REVIEW

Thyroid hormone action in mitochondria

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ABSTRACT

Triiodothyronine (T3) is considered a major regulator of mitochondrial activity. In this review, we show evidence of the existence of a direct T3 mitochondrial pathway, and try to clarify the respective importance of the nuclear and mitochondrial pathways for organelle activity. Numerous studies have reported short-term and delayed T3 stimulation of mitochondrial oxygen consumption. Convincing data indicate that an early influence occurs through an extra-nuclear mechanism insensitive to inhibitors of protein synthesis. Although it has been shown that diiodothyronines could actually be T3 mediators of this short-term influence, the detection of specific T3-binding sites, probably corresponding to a 28 kDa c-Erb Aα1 protein of the inner membrane, also supports a direct T3 influence. The more delayed influence of thyroid hormone upon mitochondrial respiration probably results from mechanisms elicited at the nuclear level, including changes in phospholipid turnover and stimulation of uncoupling protein expression, leading to an increased inner membrane proton leak. However, the involvement of a direct mitochondrial T3 pathway leading to a rapid stimulation of mitochondrial protein synthesis has to be considered.

Both pathways are obviously involved in the T3 stimulation of mitochondrial genome transcription. First, a 43 kDa c-Erb Aα1 protein located in the mitochondrial matrix (p43), acting as a potent T3-dependent transcription factor of the mitochondrial genome, induces early stimulation of organelle transcription. In addition, T3 increases mitochondrial TFA expression, a mitochondrial transcription factor encoded by a nuclear gene. Similarly, the stimulation of mitochondriogenesis by thyroid hormone probably involves both pathways. In particular, the c-erb Aα gene simultaneously encodes a nuclear and a mitochondrial T3 receptor (p43), thus ensuring coordination of the expression of the mitochondrial genome and of nuclear genes encoding mitochondrial proteins.

Recent studies concerning the physiological importance of the direct mitochondrial T3 pathway involving p43 led to the conclusion that it is not only involved in the regulation of fuel metabolism, but also in the regulation of cell differentiation. As the processes leading to or resulting from differentiation are energy-consuming, p43 coordination of metabolism and differentiation could be of significant importance in the regulation of development.
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INTRODUCTION

It is generally assumed that the fundamental influence of thyroid hormone occurs through the control of gene transcription, mediated by several triiodothyronine (T3)-binding transcription factors belonging to the nuclear receptor superfamily. Two genes encoding these receptors have been characterized, c-erb Aα and c-erb Aβ. In rats, mice and men, due to alternative splicing processes, the c-erb Aα gene encodes three proteins α1, α2 and α3; only the first binds T3 and stimulates gene transcription in response to the hormone. Shorter forms of α-receptors have also been characterized (Chassande *et al*. 1997) in mice. In the same species, the c-erb Aβ gene encodes two receptors, β1 and β2, differing

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in their pattern of tissue expression; whereas c-Erb Aβ1 is expressed in a wide range of tissues, c-Erb Aβ2 is found almost exclusively in the pituitary where it inhibits thyrotrophin (TSH) α- and β-subunit gene transcription (Abel *et al*. 1999) by binding to negative T3 response elements (REs) present on these genes (Burnside *et al*. 1989, Bodenner *et al*. 1991). Gene knock-out studies in mice have confirmed the specific involvement of the c-erb Aβ gene in T3 negative feedback on TSH synthesis (Forrest *et al*. 1996, Abel *et al*. 1999, Gauthier *et al*. 1999).

Despite spectacular progress in the knowledge of the T3 nuclear pathway over the last 14 years, a clear answer to a recurrent question is still lacking. The major involvement of thyroid hormone in the regulation of development, growth and metabolism suggests that it controls a variety of complex pathways. Through its c-Erb A receptors regulating directly or indirectly the expression of numerous genes, this unique mechanism could enable T3 to induce a broad spectrum of activities. However, is it possible to rule out the implication of other mechanisms initiated outside the cell nucleus? The pertinence of this question is emphasized by convincing reports demonstrating that iodothyronines affect calcium signalling within minutes, independently of new protein synthesis (Davis *et al*. 1989, Segal 1989), and by the observation that, *in vivo*, thyroid hormone influences thermogenesis and the lipolytic activity of catecholamines, in less than 30 min (Andrews *et al*. 1979, Lynch *et al*. 1985, Wrutniak & Cabello 1986). These last data substantiate the occurrence of non-genomic T3 pathways.

