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Reversible Epigenetic Modifications of the Two Cardiac Myosin Heavy Chain Genes During Changes in Expression

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The two genes of the cardiac myosin heavy chain (MHC) locus—alpha-MHC (aMHC) and beta-MHC (bMHC)—are reciprocally regulated in the mouse ventricle during development and in adult conditions such as hypothyroidism and pathological cardiac hypertrophy. Their expressions are under the control of thyroid hormone T3 levels. To gain insights into the epigenetic mechanisms that underlie this inducible and reversible switching of the aMHC and bMHC isoforms, we have investigated the histone modification patterns that occur over the two cardiac MHC promoters during T3-mediated reversible switching of gene expression. Mice fed a diet of propylthiouracil (PTU, an inhibitor of T3 synthesis) for 2 weeks dramatically reduce aMHC mRNA expression and increase bMHC mRNA levels to high levels, while a subsequent withdrawal of PTU diet for 2 weeks completely reverses the T3-mediated changes in MHC expression. Using hearts from mice treated in this way, we carried out chromatin immunoprecipitation-qPCR assays with antibodies against acetylated histone H3 (H3ac) and trimethylated histone (H3K4me3)—two well-documented markers of activation. Our results show that the reexpression of bMHC is associated at the bMHC promoter with increased H3ac but not H3K4me3. In contrast, the silencing of aMHC is associated at its promoter with decreased H3K4me3, but not decreased H3ac. The epigenetic changes at the two MHC promoters are completely reversed when the gene expression returns to initial levels. These data indicate that during reciprocal and inducible gene expression H3ac parallels bMHC isoform expression while H3K4me3 parallels expression of the tightly linked aMHC isoform.

Key words: Chromatin; Thyroid hormone; Heart; Histone; Acetylation; Methylation

INTRODUCTION

Myosin heavy chain (MHC) proteins are important enzymatic components of the thick filaments of the sarcomere—the fundamental unit of contraction in a muscle cell—and their intrinsic ATPase activity is responsible for transducing chemical energy into mechanical energy [reviewed in (31)]. The two MHC isoforms in the cardiac ventricle, alpha-MHC (aMHC) and beta-MHC (bMHC), are normally expressed in a developmental stage-specific manner in the mouse ventricle [reviewed in (32)]. bMHC is the main MHC isoform in the fetal ventricle. It is expressed from E8.5 until around birth, at which time its expression is reduced to very low levels. aMHC is the main isoform in the adult murine ventricle. Its expression is very low in the fetal ventricle and is increased around birth so that aMHC becomes the major isoform by neonatal day 8. During conditions of heart failure, hypothyroidism, and aging, expression of the two isoforms is reversed, so that bMHC is reexpressed in the cardiac myocytes while aMHC is downregulated [reviewed in (21)].

Expression of the two MHC genes is under the

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control of the thyroid hormone (T3), and the switch in expression from bMHC to aMHC that occurs around birth is due to a surge in T3 production. Decreasing the levels of T3 in the adult (e.g., by surgical thyroidectomy or chemical inhibition of T3 synthesis) reverses the expression levels of the two MHCs. T3 binds to thyroid hormone receptor (THR), a nonsteroidal nuclear hormone receptor [reviewed in (2)]. THR binds to its recognition sites, thyroid responsive elements (TRE), independently of the presence or absence of its ligand, T3. Whether the ligand(s) silences or activates the downstream gene depends on the type of TRE. Genes with positive TRE (pTRE) are activated by T3, while genes with negative TRE (nTRE) are silenced by T3. bMHC has a negative TRE at its promoter, and the presence of T3 consequently silences expression of bMHC. In contrast, aMHC has a positive TRE at its promoter, and presence of T3 activates expression while absence silences expression of aMHC.

The amino terminal tails of histones are the target of multiple posttranslational modifications: acetylation, methylation, phosphorylation, and ubiquitylation (15). During the past decade, the functional impact of covalent histone modifications on gene regulation has been unequivocally demonstrated (17,33). Multiple studies have revealed correlations between histone modifications and gene expression (1,9,20,30). The presence of acetylated lysine residues in histone H3 (H3ac) is associated only with active genes. Although histone methylation can be associated with either active or repressed genes, trimethylation of lysine 4 of histone 3 (H3K4me3) is only associated with active genes (1,3). Thus, H3ac and H3K4me3 are two posttranslational modifications whose presence or absence are important indicators of gene expression.

