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Thyroid hormone regulates expression of a transfected human α-myosin heavy-chain fusion gene in fetal rat heart cells

(chloramphenicol acetyltransferase assay/DNA transfection)

RICHARD W. TSIKA*, JOSEPH J. BAHL*, LESLIE A. LEINWAND†, AND EUGENE MORKIN*

†Department of Microbiology, Albert Einstein College of Medicine, Bronx, NY 10461; and *University Heart Center, University of Arizona College of Medicine, Tucson, AZ 85724

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ABSTRACT **The rat α-myosin heavy-chain (α-MHC) gene is regulated by 3,5,3′-triiodo-L-thyronine (T_3) in ventricular myocardium and is constitutively expressed in atrial tissue. Less is known about regulation of the human gene, but conservation of sequences in the 5′-flanking region between the rat and human α-MHC genes suggests that the human gene may be regulated similarly. Accordingly, T_3-responsiveness and tissue-specific expression of human and rat α-MHC/chloramphenicol acetyltransferase fusion constructs have been compared in rat fetal heart cells, L_6E_9 myoblasts and myotubes, 3T3 fibroblasts, and HeLa cells. Transient transfection assays revealed a complex series of cis-regulatory elements in the 5′-flanking sequences in the human genes, including a basal promoter element with canonical TATAA and CAAT sequences, two positive regulatory element(s), and two negative regulatory elements, which markedly diminished both constitutive and T_3-inducible activity. Interestingly, the human gene seemed to contain a proximal thyroid-hormone response element(s) not found in the rat gene. In L_6E_9 myoblasts and myotubes, the human constructs were constitutively expressed but not T_3-regulated; none of the constructs were active in 3T3 or HeLa cells. We propose that interactions among the thyroid hormone responsive elements and other cis-acting elements in the human α-MHC 5′-flanking sequences may be sufficient to explain the characteristic features of expression of this gene in cardiac tissues.**

The cardiac myosin heavy chain (MHC) isoforms are the products of two highly homologous genes, α and β, which are located in tandem and separated by 4.0 kilobases (kb) of intergenic sequence (1, 2). These genes have distinct patterns of developmental and tissue-specific expression and are subject to modulation by alterations in cardiac workload and hormone status (3–5). Of these multiple regulatory influences, the effects of thyroid hormone are the strongest and best studied (6, 7). In the ventricular myocardium of rats and rabbits, thyroid hormone causes accumulation of α-MHC mRNA and inhibits expression of β-MHC mRNA. It has been proposed that these variations in cardiac myosin isoenzyme may be physiologically important because of a direct relationship between isoform type and the intrinsic speed of muscle contraction—higher speeds of contraction being associated with a predominance of the V_1 form (4).

Recently, human α- and β-MHC mRNA sequences and their corresponding genes have been isolated and partially characterized (1, 8, 9). The 5′-flanking regions of the rat and human cardiac α-MHC genes show remarkable homology for ≈600 nucleotides upstream of the putative transcription initiation site, whereas there is little or no homology for the 1000 nucleotides in the 3′ direction of the first intron (8). There is, however, very little information available yet about regulation of the human gene. Evidence to date suggests that, like the rat and rabbit genes, the human α-MHC gene is constitutively expressed in atrium (10). Some ventricular fibers also express the α-MHC gene (11), but the extent to which the human α-MHC gene is T_3-dependent is not yet established.

The effect of thyroid hormone on expression of the α- and β-MHC genes is thought to be mediated by binding to high-affinity nuclear receptors, which recently have been identified as the products of the protooncogene, c-*erb*-A (12, 13). Direct interaction of T_3 receptors with a thyroid hormone responsive element (TRE) in the 5′-flanking sequences of the rat growth hormone gene (14) and with a similar sequence in the rat α-MHC gene (15) has been demonstrated.

In the present study, we have investigated the DNA sequence requirements for expression of human chimeric α-MHC/chloramphenicol acetyltransferase (CAT) genes containing 5′-flanking sequence. A series of important regulatory elements have been defined, which in combination may be sufficient to explain constitutive expression of the gene in atrium and T_3-dependent regulation in ventricular myocardium.