In this respect, mitochondria have a particular status in the cell. According to their postulated origin as a bacterium engulfed by the cell, they possess their own genome, a specific genetic code, and a specific apparatus involved in DNA replication, expression and protein synthesis. Despite this, the majority of mitochondrial proteins, including carriers, enzymatic subunits, transcription factors, DNA and RNA polymerases, or proteins involved in RNA translation, are encoded by nuclear genes and imported into mitochondria. Besides this nuclear contribution, the mitochondrial genome encodes 13 enzymatic subunits of the respiratory chain, two rRNAs and 22 tRNAs. Although the organelle's contribution to the synthesis of its own proteins appears to be quantitatively modest, it is an absolute requisite for life, as is clearly demonstrated by the extreme severity of diseases induced by rearrangements of the mitochondrial genome. Moreover, T3 is considered as a major regulator of mitochondrial biogenesis (Mutvei *et al*. 1989*a*), suggesting that the hormone acts on both nuclear genes and mitochondrial genome expression. In fact, it appears now that nuclear and direct mitochondrial pathways mediate this influence. In this review we have tried to define the respective contribution of each pathway in several well-established T3 influences on the organelle: stimulation of oxygen consumption, mitochondrial genome expression and mitochondriogenesis.

THYROID HORMONE INFLUENCE ON MITOCHONDRIAL OXYGEN CONSUMPTION

It has long been known that thyroid hormone administration increases oxygen consumption and heat production, and that, conversely, hypothyroidism has the opposite effects. Mitochondria extracted from the livers of hyperthyroid rats display oxygen consumption rates greater than those recorded in controls, whereas mitochondria from hypothyroid animals have a lower oxygen consumption (Brand & Murphy 1987, Hoch 1988). Similar changes have been observed in hepatocytes isolated from hyperthyroid or hypothyroid animals (Nobes *et al*. 1990). Interestingly, two kinds of thyroid hormone action have been reported: a short-term influence occurring within minutes of thyroid hormone treatment, and a more delayed influence recorded after a few hours.

Short-term influence

Several studies have reported that T3 injection in hypothyroid rats increases oxygen consumption and oxidative phosphorylations measured in isolated liver mitochondria collected less than 30 min after hormone administration (Palacios-Romero & Mowbray 1979, Sterling *et al*. 1980). In addition, this effect was not abrogated by protein synthesis inhibitors (Sterling *et al*. 1980). *In vitro* experiments also demonstrated that adding T3 to the incubation medium of isolated mitochondria from hypothyroid animals induced a similar influence within 2 min of the hormone being present (Sterling *et al*. 1977, Thomas *et al*. 1987). Moreover, Sterling & Brenner (1995) reported a stimulation of the mitochondrial carrier adenine nucleotide translocase (ANT) displaying the same features. Rapidity, refractoriness to inhibitors of protein synthesis, and occurrence in the absence of nuclei ruled out the involvement of the T3 genomic pathway. Parallel to this, several studies have demonstrated that the mitochondrion is a major

compartment of T3 accumulation in the cell (Palacios-Romero & Mowbray 1979, Sterling *et al*. 1984*b*, Hashizume *et al*. 1986, Morel *et al*. 1996). These data led to the proposition that ANT was a major T3 target involved in the short-term influence of the hormone on the organelle. In agreement with this hypothesis, Sterling (1986) reported that ANT is a high-affinity binding site for T3. However, despite the availability of the purified protein and related antibodies or expression vectors, this last result has not received any confirmation. In particular, as were others, we were unable to demonstrate significant T3 binding to purified ANT or to the protein in its mitochondrial context (Rasmussen *et al*. 1989, Wrutniak *et al*. 1995).

