Introduction

Thyroid hormones exert many physiological effects, and at the cellular level their main mechanism of action is generally believed to involve T3 binding to thyroid hormone nuclear receptors, the so-called ‘genomic action’. Thyroid hormone nuclear receptors, which belong to a large family of nuclear receptors, act as T3-inducible transcription factors. There exist many isoforms of thyroid hormone nuclear receptors that are products of different genes, the major isoforms being TRa1 and TRb1. Other isoforms created by differential splicing are TRa2 (nonhormone-binding), which acts as a dominant negative inhibitor of the actions of thyroid hormone and TRb2 (specifically expressed in brain, retina and inner ear). The expression of thyroid hormone nuclear receptor isoforms are tissue-specific and developmentally regulated, and the question arises whether they have distinct functions and, if so, what they are (for reviews see [1,2,3]).

Not all the actions of thyroid hormones can, however, be explained by invoking the genomic pathway, and it is becoming ever clearer that the actions of the thyroid hormones are exerted at several levels. Examples of the nongenomic actions of thyroid hormones are modulations of the calcium pump (Ca2+-ATPase), 2-deoxyglucose uptake, the Na+/H+ antiporter, the sodium pump, and inactivation of the Na+ current in neonatal cardiocytes (reviewed in [2]). Actually, the nongenomic actions of thyroid hormones are initiated at multiple
Mitochondria are double-membrane organelles present in most eukaryotic cells. The inner mitochondrial membrane is highly folded into cristae, which house respiratory chain complexes I–IV and ATP synthase (F1F0 ATPase – complex V), and these complexes generate the majority of cellular ATP via oxidative phosphorylation. Oxidative phosphorylation, which involves the oxidation-reduction of coenzymes such as NAD/NADH and FADH2, is carried out by the ETC. During electron transport, an active pumping of protons contributes to the creation of an electrochemical potential difference across the inner mitochondrial membrane (Δψ). This Δψ is constituted mostly by an electrical gradient (ΔΨ) and a smaller chemical proton gradient (ΔpH). The electrochemical proton gradient provides the force driving protons back to the matrix through the ATP synthase complex, thus coupling substrate oxidation to the phosphorylation of adenosine diphosphate (ADP). There is usually a tight coupling between the electron transport and the ATP synthesis, and therefore an inhibition of ATP synthase will also inhibit electron transport and cellular respiration. Under certain conditions, protons can re-enter the mitochondrial matrix without contributing to ATP synthesis, and the energy of the proton electrochemical gradient will then be released as heat. This process, known as proton leak or mitochondrial uncoupling, may be mediated by protonophores (such as FCCP) and uncoupling proteins (UCPs) [5]. Such uncoupling leads to a low ATP production together with high levels of both electron transfer and cellular respiration.

Although the primary function of mitochondria is the synthesis of ATP by oxidative phosphorylation, these organelles also play important roles in many of the processes that are critical for cell function and survival. These include calcium homeostasis, the formation of reactive oxygen species (ROS) and the initiation of apoptosis – functions that are mediated by hundreds of mitochondria-specific proteins encoded by both the nuclear and mitochondrial genomes [6]. Actually, mitochondria are responsible for the majority of cellular ROS. Even though the process of oxidative phosphorylation is efficient, a small percentage of electrons may ‘leak’ from the ETC, particularly from complexes I and III, during normal respiration and prematurely reduce oxygen, forming ROS [7]. When produced in a controlled amount, ROS may play important signalling roles in various redox-dependent processes, including apoptosis [8] and cell proliferation [9]. When overproduced, however, they can cause severe damage to macromolecules, particularly the mitochondrial proteins, lipids and DNA that are proximal to the source of superoxide. For a schematic view see Fig. 1.

