DISTRIBUTION AND STRUCTURE-FUNCTION RELATIONSHIP OF MYOSIN HEAVY CHAIN ISOFORMS IN THE ADULT MOUSE HEART*

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The two cardiac myosin heavy chain isoforms, α and β, exhibit distinct functional characteristics and therefore may be distributed regionally within the heart to match the functional demands of a specific region. In adult mouse hearts, which predominantly express α-myosin heavy chain, we observed high concentrations of β-myosin in distinct areas such as at the tip of papillary muscles and at the base close to the valvular annulus. In light of these distinct distribution patterns of the myosin isoforms, we subsequently explored the myosins’ isoform-specific structure-function relationships. The α- and β-isoforms are 93% identical in amino acid sequence, but it remains unclear which of the non-identical residues determine isoform functionality. We hypothesized that residues situated within or close to the actin-binding interface of the myosin head influence actin binding and thereby modulate actin-activated adenosine triphosphatase activity. A chimeric myosin was created containing β-sequence from amino acid 417 to 682 within the α-backbone. In mice, ~70% of the endogenous cardiac protein was replaced with the chimeric myosin. Myofibrils containing chimeric myosin exhibited adenosine triphosphatase activities that were depressed to the levels observed in hearts expressing ~70% β-myosin. In vitro motility assays showed that the actin filament sliding velocity generated by chimeric myosin was similar to that of α-myosin, almost twice the velocities observed with β-myosin. These data indicate that this region is responsible for the distinct hydrolytic properties of these myosin isoforms.

Two distinct isoforms of the myosin heavy chain (MHC), † termed V1 and V3, are expressed in the mammalian heart. V1 is a homodimer of two α-MHC molecules, whereas V3 is a ββ-homodimer. α- and β-MHC exhibit pronounced functional differences, with β-MHC characterized by slower actomyosin adenosine triphosphatase (ATPase) rates and actin filament sliding velocities in vitro, but capable of generating forces more economically in terms of energy consumption (1-3). Since cardiac MHC isoform expression is species-dependent, developmentally controlled, and sensitive to hormonal perturbations and cardiovascular stress (4-7), variations in expression patterns might play a role in fine-tuning cardiac performance.

Disease states such as cardiac failure or hypertrophy can alter the relative and absolute MHC isoform expression levels (6). For example, a shift from the normally predominant α-MHC toward β-MHC occurs in failing mouse hearts (8). We have recently shown that when transgenic (TG) mice in which 70% of the α-MHC was replaced by β-MHC were subjected to severe cardiovascular stress, the left ventricle (LV) decompensates at a faster rate than in the controls. This would imply that the α → β-MHC isoform shift observed in cardiac disease may be a maladaptive response (9). In fact, in the failing human heart, the normally small 7% α-MHC expression is also down-regulated (7, 10, 11) so that α-MHC is no longer detectable. This raises the possibility that such a small-scale shift in MHC isoform may be partially responsible for the
functional deficits observed in the failing human heart. If MHC isoform expression is not uniform throughout the entire myocardium but rather localized to specific regions of the heart, then relatively minor changes in the cardiac MHC isoform content could have significant functional impact on cardiac performance depending on where the MHC isoforms are expressed. Thus, in conjunction with studies aimed at determining the structure-function relationships of the two isoforms, it is also important to determine the regional distributions of α- and β-MHC to gain detailed anatomical insight into the heterogeneity of MHC isoform expression.

Although high-resolution crystal structures exist for several classes of myosin, myosin’s complex structure makes it difficult to understand the structural underpinnings of isoform functionality. Based on primary structure alone, the mouse α- and β-MHCs may provide a model system for addressing this question. These two isoforms are 93% identical, implying that the remaining 127 out of 1938 amino acids (i.e., 7%) that are non-identical must contribute to the distinct enzymatic and mechanical properties that characterize the two cardiac MHC’s. However, these non-identical amino acid residues are distributed throughout the molecule and may affect one or more functional domains. Two clusters of non-identical amino acids (19 in total) are found in surface loops 1 and 2, which span the nucleotide-binding pocket and the actin-binding domain, respectively (12, 13). To determine if these residues contribute to cardiac MHC isoform functionality, we previously generated chimeric MHCs, in which the sequences of either loop 1 and/or loop 2 of cardiac α-MHC, were exchanged for those of β-MHC and then expressed in vivo in a TG mouse model (14). The lack of any significant functional impact of these chimeras on enzymatic and mechanical properties at the fiber and molecular level suggested that the two surface loops alone do not confer the unique functional profiles for the α- and β-MHC, leaving the remaining 108 non-identical amino acid residues as potential candidates.

