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Review

Animal mitochondrial biogenesis and function: a regulatory cross-talk between two genomes

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Abstract

Mitochondria play a pivotal role in cell physiology, producing the cellular energy and other essential metabolites as well as controlling apoptosis by integrating numerous death signals. The biogenesis of the oxidative phosphorylation system (OXPHOS) depends on the coordinated expression of two genomes, nuclear and mitochondrial. As a consequence, the control of mitochondrial biogenesis and function depends on extremely complex processes that require a variety of well orchestrated regulatory mechanisms. It is now clear that in order to provide cells with the correct number of structural and functional differentiated mitochondria, a variety of intracellular and extracellular signals including hormones and environmental stimuli need to be integrated. During the last few years a considerable effort has been devoted to study the factors that regulate mtDNA replication and transcription as well as the expression of nuclear-encoded mitochondrial genes in physiological and pathological conditions. Although still in their infancy, these studies are starting to provide the molecular basis that will allow to understand the mechanisms involved in the nucleo-mitochondrial communication, a cross-talk essential for cell life and death.

Keywords: mtDNA; Gene expression; Retrograde communication; Hormonal regulation; Differentiation; Apoptosis

1. Introduction

Many essential metabolic pathways are compartmentalised in mitochondria, including the bulk of ATP synthesis through oxidative phosphorylation. Mitochondria are essential for practically all eukaryotic cells, but different animal tissues contain mitochondria that differ substantially in number, structure and function, reflecting their variable energetic demand. Furthermore, a specific cell type may adapt mitochondrial function in response to cellular or environmental signals such as hormones, growth factors, changes in temperature, physiological activity or developmental cues. Thus, mitochondrial biogenesis and function depend on extremely complex and well orchestrated processes that need to be precisely regulated according to the variable physiological circumstances of the cell (Cuezva et al., 1997; Enriquez et al., 1999a; Pollak and Sutton, 1980).

Mitochondria are unique structures of the animal cells because their functional activity and, specifically, their capacity to generate ATP depends on a precise cross-talk between two physically separated genetic systems (Attardi and Schatz, 1988; Grivell, 1995; Poyton and McEwen, 1996). In this review, we will briefly discuss the main features of the mitochondrial genetic system and the mechanisms of intergenomic communication involved in the control of mitochondrial biogenesis and function in cell life and death, emphasising the present state of knowledge regarding transcriptional control. More detailed discussions of other specific mitochondrial topics have been published recently, including the post-transcriptional mechanisms involved in the regulation of mitochondrial biogenesis (Cuezva et al., 1997), the role of mitochondria in development and cell differentiation (Fernández-Moreno et al., 2000b) and the expanding spectrum of diseases

Abbreviations: GABP/NRF2, nuclear respiratory factor 2/GA binding protein; MRP, mitochondrial RNA processing endonuclease; mtDNA, mitochondrial DNA; mTERF, mitochondrial termination factor; mtTFA (Tfam), mitochondrial transcription factor A; NRF1, nuclear respiratory factor 1; OXPHOS, oxidative phosphorylation system

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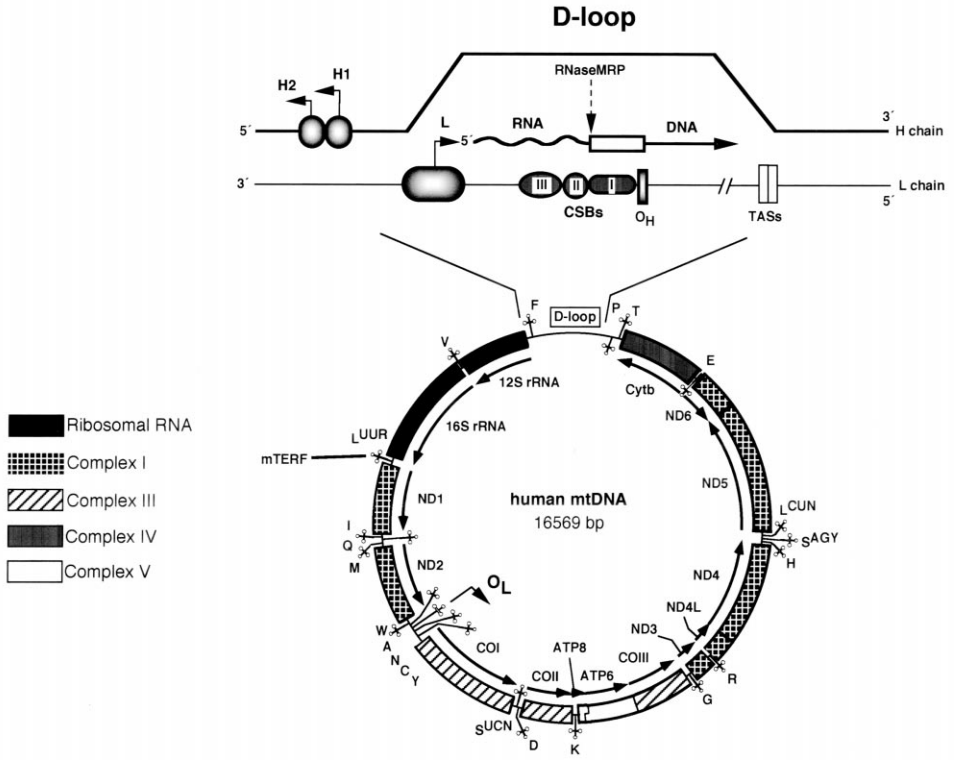

Fig. 1. Human mitochondrial DNA map. Protein and rRNA coding genes are represented by boxes. The tRNA genes are identified by the single letter code and represented by cloverleaf structures. Arrows below the genes show the direction of transcription. There are two regions containing overlapping genes: ATPase 6/8 and ND4/4L. The regulatory elements located in the D-loop are indicated: O_H: origin of replication of the heavy strand; H1 and H2: heavy strand promoters; L: light strand promoter; CSB: Conserved Sequence Blocks; TAS: Termination Associated Sequences. O_L: origin of replication of the light strand; mTERF, transcription termination factor. Replication from O_H starts with the synthesis of the primer from the L promoter by the mtRNA polymerase. A transition between replication and transcription is produced in the region containing the CSB elements, likely by RNase MRP-mediated processing of the RNA-DNA hybrids.

produced by the failure in ATP synthesis caused by mutations in mitochondrial genes (Chinnery and Turnbull, 1999; DiMauro et al., 1998; Leonard and Schapira, 2000a,b; Zeviani and Antozzi, 1997).