**Thyroid hormones: calorigenesis, mitochondria and metabolic homeostasis**

The calorigenic effect of thyroid hormones is a classic effect that is evident in adult animals and humans. It was deeply investigated at the beginning of the 1960s by Tata and co-workers [10] who, after giving a single injection of T3 to hypothyroid rats, observed an increase in basal metabolic rate. This observation stimulated many scientists to try to unravel the underlying mechanisms, and led to hopes of discovery of a way to counteract certain metabolic abnormalities, including dyslipidaemias (i.e. high serum levels of triglycerides and cholesterol), obesity and other related abnormalities such as type 2 diabetes. The past few years have seen rapid advances in basic research and novel findings related to the clinical relevance of mitochondrial function (i.e. in aging and in some socially relevant diseases such as Alzheimer’s, rare diseases, etc.). Given the known effects of thyroid hormones, it is not surprising that ‘thyroidologists’, too, have been heavily involved in investigations of basic mechanisms and of the clinical relevance of the influences that thyroid hormones exert at the mitochondrial level. As mentioned before, the calorigenic effect of thyroid hormones has long been ascribed to an uncoupling of oxidative phosphorylation, and this hypothesis initially seemed to be supported by some data obtained *in vitro* using isolated mitochondria. However, the results of such in-vitro studies were not always reproducible, and they were widely attributed to chemical artefacts. As a result, the uncoupling hypothesis was almost discarded. However, it has never been completely dropped and continues to this day to be investigated using new technical approaches, with new findings still being made. Among
others, the new findings/technical approaches of particular relevance to this article are:

1. the discovery that uncoupling proteins are not present only in brown adipose tissue but in almost all tissues and cells, and that their expressions are stimulated by T₃ [11–15];
2. the discovery of nuclear-encoded factors that affect mitochondrial biogenesis and activity, such as PGC1α and nuclear respiratory factors [6];
3. the discovery of some important uncoupling mechanisms involving adenine nucleotide translocase (ANT) [16] and mitochondrial permeability transition pores [17**]. In reference [16], the authors address the molecular nature of the basal proton conductance, and they demonstrate that ANT accounts for about 50% of the basal uncoupling;
4. the evidence that not only T₃ but also other iodothyronines, naturally occurring analogues and synthetic derivatives have important metabolic actions;
5. the use of proteomic analysis: permit to bypass discrepancies between gene expression and protein level or to discover new targets [18,19].

Thyroid hormones and uncoupling: a new dress for an old lady

Although the calorigenic effect of thyroid hormones has long been ascribed to mitochondrial uncoupling, the precise molecular mechanism is still a matter for debate. Some recent findings, however, have shed new light on this issue. In particular, the discovery that uncoupling proteins are present not only in brown adipose tissue (in which the uncoupling protein is now called UCP1) but in almost all organs and cells has assumed great importance. In particular, UCP2 (ubiquitously expressed) and UCP3 (predominantly expressed in skeletal muscle) have attracted great interest. Each of these proteins is induced by T₃. However, UCP3, because of its prevalent expression in muscle (a metabolically very active tissue), has been considered a possible candidate for the mediator of the calorigenic effects of thyroid hormones. This issue provoked studies that succeeded in demonstrating that UCP3 may be involved in the effects of thyroid hormones on resting metabolic rate [15,20]. The study reported in [15] was the first to show in vivo (in rats) that UCP3 has the potential to be a molecular determinant of the effects

Figure 1 Schematic representation of mitochondrial activities

The respiratory chain transfers electrons from reduced coenzymes (coming from intra (β-oxidation and TCA cycle) – and extramitochondrial (glycolysis) oxidative pathways) to O₂ and, pumping out H⁺ from the matrix to the intermembrane space, generates an electrochemical gradient, ΔμH⁺, which provides the driving force for ATP synthesis by FoF₁-ATPase (complex V). H⁺ can also enter the matrix by mechanisms not coupled to ATP synthesis either directly, across the lipid bilayer, or indirectly, by protein-mediated transport (mechanism not represented). I (complex I, NADH dehydrogenase), III (complex III, ubiquinone cytochrome c-reductase), IV (complex IV, cytochrome c-oxidase), V (complex V, ATP synthase), c (cytochrome c), Q (coenzyme Q). Some carriers involved in the oxidative phosphorylation and carriers mediating anion/H⁺ symport are represented individually. α-KG, α-ketoglutarate; ANT, ADP/ATP carrier; and UCP, uncoupling protein. mal, malate; cit, citrate; GC, glutamate carrier; OAA, oxalacetate; P:\, phosphate carrier; PyC, piruvate carrier; suc, succinate.
exerted by T3 on resting metabolic rate. Indeed, it was shown that after a single injection of T3 had been given to hypothyroid rats, there was a stimulation of UCP3 expression, with the maximal increase being evident at 48 h after the injection. At this time point the resting metabolic rate also reached its maximal value, whereas at the mitochondrial level there was a corresponding increase in the proton leak. However, the uncoupling properties of UCP3 have not been fully elucidated, and mitochondrial uncoupling may not be its primary function but rather a consequence of its real function.

