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Molecular and Functional Characterization of a Novel Cardiac-Specific Human Tropomyosin Isoform

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Background—Tropomyosin (TM), an essential actin-binding protein, is central to the control of calcium-regulated striated muscle contraction. Although TPM1α (also called α-TM) is the predominant TM isoform in human hearts, the precise TM isoform composition remains unclear.

Methods and Results—In this study, we quantified for the first time the levels of striated muscle TM isoforms in human heart, including a novel isoform called TPM1κ. By developing a TPM1κ-specific antibody, we found that the TPM1κ protein is expressed and incorporated into organized myofibrils in hearts and that its level is increased in human dilated cardiomyopathy and heart failure. To investigate the role of TPM1κ in sarcomeric function, we generated transgenic mice overexpressing cardiac-specific TPM1κ. Incorporation of increased levels of TPM1κ protein in myofilaments leads to dilated cardiomyopathy. Physiological alterations include decreased fractional shortening, systolic and diastolic dysfunction, and decreased myofilament calcium sensitivity with no change in maximum developed tension. Additional biophysical studies demonstrate less structural stability and weaker actin-binding affinity of TPM1κ compared with TPM1α.

Conclusions—This functional analysis of TPM1κ provides a possible mechanism for the consequences of the TM isoform switch observed in dilated cardiomyopathy and heart failure patients. **(*Circulation*. 2010;121:410-418.)**

Key Words: cardiomyopathy ■ contractility ■ heart failure ■ myocardial contraction

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The heart adapts to different challenges presented by an array of mechanical, hormonal, and nutritional signals in the process of maintaining its circulatory function. Isoform switching of sarcomeric proteins is 1 mode the heart uses to adapt to those challenges, along with alterations in the relative abundance and phosphorylation status of contractile and regulatory proteins.[1] These changes in isoform expression and phosphorylation status also play an essential role during cardiac development and in response to cardiac hypertrophy and heart failure (HF). Although sarcomeric protein isoforms are subject to developmental regulation, cardiomyopathy and HF primarily elicit changes in thick filament protein isoforms.[2] The only thin filament protein to change isoform expression in the failing human heart is troponin T.[3,4] Furthermore, altered phosphorylation of troponin I, myosin binding protein C, and other sarcomeric proteins has dramatic effects on cardiac function in the failing human myocardium.[5]

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To understand the specific role of another thin filament protein, tropomyosin (TM), in the normal and the pathological heart, it is essential to define the TM isoform expression profile. Tropomyosins comprise a family of actin-binding proteins encoded by 4 different genes (*TPM1*, *TPM2*, *TPM3*, and *TPM4*). Each gene uses alternative splicing, alternative promoters, and differential processing to encode multiple striated muscle, smooth muscle, and cytoskeletal transcripts. For example, the *TPM1* gene uses 15 exons to encode at least 9 distinct isoforms.[6,7] There are 3 primary striated muscle TM isoforms, α-TM, β-TM, and γ-TM, which are products of the *TPM1, TPM2,* and *TPM3* genes, respectively. These isoforms are highly homologous yet exhibit unique physiological properties.[8,9] Recently, a novel striated muscle isoform of the *TPM1* gene was identified in human cardiac tissue.[10] This

Received June 26, 2009; accepted October 19, 2009.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.109.889725/DC1.

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***Circulation* is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.109.889725**

isoform contains an exon pattern similar to striated muscle TPM1α mRNA (also called α-TM) except for the substitution of the smooth muscle–specific exon 2a for 2b. This TM isoform is designated TPM1κ.[10] Our quantification results of human cardiac RNA reveals that ≈50% of the striated muscle TPM1 mRNA is TPM1κ, with the remainder being TPM1α. Although TPM1κ mRNA has been identified, TPM1κ protein expression in human hearts has previously not been confirmed. To address this, we developed a TPM1κ-specific antibody and quantified protein levels in the hearts of normal patients and cardiomyopathy patients. We report here for the first time the TM protein composition in adult human hearts and determine that TPM1κ protein is expressed and incorporated into cardiac myofibrils. Interestingly, TPM1κ protein levels are differentially regulated so that cardiomyopathy patients with end-stage HF exhibit increased expression.

TMs are central to the control of calcium-regulated thin filament function and striated muscle contraction. To investigate the role of TPM1κ in sarcomeric function, we generated transgenic (Tg) mice overexpressing TPM1κ in the hearts. Results show that incorporation of TPM1κ protein in myofilaments leads to dilated cardiomyopathy (DCM). Physiological changes include decreased fractional shortening of the left ventricle, systolic and diastolic dysfunction, decreased myofilament calcium sensitivity, and weaker actin-binding affinity. Our Tg mouse studies and in vitro biophysical results demonstrate significant functional and structural differences between TPM1κ and TPM1α that provide a possible mechanism for the consequences of the TM isoform switch that is observed in DCM and HF patients.

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Methods

An expanded Methods section is available in the online-only Data Supplement.

Study Subjects and Human Samples

This study was performed in accordance with the Declaration of Helsinki as adopted and promulgated by the US National Institutes of Health and with the rules and regulations of the University of Cincinnati's Institutional Ethics Committee. The study group consisted of hearts excised from patients undergoing cardiac transplantation at the University of Cincinnati and human cardiac protein samples from previously published work.[11] The clinical data of the HF patients are presented in Table I of the online-only Data Supplement. Three healthy hearts procured from brain-dead patients/organ donors with no history of cardiac disease served as controls. Normal human RNAs from adult and fetal cardiac and skeletal muscle, uterus, and lung were procured from commercial sources (Stratagene, La Jolla, Calif).

Generation of TPM1κ Tg Mice

Tg mice (FVB/N background) were generated with a cDNA-encoding human TPM1κ cloned into the cardiac-specific α-myosin heavy-chain (α-MHC) expression vector.[12] Animal experiments were approved by the University of Cincinnati's Institutional Animal Care and Use Committee.