2. The mitochondrial genetic system

Mitochondria contain their own genome, the mitochondrial DNA (mtDNA), a covalently closed-circular double stranded DNA molecule that encodes a small number of genes, usually 37 in animal mtDNAs (Fig. 1). Twenty four encode components of the mitochondrial translational machinery: 22 tRNAs and two rRNAs (12S and 16S). The additional thirteen genes encode a set of structural proteins, all of them components of the OXPHOS system: seven subunits of the NADH: Ubiquinone oxidoreductase (Complex I; ND1-6 and ND4L), one subunit of the Ubiquinone: Cytochrome c oxidoreductase (Complex III; Cyt b), three subunits of the Cytochrome c Oxidase (Complex IV; COI-III) and two subunits of the H^+ ATP synthase (Complex V; ATPase 6 and 8). The rest of the OXPHOS subunits as well as all the factors involved in the mtDNA metabolism are nuclear-encoded, translated in cytoplasmic ribosomes and imported to their final organelle compartment by translocases located in the outer and inner mitochondrial membranes (Attardi and Schatz, 1988; Herrmann and Neupert, 2000). MtDNA is maternally inherited and contains several interesting features including a high copy number (several thousands molecules per cell; 1–10 copies per mitochondrion), very few intergenic spacing, lack of introns, overlapping genes and a genetic code that differs from the universal one (Clayton, 2000). Although traditionally considered ‘naked’, a number of proteins interacts with the mtDNA. Recently, Jacobs and colleagues have proposed that mitochondria contain a functional organised unit, the nucleoid, composed of several mtDNA molecules and proteins forming a structure, probably linked to the mitochondrial inner membrane, which functions as a segregation unit (Jacobs et al., 2000). In yeast, the proteins associated with mtDNA have been recently identified from in organello formaldehyde crosslinking experiments (Kaufman et al., 2000). About 20 proteins were found crosslinked to mtDNA, of which 11 were identified. Several of these proteins were already known to have DNA binding properties or to be involved in DNA maintenance, but others, like the mitochondrial chaperone Hsp60p or the tricarboxylic acid cycle Kgd2p, were not. Genetic experiments indicated

that these two proteins are involved in mtDNA stability. Surprisingly, Hsp60p is a single-stranded DNA binding protein that binds specifically to a putative origin of mtDNA replication.

The mitochondrial genome is replicated and transcribed within the organelle. Both mtDNA replication and transcription have been characterised mainly in mammals where the cis-elements responsible for the regulation of these processes are mostly located within a small non-coding DNA fragment, the D-loop region (see below). On the other hand, all the trans-acting factors involved in mtDNA metabolism are nuclear-encoded, including mtRNA polymerase, mtDNA polymerase (pol γ) and all the potential factors that regulate mtDNA replication, mtDNA transcription and mtRNA processing. Apart from mammals and with only few exceptions (Antoshechkin and Bogenhagen, 1995; Antoshechkin et al., 1997; Carrodeguas and Vallejo, 1997), the mitochondrial genetic system of different animal phyla are so far poorly characterised. However, it is generally assumed that the basic features of mtDNA metabolism have been conserved during evolution.

Studies in mammalian cells suggest that mtDNA replication takes place in the perinuclear space (Davis and Clayton, 1996). Although it occurs throughout the cell cycle, a link between mtDNA and nuclear DNA (nDNA) replication has been demonstrated recently in *Drosophila*. DREF, a transcription factor involved in the coordinated transcription of genes involved in nDNA replication and cell-cycle control, regulates the expression of the genes encoding the mitochondrial single-stranded DNA binding protein (mtSSB) and the accessory (β) subunit of pol γ (Lefai et al., 2000a; Ruiz de Mena et al., 2000). Thus, DREF may orchestrate the response of the mtDNA replication machinery to the cell-cycle regulated nDNA synthesis.

The mechanism of mammalian mtDNA replication was described more than twenty years ago (Kasamatsu and Vinograd, 1973) and studied in detail by the group of Clayton (Clayton, 1982, 1987), although some features still remain incompletely characterised. The two strands of the mammalian mtDNA, named on the basis of their buoyant density heavy- (H) and light- (L) strand, are replicated asynchronously and asymmetrically. DNA synthesis starts in the origin of replication of the heavy strand (O_H) located in the D-loop region and proceeds unidirectionally until the origin of replication of the light strand (O_L), located two-thirds away around the genome (Fig. 1), is reached. Once O_L is single-stranded, mtDNA synthesis starts in the opposite direction (Clayton, 1982). In addition, a coupled leading- and lagging-strand mechanism of mtDNA synthesis has been proposed to also occur in human mitochondria, suggesting that cells may use one or the other mechanism in different physiological conditions (Holt et al., 2000), a result compatible with recent data obtained in *Drosophila* (Lefai et al., 2000b).

Animal cells contain a DNA polymerase devoted to mtDNA synthesis, the DNA polymerase γ (pol γ) that has been extensively characterised in *Drosophila melanogaster* by the group of Kaguni and later in other systems (Gray and Wong, 1992; Insdorf and Bogenhagen, 1989; Kaguni and Olson, 1989; Longley and Mosbaugh, 1991; Olson et al., 1995; Wernette and Kaguni, 1986). Pol γ comprises two activities, $5' \rightarrow 3'$ DNA polymerase and a proof-reading $3' \rightarrow 5'$ exonuclease. The holoenzyme is a heterodimer composed of a large polypeptide of 125–140 kDa (pol γ-α) and a small accessory subunit of 35–55 kDa (pol γ-β). The genes encoding both subunits have recently been identified and characterised in several organisms (Carrodeguas et al., 1999; Lecrenier et al., 1997; Lewis et al., 1996; Ropp and Copeland, 1996; Wang et al., 1997; Ye et al., 1996). Both, DNA polymerase and exonuclease activity are associated with the large subunit, while accessory subunit is likely involved in primer recognition and increased enzyme processivity (Carrodeguas et al., 1999; Carrodeguas and Bogenhagen, 2000; Fan et al., 1999; Johnson et al., 2000; Lim et al., 1999). The crucial role of the catalytic subunit has been demonstrated in both *Drosophila* (Iyengar et al., 2000) and humans (Spelbrink et al., 2000). In addition, the presence of a β-like DNA polymerase activity has been recently identified in bovine mitochondria (Nielsen-Preiss and Low, 2000), opening the possibility that several DNA polymerases participate in mtDNA replication/repair. The other essential component of the replication machinery structurally and functionally characterised in several systems is the protein mtSSB (Thommes et al., 1995; Van Dyck et al., 1992; Van Tuyle and Pavco, 1985). Other factors such as helicases or topoisomerases likely involved in mtDNA replication are still poorly characterised (Clayton, 1992, 2000).

The mammalian mtDNA contains three promoters (L, H_1 and H_2) located in the non-coding D-loop region (Fig. 1). The transcription of the H-strand starts at two sites, IT_{H1} and IT_{H2} whereas L-strand transcription starts at a single site, IT_{L1} (Montoya et al., 1982, 1983). Transcripts synthesised from IT_{L1} and IT_{H2}, which is located within the $tRNA^{Phe}$ gene, are polycistronic and their corresponding transcription units encompass practically the complete L and H DNA strands, respectively (Taanman, 1999). IT_{H1}, is placed just before the $tRNA^{Phe}$ gene, and the transcription unit ends in a strong termination signal located downstream the 16SrRNA gene (within the $tRNA^{Leu(UUR)}$ gene). The termination signal is evolutively conserved (Valverde et al., 1994) and binds a trans-acting factor called mTERF (Fernandez-Silva et al., 1997). Therefore, the main function of the H_1 promoter is the regulated synthesis of the two rRNAs. The primary polycistronic transcripts are further processed to monocistronic (or exceptionally bicistronic) mRNAs with the tRNAs acting as punctuation signals (Ojala et al., 1981). The enzymatic activities responsible for the $5'$ and $3'$ processing of tRNAs have been partially characterised (Rossmanith et al., 1995), but the genes encoding the mitochondrial RNase P and precursor tRNA $3'$ endonuclease remain to be identified. Although mt-mRNAs are polyadenylated, the charac-

terisation of the mitochondrial poly (A) polymerase and potential associated factors also remains elusive.

