Expression of α-Actin Genes in Developing Skeletal Muscle of Hypothyroid Rats

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Abstract

During skeletal muscle development in the rat, both α-skeletal and α-cardiac actin mRNAs are coexpressed, but their levels of expression show different patterns of regulation. Levels of accumulation of both sarcomeric actin mRNAs during skeletal muscle development are different in euthyroid and in hypothyroid rat skeletal muscles. The amounts of both mRNAs were measured by quantitative Northern blotting. α-skeletal actin gene expression is not affected by hypothyroidism for the first 20 days after birth. The expression of the α-skeletal actin gene is up-regulated in older animals. In the absence of thyroid hormone the developmental down regulation of α-cardiac actin gene expression is retarded. The two α-actin genes are therefore affected by thyroid hormone. Transfection experiments and gel shift assays show that the rodent α-skeletal actin gene can respond directly to thyroid hormone and that a potential thyroid hormone response element is present in the first intron. Thyroid hormone receptor interferes with MyoD transactivation of this gene in the C2 muscle cell line.

Key words: sarcomeric actins, skeletal muscle, hypothyroidism, skeletal actin gene, thyroid receptor element, MyoD.

BAM 3 (1): 27-36, 1993

Actins are ubiquitous proteins which form microfilaments in non-muscle cells and thin filaments in the sarcomeres of muscle cells [28]. In mammals, there are at least six different isoforms of actin, encoded by distinct genes [36], two of which, α-cardiac and α-skeletal actin, are expressed only in striated muscles. These two sarcomeric isoforms differ by only 4 neutral amino acid changes over 375 residues, and each isoform is the product of a single gene [25, 29]. In rodents, cardiac and skeletal muscles coexpress this actin gene pair at all stages of development where, respectively, the α-cardiac and the α-skeletal actin genes predominate [37]. Accumulation of α-cardiac actin mRNA in skeletal muscle is a characteristic of embryonic [32], foetal [24] and early postnatal tissue [16]. Adult skeletal muscle contains only very low levels of α-cardiac actin mRNA in the mouse [24, 16] and in the rat [23].

Thyroid hormone levels have been shown to affect the developmental transitions which occur in striated muscle. During cardiac muscle development, thyroid hormone is required for the transition from β to α myosin heavy chains (MHC) in the ventricle. Hypothyroidism perpetuates the expression of the β isoform and prevents that of α, whereas thyroid hormone has the opposite effect [21, 17]. In skeletal muscle, the transitions of MHC isoforms (embryonic → perinatal → adult) are influenced by thyroid hormone. Hypothyroidism induces a persistence of the perinatal MHC isoform in adult skeletal muscle, and the appearance of the adult MHC is retarded [14, 5]. The same result is obtained with the dwarf mouse, which is genetically hypothyroid, and whose skeletal MHC transitions are retarded but not prevented entirely [38]. In the case of the cardiac MHC genes, thyroid hormone has been shown to have a direct effect on their transcription. The thyroid hormone receptor interacts with responsive elements in the 5' flanking region of the α-MHC gene [18, 19]. The sarcomeric

calcium ATPase promoter has also been shown to contain thyroid hormone response elements [31].

The α-actin genes have been shown to be induced by thyroid hormone in the rodent heart [18, 39], although it was not clear whether this was a direct effect [39]. More recently a thyroid hormone response element has been identified in the proximal promoter of the human α-skeletal actin gene [8]. In this paper we show that thyroid hormone affects the developmental changes in α-actin gene expression which take place in skeletal muscle. In hypothyroid rats the post natal reduction in α-cardiac actin mRNA is retarded. The accumulation of α-skeletal actin mRNA is also perturbed at a later stage. We show that the rodent α-skeletal actin gene like the human is induced by thyroid hormone and that there is another putative thyroid hormone response element in the first intron. Thyroid hormone receptor represses transactivation of this gene by MyoD.

Materials and Methods

Thyroid hormone perturbation

Hypothyroidism was induced by adding 0.05% propylthiouracil to the drinking water of pregnant rats from 3 days gestation onwards and to the water of their offspring from birth through 60 days of age. Mothers and pups were also maintained on a low iodine diet [14]. From about 10 days after birth hypothyroid mice began to exhibit an altered physical appearance. Hypothyroidism was verified by an analysis of MHC transitions where persistence of the perinatal MHC mRNA was seen in the same experiment described below [14, 5, 38].

RNA preparation and Northern blot analysis

Total RNA samples of skeletal muscle were extracted from the gastrocnemius muscle of rats. The euthyroid samples are from the gastrocnemius muscle of rats at 5, 10, 20, and 30 days and the hypothyroid samples are from the gastrocnemius of rats at 10, 20, and 30 days. RNA was prepared by homogenizing frozen tissues in 5M guanidinium isothiocyanate, 50mM Tris-HCl, pH7.5, 0.5% sodium N-lauroyl sarcosine, 0.1M β-mercaptoethanol followed by sedimentation through a CsCl gradient as described in Chirgwin et al. [7]. The concentration of polyA in each sample was determined by polyU titrations. Samples of total RNA containing the equivalent of 50 ng polyA were electrophoresed in 1% agarose, 2.5% formaldehyde gels for 4-5 h at 200 V and transferred to a diazotised Biodyne A membrane (Pall USA) in 20x SSC.