METHODS

Plasmid Construction. The human recombinant plasmid pHαMCAT-913 was constructed from a 1028-base-pair (bp) *Hin*dIII–*Stu* I fragment of the human genomic clone p8-1A (1) and subcloned into the unique *Hin*dIII site at the 5′ end of the CAT coding sequences of the transfection vector pSVOCAT. This clone contained 913 bp of 5′-flanking sequence and 115 bp of 3′ sequence to the transcriptional initiation site. The pSVOCAT plasmid contains the entire coding sequence of the prokaryotic CAT gene minus its promoter (16). Human α-MHC promoter deletion mutations were created by selecting unique restriction sites within the 5′ region of pHαMHC-913. Deletions at −754, −572, −503, −371, −303, −199, −159, −120, and −78 were made by digestion at *Bal* I, *Bst*XI, *Sca* I, *Bst*EII, *Nco* I, *Bgl* I, *Apa* I, *Dpn* I, and *Bsp*MII sites, respectively. Rat recombinant plasmids were constructed in pSVOCAT (16); the starting plasmid contained a 3.3-kb *Hin*dIII fragment of λαMHC18 (17) with 2.9 kb of 5′-flanking DNA as well as 421 bp of DNA 3′ to the transcription initiation site. Deletions at −857, −612, −540, −374, −201, −190, −162, −153, −126, and −86 were made by digestion at *Pvu* II, *Eco*RI, *Tht*111I, *Bst*EII, *Bgl* I, *Esp* I, *Mst* II, *Hph* I, *Dpn* I, and *Ava* II sites, respectively. The deletions were

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Abbreviations: MHC, myosin heavy chain; T_3, 3,5,3′-triiodo-L-thyronine; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40; TRE, thyroid hormone responsive element.

verified by restriction analysis, and in some cases, by DNA sequencing. Plasmid DNA was double banded on cesium chloride gradients and analyzed by agarose gel electrophoresis to insure that >50% of the DNA was in the supercoil form. Phage and plasmid DNA were isolated and subcloned by using standard recombinant DNA procedures (18).

Cell Culture Procedure and Transfection. Primary myocytes were cultured from 18-day (gestational age) fetal rat hearts. After differential plating to eliminate nonmuscle cells, myocytes were plated at a density of 6×10^6 cells per 100-mm dish on culture dishes coated with collagen and fibronectin. Ham's F-12 medium containing bovine serum albumin at 10 mg/ml, fetuin at 0.25 mg/ml, ascorbic acid at 20 μg/ml, epidermal growth factor at 10 ng/ml, penicillin at 100 units/ml, streptomycin at 100 μg/ml, and the mixture insulin (5 μg/ml)/transferrin (5 μg/ml)/selenium (5 ng/ml). Recombinant plasmid DNA (10 μg plus 15 μg of carrier DNA and 2.5 μg of pRSV-βgal) was introduced into myocytes by electroporation (19). This method resulted in 50% cell death, and ≈20% of the surviving cells were successfully transfected. All other cell types (L_6E_9 myoblasts and myotubes; 3T3 L1 and HeLa S3 cells) were transfected using the calcium phosphate precipitation method (20). The medium was changed after 24 hr, and 10 nM 3,5,3′-triiodo-L-thyronine (T_3) or diluent was added to the cultures. After 48 hr, cell extracts were prepared and assayed for CAT activity as described by Gorman *et al.* (16). Protein concentrations of the extracts were determined by the method of Bradford (21). CAT assays were performed as described by Gorman *et al.* (16) by using a constant amount of protein for each assay. The plasmid pRSV-βgal was used in transfection experiments as a control for transfection efficiency. β-Galactosidase activity was measured as described by Carroll *et al.* (22). Transfections in primary cardiocytes were repeated 5 to 6 times for each construct and 2 to 3 times for all other cell types with different batches of cells.

L_6E_9 myoblasts and myotube cells were cultured by the procedures of Nadal-Ginard (23) in medium from which T_3 had been removed by the method of Samuels *et al.* (24). Myoblasts were transfected at a relatively low plating density that reached confluence only on the last day of the experiment. No myotubes were detected with light microscopy. Myotubes were transfected as confluent sheets of myoblasts. By the end of the experiment myotube formation was readily observed. 3T3 L1 cells were incubated in the serum-free Ham's F-12 medium. HeLa S3 cells were cultured according to procedures described by American Type Culture Collection.

