Interaction Between Myosin Heavy Chain and Troponin Isoforms
Modulate Cardiac Myofiber Contractile Dynamics

Murali Chandra, Matthew L. Tschirgi, Steven J. Ford,
Bryan K. Slinker and Kenneth B. Campbell

Department of VCAPP, 205 Wegner Hall, Washington State University, Pullman, WA 99164

Running Head: Dynamics of myocardial contraction

Contact information:
Murali Chandra, Ph.D.
Assistant Professor
205 Wegner Hall
Department of VCAPP
Washington State University
WA 99164

Tel: (509) 335 7561
Fax: (509) 335 4650

E-mail: murali@vetmed.wsu.edu
ABSTRACT

Coordinated expression of species-specific myosin heavy chain (MHC) and troponin (Tn) isoforms may bring about a dynamic complementarity to match muscle contraction speed with species-specific heart rates. Contractile system function and dynamic force-length measurements were made in muscle fibers from mouse and rat hearts and in muscle fibers after reconstitution with either recombinant homologous Tn or orthologous Tn. The rate constants of length-mediated crossbridge recruitment \( (b) \) and tension redevelopment \( (k_{tr}) \) of mouse fibers were significantly faster than rat fibers. Both the tension cost (ATPase/tension) and rate constant of length-mediated crossbridge distortion \( (c) \) were higher in the mouse than in the rat. Thus, the mouse fiber was faster in all dynamic and functional aspects than the rat fiber. Mouse Tn significantly increased \( b \) and \( k_{tr} \) in rat fibers and, conversely, rat Tn significantly decreased \( b \) and \( k_{tr} \) in mouse fibers. Thus, the length-mediated recruitment of force-bearing XB occurs much more rapidly in the presence of mouse Tn than in the presence of rat Tn demonstrating that the speed of XB recruitment is regulated by Tn. There was a significant interaction between Tn and MHC such that changes in either Tn or MHC affected the speed of XB recruitment. Our data demonstrate that the dynamics of myocardial contraction are different in the mouse and rat hearts due to sequence heterogeneity in MHC and Tn. At the myofilament level, coordinated expression of complementary regulatory contractile proteins produces a functional dynamic phenotype that allows the cardiovascular systems to function effectively at different heart rates.

**Key Words:** myofiber dynamics, contraction speed, heart rate
INTRODUCTION

There is substantial protein sequence heterogeneity among orthologous cardiac myosin heavy chain (MHC) and troponin (Tn) isoforms across different animal species (30). This sequence heterogeneity in regulatory contractile proteins significantly affects myofilament dynamics as assessed by the force response to muscle length change in constantly activated cardiac myofibers, which exhibits two clearly separable processes (3, 5, 20, 30, 35): 1) a relatively fast force dynamic associated with myosin crossbridge (XB) distortion and 2) a relatively slow force dynamic associated with recruiting additional XB into force-bearing states. The dynamics of XB distortion are principally determined by the enzymatic kinetics of MHC and the dynamics XB recruitment are affected greatly by cooperative interactions between Tn actions and XB cycling kinetics (3, 5, 6, 30).

We showed recently that differences in Troponin T (TnT), a subunit of the Tn regulatory protein complex, affected the slow XB recruitment dynamic (9), whereas a shift from α- to β-MHC isoform in the rat heart affected both the slow XB recruitment dynamic and the fast XB distortion dynamic (5). Furthermore, there is interplay between TnT and MHC such that the effect of mutant TnT on both tension-dependent ATP consumption and the fast XB distortion dynamic were significantly influenced by a shift from α- to β-MHC isoform (34). These findings suggest that coordinated expression of species-specific contractile regulatory protein isoforms may bring about a complementarity such that the resulting myofilament dynamics produce satisfactory function when the cardiac myofiber system is driven at different resting heart rates seen across different species. For example, the high heart rate of the mouse requires faster rate of pressure generation, velocity of shortening, and rate of relaxation, compared to the rat and other larger mammals (1, 6, 12, 13, 18, 32).
The main objective of this study was to test the hypothesis that coordinated expression of species-specific regulatory contractile protein isoforms (MHC and Tn) brings about a dynamic complementarity such that muscle contraction speed is matched to species-specific heart rate (6). We used muscle fibers from mouse and rat hearts because of significant differences in the determinants of myocardial contraction in mouse and rat (1, 6, 12, 13, 18, 32), differences in resting heart rates (600 min\(^{-1}\) in the mouse vs. 300 min\(^{-1}\) in the rat), and substantial sequence heterogeneity in both MHC and Tn of mouse and rat hearts (21, 28). Furthermore, because it is relatively easy to shift the MHC isoform from \(\alpha\)- to \(\beta\)-MHC in mouse and rat hearts, these animals are ideal for probing the interplay between Tn and different MHC isoforms; in the left ventricle of both mice and rats, propylthiouracil treatment shifts the MHC isoform from predominantly \(\alpha\)- to \(\beta\)-MHC without affecting the expression of other thin filament regulatory proteins (16, 22, 24, 27, 34). An \(\alpha\)- to \(\beta\)-MHC-induced alteration in XB cycling kinetics changes cooperative interactions between XB-Tn and XB-XB, which impact contractile dynamics by modifying XB recruitment (3, 5, 6). Thus, propylthiouracil-treated mice and rats offer a unique opportunity to study the MHC-Tn interaction effects on contractile dynamics, especially given the availability of recombinant Tn for these two species.

We studied a broad range of contractile system function, including maximal Ca\(^{2+}\)-activated tension, ATPase activity, tension cost, the rate of tension redevelopment, and dynamic force-length behavior. Experiments were conducted in muscle fibers from normal mouse and rat hearts after reconstitution with either recombinant homologous Tn (i.e., from the same species) or orthologous Tn (i.e., from the other species). In addition, the dynamic complementarity among MHC and Tn was probed by repeating these experiments after shifting the MHC isoform in each species from \(\alpha\)- to \(\beta\)-MHC.
Changes in composition of both MHC and Tn significantly affected the rates of myofiber contractile dynamics. With normal MHC-Tn composition, both XB recruitment and XB distortion dynamics were faster in normal mouse fibers than in normal rat fibers. Our Tn reconstitution experiments demonstrated that Tn by itself affected contractile dynamics and that this effect varied with different MHC background. Relative to the native homolog, rat Tn decreased the speed of the XB recruitment dynamic in mouse fibers (especially when there was a β-MHC background), whereas mouse Tn increased the speed of the XB recruitment dynamic in rat muscle fibers (in fibers with both α- and β-MHC backgrounds). Thus, sequence heterogeneity in regulatory proteins confers unique myofiber functional dynamics. Such coordinated expression of complementary regulatory contractile proteins produces a dynamic phenotype that allows the cardiovascular systems to function effectively at different heart rates.
METHODS

cDNA clones for mouse and rat cardiac Tn subunits: Isolation of full-length cDNA clones for adult rat cardiac troponin I (RcTnI) and rat cardiac troponin C (RcTnC) was as described previously (9). Adult rat cardiac troponin T (RcTnT) DNA (19) was a gift from Dr. Lin JJ (University of Iowa). Adult mouse cardiac troponin I (McTnI) and mouse cardiac TnT (McTnT) were as described previously (15, 33). Mouse cardiac TnC (McTnC) sequence is identical to RcTnC. Both mouse and rat cTnT DNA constructs were tagged with the human c-myc epitope (33). Previous studies from our own and other laboratories have demonstrated that the presence of c-myc epitope in cTnT has no significant effect on cardiac muscle function (8, 23, 33, 34).

Expression and purification of recombinant mouse and rat cardiac Tn subunits: Mouse and rat recombinant cTnT, cTnI and cTnC (all in pSBETa plasmid DNA) were expressed in BL21 (DE3) cells (Novagen) and purified as described previously (9). Purified protein fractions were extensively dialyzed against deionized water containing 15 mM β-mercaptoethanol, lyophilized and stored at -80°C.

Animal treatment protocols: All procedures conformed to the guidelines laid down by the Washington State University Institutional Animal Care and Use Committee. Animals used in these studies were young, adult, Swiss-Webster mice and Sprague-Dawley rats. There were two experimental groups in each species: 1) non-treated normal animals that expressed native levels of α- and β-MHC isoforms in their left ventricles; 2) propylthiouracil-treated (PTU) animals (0.6 g L⁻¹ in drinking water for 4-5 weeks) that were made hypothyroid and, consequently, expressed primarily the β-MHC isoform.
Preparation of detergent-skinned cardiac muscle fiber bundles: Mice and rats were anesthetized with sodium pentobarbital (50 mg kg\(^{-1}\) body weight), hearts were rapidly excised and placed into ice cold high relaxing solution containing 20 mM MOPS, pH 7.0, 53 mM KCl, 10 mM EGTA, 6.81 mM MgCl\(_2\), 5.35 mM Na\(_2\)ATP, 1.0 mM DTT. Fresh cocktail of protease inhibitors (4 \(\mu\)M benzamidine-HCl, 5 \(\mu\)M bestatin, 2 \(\mu\)M E-64, 10 \(\mu\)M leupeptin, 1 \(\mu\)M pepstatin and 200 \(\mu\)M PMSF) were added to buffered solutions. Papillary muscle bundles were excised from the left ventricles of normal and PTU-treated mouse and rat hearts. Very thin muscle fiber bundles (~150 \(\mu\)m in width and 1.5-2.0 mm in length) were dissected and detergent-skinned as described previously (8).