The MHC genes provide an excellent model system for studying gene regulation, because they are reciprocally regulated in a tissue and developmental stage specific manner. Furthermore, they are both controlled by the same hormone (T3) and can be induced to dramatically change their expression levels in a reversible manner in the adult heart. The two genes are close together in the genome, with bMHC being located 5′ to aMHC and separated by a 4.5-kb intergenic region, which includes the aMHC promoter. Several studies have uncovered important *cis*-acting regulatory elements and trans-acting factors that are responsible for their expression, such as NFAT, MEF2, MCAT for bMHC [reviewed in (21)] and Ku and Yin Yang for aMHC (19,26). Recent studies have also implicated antisense- and microRNA-mediated mechanisms as playing a role in the control of MHC gene expression during development and heart failure (6–8,10,11,28,29). However, the epigenetic control of the two MHCs genes has not been studied. Thus, it unclear what specific histone modifications are associated with the two MHC genes. It is also not clear whether the histone modifications are changed when expression of the genes is altered. Consequently, we have investigated the changes in two histone modifications of the two cardiac MHC genes when their expression is altered by changes in T3 levels. We show that H3K4me3 histone methylation accompanies the changes in aMHC expression while H3 histone acetylation accompanies the changes in bMHC expression when expression of the two genes is changed reversibly in either direction by the presence or absence of T3.

MATERIALS AND METHODS

Animals

Animals used in this study were 4-month-old C57blk6/J. Animals were fed an iodine-deficient diet containing 0.15% propylthiouracil (PTU) (Harlan Teklad, TD. 97061, Rx257896) for 2 weeks, and then either sacrificed (PTU group) or fed normal chow for another 2 weeks (reversal). Control mice were fed normal chow. The mice were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International according to Institutional Animal Care and Use Committee-approved guidelines.

Tissue and Chromatin Isolation

Hearts were surgically isolated and ventricles separated from atria. Ventricles were frozen in liquid nitrogen and pulverized using a mortar and pestle. The pulverized tissue was cross-linked by resuspending in 10 ml of warm 1% formaldehyde and rotated at room temperature for 10 min. Cross-linking was stopped by the addition of 1260 μl of 1 M glycine. Tissue was pelleted at $1000 \times g$ for 5 min at 4°C, and washed with 5 ml cold PBS. Tissue from a single heart was resuspended in 5 ml of Farnham lysis buffer (5 mM PIPES, 85 mM KCl, 0.5% NP-40) with freshly added protease inhibitors (Roche Diagnostics, Cat. #14696200) and incubated for 30 min on ice. The tissue was then dounce homogenized (10 strokes) and pelleted at $1000 \times g$ for 5 min at 4°C. The pellets were resuspended in SDS buffer (1% SDS, 10 mM EDTA, 50 mM Tris) at 30 mg of heart tissue/ml of SDS buffer and incubated for 5 min on ice and stored at −80°C.

Chromatin Immunoprecipitation (ChIP) Assay

Each ChIP assay was carried out on chromatin isolated from 3 mg equivalent of heart tissue. Sonication conditions were empirically determined (8×30-s pulses with 30 s cooling between pulses) using a Branson Sonicator at 30% power to give 200–800-bp fragments, with a peak at 500 bp. After sonication, chromatin was spun 14,000 rpm for 15 min at 4°C. Five percent of chromatin was removed (5% input) and the remaining chromatin submitted to immunoprecipitation procedure exactly as described previously (4). Ten micrograms of either pan-acetylated histone H3 (Millipore, Cat. #06-599) or H3K4me3 (abcam, Cat. #ab8898) antibodies were used for immunoprecipitation. Mock IPs were carried out in parallel but without using any antibody. Values for mock IPs were 5–10-fold lower than test IPs.

