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Original Article

Immunofluorescent confocal analysis of tropomyosin in developing hearts of normal and cardiac mutant axolotls, *Ambystoma mexicanum*

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ABSTRACT Tropomyosin is a major component of the thin filaments in organized myofibrils of cardiac muscle cells. A purported role for this protein is to prevent myosin thick filaments from interacting with actin thin filaments until the cell contracts. Recently, tropomyosin has been implicated in actin filament formation/stabilization as well. In the cardiac mutant axolotl, *Ambystoma mexicanum*, heart development is arrested. Mutant embryos form hearts that fail to beat due to a lack of organized sarcomeric myofibrils. There is also a concomitant reduction in the amount of tropomyosin in mutant heart cells. Previous studies have suggested that the addition of purified tropomyosin to glycerinated homogenates of mutant hearts causes amorphous actin to polymerize (or stabilize) into thin filaments. The current study was undertaken to examine the three-dimensional distribution of tropomyosin during myofibrillogenesis in normal hearts over the course of development from the heart-beat stage (35) through the advanced embryonic stage (41) and to investigate whether myofibrils form and/or significant quantities of tropomyosin accumulate in developing mutant hearts of comparable ages.

KEY WORDS: *tropomyosin, heart development, mutant salamander, confocal microscopy*

Introduction

Tropomyosin is a major component of the thin filaments in myofibrils. Tropomyosin is situated along the helical groove of actin thin filaments and is also associated with the troponin subunits I, C and T (I= inhibitory subunit; C= calcium-binding subunit; T= tropomyosin-binding subunit). Tropomyosin molecules physically prevent myosin heads from interacting with the actin filaments until the signal for contraction. An action potential causes the release of Ca^{++} ions from the sarcoplasmic reticulum into the cytosol; these Ca^{++} ions bind with troponin C changing the conformation of the troponin molecule, which in turn causes the tropomyosin to move out from the groove of the actin filaments and exposes the actin filament binding sites so that the myosin heads can interact with the actin. The actual contraction is begun with the binding of the myosin heads to the actin filaments. Beyond this obvious role of tropomyosin in contraction, tropomyosin may also play a significant role in actin filament formation and/or stabilization in the developing heart (Lemanski, 1979; Liu and Bretscher 1989, 1992; Broschat, 1990; Weigt *et al.*, 1990).

In the cardiac mutant axolotl, embryos develop hearts in which organized myofibrils fail to form resulting in a non-beating heart. Mutant (c/c) embryos are first distinguishable from their normal (+/+ or +/c) siblings at stage 35 when the heart begins to beat rhythmically in normal embryos (Humphrey, 1972). Early morphogenesis appears to be unaffected by the mutation; the lateral plates move together across the anterior endoderm to form the heart tube in much the same way in both normal and mutant embryos. At stage 35 there are no obvious gross morphological differences between normal and mutant embryonic hearts; however, electron microscopic studies have revealed that normal hearts contain well-organized myofibrils while mutant hearts contain only amorphous proteinaceous collections (Lemanski, 1973a). We conclude from these data that cardiac lethal mutant gene results in an incomplete differentiation in the mutant myocardium which is characterized most obviously by the absence of organized sarcomeric myofibrils. At later stages (39 through death), mutant hearts become distended and thin-walled and the mutant embryos develop an ascites condition. Blood circulation is never established in mutant embryos and, consequently, they are able to survive only ~20 days beyond the heartbeat stage.

Biochemical and immunohistochemical studies on the ventricles of sectioned specimens have extended our ultrastructural data and suggest that tropomyosin is abundant in normal hearts but reduced in mutant hearts (Lemanski, 1979). These studies have shown further that mutant hearts accumulate actin in a non-filamentous amorphous state, and with the addition of purified tropomyosin to glycerinated mutant heart preparations, thin actin filaments form, apparently immediately, at the expense of these amorphous collections (Lemanski, 1979). This result indicates that

