1365.

Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, Tariq M, Wanrooij S, Garrido N, Comi G, Morandi L, Santoro L, Toscano A, Fabrizi GM, Somer H, Croxen R, Beeson D, Poulton J, Suomalainen A, Jacobs HT, Zeviani M & Larsson C (2001). Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* **28**, 223–231.

Stoneking M (1994). Mitochondrial DNA and human evolution. *J Bioenerg Biomembr* **26**, 251–259.

Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C & Schatten G (2000). Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. *Biol Reprod* **63**, 582–590.

Sutovsky P, Moreno RD & Schatten G (1999). Ubiquitin tag for sperm mitochondria. *Nature* **402**, 371.

Taanman JW (1999). The mitochondrial genome, structure, transcription, translation and replication. *Biochim Biophys Acta* **1410**, 103–123.

Takamatsu C, Umeda S, Ohsato T, Ohno T, Abe Y, Fukuoh A, Shinagawa H, Hamasaki N & Kang DC (2002). Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. *EMBO Rep* **3**, 451–456.

Tang YY, Schon EA, Wilichowski E, Vazquezmemije ME, Davidson E & King MP (2000). Rearrangements of human mitochondrial DNA (mtDNA), new insights into the regulation of mtDNA copy number and gene expression. *Mol Biol Cell* **11**, 1471–1485.

Tiranti V, Savoia A, Forti F, Dapolito MF, Centra M, Racchi M & Zeviani M (1997). Identification of the gene encoding the human mitochondrial RNA polymerase (h-mtRPOL) by cyberscreening of the expressed sequence tags database. *Hum Mol Genet* **6**, 615–625.

Tsiriyotis C, Spandidos DA & Sekeris CE (1997). The mitochondrion as a primary site of action of glucocorticoids, mitochondrial nucleotide sequences, showing similarity to hormone response elements, confer dexamethasone inducibility to chimaeric genes transfected in LATK(−) cells. *Biochem Biophys Res Commun* **235**, 349–354.

Van Dyck E, Foury F, Stillman B & Brill SJ (1992). A single-stranded DNA binding protein required for mitochondrial DNA replication in *S. cerevisiae* is homologous to *E. coli* SSB. *EMBO J* **11**, 3421–3430.

VanItallie CM (1990). Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear-encoded subunit cytocrome c oxidase. *Endocrinology* **127**, 55–62.

Virbasius JV & Scarpulla RC (1994). Activation of the human transcription factor A gene by nuclear respiratory factors – a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci U S A* **91**, 1309.

Walberg MW & Clayton DA (1981). Sequence and properties of the human KB cell and mouse L cell D-loop regions of mitochondrial DNA. *Nucleic Acids Res* **9**, 5411–5421.

Walberg MW & Clayton DA (1983). *In vitro* transcription of human mitochondrial DNA, identification of specific light strand transcripts from the displacement loop region. *J Biol Chem* **258**, 1268–1275.

Wallace DC (1992). Diseases of the mitochondrial DNA. *Annu Rev Biochem* **61**, 1175–1212.

Wallace DC (1997). Mitochondrial DNA in aging and disease. *Sci Am* **277**, 40–47.

Wallace DC, Brown MD & Lott MT (1999). Mitochondrial DNA variation in human evolution and disease. *Gene* **238**, 211–230.

Weber K, Bruck P, Mikes Z, Kupper JH, Klingenspor M & Wiesner RJ (2002). Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology* **143**, 177–184.

Wiesner RJ, Aschenbrenner V, Ruegg JC & Zak R (1994). Coordination of nuclear and mitochondrial gene expression during the development of cardiac hypertrophy in rats. *Am J Physiol* **267**, C229–235.

Wiesner RJ, Kurowski TT & Zak R (1992*a*). Regulation by thyroid hormones of nuclear and mitochondrial genes encoding subunits of cytochrome-c oxidase in rat liver and skeletal muscle. *Mol Endocrinol* **6**, 1458–1467.

Wiesner RJ, Ruegg JC & Morano I (1992*b*). Counting target molecules by exponential polymerase chain reaction, copy number of mitochondrial DNA in rat tissues. *Biochim Biophys Acta* **183**, 553–559.

Williams RS (1986). Mitochondrial gene expression in mammalian striated muscle. Evidence that variation in gene dosage is the major regulatory event. *J Biol Chem* **261**, 12390–12394.

Wolstenholme DR (1992). Animal mitochondrial DNA, structure and evolution. *Int Rev Cytol* **141**, 173–216.

Wong TW & Clayton DA (1985). *In vitro* replication of human mitochondrial DNA, accurate initiation at the origin of light-strand synthesis. *Cell* **42**, 952–958.

Wong TW & Clayton DA (1986). DNA primase of human mitochondria is associated with structural RNA that is essential for enzymatic activity. *Cell* **45**, 817–825.

Wrutniak C, Cassar-Malek I, Marchal S, Rascle A, Heusser S, Keller JM, Flechon J, Dauca M, Samarut J, Ghysdael J & Cabello G (1995). A 43-kDa protein related to c-Erb A α 1 is located in the mitochondrial matrix of rat liver. *J Biol Chem* **270**, 16347–16354.

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC & Spiegelman BM (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98**, 115–124.

Xu BJ & Clayton DA (1992). Assignment of a yeast protein necessary for mitochondrial transcription initiation. *Nucleic Acids Res* **20**, 1053–1059.

Xu BJ & Clayton DA (1996). RNA-DNA hybrid formation at the human mitochondrial heavy-strand origin ceases at replication start sites. An implication for RNA-DNA hybrids serving as primers. *EMBO J* **15**, 3135–3143.

Yoshida Y, Izumi H, Ise T, Uramoto H, Torigoe T, Ishiguchi H, Murakami T, Tanabe M, Nakayama Y, Itoh H, Kasai H & Kohno K (2002). Human mitochondrial transcription factor A binds preferentially to oxidatively damaged DNA. *Biochem Biophys Res Commun* **295**, 945–951.

Yoza BK & Bogenhagen DF (1984). Identification and *in vitro* capping of a primary transcript of human mitochondrial DNA. *J Biol Chem* **259**, 3909–3915.

Zeviani M & Antozzi C (1997). Mitochondrial disorders. *Mol Hum Reprod* **3**, 133–148.

Zhang HL, Barcelo JM, Lee B, Kohlhagen G, Zimonjic DB, Popescu NC & Pommier Y (2001). Human mitochondrial topoisomerase I. *Proc Nat Acad Sci U S A* **98**, 10608–10613.

Acknowledgements

This work was supported by the Spanish Ministerio de Ciencia y Tecnología (BMC 2001-2421 grant to J.M. and Ramón y Cajal 2001 grant to P.F.-S.) and by the Spanish Ministerio de Educación (PM-99-0082 grant to J.A.E.).