However, the possibility that T3 by itself could induce this early influence is still under debate. First, after inhibiting deiodinations by propylthiouracil, Horst *et al*. (1989) reported that diiodothyronines (T2s), but not T3, induce this short-term mitochondrial influence. In addition, in agreement with the detection of 3′,5-T2-binding sites in the organelle (Lanni *et al*. 1994, Leary *et al*. 1996), Goglia *et al*. (1994) found that 3′,5-T2 binds to a subunit of cytochrome-c-oxidase, leading to a conformational change of the enzyme and an activation of the respiratory chain. Next, as mentioned previously for T3, inhibitors of protein synthesis do not alter the influence of T2 on the organelle (O'Reilly & Murphy 1992). Yamaki *et al*. (1991) reported that 5′-deiodinase activity could be detected in mitochondria, thus suggesting that T3 to T2 conversion in the isolated organelle is not unlikely. Although controversial, this observation could explain the T3 influence recorded in this *in vitro* system. These data led to the proposition that 3′,5-T2 is actually a mediator of the short-term thyroid hormone influence.

However, in our opinion, the detection of specific T3-binding sites in mitochondria by three separate teams (Sterling & Milch 1975, Goglia *et al*. 1981, Hashizume & Ichikawa 1982) does not allow us to exclude the possibility that T3 by itself is able to induce a part of the short-term hormonal influence. In particular, Sterling *et al*. (1984*a*) found that a 28 kDa T3-binding protein was localized in the mitochondrial inner membrane. In agreement with this result, we have more recently identified a truncated form of the T3 nuclear receptor c-Erb Aα1 displaying a similar molecular mass and the same localization in the organelle as Sterling's protein (p28) (Wrutniak *et al*. 1995). According to Bigler *et al*. (1992), p28 is synthesized by alternative translational initiation at an internal AUG in the messenger encoding the full-length nuclear receptor (Fig. 1). Now, we have evidence that this T3-binding protein is actively imported in isolated mitochondria (F Casas, C Wrutniak-Cabello & G Cabello, unpublished observations). Although its exact function remains to be established, p28 could act as a receptor involved in the early mitochondrial T3 influence, taking into account its co-localization with components of the respiratory chain, uncoupling proteins (UCPs) or ANT.

Delayed influence is probably induced at the nuclear level

Studies were performed in order to identify the sites of action of T3 involved in delayed stimulation of oxygen consumption (Harper *et al*. 1993). In isolated mitochondria or in hepatocytes, they led to the conclusion that the proton leak across the inner membrane is an important target of thyroid hormone involved in its influence on oxygen consumption. In addition, studies performed in isolated mitochondria from hypothyroid rats suggested that reactions dissipating the protonmotive force like ATPase or ANT activity are also involved in this regulation, but these data were not confirmed in hyperthyroid mitochondria or in hepatocytes.

The proton leak represents about 20% of the multifactorial control of mitochondrial respiration (Brown 1992), and convergent data demonstrate that it is increased by thyroid hormone, according to several mechanisms. First, thyroid hormone increases the area of the inner membrane and alters its phospholipid composition (Brand *et al*. 1992, Bangur *et al*. 1995), leading to increased permeability to protons recorded 9–12 h after hormone administration (Horrum *et al*. 1992). More recently, the discovery of a family of mitochondrial UCPs (Ricquier & Bouillaud 2000) provided another clue to explain this influence. In contrast to initial findings indicating that UCP1 expression was restricted to brown adipose tissue, it appears that almost all tissues express at least one member of the UCP family (UCP2, 3 and brain mitochondrial carrier protein-1). Interestingly, it is now established that UCP1 (Cassard-Doulcier *et al*. 1994, Guerra *et al*. 1996, Rabelo *et al*. 1996), UCP2 (Lanni *et al*. 1997) and UCP3 (Gong *et al*. 1997, Larkin *et al*. 1997) gene expression is increased by T3. In addition, Lanni *et al*. (1999) reported the existence of a substantial correlation between UCP3 mRNA levels, mitochondrial coupling and the thyroid state, thus suggesting that control of UCPs expression is involved in the T3 regulation of the proton leak.

