Tropomyosin is an essential component of the sarcomeric thin filament in striated muscle that participates in the regulation of muscle contraction through Ca\textsuperscript{2+}-mediated activation. The two predominant tropomyosin isoforms expressed in striated muscle are \(\alpha\)- and \(\beta\)-tropomyosin, which exhibit an 86% amino acid identity between themselves. Previous studies by our laboratory utilized a transgenic mouse system to overexpress \(\beta\)-tropomyosin in the heart to address the functional differences between these two tropomyosin isoforms. Interestingly, when a high percentage of \(\beta\)-tropomyosin replaces \(\alpha\)-tropomyosin in the hearts of transgenic mice, the mice die due to severe cardiac abnormalities. In this study, we have rescued these high expression \(\beta\)-tropomyosin mice by turning off the \(\alpha\)-myosin heavy chain promoter, which is driving the \(\beta\)-tropomyosin transgene. This down-regulation of the \(\alpha\)-myosin heavy chain promoter was accomplished by the administration of 5-propyl-2-thiouracil, which disrupts thyroid hormone synthesis and inhibits promoter activity through thyroid regulatory elements located in the 5'-flanking region of the promoter. Results show that as \(\beta\)-tropomyosin expression is down-regulated, \(\alpha\)-tropomyosin expression is increased. Also, \(\alpha\)- and \(\beta\)-myosin heavy chain expression is modified in response to the changes in thyroid hormone expression. Morphological analysis of these rescued mice show a moderate pathological phenotype, characterized by atrial myocytolysis; echocardiographic analyses demonstrate altered ventricular functions, such as peak filling rates and left ventricular fractional shortening. This is the first report demonstrating that transcriptional regulatory elements located within the \(\alpha\)-myosin heavy chain promoter can be manipulated to rescue potentially lethal phenotypes, such as high expression \(\beta\)-tropomyosin transgenic mice.

Tropomyosin (TM) \(^1\) is an actin-binding protein associated with both cytoskeletal structures and thin filaments in sarcomeric and smooth muscle. In vertebrates, there are four TM genes \((\alpha, \beta, TM30, \text{and } TM4)\). These genes utilize alternative splicing mechanisms to generate multiple tissue- and development-specific isoforms. As such, TM transcripts and their associated proteins display significant nucleotide and amino acid conservation among themselves. For example, there is an 87% amino acid identity between the \(\alpha\) - and \(\beta\)-TM striated muscle isoforms. Previous investigations have established that the muscle-specific \(\alpha\)- and \(\beta\)-TM isoforms are present in different quantitative levels in various muscles (1, 2); however, little is known about the functional differences between these two striated muscle proteins.

To address the physiological differences between the \(\alpha\)- and \(\beta\)-TM striated muscle isoforms, we implemented a murine transgenic model (3). In this model, we generated transgenic mice that overexpress \(\beta\)-TM striated muscle specifically in the adult heart. Results show the \(\alpha\)-/\(\beta\)-TM ratio is critical in determining myocardial performance. Under normal conditions, there is 98% \(\alpha\)-TM and 2% \(\beta\)-TM in the murine heart; mice expressing 45% \(\alpha\)-TM and 55% \(\beta\)-TM display a significant delay in the time of myocardial relaxation and a decrease in the maximum rate of relaxation in the heart. These physiological changes occur without a net change in the total amount of TM that is produced. Additional studies demonstrate that the functional changes that occur are due to an increased sensitivity to Ca\textsuperscript{2+} exhibited by the cardiac myofibers of the transgenic mice (4). Morphological analyses show that there are no structural changes in the heart or in the sarcomere that are associated with altered 45% \(\alpha\)-TM/55% \(\beta\)-TM ratio.

In additional studies, we have recently found that when hemizygous \(\beta\)-TM transgenic mice were mated, the resulting neonatal homozygous pups die within 10–14 days (5). These mice express 75% \(\beta\)-TM protein in their myocardium and develop severe cardiac abnormalities (which include large thrombi and severely enlarged atria and ventricles) within 3–5 days after birth. Ventricular muscle strips from these high expression \(\beta\)-TM mice show altered contractile and relaxation parameters. These results clearly demonstrated that myofiber activity and cardiac function are dramatically influenced by the TM isoform population.