RESULTS

The role of 5′ cis-acting regulatory sequences in mediating the induction of the human and rat α-MHC genes by thyroid hormone was studied in fetal rat heart cells by transient expression of a series of chimeric plasmids that contained 5′-flanking sequences of α-MHC DNA linked to the CAT reporter gene. The proximal 600 bp of sequence in these clones have a high degree of homology (1, 8).

Time Course of Induction and Dose–Response Characteristics. The time course of response of the human and rat α-MHC fusion genes to T_3 was determined by transfection of ventricular cardiomyocytes with plasmids pHαMHC-503 and pRαMHC-540 that contained 5′-flanking sequences from the human and rat genes, respectively. Culture plates were analyzed for CAT activity at designated time points after the addition of T_3 (Fig. 1 *Left*). In earlier experiments (17), the time courses of thyroid hormone induction for a rat α-MHC chimeric plasmid and the endogenous α-MHC gene were found to be virtually identical, suggesting that they are regulated by similar factors. In the present experiments, the time course of induction of the rat and human fusion genes also was essentially the same, indicating that the human 5′-flanking sequences are sufficient to confer T_3 regulation in rat ventricular cardiomyocytes. However, dose–response data (Fig. 1 *Right*) indicated the human construct is more responsive to T_3.

Deletion Analysis of Human Constructs. Functional elements in the human α-MHC promoter region were located by construction of a series of deletions in the 5′-flanking sequences of pHαMHC-913. After transfection into ventricular cardiomyocytes, the resulting recombinants were analyzed for CAT activity in the presence and absence of T_3 (Fig. 2). Deletion of sequences upstream from position −78 resulted in a marked loss of overall activity, as might be anticipated, because this construct extends only 10 bp 5′ upstream from canonical CAAT and TATAA sequences. Nevertheless, the chimeric plasmid exhibited measurable constitutive activity and consistently increased in activity about two times upon

