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Anti-Sense-Mediated Inhibition of Expression of the Novel Striated Tropomyosin Isoform TPM1κ Disrupts Myofibril Organization in Embryonic Axolotl Hearts

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Abstract Striated muscle tropomyosin (TM) is described as containing ten exons; 1a, 2b, 3, 4, 5, 6b, 7, 8, and 9a/b. Exon 9a/b has critical troponin binding domains and is found in striated muscle isoforms. We have recently discovered a smooth (exon 2a)/striated (exons 9a/b) isoform expressed in amphibian, avian, and mammalian hearts, designated as an isoform of the TPM1 gene (TPM1κ). TPM1κ expression was blocked in whole embryonic axolotl heart by transfection of exon-specific anti-sense oligonucleotide. Reverse transcriptase polymerase chain reaction (RT-PCR) confirmed lower transcript expression of TPM1κ and in vitro analysis confirmed the specificity of the TPM1κ anti-sense oligonucleotide. Altered expression of the novel TM isoform disrupted myofibril structure and function in embryonic hearts. J. Cell. Biochem. 95: 840–848, 2005.

Key words: *Ambystoma mexicanum*; transfection; contractility; myofibrillar disarray

Tropomyosin (TM) is an actin binding protein found in every living cell. Diverse expression of TM isoforms in muscle and non-muscle cells is achieved through alternative splicing [Nadal-Ginard, 1990; Lees-Miller and Helfman, 1991; Pittenger et al., 1994] in a tissue specific and developmentally regulated pattern. The splicing pattern for each isoform serves to adapt TM to a specific role in a given cell. However, the mechanism by which the presence or absence of a certain exon in TM makes it more suited to a specific function is still unclear. By inhibiting the expression of TM isoforms containing a specific exon, it is possible to determine if knockdown of an isoform is of functional significance.

Recent technological advances allowed for the discovery of many unknown isoforms of TM in heart [Cooley and Bergstrom, 2001]. Most of these isoforms fall into previous categories for TM. Four TM genes have been characterized in vertebrates: α-*TM (TPM1)*, β-*TM (TPM2)*, *hTMnm (TPM3)*, and *TM-4 (TPM4)* including localization to their human chromosomes [Laing et al., 1995; Perry, 2001; Martson and Redwood, 2003]. A predominant striated muscle isoform is composed of exons 1a, 2b, 3, 4, 5, 6b, 7, 8, 9a/b from the TPM1 gene [Nadal-Ginard, 1990; Lees-Miller and Helfman, 1991; Pittenger et al., 1994]. Exon 9a/b is particularly important for the function of the striated TM [Mak and Smillie, 1981; Luque et al., 1997]. The smooth muscle specific isoform of the TPM1 gene is designated TPM1β and consists of exons: 1a, 2a, 3, 4, 5, 6b, 7, 8, and 9c/d (see Table I).

We have described an isoform of TM that bridges two of the descriptive categories [Zajdel et al., 2000, 2002]. A novel TM that contains a smooth muscle exon 2a as well as striated muscle type exon 9a/9b is expressed primarily in heart tissues of amphibian, avian, and mammalian species. This TM has been designated as TPM1κ to signify the 10th isoform to be

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TABLE I. Nomenclature Used for *TPM1* Isoforms in This Study

Nomenclature of various isoforms of TPM1 referred to in this study	Isoforms of TM currently known as	Exon composition
TPM1α	Striated muscle	1a, 2b, 3, 4, 5.6b, 7,8, 9a/b
TPM1β	Smooth muscle	1a, 2a, 3, 4, 5, 6b, 7, 8, 9d
TPM1γ	TM-2 fibroblast	1a, 2b, 3, 4, 5, 6b, 7, 8, 9d
TPM1δ	TM-3 fibroblast	1a, 2b, 3, 4, 5, 6a, 7, 8, 9d
TPM1ε	TM-5a fibroblast	1b, 3, 4, 5, 6b, 7, 8, 9d
TPM1κ	Novel striated/cardiac muscle	1a, 2a, 3, 4, 5, 6b, 7, 8, 9a/b

discovered as an alternative splice product of the TPM1 gene. No specific antibody that is distinct for all exons is currently ready to examine the protein expression of this isoform but expression of a GFP-TM construct and incorporation into myofibrils is possible [Zajdel et al., 2002]. Since we have found the TPM1κ isoform to be present in cardiac tissues of various species and capable of incorporation into myofibrils, we set out to determine its importance by inhibiting the in vivo expression.

The use of anti-sense oligonucleotide mediated inhibition of mRNA expression has proven useful in determining what role a particular protein may play in cellular functions. The current study uses an exon 2a-specific anti-sense oligonucleotide to block expression of the TPM1κ isoform in whole embryonic axolotl hearts to see what effect knocked-down expression of this isoform may have. Whole hearts are amenable to transfection procedures and maintain intercellular connections and signaling pathways. In vitro analysis was used to confirm the critical specificity of the oligonucleotide compared to other TM isoforms. Reverse transcriptase polymerase chain reaction (RT-PCR) demonstrated decreased TPM1κ expression in embryonic hearts and confocal scanning microscopy showed disruption of myofibril organization and an associated decrease in contractility.