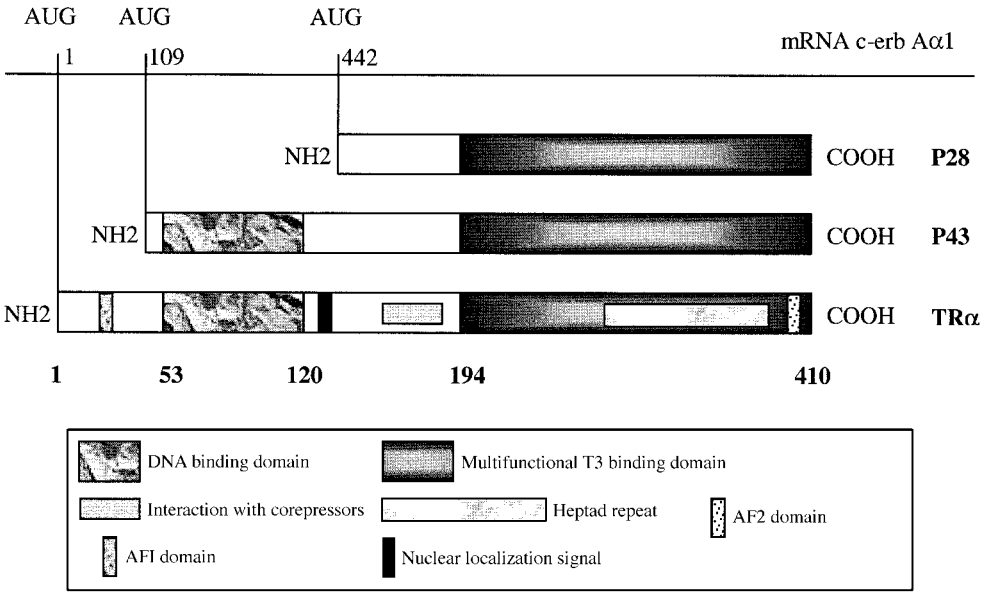

FIGURE 1. Two short c-Erb Aα1 protein isoforms (p28 and p43) are synthesized by alternative translational initiation at internal AUG in the message encoding the full-length thyroid hormone receptor (Bigler *et al*. 1992). Whereas p28 is detected in the mitochondrial inner membrane, p43 is localized in the matrix of the organelle where it stimulates mitochondrial genome transcription in the presence of T3 (Wrutniak *et al*. 1995, Casas *et al*. 1999). TRα, T3 nuclear receptor c-Erb Aα1 (47 kDa); 1, 109 and 442, number of nucleotides on the transcript (1=A of the first AUG); 1, 53, 120, 194 and 410, number of amino acids on the c-Erb Aα1 receptor.

Another finding of these studies is that thyroid hormone-induced changes in the phospholipid composition of the inner membrane include stimulation of cardiolipin synthase activity due to a rise in the mitochondrial phosphatidylglycerol pool (Hostetler 1991, Cao *et al*. 1995), thus increasing the amounts of cardiolipin (Paradies & Ruggiero 1989, 1990). As cardiolipin stimulates several mitochondrial carriers and enzymes activities (Paradies & Ruggiero 1989, 1990), this event could contribute to the delayed hormone influence on mitochondrial respiration.

Besides the proton leak, the influence of thyroid hormone on the processes involved in dissipation of the protonmotive force previously mentioned is in agreement with the observation that subunit βF1-ATPase of the mitochondrial complex synthesizing ATP (Izquierdo & Cuezva 1993) and ANT (Dummler *et al*. 1996, Schonfeld *et al*. 1997) is encoded by genes whose expression is regulated by T3. As ATPase and ANT activities contribute to the decrease in protonmotive force, these data suggest that direct or indirect induction of gene transcription is involved in delayed T3 influence.

Delayed influence of thyroid hormone on mitochondrial oxygen consumption, involving alterations in phospholipid synthesis, appeared clearly to be mainly initiated at the nuclear level. It remains to be established what is the contribution of direct mechanisms involving the c-Erb A nuclear receptors, and of indirect ones mediated by the induction of transcription factors responding to T3, such as nuclear respiratory factor 1, whose RE has been identified in several nuclear respiratory genes (for review see Pillar & Seitz 1997). However, recent data discussed below have raised the possibility that a direct mitochondrial pathway could also be involved in this delayed T3 influence.

THYROID HORMONE INFLUENCE ON MITOCHONDRIAL GENOME EXPRESSION

Besides its influence on oxygen consumption, numerous reports have established the influence of thyroid hormone on mitochondrial genome expression. Thyroid hormone administration in hypothyroid rats induces a 2- to 8-fold increase in liver mitochondrial mRNA levels (Mutvei *et al*. 1989*b*), and similar data have been reported in skeletal muscle with some tissue specificities (Wiesner *et al*. 1992). According to Mutvei *et al*. (1989*b*), this rise was accounted for by an elevated RNA synthesis.