The mitochondrial transcriptional machinery is relatively simple compared to the nuclear one and consists of a single unspecific RNA polymerase that is evolutively conserved to bacteriophage T7, T3 and SP6 RNA polymerases and at least a specificity factor, mtTFA (Tfam), a small polypeptide that belongs to the family of HMG DNA binding proteins. In addition to its role in transcription, mtTFA is essential for mtDNA maintenance in yeast (Diffley and Stillman, 1991) and most likely in mammals (Larsson et al., 1998), due to its binding capacity to non-specific DNA. Thus, mtTFA has a dual role as specificity factor / transcriptional activator and in chromosome packaging. Furthermore, the specific promoter recognition depends in *Xenopus* on a second transcription factor, mtTFB, that shares some identity to bacterial sigma factors (Antoshechkin and Bogenhagen, 1995). In this system mtTFA acts only as a weak transcriptional activator and in mtDNA wrapping. It is important to keep in mind that the mammalian mtRNA polymerase has not yet been purified to homogeneity and, therefore, the presence of a mtTFB homologue in mammals is still uncertain. A summary of the main factors potentially involved in the mtDNA and mtRNA metabolism are shown in Fig. 2.

Although it was shown long time ago that isolated mitochondria can sustain a faithful synthesis of the 13 polypeptides encoded in the mtDNA, the mitochondrial protein synthesis machinery has been so far poorly characterised. The mitoribosomes are located in the mitochondrial matrix and contain a comparative low RNA content compared to cytoplasmic and prokaryotic ribosomes. Although traditionally it has been considered that mitochondrial ribosomes contain only the mtDNA-encoded 12S and 16S rRNAs, recently the presence in the mitochondria of a 5S rRNA species of nuclear origin has been demonstrated (Magalhaes et al., 1998). From the approximately 85 ribosomal proteins that are part of mitoribosomes, only a few have been characterised so far (Taanman, 1999). Interestingly, the expression of the mammalian mitochondrial ribosomal protein L12 (mtL12) is growth regulated at the translational level (Mariottini et al., 1999), suggesting the importance of the regulation of mitochondrial protein synthesis during the cell cycle (Marty and Fort, 1996). It is interesting to point out, that even with complete genome sequences now available for two metazoans, most mitoribosomal proteins remain unidentified (O'Brien et al., 2000). This probably reflects a lack of primary sequence similarity and suggests the possible recruitment of novel proteins to mitoribosomes during the course of evolution.

The mitochondrial biosynthetic machinery has some unusual features including the use of a limited set of tRNAs and the use of several non-canonical triplets (or even a quadruplet) as initiation codons. In addition, mitochondrial mRNAs have no 5′-UTR sequence and lack the typical cap structure of cytoplasmic mRNAs. How the mitoribosomes detect the 5′ end of mRNAs and initiate translation is presently unknown. Interestingly, it has been shown recently that when the first translation initiation codon of the ND1 mRNA is absent due to a mutation, the mitochondrial ribosomes select with high efficiency the next AUG present in the sequence to initiate protein synthesis (Fernández-Moreno et al., 2000a). Initiation and elongation of translation depend on the activity of translation and elongation factors similar to their prokaryotic counterparts, including mtIF2, mtEFTu, mtEFTs and mtEFG (reviewed in Taanman, 1999). In addition, several aminoacyl tRNA synthetases are starting recently to being characterised (Jorgensen et al., 2000).

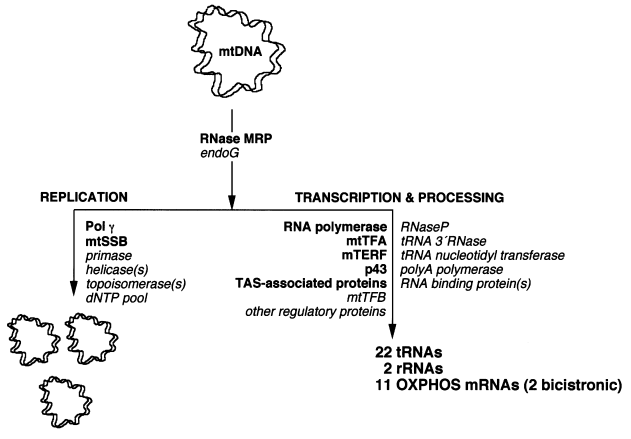

Fig. 2. Factors involved in mtDNA replication, transcription and processing. Some of the key factors involved in mtDNA replication, transcription and processing are indicated. Those well characterised in biochemical and/or molecular terms are shown in bold, whereas those still poorly characterised or unidentified, in italics.

3. Nucleo-mitochondrial communication

3.1. Nucleus to mitochondria: nuclear respiratory factors

As discussed previously, the activity of the respiratory chain depends on the expression of hundred of genes encoded in the nuclear genome and 13 genes encoded in the mitochondrial genome. This implies that a coordinate regulation of the two genetic systems of the cell is necessary for a correct mitochondrial biogenesis. With the aim to characterise the mechanisms involved in this intergenomic cross-talk, one of the most fruitful experimental approaches has been focused in the identification of transcription factors that regulate the expression of nuclear-encoded mitochondrial *respiratory* genes. Thus, the promoter of several genes encoding OXPHOS subunits and enzymes involved in mtDNA metabolism have been characterised during the last few years. In general, they lack canonical TATA and CAAT boxes, have heterogeneous transcriptional initiation sites and contain regulatory regions that are usually complex and promoter-specific, even for the genes that encode subu-

nits of the same OXPHOS complex. For example, the promoter of the mammalian ATP synthase α subunit gene is regulated by the transcription factors YY1 and USF2 (Breen and Jordan, 1998; Breen et al., 1996), while the activity of the ATP synthase β subunit promoter is dependent on two other different regulatory factors, NRF2/GABP and Sp1 (Villena et al., 1998; Zaid et al., 1999). A similar situation can be found in the genes encoding the nuclear-encoded cytochrome *c* oxidase subunits where the activity of each promoter is regulated by a different combination of transcription factors (Lenka et al., 1998). Therefore, the expression of the respiratory apparatus largely rely on gene-specific regulatory mechanisms. However and most interestingly, some DNA binding motifs are present in the promoter of several genes involved in mitochondrial biogenesis and are, therefore, candidates to participate in a coordinated response. They include the OXBOX/REBOX motif (Chung et al., 1992), the Mt motifs (Suzuki et al., 1990, 1995) (these two groups of motifs only characterised by their DNA protein-binding activity) and motifs recognised by three regulatory proteins, Sp1 (Zaid et al., 1999), nuclear respiratory factor 1 (NRF1) (Virbasius et al., 1993a) and nuclear respiratory factor 2 (GABP/NRF2) (Virbasius et al., 1993b).

The general transcription factor Sp1 belongs to the Zinc-finger family and has been demonstrated to be critical for the expression a large number of genes involved in a variety of constitutive and tissue-specific cellular functions (Suske, 1999). By contrast, NRF-1 belongs to a new class of regulatory proteins and was first identified as a transcriptional activator of the rat cytochrome c gene (Evans and Scarpulla, 1989). The DNA binding domain of NRF-1 presents homology with two developmental regulatory factors expressed in *Drosophila* (Erect Wing) and sea urchin (P3A2). Two mechanisms likely involved in the regulation of the NRF-1 activity have been reported and both are related to the ability of NRF-1 to bind DNA as homodimer. First, NRF-1 has serine residues in the amino-terminal domain which are physiologically phosphorylated, probably by casein kinase II, producing a conformational change in the dimer that enhances its DNA binding activity (Gugneja and Scarpulla, 1997). Second, a shorter NRF-1 mRNA produced by alternative splicing, and lacking the carboxy-terminal part of the DNA-binding domain, has been found at different concentrations in the various tissues (Spelbrink and Van den Bogert, 1995) suggesting a mechanism for the tissue-specific regulation of the NRF1 activity.