MATERIALS AND METHODS

Embryo Care

Axolotl embryos were obtained from the Indiana University axolotl colony and the axolotl colony at SUNY Upstate Medical University. Animals were maintained in aquaria in 50% Holfreter's solution (29 mM NaCl, 0.45 mM $CaCl_2$, 0.33 mM KCl, 0.1 mM $MgSO_4$, and 4.76 mM $NaHCO_3$) and fed commercial salmon pellets. The embryos were staged according to the standard staging system [Bordzilovskaya et al., 1989].

Preparation of Exon-Specific Oligonucleotides

Sense and anti-sense oligonucleotides were designed for exon 2 of the axolotl TPM1κ TM isoform. Axolotl TPM1α and TPM4α isoforms were used as controls to the TPM1κ isoform. The axolotl TPM4α (ATmC-3) isoform is the predominant TM isoform in embryonic axolotl hearts [Spinner et al., 2002]. The TPM1α isoform is the predominant isoform found in axolotl skeletal muscle. TPM1κ and TPM1α nucleotide sequences used for this experiment were the following: TPM1κ sense, 5′-^{f}G*C*A*C ACT GCT GAC GAG AAA *G*C*C*-3′, where *N represents a phosphorothioate blocked nucleotide and ^{f}G represents G tagged with fluorescein (FITC) at the 5′-end (IDT, Inc.). TPM1κ anti-sense, 5′-G*C*G*G CTT TCT CGT CAG CAG TG*T*G*C-3′; TPM1α sense, 5′-AGT ACT CGG AGT CCT TGA-3′; TPM1α anti-sense, 5′-A*G*G*A CTC CGA GTA CTT GTC *C*A*A-3′.

Cationic Liposome Mediated Transfection in Whole Hearts

Transfection of oligonucleotides and cDNA was performed according to previously published methods [Zajdel et al., 1998]. Stage 38 normal embryos were anesthetized and then hearts were dissected free with watchmaker's forceps and placed into Steinberg's solution. Four microliters of sense and anti-sense oligonucleotides (10 μM) were placed into 20 μl of lipofectin and 20 μl of Steinberg's solution. All hearts were beating immediately after dissection. The transfection solution was prepared and applied in a single blind method for the initial experiments examining beating. Transfected whole hearts were maintained in culture for 5 days.

RT-PCR and Southern Blot Hybridization

RT-PCR with oligo dT priming was performed to evaluate the expression of TPM1κ, TPM1α,

and TPM4α isoforms using our previously published protocol [Zajdel et al., 2000]. The strategy for RT-PCR amplification of TPM1α and TPM1κ simultaneously with the same primer-pair and subsequent detection using isoform-specific detector oligonucletide is presented in Figure 1. The sequence of PCR primers used are as follows: TPM1κ and TPM1α (common to both isoforms): (+) 5′-ATG GAC GCC ATC AAG AAG AAG-3′; and (−) 5′-ACG CTC CTG AGC ACG ATC CA-3′; TPM4α: (+) 5′-CCG CTC CCT GAC ACC GGT TCC CG-3′ and (−) 5′-GCT CTC CCT CCA GAA TAA CAA GTT-3′. Southern blot hybridization was carried out using with [^{32}P]-labeled isoform specific detector oligonucleotides, the sequence of which are as follows: TPM1κ: 5′-AGG GTG CTG GAT GAA CTG CAC AA-3′; TPM1α: 5′-AGT ACT CGG AGT CCT TGA-3′; and TPM4α: 5′-TGG CGC GCG GAC GGG GTG TTG CT-3′.

Confocal Microscopy

Whole mount immunostaining and confocal microscopy were performed according to our published procedures [Zajdel et al., 1998]. Monoclonal antibody (CH1) [Lin et al., 1985] was used for the immunodetection of TM with Lissamine Rhodamine anti-mouse used as a secondary antibody. FITC tagged oligonucleotides could be visualized without immunodetection. Specimens were viewed on a BioRad MRC 1024ES confocal laser system mounted on a Nikon Eclipse E600 microscope. Control hearts and treated hearts were examined using identical confocal settings. A simultaneous or sequential (double staining) Z-series was made for each.

In Vitro Analysis of the Isoform Specificity for Sense and Anti-Sense Oligonucleotides

Total RNA (5 μg) from axolotl heart was annealed with 10 pmoles of isoform specific

