Regulation of adenine nucleotide translocase and glycerol 3-phosphate dehydrogenase expression by thyroid hormones in different rat tissues

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Thyroid hormone (T_3)-dependent gene expression of the adenine nucleotide translocase (ANT) and the FAD-linked glycerol 3-phosphate dehydrogenase (mGPDH) was investigated in several rat tissues. Both proteins provide an important link between cytosolic and mitochondrial metabolic pathways and seem to be involved in the stimulation of mitochondrial oxygen consumption in response to T_3 . Here we show that two ANT isoforms are expressed in rat, the muscle-specific ANT1 form and the ubiquitous ANT2 form. The expression of ANT1 mRNA is not sensitive to T_3 whereas the amount of ANT2 mRNA is increased 7–9-fold in liver and heart within 12–48 h after T_3 application. Little or no effect of T_3 on ANT2 mRNA was observed in kidney and brain. The mRNA changes are paralleled by an increase in ANT protein, thus explaining the accelerated ADP/ATP exchange observed in mitochondria isolated from hyperthyroid rats. The key role of ANT2 in the control of hyperthyroid

metabolism is evident because the expression of the mersalyl-sensitive phosphate carrier and the mitochondrial creatine kinase mRNA, which are functionally linked to ANT, did not respond to T_3 . Similarly to the ADP/ATP exchange, the transfer of cytosolic NADH to the respiratory chain via the glycerophosphate shuttle is very sensitive to T_3 . Recently we demonstrated the 10–15-fold induction of mGPDH mRNA in rat liver after administration of T_3 [Müller and Seitz (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 10581–10585]. Here we show that, in contrast with ANT2, the time course of induction is fast (4–6 h). Furthermore, mGPDH mRNA is induced 6-fold by T_3 in heart and 4-fold in kidney. From these results we conclude that the T_3 -mediated transcriptional induction leading to increased activity of ANT2 and mGPDH contributes considerably to the increase in mitochondrial oxygen consumption in rat tissues.

INTRODUCTION

Thyroid hormones [3,3',5-tri-iodothyronine (T_3) and thyroxine (T_4)] are key regulators of growth, development and metabolism. The most pronounced effects can be seen in clinical situations, where serum T_3 / T_4 levels are altered. Hyperthyroid patients show an increase in basal metabolic rate and glucose turnover as well as an elevated respiratory ratio and heart rate, whereas the opposite effects occur in hypothyroid individuals.

T_3 administration to rats leads to a marked increase in oxygen consumption after 36–72 h in tissues such as heart, liver and kidney, whereas no effects occur in spleen, testes and adult brain (see, for example, [1]). This stimulation of respiration seems to be the result of an increased capacity of both the respiratory chain and oxidative phosphorylation [2]. Although the exact molecular mechanisms of these phenomena are poorly understood, there is evidence that at least some of the effects are based on the transcriptional induction of nuclear encoded mitochondrial proteins such as cytochrome *c* [3] and cytochrome *c*₁ [4].

In addition to these respiratory-chain components, proteins that connect cytosolic and mitochondrial metabolic pathways play a key role in the T_3 -mediated modulation of energy metabolism. Thus the adenine nucleotide translocase (ANT) and

the FAD-linked glycerol 3-phosphate dehydrogenase (mGPDH) are targets of thyroid hormone action [5,6].

It has been observed that the ATP/ADP exchange is enhanced in liver mitochondria isolated from hyperthyroid rats and diminished in the hypothyroid state [5]. However, there are conflicting data as to whether this alteration in ADP/ATP exchange is due to corresponding alterations in gene expression. A previous study from our laboratory [7] could not show an increase in ANT mRNA after T_3 application, whereas more recent results [4,8] show that ANT mRNA is induced in rat liver by T_3 . As three different ANT isoforms, encoded by separate genes, are expressed in humans [9–12], it was hypothesized that this discrepancy could be due to the differential expression and regulation of ANT isoforms in rats. Therefore we focused on the expression of ANT isoforms in different tissues of the rat.