In the current investigation, we have rescued the high expression \(\beta\)-TM homozygous transgenic mice and increased their survival through 8 weeks. This was accomplished by taking advantage of the thyroid regulatory elements located in the promoter of the \(\alpha\)-myosin heavy chain (MHC) gene, which was used to drive the transgene construct. By administering a diet containing 5-propyl-2-thiouracil (PTU), the \(\alpha\)-MHC promoter is shut down in the ventricle; however, this treatment simultaneously activates a complex program of genes associated with cardiac hypertrophy, such as \(\beta\)-MHC and atrial natriuretic factor. Although these transgenic mice survive with this PTU treatment, echocardiographic analysis of these rescued mice demonstrates an altered myocardial function. Moreover, when the PTU is withdrawn, the expression of exogenous \(\beta\)-TM is restored in the heart, and these mice die within 2 weeks from severe cardiac defects. This is the first report demonstrating that transcriptional regulatory elements within the \(\alpha\)-MHC promoter can be manipulated to rescue potentially lethal phenotypes, such as high expression \(\beta\)-TM or tropomodulin transgenic mice (5, 6). Thus, PTU can regulate expression of trans-
genes driven by the α-MHC promoter and effectively rescue mice from death due to overexpression of exogenous transgenes.

**Experimental Procedures**

**Animals**—The generation of β-TM overexpression transgenic mice was described previously (3). The hypothyroid condition was induced by feeding a diet containing 0.15% PTU (Teklad Premier) to pregnant female β-TM TG mice (15 days postcoitus) who were mated with male β-TM TG mice. This diet was maintained until the newborn mice reached 4 weeks of age; some litters were switched to a normal diet for 1 week following PTU treatment. We have previously used this PTU-containing diet to induce hypothyroidism in β-TM transgenic mice, as confirmed by determination of T4 serum levels (3).

**Genomic Southern Blot Analyses**—Genomic DNA was extracted from tail clips by overnight digestion at 60 °C. Purified DNA (10 μg) was digested with EcoRI, Southern blotted to nylon membrane, and hybridized with a radiolabeled probe corresponding to the SV40 3′-untranslated region. The copy number of the transgenes integrated into the genomic DNA was quantified using an ImageQuant PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). Moderate copy mice used in this study have 45–56 copies of the β-TM transgene; high copy mice have ~110 copies of the transgene.

**Northern Slot Blot Hybridization**—Serial diluted total RNA from total heart preparations was slot-blotted onto nitrocellulose membrane. Several blots were prepared and hybridized with radiolabeled isomorph-specific probes for striated muscle-specific α- and β-TM, α- and β-MHC, and glyceraldehyde-3-phosphate dehydrogenase. The α-TM probe is a 363-base pair SstI fragment, and the β-TM probe is a 299-base pair PstI/BglII fragment obtained from mouse cDNAs. The oligonucleotide used for α-MHC is 5′-CGAACGTTTATTTTATGGTGTGGCACAAGGAGGGTCGGCGAGGAGGGTCTGCTGGAGAGG-3′, and the oligonucleotide used for β-MHC is 5′-GCTTATCCTCACCCACCAAGGCTCGTGGGCAAAGGTCCCGAGGTGGAGGCGCTCTTC-3′. Each hybridization signal was quantified using an ImageQuant PhosphorImager.

**Western Blot Analyses**—Total protein homogenates were prepared from heart tissue from each experimental and control group as described (7). Quantification of protein amounts was performed using a BCA protein assay kit (Pierce), and equivalent amounts (20 μg) were subjected to Western blot analyses using a monoclonal striated muscle TM-specific antibody as described (Sigma). The intensity of the bands was quantified by PhosphorImager analysis.

**Histological Analysis**—Hearts were removed and immediately fixed in 10% neutral buffered formalin for 24–48 h. The hearts were then transferred to a solution of 70% ethyl alcohol until processed. Sections of 5 μm were prepared and stained with hematoxylin-eosin.

**In Vivo Echocardiographic Measurements of Cardiac Function**—The animals were lightly anesthetized with 2.5% avertin (0.01 ml/g of body weight) and allowed to breathe spontaneously. Two-dimensionally targeted M-mode studies were performed with a 10-MHz intraoperative scan head imaging transducer (ATL HDI 3000) using methods previously described (8). This transducer has a small offset footprint and has outstanding near field imaging quality. M-mode measurements end diastolic (EDD) and end systolic dimension, end diastolic thickness of the septum (IVSed), and posterior wall (PWed) were made online and plotted using software described (7). Quantification of protein amounts was performed using a BCA protein assay kit (Pierce), and equivalent amounts (20 μg) were subjected to Western blot analyses using a monoclonal striated muscle TM-specific antibody as described (Sigma). The intensity of the bands was quantified by PhosphorImager analysis.