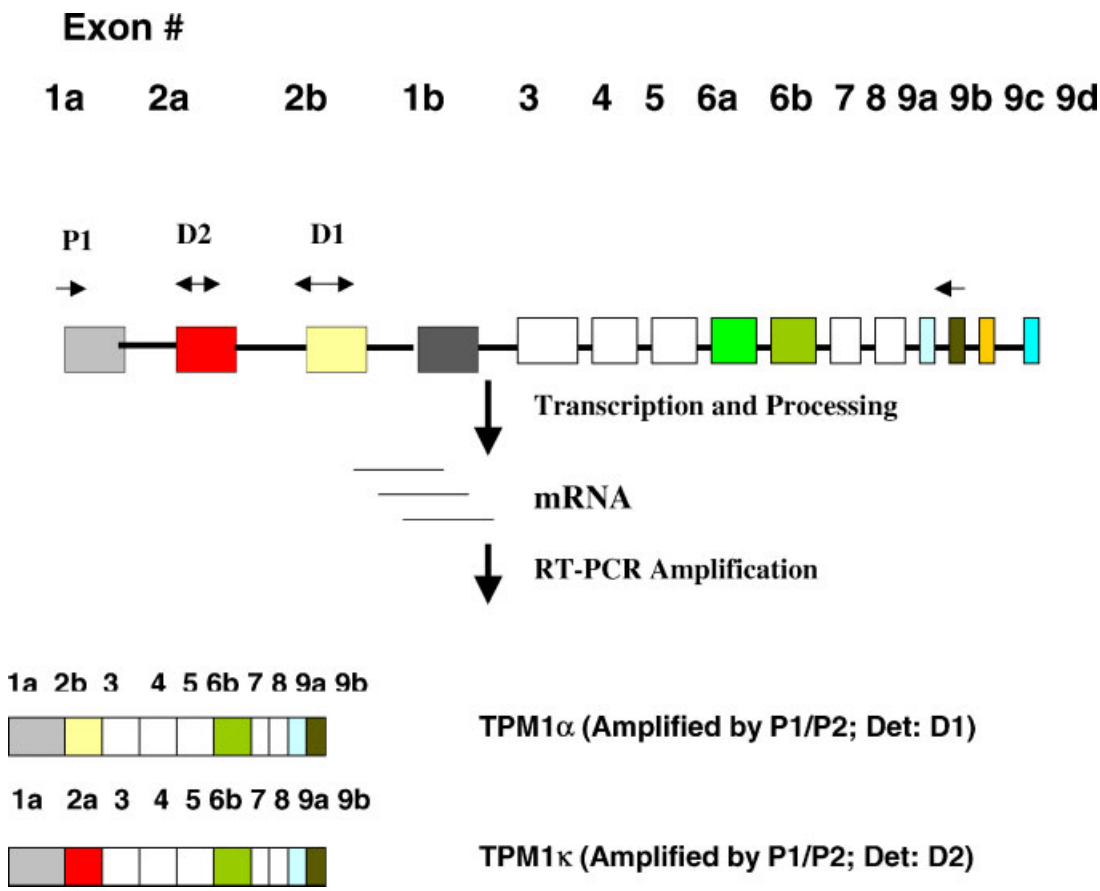

Fig. 1. Strategy for amplification of TPM1α and TPM1κ by RT-PCR using the same primer-pair and the location of isoform specific detector oligonucleotides for Southern hybridization. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sense or anti-sense oligonucleotide at 65°C for 5 min, after which the annealed mixture was chilled on ice. One microliter of RNase Inhibitor was added to the annealed mixture with 1 μl of RT-buffer, and 1 μl of RNase H (Invitrogen superscript First Strand Synthesis Kit). The mixture was incubated at 37°C for 20 min followed by denaturation at 70°C for 10 min to inactivate the enzyme. The 10-μl reaction was then chilled. RT reaction was performed using 5 μl of the above reaction mix in a total volume of 20 μl following Invitrogen's standard oligo dT protocol. Finally, PCR amplification was carried out with 2 μl of the RT product using isoform specific primer-pairs followed by the Southern blot analysis with [^{32}P]-labeled isoform specific detector oligonucleotides.

RESULTS

Specificity of TPM1κ Anti-Sense Oligonucleotide In Vitro

The specificity of the TPM1κ anti-sense oligonucleotide was examined in vitro (Fig. 3), using the strategy outlined in Figure 2. TPM4α probe was used as a control. TPM4α has a striated muscle exon 2b compared to smooth muscle type exon 2a in TPM1κ. Sense and anti-sense expression was relatively equal for the TPM4α probe. TPM1κ expression was reduced