Cardiac Function

Cardiac performance of the Tg mice was assessed by physiological studies, including echocardiography, isolated anterograde perfused heart model, and skinned fiber preparations, which are described in the online-only Data Supplement.

Quantitative Reverse-Transcription Polymerase Chain Reaction Analysis, Bacterial Recombinant Protein Expression, Circular Dichroism Measurements, Actin-Binding Assay, and Structure Modeling Analysis

Details regarding the methods used are presented in the online-only Data Supplement.

Statistical Analysis

All values are presented as mean±SD unless otherwise mentioned. Protein data were analyzed with the Wilcoxon rank-sum test. The isoproterenol response data and NEM-S1 data were analyzed using the Kruskal-Wallis test with post hoc analysis using the Wilcoxon rank sum test after adjustment of the level of significance. In addition, the NEM-S1 data were examined with a repeated-measures ANOVA with Bonferroni post hoc analysis with a significance of $P<0.05$.

Results

Expression Profile of TPM1α and TPM1κ in Human Hearts

Although the expression of TPM1κ mRNA was identified in the human heart,[10] the relative levels of TPM1κ and TPM1α transcripts are unknown. To quantify TM transcript levels in human hearts, we conducted quantitative reverse-transcription polymerase chain reaction using striated muscle TM isoform–specific primers. Results show that the TPM1α and TPM1κ isoforms are expressed in equal amounts (≈50% each) in both fetal and adult hearts (Figure 1A). Interestingly, both isoform levels increase in adult hearts by 3.1-fold compared with fetal hearts and normalized to GAPDH expression. Further analysis shows that TPM1κ is expressed only in human cardiac muscle, not in skeletal muscle, uterus, or lung (data not shown). Additional quantitative reverse-transcription polymerase chain reaction results show that β-TM is expressed at equivalent levels in fetal and adult human hearts but that γ-TM is expressed at a ≈30-fold increase in adult compared with fetal myocardium (data not shown).

To determine TM protein composition in human hearts, we conducted Western blot analyses using a striated muscle–specific TM antibody. Results reveal the ratio of TM isoforms in the adult human heart: TPM1α, 90% to 94%; TPM1κ, 3% to 5%; and β-TM, 3% to 5% (Figure 1B and 1C). Additional experiments show no expression of γ-TM protein in the adult human heart (data not shown). Interestingly, although TPM1κ and TPM1α mRNAs are expressed in equivalent amounts, 90% to 94% of the total translated TM is the TPM1α isoform, with the remaining being TPM1κ and β-TM.

To distinguish the TPM1κ protein from other TM isoforms, we developed an isoform-specific antibody with an epitope that resides in the exon 2a sequence that is encoded within TPM1κ but not in TPM1α protein. We used this antibody to quantify TPM1κ protein levels in hearts from several normal, DCM, and HF patients. The TPM1κ protein levels from human left ventricular free walls were normalized to tubulin or actin and quantified (Figure 1D through 1G). Results show that TPM1κ is expressed in normal patients and that these levels are increased in DCM and HF patients. Both control and patient samples show minimal reactivity to a smooth muscle TM antibody, thereby verifying the specificity of the TPM1κ antibody (Figure 1F). These results demonstrate the differential expression of TPM1κ protein in DCM