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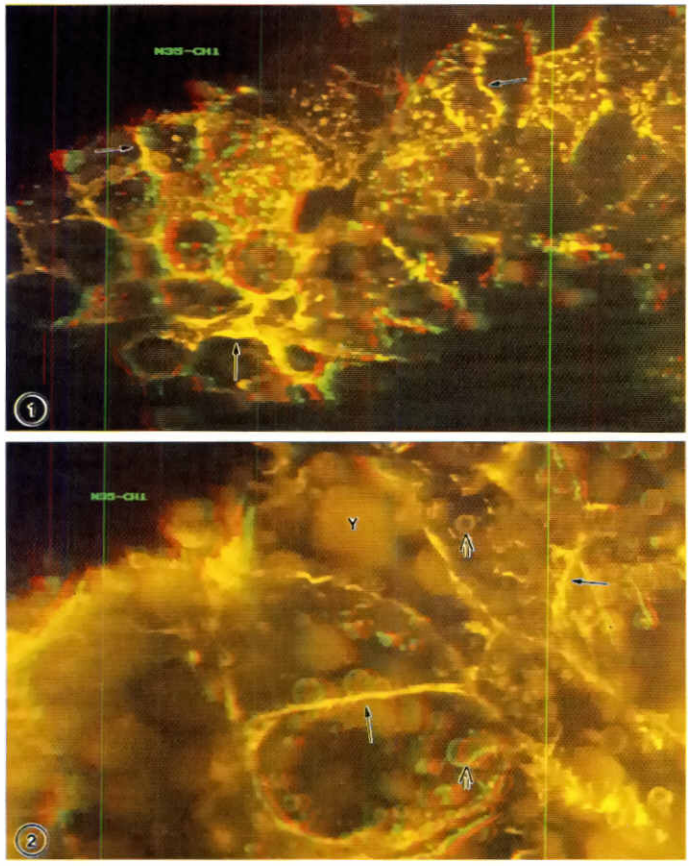


Fig. 1. Confocal 3-dimensional reconstruction of a z-series. *This z-series was taken through the ventricle of a normal stage 35 embryonic axolotl heart which was stained with CH1, a monoclonal antibody against sarcomeric tropomyosin. Note the staining mostly at the peripheries of the cells (arrows). Punctate staining is observed throughout many of the cells, perhaps representing newly-synthesized tropomyosin. Definitive myofibrils are not obvious in this micrograph. This and subsequent micrographs require red/green stereo viewing glasses to visualize the 3-dimensional images. x224.*

Fig. 2. Confocal 3-dimensional reconstructions of z-series. *The image shows a portion of the ventricle of a normal stage 35 embryonic axolotl heart stained with CH1 anti-tropomyosin antibody. Immunopositive vesicular structures can be observed (open arrows) as well as small nascent myofibrils (small arrows). Most of the staining is localized at the peripheries of the cells. Y, yolk platelets. x529.*

the presence of tropomyosin may be a necessary prerequisite for the formation and/or stabilization of actin filaments, which in turn would seem essential for the formation of organized sarcomeric myofibrils.

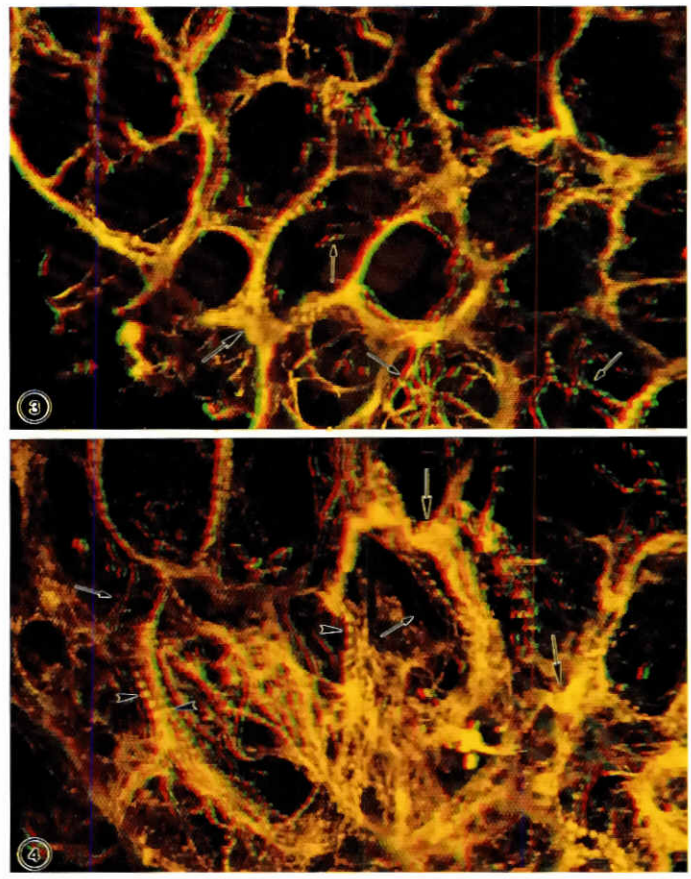
Previously it has been possible to examine only limited portions of hearts given the limitations of using sectioned material; it was not feasible by these earlier methods to determine unequivocally whether significant quantities of tropomyosin were ever present or whether myofibrils ever formed in mutant hearts. Confocal microscopy has the advantage of being able to optically section through whole-mounted hearts, yielding a high resolution, three-dimensional image of the entire heart. The present laser confocal microscope study of tropomyosin expression and accumulation in whole-mount preparations of normal and mutant hearts at various stages of development was undertaken to examine tropomyosin distribution during myofibril development and organization at the three-dimensional level in normal hearts and to determine the extent that tropomyosin is expressed in mutant hearts during development.