Reconstitution of recombinant Tn into detergent-skinned mouse and rat cardiac muscle fiber bundles: Reconstitution of endogenous Tn in normal and PTU-treated cardiac muscle fibers was based on the method described previously (9). Endogenous Tn in detergent-skinned mouse and rat cardiac muscle fibers was removed by first treating fibers with the extraction solution containing cTnT-cTnI for approximately 3-4 hours. The extraction solution contained 50 mM BES (pH 7.0 at 20°C), 180 mM KCl, 10 mM BDM, 5 mM EGTA, 6.27 mM MgCl\(_2\), 1.0 mM DTT, 0.01% NaN\(_3\), 5 mM MgATP\(^2-\) and a fresh cocktail of protease inhibitors. Fibers were washed with the extraction solution and Ca\(^{2+}\)-activated maximal tension was measured in pCa 4.3 solution to determine the residual tension. Ca\(^{2+}\)-activated tension and ATPase activity were measured at various pCa (-log of free Ca\(^{2+}\) concentration) after reconstitution with cTnC (3 mg ml\(^{-1}\)). Other details of reconstitution procedure are essentially as described previously (9). The composition of different pCa solution was calculated using the methods described by Fabiato & Fabiato (11). One set of fibers from both mice and rats, including fibers from both normal and
PTU-treated animals, was used without modification for contractile function and mechano-
dynamic studies. A second set of fibers from both mice and rats, including both normal and
PTU-treated animals, was reconstituted with recombinant homologous and orthologous Tn. For
Tn exchange experiments, controls are those in which the endogenous Tn was replaced with the
homologous recombinant Tn.

Simultaneous measurement of steady-state isometric tension and ATPase activity in
detergent-skinned cardiac muscle fibers: The muscle fiber was attached to a motor and a
force transducer using aluminum clips. Muscle fiber sarcomere length was measured, as
previously described (10). The resting sarcomere length was readjusted to 2.2 μm (after 2-3
cycles of full activation and relaxation) and monitored using a He-Ne laser diffraction system.
The muscle fiber was immersed in a 15 μl bath containing activating solutions with different
pCa. Activating solution in the bath was continuously stirred by means of motor-driven
vibration of a membrane positioned at the base of the bath. Maximum activating solution (pCa
4.3) contained: 31 mM potassium propionate, 5.95 mM Na₂ATP, 6.61 mM MgCl₂, 10 mM
EGTA, 10.11 mM CaCl₂, 50 mM BES, pH 7.0, 10 mM NaN₃, 0.9 mM NADH, 10 mM
phosphoenol pyruvate, 4 mg ml⁻¹ pyruvate kinase (500 U mg⁻¹), 0.24 mg ml⁻¹ lactate
dehydrogenase (870 U mg⁻¹) and 20 μM A₂P₅ and a cocktail of protease inhibitors.

During steady-state activation, tension and ATPase activity (20°C) were measured
simultaneously as described before (10, 31). For ATPase measurements, near UV light was
projected through the muscle chamber then split via a beam splitter (50/50) and detected at 340
nm (sensitive to changes in [NADH]) and 410 nm (insensitive to changes in [NADH]). The light
intensity at 410 nm served as a reference signal. An analog divider and a log amplifier produced
a signal proportional to the amount of ATP consumed in the muscle chamber solution. ATP regeneration from ADP was coupled to the breakdown of phosphoenol pyruvate to pyruvate and ATP catalyzed by pyruvate kinase, which was linked to the synthesis of lactate catalyzed by lactate dehydrogenase. The breakdown of NADH (which is proportional to the amount of ATP consumed), was measured on-line by UV absorbance at 340 nm (10).

**Rate of tension redevelopment (k<sub>tr</sub>):** The rate constant of tension redevelopment (k<sub>tr</sub>) was measured based on the method described previously (2) where the maximally-activated muscle fiber is unloaded by a length-changing protocol and, then, the time-course of tension redevelopment is monitored. Constantly activated muscle fiber was slackened by 10% using a high-speed length control device (Aurora Scientific Inc). After 25 ms of shortening period, the motor arm was swung rapidly (0.5 ms) backward to pass the original set point by a 10% stretch. The muscle fiber was allowed to return rapidly to its original length at which point force started to redevelop. k<sub>tr</sub> was determined by fitting the rise of tension to the following equation:

\[
F = F_{\text{obs}} (1 - e^{-k_{\text{tr}} t}) + F_0,
\]

where \(F\) is force at time \(t\), \(F_{\text{obs}}\) is observed steady-state force and \(F_0\) is the initial force from which force redevelops. Tension redevelopment in cardiac muscle was well fitted with the monoexponential equation \((R^2 > 0.97)\).

**Muscle fiber mechano-dynamics:** A protocol to determine the dynamic force-length relationship (FLR) was conducted (5, 9) during steady-state activation. Muscle fiber length \((L_M)\) was changed according to a constant amplitude (±0.5% of \(L_M\)) sinusoid of continuously varying frequency (chirp) and the resultant changes in force (\(\Delta F\)) were measured (Fig. 1). Two chirps, each of different frequencies, were administered during two sequential time periods. The first
frequency sweep during a period of 40 s emphasized low frequencies (0.1 to 2 Hz) and the
second frequency sweep during a period of 5 s emphasized high frequencies (1 to 40 Hz).
Representative muscle fiber force and length data obtained from a normal rat cardiac muscle
fiber are shown in Figs. 1A and 1B. Note the variation in force amplitude as frequency changed.
Especially, note the transition from decreasing amplitude to increasing amplitude at
approximately 1 Hz (arrow location) in the low-frequency chirp.

Myofiber force records in response to dynamic changes in length were analyzed using a
recruitment-distortion model of muscle contraction that we have used successfully in previous
studies (5). However, the model for these data analyses was modified slightly from what we
have used in the past because we consistently observed a small but systematic error in the fit by
the original model to forces associated with length changes at the highest frequencies, i.e., at
frequencies approaching 40 Hz. In brief, the model allowed prediction of change in force, \( \Delta F(t) \),
in a constantly activated muscle fiber in response to a change in its length, \( \Delta L(t) \), by the
following equation:

\[
\Delta F(t) = \frac{E_0 \eta(t)}{\text{recruitment}} + \frac{E_\infty x_1(t)}{\text{distortion 1}} + \frac{D x_2(t)}{\text{distortion 2}}
\]

where \( \eta(t) \) is a variable expressing the dynamic changes in XB recruitment with changes in
length, \( x_1(t) \) and \( x_2(t) \) are each XB distortion variables, and \( E_0, E_\infty, \) and \( D \) are scaling coefficients.

The recruitment variable, \( \eta(t) \), changes dynamically in response to \( \Delta L(t) \) according to the
differential equation:

\[
\frac{d\eta(t)}{dt} = -b[\eta(t) - \Delta L(t)]
\]
where \( b \) is the rate constant governing the speed of XB recruitment. The physical units of \( \eta(t) \) in Eq. A.2 are those of length. Thus, the scaling coefficient, \( E_0 \), possesses units of stiffness resulting in the \( E_0 \eta(t) \) product possessing units of force. This product is proportional to the incremental addition of XB to the force-bearing population following an incremental increase in muscle length, \( \Delta L(t) \), and, concomitantly, the incremental removal of XB from the force-bearing population following an incremental decrease in \( \Delta L(t) \). The nature of the differential Eq. A.2 is such that the amplitude of \( \eta(t) \) variations falls as the frequency of \( \Delta L(t) \) increases. The frequency at which the amplitude falls is determined by \( b \). The consequence is that \( \Delta L(t) \) recruits its full complement of XB to enhance force at slow frequencies (i.e., at frequencies approaching zero and \( \ll b \)) but does not recruit new force-bearing XB into the force-bearing pool at high frequencies (i.e., \( \gg b \)). This imparts to \( E_0 \) the meaning that it is the slope of the static force-length relationship and is a measure of the number of new XB added to the force-bearing population per unit increase in length under static conditions.