The nuclear-encoded mGPDH, located at the outer surface of the mitochondrial inner membrane, is part of the glycerophosphate shuttle, where it works in conjunction with a NAD⁺-linked cytosolic GPDH (cGPDH) to transfer cytosolic NADH to the respiratory chain (reviewed in [13]). The capacity of the glycerophosphate shuttle is controlled entirely by the activity of mGPDH [14]. Since the early finding of Lee and Lardy [6] that mGPDH activity is increased in several rat tissues after adminis-

Abbreviations used: ANT, ADP/ATP translocase, adenine nucleotide translocase; cGPDH, NAD⁺-linked cytosolic glycerol 3-phosphate dehydrogenase; mGPDH, FAD-linked glycerol 3-phosphate dehydrogenase; MtCK, mitochondrial creatine kinase; P_iC, phosphate carrier; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription in conjunction with PCR; sMtCK, sarcomeric MtCK; T_3 , 3,3',5-tri-iodothyronine (thyroid hormone); TRE, thyroid hormone response element; uMtCK, ubiquitous MtCK.

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tration of T_3 , mGPDH became a marker of T_3 action. We recently cloned a cDNA for mGPDH from rat liver and were able to demonstrate the dramatic increase in the amount of hepatic mGPDH mRNA in the hyperthyroid state [15].

The major aim of our present study was to determine the T_3 -dependent expression of mGPDH and ANT. In particular we wished to see whether there are tissue-specific differences in the T_3 -dependent regulation of both genes. To establish the significance of our data we also investigated whether the expression of the phosphate carrier (P_iC) and the mitochondrial creatine kinase (MtCK), which are functionally linked to ANT [16,17], is also regulated by T_3 .

MATERIALS AND METHODS

Animals and treatment

Adult male Wistar rats (Charles River Wiga) were housed under controlled conditions (22 °C, constant humidity, 12 h:12 h dark–light cycle), food and water were provided *ad lib*. Hypothyroidism was induced by intraperitoneal injection of $Na^{131}I$ (Amersham) (250 μ Ci per 100 g of body weight) 28 days before the experiments. Hyperthyroidism was provoked by a single intraperitoneal injection of T_3 (20 μ g per 100 g of body weight; Henning, Berlin, Germany). Hypo- and hyper-thyroidism were verified by measurement of serum T_3 levels (see Table 1) and of hepatic malic enzyme activity.

Enzyme assays and ANT protein measurement

Malic enzyme activity was determined as described [18]. ANT protein was quantified with the specific [3H]atractyloside binding assay essentially as described [19].

RNA isolation and Northern blotting

Total RNA was prepared as described [20]. The A_{260}/A_{280} ratios were in the range 1.8–2.0. For Northern blots 20 μ g of total RNA was separated on a 1% (w/v) agarose/formaldehyde gel and transferred on to Hybond-N (Amersham) nylon membranes by vacuum blotting with a VacuGene XL apparatus (Pharmacia). After transfer, RNA was fixed by UV cross-linking. Membranes were hybridized with either [α - ^{32}P]dCTP-labelled cDNA probes or with [γ - ^{32}P]ATP 5' end-labelled oligonucleotides. cDNA was labelled by random priming by using the Oligolabelling Kit (Pharmacia); oligonucleotides were end-labelled with polynucleotide kinase (Amersham) as described [21]. Conditions for hybridization, washing and exposure of the membranes were as described [21]. The signals were quantified by densitometric scanning. Probes were removed by pouring a boiling solution of 0.1% SDS on the membranes and allowing them to cool to room temperature.