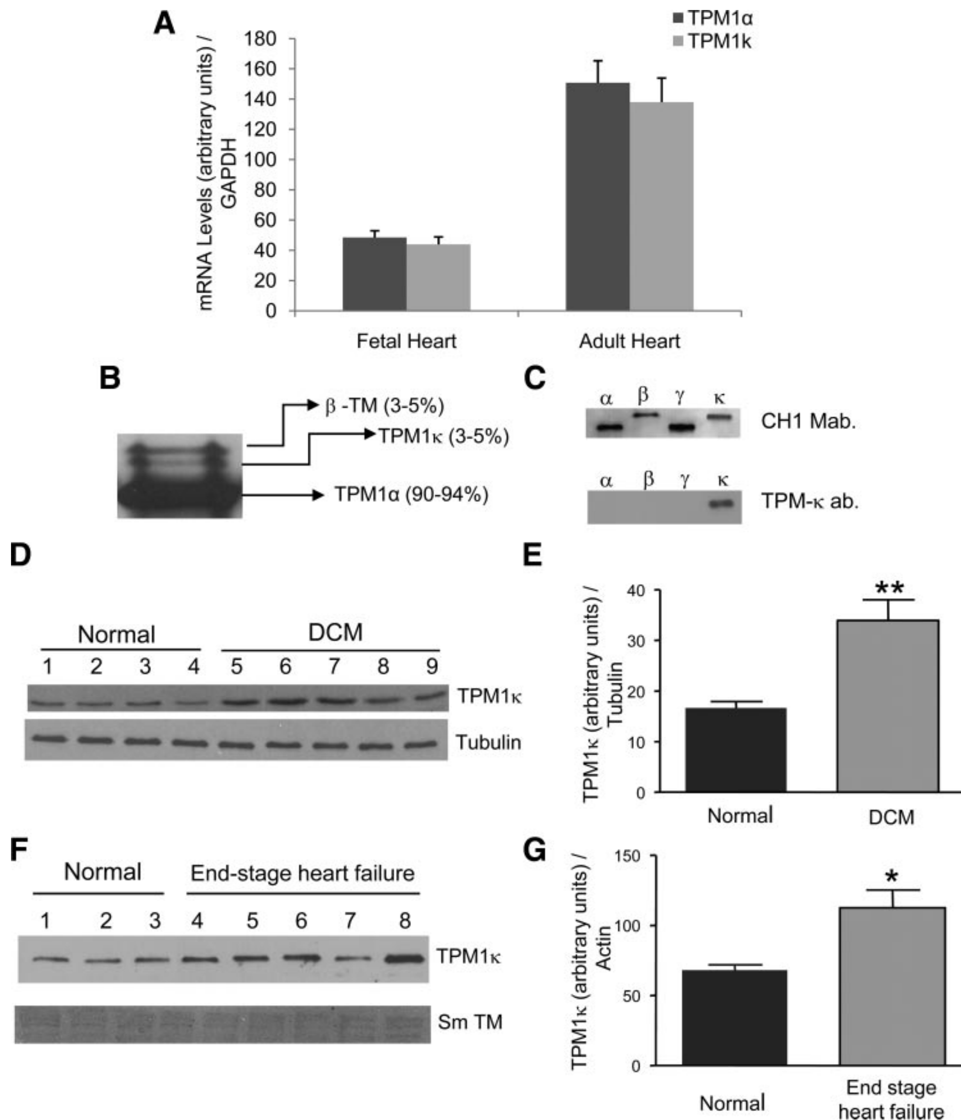

Figure 1. TPM1κ expression in human hearts. A, Real-time reverse-transcription polymerase chain reaction quantification of the TPM1α and TPM1κ mRNAs in normal fetal and adult hearts. B, TM protein profile in the normal adult human heart analyzed by Western blotting using striated muscle TM-specific antibody. C, Migration of TM isoforms and TM antibody specificity. D and E, TPM1κ expression and quantification in cardiac total protein lysate from DCM patients using anti-TPM1κ and anti-tubulin antibodies. F and G, TPM1κ protein expression and quantification in cardiac myofibrillar preparations from normal and heart failure patients using anti-TPM1κ and anti-smooth muscle TM (Sm TM) antibodies. *$P<0.02$, normal vs end-stage HF patients; **$P<0.01$, normal vs DCM. Ab indicates antibody; Mab, monoclonal antibody.

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and HF patients. We also observe a tendency of downregulation of β-TM levels in those patients (data not shown), but because of the unavailability of a β-TM–specific antibody, we are unable to quantify the exact level of change. Furthermore, the levels of TPM1α protein are so high in the heart that subtle changes in TM levels are not easily quantified. It should be noted that the relative migratory positions of β-TM and TPM1κ proteins can vary, depending on gel composition (Figure I of the online-only Data Supplement).

TPM1κ Tg Mouse Hearts Autoregulate TM Isoform Expression

To elucidate relations between sarcomeric TM isoforms and contractile behavior in striated muscle, we developed a Tg mouse model to overexpress the TPM1κ isoform in the heart. The transgene construct was generated by ligating the human TPM1κ cDNA (containing the entire amino acid–coding region and the 3′ untranslated region) into the murine α-MHC cardiac-specific expression vector. There is 100% amino acid conservation between mouse and human TPM1α, as well as exon 2a sequence. Three independent Tg mouse lines (lines 72, 77, and 80) were established that express varied levels of TPM1κ protein in the heart (90%, 32%, and 51%, respectively, of TPM1κ/total TM normalized to actin; Figure 2A and 2B). The expression of TPM1κ is coupled with a concomitant downregulation of endogenous TPM1α, with total TM levels remaining unchanged. Tg lines 72 and 80 were used for further functional analyses. Interestingly, unlike human hearts, wild-type mouse hearts do not express TPM1κ. We confirm this in various mouse strains, including FVB/N, black Swiss, C57-BL/6, and BALB/c (data not shown). Immunohistological analysis of the Tg hearts using