The XB distortion variables, \( x_1(t) \) and \( x_2(t) \), each change dynamically in response to the first time derivative of muscle length, \( \frac{dL(t)}{dt} \), according to:

\[
\frac{dx_1(t)}{dt} = -cx_1(t) + \frac{dL(t)}{dt} \quad (A.3)
\]

\[
\frac{dx_2(t)}{dt} = -dx_2(t) + \frac{dL(t)}{dt} \quad (A.4)
\]

where \( x_1(t) \) represents the average length-induced distortion of elastic regions of strong-binding (or force-bearing) XB and \( x_2(t) \) represents the average length-induced distortion of elastic regions of weak-binding (not force-bearing under isometric conditions) XB. Parameter \( c \) is the rate
constant governing XB distortion dynamics of strong-binding XB and parameter $d$ is the rate
constant governing distortion dynamics of weak-binding XB. The differential equations for $x_1(t)$
and $x_2(t)$ are such that the amplitude of $x_1(t)$ and $x_2(t)$ variations will increase from zero up to a
plateau as the frequency of $\Delta L(t)$ increases. The frequency at which the amplitude transition
from low to high is most steep is determined by $c$ and $d$ for $x_1(t)$ and $x_2(t)$, respectively. The
physical units of both $x_1(t)$ and $x_2(t)$ are those of length. Accordingly, the coefficients $E_\infty$ and $D$
in the force Eq. A.1, possess units of stiffness. These coefficients are proportional to the number
of XBs in the respective states.

The interpretation of $E_0$ as a measure of the number of new XB added to the force-bearing
XB population per unit increase in length and of $E_\infty$ as a measure of the number of XB in the
force-bearing state at the current length suggests that the ratio, $E_0/E_\infty$, is a useful index of the
fractional change in XB with a change in $\Delta L(t)$. A difference in the $E_0/E_\infty$ ratio between muscle
fibers with different contractile protein profiles would indicate a difference in the manner in
which those contractile proteins interacted during length-mediated changes in contraction.

The model was fit to the entire $\Delta F(t)$ record of Fig. 1A using $\Delta L(t)$ as an input and nonlinear
regression techniques to minimize the sum of square errors between model-predicted and
measured $\Delta F(t)$ signal. Model reproduction of the $\Delta F(t)$ in Fig. 1A and the decomposition of the
prediction in terms of the three components in Eq. A.1 are given in Figs. 1C, 1D and 1E. The
XB recruitment component of the model, $E_0 \eta(t)$, dominated the response at the lowest
frequencies ($< 1$ Hz), but its contribution to the total response progressively decreased as
frequency increased. The $x_1(t)$-XB distortion component, $E_\infty x_1(t)$, dominated the response at
frequencies $> 2$ Hz and its contribution rose until reaching a near plateau at the 40 Hz end of the
second chirp. The $x_2(t)$-XB distortion component, $Dx_2(t)$, made only small contributions to the
force response and this contribution was evident only at the highest frequencies. The effect of adding the $x_2(t)$-distortion component to the model was to minimize a small but systematic error in the model prediction of force at the highest frequencies examined in this study. In terms of reducing the un-accounted-for variation in the data by the model, the addition of the $x_2(t)$-distortion component resulted in an increase in the $R^2$ from ~0.98 to ~0.99. Because the $x_2(t)$-distortion component contributed little to the prediction of the force response and because the values of parameters $E_0$, $b$, $E_{\infty}$, $c$ were largely unaffected whether or not the $x_2(t)$-distortion component was made part of the model, we report only the $E_0$, $b$, $E_{\infty}$, $c$ parameters and do not include $D$ and $d$ values in this report. Future studies that emphasize frequencies $> 40$ Hz will require that the $x_2(t)$-distortion component be part of the model to account for the data and to allow for accurate parameter estimates. Model parameters ($E_0$, $b$, $E_{\infty}$, $c$) were typically estimated with less than 1% standard error indicating that these estimated parameter values were unambiguous and reliable for comparing one muscle fiber with another.

**Polyacrylamide gel electrophoresis:** Protein samples from muscle fibers were prepared and Western blot analysis performed, as previously described (7, 23). For Western blot analysis, proteins were transferred onto the PVDF membrane and probed (23, 33) using an anti-mouse primary antibody against the human $c$-myc epitope (Fitzgerald, 10-T85) or an antibody against cTnT (Satacruz, SC-40 HRP). MHC from normal and PTU-treated mouse and rat heart left ventricles were prepared and run on 8% SDS-PAGE (27).

**Protein sequence comparison:** Protein sequence alignment and comparisons were performed using the ClustalW multiple sequence alignment program on the BCM search launcher, Baylor
College of Medicine website (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html)

Mouse β-MHC (accession # Q91Z83), rat α-MHC (accession #P02563), rat β-MHC (accession #P02564) and mouse α-MHC (accession #Q02566), rat cTnT (accession #P50753), rat cTnI (accession #P23693), rat cTnC (accession #Q4PP99), mouse cTnT (accession #P50752), mouse cTnI (accession #P48787) were from the Swiss Bank.

**Data analysis:** Contractile function parameters (Ca$^{2+}$-activated tension, Ca$^{2+}$-activated maximal ATPase activity, tension cost) and mechano-dynamic parameters (model parameters $E_{0}$, $b$, $E_{\infty}$, $c$ and the non-model-dependent parameter, $k_{tr}$) of unmodified fibers from mouse and rat hearts, including normal and PTU-treated animals, were compared using two-way analysis of variance (ANOVA), where one factor was species and the second was PTU treatment. In reconstituted muscle fibers from normal and PTU-treated mouse and rat hearts, contractile-function parameters and mechano-dynamic parameters were compared with respect to the effect of reconstitution with mouse Tn vs. rat Tn. Analysis was by three-way ANOVA with one factor being cardiac Tn (mouse Tn or rat Tn), the second factor being species and the third factor being PTU treatment (α-MHC or β-MHC). Our analysis focused on the main effect due to reconstitution with mouse Tn vs. rat Tn and the two-way interaction effect due to the influence of MHC background on effects attributable to species-specific Tn reconstitution. In addition, a three-way interaction represented species difference in the two-way interaction of MHC background (i.e., PTU treatment) and species-specific Tn reconstitution. When this three-way interaction was significant, separate two-way ANOVA was then performed to dissect the PTU and species-specific troponin effects within both rats and mice. When the interaction effects in these two-way ANOVA were significant, main effects were not interpreted and multiple pairwise
Bonferroni-corrected $t$-test was used to compare Tn-reconstitution treatment groups (normal or PTU treated). On the other hand, if the three-way interaction was not significant, then we concluded that the responses to PTU and/or species-specific Tn reconstitution were similar for both rat and mouse fibers, and the two-way interactions of species-specific Tn reconstitution and PTU effects were interpreted in the three-way ANOVA. Data from the normalized pCa-tension measurements were fitted to the Hill equation using a nonlinear least-square regression procedure to obtain the pCa$_{50}$ (pCa required for half maximal activation) and the Hill coefficient ($n$). pCa$_{50}$ and $n$ were determined separately from each muscle fiber experiment and the values were averaged. Data are expressed as mean ± SEM.
RESULTS

The high heart rate of the mouse requires faster rate of pressure generation, velocity of shortening, and rate of relaxation, compared to the rat and other larger mammals (1, 12, 13, 18, 32). We hypothesize that coordinated expression of species-specific regulatory contractile protein isoforms (MHC and Tn) bring about a dynamic complementarity such that contraction speed is well matched to species-specific heart rates. Therefore, we studied: 1) muscle fibers from native mouse and rat hearts to show how contractile function is affected by different isoforms of α-MHC; 2) muscle fibers from PTU-treated mouse and rat hearts to show how contractile function is affected by different isoforms of β-MHC; and 3) the effect of homologous and orthologous Tn on speed of contraction in fibers from both normal (α-MHC) and PTU-treated (β-MHC) mouse and rat hearts to understand how altered MHC-Tn interactions affect contractile dynamics.

Myofiber mechano-dynamics and contractile function of muscle fibers from mouse and rat hearts: Because mouse and rat cardiac α-MHC differ by 28 amino acids (21), we first compared mechano-dynamic parameters and contractile function differences between normal mouse and rat cardiac muscle fibers (Table 1). Normal mouse and rat cardiac muscle fibers differed in contractile parameters. Ca\(^{2+}\)-activated maximal tension was approximately 10\% lower in the mouse than in the rat (\(P = 0.01\); Table 1). In contrast, the Ca\(^{2+}\)-activated maximal ATPase activity in the mouse was 27\% higher than that in the rat (\(P < 0.001\); Table 1). This combination of higher ATPase activity and lower tension resulted in the higher tension cost in the mouse. We determined the tension cost from the mean slope of the tension-ATPase relationship of several muscle fibers (9, 34). The tension cost in the mouse was 51\% higher than that in the rat (\(P <
0.001; Table 1). Myofilament Ca$^{2+}$-sensitivity, as measured by pCa$_{50}$ from the pCa-tension relationship, was not significantly different between normal mouse and rat fibers (Table 1). Myofilament cooperativity, as measured by the Hill coefficient ($n$), in the mouse was significantly lower than that in the rat ($P < 0.001; Table 1$).