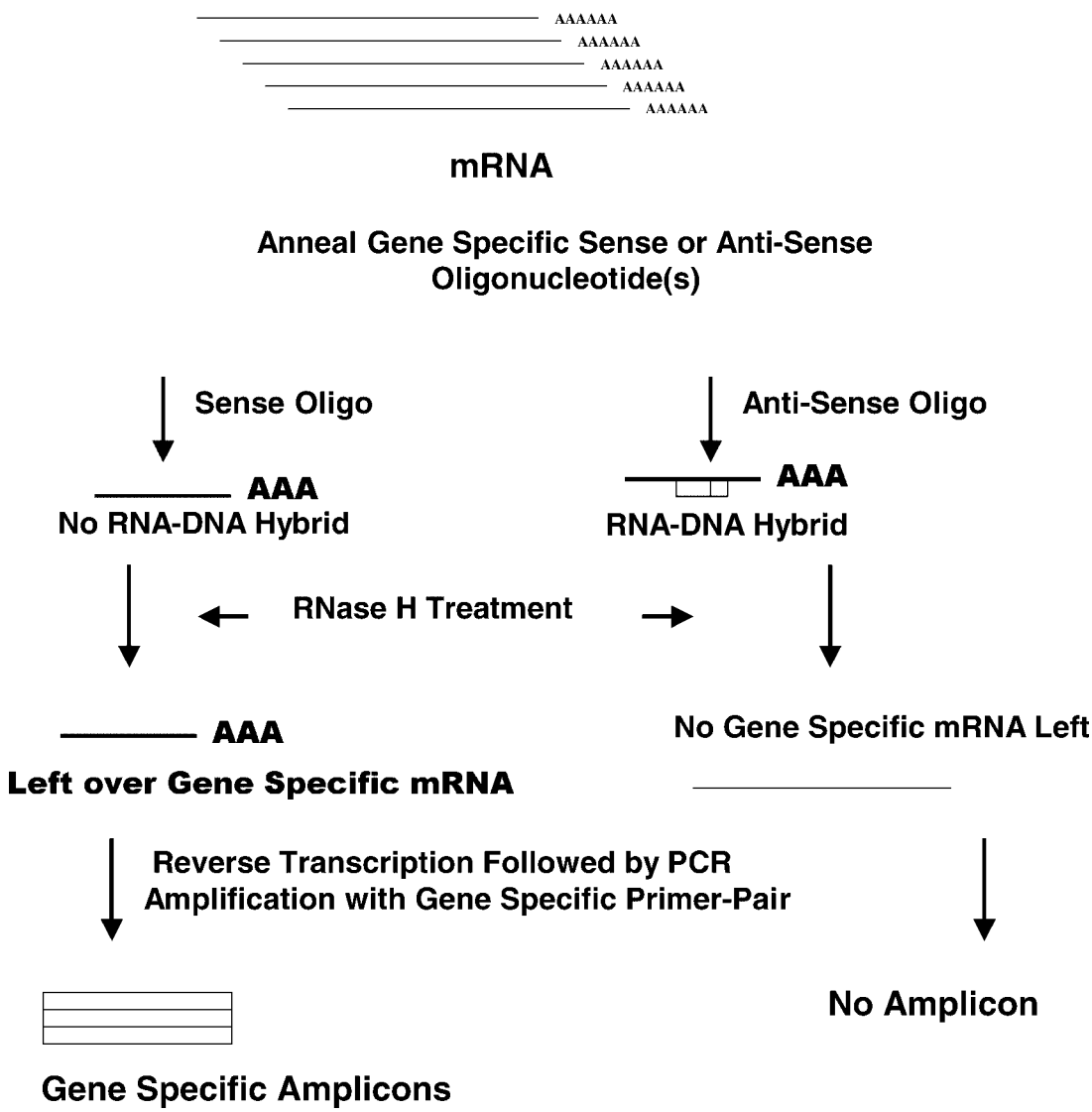

Fig. 2. Scheme for testing the in vitro specificity of sense and anti-sense oligonucleotides.

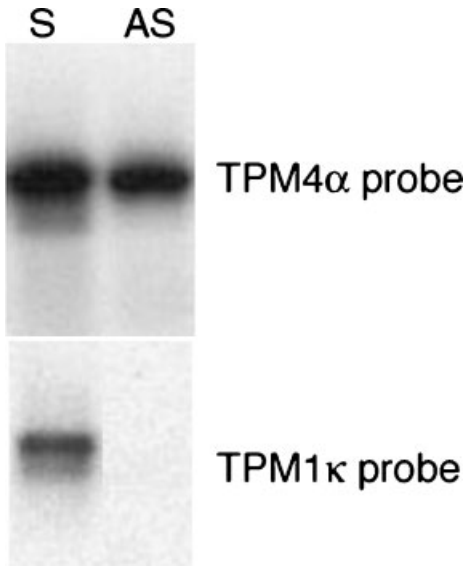

Fig. 3. RT-PCR showing specificity of TPM1κ anti-sense oligonucleotide in vitro. TPM1κ sense (**lane 1**) and anti-sense (**lane 2**) treated axolotl heart RNA. Primer control lane was blank (not included). The Southern hybridization of PCR products amplified with either TPM4α or TPM1κ specific primer pairs and probed with isoform specific oligonucletides is shown. TPM4α normally found in axolotl embryonic hearts was used as the control. The amount of TPM1κ transcripts was drastically reduced in the anti-sense treated RNA lane demonstrating the specificity of the TPM1κ anti-sense oligonucleotide compared to another predominant cardiac isoform, TPM4α.

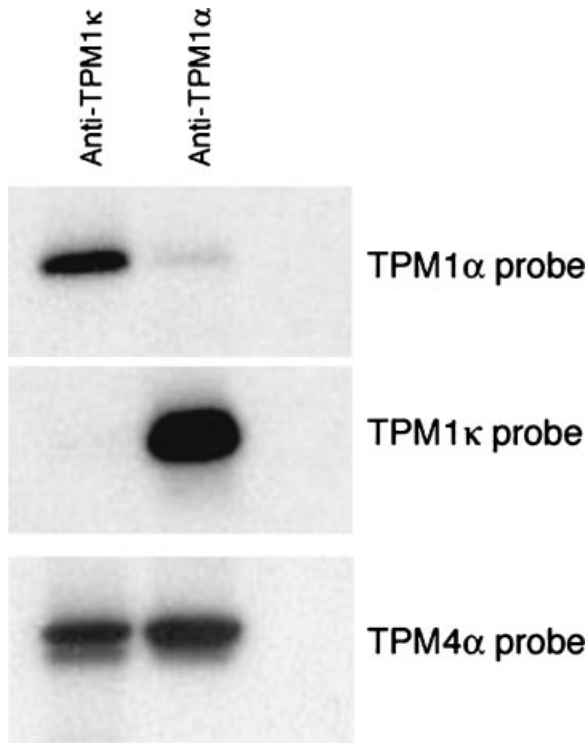

Fig. 4. Anti-sense TPM1κ and anti-sense TPM1α specificity in vitro. **Lane 1**, Anti-sense TPM1κ treated RNA with TPM1α, TPM1κ, and TPM4α axolotl tropomyosin probes. **Lane 2**, Anti-sense TPM1α treated RNA with TPM1α, TPM1κ, and TPM4α axolotl tropomyosin probes. **Lane 3**, Primer control. Shown is the Southern hybridization of PCR products amplified with either TPM1α/TPM1κ or TPM4α specific primers and probed with isoform specific oligonucleotides. TPM1κ expression was reduced with TPM1κ anti-sense treatment and TPM1α expression was reduced with TPM1α anti-sense treatment. TPM4α was unaffected by either anti-sense oligonucleotide and was again used as a control. This demonstrates the specificity of the TPM1κ anti-sense oligonucleotide compared to another TM isoform (TPM1α) from the same gene.

for TPM1κ antisense oligonucleotide with the TPM1κ probe. The sense oligonucleotide did not reduce expression as seen with the TPM1κ probe.