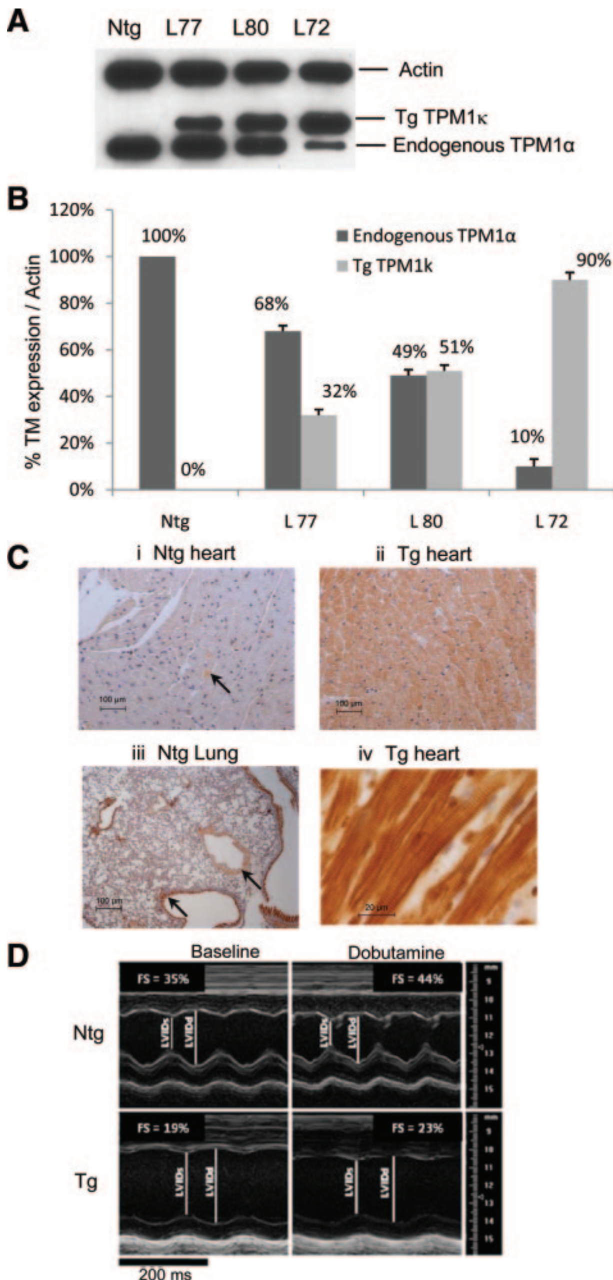

Figure 2. Characterization of the TPM1κ Tg mice. A and B, Western blot analysis of myofilament preparations from TPM1κ Tg mouse hearts using striated muscle TM and actin antibodies. The TM level found in Ntg hearts was set to 100%, and the signal intensity of the TM isoforms in Tg mice was quantified and normalized to striated muscle α-actin. C, Immunohistochemistry of ventricular sections of Ntg and TPM1κ Tg hearts using anti-TPM1κ antibody. Arrows indicate the regions of expression for vascular smooth muscle TM in the Ntg tissue sections. Bottom right, Incorporation of TPM1κ protein into sarcomeres as reflected by the striated staining pattern. D, Representative AM-mode echocardiographic images of adult left ventricular (LV) response to dobutamine. Short-axis view of AM-mode recording of the LV from Ntg and Tg mice before and after infusion of dobutamine. LVIDs indicates LV end-systolic internal dimension; LVIDd, LV end-diastolic internal dimension; and FS, fractional shortening.

the anti-TPM1κ antibody reveals incorporation of the TPM1κ protein into organized myofibrillar bundles, which is absent in non-Tg (Ntg) mouse hearts (Figure 2C). The epitope of TPM1κ against which the antibody was raised is also found in smooth muscle TM; because of antibody cross-reactivity, there is a positive reaction in vascular smooth muscle regions of Ntg hearts, which is more prominent in mouse lung sections (Figure 2C).

TPM1κ Mice Exhibit Altered Cardiac Morphology and Function

None of the founder TPM1κ mice or their progeny demonstrate any differences in percentage heart-weight-to-body-weight ratio or reduced viability compared with Ntg controls. Histological analysis of hearts from 3-, 6-, and 12-month-old Tg mice reveals no detectable changes in gross chamber morphology, interstitial fibrosis, or myocyte disarray compared with age- and sex-matched Ntg littermates (data not shown); however, echocardiographic analyses show that the TPM1κ mice have increased end-systolic and end-diastolic left ventricular dimensions (Figure 2D, and Table II of the online-only Data Supplement).

To obtain a better understanding of the physiological significance of TPM1κ protein in the regulation of cardiac contractility, we implemented echocardiography, the work-performing heart model, and skinned fiber preparations. In vivo physiological assessment of cardiac function in young (2 months) and adult (5 months) mice using Doppler echocardiographic analyses demonstrated a progressive decrease in fractional shortening and ejection fraction (Table I of the online-only Data Supplement). Stroke volume and cardiac output were maintained at the expense of an increased end-diastolic volume. To test whether impairment of cardiac function could be reversed by stimulation of β-adrenergic receptors, we subjected the hearts to dobutamine, a positive inotropic agent (Figure 2D). Results show the Tg hearts responded appropriately to dobutamine but maintained their inherent physiological impairment (Table III of the online-only Data Supplement).

To assess cardiac function ex vivo, we implemented the work-performing heart model in the absence of hormonal stimulation and under controlled loading conditions. Studies show that overexpression of wild-type TPM1α in Tg mouse hearts does not lead to alterations in morphology or physiological performance of the heart.[13–15] As summarized in Table IV of the online-only Data Supplement, the TPM1κ Tg hearts show decreased baseline contraction (dP/dt) and relaxation (−dP/dt). The magnitude of impairment was greater for line 72 compared with 80, consistent with higher levels of transgene expression. Heart rates were unaltered in either line compared with Ntg. An increased time-to-peak pressure in both lines and half-time to relaxation in line 72 were observed, consistent with systolic and diastolic dysfunction.

Because there is systolic and diastolic dysfunction, we determined how hearts from the TPM1κ mice would respond to acute functional stress induced by stimulation of the β-adrenergic cardiostimulatory pathway. The reduced contractile and lusitropic performance by TPM1κ Tg hearts was assessed during stimulation with isoproterenol, a β-adrenergic agonist that augments muscle contraction and relaxation by increasing the rate of Ca^{2+} cycling. Although Tg hearts show an isoproterenol dose response, they are unable to reach control levels of contraction or relaxation (Figure 3). Thus, in