qPCR

Purified chromatin from each IP was resuspended in 25 μl of water, and qPCR carried out on 1 μl purified DNA using SYBR green and real-time PCR procedure. The qPCR data were calculated using ddCT method relative to the sample representing 5% input, which was tested simultaneously on the same plate. Fold enrichment relative to 100% input was calculated by dividing the fold enrichment (relative to 5%) input by 20. Six independent ChIPs (2 ChIPs/heart and 3 hearts/condition) were conducted for each condition (control, PTU, and reverse) for histone acetylation. H3k4me3 ChIP-qPCRs involved a total of seven independent ChIPs from four animals for each condition. Data are presented as mean ± SEM for each data point. The *p*-values were calculated using Student *t*-test between control and PTU groups (denoted by *) or between PTU and reversal groups (denoted by #). Primer sets were designed at the designated position on the bMHC and aMHC genes to give ~100–120-bp products. Each primer set was tested against genomic DNA for product specificity and purity. The primer sequences used are shown in Table 1. Gene expression for bMHC and aMHC was conducted as described previously (22,23).

RESULTS

PTU Treatment Changes the Expression of the Two MHC Genes in Reverse Direction

The expression of aMHC and bMHC in the mouse heart is dependent on the actions of thyroid hormone T3. PTU is an inhibitor of the terminal step of T3 synthesis, and has been previously demonstrated to switch the expression of aMHC and bMHC in the adult mouse heart (24). Accordingly, 4-month-old C57BLK6/J mice were fed an iodine-free diet containing 0.15% PTU for 2 weeks. To confirm that this PTU treatment changed the expression levels of the two MHC we conducted real time-qPCR on the heart tissue from control and PTU-treated mice. Figure 1 depicts the mRNA expression levels of aMHC (Fig. 1A) and bMHC (Fig. 1B) in the hearts of PTU-treated animals (closed bars) and untreated animals (open bars). As illustrated in the figure, PTU treatment has a dramatic effect on the expression levels of the two MHC: aMHC, which is typically expressed at high levels, is significantly reduced (approximately 300-fold relative to untreated animals, $p < 0.0001$); bMHC, which is typically expressed at low levels, is dramatically increased (approximately 50-fold relative to untreated animals, $p < 0.001$). Thus, the expression levels of the two MHC genes are dramatically altered in opposite directions, thereby allowing a close to ideal model system in which to study the changes in epigenetic modifications occurring at two genes of the same locus.

PTU Treatment Changes H3ac at bMHC But Not at aMHC

Because acetylation of histone H3 has been demonstrated to be an important marker for active gene expression, we first investigated the H3ac levels in the promoters and 5′ regions of the two MHC genes. We conducted ChIP assays on whole heart tissue using a pan-acetyl histone H3 antibody that recognizes acetylated lysine 9 and acetylated lysine 14 of histone H3. This antibody has been used extensively by others to test H3 lysine acetylations, hereafter referred to as H3ac. We used quantitative real-time PCR (qPCR) to quantify the enrichment of acetylated histone H3 at multiple locations of the two MHC genes. Figure 2 illustrates the locations of the primer pairs used for qPCR analyses at the bMHC and aMHC genes. Histone acetylations were assessed at seven individual locations for bMHC: −5 kb, −3 kb, −1 kb, −0.5 kb, 0 kb, +3 kb, and +5 kb, and six individual locations for aMHC: −3 kb, −1 kb, −0.5 kb, 0 kb, +3 kb, and +5 kb, each relative to its transcription start site at 0 kb.

The results of the ChIP qPCRs for bMHC using the pan-acetylated histone H3 (H3ac) are depicted in Figure 3. Hearts from untreated animals (open circles) show levels of H3ac that are similar at all locations within the bMHC gene. Hearts from animals treated with PTU (Fig. 3, open squares) show increased levels of H3ac at multiple locations within