Anti-Sense TPM1κ and Anti-Sense TPM1α Specificity In Vitro

Three isoforms of TM have currently been identified in embryonic axolotl heart. TPM1α and TPM1κ are derived from the same TPM1 (α-*TM*) gene [Zajdel et al., 2000] while TPM4α is from a TM-4 type gene [Luque et al., 1994; Spinner et al., 2002]. Figure 4 illustrates the expression of these three isoforms in anti-sense TPM1κ- and TPM1α-treated heart RNA. Lane 1 shows that expression of TPM1α or TPM4α was not reduced with the use of the anti-sense TPM1κ oligonucleotide for each probe, respectively. The use of the TPM1κ probe clearly demonstrates reduced expression of this isoform with anti-sense TPM1κ oligonucleotide treatment. Likewise, TPM1α anti-sense oligonucleotide treatment reduced expression of TPM1α as indicated by that probe. TPM1α and TPM4α expression was not reduced with anti-sense TPM1κ oligonucleotide.

Sense and Anti-Sense TPM1κ Specific Oligonucleotide In Vivo

TPM1κ exon 2 specific oligonucleotides were transfected into whole normal hearts. Whole hearts were used to maintain intercellular pathways and connections. After 5 days, the expression of mRNA was examined by TPM1κ and TPM4α probes. TPM4α, a TM-4 type TM is the predominant TM found in these embryonic hearts and this probe was used as a control. Figure 5 demonstrates a reduction in TPM1κ expression in TPM1κ exon 2 anti-sense-treated hearts. Expression was not decreased in the sense treated normal whole hearts. TPM4α

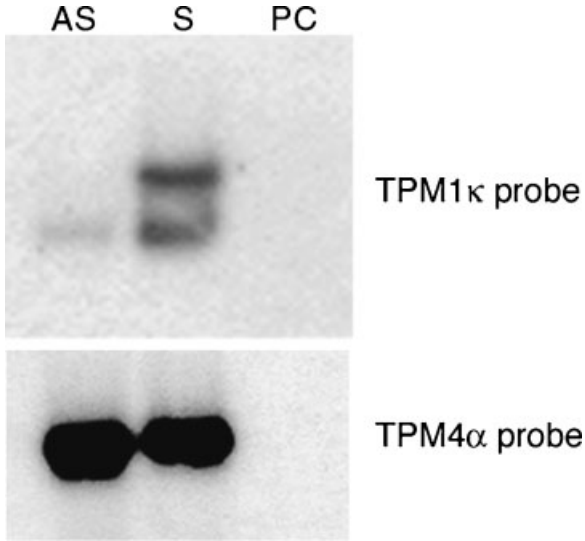

Fig. 5. Effect of TPM1κ sense and anti-sense oligonucleotides on transcript levels in vivo. TPM1κ anti-sense (**lane 1**) and sense (**lane 2**) treated whole axolotl heart. TPM4α probe was used as a control. **Lane 3**, Primer control. TPM1κ mRNA expression was reduced in whole normal hearts transfected with TPM1κ anti-sense oligonucleotide. The expression of TPM1κ was not affected when the hearts were transfected with the sense oligonucleotide. The expression of TPM4α was again unaffected by transfection of either oligonucleotide.

expression was unaffected in TPM1κ sense or anti-sense oligonucleotide-treated normal hearts.

Confocal Laser Scanning Microscopy of TPM1κ Sense- and Anti-Sense-Treated Whole Hearts

Confocal laser scanning microscopy was used to examine the thickness of the whole normal hearts. Normal hearts transfected with anti-sense TPM1κ oligonucleotides (Fig. 6a) demonstrated a disruption of myofibril organization with few sarcomeric myofibrils compared to normal hearts transfected with FITC-tagged TPM1κ sense oligonucleotides that showed no disruption in myofibrils (red) (Fig. 6b). The presence of the sense oligonucleotide was confirmed by FITC detection (green) localized to the nucleus of the myocytes.

Contractions in TPM1κ Anti-Sense Oligonucleotide Treated Hearts

Synchronous contractions of the whole hearts were monitored during the course of the experiments in a single blind method (Table II). Contractions were disrupted in the hearts during the course of the experiments. At 4 and 5 days of treatment, 3 of 11 hearts were not beating, which coincides with the reduction in TPM1κ RNA expression. There appeared to be an partial recovery or compensation of the TPM1κ anti-sense treated hearts at day 2 and 3, but by day 4 and 5, the number of beating hearts was again reduced. The heart rate of the hearts still beating was reduced compared to the normal controls. Even during this period of possible recovery, the heart rate of the beating hearts averaged below the control hearts. Normal and TPM1κ sense whole hearts were used as a control and had no reduction in beating with contractions in 11 of 11 in both categories. The normal control hearts and oligonucleotide treated hearts were not descriptively labeled for the examiner and were examined blindly.