Functional NRF1 sites has been identified in the promoter of several genes encoding OXPHOS subunits, the rate-limiting enzyme in the synthesis of heme, delta amino levulinate synthase, factors involved in mtDNA replication and transcription and in the promoter of the mitoribosomal protein S12 (Johnson et al., 1998; Virbasius et al., 1993a). This suggest that NRF1 plays a key role in the mitochondrial biogenesis process (Scarpulla, 1997; Virbasius and Scarpulla, 1994), a view supported by several lines of evidence. For example, after partial hepatectomy, the oxidative phosphorylation capacity increases in the remnant liver. In this situation, the content of NRF-1 mRNA increases quickly, reaching a maximum level 1 h later and preceding the response of mtDNA or cytochrome b mRNA that reach their maximum concentration after 3 h (Koyama et al., 1998). Other data suggesting that the induction of NRF-1 is a prerequisite for the expression of genes associated with mitochondrial proliferation was obtained after the electrical stimulation of neonatal cardiac myocytes (see below). A temporal pattern of mRNA expression was observed in which NRF-1 preceded Cyt c by about 20 h (Xia et al., 1997).

The third transcription factor critical for the expression of several nuclear respiratory genes is GABP/NRF2, which was identified as a transcriptional activator of genes encoding cytochrome *c* oxidase subunits (Virbasius et al., 1993b). GABP/NRF2 is composed of three subunits, α, β, and γ, in the NRF nomenclature (Virbasius et al., 1993b) or otherwise α, β1, and β2, in the GABP one (Thompson et al., 1991). Only the α subunit, that belongs to the ETS family of regulatory proteins (Wasylyk et al., 1993), has DNA binding activity. It binds DNA weakly, but the active form of GABP/NRF2, which is an α2β2 heterotetramer, has the ability to bind avidly to tandem GGAA (ets) sites (Thompson et al., 1991; Virbasius et al., 1993b). The regulation of the GABP/NRF2 activity has been less characterised compared to NRF1. Northern and Western analysis (Escriva et al., 1999; Vallejo et al., 2000) indicated possible levels of transcriptional and post-transcriptional regulation in the expression of GABP/NRF2 subunits. The mRNA abundance of the different subunits is tissue-specific and suggests that the α subunit may be limiting for complex assembly. Furthermore, its binding activity appears to be redox-regulated in vivo possibly by thioredoxin-mediated reduction and GSSG-mediated oxidation of the cysteines present in the DNA binding and dimerization domains (Martin et al., 1996).

Two main factors involved in mtDNA replication/transcription are mtTFA and MRP. Interestingly, the expression of both is regulated by NRF1 (Virbasius and Scarpulla, 1994). MtTFA contains in addition a GABP/NRF2 site, a characteristic also reported for the COX Vb subunit and S12 (Scarpulla, 1997; Johnson et al., 1998). Therefore, NRF1 links the expression of nuclear-encoded OXPHOS genes with the increase of mtDNA and mtRNA concentration, and thus is an excellent candidate to play a key role in nucleo-mitochondrial communication (Fig. 3). In contrast to the simplicity of this model, the actual picture is likely more complex for several reasons. First, there are respiratory genes that do not have NRF1 sites in their promoters and many other genes not related to mitochondrial function that do contain NRF1 sites (Scarpulla, 1997), a situation also found with GABP/NRF2. This indicates that NRF1 is involved in the integration at the transcriptional level of mitochondria with other cellular functions including cell

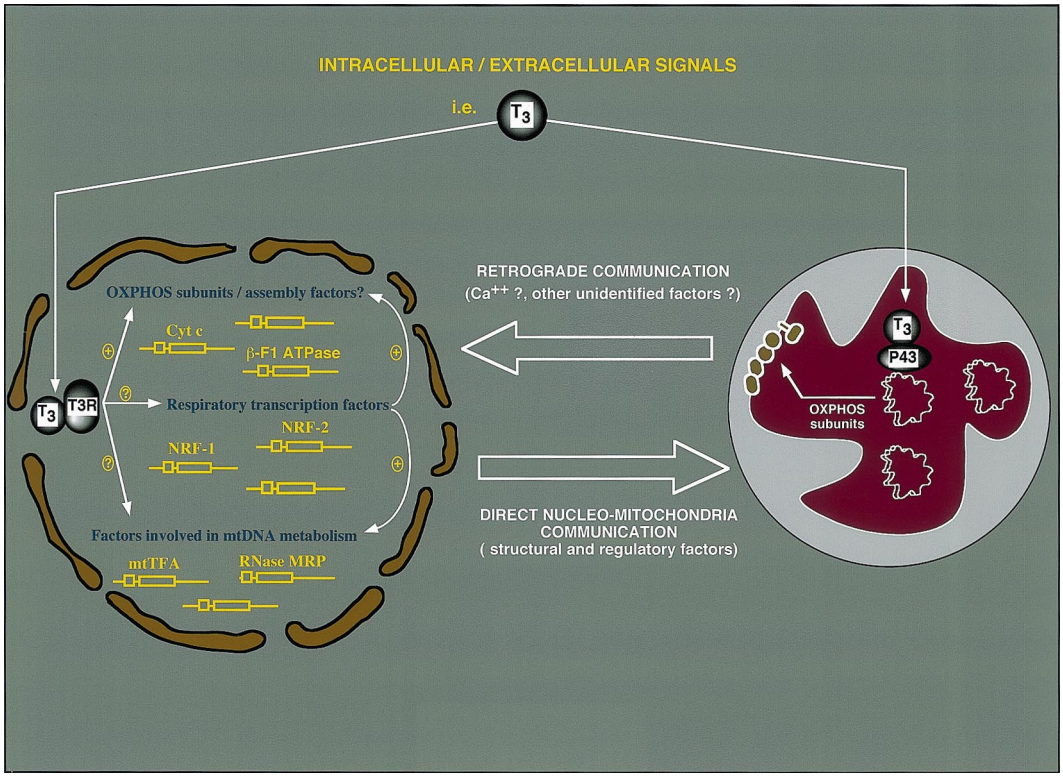

Fig. 3. Nucleo-mitochondrial communication. Schematic representation of the mechanisms potentially involved in nucleo-mitochondrial communication. NRF-1 and/or NRF-2 regulate the expression of OXPHOS structural proteins and factors involved in mtDNA metabolism such as mtTFA and the RNA component of the RNase MRP. OXPHOS structural and regulatory factors are imported to mitochondria and participate in the biogenesis of the respiratory chain directly or by controlling the replication /transcription of the mtDNA (direct nucleo-mitochondrial communication). Intracellular and extracellular signals such as hormones, growth factors or environmental stimuli are transmitted directly to the nucleus and/or mitochondria by unidentified or poorly characterised pathways. One exception is the thyroid hormone. T_3 regulates the expression of the mitochondrial-encoded genes, an effect mediated by the mitochondrial T3 receptor p43, and at least some nuclear-encoded respiratory subunits through T3R. Whereas the stimulatory effect of T_3 on the mitochondrial transcription is direct, the activation of the nuclear-encoded respiratory genes expression may be indirect. An open question remains whether T_3 activates the respiratory factors NRF-1 and/or NRF-2 directly. The unnamed boxes within the nucleus refer to other identified, or yet-to-identify factors involved in mitochondrial biogenesis. Although the retrograde pathway is still poorly characterised, calcium is most likely involved in the cross-talk in the mitochondria-to-nuclear direction.