In this study, we took a more global approach to the general problem of cardiac MHC functionality by switching ~14% of the non-identical amino acids (i.e., 24) in the α-MHC backbone to that of β-MHC, all of which are contained in a critical region of the catalytic domain that interacts with actin and undergoes substantial movements during the actomyosin ATPase cycle (15, 16). The primary sequence bounded by these substitutions begins with the first substitution at residue 417 and ends with the final substitution at residue 682. This chimeric MHC, termed ABC (for α/β-chimera), was then expressed specifically in mouse cardiomyocytes in order to replace the endogenous protein with the genetically engineered protein. The mechanics of isolated papillary fibers were characterized while myofibrils and myosin were isolated from the TG hearts to assess the mechanical and biochemical characteristics of ABC in terms of myofibrillar ATPase activity and in vitro actin sliding velocities.

**EXPERIMENTAL PROCEDURES**

**Immunofluorescence**—Hearts of 5–13 month-old male and female FVB/N mice were fixed by perfusion with 4% paraformaldehyde in cardioplegic buffer (phosphate-buffered saline, dextrose, 50 mM KCl), cut in half longitudinally or transversely and further fixed in 4% paraformaldehyde in PBS for 24 hrs, infused with 30% sucrose, embedded, and frozen. Four to five µm cryostat sections were immunolabeled with α-MHC monoclonal antibody (harvested from BA-G5 cells, American Type Culture Collection, Manassas, VA). As a secondary tag, Streptavidin/Alexa Fluor 568 (Molecular Probes, Eugene, OR) was employed. For double immuno-labeling, a custom-made polyclonal anti-loop-2 of cardiac β-MHC (Genemed Synthesis Inc., San Francisco, CA) was used. It was visualized with a goat anti-rabbit IgG/Alexa Fluor 488 (Molecular Probes). Images were acquired using a PCM 2000 Nikon confocal microscope equipped with Argon lasers and Simple PCI software.

**Generation of TG animals**—ABC cDNA was produced using a previously generated DNA construct (14) and site-directed mutagenesis. Starting with the L2 construct, which consists of an α-MHC backbone with the sequence of the surface loop 2 switched to the respective amino acid sequence of β-MHC (containing 13 non-identical amino acids), an additional 11 amino acids residues were switched: S417N, Y422S,
S424A, E510T, S545T, M592L, E596Q, K665R, T666S, R680T, and A682S. All residue positions here and in the following text are given with reference to mouse α-MHC. The resulting chimeric protein contained β-MHC sequence from amino acid 417 to 682 in the α-MHC backbone (Fig. 2). Cardiac expression of the ABC protein was driven by the mouse α-MHC promoter to a level of ~43%. To enhance the degree of replacement of the endogenous α-MHC with the chimeric protein, the TG line chosen was bred into the heterozygous α-MHC null background (17), resulting in 70% replacement with the chimeric MHC. In addition to the ABC mouse, a previously developed β-MHC TG mouse was used as well as propylthiouracil (PTU) -treated mice expressing 95% β-MHC (14).