DISCUSSION

The predominant TM isoform expressed in striated muscle has been considered to be TPM1α from the TPM1 gene [Lees-Miller and Helfman, 1991]. TPM1α consists of exons 1a, 2b, 3, 4, 5, 6b, 7, 8, and 9a/b. We have cloned and sequenced cDNAs for three striated muscle isoforms of TM from axolotl hearts. These isoforms are designated as TPM1α, TPM1κ, and TPM4α. Of these, TPM1κ is the novel isoform, which unlike TPM1α contains smooth muscle type exon 2a. We believe TPM1α and TPM1κ are alternatively spliced products of the TPM1 gene. This novel splicing is not unique to the Mexican axolotl. We have cloned and sequenced the TPM1 isoform from avian as well as mammalian species including human [Luque et al., 1997; Zajdel et al., 2003; Denz et al., 2004].

The cardiac mutant axolotl heart has specific advantages for determining the structural/functional relationships of TM because mutant hearts are deficient in sarcomeric TM protein [Lemanski, 1979; Lemanski et al., 1996; Zajdel et al., 1998]. A lack of isoform specific antibodies limits the study of which isoform is essential for the formation of myofibrils in vivo or, whether various combinations of the three isoforms may play a crucial role in forming myofibrils as well as affecting the contractility of cardiac muscle in vivo. In addition, gene knockout technology is not well established in amphibian systems, nor would it be able to knockout a single isoform. It is even difficult to utilize a transgenic mouse model, since α-TM (*TPM1*) knockout has been found to be embryonic lethal [Rethinasamy et al., 1998]. Additionally, one cannot use

knockout technology to eliminate exon 2a because it is a component of both TPM1κ and TPM1γ, which is the smooth muscle isoform. Because exon 2a is required for another essential TM isoform, simply knocking out this exon will not provide the information as to whether TPM1κ is essential for cardiac contractility and cardiogenesis.

This issue has been addressed by inhibiting the expression of TPM1κ using an exon 2a specific anti-sense oligonucleotide. The specificity of this oligonucleotide was determined in vitro (Figs. 3 and 4). Annealing of the anti-sense oligonucleotide specific for exon 2a to total heart RNA and subsequent RNase H treatment allowed the degradation of TPM1κ but not TPM1α or TPM4α. On the other hand, the exon 2a specific sense oligonucleotide did not have any effect. Additionally, transfection of the exon 2a anti-sense oligonucleotide into normal axolotl hearts in organ culture affected the concentration of TPM1κ transcripts but not TPM4α, which has already been reported to play an essential role in the cardiac contractility in axolotl (Fig. 4) [Spinner et al., 2002]. On the contrary, anti-sense TPM1α was previously found to have an insignificant effect on the myofibril formation in axolotl heart in culture [Zajdel et al., 2000].

The TPM1κ specific anti-sense oligonucleotide, but not its sense counterpart, decreased cardiac contractility (Table II). At 24 h post-treatment with TPM1κ anti-sense (day 1), the number of hearts beating (3/12) was significantly lower than either the control or TPM1κ sense treated hearts (11/11). By day 2 and 3, the number of anti-sense treated hearts beating