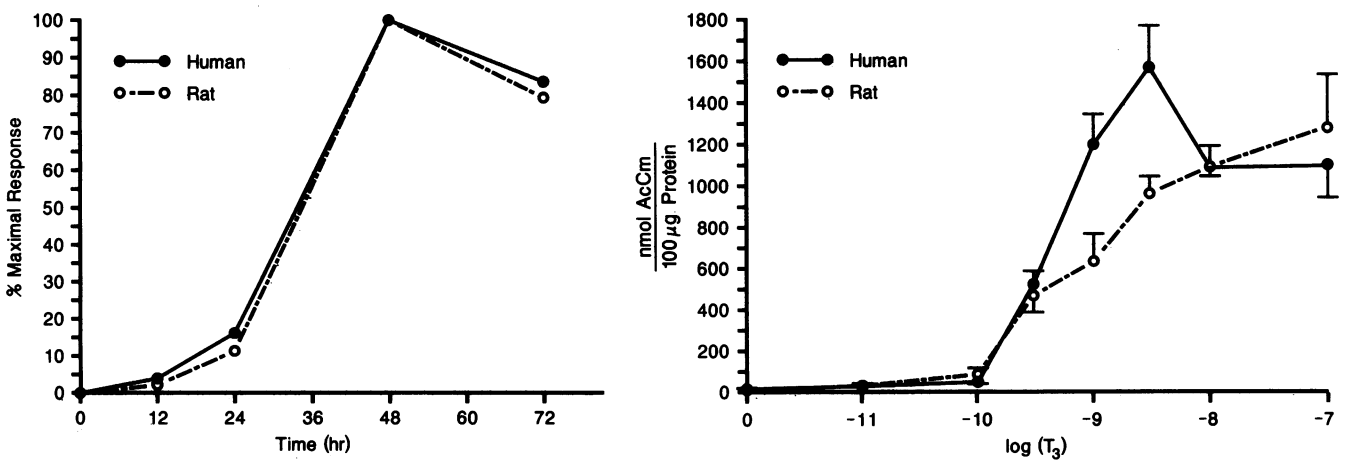

FIG. 1. Comparison of time course (*Left*) and dose–response (*Right*) curves for pRαMHC-540 and pHαMHC-503 in primary cultures of rat fetal cardiomyocytes. (*Left*) At the times indicated after T_3 addition (final concentration, 10 nM), cells from duplicate plates were analyzed for conversion of [^{14}C]chloramphenicol (Cm) to its acetylated derivatives (AcCm). Data are expressed as percent maximum activity. Zero time point refers to values obtained from untreated plates (≈14 nmol of AcCm/100 μg of protein). Maximum values at 48 hr were 800 and 1100 nmol of AcCm/100 μg of protein for rat and human constructs, respectively. (*Right*) Cardiocyte cultures were treated for 48 hr with the indicated T_3 concentrations and assayed for CAT activity. Zero dose refers to values from plates to which only diluent was added. Each point represents the mean ± SE of three experiments. Cotransfection with pRSV-βgal was used as control for transfection efficiency.

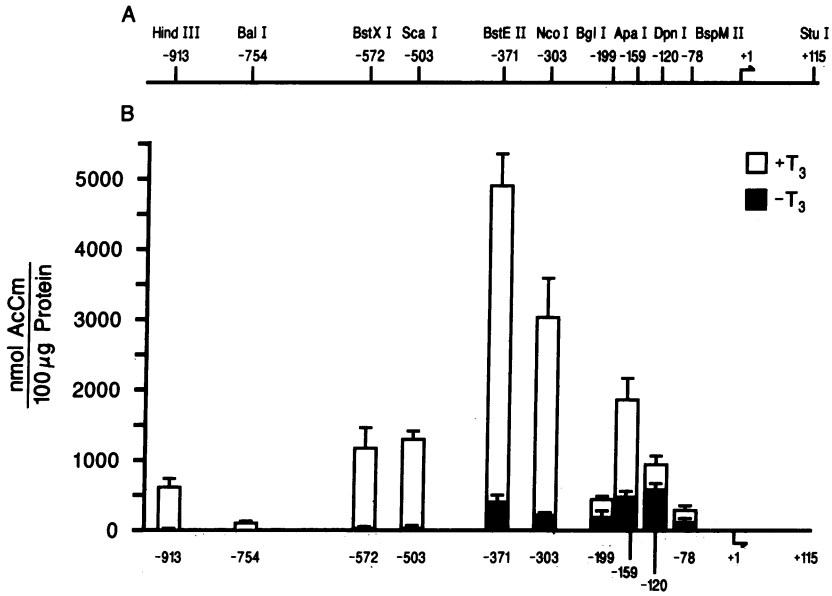

FIG. 2. Analysis of activity of the human α-MHC/CAT recombinant plasmids after transfection into primary cultures of cardiac myocytes. (*A*) Restriction sites used in construction of recombinants. All constructs contained a common 3′ *Stu* I site located 115 bp in the 3′ direction downstream from the transcriptional start site and differed only in the length of their 5′ upstream sequences. (*B*) Transient expression assays were done as described. Values represent mean ± SE for five or six experiments. Cotransfection with pRSV-βgal was used as a control for transfection efficiency.

T_3 addition. Chimeric plasmids pHαMHC-120 and pHαMHC-159 showed higher levels of both constitutive expression and T_3-inducible activity. Surprisingly, clone pHαMHC-199 which is only 40 bp longer than pHαMHC-159 showed a dramatic reduction in T_3 inducibility. The rise and fall in T_3-inducible CAT activity by the more proximal constructs was repeated by plasmids pHαMHC-371, pHαMHC-503, and pHαMHC-754. Constructs pHαMHC-371 and pHαMHC-503 demonstrated high T_3 inducibility, pHαMHC-371 having the highest T_3 activity of all the human constructs. The longest human construct examined, plasmid pHαMHC-913, also showed a marked increase in activity upon T_3 addition.

It should be noted that the constitutive expression of this series of deletion constructs varied much less than the degree of T_3 inducibility. In experiments in which pRSV-βgal was cotransfected, correction for variations in transfection efficiency resulted in minor quantitative changes, but the overall pattern of activity was the same.