Hybridizations were performed using single-stranded DNA probes containing the 5' region of the α-cardiac or the α-skeletal actin genes from mouse [32] prepared from M13mp8 recombinants. As such, each probe contained the first non-coding exon of either the α-skeletal gene (58 nucleotides) or the α-cardiac gene (50 nucleotides). Our mouse probes hybridize to rat RNA because of the high degree of sequence conservation for actin genes between species. Between the rat [41] and mouse [32] α-skeletal actin genes, there is just one nucleotide difference over the 58 nucleotides in the first exon. Although the sequence of the first exon in the rat α-cardiac actin gene has not been determined, it is likely that it is very similar or identical to the corresponding sequence in the mouse gene. Even between human [26] and mouse [32] α-cardiac actin genes, the 5' untranslated regions remain 65% homologous. Probes were labelled by incubation of 3.7 μg of the single stranded DNA with 12.5 ng of M13 universal primer (17 mer) using the Klenow fragment of DNA Polymerase I (5 units) in the presence of 20 mCi (α ^{32}P) dCTP (800 Ci/mmol) and 20 μCi (α ^{32}P) TTP (800 μCi/mmol) and cold dATP (0.75 mmol) and dGTP (0.75 mmol) in a final volume of 150 μl at room temperature for 15 min. The resulting double stranded DNAs were cut either with Sac I for the α-skeletal actin sequence or with Bst NI for the α-cardiac actin sequence, and the DNA fragments labelled on a single strand, after gel purification, were denatured by heating at 100°C for 3 min before use as probes. 30 to 60x10^6 cpm per 50ng of DNA template were obtained routinely and used for hybridization. Hybridization conditions were as described in Alonso et al. [1]; blots were washed in 0.1xSSC, 0.1% SDS at 60°C. Quantification was based on comparison with mRNA signals from a standard sample of RNA from C57BL/6 mouse skeletal muscle, on the same blot in the same hybridization. It had been previously determined that this sample of total RNA, representing the equivalent of 50 ng PolyA, contains 0.21 ng of α-skeletal actin mRNA and 0.01 ng of α-cardiac actin mRNA [1]. Quantification was achieved by the scanning of a variety of exposures using a model 620 Bio-Rad densitometer. Blots were dehybridized by washing at 80°C for 30min in 0.1xSSC, 0.1% SDS and checked by autoradiography before hybridization to the second probe.

Cell culture and transfection

Mouse myogenic C2/7 cells (see 10) derived from the adult C2 cell line [40] were cultured in DMEM supplemented with 20% FCS in 7% CO_2. Myoblast differentiation into multinucleate myotubes is obtained by reducing serum to 2% and is almost complete within 72 to 96 h. COS fibroblast-like cells derived from the CV1 monkey kidney cell line were grown in DMEM supplemented with 7% FCS in 7% CO_2.

Cells were grown until about 50% confluence in 35 mm diameter Falcon dishes. The day before transfection, cells were cultured in thyroid-deficient medium containing 7% charcoal-stripped FCS in DMEM and medium was changed 3 h before transfection. Each dish of cells was transiently transfected with 5 μg of pBL-Act-CAT3 plasmid DNA expressing CAT, mixed with 1.5 μg of thyroid receptor expression vector CDM62 [30, 3] or the pUC118 vector DNA. For transactivation experiments 1 μg of MyoD expression vector was added to the transfection [11]. In order to keep constant the total amount of DNA in each transfection, variable amounts of pUC118 DNA were added. For each plate we co-transfected with 1 μg of RSV-βGal in order to normalize the transfection effi-

ciency. The DNA mixtures were cotransfected into C2/7 myoblasts and COS fibroblasts by the $CaPO_4$-DNA coprecipitation method (see 10). After 24 h, fresh medium with or without triiodothyronine (T3) (10 nM) was added to the cultures. Myoblasts and myotubes were harvested for CAT activity respectively 24 h and 48 h after the transfection period. COS cells were harvested 24 h after transfection.

CAT assays

50 µl of the cell extracts were incubated at 37°C with 0.05 µCi of ^{14}C chloramphenicol (53 mCi/mmol) (Amersham CFA754) in the presence of 0.53 mM butyryl-CoA (Sigma B1508) and 250 mM Tris-HCl, pH 7.5. After one hour incubation at 37°C, we extracted butyrylated products with 200 µl of Tetramethylpentadecan (TMPD): Xylene (2:1) and we counted 170 µl of the organic phase in 6 ml of organic counting scintillant. Counts were normalized with the corresponding figure for β-galactosidase activity to attempt to standardise for the variability in transfection efficiency.

Oligonucleotides

αsk-TRE1 : TCGACTGGGGAGGGTGACCTGGAGACCCAGCAAG
αsk-TRE2 : GATCCTTGCTGGGTCTCCAGGTCACCCTCCCCAG
Pal-TRE: GATCCTCAGGTCAGGTCATGACCTGACCTGAG

Nuclear extracts and gel mobility shift assays

Nuclear extracts were prepared according to the method of Dignam et al. [12] with slight modifications. C2/7 cells were lysed by 10 to 20 strokes of a Kontes all glass Dounce homogenizer and COS cells were lysed using 0.4% Nonidet P-40. The final concentrations of the extracts were 5 µg/µl protein for COS cells, and 3 µg/µl for C2 cells.