Results

It is essential to use red/green stereo viewing glasses when observing the confocal micrographs presented in this paper in order to visualize the images in three-dimensions.

Stage 35 normal hearts

Normal hearts have very small, tropomyosin-positive myofibrils which are seen primarily at the cell peripheries; most cells exhibit continuous lines of staining in these areas (Fig. 1). Well-defined, discreet myofibrils are rarely seen at stage 35 (Fig. 2). Yolk platelets are abundant at this early developmental stage. Immunopositive vesicle-like structures are present throughout the



Figs. 3 and 4. Confocal 3-dimensional reconstructions of z-series through stage 38 normal embryonic axolotl hearts. *Shown are portions of ventricles stained with CH1 anti-tropomyosin antibody. Small nascent myofibrils (small arrows) can be seen at the basal surface (away from the viewer) while large, more mature myofibrils are seen at the apical (luminal) surface (toward the viewer). Large myofibrils are generally arrayed parallel to the long axis of the cells while nascent myofibrils appear to be random in their organization. Also, large myofibrils are found mostly at the peripheries of cells while nascent myofibrils are located in the basal-central cytosol. Many large myofibrils are aligned in register (arrow heads). What appear to be amorphous areas stained with anti-tropomyosin are evident (large arrows). x529.*

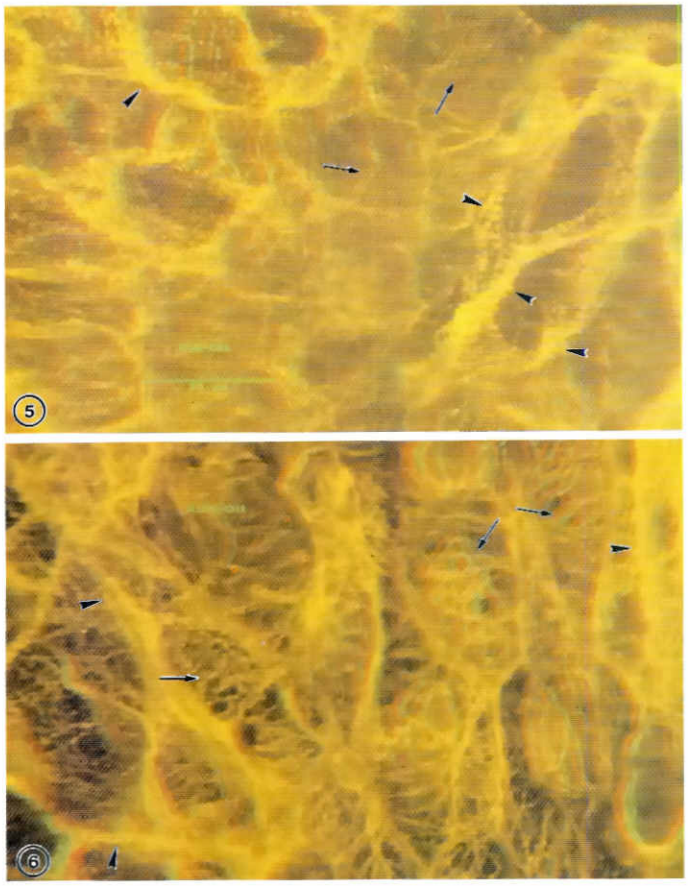


Fig. 5. Confocal 3-dimensional reconstructions of z-series through stage 40 normal embryonic axolotl hearts. *Most of the tropomyosin has been assembled into large, mature myofibrils which form thick cables (arrow heads) near the luminal surface of the cells. Small, nascent myofibrils (small arrows) are localized at or near the basal surface (away from the viewer) of the cells. x529.*

Fig. 6. Confocal 3-dimensional reconstruction of a z-series through a stage 40 normal embryonic axolotl heart. *Small, nascent myofibrils (small arrows) can be seen in deep pockets representing the basal surface of the cells. Large, more mature myofibrils (arrow heads) are seen at the apical (luminal) surface of cells. x529.*

myocardium as visualized in three dimensions. These vesicle-like structures are variable in size and are seen in all experimental hearts examined at stage 35. The vesicles are not observed in control hearts where the primary antibody has been omitted; these controls show virtually no background staining (not shown).