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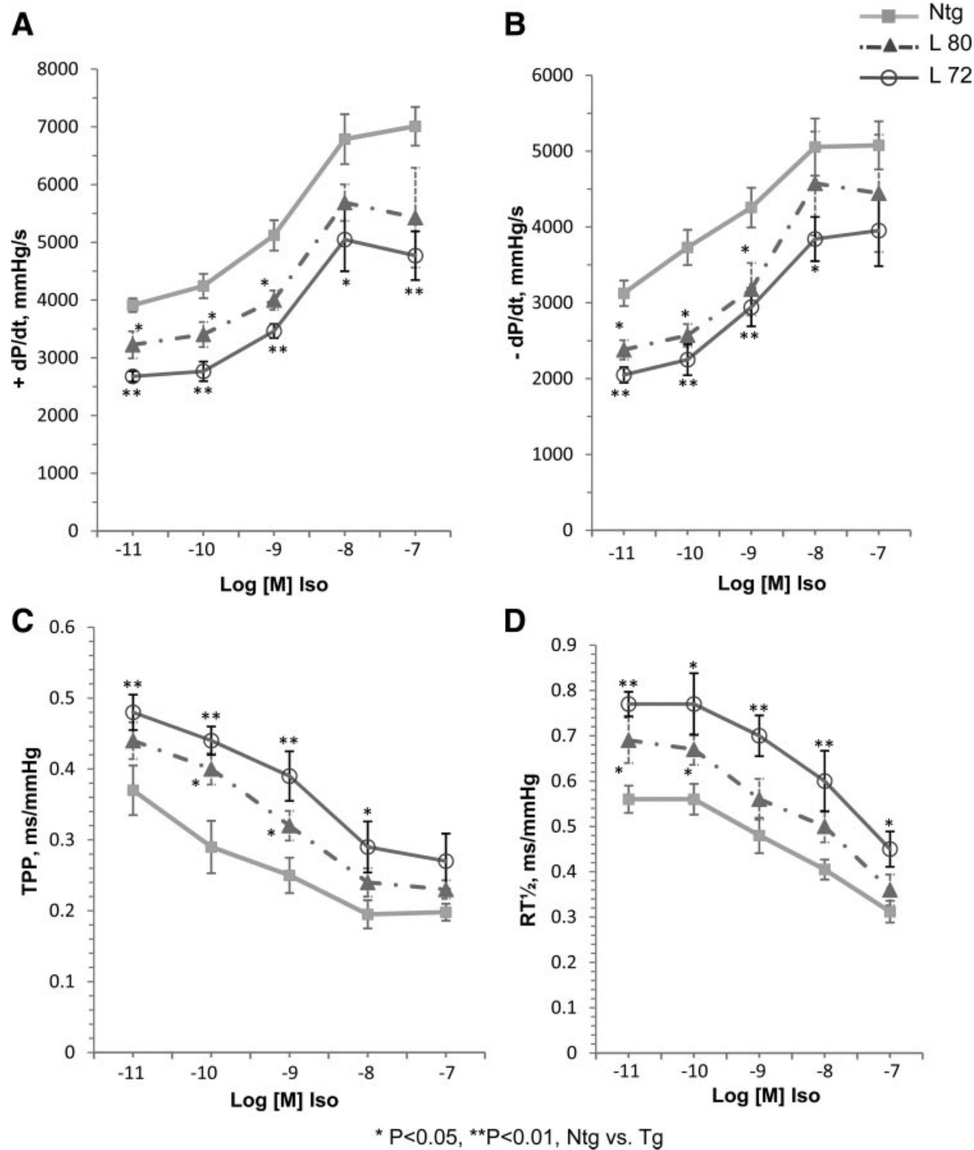

Figure 3. Isoproterenol dose-response curves in Ntg and TPM1κ Tg mouse hearts at 5 months of age. A through D, Hearts from Ntg and Tg lines 72 and 80 were subject to isolated heart analyses with increasing concentrations of isoproterenol (10^{-11} to 10^{-7} mol/L). TPP indicates time to peak pressure; RT½, half-time to relaxation.

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isolated heart preparations, the cumulative dose response to isoproterenol of both contractile (dP/dt, time to peak pressure) and relaxation (−dP/dt, half-time to relaxation) parameters is similar in the Tg and Ntg hearts even though functional differences remain. These results show that although TPM1κ mice demonstrate significantly impaired indicators of contractility at basal loading conditions, this magnitude of hemodynamic impairment is not sufficient to provoke compensatory chronic activation of sympathetic drive and desensitization of β-adrenergic signal transduction mechanisms.

TPM1κ Mice Exhibit a Decreased Myofilament Ca^{2+} Sensitivity

We compared the tension-PCA relation of myofilaments from Ntg and Tg hearts (line 72). Results (Figure 4A) show a significant decrease in the Ca^{2+} sensitivity of Tg myofilaments (Ntg: pCa_{50}=5.91±0.012; Tg: pCa_{50}=5.76±0.006; $P<0.05$) with no change in the maximum developed tension (Ntg: Fmax=29.59±0.76 mN/mm^2; Tg: Fmax=30.84±0.93 mN/mm^2). Compared with Ntg, the Hill coefficient of Tg myofilaments was significantly increased (Ntg: n_H=4.22±0.09; Tg: n_H=5.84±0.21; $P<0.05$).

To assess cooperative activation of the myofilaments, we used NEM-S1 to mimic the effect of strong crossbridge binding. NEM-S1 is known to probe cooperative processes and to enhance endogenous crossbridge cycling.[16] In the presence of NEM-S1, no change in maximum developed tension was observed between the 2 groups (Ntg: Fmax=29.35±0.75 mN/mm^2; Tg: Fmax=30.45±0.97 mN/mm^2; Figure 4B). In addition, Tg myofilaments are less sensitive to Ca^{2+} than Ntg (Ntg+NEM-S1: pCa_{50}=5.95±0.013; Tg+NEM-S1: pCa_{50}=5.78±0.009; $P<0.05$). At low Ca^{2+} values (pCa 6.5), NEM-S1 induces activation of the thin filament to a greater extent in Ntg than in Tg myofilaments (Ntg+NEM-S1: 3.18±1.3;