This conclusion is substantially supported by a recent observation indicating that T3 decreases mitochondrial mRNA half-life (Enriquez *et al*. 1999), thus ruling out the possibility that thyroid hormone could raise mitochondrial (mt) RNA levels by improving their stability.

This transcriptional influence has been explained by the finding that T3 increases mitochondrial transcription factor (mt-TFA) mRNA levels in rats (Garstka *et al*. 1994). As mt-TFA acts in mitochondria to stimulate mt-DNA replication and expression (Clayton 1991), this result suggested that the T3 transcriptional influence was essentially elicited at nuclear level. However, studies using isolated mitochondria from hypothyroid or control rat liver led to the conclusion that this mechanism was not exclusive. First, Martino *et al*. (1986) observed that *in vitro* addition of T3 stimulates mt-RNA polymerases in the absence of nuclear influence, with a latency period of less than 5 min. Secondly, Enriquez *et al*. (1999) demonstrated that addition of minute amounts of the hormone to isolated mitochondria influenced mitochondrial transcription, and particularly the mRNA/rRNA ratio, in relation to changes in the pattern of protein binding to the mitochondrial genome. These data demonstrated that thyroid hormone influence on mitochondrial transcription involves direct action on the organelle transcription machinery.

In support of this result, we have previously identified, in the matrix of rat liver mitochondria, a second truncated form of the c-Erb Aα1 nuclear receptor with a molecular mass of 43 kDa (p43) (Wrutniak *et al*. 1995), synthesized by alternative translational initiation at another internal AUG in the messenger encoding the full-length nuclear receptor (Fig. 1). This protein, which, like p28, is not detected in the nucleus (Wrutniak *et al*. 1995), binds T3 with an affinity unsurprisingly similar to that reported for c-Erb Aα1 (Casas *et al*. 1999). Moreover, in contrast to p28, this protein harbours the DNA-binding domain of the T3 nuclear receptor. Interestingly, gel shift experiments established that p43 efficiently bound to four sequences of the mitochondrial genome previously identified (Wrutniak *et al*. 1998, Casas *et al*. 1999), sharing strong homologies with T3 REs described on nuclear genes. Last, *in organello* transcription experiments demonstrated that p43 strongly increases mitochondrial genome transcription, and, as a consequence, mitochondrial protein synthesis (Casas *et al*. 1999). In agreement with Martino *et al*. (1986), this influence was detected as soon as the hormone had been present for 5 min. Complementary studies were performed in cultured cells. We found that p43 overexpression raises the level of mt-RNAs in a myoblast model in which mt-TFA is not a transcriptional T3 target (Casas *et al*. 1999). In addition, it stimulates cytochrome-c-oxidase activity and increases mitochondrial membrane potential assessed by rhodamine 123 uptake (Wrutniak *et al*. 1995). According to the short latency period recorded in our experiments, we suggest that this mechanism is in particular involved in the influence of T3 on mitochondrial oxygen consumption culminating in some hours, by increasing mitochondrial protein synthesis and consequently the activity of the respiratory chain as experimentally demonstrated (Wrutniak *et al*. 1995, Casas *et al*. 1999).

From a molecular point of view, we obtained indications that p43 monomer does not bind to mt-DNA (Casas *et al*. 1999), which led us to search for dimerization partners of this receptor. We recently found that p43 binds to one particular T3RE located in the mitochondrial D-loop by forming a complex with a 45 kDa truncated form of another member of the nuclear receptor superfamily, PPARγ2 (peroxisome proliferator activated receptor), whose expression is induced by peroxisome proliferators (mt-PPAR) (Casas *et al*. 2000*a*,*b*). Although devoid of any mitochondrial activity by itself, due to the absence of a ligand-binding domain (Casas *et al*. 2000*a*), co-expression of mt-PPAR with p43 significantly enhanced the stimulation of mitochondrial activity induced by p43 alone (F Casas, C Wrutniak-Cabello & G Cabello, unpublished observations). These results provide an interesting explanation of the thyromimetic influence of fibrates reported in several studies (Hertz *et al*. 1991, Cai *et al*. 1996). In addition, they also suggest that p43 binds to the three other mitochondrial T3RE sequences by forming homodimerical or heterodimerical complexes with unidentified partners. Taken together, our data raise the possibility that other members of the nuclear receptor superfamily could be imported into the organelle. This hypothesis is already well supported by our data demonstrating that a particular c-Erb Aβ isoform (β0), expressed in non-mammalian species, is actively imported into mitochondria where it plays the same role as p43 (Casas *et al*. 1999), and by the finding that the glucocorticoid receptor is addressed into mitochondria (Scheller *et al*. 2000).