Major differences in mechano-dynamic parameters between normal mouse and rat cardiac muscle fibers were observed as described below. Muscle length-mediated XB recruitment of mouse fibers was dramatically faster than that of rat fibers. For example, the model-estimated rate constant of muscle length-mediated XB recruitment ($b$) was 3.5-fold greater in the mouse than in the rat ($P < 0.001; Table 1$). Thus, the rate by which XB is recruited from a noncycling (weak XB) pool to the cycling pool (strong XB) is faster in the mouse than in the rat. Consistent with this model-based mechano-dynamic assessment, the non-model-dependent variable, $k_{tr}$, was 67% faster in the mouse ($P < 0.001; Table 1$). The $k_{tr}$ is a measure of the rate by which XB are recruited from a pool of non-force bearing to force-bearing state (2). Recovery from the length-mediated XB distortion occurred more rapidly in the mouse than in the rat cardiac muscle fiber. The model-estimated rate constant of XB distortion ($c$) was 15% higher in the mouse ($P < 0.02; Table 1$). Because both the tension cost and $c$ reflect the rate constant of XB detachment (2, 17), our observations suggest that the kinetics of XB detachment are faster in the mouse. Thus, both the model-dependent and non-model-dependent estimates show that the normal mouse cardiac muscle fiber is faster in all kinetic and dynamic dimensions than the normal rat cardiac muscle fiber. Because the speed of length-mediated XB recruitment was significantly different in mouse and rat fibers, we wanted to test if the magnitude of length-mediated XB recruitment in mouse and rat cardiac muscle fibers was different. The magnitude of length-mediated XB recruitment can be seen in the effect on $E_0/E_{\infty}$ ratio. $E_0/E_{\infty}$ is a useful
index of the fractional change in XB with a change in $\Delta L(t)$ because $E_0$ is a measure of the number of new XB added to the force-bearing XB population per unit increase in length and $E_\infty$ is a measure of the number of XB in the force-bearing state at the current length. The magnitude of length-mediated XB recruitment in mouse and rat cardiac muscle fibers is listed in Table 1. Roughly speaking, the ratio of $E_0/E_\infty$ expresses the number of new XB added to the force-bearing XB population per unit increase in length, relative to the number of force-bearing XB prior to length change. Differences in $E_0/E_\infty$ between mice and rat was marginally significant ($P = 0.08$).

Next, we compared mechano-dynamic parameters and contractile function differences between muscle fibers from PTU-treated mouse and rat hearts, because mouse and rat cardiac $\beta$-MHC differ by 15 amino acids (21). PTU treatment shifted the MHC composition in both rat and mouse cardiac muscle fibers to 100% $\beta$-MHC. An $\alpha$- to $\beta$-MHC isoform shift in both mouse and rat is shown in Figs. 2B and 2C, respectively. After a shift from $\alpha$- to $\beta$-MHC, Ca$^{2+}$-activated maximal tension in mouse and rat cardiac muscle fibers was not affected significantly (Tables 1 and 2). An $\alpha$- to $\beta$-MHC isoform shift reduced the Ca$^{2+}$-activated maximal ATPase activity in both mouse and rat fibers by more than two-fold ($P < 0.001$, Tables 1 and 2) with a larger reduction in the mouse than in the rat ($P < 0.01$). Although ATPase activity in mouse fibers was 18% higher than in rat fibers after the MHC isoform was shifted from $\alpha$- to $\beta$-MHC, this was not significant ($P = 0.41$). This 18% increase in ATPase activity resulted in the tension cost in $\beta$-MHC-containing mouse fibers being higher than that in the rat, indicating that the kinetics of XB detachment continued to be faster in the mouse. Thus, the mouse fiber continued to be faster in most kinetic and dynamic dimensions than the rat fiber even after the shift from $\alpha$- to $\beta$-MHC. Myofilament Ca$^{2+}$ sensitivity (pCa$\text{50}$) in mouse and rat fibers were not affected.
significantly by β-MHC (Tables 1 and 2). The Hill coefficient \( n \) remained significantly lower in β-MHC-containing mouse fibers than in β-MHC-containing rat fibers \( (P < 0.001; \text{ Table 2}) \).

Mechano-dynamic parameters and contractile function of β-MHC-containing fibers from mouse and rat hearts are summarized in Table 2. Relative to normal animals, all three mechano-dynamic rate constants \( (b, c, k_{tr}) \) in fibers from PTU-treated animals were reduced by a factor of \(~2\) (Tables 1 and 2). However, the dramatic differences in the rate constant of muscle length-mediated XB recruitment between mouse and rat fibers continued to persist. For example, the dynamic rate constant \( b \) in β-MHC-containing mouse fibers was 3.4-fold faster than β-MHC-containing rat fibers (Table 2). There was no significant difference in \( k_{tr} \) measured in fibers from PTU treated mice and rats. After PTU treatment, the species difference in length-mediated XB distortion \( (c) \) continued to be higher in the mouse than in the rat by about the same amount (Table 2). Finally, although the difference in the tension cost between mouse and rat was reduced in β-MHC-containing muscle fibers \( (P < 0.001) \), the mouse remained ~28% higher than rat \( (P < 0.005; \text{ Table 2}) \). The effect of PTU on \( E_\ell/E_x \) in mice vs. rat fibers was nonsignificant \( (P = 0.28; \text{ Table 2}) \). Species-PTU interaction effect was significantly different in mouse vs. rat fibers \( (P < 0.001 \text{ for two-way interaction}) \). This suggests that the effect of species-specific regulatory proteins on \( E_\ell/E_x \) was different with different MHC background and this effect in mouse fiber was opposite to that of rat fiber (Tables 1 and 2).

Despite the β-MHC-induced slowing of both XB recruitment and XB distortion dynamics in mouse and rat cardiac muscle fibers (Tables 1 and 2), major differences in XB recruitment dynamics between mouse and rat still persisted. This indicated that a regulatory contractile protein, other than the MHC alone, participated in promoting much faster dynamics in the mouse after the MHC isoform was shifted from the faster to the slower isoform. Because the XB
recruitment response is significantly affected by an interplay between Tn actions on thin filament activation and XB cycling kinetics (6, 9, 14, 34), our expectation is that species-dependent sequence heterogeneity in Tn is also involved in regulating speed of cardiac muscle contraction.

**Effect of cardiac Tn switching on contractile function and myofiber mechano-dynamics:**

Of the three subunits in the cardiac Tn complex, cTnT is the most dissimilar between mouse and rat. There are 15 amino acid differences between mouse and rat cTnT, two amino acid differences between mouse and rat cTnI, and no differences between mouse and rat cTnC. Contractile functions and mechano-dynamic parameters were measured in muscle fibers from both normal and PTU-treated mouse and rat hearts after reconstitution with either recombinant homologous Tn (i.e., from the same species) or orthologous Tn (i.e., from the other species). As we have demonstrated previously, the endogenous Tn complex is removed as a whole (7, 9) by the exogenously added Tn. To verify exchange of Tn, we examined the incorporation of c myc-tagged cTnT in reconstituted mouse and rat cardiac muscle fibers (Fig. 3A). No immunoreactivity was evident for the endogenous cTnT in reconstituted muscle fibers (Fig 3B), demonstrating that the endogenous cTnT was completely replaced by c myc-tagged cTnT. The residual tension (pCa 4.3) in both mouse rat cardiac muscle fibers was minimal (~1.0 mN mm⁻²), suggesting that the endogenous Tn was removed. Comparisons of mechano-dynamic and contractile function parameters were made with respect to the effects of mouse Tn vs. rat Tn in reconstituted cardiac muscle fibers from both normal (α-MHC) and PTU-treated (β-MHC) animals. As described in the methods section, analysis was by three-way ANOVA with one factor being cardiac Tn (mouse Tn or rat Tn), the second factor being species and the third factor being PTU treatment (α-MHC or β-MHC). When this three-way interaction was significant,
separate two-way ANOVA was performed to dissect the PTU and species-specific troponin effects within both rats and mice.

\[ \text{Ca}^{2+} \text{-activated maximal tension} \]

Ca\[^{2+}\]-activated maximal tension in Tn reconstituted muscle fibers from normal (\(\alpha\)-MHC) and PTU-treated (\(\beta\)-MHC) mouse and rat hearts are listed in Tables 3 and 4, respectively. As was the case for fibers from normal mouse and rat hearts (Table 1), Ca\[^{2+}\]-activated maximal tension in Tn-reconstituted mouse fibers was generally lower than that in Tn-reconstituted rat fibers regardless of the type of Tn exchanged (Tables 3 and 4). The three-way interaction was significant \((P = 0.001)\), meaning that the species-specific Tn interaction with MHC background in rat fibers was different than that in mouse fibers. Separate two-way ANOVA showed that the effect of species-specific Tn reconstitution on Ca\[^{2+}\]-activated tension in \(\alpha\)-MHC background was opposite to the effect in \(\beta\)-MHC background in both mouse and rat fibers; the Tn-MHC interaction effect was statistically significant in both mouse \((P < 0.05)\) and rat fibers \((P < 0.01)\).