αsk-TRE1 and αsk-TRE2 were annealed together and Pal-TRE oligonucleotides were auto-annealed. Oligonucleotide duplexes were labeled by filling-in using dCTP $\alpha^{32}P$ and dTTP $\alpha^{32}P$ in the presence of the Klenow enzyme. Oligonucleotides were passed through a push column (Stratagene), precipitated and diluted in 50 µl H_2O. The concentration of labeled duplex is approximately 1 ng/µl.

Binding assays were performed in 16 µl with approximately 1 ng of Klenow-labeled oligo duplex, 4 µl of binding buffer 4x concentrated (binding buffer is 50 mM Tris-HCl, pH 7.9; 60 mM KCl; 5 mM DTT; 4% PEG-8000; 5% glycerol; 1 mM EDTA) 2 µl of nuclear extract and 2 µl of polydeoxyinosinic-deoxycytidylic acid (poly dIdC) as a non specific competitor. The binding mixtures were incubated on ice and DNA-protein complexes were electrophoresed on a 5% non denaturating acrylamide gel in TBE 0.5x. (x1 TBE is 90 mM Tris-borate, 2 mM EDTA, pH 8.0). The gel was subsequently processed for autoradiography.

Results

1) Effects of hypothyroidism

We examined α-actin gene expression during postnatal development of the gastrocnemius muscle in euthyroid and hypothyroid rats. We have quantified both α-cardiac and α-skeletal mRNA levels in rats of 5, 10, 20, and 30 days of age. A Northern blot of total RNA from these age groups was hybridized to single-stranded DNA probes homologous to the 5' non-coding exon of either α-skeletal or α-cardiac mRNA (See Materials and Methods for probes and quantification). The results, shown in Fig. 1 and summarized in Table 1, are expressed for an equivalent of 50 ng poly(A) loaded on the gel for each total RNA sample. The proportion of poly(A) per total RNA showed small variations (0.16 to 0.21%) between rats of different ages; only the 5 day euthyroid rat had a higher percentage of poly(A) (0.29%) which may represent a higher level of mRNA transcription just after birth. This range of values is of the same order as that found in skeletal (total hindlimb) muscle RNA from different recombinant inbred mouse lines (0.2 to 0.35%) and clearly different from the percentage of poly(A) in the heart (0.35 to 0.6%) (see 1). Quantification was based on the comparison of the signal obtained with a standard sample of RNA from mouse (C57BL/6) skeletal muscle to the signal given by the rat mRNA samples on the same blot in the same hybridization. The amounts (in nanograms) of α-skeletal and α-cardiac actin mRNAs in the skeletal muscle of the C57BL/6 mouse had been previously quantified [1]. In an equivalent of 50 ng poly(A) of this control RNA, there is 0.21 ng of α-skeletal actin mRNA and 0.01 ng of α-cardiac actin mRNA.

During skeletal muscle development in euthyroid rats, the level of α-skeletal actin mRNA rapidly increases between 5 days (0.12 ng) and 20 days (0.61 ng) after birth. Between 20 and 30 days (0.63 ng), the rate of accumulation begins to level off. These results are similar to the increase observed during skeletal muscle development for the C3H mouse [15]. The levels of expression of this gene in hypothyroid rats at 10 days (0.26 ng) and 20 days (0.58 ng) after birth are very similar to what is observed in euthyroid rats. However, at 30 days there is an increase in α-skeletal mRNA accumulation (1.42 ng) in hypothyroid animals amounting to more than two fold the level measured in the 20 day euthyroid rat.

The expression of the α-cardiac actin gene during skeletal muscle development in euthyroid rats, examined from 5 days after birth, undergoes a rapid decrease to virtually undetectable levels of this mRNA by 20 days. The C57BL/6 mouse line shown here contains a relatively high proportion (4.5%) of α-cardiac actin mRNA in the adult compared to other mouse lines [1]. In hypothyroid rats, firstly the level of α-cardiac actin mRNA is approximately three fold higher at 10 days after birth, than that in normal animals. Secondly the disappearance of α-cardiac actin transcripts is retarded, such that the level at 20 days is similar to that at 10 days in euthyroid animals, and only