One postulated function of UCP3 is its involvement in the export of fatty acid peroxides (LOOH) from the inner to outer mitochondrial membrane leaflet [21]. This is important to prevent damage being induced by these very aggressive molecules. The stimulation of UCP3 by T3 would, on these grounds, perform a double function: it would mediate, at least in part, the uncoupling effect of T3 while at the same time counteracting the deleterious effects due to the increased ROS production that follows T3 administration. However, to date there is no published evidence from studies of isolated mitochondria showing either that UCP3 is involved in the extrusion of LOOH from the mitochondrial matrix or that this process is associated with mitochondrial uncoupling.

In a very recent study, Lombardi et al. [22**, using mitochondria isolated from UCP3-null mice or their wild-type littermates, addressed the putative roles of UCP3. They showed that UCP3 is involved in both fatty acid peroxides induced mitochondrial uncoupling and LOOH export across the mitochondrial inner membrane (MIM). The authors also hypothesized as follows regarding how UCP3-mediated LOOH export might be related to UCP3-mediated uncoupling. When mitochondria produce and release a high level of O2− at the matrix side of their inner membrane, a high level of LOOH is also produced at the same site. These LOOH are then translocated across the mitochondrial inner membrane. Once in the outer leaflet part of MIM, some LOOH are metabolized by lipid glutathione peroxidase (GPX) to LOH, whereas the remainder, in protonated form, flip back into the matrix. Such LOOH extrusion and the associated flip back of LOOH into the matrix would lead to uncoupling (Fig. 2). However, other mechanisms may underlie the uncoupling effect of T3. Interestingly, a recent study showed that the mitochondrial uncoupling induced by T3 is transduced (in rats in vivo and in cultured Jurkat cells) by a gating of the mitochondrial permeability transition pore (PTP) [17**]. This T3-induced PTP gating was abrogated in inositol 1,4,5-trisphosphate [IP(3)] receptor 1 [IP(3)R1]−/− cells, indicating that the endoplasmic reticulum IP(3)R1 may serve as the upstream target for the mitochondrial activities of T3.

Figure 2 Proposed mechanism of action of uncoupling protein-3

When a high level of lipid hydroperoxides is present at the inner leaflet of the mitochondrial inner membrane, because of high release of O2− into the matrix, they are translocated in the anionic form (LOO−) across the mitochondrial inner membrane. Once in the outer leaflet, some lipid hydroperoxides in the protonated form (LOOH) flip back across the membrane towards the matrix. This cycle protects the vitally important mitochondrial matrix-localized components against lipid hydroperoxide-induced oxidation, at the same time it causes dissipation of proton-motive force, PUFA, polymersaturated fatty acid.

Beneficial effects of thyroid hormone derivatives

The metabolic effects of thyroid hormones have long been the focus of research into the potential use of these hormones as drugs to stimulate body weight loss and lipid metabolism. However, the simultaneous induction of a thyrotoxic state (tachycardia, muscle wasting, bone loss) effectively terminated the use of thyroid hormones for these purposes. Potentially, newly discovered analogues and derivatives, however, may have similar effects without deleterious side-effects. Indeed, since the middle of the last century much effort has been devoted to the development of analogues of thyroid hormones that might improve serum lipid profiles (i.e. plasma cholesterol, lipoprotein, etc.) without undesirable cardiac effects. In the past few years, the attention of scientists has been focused on the study of agents that are both tissue and TRβ-selective (β receptors are barely expressed in cardiomyocytes) with the principal aim of addressing such large medical problems as obesity, ectopic fat accumulation and atherosclerosis (for review see [23*,24**]). Representatives of such compounds are T2, GC-1 (or sobetirome) and KB2115 (or eprotirome).