TABLE 1
PRIMER SEQUENCES

	Forward	Reverse
bMHC −5.0 kb	5′-TAAGTCCAATATTAGAGATTCCAATTGAAGAT-3′	5′-GGTGTGGGGGAGCTGGCTTTTAG-3′
bMHC −3.0 kb	5′-GGAGCAGCTGCTTGTTAAAGAGAAACC-3′	5′-GGAGTGGTAGCCAGCACTTGCCCC-3′
bMHC −1.0 kb	5′-CTAGGAAGAACAAAGATTGCACCCACG-3′	5′-CCTGCAGATTTTCGAGGCGCAAGG-3′
bMHC −0.5 kb	5′-CCAACTCTGCCCTGCCCCTCT-3′	5′-TTGCCCCCAGTGCTAGCCTGG-3′
bMHC 0 kb	5′-AAATCTGGCTAGGGACTGG-3′	5′-CCTACCTGAGGGTAGCAAGTA-3′
bMHC +3 kb	5′-CCCTGATCCCAGAATCCCCTGAGA-3′	5′-GGGAGAGAGGCACAAAGAAAAGAGTTG-3′
bMHC +5 kb	5′-GGAGAGCCCTACAGACCCTGGG-3′	5′-CTCTGTAGCTGCTGTGGGCTACC-3′
aMHC −3.0 kb	5′-CCCAAGCTGACCCAATGTTCTCAGTA-3′	5′-GGCCACCTGTCTGCAGCTGTGT-3′
aMHC −1.0 kb	5′-CACCCACACCAGACCTCTCCC-3′	5′-CCTCCTGCCACTGAGAACTTCCCA-3′
aMHC −0.5 kb	5′-GAGTGCTGAGAATCACACCTGGGG-3′	5′-ATGGAACAGGACAAGCCAGGG-3′
aMHC 0 kb	5′-CTCCAAATTTAGGCAGCAG-3′	5′-CGAAACTCCCTCTTACCTG-3′
aMHC +3.0 kb	5′-TGCAGGCATACACAGATAAACAGACAC-3′	5′-GGCCTTTGTCCCTCAAACATGACT-3′
aMHC +5.0 kb	5′-CAAAGGAATGGAGGTACTGAAAATGCTT-3′	5′-CCACCTTCACCCGAGGGTGACA-3′

the bMHC gene, with the levels reaching significance at −1 kb ($p < 0.005$), +3 kb ($p < 0.01$), and +5 kb ($p < 0.05$) positions. These increases in the pan-acetylation levels of histone H3 at the bMHC gene during PTU treatment, which results in increased levels of expression, are consistent with the role of H3 acetylation as a marker of active gene expression.

We next investigated the changes in H3ac levels at the aMHC in the same samples. Figure 4 illustrates the results of the ChIP qPCRs done on the hearts from untreated (open circles) and PTU-treated (open squares) animals at multiple locations within aMHC promoter. Strikingly, no significant changes were found in the H3ac levels at any location between the control and PTU-treated animals, even though PTU treatment decreased the expression of aMHC by almost 300-fold. These data show that H3ac levels change only at the bMHC gene, but not the aMHC gene, in response to PTU treatment, even though the expression levels of both genes show dramatic changes in gene expression.

PTU Treatment Changes H3K4me3 at aMHC But Not bMHC

To test if the two MHC genes show discordant changes with respect to other epigenetic markers, we next considered H3K4me3. Genome-wide studies have shown that H3K4me3 is a widespread marker of active gene expression (1,3). We conducted ChIP qPCR analyses on the hearts from the same two groups described above using an antibody to H3K4me3. Figure 5 depicts the levels of H3K4me3 at multiple locations of bMHC gene in the hearts of control (open circles) and PTU-treated (open squares). No differences were found in the levels of H3K4me3 between the control and PTU-treated hearts at the bMHC promoter, although bMHC expression and the levels of H3ac were significantly increased in the same samples. Thus, changes in H3K4me3 levels do not follow the changes in expression of the bMHC gene.

To test if this modification is altered at the aMHC, we conducted qPCRs for the different aMHC locations on the same ChIP/H3K4me3 samples as were used for bMHC experiments. Figure 6 illustrates the results of qPCR on the aMHC promoter for untreated hearts (open circles) and PTU-treated hearts (open squares). The data show substantial decreases in the H3K4me3 levels at multiple locations with the decreases reaching significance at +3 kb ($p < 0.05$) and +5 kb ($p < 0.05$). This decrease in H3K4me3 modification in the PTU-treated hearts is concordant with the substantial decrease in aMHC gene expression. Together, our data show that, in response to PTU treatment, histone H3K4me3 levels are substantially changed at aMHC but not at bMHC, even though the expression levels of both genes change dramatically.