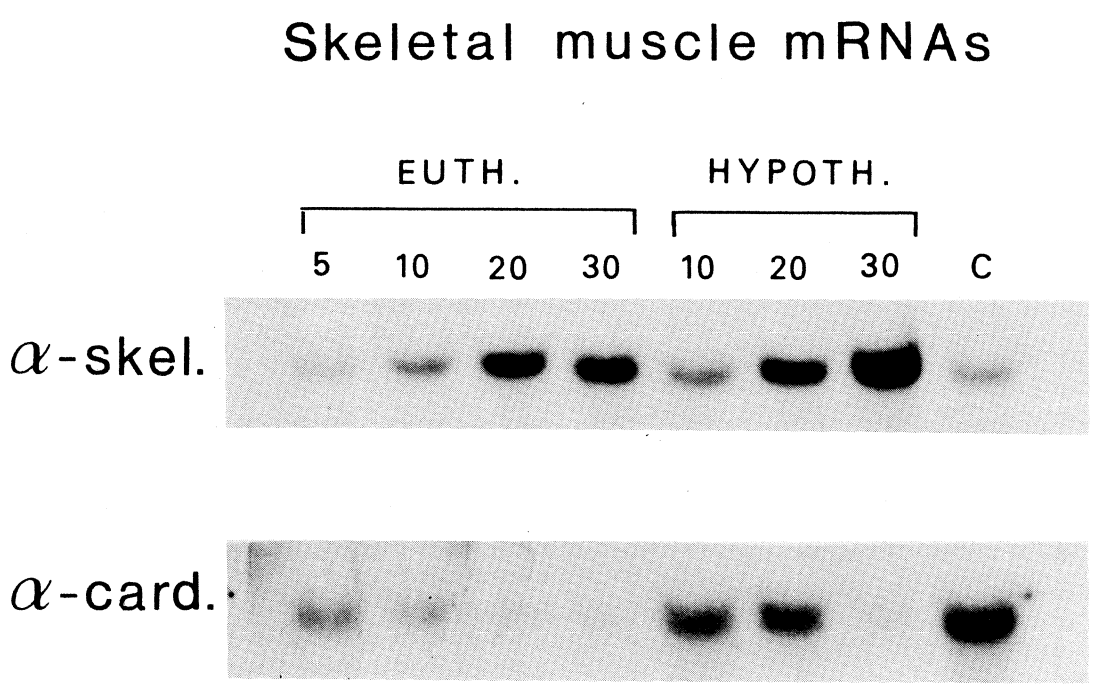

Figure 1. Northern blot of total skeletal muscle RNA

Samples of total skeletal muscle RNA containing the equivalent of 50 ng of PolyA were run on an agarose formaldehyde gel and blotted onto DBM paper. The filter was sequentially hybridized first to an α-skeletal and, after removal of this signal, second to an α-cardiac actin mRNA specific probe. Total RNA was extracted from the gastrocnemius of euthyroid rats at 5, 10, 20 and 30 days and also of hypothyroid rats at 10, 20 and 30 days. Rat muscle mRNA signals were quantified by comparison with a standard RNA sample from adult C57BL/6 mouse skeletal muscle (C). The α-actin content of this sample had been quantified previously [1]. The two α-actin sequences are highly conserved between rat and mouse (see Materials and Methods). Exposure times were one day for the α-skeletal mRNA probe and seven days for the α-cardiac mRNA probe. On comparable exposures α-skeletal actin mRNA is the major transcript in all samples (see Table 1).

Table 1. Sarcomeric actin mRNAs in rat skeletal muscle

Quantification of α-skeletal and α-cardiac actin mRNAs present in 50 ng PolyA equivalents of total skeletal RNA. The age of euthyroid and hypothyroid rats is given in the first column. Columns 2 and 3 give the numbers of nanograms of α-skeletal actin mRNA present in euthyroid and hypothyroid rats. Columns 4 and 5 give the numbers of nanograms of α-cardiac actin mRNA present in euthyroid and hypothyroid rats. Columns 6 and 7 give the percentages of total sarcomeric actin mRNA that is α-cardiac actin mRNA in euthyroid and hypothyroid rats. Some signals were not detectable (-) and some values have not been determined (N.D.).

	ng α skel. mRNA		ng α card. mRNA		% α card. mRNA	
	Euthyr. rats	Hypoth. rats	Euthyr. rats	Hypoth. rats	Euthyr. rats	Hypoth. rats
30 days	0.63	1.42	-	-	-	-
20 days	0.61	0.58	-	0.005	-	0.8
10 days	0.27	0.26	0.0015	0.005	0.5	1.9
5 days	0.12	N.D.	0.002	N.D.	1.6	N.D.

falls by 30 days. Thus α-cardiac actin mRNA which can be regarded as a foetal transcript in skeletal muscle [24] persists over a longer period after birth in hypothyroid rats.

2) Examination of the effects of thyroid hormone on transcription of the α-skeletal actin gene

As far as the transcriptional regulation of these α-actin genes by thyroid hormone is concerned, there is no very obvious thyroid hormone response element in the proximal promoter region of the mouse α-actin genes [15, 1]. In the case of the α-skeletal actin gene [1], we noted the presence of a good consensus sequence [19, 20] at the beginning of the first intron.

Transfection experiments were carried out with the promoter sequence of the mouse skeletal actin gene from -879 to +125 which includes the TRE element in the first intron linked to the chloramphenicol acetyl transferase (CAT) reporter gene. This plasmid (pAct-CAT3) shows regulated expression between dividing myoblasts and differentiated myotubes when transfected into the C2/7 muscle cell line. Its level of expression in non-muscle COS cells is similar to pBLCAT3. In order to test for a thyroid hormone response, C2/7 cells were grown in thyroid hormone-depleted medium and then co-transfected with pAct-CAT3 and an expression plasmid (CDM62) for thyroid hormone receptor (TRβ1 isoform) [30, 3] in the presence or absence of thyroid hormone T3. The CAT activity due to pAct-CAT3 increased in the presence of TR and T3 in myotubes, but not in myoblasts (Fig 2). This effect was more striking in C2 cells transfected with a MyoD expression vector [11] which increases the extent of differentiation. In the absence of T3, when TR and MyoD are present, about two thirds of the stimulation persist, probably as a result of contaminating T3 still present in the medium. In non-myogenic COS cells co-expression of TR with pAct-CAT3 did not result in a stimulation of activity (results not shown). This is probably due to the absence of muscle specific factors necessary for the transcriptional activation of this gene and its response to thyroid hormone. These results suggest that the rodent α-skeletal actin gene can respond directly to thyroid hormone.