Stage 38/39 normal hearts

Normal hearts at this intermediate stage of development have larger, more well-developed myofibrils than at stage 35 (Figs. 3,4). The continuous lines of tropomyosin staining seen in hearts at stage 35 are no longer observed in stage 38 hearts; instead, discreet myofibrils loosely fill the periphery as well as the more central cytosol of the myocytes. Well-developed, more mature myofibrils are found toward the cell periphery while nascent myofibrils are found toward the center of the cell (Fig. 3). A basal-to-apical organization is observed within the myocardium. That is, nascent myofibrils are found mostly near the basal surfaces of cells while large, mature myofibrils are found near the apical (or luminal) surfaces of cells (Figs. 3,4). Amorphous, apparently unassembled tropomyosin is observed in some cells (large arrows; Figs. 3, 4). Nascent myofibrils form a loose network of strands whereas large, mature myofibrils form dense networks of thick cables of myofibrils. These thick cables represent myofibrillar tracts which have begun to align into register. Such tracts of aligned myofibrils often span several cells (Fig. 4). There are fewer yolk platelets in stage 38 normal hearts than in stage 35 normal hearts. The immunopositive vesicle-like structures seen in normal hearts at stage 35 are rarely seen in normal hearts by stage 38.

Stage 40 normal hearts

Normal hearts at stage 40 exhibit a dense meshwork of well-developed myofibrils positive for tropomyosin staining (Figs. 5,6). Even at this stage, where there are numerous myofibrils, one can see that the basal-apical organization is still exhibited. Tiny beads of staining representing nascent myofibrils can be found in concave areas at/near the basal surface of the cardiomyocytes (best seen in Fig. 6). Large myofibrils are often aligned in register (Fig. 5) along the peripheries. Tracts of myofibrils are now seen to span as many as six cells (Fig. 6). Figure 6 demonstrates an interesting view of the relationships between nascent and mature myofibrils. There appears to be a central circumferential organization of the nascent myofibrils that is 90° with respect to the long axis of the cells. Large mature myofibrils are found at the cell peripheries and are parallel with the long axis of the cell. Normal hearts at this late developmental stage contain very few yolk platelets and the immunopositive vesicle-like structures are no longer present.

Stage 41 normal heart

The myocardium is filled with large myofibrils aligned in register (arrow heads) but still contains a few nascent myofibrils (small arrows) as seen in Fig. 7. By stage 41, yolk platelets are no longer detected in normal hearts. A putative necrotic cell (Fig. 7, asterisk) was observed in this portion of the ventricular myocardium.

Stage 35 mutant hearts

Stage 35 mutant hearts exhibit slight tropomyosin staining in the form of small amorphous collections in some but not all cells (Fig.

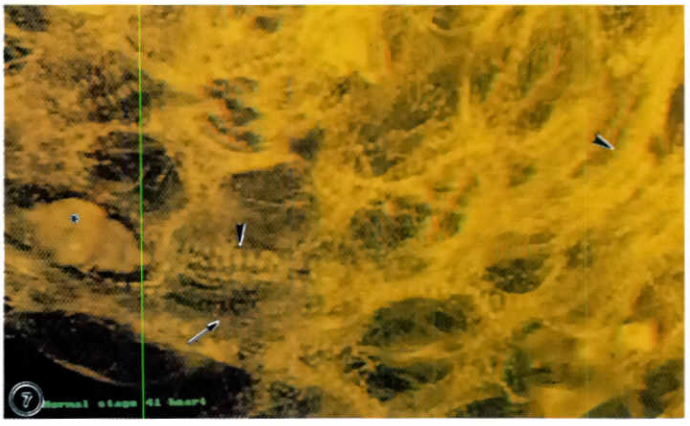


Fig. 7. Confocal 3-dimensional reconstruction of a z-series through a stage 41 normal embryonic axolotl heart. *This is a ventricular area of a heart which has been stained with CH1 anti-tropomyosin antibody. Small nascent myofibrils (small arrows) are still observed in basal areas of the cells while large mature myofibrils (arrow heads) are aligned in register. What appears to be a necrotic cell (asterisk) is seen in this micrograph. x529.*