This possibility acutely raises the question of the process involved in the mitochondrial import of these receptors. We have not recorded putative mitochondrial localization signals in p43. However, we observed that deletion of the DNA-binding domain abrogates p43 import (F Casas,

C Wrutniak-Cabello & G Cabello, unpublished observations), thus emphasizing the importance of this well-conserved sequence among members of the nuclear receptor superfamily. In addition, studies of p28 import indicated that this receptor devoid of the DNA-binding domain is addressed into the organelle only in the presence of T3 (F Casas, C Wrutniak-Cabello & G Cabello, unpublished observations). This last observation suggests that conformational changes consequent to T3 binding allow unmasking of a sequence inducing mitochondrial import. Overall, it appears that, at least for c-Erb A mitochondrial proteins, translocation in the organelle involves two domains with constitutive or T3-dependent activities. Besides their interest in endocrine regulation of mitochondrial activity, such studies could bring new original data concerning mitochondrial protein import.

As nuclear receptors exert their activity by interacting with transcriptional cofactors, other interesting questions are raised. In particular, the occurrence in the organelle of coactivators or corepressors has to be questioned. Today, no evidence has been provided that histone acetylation and deacetylation are important processes for mitochondrial genome transcription, according to the organization of the circular mt-DNA molecule. Therefore, it is unlikely that coactivators with histone acetylase activity, or interacting with histones acetylases, could play an important role in the regulation of mitochondrial transcription. Despite that, a search should be made for the presence of PPARγ coactivator, a common c-Erb A and PPARγ coactivator (Puigserver *et al*. 1998) interacting with steroid receptor coactivator histone acetylase (Puigserver *et al*. 1999), taking into account its involvement in the regulation of mitochondrial biogenesis (Wu *et al*. 1999). However, a more systematic study of p43 interactions with known mitochondrial proteins, such as mt-TFA or mt-RNA polymerase, could bring interesting data.

Although numerous questions remains unsolved, these studies, which include the characterization of a new mitochondrial T3 receptor, have clearly established the existence of a T3 extra-nuclear pathway. As we have detected p43 in all tested vertebrate species (human, rat, mouse, rabbit, chicken, *Xenopus*), we suggest that this well-conserved pathway is of significant physiological importance. More generally, these results are of further interest; as a specific stimulation by p43 overexpression of the synthesis of enzyme subunits encoded by the mitochondrial genome is sufficient to induce stimulation of the organelle activity (Wrutniak *et al*. 1995), we suggest that the expression of subunits encoded by nuclear genes is not rate-limiting. This observation is consistent with previous data indicating the occurrence of an unassembled cytochrome-c-oxidase subunit pool in the cytosol of rat liver (Hundt *et al*. 1980). Similarly, in synchronous cultures of yeast, whereas nuclear-encoded cytochrome-c-oxidase subunits accumulate during the G1 and early S phases, they are integrated into the inner membrane in the late S phase only after the mitochondrially made subunits have accumulated (Somasundaram & Jayaraman 1981), suggesting the latter could have rate-limiting importance for enzyme functioning. Moreover, it emphasizes the importance of the rapid regulation of mitochondrial transcription for organelle activity.

THYROID HORMONE STIMULATES MITOCHONDRIOGENESIS

Another well-established influence of thyroid hormone concerns the stimulation of mitochondriogenesis, considered as a long-term influence detected after a latency period much longer than 24 h (Gross 1971). Mitochondriogenesis is the result of numerous events leading to membrane phospholipid synthesis and assembly, DNA replication and stimulation of the expression of the mitochondrial genome and of nuclear genes encoding mitochondrial proteins. This apparent complexity is probably the reason for the length of the T3 latency period.