The promoter of the gene contains a number of E boxes [1] and is transactivated by MyoD. Surprisingly, the stimulation of transcriptional activity seen in myoblasts and also in differentiated myotubes, when MyoD is overexpressed

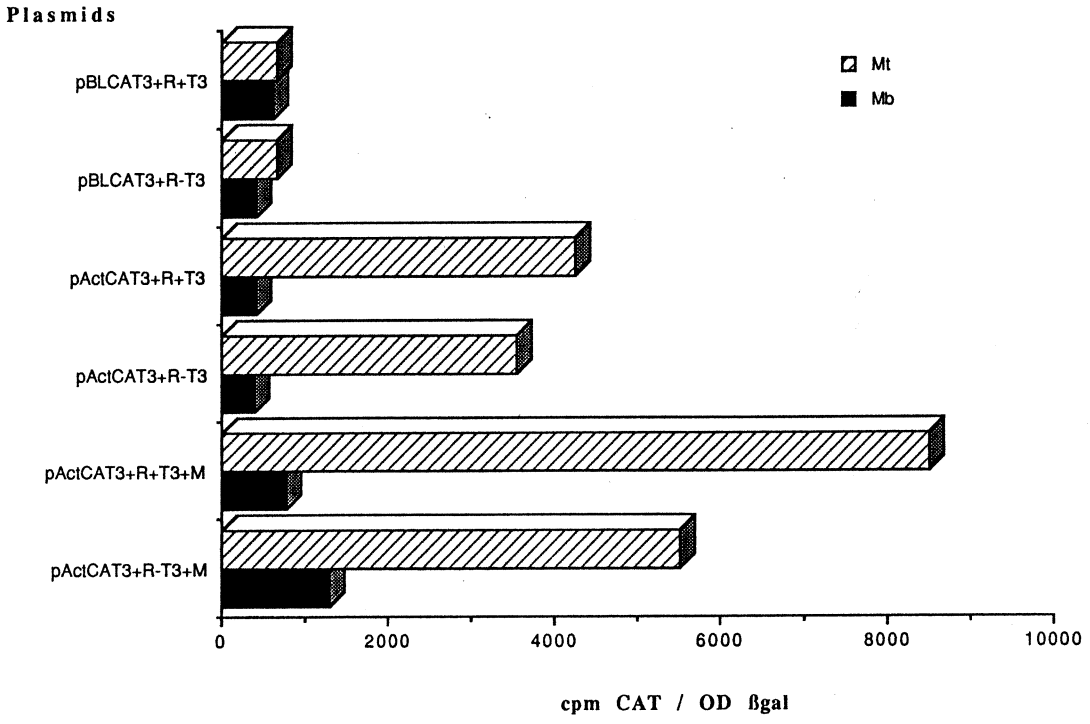

Figure 2. The effects of thyroid hormone on the activity of a skeletal actin-CAT reporter gene transfected into muscle cells. The averaged results of several transfection experiments in myoblasts and myotubes of the C2 muscle cell line are shown, after co-transfection of the CAT reporter gene under control of regulatory sequences from the α-skeletal actin gene (pActCAT3) or as a promoterless control vector (pBLCAT3), with the expression vector containing the sequence for the thyroid hormone receptor (R), with or without added thyroid hormone (T3) and in presence or absence of MyoD expression vector. Results are expressed as radioactive butyrylchloramphenicol converted by the CAT enzyme activity, normalized to ß-galactosidase activity in the same sample. All vectors were co-transfected with an RSV-ßGalactosidase plasmid in order to standardize for transfection efficiency.

in these cells, is substantially decreased in co-transfection experiments when the thyroid hormone receptor is also overproduced (Fig. 3). The addition of T3 had little effect on this phenomenon. This preliminary finding may point to interference between these two proteins.

3) Identification of a putative thyroid hormone response element in the first intron of the α-skeletal actin gene

The sequence present at the beginning of the first intron in the mouse α-skeletal actin gene shows high sequence homology to the thyroid hormone response element motif as defined in the cardiac α-MHC promoter [19]. This element is also conserved in the human [35] and rat [41] α-skeletal actin genes (Fig. 4).

Electrophoretic mobility shift assays were performed to determine if this putative TRE interacts with a nuclear factor from muscle cells. Nuclear extracts were prepared from non-muscle COS cells and from myoblasts and myotubes of the mouse muscle cell line, C2/7. Two different oligonucleotides were used in these assays : the putative mouse α-skeletal thyroid hormone response element (αsk-TRE) and the palindromic thyroid hormone response element (Pal-TRE), which represents the standard thyroid hormone binding site [20].