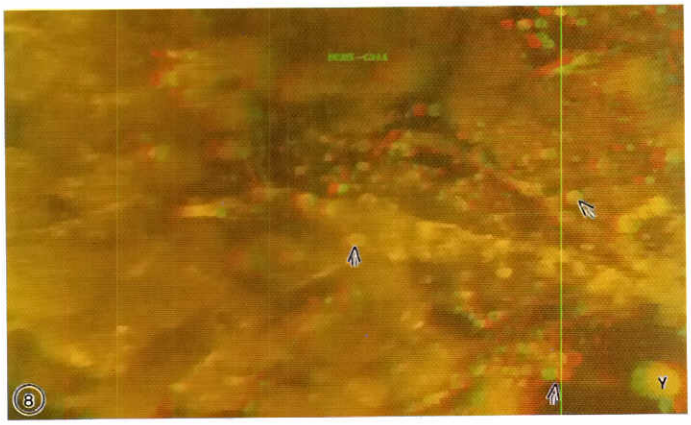


Fig. 8. Confocal 3-dimensional micrograph of a z-series through a stage 35 mutant (c/c) embryonic axolotl heart. *This portion of the ventricle is stained with CH1 anti-tropomyosin antibody. Little specific staining is observed with the exception of the vesicular structures (open arrows). Y, yolk platelets. x529.*

8). Immunopositive vesicle-like structures are present and appear similar in amount and distribution to those seen in stage 35 normal hearts. Yolk platelets are abundant.

Stage 38/39 mutant hearts

The amount and distribution pattern for tropomyosin in stage 38/39 mutant hearts appears to be very similar to those observed in stage 35 mutant hearts. There is very little tropomyosin staining in the mutant myocardium at this stage and no discreet organization of myofibrillar components is found (Fig. 9). Immunopositive vesicle-like structures are no longer observed. Yolk platelets continue to be abundant and are present in greater numbers than in stage 38/39 normal hearts.

Stage 40 mutant hearts

Tropomyosin is nearly undetectable in mutant hearts at stage 40. Faint lines of staining are visible in the cells of some specimens while many mutant hearts exhibit very little if any tropomyosin staining (Fig. 10). Yolk platelets, which have almost disappeared in normal hearts by this stage, are still present throughout the myocardia of stage 40 mutant hearts. As in normal hearts at stage 40, mutant hearts at this stage also do not contain the immunopositive vesicular structures previously described.

Discussion

Cardiac lethal gene results in an incomplete differentiation of the myocardium of homozygous embryos. Specifically, mutant embryos form hearts which fail to beat, lack organized sarcomeric myofibrils and contain significantly reduced amounts of tropomyosin. While actin appears to be present in normal amounts in mutant hearts, early studies indicated that tropomyosin was present in significantly reduced amounts, compared to normal hearts, after stage 35 (Lemanski *et al.*, 1976; Lemanski, 1979). Radioimmunoassays quantitatively showed that, even as early as stage 35, mutant hearts contained only about 1/4 as much tropomyosin as wild type hearts (Moore and Lemanski, 1982). Although other studies indicated that tropomyosin levels might be normal in mutant hearts at stage 35 (Starr *et al.*, 1989), or even later (Fuldner *et al.*, 1984), these conclusions were based on qualitative assessments of the intensity of bands on electrophoretic gels. Thus, it was not possible to draw unequivocal conclusions about the relevant amounts of tropomyosin in wild type and mutant hearts. Results from earlier studies have strongly suggested that the addition of purified tropomyosin to glycerinated mutant heart homogenates causes non-filamentous actin present in amorphous collections at the mutant cells peripheries to form into or to stabilize the thin filaments (Lemanski, 1979).

Studies on skeletal muscle suggest the possibility that actin filament length within myofibrils is regulated in part by tropomyosin. Tropomyosin, when bound to actin thin filaments *in vitro*, stabilizes the pointed end of thin filaments, which reduces the rate of polymerization/depolymerization (Broschat, 1990; Weigt *et al.*, 1990). Recently, tropomyosin has been reported to be essential for actin cable formation in yeast (Liu and Bretscher, 1989, 1992); disruption of the tropomyosin gene in yeast results in a reduced