The three-way interaction effect for pCa\(_{50}\) was not significant \((P = 0.32)\), meaning that the effect was similar in both mice and rats (Fig. 4; Tables 3 and 4). The two-way species specific Tn-MHC interaction effect was significant \((P < 0.03)\), which suggested that Tn impact on pCa\(_{50}\) was affected differently by \(\alpha\)- and \(\beta\)-MHC isoforms. The Hill coefficient \((n)\) in mouse fibers reconstituted with either the mouse or rat Tn was lower than that in rat fibers (Tables 3 and 4) regardless of the type of Tn exchanged. Because the three-way interaction was not significant \((P = 0.35)\), the species-specific Tn-MHC interaction was similar in fibers of both species.

Averaged across fibers of both species, the species-specific Tn-MHC interaction in the three-way ANOVA was borderline significant \((P = 0.08)\). Although rat Tn increased \(n\) in both \(\alpha\)-MHC and \(\beta\)-MHC-containing mouse fibers, the magnitude of this increase was larger in \(\beta\)-MHC-containing
mouse fibers (Tables 3 and 4); taken together, these results suggest that the effect of species-specific Tn on $n$ increased after a switch from $\alpha$- to $\beta$-MHC.

As in native $\alpha$-MHC (Table 1) and $\beta$-MHC-containing (Table 2) mouse cardiac muscle fibers, Ca$^{2+}$-activated maximal ATPase activity in Tn-reconstituted mouse cardiac muscle fibers was generally higher than that in Tn-reconstituted rat cardiac muscle fibers (Tables 3 and 4). The species-specific Tn-PTU interaction was different in mouse fibers vs. rat fibers ($P < 0.001$ for three-way interaction). Separate two-way ANOVA for rat and mouse fibers showed a significant species-specific Tn-MHC interaction effect for both mouse ($P < 0.05$) and rat ($P < 0.001$) fibers. Taken together these results suggest that in both species the effect of Tn on Ca$^{2+}$-activated maximal ATPase activity was different with different MHC isoforms, and that this effect in mouse fibers was opposite the effect in rat fibers.

Because Ca$^{2+}$-activated maximal ATPase activity in Tn-reconstituted mouse cardiac muscle fibers (Tables 3 and 4) was significantly higher than that in Tn-reconstituted rat cardiac muscle fibers, we wanted to know not only whether orthologous Tn had any effect on the tension cost, but also whether the Tn effect was modulated by different MHC backgrounds in both mouse and rat fibers. After reconstituting with either the homologous Tn or the orthologous Tn, the tension cost was measured in mouse (Fig. 5A) and rat (Fig. 5B) cardiac muscle fibers from both normal ($\alpha$-MHC) and PTU-treated ($\beta$-MHC) animals. Because the three-way interaction was not significant ($P = 0.72$), the species-specific Tn-MHC interaction was similar in fibers of both species. The species-specific Tn-MHC interaction in the three-way ANOVA was also not significant ($P = 0.89$). The main effect of MHC was significant ($P = 0.05$), but the main effect of species-specific Tn reconstitutions was not ($P = 0.35$). Thus, the balance of effects is such that
tension cost was significantly lower in β-MHC containing fibers, regardless of which species fibers came from or the species-specific source of Tn (Tables 3 and 4).

Next, we compared the tension cost results with the rate constant of the length-mediated XB distortion dynamic ($c$), because the dynamic rate constant $c$ also has a strong dependence on the rate of XB detachment kinetics (5). The rate constant $c$ of mouse and rat cardiac muscle fibers reconstituted with different Tns (in both normal and PTU-treated groups) is listed in Tables 3 and 4, respectively. These results are largely in agreement with the tension cost results. Although the pattern of effect on $c$ is the same as observed with tension cost, the three-way interaction was borderline significant ($P = 0.07$). Subsequent two-way ANOVA showed that in mouse fibers, neither the main effect of Tn ($P = 0.59$) nor the interaction effect ($P = 0.71$) were significant (Fig. 5C); whereas in rat fibers, the interaction effect was significant ($P = 0.02$), indicating that the effect of species-specific Tn on XB detachment kinetics in rat fibers did depend on the MHC background (Fig. 5D). Overall the data suggests that mouse Tn increased the rate of XB detachment kinetics in rat fibers when α-MHC was present and, conversely, decreased the rate of XB detachment kinetics in rat fibers when β-MHC was present.

Because major differences in length-mediated XB recruitment rate ($b$) between mouse and rat cardiac muscle fibers (Tables 1 and 2) still persisted even after the MHC isoform was shifted from α-MHC (fast) to β-MHC (slow), we wanted to determine if species-dependent differences in Tn were contributing to this faster recruitment dynamic in the mouse. Therefore, we tested the effects of homologous and orthologous Tn on the speed of XB recruitment against a background of either α-MHC or β-MHC isoform. The rate constant $b$ in mouse and rat fibers reconstituted with different Tns in both normal and PTU-treated groups is listed in Tables 3 and 4, respectively. Neither the three-way interaction nor the two-way species-specific Tn-MHC
interaction were significant ($P = 0.22$ and $P = 0.13$, respectively). The single most profound impact of Tn replacement in both mouse and rat fibers was the effect of orthologous Tn on $b$. Whether fibers were from normal ($\alpha$-MHC; Table 3) or PTU-treated ($\beta$-MHC; Table 4) rats or mice, rat Tn significantly decreased $b$ compared to mouse Tn ($P < 0.01$, Fig. 6A). Conversely, $b$ was significantly higher when mouse Tn was reconstituted in rat fibers ($P < 0.01$, Fig. 6B).

Thus, regardless of species of fiber origin or MHC background, the speed of XB recruitment is slower when regulated by rat Tn and is faster when regulated by mouse Tn. Although not statistically significant, the effects of Tn on $b$ were larger in $\beta$-MHC-containing fibers from both mouse and rat: in mouse fibers reconstituted with rat Tn, 7% lower when $\alpha$-MHC was present vs. 53% lower when $\beta$-MHC was present; in rat fibers reconstituted with mouse Tn, 30% higher when $\alpha$-MHC was present vs. 84% higher when $\beta$-MHC was present.

Because orthologous Tn had a significant impact on the rate of XB recruitment ($b$), we wanted to test whether the model-independent variable $k_{tr}$ (rate constant of force redevelopment) was also affected by the type of Tn. The $k_{tr}$ in mouse and rat cardiac muscle fibers reconstituted with different Tns (in both normal and PTU-treated groups) is listed in Tables 3 and 4, respectively. The species-specific Tn-MHC interaction differed in mouse fibers vs. rat fibers (three-way interaction was significant; $P < 0.01$). Separate two-way ANOVA then showed that in reconstituted mouse fibers, the magnitude of the small decrease in $k_{tr}$ induced by rat Tn was similar in both $\alpha$- and $\beta$-MHC-containing fibers (Fig. 6C). Thus, both the main effect ($P = 0.43$) and the species-specific Tn-MHC interaction ($P = 0.11$) were not significant in mouse fibers (Table 3 and 4). In contrast, this interaction effect was significant in rat fibers ($P < 0.01$; Table 3 and 4), suggesting that the effect of species-specific Tn reconstitution on $k_{tr}$ depended on the MHC isoform in rat fibers (Fig. 6D). When $\alpha$-MHC was present, $k_{tr}$ was higher in rat fibers.
reconstituted with mouse Tn. Conversely, when β-MHC was present, $k_{tr}$ was lower in rat fibers reconstituted with mouse Tn.