In Fig. 5 the results of such an experiment are shown. With COS cell extracts a single clear intense band is seen only with the labelled Pal-TRE. A second upper faint band is seen with both the Pal-TRE and αsk-TRE while a third one is only detected with αsk-TRE. In C2 muscle cells two distinct bands are seen in both myoblasts (lanes 4, 6) and differentiated myotubes (lanes 8, 13) with the αsk-TRE and the Pal-TRE respectively. In myoblasts the complex formed with the labelled αsk-TRE (lane 5) or the labelled Pal-TRE (lane 7) is competed by adding excess of the other cold probe. In myotubes the complex formed by the labelled αsk-TRE is competed by itself (lane 9) and by the Pal-TRE (lane 12). The reverse is also true for the labelled Pal-TRE (compare lane 10 with lane 13). From these experiments we conclude that thyroid hormone receptor is present both in myoblasts and myotubes. It is probably complexed with other muscle cell proteins, since the size of the major bands are not the same with COS cell extracts. The same protein complexes are formed with the putative αsk-TRE as shown by their size and by the competition experiments. It is clear that the Pal-TRE binds more strongly, but it is also clear that the intronic sequence in the mouse α-skeletal actin gene is a thyroid hormone binding element and hence a potential target sequence for the effects of the hormone on this gene.

Discussion

We show here that thyroid hormone affects α-actin levels during the development of skeletal muscle. In the gastroc-

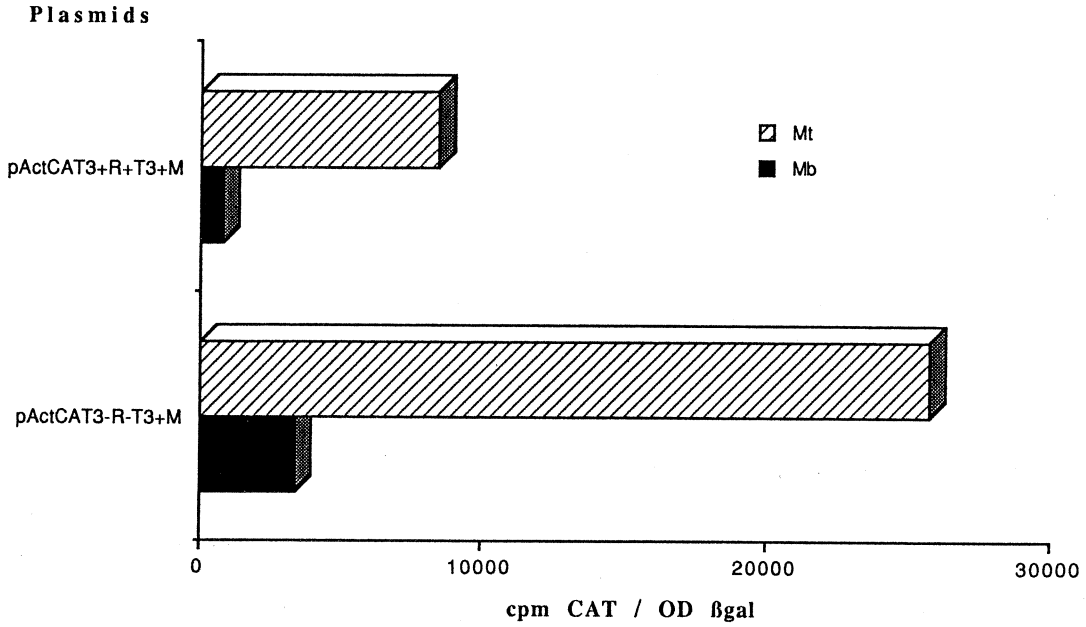

Figure 3. The effects of thyroid hormone receptor on MyoD stimulation of the activity of a skeletal actin-CAT reporter gene, transfected onto muscle cells. The averaged results of several transfection experiments in myoblast and myotubes of the C2 muscle cell line are shown after co-transfection of the CAT reporter gene under control of regulatory sequences from the α-skeletal gene (pActCAT3) with a MyoD expression vector (M) and with or without the expression vector for thyroid hormone receptor (R) and added thyroid hormone (T3). Results are expressed as radioactive butyryl chloramphenicol converted by the CAT enzyme activity, normalized to ß-galactosidase activity in the same sample. All vectors were co-transfected with an RSV-ßGalactosidase plasmid in order to standardize for transfection efficiency.

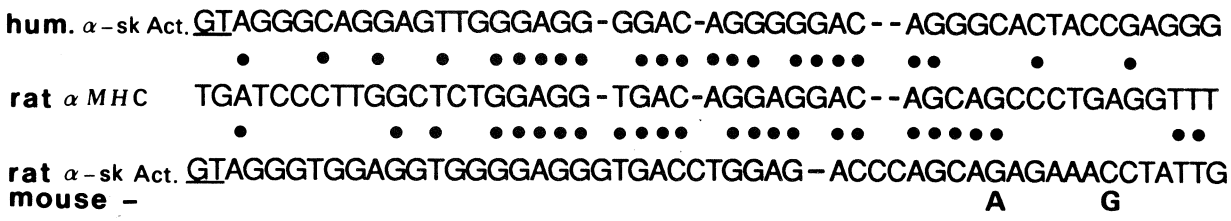

Figure 4. A putative thyroid hormone receptor binding site in the first intron of the α-skeletal actin gene. The region of the rat cardiac α MHC gene promoter (21 containing the 13 nucleotide core sequence element required for thyroid hormone receptor binding [19] (5'-GGAGGTGACAGGA-3', located at positions -132 to -144 of the anti-sense strand) is compared with a homologous sequence located in the first intron of the α-skeletal actin gene which shows 17/19 matched bases (indicated by black dots) in the case of the human sequence [35], 20/26 with the rat [41] and 19/25 with the mouse gene [1]. The homology in both cases extends 3' of the published consensus [19]. The dinucleotide GT at the beginning of the intron is underlined. (-) introduces a space to optimize the homology.