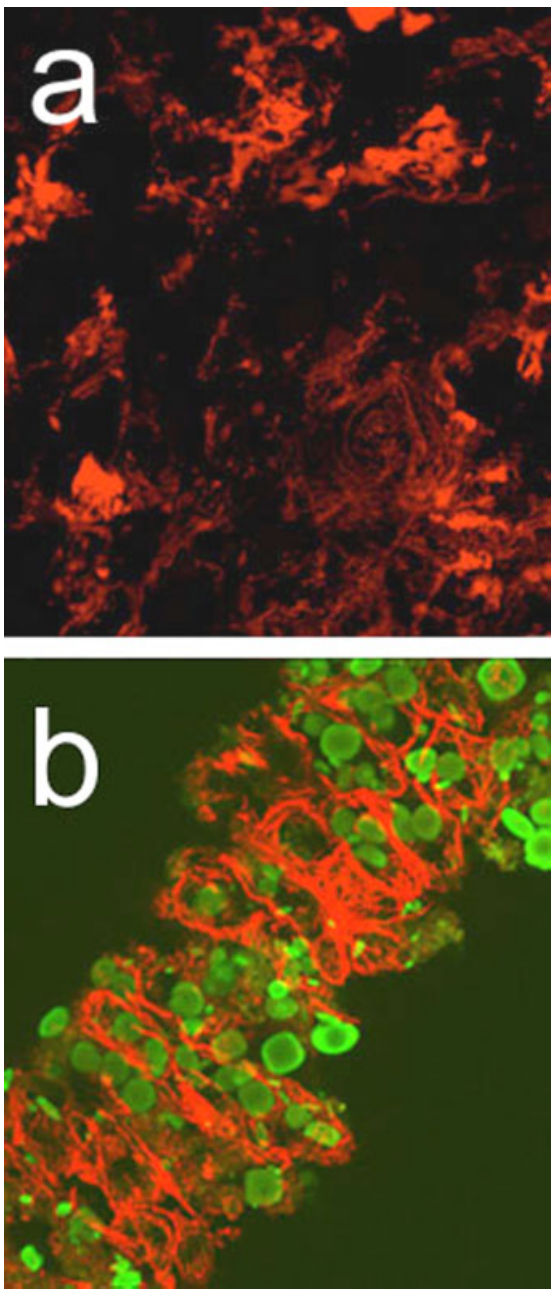

Fig. 6. Myofibril organization and entry of FITC-tagged oligonucleotide into cells of whole hearts. Confocal z-series images of stage ~38 embryonic axolotl hearts transfected with either TPM1κ anti-sense or sense oligonucleotides. Immunodetection of sarcomeric tropomyosin using CH1 monoclonal antibody is shown in red. **a**: TPM1κ anti-sense transfection disrupted the myofibril organization in normal axolotl heart compared to TPM1κ sense transfection. Very little organized structure is seen when examined by the tropomyosin staining. The secondary antibody is contained within amorphous areas in the cells. In (a), the cells are identifiable by black circular areas surrounded by staining. The amorphous staining can be seen at the periphery of the black circular areas or at the cell membrane. Since this is a three dimensional projection, not all the cells and surrounding staining is the plane of focus. **b**: It shows that the TPM1κ sense oligonucleoides did not affect the structure. Since no effect on myofibril structure with TPM1κ sense was observed, we tagged the oligonucleotide with FITC (green) to verify its presence and found it to be within the myocytes (panel b). Double staining of the nucleus (green) and the myofibrils at the periphery of the cells (red) can be seen. The green staining is ovoid in shape and primarily located at the center of the cells [Zajdel et al., 2000]. Cells can be identified by the consistent myofibril linear and circular staining below the membrane, by the black non-staining area below the myofibril staining, and the green nuclear staining in the middle of the cells. This figure shows that TPM1κ plays a critical role in maintaining the myofibrillar structure in embryonic axolotl hearts. Note: panel (a) is at 60× to show the lack of myofibril structure at a higher resolution while panel (b) is at 20× to show the clear structure in conjunction with presence of the FITC tagged sense oligonucleotide. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II. Five Day Comparison of Synchronous Contractions in Control, Sense, and Anti-Sense Treated Whole Hearts

	Control	Sense TPM1κ	AS TPM1κ
Day 1 (beats/min)	11/11 (17/min)	11/11 (15/min)	3/12 (13/min)
Day 2 (beats/min)	10/11 (30/min)	11/11 (38/min)	9/12 (25/min)
Day 3 (beats/min)	11/11 (36/min)	11/11 (38/min)	9/11 (28/min)
Day 4 (beats/min)	10/11 (36/min)	11/11 (38/min)	3/11 (12/min)
Day 5 (beats/min)	11/11 (37/min)	11/11 (32/min)	3/11 (11/min)

increased, but not to the level seen in the controls nor the same rate (25 and 28 beats/min, respectively, compared to 38 beats/min for the controls). At day 4 and 5 post-treatment with TPM1κ anti-sense, the number of beating hearts was again reduced to levels seen at day 1 (3/11 for both) as well and the rate of beating for those 3 hearts (12 and 11 beats/min, respectively). The rate of contraction and number of beating hearts stayed consistent in the control and TPM1κ sense treated hearts. The partial recovery seen in the anti-sense-treated hearts could be an attempt to compensate for the TPM1κ reduction, possibly through upregulation of another TM isoform, but that is ultimately insufficient to maintain proper contraction. After 5 days, some of the hearts restarted beating possibly suggesting the ability to overcome the anti-sense inhibition.

The anti-sense oligonucleotide specific for TPM1κ also causes disorganization of the myofibrils in normal axolotl heart (Fig. 6). On the contrary, the FITC labeled sense oligonucleotide, although it can be detected within the myocardium of transfected axolotl heart, does not affect the existing myofibrils or cardiac contractility. Hence, it is tempting to conclude that TPM1κ plays a critical role in the contractility as well as myofibrillogenesis of axolotl hearts. Our results do not permit us to conclude that the TPM1κ specific anti-sense oligonucleotide that has been used in this study does not affect other important isoforms, such as TPM1γ, because exon 2a is also a part of this isoform. However, the smooth muscle isoform is not known to play any significant role in cardiac contractility or cardiac myofibrillogenesis.

Another important question that remains is how does TPM1κ affect the process of myofibril formation in striated muscles, cardiac, in particular. We have evidence of TPM1κ being cardiac specific in human [Denz et al., 2004], chicken [Zajdel et al., 2003], and rat (unpublished results). We believe that the mechanism for formation of a mature myofibril in cardiac muscles follows the proposed three-step process of myofibrillogenesis [Rhee et al., 1994]. According to this model, the formation of mature myofibrils is mediated by the initial deposition of pre-myofibrils in close association with the cell surface. Pre-myofibrils then give rise to nascent myofibrils that subsequently help to form mature myofibrils. Each of these stages is associated with specific sarcomeric protein markers; for example, non-muscle myosin IIB is present in pre-myofibrils but is replaced by myosin in nascent myofibrils. The costameric proteins, talin and vinculin, first appear in mature myofibrils. The pre-myofibril model of myofibrillogenesis also appears to be applicable to myofibrillogenesis in skeletal muscle cells [Rhee et al., 1994; Rethinasamy et al., 1998].