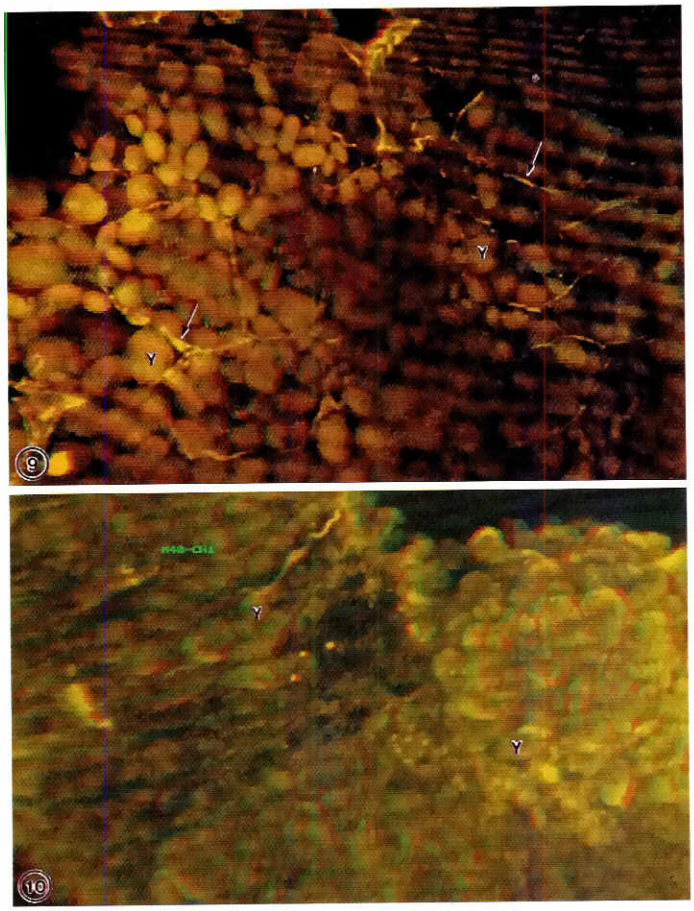


Fig. 9. Confocal 3-dimensional reconstruction of an immunofluorescent z-series through a stage 38 mutant (c/c) embryonic axolotl heart. *This heart was stained with CH1 anti-tropomyosin antibody. Some specific staining can be seen (arrows) but it does not appear to exhibit distinct myofibrils. The amount of tropomyosin is greatly reduced compared to normal hearts of the same stage. Yolk platelets (Y) are abundant. x224*

Fig. 10. Confocal 3-dimensional reconstruction of a z-series through a stage 40 mutant (c/c) embryonic axolotl heart. *This heart was stained with CH1 anti-tropomyosin antibody. Some specific staining can be seen (arrows) but it does not appear to exhibit distinct myofibrils. The amount of tropomyosin is greatly reduced compared to normal hearts of the same stage. Yolk platelets (Y) are abundant. x224.*

growth rate and the disappearance of actin cables. Correlatively, the introduction of an additional gene for tropomyosin and the overexpression of tropomyosin in a mutant strain of yeast *(act1-2)* which lacks actin cables, causes rescue and partial restoration of actin cables in the yeast cells (Liu and Bretscher, 1989). These studies, when taken together, imply that tropomyosin may be required for the formation of actin thin filaments or filamentous structures as in the case of actin cables in yeast. In the cardiac mutant axolotl, it seems possible that the failure of actin to form filaments is related to the reduction of tropomyosin and, hence, the failure of mutant heart cells to form well-organized myofibrils.

The present *in vivo* confocal immunofluorescent study examines tropomyosin expression in normal and mutant hearts during their development from the heartbeat stage (stage 35) until shortly before death of the mutant embryo. Stage 40 hearts were chosen for the study to examine mutant myocardia at an advanced stage that does not yet show severe pathology. By stage 40, the embryos have just hatched from their jelly coats and the mutants, while exhibiting an ascites condition, appear relatively healthy (i.e. they are actively swimming and show no obvious signs of energy or oxygen deficiency). Stage 38/39 embryos were selected as an intermediate stage of development. Confocal microscopy made it possible to sample the entire heart using a whole-mount method as described in Materials and Methods. Thus, the present study was undertaken to determine whether mutant hearts, at any time during their development, express significant quantities of tropomyosin and/or whether myofibrils ever form.

Our results show that tropomyosin is expressed at very low levels throughout development of the mutant heart when compared to normal, and that well-organized sarcomeric myofibrils do not form in mutant hearts. There does, however, appear to be some expression of sarcomere-specific tropomyosin that is detected by the monoclonal antibody CH1 prepared initially by Lin *et al.* (1985). This monoclonal antibody has been well characterized and recognizes sarcomere-specific tropomyosin; moreover, it does not appear to stain any of the cytoskeletal tropomyosin in the overlying epicardium of stage 40 embryonic hearts in the present study. We observed intense sarcomeric striated staining in the myocardium of normal hearts but only faint amorphous staining in the myocardium of mutant hearts. No staining was observed in control hearts where CH1 was omitted from the staining procedure and hearts were incubated in fluorescently-labeled secondary antibody only.