proliferation, chromosome maintenance and cell cycle regulation (Scarpulla, 1997), and therefore may be better considered a regulator of cell growth. Second, the analysis of the homologous gene in mouse, Nrf1 (Schaefer et al., 2000), has revealed a rather specific pattern of expression. Nrf1 is highly expressed in the central nervous system, somites, first branchial arch, optic and otic vesicle, a result compatible with the phenotype obtained by the lack of *nrf1* expression in zebrafish that produces post-embryonic lethality with severe defects in the brain and eye (Becker et al., 1998). It is important to keep in mind that mutations in the *Drosophila erect wing* gene that encodes a protein of the NRF1 family also produce severe alterations in the development of the nervous and muscle systems (DeSimone et al., 1996; DeSimone and White, 1993). Therefore, NRF1 function seems to be very complex and the understanding of its in vivo function is only in its early stage. In sum, the current view is compatible with the idea that the expression of OXPHOS subunits and other important factors involved in mitochondrial biogenesis is transcriptionally controlled by a combination of constitutive transcription factors, promoter-specific, that warrant a proper level of expression. In addition, a second level of regulation involves a more reduced and sophisticated group of regulatory proteins that coordinate the expression of key nuclear-encoded mitochondrial genes with the replication and transcription of the mtDNA, at least in energy-demanding tissues and in response to environmental changes.

3.2. Mitochondria to nucleus: retrograde communication

Cells can respond to alterations in mitochondrial activity with changes in nuclear gene expression, a process called retrograde communication (Poyton and McEwen, 1996). This important phenomenon has been characterised during the last few years mainly by the group of Butow in yeast, where the changes in the mitochondrial status modulate a variety of metabolic pathways (Liao and Butow, 1993; Rothermel et al., 1995, 1997). For example, in yeast lacking mtDNA (ρ^0) several enzymes are induced, including the

peroxisomal isoform of citrate synthase encoded in the gene *CIT2*. Three members of the transduction pathway involved in this intergenomic communication, Rtg1–3, have been characterised. Rtg1 and Rtg3 are transcription factors of the bHLH family, while Rtg2 is a member of the heat shock protein family involved in the translocation of Rtg1 and Rtg3 to the nucleus (Sekito et al., 2000). When the cell monitors a defect in mitochondrial activity, likely detecting signals as glutamate sent by deficient mitochondria, the bHLH transcription factors are translocated to the nucleus where strongly transactivate a battery of genes including *CIT2*. These genes encode proteins important for the adjustment of cellular homeostasis in an energy-deficient situation (Sekito et al., 2000).

In vertebrates, no genes homologous to *RTGs* have been identified. However, several studies have shown that in cells with compromised ATP synthesis the expression of several genes encoding OXPHOS proteins is misregulated (Marusich et al., 1997; Wang and Morais, 1997). Furthermore, myocytes with low mtDNA content and/or decreased mitochondrial membrane potential generate a complex nuclear response including important variations in the levels of calcineurin dependent NFATc, JNK-dependent ATF2 and NF-κB transcription factors and an enhanced transcription of the gene encoding cytochrome *c* oxidase Vb (Biswas et al., 1999). The signalling molecule involved in this retrograde communication is Ca^{2+}. In particular, the disruption of the membrane potential and the decreased ATP synthesis capacity of altered mitochondria generate an increase in the cytosolic Ca^{2+} concentration that is likely responsible for the cellular response (Biswas et al., 1999).

One interesting point to be discussed on the light of retrograde signalling is the relationship between mitochondrial function and cell differentiation. In myoblasts, the elimination of mtDNA by ethidium bromide treatment inhibits differentiation but not proliferation (Herzberg et al., 1993). In many cells including myoblasts, treatment with chloramphenicol, an inhibitor of mitochondrial protein synthesis, suppresses differentiation but not cell proliferation (Herzberg et al., 1993; Korohoda et al., 1993; Laeng et al., 1988). In contrast, treatment with drugs that induce differentiation induces mitochondrial proliferation (Mancini et al., 1997), suggesting that mitochondria act as a differentiation promoting cellular component (Von Wangenheim and Peterson, 1998). In this connection, it has been reported recently (Rochard et al., 2000) that the inhibition of mitochondrial translation leads to the block of myoblast differentiation. Myogenin mRNA and protein levels are strongly upregulated during muscle terminal differentiation, but the inhibition of mitochondrial translation drastically reduces the transcription of myogenin although not of other myogenic regulatory factors, thus resulting in block of myoblast differentiation (Rochard et al., 2000). This result clearly shows that the alteration in mitochondrial activity interferes with the ability of myogenic factors to induce terminal differentiation and suggests that myogenin is a specific target of animal mitochondrial activity. Further analysis will be needed in order to better delineate the mitochondria-to-nucleus retrograde pathway that appears to be involved in the regulation of animal cell differentiation.

4. Regulation of mitochondrial biogenesis

4.1. Regulation of mitochondrial replication, transcription and translation

Animal cells contain a defined number of mitochondria to cope with the variable energetic demand of the different tissues, and thus the abundance of mitochondria and mtDNA is strictly maintained for a defined cell type. The primer for DNA synthesis from O_H is synthesised by the mitochondrial RNA polymerase from the L-strand promoter. The transition between RNA and DNA synthesis takes place in a DNA region containing a series of specific motifs, the conserved sequence blocks I, II and III and needs the activity of a trans-acting factor called RNA processing endonuclease (RNase MRP), an enzyme that also participate in the processing of the nucleolar 5.8S rRNA (Clayton, 1994, 1998). RNase MRP is a ribonucleoprotein likely critical for the regulation of the replication/transcription balance in mitochondria, although its precise role in this process is practically unknown and remains controversial (Clayton, 2000). A potential second mechanism of control has been also suggested in mammals, where most nascent H-strand are arrested after initiation close to a conserved region denominated termination-associated sequence (TAS) and remains annealed to the template. This creates a triplex DNA structure, the D-loop, that encompasses the majority of the non coding region. Although the mechanisms controlling the number of molecules arrested in the TAS elements compared to that continuing the complete DNA synthesis remain unknown, it is attractive to speculate that it may be critical for the regulation of mtDNA abundance in animal cells. In connection, two trans-acting factors have been identified in bovine and sea urchin mitochondria that are strong candidates to play a key role in regulating the elongation of the H-strand synthesis. The bovine factor, a 48 kDa protein, binds with high specificity a TAS element (Madsen et al., 1993), whereas the sea urchin factor, a 40 kDa protein with some identity to mTERF, binds to the 3′ end of the D-loop and to the ND5-ND6 boundary, a result that suggests a dual role in the control of replication and in transcription termination (Polosa et al., 1999).