cDNA probes and oligonucleotides for Northern blotting

Bovine ANT1 cDNA was kindly provided by H. Wohlrab (Harvard Medical School, Boston, MA, U.S.A.). The P_iC cDNA was a gift from P. Pederson (The Johns Hopkins School of Medicine, Baltimore, MD, U.S.A.). Oligonucleotides directed against ANT2 and ANT3 were derived from the human ANT2 [10] and ANT3 [11] cDNA (the corresponding nucleotides are indicated): ANT2I, 5'-CCACTCCACCTGCCAGGAAGTCCT (97–120); ANT2II, 5'-CCTTTCAGCTCCAGCTTACCCACATCA (497–524); ANT3I, 5'-GCGCTCTGTGCCTGACTTTCCCACGTCC (321–348); ANT3II, 5'-CCCTCATCTCTGAAGATCTTCCCTCAACA (668–689). A 369 bp fragment of the human ANT3 cDNA was amplified by reverse transcription in

conjunction with PCR (RT–PCR) from HeLa mRNA with a primer complementary to ANT3I as forward primer and ANT3II as reverse primer.

ANT2 cDNA isolation

ANT2 cDNA amplification with the 3' rapid amplification of cDNA ends (3' RACE) system (Gibco BRL) and 5' ligation-anchored PCR was done as described [15,22,23] with primers derived from the human ANT2 cDNA sequence. For 3' RACE, first-strand cDNA was synthesized from 5 μ g of total RNA with the oligo(dT) adaptor primer [5'-GGCCACGCGTCGACTAGTAC(T)₁₇]. Gene-specific forward primers for 3' RACE were 5'-ACAGATGCCGCATTGTCCTTCGCCA (72–96) for the first round of amplification and 5'-AGGACTTCCTGGCAGGTGGAGTGG (97–120) for the second round (nested). For ligation-anchored PCR the RT reaction was done with the primer 5'-TGTAATTCTTGATTTCATCATACAAGAC (938–960). After ligation of the 5' phosphorylated and 3' ddATP-blocked oligonucleotide anchor (5'-GATCCAGGTCACCTTCAGGTCA) [15,22,23], the first and second rounds of amplification were done with the reverse primers ANT2II and ANT2I in combination with the complementary anchor primer 5'-TGACCTGAAGTGACCTGGATC.

Cloning and sequencing

PCR products were recovered from low-melting agarose with Magic PCR Preps Purification system (Promega) and cloned into pT7 Blue T-vector with the pT7 Blue T-vector kit (Novagen). Double-stranded sequencing was done with the Sequenase 2.0 kit (United States Biochemical) with either universal sequencing primers or internal primers. For each fragment three clones from independent PCR reactions were sequenced in both directions.

Detection of MtCK isoforms by RT–PCR

RT–PCR was done with random-hexamer-primed (5 μ g of total RNA) first-strand cDNA from different tissues. For the detection of the sarcomeric and ubiquitous MtCKs (sMtCK and uMtCK respectively), primers were derived from the rat cDNA sequences [24]. A 323 bp fragment of sMtCK cDNA was amplified. The forward primer 5'-CAGAAGGTATCTGTGATGCC matches positions 174–194; the reverse 5'-GGATGCTTCATCAGCC-TAGG primer corresponds to nucleotides 477–496. uMtCK (353 bp) was amplified with the forward primer 5'-AATGAGGAGGACCACACACG (899–919) and the reverse primer 5'-ACCTCTGACTTGCCAAGTCG (1227–1251).

Quantitative RT–PCR for mGPDH and MtCK mRNA measurement

mGPDH mRNA was quantified by RT–PCR as previously described [15]. Total RNA (5 μ g) from hypothyroid, euthyroid and hyperthyroid animals was reverse-transcribed with 300 ng of random hexamer primers (Pharmacia) in a 20 μ l reaction volume. For quantification of mGPDH mRNA a 346 bp mGPDH cDNA fragment was co-amplified with a 577 bp cDNA fragment of the non- T_3 -responsive cGPDH as an internal control. The primers have been described previously [15]. To compensate for the differences in the expression of mGPDH and cGPDH, cGPDH primers were added three (heart, kidney) or four (liver) cycles after PCR was started. One-tenth of the first-strand cDNA was used in a standard PCR reaction mixture containing 15 μ Ci of [α - ^{32}P]dCTP and 10 pmol of each primer. Samples (5 μ l) of the reaction were removed after 21, 23, 25 and 27 cycles and electrophoresed on a non-denaturing 4% (w/v) polyacrylamide