T2: About 20 years ago, surprising results were published showing that (among a large number of iodothyronines tested) T2, like T3, was able at pM concentration to induce a rapid stimulation of oxygen consumption in perfused livers isolated from hypothyroid rats [25]. In the same study, it was shown that the effect of T3 was largely inhibited by the addition of an inhibitor of D1 deiodinase, whereas the effect of T2 was not. Moreover,
in further studies it was shown that, after a single injection, T₂, like T₃, rapidly stimulated both mitochondrial activity [26] and resting metabolic rate [27]. These findings prompted researchers to try to demonstrate possible effects of T₂ on fat accumulation, obesity and the serum lipid profile. Within the last few years, it has been shown that T₂, when administered to rats simultaneously receiving a high-fat diet (HFD), can prevent excessive body weight gain and the development of liver steatosis, without inducing a thyrotoxic state [28,29]. Lanni et al. [28] were the first to demonstrate that T₂ is able to prevent the fat accumulation that occurs upon a consumption of a HFD. Indeed, rats that had received HFD + T₂ displayed a lower body weight, less visceral fat and significant reductions in the serum triglyceride and cholesterol levels (versus rats receiving HFD alone). The biochemical mechanism underlying these effects of T₂ involved a stimulation of the mitochondrial uptake of fatty acids and an increase in their oxidation rate, associated with a less efficient utilization through an increase in mitochondrial uncoupling (proton leak). Interestingly, T₂ administration is able not only to prevent but also to reduce adiposity, and to reverse a hepatic steatosis that has already been induced by feeding a HFD [30**] (Fig. 3). Moreover, T₂ ameliorates mitochondrial oxidative stress, which is considered to be an important causal factor in the hepatocyte injury associated with nonalcoholic fatty liver disease [21]. The published studies on T₂ seem to support the idea that its actions are exerted through pathways that are independent of protein synthesis. Recent results, however, demonstrating an effect of T₂ on mitochondrial ATP synthase, suggest that T₂ may exert some of its actions by stimulating the expression of specific proteins [31**].

Thyroid hormone derivatives: A series of compounds have recently been synthesized with thyroid hormone-like effects but mechanisms of action that differ from those of thyroid hormones [24**]. A representative is the GC-1 (sobetirome) that has the potential to inhibit or reverse the hepatic steatosis present in a nutritional model such as rats fed a choline-methionine-deficient diet [32] with no significant side-effects on heart rate. Interestingly, GC-1 has the potential to stimulate energy expenditure too [33], a characteristic not shown by all the other structural analogues of thyroid hormones so far studied [24**]. Indeed, GC-1 is able to stimulate mitochondrial oxidative processes even if to a lesser extent compared to those elicited by T₃ [34*]. GC-1 also has the capacity to reduce the serum levels of cholesterol and triglycerides, and phase 1 clinical trials began to evaluate these effects [24**]. Another representative of thryomimetic agents is KB2115 (or eprotirome). In a trial, this compound was shown to be effective in reducing LDL cholesterol and triglycerides avoiding an adverse effect attributable to thyroid hormone [35**].

**Conclusion**

The recent findings discussed in this article have generated some important new concepts that could help towards a better understanding of some of the effects of thyroid hormones and those of their analogues/derivatives. Elucidation of the cellular/molecular mechanisms of action of these agents should not only help us to understand their physiology but also stimulate efforts to discover new agents that may prove effective at counteracting certain major diseases that are growing in importance worldwide. Agents that both act in a tissue-specific manner and are selective in their binding to thyroid hormone nuclear receptors seem particularly attractive and promising. Agents that target mitochondrial uncoupling may be particularly intriguing because of the possibility of modulating this process through a nongenomic pathway.

**Acknowledgement**

The work was supported by Grant MIUR-COFIN 2008 Prot 20089SRS2X.

**Figure 3** Histological analyses of livers from N, D, and DT rats

Livers and representative Sudan Black staining of liver section from N (a and b), D (c and d), and DT (e and f) rats. N = rats fed for 10 weeks with a standard diet; D = rats fed for 10 weeks with a high-fat diet and sham-injected throughout the last 4 weeks; DT = rats fed for 10 weeks with a high-fat diet with T₂ being administered throughout the last 4 weeks.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 486–487).

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38 This study describes the T(2)-induced gating of the PTP and shows that the endoplasmic reticulum inositol 1,4,5-triphosphate [IP3][Pi] receptor 1 [IP3R1] may serve as an upstream target for T(3)-induced mitochondrial activity. The study is important because it may indicate novel targets that will inform the design of new thyromimetics intended to modulate energy expenditure.