In addition to the effect on the speed of length-mediated XB recruitment, orthologous Tn also affected the magnitude of length-mediated XB recruitment in both mouse and rat cardiac muscle fibers. This effect of rat Tn on the magnitude of length-mediated XB recruitment can be seen in the effect of Tn replacement in mouse and rat fibers on the $E_0/E_\infty$ ratio (Tables 3 and 4). In general, $E_0/E_\infty$ was greater in fibers with rat Tn than in those with mouse Tn (Fig. 7). Because the three-way interaction was not significant ($P = 0.47$), the species-specific Tn-MHC interaction was similar in fibers of both species. The effect of rat Tn on $E_0/E_\infty$ was larger in the face of β-MHC vs. α-MHC (the species-specific Tn-MHC interaction in the three-way ANOVA was significant ($P < 0.05$)). Except for the normal mouse fiber, on an average $E_0/E_\infty$ was ~0.20 in the presence of mouse Tn and ~0.25 in the presence of rat Tn, representing a 25% increase in XB recruitment with rat Tn.

Small but significant differences in some of the contractile function parameters were observed when data from mouse normal fibers reconstituted with mouse Tn (Table 3) were compared with those of normal untreated mouse fibers (Table 1). On the other hand, muscle fiber mechanics, such as length-dependent XB recruitment rate ($b$) and XB distortion rate ($c$), showed no apparent changes in mouse normal fibers reconstituted with mouse Tn. Because the comparison of mechano-dynamic and contractile function parameters were made with respect to the effects of mouse Tn vs. rat Tn in reconstituted cardiac muscle fibers from both normal (α-MHC) and PTU-treated (β-MHC) animals, we do not expect that these small differences will affect our conclusion about the effects of homologous and orthologous Tn on muscle fiber mechanics.
DISCUSSION

The high heart rate of mice requires fast myocardial contractile dynamics to produce fast rates of pressure generation, shortening and relaxation. These dynamic dimensions of functional differences would be expected to compliment the species-specific resting heart rate (600 min\(^{-1}\) in the mouse vs. 300 min\(^{-1}\) in the rat) because the dynamic response of cardiac muscle contraction involves interactive feedback between the thin-filament-activating effects of Tn and MHC cycling kinetics (4, 6, 14, 26). Therefore, changes in the composition of MHC and Tn, as in hearts of different species, will likely impact the overall dynamic response of the heart. We hypothesize that sequence differences between orthologous MHC and Tn in mice and rats confer selective changes in myofilament dynamics that are consistent with the need for different speeds of contractile function in these two species.

Sequence heterogeneity in cardiac MHC isoforms: implications for species-dependent changes in speeds of XB recruitment and XB detachment dynamics: We imposed small amplitude length changes in maximally Ca\(^{2+}\)-activated muscle fibers to elicit mechano-dynamic behaviors in cardiac muscle fibers from mice and rats. In fibers from both species, the mechano-dynamic behaviors exhibited two distinct processes: a XB distortion dynamic (fast process), which is determined principally by the enzymatic kinetics of MHC, and a XB recruitment dynamic (slow process), which is affected greatly by cooperative interactions between Tn actions and XB cycling kinetics (3, 5, 30). In accord with our expectations, we found that the normal mouse cardiac fiber was faster in all kinetic and dynamic dimensions than the normal rat cardiac muscle fiber (Table 1). Specifically, the rate constants of length-mediated XB recruitment \((b)\) and force redevelopment \((k_{tr})\) of mouse fibers were significantly faster, concordant with our
expectation that rapidly beating hearts of mice require faster rates of contractile activation and pressure development. In addition, rapidly beating hearts of mice have shorter systolic periods, which require increased velocity of myofilament shortening and mechanical relaxation. Our observation that XB detachment rate was significantly faster in the mouse than in the rat is consistent with this idea. For example, the tension cost measurements and model-dependent rate constant of length-mediated XB distortion \(c\) of mouse fibers were significantly faster than those of rat fibers (Table 1). The tension cost and \(c\) (both proportional to XB detachment rate constant \(g\)), are principally determined by the MHC enzyme kinetics. Thus, a significant difference in the ATPase activity of mouse and rat \(\alpha\)-MHC is a key element in determining species-dependent changes in rates of XB recruitment and XB detachment dynamics.

Different ATPase activity of mouse and rat cardiac \(\alpha\)-MHC would be expected to result from species-dependent differences in the primary structures of MHC isoforms. Whereas both mice and rats express predominantly the \(\alpha\) isoform of MHC \((\sim 100\% \alpha\)-MHC in the mouse; \(\sim 90\% \alpha\)-MHC in the rat), there are substantial amino acid sequence differences in the \(\alpha\)-MHC orthologs of these two species; that is, rat and mouse \(\alpha\)-MHC differ by 28 amino acids. Of these 28 amino acids, 10 are in the heavy chain S1 head region. None of the differences are in the structural loops (25) that are thought to be important for modulating the kinetics of acto-myosin interactions. The converter domain, a key element that experiences elastic distortion, is similar in the mouse and rat \(\alpha\)-MHC except for one charge substitution at position 740 (Gly to Arg in the mouse). Thus, it is likely that one source for differences in contractile mechano-dynamics in these species lies in differences in the enzymatic kinetics of the species-specific \(\alpha\)-MHC.

Sequence heterogeneity in \(\beta\)-MHC of mouse and rat also resulted in significant differences in contractile function between the two species (Table 2). Concordant with the shift
to the slower \( \beta \)-MHC isoform, there was a marked slowing of XB recruitment and XB detachment dynamics in both mouse and rat fibers (Tables 1 & 2). Importantly, however, there remained a 3.4-fold difference in the rate constant for XB recruitment dynamics. This implies that, in addition to the role of MHC to determine XB recruitment dynamics, another contractile regulatory protein complex participated in promoting a much faster dynamic in the mouse than in the rat after MHC was shifted from the faster \( \alpha \) to the slower \( \beta \)-MHC isoform. Species-related differences in the speed of XB recruitment dynamics may be significantly impacted by the Tn complex, because a well coordinated interplay between Tn and XB cycling kinetics affect XB recruitment dynamics by modulating XB recruitment response (6, 9, 14, 34). For example, we recently showed that changes in the composition of cardiac Tn affected the speed of XB recruitment dynamics in rat cardiac fibers (9) and the impact of mutant cTnT on both the tension cost and XB distortion dynamics were significantly altered by a shift from an \( \alpha \)- to \( \beta \)-MHC isoform (34). Thus, species-related changes in the primary structure of Tn may also play a role in conferring selective advantages that are consistent with the need for different speeds of myofilament function in hearts of different species.

**Sequence heterogeneity in cardiac Tn isoforms: implications for species-dependent changes in XB recruitment dynamics:** Comparison of cTnT sequence from the mouse and rat hearts revealed 15 amino acid differences. Fourteen of these substitutions are in the N-terminus of cTnT, which is thought to be important for regulating the size of the thin filament functional unit (14, 29). There are only two amino acid differences between mouse and rat cTnI, but both of these are charge substitutions: Ser78 and Val85 in rat cTnI are substituted by Arg and Glu in the mouse. The functional effects of these differences became evident either when rat Tn was
substituted for mouse Tn in mouse fibers or when mouse Tn was substituted for rat Tn in rat fibers. Whether in mouse or rat fibers, the XB recruitment dynamic was faster when regulated by mouse Tn than when regulated by rat Tn (Fig. 6). These results demonstrate that the Tn complex plays a significant role in regulating the speeds of XB recruitment in different species. Thus, changes in the primary structure of subunits of the Tn complex, as occurs in orthologous forms of Tn between species, would have a functionally important effect on cardiac myofiber mechano-dynamics.

In cardiac myofilaments, cooperatively-coupled effects of XB-Tn and XB-XB affect XB recruitment dynamics by modifying XB recruitment responses to changes in muscle length (6). For example, the XB recruitment rate constant $b$ is slowed when the myofilament cooperativity is more prominent, whereas $b$ is speeded when the myofilament cooperativity is less prominent. In mouse and rat fibers, cooperatively-coupled effects on the recruitment dynamics may be different due to variation in either MHC or Tn or both. The Hill coefficient, $n$, which is an aggregate measure of cooperativity in $\text{Ca}^{2+}$ binding to Tn and near-neighbor interactions between Tn-Tn, XB-Tn, and XB-XB (6, 26), was significantly lower in mouse fibers (Tables 1-4).

Because XB-related cooperativity has the greatest impact on length-mediated XB recruitment dynamics (6), subtle changes in the strength of such cooperativity could have major implications for differences in the XB recruitment dynamics of mouse and rat cardiac muscle fibers.