gel. Gels were dried and exposed to X-ray film. The bands corresponding to mGPDH and cGPDH were excised from the gel and incorporated activity was measured by scintillation counting. The same procedure was used for measurement of sMtCK and uMtCK mRNAs. The mGPDH primers were replaced with the relevant MtCK primers (see above under 'Detection of MtCK isoforms'). As sMtCK and uMtCK mRNA levels are higher than cGPDH mRNA levels, the PCR reactions were started with cGPDH primers and after two PCR cycles sMtCK or uMtCK primers respectively were added.

RESULTS

Tissue-specific expression of ANT isoforms

To examine the expression pattern of ANT isoforms in different tissues of the rat, multi-tissue Northern blots were probed with a fragment of the bovine ANT1 cDNA and with 5'-end-labelled oligonucleotides derived from the human ANT2 and ANT3 isoforms (see the Materials and methods section). Two oligonucleotides for each ANT isoform were derived from non-homologous regions of the human cDNAs to enable isoform specific ANT detection. The results are shown in Figure 1. The ANT1-specific probe hybridized to a 1.3 kb mRNA in rat heart and muscle, but did not detect an RNA species in any other tissue examined. In contrast, the ANT2 probes hybridized to a 1.4 kb RNA in all tissues examined (kidney, heart, liver, muscle and brain). None of the ANT3-derived oligonucleotides hybridized to an mRNA in any rat tissue. The obvious lack of ANT3 expression in rat was confirmed by hybridization with a 369 bp human ANT3 cDNA fragment amplified by RT-PCR from RNA from HeLa cells. As with the ANT3 oligonucleotides no hybridization signal occurred, whereas strong signals could be seen in the positive control HeLa RNA. To characterize the ubiquitous ANT2 form further, a full-length cDNA for this isoform was isolated from a rat liver cDNA pool with a combination of RT-PCR methods (see the Materials and methods section). As confirmed by Shinohara et al. [25], the 1236 bp cDNA sequence is 89% identical with human ANT2: the deduced amino acid sequence differs from human ANT2 in only five positions.

T₃-dependent expression of ANT isoforms

T₃-mediated gene expression of ANT1 and ANT2 in heart was investigated by Northern blotting on RNA isolated from hypothyroid, euthyroid and hyperthyroid rats. The membranes were first hybridized with a probe corresponding to the ANT1 isoform. After exposure the membranes were stripped and rehybridized with an ANT2-specific probe. A representative Northern blot showing ANT mRNA levels in hypothyroid and hyperthyroid

Figure 1 Tissue-specific expression of ANT isoforms

Total RNA (20 µg) was separated on a 1% (w/v) formaldehyde/agarose gel, blotted and hybridized to a ³²P-labelled bovine ANT1 cDNA probe and ³²P-end-labelled ANT2-specific oligonucleotides. Identical results were obtained from four different animals.