At stage 35, when normal hearts have just begun to beat, immunopositive vesicle-like structures are present in both normal and mutant hearts. These structures are not seen in control specimens that have been stained with fluorescently labeled secondary antibody alone. The function of the vesicle-like structures is not known; it is possible that they represent newly synthesized tropomyosin that has not yet been incorporated into organized myofibrils. The vesicle-like structures were observed only in the stage 35 hearts and not at stages 38 or 40. Tropomyosin-positive vesicles have not been reported in our previously published immunocytochemical data; this may be due to the previously-mentioned sampling problem using sectioned material.

The overall organization of myofibril formation during heart development was examined in normal hearts at stages 35, 38/39 and 40. Small, nascent myofibrils appear to form at the basal peripheral areas of the cells in a looser, less organized arrangement than the large mature myofibrils found at the luminal peripheries of the cells. Possibly, newly-formed nascent myofibrils are formed in the basal cytosol and translocated to the luminal side of the cell where they become incorporated into larger myofibrillar structures. Such myofibrils are capable of increasing their force as the organ needs to pump greater amounts of blood. This organization is observed even as late as stage 40, when normal hearts are fully functional and vigorously pumping blood. Three dimensional confocal analysis of thick sections of adult axolotl heart, or whole-mounts of juvenile axolotl heart, would be required to determine if this apical-to-basal organization persists throughout adulthood. Further, the circumferential organization of nascent myofibrils versus the longitudinal organization of the mature myofibrils was observed. Presumably, the centrally-located, circumferential, nascent myofibrils (while being translocated to the luminal portion of the cell) move toward the periphery and become longitudinally aligned. During this process, nascent myofibrils become incorporated into myofibrillar tracts and are also aligned into register.

By stage 41, extensive trabeculation is occurring. It has been previously reported that programmed selective cell death is possibly associated with the phenomenon of trabeculation in developing axolotl heart (Lemanski, 1973b). Figure 7 illustrates an example of what appears to be a necrotic cell in the ventricle of a stage 41 normal embryonic heart, which is at a stage when extensive trabeculation is taking place.

The data presented in this paper demonstrate that mutant hearts do not, at any time during their development, express tropomyosin in normal quantities nor do they form organized myofibrils except occasionally in the conus area (an area known to sometimes contract in mutant hearts while the ventricle remains quiescent). These data corroborate our previous electron microscopic, immunohistochemical and biochemical data (Lemanski, 1973a,b, 1976, 1979; Lemanski *et al.*, 1976, 1980; Moore and Lemanski, 1982), which, when taken together, suggest that there is insufficient tropomyosin present in mutant hearts to support the formation/stabilization of thin actin filaments and, hence, organized myofibrils. Further, with respect to our three-dimensional data, we have observed a previously undescribed pattern of myofibril formation within the myocardia of normal embryos which suggests that large, mature myofibrils are found at the luminal side of the cells where they can exert the greatest amount of force on the blood within the lumen of the developing organ. Newly formed myofibrils, on the other hand, form at the basal periphery of cells and later become incorporated into the large mature myofibrils.

Materials and Methods

Tissue procurement

Heterozygous (+/c x +/c) adult axolotls, *Ambystoma mexicanum*, were mated and the fertilized eggs were grown at 17°C until the embryos reached stages 35, 38/39 and 40. The staging systems of Bordzilovskia *et al.* (1989) was used. Sibling normal and mutant embryos were used in each individual experiment.

Confocal microscopy

Normal and mutant hearts were fixed according to a method modified after Bell *et al.* (1987). This method allows simultaneous permeabilization and fixation using the crosslinker dithiosulfylpropionate (DTSP) and DMSO in the initial fixation step. All steps took place at room temperature with gentle agitation on an orbital shaker. Following a 20 min fixation in DTSP in 0.01% DMSO and Steinberg's solution, whole hearts were further permeabilized for 15 min in 0.5% NP-40 and the reaction stopped by 0.1 M glycine. Hearts were then incubated in mouse monoclonal antibody CH1 concentrate against chicken heart sarcomeric tropomyosin (Lin *et al.*, 1985) at a 1:30 dilution in Steinberg's solution for 40 min, washed several times and incubated 20 min in 0.1 mg/ml BSA solution in Steinberg's

solution to block non-specific binding. Hearts were transferred to rabbit anti-mouse lissamine rhodamine antibody at a 1:125 dilution for 40 min. Control hearts were not incubated in primary antibody but were incubated in secondary fluorochrome-conjugated antibody only. Following several washes in buffer, the hearts were post-fixed in 2% glutaraldehyde for 30 min and quenched in 0.1 M glycine for 15 min. Finally, the hearts were mounted on slides in 50% glycerol containing 2% n-propyl gallate. Parafilm bridges were placed along the sides of the long axis of the slides before applying a coverslip to prevent compression of the hearts. The coverslips were sealed over the tissues on the slides using boatsealer and viewed on a BioRad MRC-600 Confocal Laser Scanning Microscope. The black and gain levels were set using tropomyosin-stained normal hearts, and all other hearts were examined under identical conditions. A z-series was made for each sample and the images were displayed on a monitor. The images were stored on optical discs and a Focus Graphics Imagecorder was used to record the images on 35 mm color slide film for subsequent photographic processing.