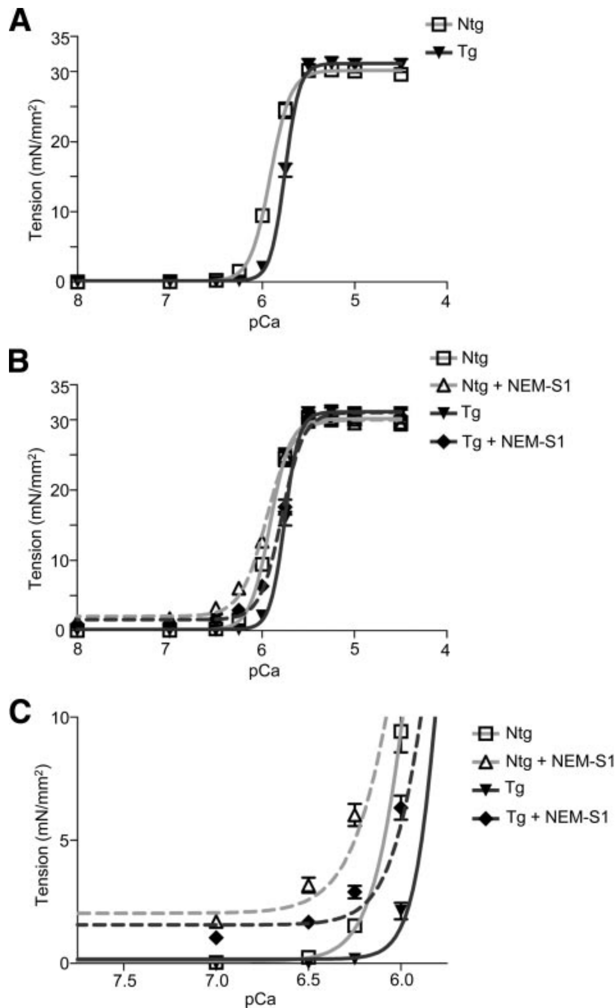

Figure 4. Tension-pCa relation of myofiber preparations from Ntg and TPM1κ Tg hearts. A, Tension-pCa relation with a sarcomeric length set at 2.0 μm. B, Tension-pCa relation before and after treatment with NEM-S1. C, Expanded scale of tension generated at low Ca^{2+} concentrations that enhances recruitment of crossbridges by NEM-S1.

Tg+NEM-S1: 1.68±0.81; $P<0.0001$; Figure 4C). Although decreased in both cases, the Hill coefficient remained significantly different ($P<0.05$): $n_H=3.17\pm0.06$ for Ntg+NEM-S1 versus $n_H=3.98\pm0.17$ for Tg+NEM-S1.

Altered Thermal Stability and Actin Binding of TPM1κ

To investigate whether the physiological alterations in TPM1κ Tg hearts are due to an altered protein structure, we used circular dichroism to study the thermal stability of recombinant TPM1κ protein. Results show a decreased TPM1κ homodimer stability compared with TPM1α at temperatures >37°C, which may influence actin binding and myofilament Ca^{2+} sensitivity (Figure 5A).

We conducted a series of cosedimentation assays to assess the ability of TM to bind actin. Results show that the binding of TPM1κ protein to actin was barely detectable with a much weaker affinity than TPM1α (Figure 5B). This result is in agreement with previous observations using unacetylated recombinant TMs.[17] The addition of human cardiac troponin dramatically increased the actin-binding affinity of TPM1κ protein (Figure 5C), which agrees with previous observations that troponin increases the affinity of TM for actin.[18] The dependence of TPM1κ on troponin for binding to actin and its increased expression in failing hearts suggest that the TM isoform switch is commensurate with the isoform switch of troponin T that occurs in HF.

Discussion

In this study, we report for the first time that the normal adult human heart expresses TPM1α, TPM1κ, and β-TM proteins and that the TPM1κ level is differentially regulated in cardiomyopathy patients. Previous results have demonstrated there are functional differences among various striated muscle TM isoforms[19,20]; our results provide insight into the structure-function relations of TPM1κ and its influence on cardiac function. Cardiac-specific overexpression of β-TM leads to a phenotype with diastolic dysfunction and increased myofilament calcium sensitivity.[15,20] Overexpression of γ-TM leads to a hyperdynamic effect on systolic and diastolic function but decreased myofilament calcium sensitivity without any morphological abnormalities in the heart.[21] Interestingly, TPM1κ mouse hearts elicit functional properties that are a combination of β-TM and γ-TM isoforms: systolic and diastolic impairment coupled with a decrease in myofilament calcium sensitivity. This physiological profile is similar to the α-TM E54K mouse model that causes DCM[14]; the TPM1κ Tg hearts also exhibit a dilated cardiac chamber phenotype.

The relative amounts of the different TM isoforms that are expressed in a muscle cell are characteristic of the species, muscle fiber type, stage of development, and other factors.[22] Exons 2a and 2b of the *TPM1* gene are spliced in a mutually exclusive manner with exon 2b being the default exon in the mRNA of most cell types; exon 2a had previously been restricted to smooth muscle TM.[6,7] That TPM1κ, a striated muscle TM that includes exon 2a, is incorporated into the sarcomere is a novel finding. The significance of this TM region (amino acids 41 to 80) is illustrated by the occurrence of various cardiomyopathies in humans, with mutations found in exon 2b leading to familial hypertrophic cardiomyopathy (Glu62Gln, Ala63Val, and Lys70Thr) and DCM (Glu40Lys and Glu54Lys).[9,14] Furthermore, both DCM mutations and a familial hypertrophic cardiomyopathy mutation (Glu62Gln) found in exon 2b have conserved amino acids in the same positions in exon 2a. Hence, the potential of the TPM1κ isoform replacing the TPM1α isoform containing mutant exon 2b in such cardiomyopathic conditions may be critical.

An intriguing question is why high levels of TPM1κ mRNA are present in adult human hearts when the protein levels are low. Similar discordant mRNA and protein levels were previously found with α-MHC expression in human ventricles.[23,24] Possible explanations for this mismatch between mRNA and protein levels may be associated with translational regulation or the involvement of micro-RNAs.[25]

Our results demonstrate that the TPM1κ protein affects tension developed at submaximal Ca^{2+} concentrations, the level at which normal heart function occurs, without altering maximum tension. The decreased binding affinity of TPM1κ to actin in the absence of troponin may contribute to the