nemius muscle of the rat, transcripts of the α-cardiac actin gene are undetectable by 20 days after birth whereas this is not the case until 30 days in the hypothyroid animals where the postnatal reduction of this mRNA is retarded. A similar delay in a postnatal developmental transition has been reported for the myosin heavy chains where hypothyroidism retards the appearance of adult MHC isoforms and the disappearance of the neonatal MHC isoform [14, 38, 9]. Maturation of the skeletal muscle phenotype also depends on innervation. In the case of the α-actins, the reduction in α-cardiac actin mRNA is not observed in the denervated pectoralis muscle of chickens after hatching [34]. In contrast to the situation with thyroid hormone, denervation appears to block, rather than delay, the developmental progression towards the adult phenotype for the actins. Myosin heavy chain transitions, on the other hand are perturbed, but the appearance of the adult phenotype is not blocked [14, 4].

A second effect of hypothyroidism on α-skeletal actin transcripts is observed after longer periods in our experiments. While normal and hypothyroid animals show a similar increase in levels of α-skeletal actin mRNA over the first time points, at 30 days the hypothyroid muscle contains more than twice the quantity seen in control muscles. At this stage the control muscle has attained an adult contractile protein phenotype. The effects of thyroid hormone injection on adult skeletal muscle have been documented, particularly where the myosins are concerned (e.g. 18). In adult rat skeletal muscle, no effect on α-actin mRNA levels was detected. In adult cardiac muscle on the other hand, Gustafson, et al. [17] report a transitory increase in α-cardiac actin mRNA; a rapid transitory increase in α-skeletal actin mRNA has also been reported [39]. This transitory increase resembles that seen during the initial stages of cardiac hypertrophy [33]. In adult heart the effect of thyroid hormone on α and β myosin heavy chain gene expression is well documented [21, 17, 13]. Expression of the cardiac α-myosin heavy chain gene in the heart, for example, is induced by thyroid hormone and this has been shown to be due to a direct transcriptional effect mediated by the thyroid hormone receptor interacting with a 5' flanking sequence of this gene [19]. It is less clear whether thyroid hormone has a direct effect on the transcription of myosin heavy chain genes in skeletal muscle. As far as the actin genes are concerned, most studies, like that reported here, concern mRNA accumulation. It seems likely that protein levels of the two α-actins follow what is observed at the mRNA level [37]. Estimations of transcriptional rates, based on nuclear run on experiments, in cell culture and during skeletal muscle development *in vivo* suggest that the initial level of the α-actin mRNAs is transcriptionally regulated, but that post-transcriptional modulation, probably via mRNA stability, has a considerable effect on the final level of accumulated mRNA, particularly where the major α-skeletal actin transcript is concerned.

As far as transcriptional regulation of these α-actin genes is concerned, there is no evident thyroid hormone receptor binding site on the sense or anti-sense strand in the proximal promoter (from +1 to -700) of these genes [15, 1] although there are several regions with some homology to the TRE consensus. Collie and Muscat [8] have shown that a sequence present between -173 and -149 in the promoter of the human skeletal actin gene can act as a TRE. In both human and rodent striated α-actin genes, potential TREs are present in the introns. There is a sequence in the first intron of the rat [41], mouse [1] and human [35] α-skeletal actin genes which shows high sequence homology to the motif defined in the cardiac α-MHC [19, 22]. The α-cardiac actin gene also has a less well conserved consensus sequence within the second intron, although direct stimulation of the transcriptional activity of this gene has yet to be demonstrated. We show here that the rodent (mouse) α-skeletal actin gene like that of the human [8] responds in transfection assays to thyroid hormone receptor stimulation in the presence of T3. We also show that the intronic sequence behaves like a TRE in gel mobility shift assays, in that it forms complexes with nuclear proteins in muscle cells of similar size to those formed with the standard TRE, and that these complexes are specifically competed by the TRE. In order to demonstrate the precise role of this and of other putative TREs in the promoter region, to the