**Results**

**Identification and PTU Treatment of High Copy β-TM Mice**

High level expression β-TM transgenic mice were generated by mating hemizygous β-TM transgenic mice within identical or different lines (3, 5). At 15 days postcoitus, pregnant hemizygous mothers were fed a diet containing PTU to down-regulate expression of thyroid hormone in the developing fetuses. This diet was continued while the newborn pups were nursed by the mother. One effect of PTU is to down-regulate the α-MHC promoter, which drives the β-TM transgene (3).

With a diet containing PTU, the newborn mice were uniformly similar in size and weight compared with wild type. At 2 weeks postnatal, the mice were genotyped by genomic Southern blot analyses using DNA isolated from the tail. As shown in Fig. 1, results from the autoradiograph show that a 6.8-kilobase pair (kb) hybridizing band was obtained in the transgenic genomic DNA with variable intensity according to the number of integrated copies (see “Experimental Procedures”).

By giving PTU to the expectant mothers and continuing this treatment following delivery, the high expression β-TM transgenic mice were able to survive past their normal life span of 10–14 days up to 8 weeks. However, hypothyroidism causes pleotrophic effects upon biological systems, including growth retardation and weight loss (9). This phenomenon was exhibited by the mice receiving PTU; after 4 weeks, the weanlings, regardless of genotype, were phenotypically similar in size and weight (7–8 g); this weight is approximately half the expected weight of 15 g for a nontransgenic, non-PTU-treated mouse. Interestingly, hypothyroidism is also known to cause cardiac hypertrophy and reactivation of the fetal gene program (10). Initial results show that moderate copy TG and control mice exhibit a similar heart weight/body weight ratio at 30 days (Fig. 2). This result is in agreement with previous studies conducted by our laboratory showing no gross morphological differences exist between moderate β-TM TG mice and their wild type controls (3). Mice treated with PTU show an increased heart/body weight ratio over nontreated animals. Within the PTU group, there is a tendency for a higher ratio in the moderate and high copy β-TM transgenic mice. If the mice are switched back to a normal diet (non-PTU diet) after 30 days of PTU treatment, by 7 days there is a significant difference in the heart weight/body weight ratio between the high copy β-TM transgenic mice and wild type controls. The primary reason for this difference appears to be a more rapid increase in body weight of the wild type mice compared with the transgenic mice. For the transgenic mice, the switch back to a normal diet allows the reoccurrence of exogenous β-TM protein. In the high copy mice, this increased β-TM results in the development of severe pathological cardiac abnormalities, similar to the abnor-
malities that develop in the non-PTU-treated high β-TM expression neonatal mice (5). Thrombi formation in these mice restricts blood flow to the systemic circulation and severely curtails growth and development, thus leading to an increased heart weight concomitant with a reduced body size and weight. Once these mice are switched from a PTU-containing diet to normal feed, they die within 7–10 days.

**Regulation of Gene Expression in Rescued β-TM Mice**

**Regulation of Tropomyosin mRNA Expression in PTU Transgenic Mice—**Previous work by ourselves and others has shown that endogenous TM expression is not regulated by thyroid hormone levels (3, 10). To quantify levels of TM expression in the β-TM mice and their controls, total RNA from cardiac tissue was isolated, slot-blotted, and hybridized with radiolabeled, isomorph-specific probes. A 299-base pair striated muscle-specific β-TM cDNA probe was generated using polymerase chain reaction. Results in Fig. 3 show that the β-TM is not expressed in hearts from non-TG mice, regardless of their PTU status. Moderate copy β-TM mice do express significant levels of β-TM in their hearts because of the α-MHC-driven transgene. These levels decrease upon the addition of PTU to the diet and return to high levels following the switch to the normal diet. High copy mice subject to PTU have low levels of exogenous β-TM expression that are increased following the return to the normal diet after 30 days. These results show that PTU is effective in down-regulating the expression of the α-MHC promoter/β-TM transgene, which results in prolonged viability of the high copy β-TM mice.