We interpret these data to suggest that the human α-MHC 5′-flanking sequences contain four types of cis-acting elements: (*i*) a basal promoter element represented by pHαMHC-78; (*ii*) positive regulatory elements located approximately from positions −78 to −159, −199 to −572, and −754 to −913; (*iii*) negative regulatory regions from −159 to −199 and −572 to −754; and (*iv*) a minimum of two TREs located 3′ downstream from position −159.

Transient expression of human α-MHC/CAT fusion constructs also was examined in L_6E_9, a permanent skeletal muscle cell line (Fig. 3). The series of human deletion constructs were expressed surprisingly well in both L_6E_9 myoblasts and myotubes, but the activity was not increased by addition of T_3 to the medium. Although the pattern of activity of the clones was qualitatively similar to that seen in primary cardiocytes, the CAT activity expressed either per μg of protein or per unit of β-galactosidase activity was always less than levels at which these constructs were expressed constitutively in primary cardiomyocytes (Fig. 3). This result suggests that the transcriptional factors required for T_3 inducibility but not those required for activation of the upstream positive and negative elements are tissue specific.

In this regard, it is worth noting that the sequence (5′-TTCCCGTTTAGTCCCC-3′) located at positions −177 to −161 in the human α-MHC gene, is similar (15/17 bp) to an element found in the genes encoding the muscle form of mouse creatine kinase, rat and chicken α-actins, and rat myosin α-MHC (26). In the rat gene, this muscle-specific element has been reported to restrict expression to muscle cells (25). This was not the case in the present experiments with the human gene. Deletion of this element as, for exam-

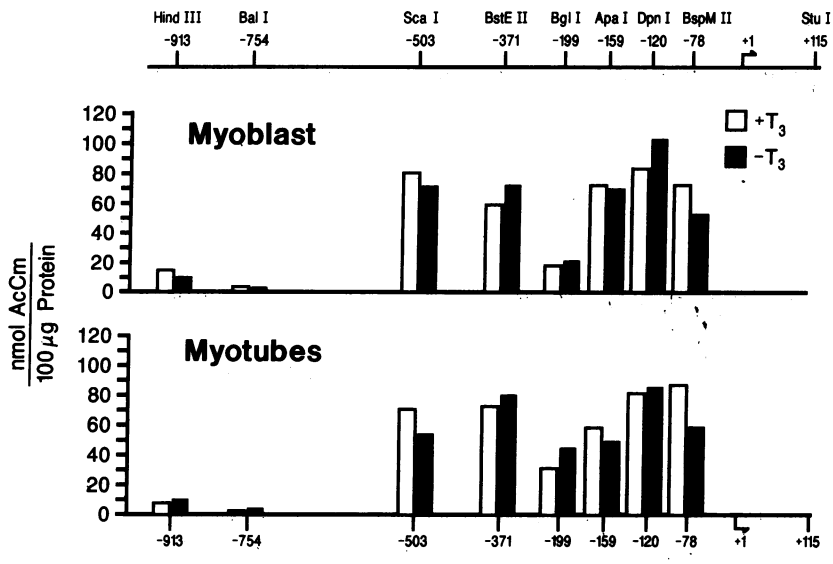

FIG. 3. Analysis of CAT activity of human α-MHC/CAT recombinant plasmids after transfection into L_6E_9 myoblasts and myotubes. Approximately 48 hr after T_3 addition (final concentration, of 10 nM), cells from duplicate plates were analyzed for CAT activity. Cotransfection with pRSV-βgal was used as control for transfection efficiency. Data are expressed as the average of duplicate plates from two experiments.