In conclusion, our data demonstrate that normal mouse cardiac myofilaments are significantly faster than normal rat cardiac myofilaments in all kinetic and dynamic dimensions. Species-related differences in the primary structures of MHC and Tn are likely to be responsible for these different myofiber system mechano-dynamics. Length-mediated XB recruitment dynamics in mouse fibers is dramatically faster than that of rat fibers. Part, but not all, of this
difference in the speed of XB recruitment between the mouse and rat is due to the effect of Tn. Moreover, the effect of rat Tn to decrease the speed of XB recruitment in mouse fibers or, conversely, the effect of mouse Tn to increase the speed of XB recruitment in rat fibers is influenced strongly by the interaction of MHC kinetics with the thin-filament activating effects of Tn. Our data suggest that orthologous proteins have evolved in different species to bring about dynamic complementarity among the several kinetic processes that underlie cardiac muscle contraction so that muscle contraction speeds complement species-specific heart rates.
ACKNOWLEDGEMENTS

This work was supported by the grants R01 HL075643-02 from the National Institute of Health (MC) and 0555548Z from the American Heart Association (KBC). We would also like to thank Robert Kirkpatrick for his support with the instrumentation and software programming.
REFERENCES


FIGURE LEGENDS

Figure 1. Measurement and model prediction of force response, \( \Delta F(t) \), to muscle length change, \( \Delta L(t) \). Panel A: Measured \( \Delta F(t) \) (light gray trace) is in response to double-chirp \( \Delta L(t) \) protocol shown in Panel B. Model-predicted \( \Delta F(t) \) (black trace) is overlaid on measured \( \Delta F(t) \) and distinction between these two traces can not be made at this scale (\( R^2 \) of fit = 0.99). Panel B: \( \Delta L(t) \) was administered in two chirp sequences: from 0.1 to 2 Hz over 40 s in chirp 1 and from 1 to 40 Hz over 5 s in chirp 2. Panels C-E: Decomposition of model prediction in terms of the three components in Eq. A.1. Panel C. The XB recruitment component \( (E_0 \eta(t)) \) dominated the force response at lowest frequencies (< 1 Hz) but its contribution decreased as the frequency of \( \Delta L(t) \) increased. Panel D. The strong-XB distortion component \( (E_\infty x_1(t)) \) made only small contributions at low frequencies but dominated the force response at frequencies above 2 Hz where it reached a plateau early (~10 Hz) during chirp 2. Panel E. The weak-XB distortion component \( (D x_2(t)) \) made only minor contributions to the force response, which was evident only at the highest frequencies. Arrows indicate a landmark transition in \( \Delta F(t) \) amplitude and phase where the dominance of the XB recruitment component in the \( \Delta F(t) \) response gives way to dominance of the strong-XB distortion component (5).
**Figure 2. SDS-PAGE analysis of proteins from cardiac muscle fibers.** Muscle protein preparation for gel analysis and SDS-PAGE were performed as described previously (7, 23). 

A. Analysis of MHC composition from the left ventricle of normal mouse and rat hearts. 

B. MHC from the left ventricle of normal mouse and PTU-treated mouse hearts. 

C. MHC from the left ventricle of normal rat and PTU-treated rat hearts. In both B and C, there is a complete shift from α- to β-MHC in PTU-treated mouse and rat heart muscle preparations. SDS-gels were run at least once and stained with Coomassie blue as described before (7, 23, 27).

**Figure 3. Western blot analysis of cardiac muscle fibers reconstituted with recombinant Tn.** Tn reconstitution was performed as described previously (9). 

A. Western blot analysis of reconstituted muscle fibers with an antibody against human c-myc epitope. 

B. Western blot analysis of reconstituted muscle fibers with an antibody against human cTnT. For both A and B, lane identifications are as follows: Lane 1, pure recombinant c-myc WT-cTnT; lane 2, mouse cardiac muscle fibers reconstituted with mouse c-myc WT-cTnT+cTnI+cTnC and lane 3, rat cardiac muscle fibers reconstituted with rat c-myc cTnT+cTnI+cTnC. Panel A demonstrates that c myc-tagged cTnT is incorporated in muscle fibers. As shown in B, lack of immunoreactivity for the endogenous cTnT in both mouse and rat cardiac muscle fibers is indicative of the removal of the endogenous Tn.
**Figure 4**: pCa$_{50}$ in Tn reconstituted muscle fibers from normal and PTU-treated mouse and rat hearts. Data from the normalized pCa-tension measurements were fitted to the Hill equation using a nonlinear least-square regression procedure to obtain pCa$_{50}$. Symbols for Tn-reconstituted fibers from normal animal hearts (α-MHC) are: mouse Tn (Δ) and rat Tn (□) and symbols for Tn-reconstituted fibers from PTU-treated animal hearts (β-MHC) are: mouse Tn (▲) and rat Tn (■). 

A. pCa$_{50}$ in Tn-reconstituted mouse fibers. 

B. pCa$_{50}$ in Tn-reconstituted rat fibers. pCa$_{50}$ values and number of fibers for each experimental group are listed in Tables 3 and 4. Standard error bars are smaller than symbols in some cases. Data were analyzed using three-way ANOVA. Statistical differences between different groups are listed in the Results section.

**Figure 5. Tension cost and XB distortion rate constant in Tn reconstituted muscle fibers from normal and PTU-treated mouse and rat hearts.** Tension cost (in pmol mN$^{-1}$ mm$^{-1}$ s$^{-1}$) was determined from the mean slopes of tension-ATPase relationship of several fibers (9, 34). XB distortion rate constant (c, s$^{-1}$) was determined at pCa 4.3 (5, 9). Symbols for Tn-reconstituted fibers from normal animal hearts (α-MHC) are: mouse Tn (Δ) and rat Tn (□) and symbols for Tn-reconstituted fibers from PTU-treated animal hearts (β-MHC) are: mouse Tn (▲) and rat Tn (■). 

A. Tension cost in Tn-reconstituted mouse fibers. 

B. Tension cost in Tn-reconstituted rat fibers. 

C. Rate constant c in Tn-reconstituted mouse fibers. 

D. Rate constant c in Tn-reconstituted rat fibers. Tension cost, c and number of fibers for each experimental group are listed in Tables 3 and 4. Standard error bars are smaller than symbols in some cases. Data were analyzed using three-way ANOVA. Statistical differences between different groups are listed in the Results section.
Figure 6. The rate constants for XB-recruitment and tension redevelopment in Tn reconstituted muscle fibers from normal and PTU-treated mouse and rat hearts. XB recruitment rate constant ($b$, s$^{-1}$) and the rate constant for tension redevelopment ($k_{tr}$, s$^{-1}$) were determined at pCa 4.3 (2, 5, 9). Symbols for Tn-reconstituted fibers from normal animal hearts ($\alpha$-MHC) are: mouse Tn (Δ) and rat Tn (□) and symbols for Tn-reconstituted fibers from PTU-treated animal hearts ($\beta$-MHC) are: mouse Tn (▲) and rat Tn (■). A. Rate constant $b$ in Tn-reconstituted mouse fibers. B. Rate constant $b$ in Tn-reconstituted rat fibers. C. $k_{tr}$ in Tn-reconstituted mouse fibers. D. $k_{tr}$ in Tn-reconstituted rat fibers. $b$, $k_{tr}$ and number of fibers for each experimental group are listed in Tables 3 and 4. Standard error bars are smaller than symbols in some cases. Data were analyzed using three-way ANOVA. Statistical differences between different groups are listed in the Results section.

Figure 7. The ratio of magnitude scaling parameter for length-mediated XB recruitment ($E_0$) to that of XB distortion ($E_\infty$). Magnitudes of XB recruitment ($E_0$) and XB distortion ($E_\infty$) were estimated (pCa 4.3) from fitting the recruitment-distortion model to double chirp protocol as explained in Fig. 1 and the Methods. Symbols for Tn-reconstituted fibers from normal animal hearts ($\alpha$-MHC) are: mouse Tn (Δ) and rat Tn (□) and symbols for Tn-reconstituted fibers from PTU-treated animal hearts ($\beta$-MHC) are: mouse Tn (▲) and rat Tn (■). A. $E_0/E_\infty$ in Tn-reconstituted mouse fibers. B. $E_0/E_\infty$ in Tn-reconstituted rat fibers. Standard error bars are smaller than symbols in some cases. $E_0/E_\infty$ and number of fibers for each experimental group are listed in Tables 3 and 4. Data were analyzed using three-way ANOVA. Statistical differences between different groups are listed in the Results section.
Table 1. Contractile function and mechano-dynamic parameters of detergent-skinned muscle fibers from normal (α-MHC) mouse and rat hearts.