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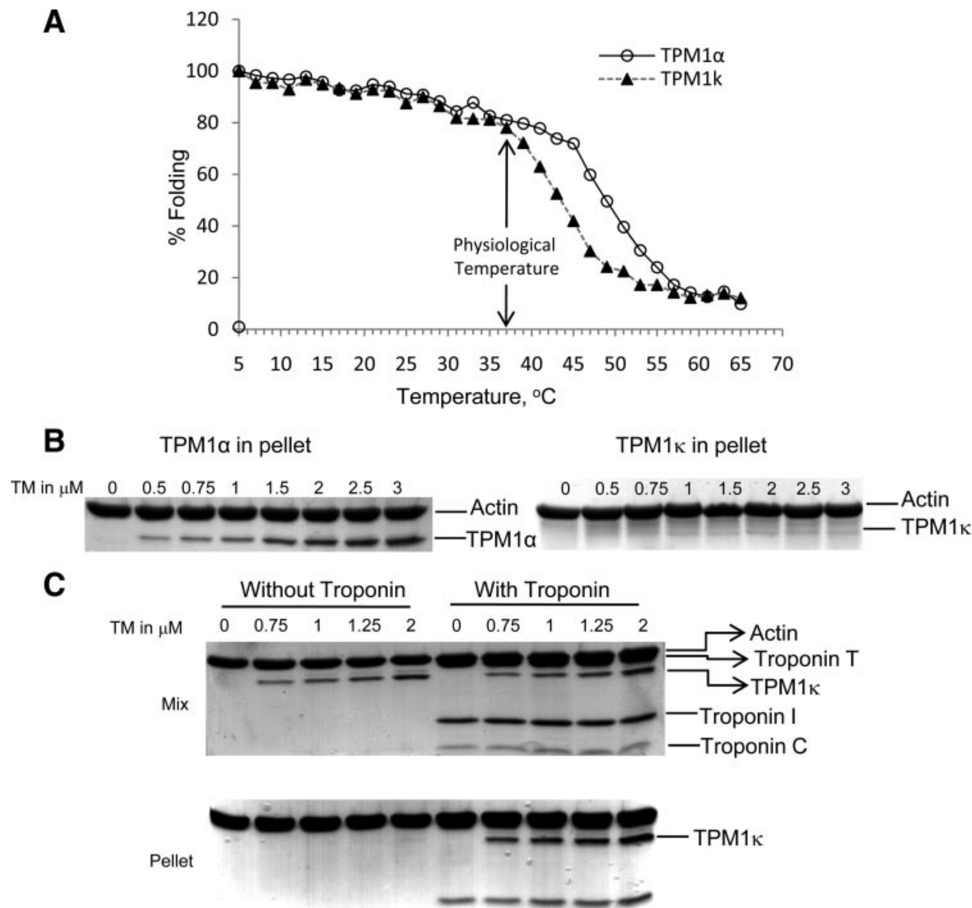

Figure 5. Biochemical analyses of TPM1κ. A, Thermal denaturation profiles of recombinant TPM1α and TPM1κ proteins monitored by circular dichroism measurements. The curves show the percentage folding vs temperature and were calculated on the basis of the ellipticity at a wavelength of 222 nm. B, Cosedimentation assay of TPM1α and TPM1κ binding to cardiac actin. C, Cosedimentation assay of TPM1κ binding to actin with and without human cardiac troponin. The mixture and the pellet fractions were analyzed by SDS-PAGE and Coomassie blue staining.

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decrease in myofilament Ca^{2+} sensitivity. Substitution of exon 2a for 2b, which happens with TPM1κ, may alter the propagation of force through the myofilament. Interestingly, with DCM TM mutations that occur in exon 2b, the same region of the TM protein, myofilaments also exhibit decreased sensitivity to Ca^{2+}.[14,26] In fact, TPM1κ expression is increased in human patients with DCM and HF. Although the properties of the DCM α-TM E54K and TPM1κ isoforms are similar in decreasing myofilament Ca^{2+} sensitivity, the novel TM isoform does not affect maximum tension (Figure 4A), which contrasts with the E54K mutation.[14] This difference in tension development could be due to structural effects of the E54K mutation/TPM1κ isoform. Previous studies demonstrate that TM mutations can result in structural alterations that affect TM stability[27] and binding to actin,[17,28] which can influence myofilament function. With TPM1κ, saturation of troponin C with high Ca^{2+} concentrations is able to fully activate the myofilaments.

Our evidence points to a mechanism in which thin filaments regulated by TPM1κ demonstrate reduced activation by both Ca^{2+} and strong crossbridges in the form of NEM-S1. The steeper dependence of force on Ca^{2+} in the Tg fibers indicates a greater reliance on crossbridge-dependent activation, a process of higher cooperativity in the face of little change in Ca^{2+} binding, a relatively noncooperative process. Evidence that crossbridge-dependent activation is blunted in the Tg fibers comes from our data of thin filament activation at low Ca^{2+} (Figure 4C). The data show that induction of force at or near relaxing levels of free Ca^{2+} was greater in Ntg than in Tg fibers. This effect is likely due to lower stability and weaker actin-binding affinity of TPM1κ compared with TPM1α.

TM is an α-helical, coiled-coil dimer characterized by a heptapeptide repeat motif (a-b-c-d-e-f-g) in which residues in the hydrophobic core positions a and d are the primary determinants of folding and stability.[29] The difference between TPM1κ and TPM1α is a stretch of 40 amino acids encoded by exon 2a/b, which contains a number of substituted residues (26 of 40 amino acid differences). Alanine residues in TPM1α promote flexibility in the helical structure and enhance binding to actin.[30] Replacement of these interface alanines with other residues would be expected to decrease actin affinity as a result of localized helix stabilization and loss of flexibility. Structural modeling of the TPM1κ isoform reveals several regions where substituted amino acids are likely to decrease the overall stability of the TPM1 coiled-coil dimer, as well as a few potentially stabilizing substitutions (Figure 6 and Table V of the online-only Data