PTU-Induced Changes Are Completely Reversible at Each MHC

To test whether the epigenetic changes at the two MHC genes could be reverted to their original states when the gene expression changes are reversed, animals previously treated with PTU for 2 weeks (solid black bars in Fig. 1) were returned to a diet of regular chow for a further 2 weeks (cross-hatched bars in Fig. 1). The data show that the expression of aMHC in the hearts from these animals is restored to a high level indistinguishable from that of the untreated control animals ($p = 0.8$ for reversal verses untreated), while the expression of bMHC is restored to a low level indistinguishable from that of the untreated control animals ($p = 0.7$ for reversal verses untreated). Thus, withdrawal of the PTU treatment for 2 weeks completely restores the expression profile of aMHC and bMHC back to control levels.

ChIP qPCR analyses were conducted on chromatin

isolated from these hearts using antibodies against H3ac and H3K4me3. Figure 3 shows that the PTU-induced increases in H3ac levels at the bMHC promoter are reduced back to levels that are indistinguishable from untreated levels (compare open squares with closed squares), in parallel with the decrease in expression of bMHC. Similarly, withdrawal of PTU increases H3K4me3 levels at the aMHC promoter back to levels indistinguishable from untreated levels (Fig. 6, compare open squares with closed squares), in parallel with the increases in expression of aMHC. In contrast, histone acetylation and H3K4me3 levels remain unchanged at the aMHC and bMHC, respectively (Figs. 4 and 5, compare open squares with closed squares), even though the PTU withdrawal substantially changed the expression of these genes. Together, these data show that PTU-induced increases of H3ac at bMHC and decreases in H3K4me3 at aMHC are completely reversible.

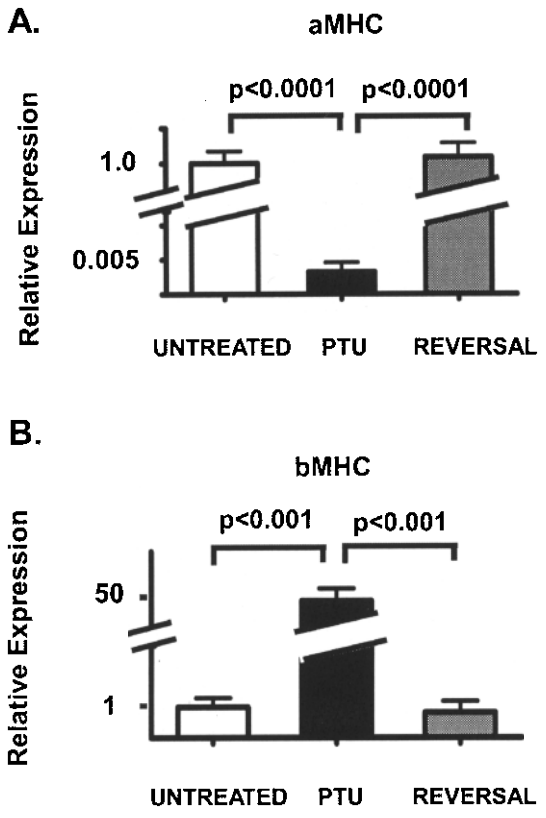


Figure 1. Reversible changes in mRNA expression levels of MHCs in response to PTU treatment. Comparisons of mRNA expressions relative to the mean of untreated animals (Relative Expression) for (A) aMHC, and (B) bMHC from hearts of untreated (white bars), PTU-treated (black bars), and PTU withdrawal (reversal, gray bars) animals. The mean for untreated group was set at 1, and the values for other groups are relative to this value. Animals were fed either regular chow (untreated), PTU diet for 2 weeks (PTU), or PTU diet for 2 weeks followed by regular chow for a further 2 weeks (reversal), and hearts isolated for analyses. Quantitative gene expressions were determined by Taqman real-time PCR.

DISCUSSION

Changes in histone modification have been recognized as being intricately associated with gene regulation for over 10 years (9,15,17,20,33). The two most conserved markers of gene activation are increased acetylation of multiple lysine residues and increased trimethylation of lysine 4 of histone H3. There is, however, much less documentation of the plasticity of these modifications in the face of reversible gene expression. Thus, although it is clear that gene activation is correlated with increases in the levels of these modifications, it is not clear to what degree these active modifications are lost when gene expression is subsequently silenced. The MHC locus is an excellent model system for studying inducible expression of reciprocally regulated genes, because the same stimulus (T3) dramatically regulates the expression of the two MHC genes in opposite directions. In this report we analyze the changes in the levels of histone acetylation (H3ac) and H3K4me3 at multiple sites within the promoters of the cardiac aMHC and bMHC genes.