Figure 2 T₃-dependent expression of ANT isoforms

RNA from hypothyroid and hyperthyroid animals (48 h after a single injection of 20 µg of T₃ per 100 g of body weight) was prepared from the tissues indicated. Total RNA (20 µg) from three different animals of each group was loaded, subjected to electrophoresis, blotted and hybridized to the ANT1- and ANT2-specific probe.

rats is given in Figure 2. The quantitative data are summarized in Table 1. Whereas changes in the T₃ status of the animals have no influence on the amount of ANT1 mRNA, the ANT2 mRNA is barely detectable in the hypothyroid state and increased 7–9-fold in the hyperthyroid state. Euthyroid mRNA levels were about three times the hypothyroid values. In liver, where only ANT2 is expressed, the alteration of ANT2 expression after T₃ administration was comparable with that seen in heart (Figure 2 and Table 1). The time course of hepatic ANT2 induction is depicted in Figure 3(A). A significant increase in ANT2 mRNA occurred 12 h after T₃ administration; maximal levels were reached after 48 h. In addition to ANT2 mRNA, the hepatic ANT protein content in isolated mitochondria was measured in various thyroid states with a specific radioactive-ligand-binding assay [19]. It was shown that the increase in ANT2 mRNA from hypothyroidism to hyperthyroidism is paralleled by a 2.3-fold increase in the amount of ANT protein. In euthyroid animals the protein content was elevated 1.5-fold (Table 1). In contrast with rat heart and liver, investigation of T₃-regulated ANT2 expression in kidney and brain showed a minor change (kidney; less than 2-fold) or no change (brain) in the amount of ANT2 mRNA in the different thyroid states (Figure 2 and Table 1).

T₃-dependent expression of P_iC and MtCK

The major transport system for the translocation of P_i across the mitochondrial inner membrane is the mersalyl-sensitive P_iC [16]. T₃-dependent P_iC expression was measured in heart, kidney, liver and brain by Northern blotting with a fragment of the bovine P_iC cDNA. With the exception of liver, where the P_iC mRNA content is doubled from hypothyroidism to hyper-

Table 1 Effects of T_3 on the relative amount of ANT mRNA in different tissues

Hypothyroidism and hyperthyroidism were induced as described in the Materials and methods section. Serum T_3 concentrations are given in $\mu\text{g/l}$. The mRNA contents were determined by Northern blot hybridization as described in the legend to Figure 2. mRNA results are presented in densitometric units relative to the respective hypothyroid value. Hepatic ANT protein was measured by [^3H]atractyloside binding [19]. The amount of bound radioactivity is given relative to the hypothyroid value. Each group consisted of four animals. Values are given as means $\pm$ S.D.

	Heart		Liver		Kidney	Brain
	ANT1 mRNA	ANT2 mRNA	ANT2 mRNA	ANT2 protein	ANT2 mRNA	ANT2 mRNA
Hypothyroid (0.42 $\pm$ 0.02)	1.0 $\pm$ 0.2	1.0 $\pm$ 0.15	1.0 $\pm$ 0.3	1.0 $\pm$ 0.05	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2
Euthyroid (1.24 $\pm$ 0.12)	1.2 $\pm$ 0.1	2.5 $\pm$ 0.3	2.2 $\pm$ 0.6	1.5 $\pm$ 0.1	1.4 $\pm$ 0.3	1.1 $\pm$ 0.1
Hyperthyroid (32.4 $\pm$ 4.2)	1.3 $\pm$ 0.3	7.9 $\pm$ 1.3	7.4 $\pm$ 2.2	2.3 $\pm$ 0.3	1.8 $\pm$ 0.4	0.9 $\pm$ 0.4

Table 2 Effects of T_3 on the relative amount of mGPDH mRNA in different tissues

Experimental conditions were as described in Table 1. T_3 levels for each group of rats are given in $\mu\text{g/l}$. mRNA was quantified by the RT-PCR assay described in the Materials and methods section. Incorporated radioactivity is given relative to the hypothyroid value. Each group consisted of four animals. Values are given as means $\pm$ S.D.