It is important to understand whether TPM1α and TPM1κ play the same role in myofibril formation in cardiac tissues in a competitive manner. Or, do both isoforms take part in the process of myofibrillogenesis in a very specific manner where each of them may play a critical role at different stages of myofibril formation. Again, such is the case with non-muscle myosin IIB, which is essential in pre-myofibril formation and then is replaced by the muscle specific myosin. Currently, we are planning to evaluate the progression of myofibrillogenesis in axolotl heart where one can control the process of cardiogenesis by regulating the environmental temperature. Differential epitope tagging of various TM isoforms will facilitate the identification and localization of the ectopically expressed TM protein molecules on sarcomeres using epitope specific antibodies and subsequent confocal microscopy and/or immunoelectron microscopy.

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REFERENCES

Bordzilovskaya NP, Detlaff TA, Duhon ST Malacinski GM. 1989. Developmental stage series of axolotl embryos. In: Armstrong JB, Malacinski GM, editors. Developmental biology of the axolotl. Oxford: Oxford Press. pp 201–219.

Cooley BC, Bergstrom G. 2001. Multiple combinations of alternatively spliced exons in rat tropomyosin-α gene mRNA: Evidence for new isoforms in adult tissues and cultured cell. Arch Biochem Biophys 390:71–77.

Denz CR, Narshi A, Zajdel RW, Dube DK. 2004. Expression of a novel cardiac specific tropomyosin isoform in humans. Biochem Biophys Res Comm 320:1291–1297.

Laing NG, Wilton SD, Akkari PA, Dorosz S, Boundy K, Kneebone C, Blumbergs P, White S, Watkins H, Love DR. 1995. A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. Nat Genet 9(1):75–79.

Lees-Miller J, Helfman D. 1991. The molecular basis for tropomyosin diversity. BioEssays 13:429–437.

Lemanski LF. 1979. Role of tropomyosin in actin filament formation in embryonic salamander heart cells. J Cell Biol 82:227–238.

Lemanski LF, Nakatsugawa M, Bhatia R, Erginel-Unaltuna N, Spinner B, Dube DK. 1996. Characterization of an RNA which promotes myofibrillogenesis in embryonic cardiac mutant axolotl hearts. Biochem Biophys Res Comm 229:974–981.

Lin JJ, Chou C-S, Lin JL-C. 1985. Monoclonal antibodies against chicken tropomyosin isoforms: Production, characterization, and amplification. Hybridoma 4:223–242.

Luque EA, Lemanski LF, Dube DK. 1994. Molecular cloning, sequencing and expression α-tropomyosin from cardiac muscle of the Mexican axolotl, *Ambystoma mexicanum*. Biochem Biophys Res Comm 203:319–325.

Luque EA, Spinner BJ, Dube S, Dube DK, Lemanski LF. 1997. Differential expression of a novel isoform of alpha-tropomyosin in cardiac and skeletal muscle of the Mexican axolotl (*Ambystoma mexicanum*). Gene 185:175–180.

Mak AS, Smillie LB. 1981. Structural interpretation of the two-site binding of troponin on the muscle thin filament. J Mol Biol 149:541–550.

Martson SB, Redwood CS. 2003. Modulation of thin filament activation by breakdown or isoform switching of thin filament proteins: Physiological and pathological implications. Cir Res 93:1170–1178.

Nadal-Ginard B. 1990. Muscle cell differentiation and alternative splicing. Curr Opin Cell Biol 2:1058–1064.

Perry SV. 2001. Vertebrate tropomyosin, properties and function. J Muscle Res Cell Motil 22:5–49.

Pittenger MF, Kazzaz JA, Helfman DM. 1994. Functional properties of non-muscle tropomyosin isoforms. Curr Opin Cell Biol 1:96–104.

Rethinasamy R, Muthuchamy M, Hewett T, Bovin G, Wolska BM, Evans C, Solaro RJ, Wieczorek DF. 1998. Molecular and physiological effects of α-tropomyosin ablation in the mouse. Cir Res 82:116–123.

Rhee D, Sanger JM, Sanger JW. 1994. The premyofibril: Evidence for its role in myofibrillogenesis. Cell Motil Cytoskel 28:1–24.

Spinner BJ, Zajdel RW, McLean MD, Denz CR, Dube S, Mehta S, Choudhury A, Nakatsugawa M, Dobbins N, Lemanski LF. 2002. Characterization of a TM-4 type tropomyosin that is essential for myofibrillogenesis and contractile activity in embryonic hearts of the Mexican axolotl. J Cell Biochem 85:747–761.

Zajdel RW, McLean MD, Lemanski SL, Muthuchamy M, Wieczorek DF, Lemanski LF, Dube DK. 1998. Ectopic expression of tropomyosin promotes myofibrillogenesis in mutant axolotl hearts. Dev Dynamics 213:412–420.

Zajdel RW, McLean MD, Lemanski LF, Dube DK. 2000. Alteration of cardiac myofibrillogenesis by lipofectin-mediated delivery of exogenous proteins and nucleic acids into whole embryonic hearts. Anat Embryol 210: 217–222.

Zajdel RW, Sanger J, Denz CR, Lee S, Dube S, Poiesz BJ, Sanger JW, Dube DK. 2002. A novel striated tropomyosin incorporated into organized myofibrils of cardiomyocytes in cell and organ culture. FEBS Lett 520:35–39.

Zajdel RW, Denz CR, Lee S, Dube S, Ehler E, Perriard E, Perriard J-C, Dube DK. 2003. Identification, characterization, and expression of a novel α-tropomyosin isoform in cardiac tissues in developing chicken. J Cell Biochem 89:427–439.