Western Blots, 2D-electrophoresis, and RNA dot blots—Protein replacement was measured using a custom-made antibody specific for the β-MHC sequence of loop 2 (14). The relative amount of α- and β-MHC in standards from PTU-treated animals was determined using gel retardation as described (14). Protein extracts from the TG mice expressing ABC were run alongside standards on 7% acrylamide gels and transferred to PVDF membranes for immunoblotting. Signal intensities with the loop 2-antibody were normalized to the Coomassie stain of the MHC band and the relative transgene content calculated. For detection of tropomyosin and TnT isoforms, total protein homogenates were electrophoresed on 12% (29:1 acrylamide/bis) and 14% (180:1 acrylamide/bis) gels, respectively. After transfer, membranes were probed with mouse monoclonal antibodies (Sigma) against tropomyosin (clone CH1) and TnT (clone JLT-12). For 2D-electrophoresis, myofibrillar preparations (18) were loaded onto pH 4.7–5.9 micro-range strips. After isoelectric focusing (protocol modified from (19)), proteins were run out on 8-16% tris-HCl gels and visualized using SyproRuby stain (Biorad). Transcript analysis was performed with transcript-specific probes as described (17).

ATPase and Motility Assays—Ca2+-stimulated Mg2+-ATPase activity of myofibrillar preparations from ventricular tissue (18) was determined as previously described (20). MHC was purified from individual mouse hearts and actin-activated ATPase activity (40µM chicken skeletal actin) determined as described (14). Ca2+-activated ATPase activity in the absence of actin was measured in a reaction buffer consisting of 400mM KCl, CaCl2 10 mM, Tris 10 mM, pH 8.0) (21). In vitro motility assays were conducted as described (22, 23).

**Isometric force measurements**—Ca2+-activated force of ventricular papillary muscle fibers was measured as described (14). Sarcomere length at resting tension was ~2.2 µm at 27°C. Strip force (mN/mm²) was measured at free Ca2+ concentrations ranging from pCa 8.0 to pCa 5.0 and the rate of force development determined. Individual values of normalized isometric force were fit to the Hill equation:

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\frac{[\text{Ca}^{2+}]}{([\text{Ca}^{2+}])_{50} + [\text{Ca}^{2+}]} \approx \text{mean ± S.E.M. unless otherwise indicated. Comparisons between wild type (WT) and TG animals were evaluated using Student’s t-test, and a p value of < 0.05 was considered significant.}
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### RESULTS

**Anatomical Location of MHC Isoforms**—Both adult mice and humans normally express a high proportion of a single cardiac MHC isoform (β-MHC in mice; α-MHC in humans) with small amounts of the other cardiac isoform present. In the FVB/N mouse strain used in our laboratory, the β-MHC transcript makes up only 3–7% of the total MHC mRNA in the adult heart.2 This percentage is not constant and may be shifted by certain stimuli such as thyroid status or hemodynamic load (24, 25). It is not known, however, if or where in the heart the β-MHC is localized, that is, in a specific cell population or scattered randomly. Therefore, we used immunohistological techniques to characterize the regional distribution of α- and β-MHC in adult mouse hearts.

These studies revealed that β-MHC-containing cells are preferentially situated in distinct areas of the heart (Fig. 1). Interestingly, their location was almost identical in every heart examined. The most striking locations were clusters of β-MHC-containing cells at the base of the heart (Fig. 1A - E) and at the tip of papillary muscles (Fig. 1F - H). Examination of numerous hearts sectioned at
various angles allowed us to establish that the β-MHC-containing cells at the base of the heart form a ring at the atrio-ventricular junction where mitral and aortic valves are anchored (overview in Fig. 1A and 1B; higher magnification in Fig. 1C - E). β-MHC-containing cells at the tip of the papillary muscle form a cap that is continuous with the chordae tendineae (Fig. 1F). The β-MHC-positive cardiomyocytes were also found in the proximal apex where the septum and the right ventricular free wall meet (Fig. 1I and 1J), as well as in the distal apex (Fig. 1K). In the proximal apex, β-MHC-containing cells appeared to form a sub-endocardial lining (Fig. 1f). β-MHC-containing cells in the apex formed a distinct group in some hearts while they were irregularly distributed in others. Additionally, there were also small clusters of cells or single cells in sub-endocardial layers of the septum and left ventricular free wall (Fig. 1D and E), at the origin of papillary muscles (Figure 1G) and also in the sub-epicardial layer of the LV. No gender differences were observed in either the intensity or distribution of the β-MHC-expressing cardiomyocytes. We also studied MHC isoform distribution as the animals aged and in the C57/B16 strain and found similar distributions.