We first used PTU-based inhibition of thyroid hormone synthesis to induce reversible changes in the expression of the aMHC and bMHC in the heart. Mice fed a PTU diet for 2 weeks dramatically reduce their expression of aMHC by about 300-fold and increase bMHC expression about 50-fold. Subsequent replacement of the PTU diet with normal chow for 2 weeks results in a complete return of the gene expression levels of aMHC and bMHC to their initial levels. These dramatic and reversible changes in expression of two linked genes in response to the same stimulus provided us with an excellent model system with which to investigate reversible shifts in epigenetic modifications during gene expression.

The change in expression of aMHC in response to T3 is an example of a pTRE form of regulation. THR constitutively binds to TRE sequences in promoters and, in the absence of T3, represses expression of the downstream gene (5), acting through a co-repressor complex that is formed in the absence of TH. This co-repressor complex consists of NCoR/SMRT proteins that recruit a complex of histone deacetylases (including HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC7, HDAC9) that deacetylate the histones surrounding a responding gene, resulting in its repression (14). The THR-mediated repression in the absence of TH is also associated with decreases in the levels of histone methylation including H3K4me3

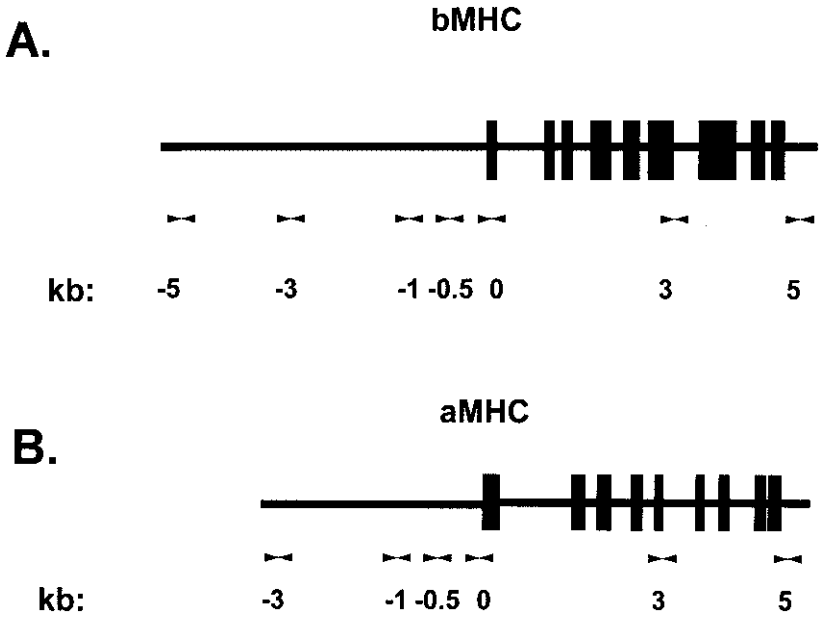


Figure 2. Locations of the primers used for ChIP qPCRs. Schematic of primer pairs (each arrowhead denotes a single primer) for multiple locations at the bMHC (A) and aMHC (B). Location nomenclature is relative to the transcription start site (0 kb). Boxes represent exons. Each primer pair amplified a fragment of approximately 100 to 120 bp.