It is likely that T3 regulation of mitochondriogenesis involves both nuclear and mitochondrial receptors. As previously discussed, *de novo* lipid synthesis and mobilization in membranes probably result from the general influence of the hormone on lipid turnover assumed to be elicited at the nuclear level. Moreover, T3 stimulation of mt-TFA expression (Garstka *et al*. 1994) is probably a major mechanism involved in mitochondriogenesis as this factor stimulates mitochondrial genome expression and replication. The expression of several nuclear genes encoding mitochondrial proteins is T3-regulated, as shown for β-F1ATPase, ANT, cytochrome c1, mt-TFA, UCPs and several sub-units of the respiratory chain (for review see Pillar & Seitz 1997). In addition, improvement in the mitochondrial import of nuclear-encoded proteins has been observed in cardiac muscle cells (Craig *et al*. 1998), in agreement with the study of Schneider & Hood (2000) indicating that mt-heat shock protein 70 expression, a chaperone involved in import, is increased by thyroid hormone. However, the work of Alvarez-Dolado *et al*. (1999), reporting that expression of mitochondrial

preprotein translocase of outer membrane 70, a component of the organelle import apparatus encoded by a nuclear gene, is negatively regulated by T3 in several regions of the brain, points to the existence of differential regulation depending on the relevant tissue. Lastly, at the mitochondrial level, by activating p43 the hormone directly increases mitochondrial genome transcription and synthesis of the corresponding proteins (Casas *et al*. 1999).

Therefore, it appears that mitochondriogenesis needs some coordination between nuclear and mitochondrial genome expression. Interestingly, the c-erb Aα gene simultaneously encodes a nuclear and a mitochondrial T3 receptor, thus providing an efficient system to coordinate expression of a number of nuclear genes encoding mitochondrial proteins, and expression of the mitochondrial genome (Casas *et al*. 1999). This dual influence not only explains the major role of T3 in the regulation of mitochondriogenesis, but also underlines the complementarities between the nuclear and direct mitochondrial T3 pathways. As other members of the nuclear receptor superfamily have been characterized in the organelle (PPAR, glucocorticoid receptor), it is likely that they could also contribute to this coordination, thus ensuring fine regulation of mitochondriogenesis in response to physiological stimuli.

PHYSIOLOGICAL IMPORTANCE OF THE DIRECT MITOCHONDRIAL T3 PATHWAY

In this review, it clearly appears that a direct T3 mitochondrial pathway does indeed exist, mediated by at least one receptor encoded by the c-erb Aα gene. As p43 is the first T3 receptor identified at the origin of an extra-nuclear action of thyroid hormone, this raises the question of the exact physiological importance of this new hormonal pathway.

T3 influence at the mitochondrial level initially suggested that the pathway was essentially involved in the regulation of fuel metabolism and thermogenesis. This possibility is consistent with the observation that p43 overexpression induces stimulation of mitochondrial activity (Wrutniak *et al*. 1995). In addition, whereas high amounts of this receptor are present in mitochondria from brown adipose tissue implicated in non-shivering thermogenesis, p43 is not detected in brain organelles, a tissue considered as not responsible for the calorigenic influence of thyroid hormone (Wrutniak *et al*. 1995, Casas *et al*. 2000*a*). These data argue in favour of an involvement of this pathway in T3 thermogenic effects. This possibility agrees well with the observation that body temperature is specifically altered by disruption of the c-erb Aα gene, encoding a nuclear and a mitochondrial receptor, whereas knock-out of the c-erb Aβ gene only encoding nuclear T3 receptors is without influence (Wikstrom *et al*. 1998, Johansson *et al*. 1999).

However, the importance of mitochondrial activity in other important physiological processes is currently emerging. The organelle function in particular seems strongly implicated in the processes of development. First, mitochondria play a key role in the induction of apoptosis (for review see Loeffler & Kroemer 2000). In addition, several studies have established that inhibition of mitochondrial activity, either by deleting mt-DNA (Rho^0 cells) or by blocking translation in the organelle, stops or decreases proliferation of different cell lines (Leblond-Larouche *et al*. 1979, Morais *et al*. 1980, Van den Bogert *et al*. 1992). Furthermore, the general activity of the organelle, not restricted to energy production, is implicated in such regulation (Grégoire *et al*. 1984, Buchet & Godinot 1998). Lastly, mitochondrial protein synthesis inhibition is associated with the impairment of differentiation in different cell types, such as mouse erythroleukaemia (Kaneko *et al*. 1988) and mastocytoma cells (Laeng *et al*. 1988), neurons (Vayssière *et al*. 1992), and human (Herzberg *et al*. 1993), avian (Korohoda *et al*. 1993) or murine myoblasts (Hamai *et al*. 1997). In agreement with this set of data, mt-TFA gene knock-out in mice is associated with embryonic lethality (Larsson *et al*. 1998). Despite these reports, it was not clear if these adverse influences were a non-specific consequence of impairment in cell viability due to insufficient ATP stores, or attested to the occurrence of an actual physiological regulation of cell proliferation and differentiation by the organelle activity.