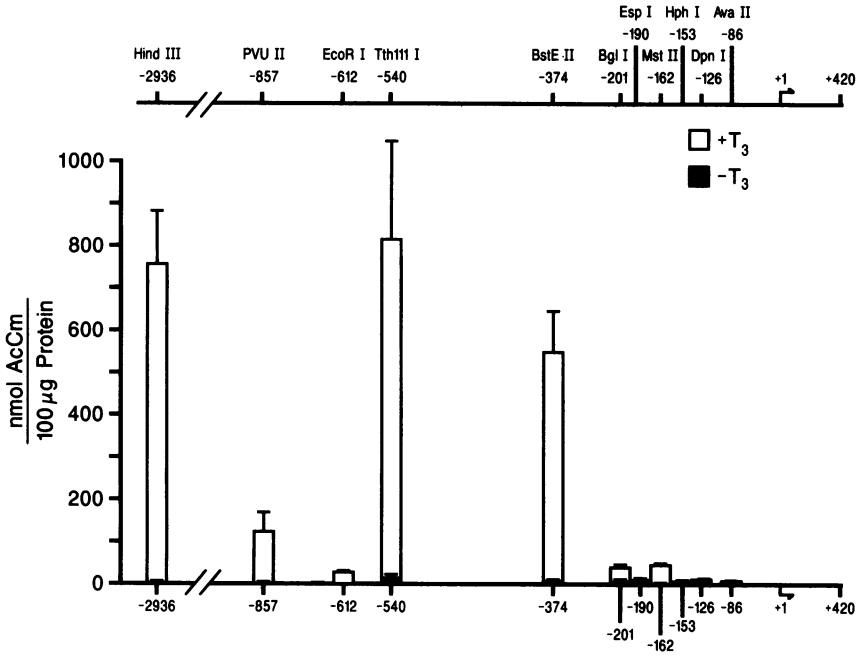

FIG. 4. Activity of rat α-MHC recombinants containing deletions in the 5'-flanking sequences after transfection into primary cardiomyocytes. (*Upper*) Restriction sites used in construction of recombinant plasmids. All constructs contained a common 3' *Hin*dIII site located +421 bp 3' downstream from the cap site and differed only in length of their 5' upstream sequences. (*Lower*) Transient expression analysis was done as described. Cotransfection with pRSV-βgal was used as control for transfection efficiency. Values represent mean ± SE for five or six experiments.

ple, in pHαMHC-159 did not permit the clone to be expressed in either fibroblasts or HeLa cells.

Deletion Analysis of Rat Constructs. Important cis-acting regulatory elements in the 5'-flanking sequences of the rat α-MHC gene were demonstrated by transient transfection assay of a series of fusion constructs in cardiomyocytes (Fig. 4). The rat clones had less constitutive expression than the human constructs, but exhibited a similar rise and fall in T_3-inducible activity, suggesting that the 5'-flanking sequences of the rat gene also contain a series of positive and negative elements. Interestingly, the activity of pRαMHC-153, which ends 5' upstream from the TRE described by Izumo and Mahdavi (15), was not T_3-inducible, whereas pRαMHC-162 exhibited a 14-fold induction in plates treated with the hormone. Apparently, the sequences critical to T_3 receptor binding are located more distally than initially indicated. Also, the activity of the rat gene fusion constructs, pRαMHC-86 and pRαMHC-126, which terminate 3' downstream from the putative TRE, were not stimulated by addition of T_3 to the medium. The latter finding contrasts with results obtained with the human chimeric plasmids from the same region, pHαMHC-78 and pHαMHC-120, which exhibited ≈2-fold stimulation of activity by T_3.

The rat α-MHC/CAT constructs had low levels of expression in L_6E_9 myoblasts and myotubes but, like the human constructs, they were not regulated by T_3 (Fig. 5). The rat α-MHC clones also were not expressed in either 3T3 fibroblast or HeLa cells.

DISCUSSION

In the present study, we have used chimeric plasmids containing varying lengths of the 5'-flanking region of the human α-MHC fused to the CAT reporter gene to locate important regulatory sequences. Activation of the human chimeric plasmids with T_3 was compared with chimeric plasmids containing 5'-flanking sequences of the rat α-MHC gene. Overall, there were similarities in the time course of induction of the human and rat genes as well as in the general pattern of positive and negative regulatory elements in their promoter regions. However, the human gene was more sensitive to the effects of T_3, possibly because it contains an additional proximal TRE with a 3' boundary extending into the basal promoter area. These observations indicate that the unique features of the cis-acting elements of the human α-MHC gene can be observed within the intracellular milieu of the rat heart cell.