To ascertain whether overexpression of β-TM affected the expression of the endogenous α-TM striated muscle isoform, we probed RNA slot blots for α-TM striated muscle message. A 363-base pair α-TM cDNA fragment was purified, radiolabeled with 32P, and hybridized with cardiac RNA from non-TG, moderate copy TG, and high copy TG mice. As seen in Fig. 3, α-TM striated muscle message is detected in all samples. The non-TG control mice exhibit a greater amount of α-TM message than moderate copy TG mice, which is in agreement with previous studies by our laboratory (3). As previously mentioned, PTU treatment does not affect endogenous α-TM expression. When the TG mice are switched from a PTU diet to a normal diet, the level of α-TM expression decreases, presumably due to the increased expression of β-TM from the transgene, which causes a TM feedback mechanism to decrease the production of α-TM striated muscle. This lower quantifiable level of α-TM expression is also evident in the adult control TG mice.

**Regulation of TM Protein Levels in PTU-treated TG Mice—**Previous studies have established that translational mechanisms are operative in the regulation of TM protein synthesis (11, 12). To examine whether the changes in TM expression at the transcript level were reflected at the translational level, total protein extracts were prepared from the hearts of the various experimental groups. These protein preparations were electrophoresed on SDS-polyacrylamide gels and Western blotted using a striated muscle-specific TM antibody that immunologically reacts with both α- and β-TM. Results show that both α- and β-TM are distinguishable and are expressed at quantifiable levels reflective of the dietary treatment to which the mice were exposed (Fig. 4A). As expected, wild type control mice do not produce β-TM, either at 1 month or in adulthood (lanes 1 and 9, respectively), whereas transgenic mice express β-TM protein at 1 month and in the adult (lanes 2 and 10). PTU treatment decreases the α-MHC promoter activity, which results in lowered β-TM production in both TG and high copy mice (lanes 4 and 5); as stated previously, endogenous TM levels are not altered by PTU treatment (lane 3). Switching to the normal diet in the 30-day-old mice reactivates the promoter, and expression of β-TM protein is increased (lanes 7 and 8). These results clearly demonstrate that the changes exhibited at the transcript level are also reflected at the translational level. A PhosphorImager quantitative analysis shows that total TM protein in these mice remains relatively constant (Fig. 4B). Although the ratio of α- and β-TM dramatically changes with transgene copy number and the regulation of the promoter by PTU treatment, translational control mechanisms appear operative to ensure that a constant level of TM is produced within the cardiomyocyte. Similar results have been obtained with overexpression of other TM isoforms (3, 13).

**Regulation of MHC in PTU-treated TG Mice—**Cardiac muscle α-MHC is the predominant MHC isoform expressed in the adult murine myocardium. Moreover, the ventricular expression of this myosin heavy chain isoform is regulated by thyroid hormone regulatory elements. To determine the effect of the PTU treatment on α-MHC expression, we generated an
α-MHC-specific oligonucleotide probe corresponding to 3′-untranslated region sequence. This probe was radiolabeled and hybridized to RNA from hearts of the various experimental groups. Results in Fig. 3 show that in the absence of PTU, both non-TG and TG mice have equivalent levels of α-MHC expression. With the addition of PTU to the diet, α-MHC expression decreases; this reduction is most pronounced in the non-TG mice but also occurs in the moderate copy β-TG mice. Interestingly, α-MHC expression appears slightly increased in the high copy β-TG mice; we speculate that continued expression of α-MHC in the presence of PTU may be due to increased numbers of thyroid hormone regulatory elements present in these mice and nonsaturating conditions of the PTU in blocking all of the thyroid hormone regulatory elements (see “Discussion”).

With the return to a normal diet, α-MHC expression switches back to control levels in the non-TG and moderate mice; interestingly, the high copy mice do not alter expression of α-MHC after switching back to normal feed.

Numerous studies have demonstrated that α- and β-MHC are antithetically regulated in the myocardium. The β-MHC gene is not expressed in the hearts of control non-TG mice (Fig. 3); however, there is low expression of β-MHC message in the β-TM TG mice at 30 days. When the mice are subject to PTU treatment, β-MHC is dramatically increased in non-TG mice. When the PTU diet mice are switched back to normal feed, β-MHC expression decreases for the non-TG and high copy TG mice; there is a slight increase in β-MHC expression in the moderate TG mice following the change in diet from PTU to normal feed. The results of α- and β-MHC expression in the presence of PTU reflect the complex pattern of cardiac muscle gene expression that results following the administration of PTU to mice. Furthermore, following the switch from PTU to normal feed, the β-TM transgene is reactivated, and in the case of the high copy mice, develops severe dilation and hypertrophy, which culminates in lethality.