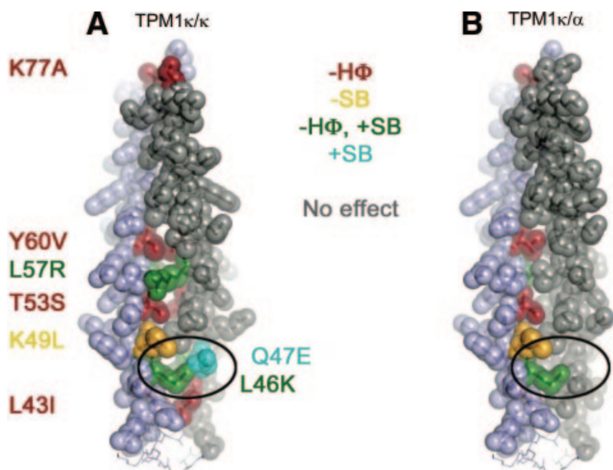

Figure 6. Structural models of TPM1κ/κ and κ/α isoforms. A and B, Substitutions in the κ isoform that are predicted to alter the stability of the *TPM1* coiled coil are highlighted in color. Red and yellow indicate amino acid substitutions that are likely to destabilize the coiled coil; green indicates a mixed effect; and cyan indicates putative stabilizing changes. The oval highlights residues that are predicted to form a salt bridge in a κ/κ coiled coil but would be destabilizing in the κ/α isoform. −HΦ indicates loss of hydrophobic contact; −SB, loss of salt bridge; and +SB, potential gain of a salt bridge.

Supplement). The destabilizing substitutions include several residues located in the hydrophobic central region of the coiled-coil (the a and d positions, including L43I, L46K, T53S, L57R, and Y60V) that lose well-packed hydrophobic interactions in the TPM1κ isoform (Table V of the online-only Data Supplement). In addition, there are a few destabilizing substitutions in the e and g positions that typically form polar or salt-bridge interactions; these include K49L, A63E, Q68E, and K70S. The few substitutions predicted to stabilize the dimer (Q47E, S61L, and K77A) occur in the e or g position. Substitutions on the outer surface of the TPM1 dimer (positions b, c, and f) are not involved in the dimer interface but could have direct effects on the interaction with actin or troponin. There are also a few differences in predicted stability between the TPM1κ/TPM1κ (least probable in vivo dimer) and the TPM1κ/TPM1α isoforms (most probable in vivo dimer). For example, the Q47E substitution is predicted to stabilize the homodimer κ/κ isoform through the addition of a salt bridge with L46K from the opposing helix, but this salt bridge would not form in the heterodimer κ/α isoform. Thus, the variation in amino acid sequence between exons 2a and 2b may contribute to differences in thermal denaturation and actin binding of TPM1κ compared with TPM1α.

What is the biological significance for expressing the TPM1κ isoform? In terminally differentiated cells such as cardiomyocytes, there is a need for adaptation to changing environments. Increasing protein isoform diversity through processes such as alterative splicing meets this need. The TPM1κ isoform, 1 of 10 distinct products of the *TPM1* gene, provides the opportunity to modulate sarcomeric performance during changing conditions such as exercise, stress, or cardiac disease. Several studies reported that during end-stage HF, the contractile apparatus is characterized by an increased Ca^{2+} sensitivity.[5,31,32] That TPM1κ compensates by decreasing calcium sensitivity without affecting maximum tension is logical for patients exhibiting chronic DCM. Although the increased level of TPM1κ is low compared with the total TM, the relative percentage of TPM1κ increase is significant. In addition, there is a possibility of a regional distribution of TPM1κ expression within the heart to match the functional demands of a specific region. Similar regional preference of β-MHC isoform expression compared with α-MHC was previously demonstrated to improve cardiac function.[33] The fact that human patients with chronic DCM and HF symptoms exhibit a cardiac pathology and physiology similar to TPM1κ Tg mice is striking considering the small but significant increase in TPM1κ protein levels in these patients. We speculate that during normal cardiac function, the low levels of TPM1κ (which confers decreased myofilament Ca^{2+} sensitivity) are offset by the low levels of β-TM (which confers increased Ca^{2+} sensitivity). Recent studies found that normalization of myofilament calcium sensitivity can rescue mice exhibiting cardiac hypertrophy.[34,35] In DCM and HF patients, there is decreased cardiac performance, which correlates with increased TPM1κ levels; whether this increased expression is the cause or a consequence of the DCM/HF phenotype remains to be determined.

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Acknowledgments

Dr Brandon Biesiadecki graciously provided the human cardiac troponin. We thank Jon Neumann for production of the Tg mice, Maureen Bender for her daily care of the animals, and Drs R. Shukla and A. Dwivedi of the University of Cincinnati Center for Biostatistical Services. We also thank Emily Schulz and Vikram Prasad for editorial assistance.

Sources of Funding

This work was supported in part by National Heart, Lung, and Blood Institute HL081680 awarded to Dr Wieczorek, HL79032 to Dr Wolska, and HL22231 and HL062426 to Dr Solaro.

Disclosures

None.

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CLINICAL PERSPECTIVE

Understanding the function of tropomyosin (TM) is important from a clinical perspective because mutations in the protein can result in familial hypertrophic cardiomyopathy and dilated cardiomyopathy. Four TM genes have been identified, each of which undergoes differential promoter usage and alternative splicing to generate multiple isoforms. In the heart, TM is an essential sarcomeric protein that controls calcium-regulated muscle contraction. Although much is known about TM, little is known about its expression in human hearts. In this study, we determine and quantify the expression levels of striated muscle TM isoforms in the human heart, including a novel TM isoform called TPM1κ. Our data show that TPM1κ is incorporated into cardiac myofilaments, and its level increases by 100% during human dilated cardiomyopathy and heart failure. We also develop a mouse model to understand the physiological function of TPM1κ. Because there is 100% amino acid sequence identity between human and mouse TM, these studies have direct relevance to TM function. Our mouse model shows that increased expression of TPM1κ results in increased end-systolic and end-diastolic left ventricular dimensions, similar to patients with dilated cardiomyopathy. Physiological assessment of the hearts from our mouse model show decreased fractional shortening, systolic and diastolic dysfunction, and decreased myofilament calcium sensitivity, which is similar to patients with heart disease. These studies provide a possible mechanism for the clinical features that are observed in dilated cardiomyopathy and heart failure patients.

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