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References

BELL, P.B., RUNDQUIST, I., SVENSSON, I. and COLLINS, V.P. (1987). Use of cytofluorotomy to evaluate binding of antibodies to the cytoskeleton of cultured cells. *J. Histochem. Cytochem. 35:* 1381-1388.

BORDZILOVSKAYA, N.P., DETLAFF, T.A., DUHON, S. and MALACINSKI, G.M. (1989). Developmental-stage series of axolotl embryos. In *Developmental Biology of the Axolotl* (Eds. J.B. Armstrong and G.M. Malacinski). Oxford University Press, New York, pp. 201-219.

BROSCHAT, K.O. (1990). Tropomyosin prevents depolymerization of actin filaments from the pointed end. *J. Biol. Chem. 265:* 21323-21329.

FULDNER, R.A., LIM, S.S., GREASER, M.L. and LEMANSKI, L.F. (1984). Accumulation and localization of troponin-t in developing hearts of *Ambystoma mexicanum*. *J. Embryol. Exp. Morphol. 84:* 1-17.

HUMPHREY, R.R. (1972). Genetic and experimental studies on a mutant gene determining absence of heart action in embryos of the mexican axolotl *(Ambystoma mexicanum). Dev. Biol. 27:* 365-375.

LEMANSKI, L.F. (1973a). Morphology of developing heart in cardiac lethal mutant mexican axolotls, *Ambystoma mexicanum. Dev. Biol. 33:* 312-333.

LEMANSKI, L.F. (1973b). Heart development in the mexican salamander, *Ambystoma mexicanum* II. Ultrastructure. *Am. J. Anat. 136:* 487-525.

LEMANSKI, L.F. (1976). Morphological and biochemical abnormalities in hearts of cardiac mutant salamanders *(Ambystoma mexicanum). J. Supramol. Struct. 5:* 221-238.

LEMANSKI, L.F. (1979). Role of tropomyosin in actin filament formation in embryonic salamander heart cells. *J. Cell Biol. 82:* 227-238.

LEMANSKI, L.F., FULDNER, R.A. and PAULSON, D.J. (1980). Immunofluorescence studies for myosin, α-actinin and tropomyosin in developing hearts of normal and cardiac lethal mutant Mexican axolotls, *Ambystoma mexicanum. J. Embryol. Exp. Morphol. 55:* 1-15.

LEMANSKI, L.F., MOOSEKER, M.S., PEACHEY, L.D. and IYENGAR, M.R. (1976). Studies of muscle proteins in embryonic myocardial cells of cardiac lethal mutant Mexican axolotls *(Ambystoma mexicanum)* by use of heavy meromyosin binding and sodium dodecyl sulfate polyacrylamide gel electrophoresis. *J. Cell Biol. 68:* 375-388.

LIN, J.J.C., CHOU, C.S. and LIN, J.L.C. (1985). Monoclonal antibodies against chicken tropomyosin isoforms: production, characterization, and application. *Hybridoma 4:* 223-242.

LIU, H. and BRETSCHER, A. (1989). Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell 57:* 233-242.

LIU, H. and BRETSCHER, A. (1992). Characterization of TPM1 disrupted yeast cells indicates an involvement of tropomyosin in directed vesicular transport. *J. Cell Biol. 118:* 285-299.

MOORE, P.B. and LEMANSKI, L.F. (1982). Quantification of tropomyosin by radioimmunoassay in developing hearts of cardiac mutant axolotls, *Ambystoma mexicanum. J. Muscle Res. Cell Motil. 3:* 161-167.

STARR, C.M., DIAZ, J.G. and LEMANSKI, L.F. (1989). Analysis of actin and tropomyosin in hearts of cardiac mutant axolotls by two-dimensional gel electrophoresis, western blots, and immunofluorescent microscopy. *J. Morphol. 201:* 1-10.

WEIGT, C., SCHOEPPER, B. and WEGNER, A. (1990). Tropomyosin-troponin complex stabilizes the pointed ends of actin filaments against polymerization and depolymerization. *FEBS Lett. 260:* 266-268.

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