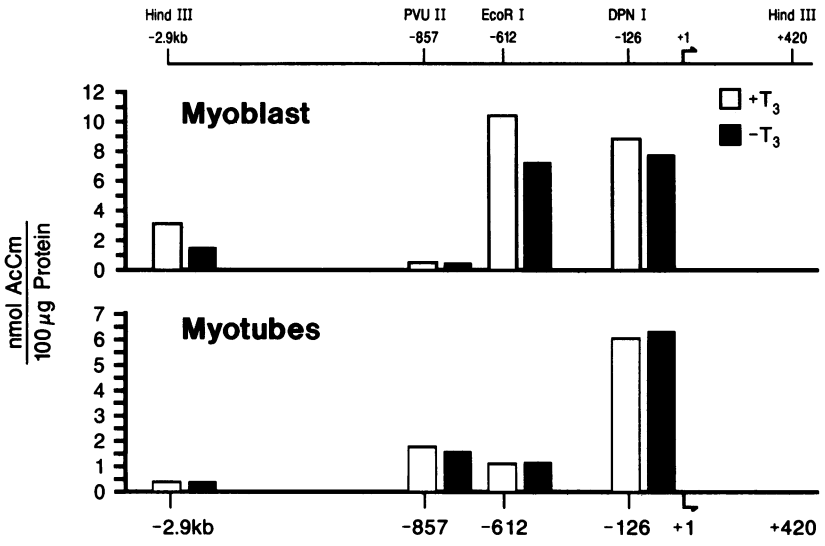

FIG. 5. Analysis of CAT activity of rat α-MHC deletion mutants after transfection into L_6E_9 myotubes and myoblast. Approximately 48 hr after T_3 addition (final concentration, 10 nM), cells from duplicate plates were analyzed for CAT activity. Data are expressed as average of duplicate plates from two experiments. Cotransfection with pRSV-βgal was used as control for transfection efficiency.

Recent experiments with the rat growth hormone (14) and α-MHC genes (15, 17, 25) have indicated that sequences within the 5′-flanking region are sufficient for induction by T_3. Results obtained with deletion analysis of the rat α-MHC are consistent with the presence of a T_3-receptor binding sequence (14, 15), 5′-GGAGGTGACAGGA-3′, within clone pRαMHC-162. (According to our data this sequence is located at positions −140/−153.) An identical sequence was found in the human α-MHC gene from positions −151 to −138, which probably explains the strong T_3-inducible activity of plasmid pHαMHC-159 (Fig. 2). Interestingly, the two shortest human constructs, pHαMHC-78 and pHαMHC-120, exhibited T_3 sensitivity, suggesting the existence of at least one additional TRE in the human gene. In contrast, rat deletion constructs from the same region, pRαMHC-86 and pRαMHC-126, were not T_3 responsive. The presence of multiple functional TREs within a single promoter is unusual but has been described recently for the rat growth hormone gene (27). The location of the additional putative TRE(s) in the human gene is uncertain because the more proximal sequences (−120 to +115) in our constructs do not contain elements with extensive homology to the rat α-MHC or growth hormone TREs.

These results are consistent with a minimum model for T_3 regulation of gene expression proposed for the growth hormone gene (14), which consists of a basal promoter followed by a TRE and nearby upstream elements that act to amplify the activity induced by the hormone. The human and rat α-MHC genes are more complex, however, with a pattern of positive and negative cis-acting elements extending over a distance of at least 1 kb. Although the upstream elements have not been characterized in detail, they have been found to be inactive when fused to a heterologous promoter (28), supporting the concept that induction of activity by T_3 requires cooperative interactions between the upstream elements and the more proximally located T_3-receptor binding sequence(s).

The significance of the negative regulatory regions found in both the human and rat 5′-flanking sequences is unclear. The negative regions in both genes are flanked by strong positive elements. Possibly, the negative elements may serve to control the number of positive elements engaged in stimulation of transcriptional activity. This arrangement may represent a duplication of regulatory elements that are selectively expressed either during development or in atrial and ventricular tissues.

Finally, the complex array of cis-acting elements found in the 5′-flanking sequences may be sufficient to explain the characteristic pattern of expression of the α-MHC gene *in vivo*. Thus, the proximal elements that are active in the absence of T_3 could be responsible for the constitutive expression of the α-MHC gene in atrial tissue (10). These elements may overlap with one or more TREs in the proximal area to induce expression of the gene in ventricular tissue when stimulated by T_3 (5, 6). One or more positive elements in the sequences 5′ upstream from the TRE(s) clearly are needed for maximum expression of activity. The role of the two negative elements in the 5′ upstream sequences is uncertain, but they may modulate the activity of the adjacent positive elements. Quantitative differences between rat and human in the level of expression of the gene in the ventricular tissue (10, 11) may be the result of differences in trans-acting factors.