In addition to the potential regulatory events discussed above, other mechanisms could be involved. Different lines of evidence have shown that the concentration of the catalytic subunit of pol γ is in excess to cellular demand, but the amount of mtTFA and mtSSB correlates with the mtDNA copy number (Schultz et al., 1998). However, it is not clear whether mtDNA abundance determines the amounts of these proteins or simply the proteins are unstable when they are not

bounded to mtDNA. One promising candidate to be involved in the control of mtDNA dosage is the accessory subunit of pol γ. Most likely, this protein is essential for priming recognition and has been shown that its mRNA (but no the mRNA encoding the pol γ catalytic subunit) is upregulated in the periods of active mtDNA replication during *Drosophila* development (Lefai et al., 2000a). On the other hand, in an elegant series of experiments using cell lines harbouring mtDNAs of different sizes, Tang and colleagues have recently shown that cells regulate strictly not the mtDNA copy number but the mtDNA mass (Tang et al., 2000). This suggests the interesting possibility that the pool of dNTPs in the mitochondria would be strictly controlled. Supporting this view, a disease of mitochondrial origin, the mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is caused by mutation in the gene encoding the cytoplasmic thymidine phosphorylase (Nishino et al., 1999). Furthermore, it has been shown recently that the gene encoding the heart/skeletal muscle adenine nucleotide translocator ANT1 (Kaukonen et al., 2000) is affected in a family suffering autosomal dominant progressive external ophtalmoplegia, a genetically heterogeneous disease characterised by multiple mtDNA deletions. Therefore, unexpectedly, ANT1 has turned out to be critical for mtDNA replication/maintenance. In summary, mtDNA replication, a process essential for mitochondrial proliferation, is likely regulated by a complex variety of mechanisms.

The relative simplicity of the mtDNA structure and organisation, with three polycistronic transcription units and the promoters located in a short region, has extended the idea that the mtDNA transcriptional control is exerted by controlling the level of mtTFA (or mtTFB) for mRNAs / tRNAs, and mtTFA / mTERF for rRNAs. Although attractive, this hypothesis has not yet received definitive experimental support and the potential presence of additional trans-acting regulatory factors has been suggested. In fact, recent data indicate that the reduction in the physiological levels of mtTFA does not produce significant changes in the mitochondrial transcript levels (Escriva et al., 1999; Larsson et al., 1998). On the other hand, the recent demonstration that thyroid hormones regulate mtDNA transcription acting directly in mitochondria through an isoform of the erbA receptor (see below) indicates that mtDNA may by transcriptionally regulated by a variety of transcription factors other than mtTFA. Finally, post-transcriptional mechanisms and, in particular, mtRNA stability most likely play a major role in the regulation of mtDNA expression in a variety of physiological conditions.

4.2. Mitochondrial activation in the cell cycle

Although cells need to adjust mitochondrial biogenesis and function in response to the changing physiological conditions, the mechanisms involved in the regulation of mitochondrial activity by extracellular signals are practically unknown (Poyton and McEwen, 1996; Scarpulla, 1997). Two exceptions are the transcriptional regulation of the gene encoding cytochrome c (Cytc) by the CRE binding protein (CREB), a transcription factor that recognises cAMP response elements (CRE), and the regulation of mitochondrial activity by thyroid hormone (see below). Interestingly, it has been recently shown that the increase in mitochondrial respiratory capacity produced when cells entry the cell cycle after serum treatment is mediated by an increase in the expression of the Cytc gene (Herzig et al., 2000). The primary effect induced by the serum is the phosphorylation of both CREB and NRF1 that contribute equally to the Cytc transcriptional activation. Therefore, the enhanced expression of a specific key subunit of the OXPHOS system may be responsible for the increase in ATP production needed for cell cycle progression (Herzig et al., 2000).

4.3. Hormonal regulation of mitochondrial activity

The mitochondrial respiratory rate is modulated by thyroid hormone in response to the energy demands of the different tissues. In general, increased amounts of thyroid hormone stimulate mitochondrial respiratory activity in contrast with the low level of cellular respiration observed in hypothyroidism. The mRNA steady-state level of several mitochondrial genes encoded in both the nuclear and mitochondrial genomes has been shown to change drastically in response to the level of thyroid hormones in several tissues, including liver and brain (Alvarez-Dolado et al., 1999; Izquierdo and Cuezva, 1993; Joste et al., 1989; Luciakova and Nelson, 1992; Vega-Nuñez et al., 1995; Wiesner et al., 1992). Most interestingly, the stimulatory effect of thyroid hormone on mitochondrial respiration is exerted at the level of the two genomes (Fig. 3). The direct regulation of mitochondrial RNA synthesis by thyroid hormone has been demonstrated recently (Enriquez et al., 1999b), both in vivo and in organello. After hormonal treatment, two main effects were observed in mitochondria: an increase in the overall mtDNA transcriptional rate, and a selective synthesis of mitochondrial mRNAs versus mitochondrial rRNAs, as a result of the selective use of the L and H2 promoters versus H1 (see Fig. 1). In agreement with these results, an isoform of the T_3 nuclear receptor c-Erb A α_1 lacking its amino-terminal domain has been found in the mitochondrial matrix (Wrutniak et al., 1995). This protein (p43) binds to thyroid hormone with similar affinity than the full-length receptor, which is not transported to mitochondria (Casas et al., 1999). Interestingly, mtDNA contains four motifs with high similarity to nuclear thyroid hormone response elements (TRE), two of them located in the D-loop region. The p43 receptor binds to the response elements present in the D-loop region and activates mitochondrial transcription in a way compatible with the in vivo and in organello studies (Wrutniak et al., 1995). Therefore, p43 could be a thyroid hormone-dependent mitochondrial transcription factor which binds to elements of the D-loop

different of those recognised by mtTFA. Interestingly, the overexpression of the mitochondrial thyroid hormone receptor in myoblasts stimulated mitochondrial activity and myoblast differentiation (Rochard et al., 2000). Thyroid hormone seems therefore to influence directly mitochondrial DNA transcription probably by allowing accessibility of protein factor(s) required for transcription. Finally, it is important to emphasise that the thyroid status also affects the stability of mitochondrial RNAs (Enriquez et al., 1999b), and therefore the direct effect of T3 on mitochondria is exerted at different levels.

The transcriptional activation by thyroid hormone of the expression of certain nuclear-encoded respiratory subunits is known from a long time, but the overall mechanism of activation is far from being completely understood (Pillar and Seitz, 1997). Of the more than one hundred nuclear genes that are necessary for the biogenesis of the mitochondrial respiratory apparatus, only a few have been reported to respond to thyroid hormone; these genes might have an organising function in the assembly of the respiratory complexes (Joste et al., 1989; Pillar and Seitz, 1997). Although thyroid hormone treatment activates the expression of some OXPHOS genes (i.e. cytochrome c_1 and ANT-2) in transient transfection experiments (Li et al., 1997), the low sequence conservation of the TREs present in the promoters of these genes makes more likely the interpretation that the observed activation is due to a secondary event induced by the hormone. Thus, a transacting gene (for example NRF1 or GABP/NRF2) might be activated by thyroid hormone and in turn would transactivate the OXPHOS promoters (Fig. 3). In this context, GABP/NRF2 regulates the transcription of the ATP synthase β subunit (Villena et al., 1998), a gene shown to respond to thyroid hormones (Cuezva et al., 1997; Izquierdo and Cuezva, 1993). In contrast, NRF1 and GABP/NRF2 binding sites are absent from other thyroid-responding genes like cytochrome c_1 or ANT2 (Nelson et al., 1995). Furthermore, the potential transcriptional regulation of the nuclear respiratory factors by thyroid hormones has not been demonstrated to date and therefore it remains an open issue to be investigated in the near future.