We conclude that the small proportion of expressed β-MHC is localized to discrete regions of the heart as shown in a schematic overview in Fig. 1L.

**Chimeric Myosin Functionality**—Our aim was to investigate whether replacing ~14% of the non-identical amino acids within a linear segment of the α-MHC primary sequence with that of the β-MHC would effect a switch of the mechanical and/or enzymatic characteristics of α-MHC to β-MHC. The experimental model was a TG mouse in which the majority of the endogenous α-MHC was replaced with chimeric α-MHC containing 24 β-MHC amino acid substitutions from amino acid 417 to 682 (presumed structure based on chicken myosin subfragment-1 shown in Fig. 2 (26)). TG mice were analyzed for ventricular MHC protein content by Western blot at 10-12 weeks of age (Fig. 3). For quantification, we used heart tissue from hypothyroid mice treated with propylthiouracil (PTU) that contained 95% β-MHC. Under the conditions used for western blotting of tissue homogenates from the apex of the hearts, the level of endogenous β-MHC was below the detection threshold (WT and line 78 hearts, Fig. 3A). In the highest-expressing TG line, line 104, we achieved 43% replacement of the endogenous protein with ABC (Fig. 3A). Breeding line 104 into the heterozygous α-MHC knockout background resulted in 70% replacement (Fig. 3B), a level commensurate with our previous replacements of endogenous MHC with our TG MHC constructs (14). Cardiac myofibrils or purified MHC were then used for biochemical and mechanical assays in order to determine the functional properties of the chimeric protein as compared to preparations consisting of primarily α-MHC (WT hearts) or β-MHC (PTU hearts) as well as preparations from a β-MHC transgenic mouse in which ~70% replacement of α-MHC with β-MHC was obtained (14).

We first tested whether any secondary changes in other important sarcomeric proteins were induced by the expression of ABC. Using RNA dot blots, 2D gel electrophoresis, and western blots, we did not find any evidence of increased expression of α-skeletal actin, embryonic TnT isoforms, or β-tropomyosin (Fig. 2 C-F). No change in the pattern of myosin light chain phosphorylation could be detected by 2D-electrophoresis (Fig. 2D).

As expected (27), a twofold difference in myofibrillar Ca²⁺-stimulated Mg²⁺-ATPase activity was observed between α-MHC WT and PTU hearts (Fig. 4A). Myofibrillar proteins isolated from β-MHC TG hearts showed depressed ATPase activities consistent with the ~70% replacement of α-MHC with β-MHC. Interestingly, extracts from ABC hearts had myofibrillar ATPase values very close to that of β-MHC TG hearts. Additionally, we determined the actin-activated ATPase activity of purified myosins. Consistent with the results from the myofibrillar assays, ABC demonstrated ATPase activities very similar to that of β-MHC (Fig. 4B). In contrast, in the absence of actin and stimulated by 10 mM Ca²⁺ in the presence of 400 mM KCl, the ATPase activity of ABC was significantly different from that of β-MHC, but not from α-MHC (Fig. 4C). Although these high salt Ca²⁺-activated ATPase data suggest that the ABC is inherently more like the α-MHC in the absence of actin, actin’s mode of activating the ABC can
apparently modulate its hydrolytic activity to that of β-MHC.

To determine the effect on myosin molecular mechanics, in vitro actin filament motility assays, a model system for unloaded muscle shortening, were performed using MHC that had been isolated from WT, ABC, β-MHC, and PTU-treated animals. Interestingly, filament sliding velocity of the ABC was not statistically different from that of WT α-MHC and thus faster than β-MHC (Fig. 5A).