	Heart	Liver	Kidney	Brain
Hypothyroid (0.37 $\pm$ 0.06)	1.0 $\pm$ 0.3	1.0 $\pm$ 0.15	1.0 $\pm$ 0.3	1.0 $\pm$ 0.05
Euthyroid (1.47 $\pm$ 0.18)	1.5 $\pm$ 0.1	1.6 $\pm$ 0.3	1.7 $\pm$ 0.2	0.7 $\pm$ 0.4
Hyperthyroid (38.8 $\pm$ 7.2)	5.7 $\pm$ 0.3	9.4 $\pm$ 1.3	4.1 $\pm$ 0.3	1.2 $\pm$ 0.3

the T_3 -dependent expression of MtCK in heart. The very sensitive quantitative RT-PCR assay system was used, to detect not only the highly expressed (muscle specific) sMtCK in heart, but also the uMtCK transcript, which is below the detection limit of a conventional Northern blot [24]. Neither the sMtCK nor the uMtCK mRNA responded to T_3 (results not shown).

T_3 -dependent expression of mGPDH

We recently cloned a cDNA for mGPDH from rat liver and demonstrated the marked induction of hepatic mGPDH mRNA, when hypothyroid animals were rendered hyperthyroid ([15]; see Table 2). The kinetics of this induction was followed after a single injection of T_3 . RNA was isolated, reverse-transcribed and measured by the quantitative RT-PCR assay described ([15]; see the Materials and methods section). By 2 h after the application of T_3 the mRNA level had increased 5-fold; maximal induction levels (8–10-fold) were reached 4–6 h after T_3 administration (Figure 3B).

The T_3 -dependent expression of mGPDH in rat heart, kidney and brain is shown in Table 2. For comparison the results for liver are also included. As in liver, mGPDH mRNA in heart and kidney is expressed at a very low level in the hypothyroid state, whereas hyperthyroid animals showed a 4-fold increase in mGPDH mRNA in kidney and a 6-fold increase in heart. Euthyroid mGPDH mRNA levels in both tissues are only slightly higher (1.5-fold) than hypothyroid levels (Table 2). These mRNA results correlate well with the reported alteration in enzyme activity after administration of T_3 [6]. As with ANT2, the expression of mGPDH in brain is not sensitive to T_3 (Table 2).

DISCUSSION

Expression and T_3 -dependent regulation of ANT

In rat, two ANT isoforms, homologous with human/bovine ANT1 and human ANT2, are expressed. As in human and bovine tissues [9–12,28], the expression of rat ANT1 is restricted to heart and skeletal muscle. In contrast, ANT2 is expressed ubiquitously in rat tissues, whereas ANT3, the major ANT form in human and bovine tissues [28], was not detected. Although it is not yet known whether different ANT isoforms have distinct physiological functions, the expression of ANT isoforms might facilitate the specific regulation of energy metabolism in response to various metabolic situations. The results shown here support this view, as the expression of ANT2 is under the control of T_3 , whereas ANT1 is not influenced by T_3 . The increase in the amount of hepatic ANT2 protein in response to

Figure 3 Time course of induction of hepatic ANT2 (A) and mGPDH (B) mRNA by T_3

Hypothyroid animals received a single intraperitoneal injection of T_3 (20 μg per 100 g of body weight). RNA was prepared at the time indicated and quantified by either Northern blotting (ANT2) or quantitative RT-PCR (mGPDH). Values represent means $\pm$ S.D. ($n = 4$).

thyroidism, in none of the other tissues were alterations in mRNA expression observed (results not shown).

Because the rate of mitochondrial phosphocreatine synthesis in cardiac muscle is altered with the T_3 state [26,27], we studied

T_3 strongly suggests that the changes in ANT carrier activity are based on this mRNA up-regulation. The earlier study done in our laboratory [7] used only the ANT1-specific probe and therefore failed to detect an mRNA up-regulation in response to T_3 .

The most prominent effects of T_3 on ANT2 mRNA expression were observed in heart and liver, whereas weak or no responses occurred in kidney and brain. This tissue-specific T_3 response on ANT mRNA shows a striking similarity to the tissue-specific changes in respiratory metabolism [1,2] and thus suggests that ANT2 is important in modulating T_3 -induced oxygen consumption.