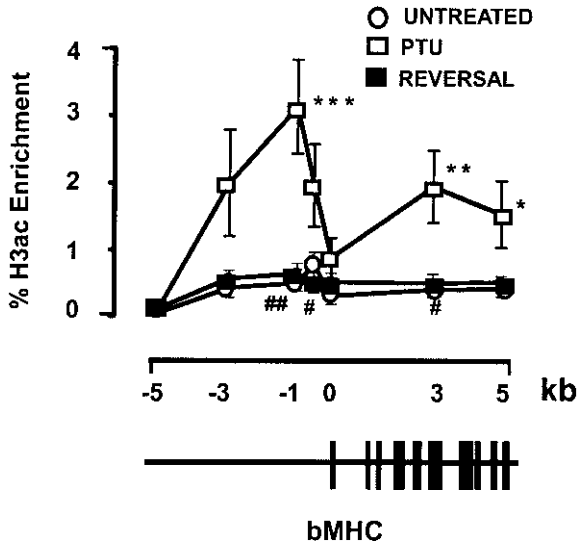
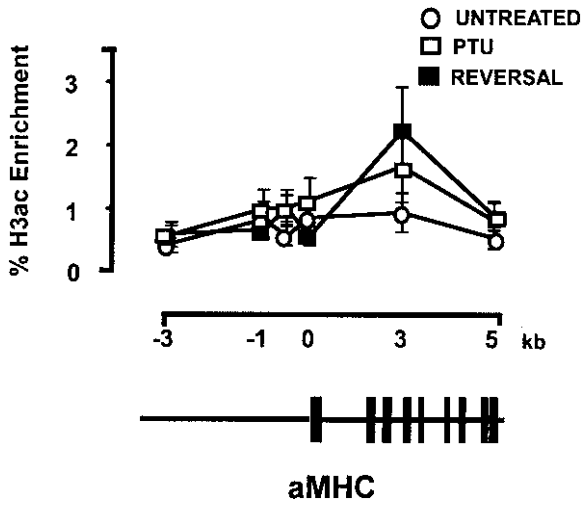


Figure 3. ChIP qPCRs analyses for pan-acetylation of histone H3 at the bMHC gene. Percent enrichment (relative to 100% input) of immunoprecipitated DNA is shown on the *y*-axis and the various locations of bMHC are shown on the *x*-axis. ChIP PCRs were conducted using anti-pan-acetylated H3 antibody (recognizing acetylated lysines 9 and 14) from chromatin isolated from untreated (open circles), PTU-treated (open squares), or reversal (closed squares) animals. Each data point represents mean of six independent ChIP experiments from three individual animals within each group. Error bars represent SEM. $*p < 0.05$ (relative to untreated group), $**p < 0.01$ (relative to untreated group), $***p < 0.005$ (relative to untreated group), $\#p < 0.05$ (relative to PTU group), $\#\#p < 0.01$ (relative to PTU group).

(18). Binding of TH to THR induces a conformational change that causes the dissociation of the co-repressor complex, and recruitment of co-activator complex that includes p160/steroid receptor proteins,



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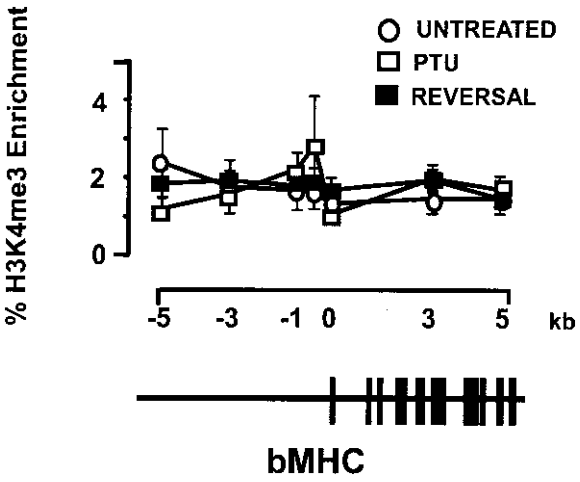
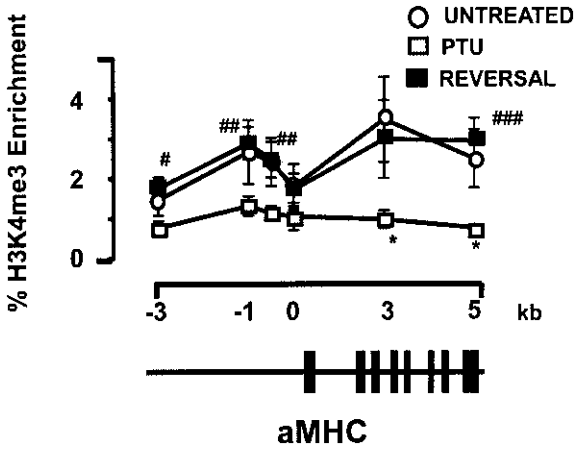