We performed a series of experiments to control for the potential that the ABC does not effectively interact with actin in the motility assay, so that the observed actin filament velocities would reflect the properties of the remaining 30% endogenous α-MHC. In the first experiment, purified α- and β-MHC were mixed in varying proportions and actin filament velocity as a function of the α/β mixture determined.Velocities were directly proportional to the α/β mixture (Fig. 5B) as previously observed (23). The velocity of the 30/70 α/β mixture was not different from that of the β-MHC mouse, with its MHC expression at a similar level, both of which were significantly slower than the ABC. Finally, we obtained velocities using only α-MHC at 30% of the normal concentration and as expected (28) the velocity at sub-saturating surface densities was less than WT. These controls collectively indicate that there is no preferential binding of the actin filaments to the faster α-MHC isoform and that the ABC does not have β-MHC-like motion generating capacity, otherwise the observed velocity for the ABC myosin should have been similar to the 30/70 α/β mixture.

We next examined the isometric force development using skinned left ventricular papillary fibres from WT, ABC with 43% protein replacement (therefore labeled low-level ABC), and β-MHC hearts (Fig. 5C). Maximum calcium-activated isometric forces, calcium sensitivity (pCa_{50}) and Hill coefficients were no different in all three cases. These data suggest that the inherent force generating capacity of the ABC has not been compromised by the domain switch and must equal that of both the α- and β-MHC, which are similar for the mouse cardiac MHC isoforms (14, 23).

**DISCUSSION**

1. **Isoform Distributions**—Detailed analysis of MHC isoform expression in adult mouse hearts revealed distinct areas of β-MHC predominance, with the isoform abundantly expressed at the tips of the papillary muscles and at the base of the heart close to the valvular annulus (Fig. 1). These unique distributions were conserved across both different strains of mice and throughout the adult stage. These findings indicate that even in hearts that contain predominantly α-MHC, expression of β-MHC in specialized areas could reflect the unique functional demands of a particular region. These regions would have different mechanical and metabolic demands than the rest of the myocardium, which are related to the enzymatic and mechanical differences between the α-MHC and β-MHC. For example, in the mouse, β-MHC may be more suitable for maintaining appropriate tension on the atrioventricular valve leaflets. Therefore, preference of β- over α-MHC in these areas could play a critical role for fine-tuning overall contractile performance of the mouse heart. By extension, our data from the mouse hearts are consistent with the hypothesis that relatively minor changes in overall MHC isoform content, such as occurs in human heart failure, could have significant functional consequences.

2. **MHC Molecular Structure and Function**—Despite extensive structural (29) and biochemical (2, 3, 23) data, it is still unclear how subtle differences that must exist in the MHC isoform structure relate to differences in their functional capacities. A simplistic model is that isoform-specific differences in distinct regions of the myosin molecule may determine a particular biochemical and/or mechanical characteristic. On the basis of structural mutagenesis in Dictyostelium MHC II, Spudich and colleagues postulated that the primary sequence of a surface loop (loop 2) spanning the actin-binding domain determines the actomyosin ATPase rate in an isoform-specific manner (30, 31). This conclusion was based on a series of elegant experiments in which loop 2 from various muscle myosins was exchanged into the Dictyostelium MHC II, with the chimera displaying ATPase activities that were dictated by the properties of the donor myosin (30, 31). However, this concept cannot be generalized to all MHC species. Rovner et al. demonstrated...
that loop 2 substitutions from skeletal and β-MHC into the smooth muscle heavy meromyosin did not result in ATPase activities comparable to that of the donor myosins (32). Similarly, we found that the loop 1 and loop 2 regions are not major modulators of isoform-specific functionality in the mouse cardiac MHCs (14).

Since exchanging the β-MHC surface loops 1 and 2 into the mouse α-MHC did not affect actomyosin ATPase and actin filament velocities, we reasoned that a more substantial region of the catalytic domain must be involved in determining the distinct properties of these two isoforms. Thus, in the present study 24 β-MHC specific residues were introduced into the α-MHC backbone between residues 417 to 682, a region that undergoes significant rearrangement in response to both actin binding and nucleotide state within the active site. Myofibrils isolated from TG hearts had significantly slower ATPase activities that were similar to that of the β-MHC. Thus, the amino acid substitutions successfully switched the α- to a β-MHC, at least with respect to its actin-activated enzymatic activity. In contrast there was no effect on actin filament sliding velocity for purified chimeric myosin compared to the α-MHC or in its force generating capacity as observed in skinned fibers. With the β-MHC substitutions affecting the enzymatic but not the mechanical performance of the molecule, it appears that: 1) structural domains may be associated with specific functions such that the region defined by residues 417 to 682 determines ATPase activity; 2) actomyosin ATPase activity and actin filament velocity are not absolutely linked properties as originally proposed by Barany (33). This is not surprising since ATPase activity and actin filament velocity have different rate limiting steps, where the weak to strong-binding transition is rate limiting for actomyosin ATPase activity while myosin detachment limits filament velocity (34).