Functionally linked to ANT are the P_i C [16] and, in muscle tissues, the MtCK (reviewed in [17]). To allow stoichiometry and electroneutrality of the exchange, co-transport of P_i and H^+ is required. This proton, together with a molecule of P_i , re-enters the matrix space via the mersalyl-sensitive P_i C. The approx. 2-fold increase in hepatic P_i C mRNA after application of T_3 is consistent with the slight increase in carrier activity reported previously [29]. As this increase is small compared with ANT, it is suggested that the P_i C is not an important site of thyroid hormone action.

In addition it has been postulated that in oxidative slow-twitch and cardiac muscle, where phosphocreatine represents the major storage form of high-energy phosphates, MtCK is functionally coupled to oxidative phosphorylation [17]. In this complex MtCK is located at contact sites between the outer and inner membrane and interacts directly with ANT. After transfer through the inner mitochondrial membrane, ATP is transphosphorylated by MtCK to give phosphocreatine [17]. Our results show that the expression of MtCK is not regulated by T_3 , thus underlining the significance of the induction of ANT for the control of cardiac high-energy phosphate metabolism in response to T_3 .

T_3 -dependent regulation of mGPDH

The well-known increase in mGPDH activity in response to T_3 in rat tissues such as liver, heart and kidney [6] is due to the induction of mGPDH mRNA after administration of T_3 . The higher capacity of the glycerophosphate shuttle, resulting from this up-regulation, prevents the accumulation of reducing equivalents derived from T_3 -stimulated glycolysis. Mitochondrial oxidation of these reducing equivalents might account for a considerable part of the increased mitochondrial oxygen uptake [30]. The tissue-specific up-regulation of mGPDH mRNA is similar to ANT2 mRNA, with the highest levels observed in liver and heart, a lower induction in kidney and no T_3 response in brain. However, in contrast with the delayed induction of ANT2 and all other T_3 -regulated mitochondrial proteins investigated so far, the induction of mGPDH occurs rapidly (4–6 h) and is by far the earliest T_3 response observed for a nuclear-encoded mitochondrial protein. The time course of induction is similar to cytosolic T_3 -regulated enzymes, e.g. hepatic malic enzyme [31] or glucokinase [32]. It is tempting to speculate that the rapid induction of mGPDH is based on a direct interaction of the T_3 receptor with specific *cis*-acting elements (thyroid hormone response elements; TREs) [33] in the mGPDH promoter region.

Conclusion and perspectives

The results presented here demonstrate that only a subset of nuclear-encoded mitochondrial proteins are regulated by thyroid hormones at the transcriptional level. Together with cytochrome *c* [3] and cytochrome c_1 [4], ANT2 and mGPDH show the most pronounced induction of all mitochondrial proteins yet investigated. These proteins seem to play a key role in the T_3 -mediated

stimulation of oxygen consumption. For several subunits of cytochrome *c* oxidase [34] and ATPase [35,36] mRNA increases have been observed; however, the level of induction is weak (approx. 2-fold). As we observed a similar increase in hepatic P_i C mRNA and hepatic uMtCK (results not shown) in response to T_3 , but also for non-mitochondrial proteins (e.g. β -actin and tubulin), we suggest that these borderline effects do not reflect T_3 -dependent regulation of selected genes and are not sufficient to explain the increase in mitochondrial metabolic activity.

Promoter studies should help to delineate the sequences that mediate the T_3 -dependent transcriptional activation of ANT and mGPDH. With regard to the delayed time course of ANT, cytochrome *c* [3] and cytochrome c_1 [4] induction, it is questionable whether T_3 directly stimulates transcription via TREs in the promoter region of these genes. Consistent with this hypothesis, no TRE sequences have so far been identified in the promoter regions of rat cytochrome *c* [37] and c_1 [4]. This observation favours the involvement of an as yet unidentified *trans*-acting factor, which regulates the co-ordinated induction of these genes.

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