4.4. Mitochondrial activation by environmental stimuli

In response to energy demands, mitochondrial biogenesis can be activated by various environmental stimuli as the increased contractile activity in skeletal muscle (Williams, 1986; Williams et al., 1986), the direct electrical stimulation of cardiomyocites (Xia et al., 1997), or the adaptive thermogenesis in response to low temperature or excessive caloric intake (Wu et al., 1999). The chronic stimulation of mammalian skeletal muscle induces an increase in mitochondrial mass and several enzymes of the oxidative metabolism. This response is complex and involves an increase in gene dosage and in the steady-state level of mitochondrial and nuclear-encoded OXPHOS transcripts (Hood et al., 1989; Williams, 1986). Interestingly, the RNA subunit of the RNase MRP processing enzyme is also induced rapidly after electrical stimulation of the skeletal muscle, paralleling mitochondrial biogenesis (Ordway et al., 1993). Also the electrical stimulation of neonatal cardiac myocytes in culture produces an increase in mitochondrial content and activity. Interestingly, NRF1 has an early response and is responsible for the activation of the expression of other downstream genes like Cyt b, thus reinforcing the role of NRF1 in mitochondrial biogenesis under a variety of physiological circumstances (Xia et al., 1997).

Adaptive thermogenesis is the process that allows energy dissipation in the form of heat, a physiological defence against obesity, in response to stimuli such as exposure to cold or high-calorie diets. Adaptive thermogenesis occurs mainly in the mitochondria of brown fat in small mammals or in skeletal muscle in large animals like humans. It is a complex process which comprises mitochondrial uncoupling combined with an increase in mitochondrial mass and respiration. The occurrence of this program is probably related to the need to increase the fuel oxidation rate while maintaining the normal ATP/ADP ratios. A key role in thermogenesis is played by the uncoupling proteins UCPs which are small intramembranous proteins that are expressed in a tissue-specific manner. UCP-1 is expressed in brown fat, UCP-3, in brown fat and skeletal muscle and UCP-2, in most tissues. UCP-1 is the primary molecule that induces thermogenesis in brown fat by uncoupling proton entry from ATP synthesis. However, because UCP-2 and UCP-3 are homologous to UCP-1, have uncoupling activity and are expressed widely, it has been hypothesised that they would contribute to adaptive thermogenesis as well (Lowell and Spiegelman, 2000). Recent studies have shown that a novel transcriptional co-activator, PGC-1 (Puigserver et al., 1998), plays a central role in this complex response. This coactivator is specifically induced in brown fat and skeletal muscle upon exposure to cold. Subsequently to PGC-1 induction, up-regulation of UCPs is observed in parallel with increase in the amount of mitochondrial DNA, mitochondrial transcripts and respiratory rate, as well as proliferation of mitochondria. Interestingly, PGC-1 stimulates a powerful induction of NRF-1 and GABP/NRF2 (Wu et al., 1999). In addition, the overexpression of PGC-1 in cells highly increased the activity of the mtTFA promoter, activation that was completely abolished by mutation of NRF-1 binding site but only moderately by that of GABP/NRF2. Although these results unveil mechanisms linking environmental stimuli to mitochondrial function in the tissues where PGC-1 is expressed, it remains to be seen whether other coactivators can do a similar work in other tissues or physiological circumstances.

4.5. Regulation by post-transcriptional mechanisms

The regulation of the expression of the nuclearly encoded mitochondrial genes at the level of translation has not yet been thoroughly investigated, but it likely represents an

important point of control. In this regard, the group of Cuezva has investigated the translational regulation of the β subunit of ATP synthase, during the postnatal differentiation of rat liver mitochondria. This occurs through the variation in the translational efficiency of β-ATP synthase mRNA (Ostronoff et al., 1996), which can be masked in cytoplasmic clusters (Egea et al., 1997) found in the vicinity to mitochondria where the control of the transcript translation occurs (Ricart et al., 1997). The translational control is performed by a regulatory protein that binds the 3′UTR of the β-ATP synthase transcript (Izquierdo and Cuezva, 1997). It should be worth-investigating whether similar regulatory post-transcriptional mechanisms apply to other key nucleus-encoded mitochondrial proteins.

5. Mitochondrial dynamics

Mitochondria are found as dynamic reticular networks in growing cells. In terminal differentiated cells, mitochondria are not found distributed at random in the cytoplasm but rather localised to specific cytoplasmic regions, where a high concentration of ATP is likely needed. The shape and distribution of mitochondria are characteristics of a differentiation stage and their alterations are associated with pathologies like muscular dystrophy, cardiomyopathy, liver disease or cancer. It is now recognised that a cellular machinery is needed to coordinate the observed movements of mitochondria during cell division and differentiation (Yaffe, 1999), and although the molecular apparatus involved in the intracellular localisation of mitochondria remains largely unknown, recent evidence implicates both microtubule and actin filaments in their cellular distribution (Boldogh et al., 1998; Morris and Hollenbeck, 1995; Yaffe et al., 1996). Different members of the kinesin family, the motor proteins that bind to microtubules, are associated with mitochondria and the disruption of one of the kinesin genes in mice resulted in embryonic death (Tanaka et al., 1998). Also another class of proteins, the dynamin superfamily of large GTP-binding proteins, appears to act in concert with molecular motors to modify the location and/or morphology of mitochondria. One of these proteins seems to mediate mitochondrial fusion and was identified through the analysis of a *Drosophila* mutant defective in sperm development (Hermann et al., 1998). These interesting observations emphasise the essential role of mitochondria in development and the role of the cytoskeleton in the mitochondrial movements that take place in regions of cell growth or increased metabolic needs. To determine how the machinery that allows mitochondrial distribution is regulated during growth and development is a challenge for future research.

6. Mitochondria and cell death

Programmed cell death or apoptosis is an essential physiological process for cell and tissue homeostasis (Desagher and Martinou, 2000). The role of mitochondria in apoptosis was suggested six years ago (Newmeyer et al., 1994), and later on unequivocally demonstrated in a variety of biological systems. Apoptosis triggered by many stimuli needs the presence of mitochondria and the release from these organelle of cytochrome c (Green and Reed, 1998). The release of cytochrome c can be inhibited by the presence of Bcl-2 in the outer mitochondrial membrane or activated by translocation to mitochondria of cytosolic proteins of the Bax/Bid families (Desagher and Martinou, 2000). In the cytosol, cytochrome c binds to the adapter protein Apaf-1 which together with procaspase-9 and dATP form the apoptosome death complex. This complex triggers the activation of caspase-9 that starts the proteolytic cascade that ends in apoptosis (Fig. 4). Caspases, the effectors of apoptosis, are cysteinil aspartate-specific proteases which cleave key intracellular substrates with the result of cell death. The structural similarity of the proteins Bcl-2 and Bax with the bacterial colicins synthesised by bacteria to kill other competing bacteria suggests that these proteins have been appropriated by animal cells and incorporated to their cell death mechanisms. It is interesting that CED-9, the Bcl-2 homologue of C. elegans, is translated from a bicistronic mRNA where it is encoded together with cytochrome c_1, a subunit of complex II of the mitochondrial respiratory chain. This suggests that CED-9 may originate from the genome of the protomitochondrial symbiont from where it was transferred along with other mitochondrial genes to the nuclear genome (Hengartner and Horvitz, 1994). It is not however clear the role that cytochrome c plays in the apoptosis of cells from nematodes or insects, posing the question of whether the mechanism is a late sophistication or was lost in more primitive organisms.