To clarify this point, we studied the influence of the direct mitochondrial T3 pathway on myoblast differentiation (Rochard *et al*. 2000). First, T3 is a major regulator of myoblast differentiation (Marchal *et al*. 1993), through mechanisms involving its c-Erb A nuclear receptors (Cassar-Malek *et al*. 1996, Marchal *et al*. 1996). Secondly, the molecular events inducing terminal differentiation in this cell type are relatively well known, with a major influence of myogenic factors acting as muscle-specific transcription factors (Myf 5, MyoD, myogenin and MRF4). Gene knock-out in mice provided evidence that Myf 5 and MyoD are more involved in the acquisition of the muscle phenotype, whereas myogenin is involved in terminal differentiation by inducing myoblast fusion and expression

of muscle-specific proteins. In addition, overexpression of only one of these transcription factors in cells other than myoblasts can induce expression of a myogenic phenotype.

We first observed that p43 overexpression increases myoblast withdrawal from the cell cycle, an important step in myogenic differentiation, and stimulates their differentiation. In contrast, chloramphenicol, a drug inducing the opposite influence to p43 by reducing mitochondrial protein synthesis, inhibits myoblast withdrawal from the cell cycle and their differentiation. In this study (Rochard *et al*. 2000), we obtained good evidence that changes in ATP production were not involved in this myogenic influence. More interestingly, we found that myogenin expression was increased by p43 overexpression and decreased by chloramphenicol, events elicited at transcriptional level, thus establishing the existence of an actual regulation of myoblast differentiation by mitochondrial activity. The signalling at the origin of the influence of the organelle on nuclear gene expression remains to be identified, but recent data already suggest that calcium signalling is probably involved (Luo *et al*. 1997, Biswas *et al*. 1999). We also observed that the ability of myogenic factors to induce terminal differentiation was under the control of mitochondrial activity.

These data shed new light on the sharp increase in mitochondrial activity spontaneously occurring just before myoblast differentiation, already proposed as a possible mechanism involved in the commitment of these cells in the differentiation programme (Rochard *et al*. 1996). As we have recently observed a similar regulation of preadipocyte differentiation (A Fraysse, C Wrutniak-Cabello, L Daury, A Rodier, F Casas, P Rochard, G Cabello & J Charrier, unpublished observations), this set of data demonstrates that, like the nuclear pathway, the direct mitochondrial T3 pathway is involved in the regulation of cell differentiation.

CONCLUSIONS

In this review, we have stressed that the complex regulation of mitochondrial activity by thyroid hormone involves several pathways. Besides the well-established nuclear pathway involving the c-Erb A receptors, it appears that a recently identified direct mitochondrial pathway also plays a significant role by stimulating mitochondrial genome transcription with a very short latency period. Interestingly, both mechanisms could contribute to setting up efficient coordination in the induction of transcription of the mitochondrial genome and of nuclear genes encoding mitochondrial proteins needed for mitochondriogenesis. Moreover, it appears that by its short-term action, T3 and/or T2 could immediately adapt mitochondrial activity to abrupt changes in environmental conditions, whereas when acting through p43, T3 rapidly increases the efficiency of the mitochondrial apparatus to respond to these changes within minutes. In association with more delayed responses through the nuclear pathway leading to a stimulation of mitochondriogenesis, these mechanisms provide an efficient mitochondrial response to abrupt and/or prolonged changes in physiological conditions.

Lastly, it also appears that, like the nuclear T3 pathway, the direct mitochondrial pathway is deeply involved in the regulation of cell differentiation, further underlining the complementarities of these mechanisms initiated at different levels. In addition, we would emphasize that thyroid hormone regulation of mitochondrial activity, simultaneously influencing ATP production and cell differentiation, an energy-expensive process, could be a major link between metabolism and development. All these considerations led us to the conviction that studies on mitochondrial T3 regulation will shed new light on major interactions between endocrinology, metabolism and development.

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