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1. Saez, L. J., Gianola, K. M., McNally, E. M., Feghali, R., Eddy, R., Shows, T. B. & Leinwand, L. A. (1987) *Nucleic Acids Res.* **15,** 5443–5459.
2. Mahdavi, V., Chambers, A. O. & Nadal-Ginard, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2626–2630.
3. Lompre, A.-M., Nadal-Ginard, B. & Mahdavi, V. (1984) *J. Biol. Chem.* **259,** 6437–6446.
4. Swynghedauw, B. (1986) *Physiol. Rev.* **66,** 710–771.
5. Izumo, S., Lompre, A.-M., Matsuoka, R., Koren, G., Schwartz, K., Nadal-Ginard, B. & Mahdavi, V. (1987) *J. Clin. Invest.* **79,** 970–977.
6. Izumo, S., Nadal-Ginard, B. & Mahdavi, V. (1986) *Science* **231,** 597–600.
7. Gustafson, T. A., Markham, B. E. & Morkin, E. (1986) *Circ. Res.* **59,** 194–201.
8. Yamauchi-Takihara, K., Sole, M. J., Liew, J., Ing, D. & Liew, C.-C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3504–3508.
9. Jandreski, M. A. & Liew, C.-C. (1987) *Hum. Genet.* **76,** 47–53.
10. Kurabayashi, M., Tusuchimochi, I. K., Takaku, F. & Yazaki, Y. (1988) *J. Clin. Invest.* **82,** 524–531.
11. Bouvagnet, P., Leger, J., Pons, F., Dechesne, C. & Leger, J. J. (1984) *Circ. Res.* **55,** 794–804.
12. Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysadel, J., Leutz, A., Beug, H. & Vennstroem, B. (1986) *Nature (London)* **324,** 635–640.
13. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M. (1986) *Nature (London)* **324,** 641–646.
14. Glass, G. K., Franco, R., Weinberger, C., Albert, V. R., Evans, R. M. & Rosenfeld, M. G. (1987) *Nature (London)* **329,** 738–741.
15. Izumo, S. & Mahdavi, V. (1988) *Nature (London)* **334,** 539–542.
16. Gorman, C. M., Moffat, L. F. & Howard, B. M. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
17. Gustafson, T. A., Markham, B. E., Bahl, J. J. & Morkin, E. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3122–3126.
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 189–195.
19. Chu, G., Hayakawa, H. & Berg, P. (1987) *Nucleic Acids Res.* **15,** 1311–1326.
20. Graham, F. & van der Eb, A. (1973) *Virology* **52,** 456–467.
21. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
22. Carroll, S. L., Bergsma, D. J. & Schwartz, R. J. (1988) *Mol. Cell. Biol.* **8,** 241–250.
23. Nadal-Ginard, B. (1978) *Cell* **15,** 855–864.
24. Samuels, H. H., Stawley, F. & Casanova, J. (1979) *Endocrinology* **105,** 80–105.
25. Mahdavi, V., Koren, G., Michaud, S., Pinset, C. & Izumo, S. (1989) in *Cellular and Molecular Biology of Muscle Development*, UCLA Symposia on Molecular and Cellular Biology, New Series, eds. Kedes, L. H. & Stockdale, F. E. (Liss, New York), Vol. 93, pp. 369–379.
26. Jaynes, J. B., Chamberlain, J. S., Buskin, J. N., Johnson, J. E. & Hauschka, S. D. (1986) *Mol. Cell. Biol.* **6,** 2855–2864.
27. Norman, M. F., Lavin, T. N., Baxter, J. D. & West, B. L. (1989) *J. Biol. Chem.* **264,** 12063–12073.
28. Morkin, E., Bahl, J. J. & Markham, B. E. (1989) in *Cellular and Molecular Biology of Muscle Development*, UCLA Symposia on Molecular and Cellular Biology, New Series, eds. Kedes, L. H. & Stockdale, F. E. (Liss, New York), Vol. 93, pp. 381–389.