<table>
<thead>
<tr>
<th></th>
<th>Mouse Fibers (n=11)</th>
<th>Rat Fibers (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contractile Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Tension</td>
<td>48.1±1.8</td>
<td>53.4±2.0</td>
</tr>
<tr>
<td>Max ATPase activity</td>
<td>313±11</td>
<td>229±14</td>
</tr>
<tr>
<td>Tension Cost</td>
<td>6.49±0.18</td>
<td>4.29±0.20</td>
</tr>
<tr>
<td>pCa50</td>
<td>5.75±0.01</td>
<td>5.72±0.02</td>
</tr>
<tr>
<td>n</td>
<td>2.5±0.2</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td><strong>Mechano-Dynamic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b (s⁻¹)</td>
<td>18.82±0.91</td>
<td>5.31±0.32</td>
</tr>
<tr>
<td>k_tr (s⁻¹)</td>
<td>10.97±0.50</td>
<td>6.57±0.16</td>
</tr>
<tr>
<td>c (s⁻¹)</td>
<td>38.11±1.22</td>
<td>33.34±1.44</td>
</tr>
<tr>
<td>E₀/E∞</td>
<td>0.33±0.01</td>
<td>0.22±0.01</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Ca²⁺-activated maximal tension (mN mm⁻²) and ATPase activity (pmol mm⁻³ s⁻¹) were measured in pCa 4.3 (9, 10). Data from the normalized pCa-tension measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa₅₀ and Hill coefficient (n) values. The tension cost (pmol mN⁻¹ mm⁻¹ s⁻¹) was determined from the mean slopes of tension-ATPase relationship of several muscle fibers, as described previously (9). b, c, k_tr and E₀/E∞ were determined in pCa 4.3 solution as described in the Methods. Multiple groups were analyzed by two-way ANOVA and statistical significances are discussed in the Results. n indicates number of different muscle fibers for each experimental group.
Table 2. Contractile function and mechano-dynamic parameters of detergent-skinned muscle fibers from PTU treated (β-MHC) mouse and rat hearts.

<table>
<thead>
<tr>
<th></th>
<th>Mouse Fibers (n=12)</th>
<th>Rat Fibers (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contractile Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Tension</td>
<td>47.2±1.6</td>
<td>52.1±2.2</td>
</tr>
<tr>
<td>Max ATPase activity</td>
<td>134±11</td>
<td>114±15</td>
</tr>
<tr>
<td>Tension Cost</td>
<td>2.88±0.16</td>
<td>2.24±0.15</td>
</tr>
<tr>
<td>pCa50</td>
<td>5.73±0.02</td>
<td>5.74±0.03</td>
</tr>
<tr>
<td>n</td>
<td>2.1±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td><strong>Mechano-Dynamic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b (s⁻¹)</td>
<td>9.43±0.1</td>
<td>2.75±0.1</td>
</tr>
<tr>
<td>kₜ (s⁻¹)</td>
<td>4.84±0.12</td>
<td>4.80±0.14</td>
</tr>
<tr>
<td>c (s⁻¹)</td>
<td>16.52±0.45</td>
<td>15.31±0.96</td>
</tr>
<tr>
<td>E₀/E∞</td>
<td>0.26±0.01</td>
<td>0.32±0.02</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Ca²⁺-activated maximal tension (mN mm⁻²) and ATPase activity (pmol mm⁻³ s⁻¹) were measured in pCa 4.3 (9, 10). Data from the normalized pCa-tension measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa₅₀ and Hill coefficient (n) values. The tension cost (pmol mN⁻¹ mm⁻¹ s⁻¹) was determined from the mean slopes of tension-ATPase relationship of several muscle fibers, as described previously (9). b, c, kₜ and E₀/E∞ were determined in pCa 4.3 solution as described in the Methods. Multiple groups were analyzed by two-way ANOVA and statistical significances are discussed in the Results. n indicates number of different muscle fibers for each experimental group.
Table 3. Contractile function and mechano-dynamic parameters of muscle fibers from normal (α-MHC) mouse and rat hearts reconstituted with mouse and rat Tn.

<table>
<thead>
<tr>
<th></th>
<th>Mouse Fibers (α-MHC)</th>
<th>Rat Fibers (α-MHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reconstituted with Mouse-Tn (n=10)</td>
<td>Reconstituted with Rat-Tn (n=15)</td>
</tr>
<tr>
<td><strong>Contractile Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Tension</td>
<td>39.66±0.95</td>
<td>45.20±1.78</td>
</tr>
<tr>
<td>Max ATPase activity</td>
<td>272±11</td>
<td>286±10</td>
</tr>
<tr>
<td>Tension Cost</td>
<td>6.8±0.2</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>pCa50</td>
<td>5.64±0.01</td>
<td>5.65±0.01</td>
</tr>
<tr>
<td>n</td>
<td>2.6±0.2</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td><strong>Mechano-Dynamic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b (s⁻¹)</td>
<td>21.05±1.66</td>
<td>19.57±1.66</td>
</tr>
<tr>
<td>k_tr (s⁻¹)</td>
<td>12.69±0.42</td>
<td>12.33±0.41</td>
</tr>
<tr>
<td>c (s⁻¹)</td>
<td>43.21±2.18</td>
<td>42.87±1.87</td>
</tr>
<tr>
<td>E₀/E∞</td>
<td>0.25±0.02</td>
<td>0.24±0.02</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Muscle fibers were reconstituted with either recombinant mouse Tn or rat Tn as indicated. Ca²⁺-activated maximal tension (mN mm⁻²) and ATPase activity (pmol mm⁻³ s⁻¹) were measured in pCa 4.3 (9, 10). Normalized pCa-force measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa₅₀ and Hill coefficient (n) values. The tension cost (pmol mN⁻¹ mm⁻¹ s⁻¹) was determined from the mean slopes of tension-ATPase relationship of several fibers (9). b, c, k_tr and E₀/E∞ were measured in pCa 4.3. Multiple groups were analyzed by three-way ANOVA and statistical significances are discussed in the Results. n indicates number of different muscle fibers for each experimental group.
Table 4. Contractile function and mechano-dynamic parameters of muscle fibers from PTU-treated (β-MHC) mouse and rat hearts reconstituted with mouse and rat Tn.

<table>
<thead>
<tr>
<th></th>
<th>Mouse Fibers (β-MHC)</th>
<th>Rat Fibers (β-MHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reconstituted with Mouse-Tn (n=6)</td>
<td>Reconstituted with Rat-Tn (n=9)</td>
</tr>
<tr>
<td><strong>Contractile Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Tension</td>
<td>45.18±1.33</td>
<td>43.90±1.81</td>
</tr>
<tr>
<td>Max ATPase activity</td>
<td>118±10</td>
<td>88±4</td>
</tr>
<tr>
<td>Tension Cost</td>
<td>2.6±0.1</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>pCa50</td>
<td>5.67±0.02</td>
<td>5.60±0.03</td>
</tr>
<tr>
<td>n</td>
<td>2.6±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td><strong>Mechano-Dynamic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b (s⁻¹)</td>
<td>10.93±1.13</td>
<td>5.78±0.91</td>
</tr>
<tr>
<td>kₜ (s⁻¹)</td>
<td>5.32±0.29</td>
<td>4.31±0.21</td>
</tr>
<tr>
<td>c (s⁻¹)</td>
<td>17.83±0.67</td>
<td>16.02±1.29</td>
</tr>
<tr>
<td>E₀/E∞</td>
<td>0.21±0.02</td>
<td>0.28±0.01</td>
</tr>
</tbody>
</table>

Values are means ±SEM. Muscle fibers were reconstituted with either recombinant mouse Tn or rat Tn as indicated. Ca²⁺-activated maximal tension (mN mm⁻²) and ATPase activity (pmol mm⁻³ s⁻¹) were measured in pCa 4.3 (9, 10). Normalized pCa-force measurements were fitted to the Hill equation by using a nonlinear least square regression procedure to derive the pCa₅₀ and Hill coefficient (n) values. The tension cost (pmol mN⁻¹ mm⁻¹ s⁻¹) was determined from the mean slopes of tension-ATPase relationship of several fibers (9). b, c, kₜ and E₀/E∞ were measured in pCa 4.3. Multiple groups were analyzed by three-way ANOVA and statistical significances are discussed in the Results. n indicates number of different muscle fibers for each experimental group.
FIGURE 2
FIGURE 3

A

1 2 3

B
FIGURE 4

A  Mouse Fiber

B  Rat Fiber

pCa_{50}

5.75

5.70

5.65

5.60

5.55

Mouse Tn  Rat Tn

Mouse Tn  Rat Tn
FIGURE 5

A. Mouse Fiber

B. Rat Fiber

Mouse Tn
Rat Tn

Tension Cost (pmol mN⁻¹ mm⁻¹ s⁻¹)

C. Mouse Fiber

D. Rat Fiber

Mouse Tn
Rat Tn

c (s⁻¹)
FIGURE 7

A  Mouse Fiber

B  Rat Fiber

$E_0/E_\infty$

Mouse Tn  Rat Tn

Mouse Tn  Rat Tn