To demonstrate that equivalent amounts of total RNA were loaded for the various experimental groups, we stripped the RNA slot blot free of probes and rehybridized to a radiolabeled mouse glyceraldehyde-3-phosphate dehydrogenase cDNA fragment. As seen in Fig. 3, equivalent levels of signal are detected in all of the lanes for the various experimental groups. These results demonstrate that the changes detected for TM and MHC isoforms are due to alterations in expression for these genes and not due to differential loading of RNA samples.
Histological and Physiological Analyses of the Rescued High Copy Transgenic Mice

Previous studies show that there are no morphological differences between control and moderate copy β-TM TG mice (3). On the other hand, high copy β-TM TG mice exhibit several pathological abnormalities, including thrombus formation in the lumen of the atria and ventricles, along with chamber dilation, fibrosis, and diffuse myocyteolysis (5). As stated previously, these high copy mice succumb by day 10–14, postpartum. To morphologically examine the hearts following reversal of lethality by PTU treatment followed by a return to the normal diet prior to examination. A comparative pathological analysis demonstrates that both moderate and high copy TG mice undergo similar diffuse cytoplasmic changes, which are most severe in the atria (Fig. 5). These alterations are characterized by decreased or lost striations, decreased staining intensity, vacuolization, separation of the cytoplasm from the cell membrane, and waviness of the myofibers. A high percentage (70–100%) of atrial cells are affected, whereas there is characteristically only ~20–40% ventricular involvement, with the apex being primarily affected. The non-TG control animals did not exhibit these dramatic cytoplasmatic alterations; however, mild myocyte hypertrophy was present in all PTU-treated mice.

To assess the in vivo cardiac performance of the PTU-treated mice, we performed echocardiographic analyses. Heart rate, peak filling rate, and percentage fractional shortening measurements were conducted in control and moderate copy TG mice. (The high copy mice treated with PTU or on the “switched” diet were unable to tolerate the light anesthesia associated with echocardiography and succumbed during the procedure; this result is surprising, since the pathological abnormalities found in the high copy PTU diet mice were mild and similar to those found in the moderate copy TG mice (see “Discussion”).) The mice were evaluated after 30 days of PTU treatment and also after switching them to a normal diet for 7 days. The measured values provided a functional assessment of cardiac performance following the changes in gene expression associated with PTU administration and following the re-expression of the β-TM transgene.

There were no significant differences in heart rate between wild type and TG mice when treated with PTU (207 ± 21 versus 194 ± 19 beats/min, respectively) (Table I). However, when the mice are switched back to a normal diet not containing PTU, significant differences in heart rate appear; the heart rate of wild type mice on a normal diet is 327 ± 23 beats/min versus 222 ± 23 beats/min for TG mice. This increased heart rate for wild type mice on the normal diet is also significantly higher than wild type mice treated with PTU, which may be reflective of changes in myosin isozymes and sarcoplasmic reticulum Ca2+-ATPase, which are transcriptionally regulated by T3 levels. The inability of the TG mice to increase their heart rate after the removal of PTU may reflect the re-expression of the β-TM transgene and its influence on sarcomeric function and diastole.

Ventricular diastole is composed of an early rapid phase due to ventricular relaxation and the left atrioventricular pressure gradient and late diastolic filling due to atrial contraction and ventricular compliance. We measured the early rapid phase from digitized M-mode echocardiograms. Results show that there is no difference between wild type and moderate TG cardiac peak filling rates when on PTU (13.0 ± 1.5 versus 11.5 ± 1.4 mm/s). However, when the mice are shifted to a normal diet, the wild type mice increase their peak filling rate to 15.1 ± 1.8 mm/s, whereas the TG mice decrease their peak filling rate to 10.2 ± 1.2 mm/s (p < 0.05). This decrease in filling rate for the TG mice may reflect the delay in relaxation associated with diastole of β-TM TG mice (3).

In PTU-treated mice, there were no significant differences in left ventricular dimensions, fractional shortening, peak filling rate, left ventricular mass, or heart rate. However, after diets were switched, fractional shortening, peak filling rates, and heart rates were significantly different in transgenic than wild type animals.