In many apoptotic scenarios the permeabilization of the mitochondrial membrane due to the opening of the permeability transition pore (PT) is an early essential step. Multiple stimuli such as Ca^{+2} overload, oxidants, or the incorporation to the mitochondrial membrane of the protein Bax activate PT, thus triggering the release of cytochrome c and possibly other apoptosis-inducing factors like AIF and Diablo/Smac. AIF is a flavoprotein homologous to bacterial oxidoreductases, which is normally localised in the mitochondrial intermembranous space but translocates to the nucleus when apoptosis is induced (Susin et al., 1999). The exit to the nucleus is prevented by Bcl-2 and the translocation is activated by ATP depletion (Daugas et al., 2000). Once in the nucleus, it induces directly chromatin condensation and DNA fragmentation. These effects cannot be prevented by caspases inhibitors and therefore AIF can be considered as a caspase-independent death factor (Fig. 4). Though much remains to be learned, the identification of gene families that control the physiological cell death pathway has provided a basis to improve the understanding of tumour biology (Kroemer and Reed, 2000). In this regard, IAPs are apoptosis-inhibiting proteins that bind to procaspases inhibiting their activation and to caspases, inhibiting

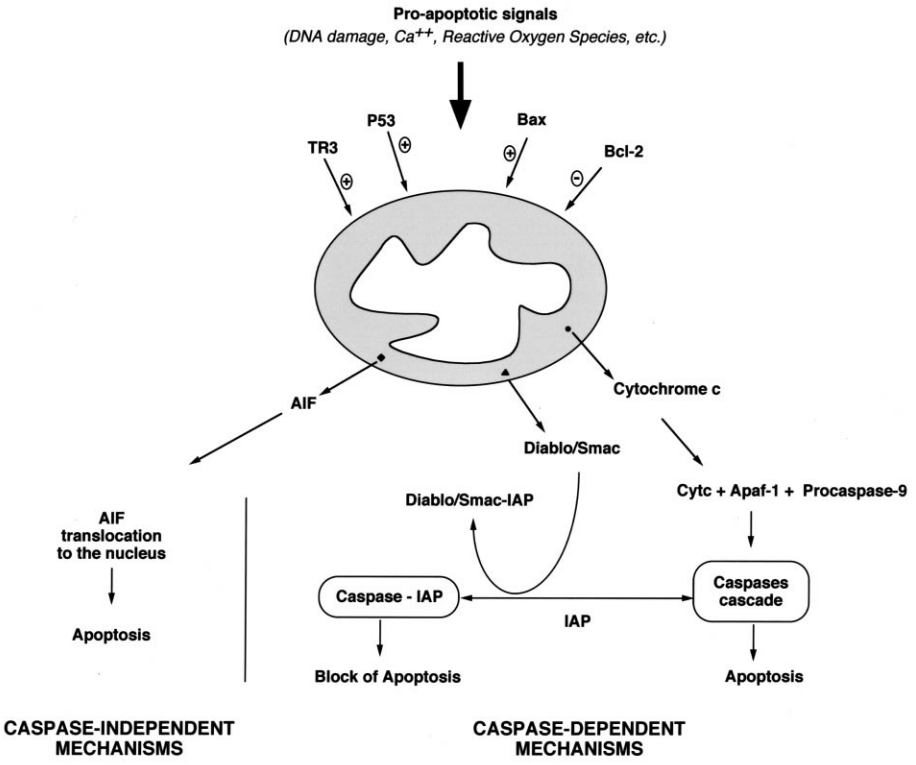

Fig. 4. Mitochondria control cell death by caspase-dependent and -independent mechanisms. Multiple pro-apoptotic signals like Ca^{+2}, oxidants or DNA damage induce the translocation to mitochondria of factors like Bax, TR3 or p53, that induce mitochondria to release proteins as cytochrome c, Diablo/Smac or AIF from the intermembranous space. This is achieved by the opening of the mitochondrial permeability transition pore or as a consequence of mitochondrial swelling and outer membrane rupture induced by the stimuli. Once in the cytosol, Cyt c joins the adapter protein Apaf and then procaspase 9, forming the apoptosome death complex where procaspase 9 is cleaved to active caspase 9. Caspase 9 in turn activates caspases 3, 6 and 7 which cleave intracellular substrates ensuring cell death. There are points in the apoptotic webb at which cell death can be regulated. For instance, Bcl-2 proteins inhibit the release of proteins from mitochondria, and IAPs (inhibitor-of-apoptosis proteins) inhibit both the activation of procaspases and the activity of caspases by binding these molecules. The inhibition of caspases action can be lifted by Diablo/Smac binding to IAPs, thus providing the capacity to modulate the initial apoptotic signal. Mitochondria are also involved in caspase-independent apoptosis through the direct action of AIF (apoptosis-inducing factor) translocated to the nucleus where produces nuclear disassembly.

their activity. These proteins may favour the proliferation of cancer cells by preventing their entrance into the apoptotic pathway. Interestingly, Diablo/Smac, a caspase co-activator that has been identified recently (Du et al., 2000; Verhagen et al., 2000) remains in the mitochondrial intermembrane space until it is released at the same time as cytochrome c. Diablo lifts the block to apoptosis by binding IAPs. Thus, the compartimentation of death-inducing proteins in mitochondria endows these organella with control over cell death. Two other unexpected discoveries in the fascinating relationship between mitochondria and apoptosis have been published very recently. TR3, an orphan receptor that forms homodimers or heterodimers with RXR, is a proapoptotic transcription factor of the steroid/thyroid receptor superfamily that is normally present in the nucleus from where, in some circumstances, it can be translocated to mitochondria to induce membrane permeabilization and trigger apoptosis (Li et al., 2000). On the other hand, the mechanism of p53-mediated apoptosis occurring after stress signals like DNA damage or hipoxia is not well understood. It has now been demonstrated in vivo (Marchenko et al., 2000) that a fraction of p53 is translocated to mitochondria prior the onset of apoptosis. The mitochondrial p53 localisation is specific for p53-dependent apoptosis but not for p53-independent apoptosis or the p53-dependent cell cycle arrest. Accordingly, the direct targeting of p53 to mitochondria is sufficient to induce apoptosis in p53-deficient cells. The accumulation of p53 in mitochondria precedes changes in mitochondrial membrane potential, cytochrome c release and procaspase-3 activation. These two factors add to the effectors that exert their apoptotic effects not by controlling nuclear transcription but by interacting directly with mitochondria.

7. Concluding remarks

The integrity of the mitochondrial genetic system is essential for the energy production of the cell because it encodes 13 subunits of the enzyme respiratory chain. The rest are encoded in the nucleus, as well as all the factors involved in mtDNA replication and expression. Mitochondria also play a pivotal role in other processes such as cell

differentiation or apoptosis, and therefore mitochondrial function is strictly controlled at the cellular level. However, despite its central role in cellular homeostasis, the regulation of mitochondrial biogenesis and function is still poorly understood. A variety of complex mechanisms are used by the cells to communicate nucleus and mitochondria, including the coordinated transcriptional regulation of many structural and regulatory mitochondrial genes. At least two transcription factors NRF-1 and GABP/NRF2 are involved in this cross-talk, and other regulatory proteins including hormone-regulated transcription factors also play a critical role. The great impulse that the field has received in the last few years, in part mediated by the study of the pathophisiology of mitochondrial diseases, will allow to identify and characterise in detail new essential factors involved in mtDNA maintenance and expression, the regulation of nuclear-encoded mitochondrial genes, and the nucleo-mitochondrial communication. This will be essential to understand the molecular basis of the biogenesis of mitochondria, a central process in cell life and death.

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