Figure 5. ChIP qPCRs analyses for H3K4me3 at the bMHC gene. Percent enrichment (relative to 100% input) of immunoprecipitated DNA is shown on the *y*-axis and the various locations of bMHC are shown on the *x*-axis. ChIP PCRs were conducted using anti-H3K4me3 antibody from chromatin isolated from untreated (open circles), PTU-treated (open squares), or reversal (closed squares) animals. Each data point represents mean of seven independent ChIP experiments from four individual animals within each group. Error bars represent SEM.

p300, and TRAP/DRIP, which together directly recruits the RNA Pol II and other members of the basal transcriptional machinery and leads to the formation of preinitiation complex and to transcriptional activation (13,16). Many of these proteins have intrinsic



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HAT activity that increases the acetylation of lysine residues on the histones. Our ChIP qPCR analyses show that the 300-fold decrease in expression of aMHC is associated with highly significant decreases in the levels of H3K4me3 at the aMHC promoter. Surprisingly, however, this decrease in expression of aMHC is not associated with changes in levels of histone h3 acetylation, because ChIP qPCR analyses done on the same samples show no differences in the levels of histone acetylation at the aMHC gene. Thus, PTU-mediated changes in aMHC gene expression are associated with changes in H3K4me3 levels but not in H3ac levels showing that histone H3ac is not obligatory for TH-mediated activation of pTRE-associated gene activation.

The response of bMHC to T3 is an example of a nTRE form of regulation (2). Thus, in the absence of T3, the THR binds to the TRE site of bMHC and activates its transcription. In the presence of T3, the receptor becomes a repressor, presumably via the loss of co-activators and concomitant recruitment of co-repressors. We find that treatment with PTU increases the expression of bMHC approximately 50-fold, and that this increase in expression of bMHC is associated with increases in histone acetylation at multiple sites within the bMHC promoter. These observations are consistent with previous reports implicating increases in histone acetylation in other nTRE-mediated increases in gene expression. For example, it has been demonstrated that the co-repressors NCoR/SMRT/SRC associate with the histone deacetylase HDAC1 and HDAC2, leading to decreases in histone acetylation levels at the promoters of thyroid-stimulating hormone and thyrotropin-releasing hormone (12,25,27). Consistently, the activation of these genes in the presence of T3 leads to the dissociation of HDAC2/HDAC3. Surprisingly, however, little is known, regarding the identity of the HATs that are responsible for the ligand-mediated increases in the acetylation of nTRE genes. There is one report demonstrating that T3-dependent repression of a nTRE gene is associated with decreases in H3K4me3 (27). However, our data show that T3-mediated changes in bMHC expression are not associated with changes in H3K4me3. Thus, decreases in H3K4me3 are not obligatory for T3 regulation of all nTRE-responsive genes.

Our data also demonstrate that the changes in histone modifications induced by PTU are completely reversible at both MHC when the PTU is withdrawn, as are the corresponding changes in gene expression. Thus, the PTU-induced increase in histone acetylation at the bMHC promoter is completely reversed when the animals are returned to a normal diet for 2 weeks. Similarly, the decrease in histone methylation

induced at the aMHC promoter by PTU treatment is completely restored to the higher initial levels after PTU withdrawal. It is important to note that in cardiac myocytes, which do not divide appreciably in the adult, any decreases in histone modification levels must be the result of active removal of the modifications rather than dilution by new unmodified histones synthesis during cell replication. The identity of the different HDACs and demethylases involved in bMHC and aMHC silencing, respectively, is not known, and further work needs to done to identify the temporal steps in MHC gene regulation.

Genome-wide studies have demonstrated that the promoters of active genes are generally associated with increased levels of both H3 acetylation as well as with increased trimethylation of H3K4. However, our data showing that the increase in H3ac occur only at bMHC while the increase in h3K4me3 occurs only at aMHC demonstrated that these two posttranslational modifications are not both required for expression of the two closely linked MHC genes. At least one other active promoter has been identified that does not show both modifications simultaneously: the promoter of gamma inferferon in T cells has high levels of H3K4me3 but not of H3ac (both K9 and K14) (30). Thus, joint occurrences of these two modifications are not universally associated with gene activation even with two reciprocally expressed tightly linked isoforms.

In summary, we show that the T3-responsive changes in aMHC and bMHC gene expression in the adult murine heart involve reversible histone modifications at the promoter, with H3ac accompanying changes in expression of the bMHC isoform, and H3K4me3 accompanying changes in the tightly linked aMHC isoform.

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