Results from the M-mode echocardiographic analysis of the wild type and transgenic mice showed that the fractional shortening was similar in wild type mice on PTU or the switched diet (45.9 ± 1.2 versus 46.8 ± 1.5). Also, there was no significant difference in fractional shortening between wild type and moderate TG mice when on PTU (45.9 ± 1.2 versus 38.9 ± 3.4); however, there was a difference in fractional shortening (p < 0.05) between wild type and moderate TG mice on the switched diet (46.8 ± 1.5 versus 33.9 ± 2.5).

**DISCUSSION**

Exploration of protein isoform diversity is essential to understand sarcomeric function in striated muscle. Tropomyosin, an essential component of the thin filament, is composed of α- and β-TM isoforms, which exhibit an 86% amino acid identity between themselves (14). This highly conserved homology extends throughout the entire protein, with amino acid substitutions scattered throughout the molecules. Defining the functional significance of α- versus β-TM has been accomplished through the usage of transgenic mice, where our laboratory has utilized the α-MHC promoter to overexpress the β-TM isoform.
in the heart. Results show that functional differences exist between these two TM isoforms, which principally alter cardiac relaxation properties. High levels of β-TM lead to severe cardiac abnormalities, which culminate in lethality within 10–14 days of expression. In this study, we have successfully extended the survival of these high expression TG mice by the down-regulation of the α-MHC promoter, which drives the transgene expression. Results presented here provide the first example of rescuing a lethal cardiac phenotype by modulation of the α-MHC promoter through two thyroid regulatory elements. This down-regulation is accomplished by disrupting thyroid hormone synthesis by PTU administration. Ventricular expression of α-MHC is dependent upon thyroid hormone binding to thyroid hormone regulatory elements located within the 5′ regulatory region. These thyroid hormone regulatory elements are highly conserved transcriptional regulatory elements that assist in the modulation of α-MHC gene transcription during development of the heart (15, 16). Inducing hypothyroidism using PTU decreases the transcriptional activity of the α-MHC promoter, coupled with a concomitant increase in β-MHC promoter activity (17). In our study, removal of PTU from the diet and re-expression of the β-TM transgene leads to severe cardiac abnormalities, which result in lethality.

Previous studies have established that thyroid hormone influences myocardial contractility through several distinct mechanisms, including regulation of gene expression and interactions with the sympathetic nervous system (18). In hypothyroidism, myosin isoforms are predominantly present in slower contracting forms (i.e. β-MHC), as well as decreased activity of sarcoplasmic reticulum Ca2+ -ATPase, which affects the velocity of diastolic relaxation (19). Also, thyrotropin-releasing hormone, which stimulates sympathetic outflow within the central nervous system, is elevated in hypothyroidism (20, 21). The effect of thyroid hormones on stimulation of protein synthesis in cardiac tissue appears to be secondary to the effect of protein synthesis on hemodynamics and cardiac work (9). Certain cardiac functional measures appear unaffected by hypothyroidism, including left ventricular ejection fraction, left ventricular end diastolic dimension and end systolic wall stress (19). This may be the reason that results from our investigation showed that there were no significant changes in whole animal cardiac performance for peak filling rate or fractional shortening between control mice on a normal or PTU diet. Interestingly, heart rate measurements in this study of wild type control mice were decreased in PTU-treated animals, which is similar to the result found with intact closed-chest measurements of murine cardiac function using in situ Millar transducers (22). With PTU, the heart rate is similar in both NTG and TG mice, since the transgene is shut off, whereas in the ers (22). With PTU, the heart rate is similar in both NTG and

The usage of conditional expression systems can play a unique role in the assessment of exogenous protein function. For bacterial and cell culture expression systems, inducible promoters can be employed to transcriptionally regulate expression of genes. Regulated mammalian in vivo expression systems are more limited, with the cre-lox system being operative but technically complicated to establish. This current study utilizes the extensively used α-MHC promoter to drive transcription of a transgene for cardiac tissue-specific expression. Since this promoter is controlled by thyroid regulatory elements, the hypothyroid condition will down-regulate its activity. Although hypothyroidism affects the expression of numerous genes and hormonal systems, our ability to induce this condition through PTU allows us to rescue the high copy β-TM mouse for survival past its normal life span of 10–14 days to at least 8 weeks. Although one must be judicious in the analyses, this ability to manipulate the α-MHC promoter is invaluable for conducting analyses past the newborn developmental stage and may serve to further research on other lethal phenotypes that result from overexpression of α-MHC-driven transgenes.

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