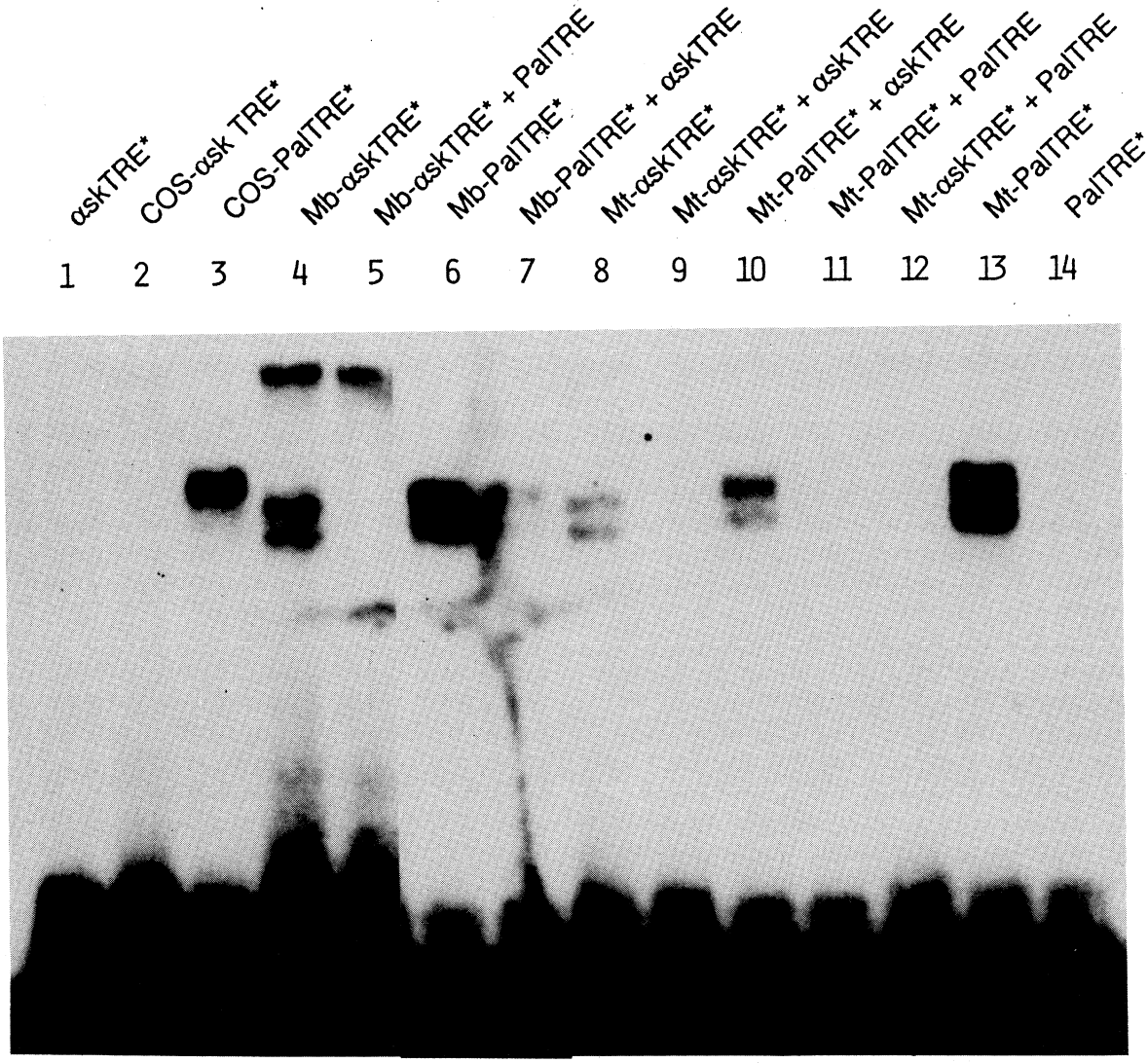

Figure 5 : Electrophoretic mobility shift analysis of the putative thyroid hormone receptor binding element in the first intron of the mouse skeletal α-actin gene.
Extracts from COS cells (COS) and from myoblasts (Mb) and myotubes (Mt) of the mouse muscle C2 cell line were incubated with radioactive (*) oligonucleotides corresponding to the consensus binding element (Pal-TRE) and to the element in the α-skeletal actin gene (αsk-TRE), and the complexes formed analysed on gels, and visualized by autoradiography. In competition experiments, excess unlabelled probe was added (lanes 5, 7, 9, 10, 11, 12).

response of the rodent α-skeletal actin gene to thyroid hormone, site directed mutagenesis, followed by functional transfection assays will be necessary. The mouse α-skeletal actin promoter contains a number of E boxes, including tandem MyoD consensus sites at -630 and -650 [1], so that it was not surprising to see transactivation of this promoter by MyoD. Carnac et al. (1992) have shown that addition of T3 to C2 cells grown in thyroid hormone depleted medium increases MyoD protein level by two-fold with consequent stimulation of differentiation. However it was surprising that co-transfection with thyroid hormone receptor, in the presence or absence of T3 considerably reduced this effect. It is unlikely that this phenomenon is simply due to competition between the promoters of the expression vectors for common transcriptional factors, since the RSV-lacZ plasmid co-transfected in all these assays as an indicator of transfection efficiency showed no diminution in expression. It also seems unlikely that there is direct competition for binding to overlapping TRE and MyoD sites, since the best consensus MyoD sites

and TREs examined are not adjacent. We would suggest tentatively that there may be interference between MyoD and thyroid hormone receptor under some conditions. In these experiments firstly excess receptor was produced by an expression vector, and secondly it was the β1 form not the α1 form which has been reported in C2 cells [6]. Cross-talk between different transcriptional regulatory circuits has already been demonstrated and MyoD/AP1 interaction [2] provides an example. This preliminary observation on MyoD and the thyroid hormone receptor may point to a phenomenon of this kind and merits further investigation.

Acknowledgements

This work was supported by grants from the Pasteur Institute, the CNRS, the AFM and the MDA. During this work I. Garner and G. Lyons were recipients of fellowships from the AFM. We thank David Moore for the gift of the clone CDM 62 and Bernd Kirschbaum for help with gel shift assays.

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