With the conversion of α-MHC's ATPase activity to that of β-MHC, does the location of the 24 substituted amino acids offer any insight into myosin’s molecular structure and function? Since 13 of these non-identical amino acids exist within loop 2, which when substituted previously had no effect on actomyosin ATPase activity, it would appear that these 13 amino acids alone are not sufficient to convert α- to a β-like MHC. Of the remaining 11 non-identical amino acid residues that were substituted in the present study, 5 are conservative substitutions (S545T, M592L, E596Q, K665R, T666S) and most likely do not contribute to the observed effects on ATPase activity. Therefore, the following discussion will focus on the remaining 6 non-identical amino acids (S417N, Y422S, S424A, E510T, R680T, A682S) with regards to their location within the molecule and their potential for modulating myosin’s hydrolytic activity.

Presuming that the three-dimensional structure of mouse cardiac MHC is similar to that of the chicken myosin subfragment-1 (29), these 6 amino acids are localized to three important regions of myosin’s catalytic domain. Residues S417N, Y422S, and S424A are located in the upper 50-kDa segment of the MHC molecule as part of or adjacent to a long α-helix that borders the large cleft between the upper and lower 50-kDa segments of the myosin head (Fig. 2) (29). The closure of this cleft is believed to be required for myosin strong binding to actin while ATP-binding within the active site opens the cleft and disrupts the actomyosin interaction to allow progression through the actomyosin ATPase cycle (15). The potential that these 3 residues affect cleft opening and closure may provide a mechanism for modulating ATPase activity in an isoform-dependent manner. Previously, we came to a similar conclusion when comparing the primary sequences for rodent and rabbit α-MHC (23). Interestingly, the mouse and rabbit α-MHC differ by a factor of 2 in enzymatic and mechanical performance but only differ by 3 amino acids (210, 442, and 452) within the myosin head, where residue 442 is in the same upper 50 kDa helix while residue 452 is in a linker that exits the helix. In support of this, the nearby R453C mutation found in patients with familial hypertrophic cardiomyopathy, also leads to lower actomyosin ATPase rates for myosin isolated from a knock-in mouse model (35, 36).

Residue E510T is located in the rigid relay loop (37). This loop structure forms a critical link between the active site and the converter domain, which is directly coupled to the myosin lever arm. Thus, nucleotide-dependent conformational changes originating in the active site are amplified to generate motion at the end of the myosin lever arm by way of the rigid relay loop’s interactions.
with both the active site and converter domain. Although the structural importance of the relay loop is obvious, how the Glu to Thr substitution might affect the kinetics of hydrolysis are not as clear.

Finally, substitutions R680T and A682S are situated along a surface loop that forms part of the opening to the nucleotide-binding pocket (29). The Arg→Thr exchange removes a basic group and inserts a nucleophilic group, whereas the Ala→Ser exchange introduces a second nucleophilic group. These changes may alter the microenvironment of the nucleotide-binding pocket so that the rates of nucleotide entry and exit from the pocket may ultimately affect the kinetics of the ATPase cycle.

Although we have focused on 6 non-identical amino acids as potential determinants for converting the α-MHC ATPase activity to that of β-MHC, the question whether any one residue or a more global structural perturbation due to all 24 substituted amino acids was responsible for the observed slowing of ATPase activity remains unresolved. However, the fact that actin filament velocities were unaffected by the substitutions suggests that molecular structure and function may be compartmentalized. Even though in this study the substitutions occurred over a range of primary sequence that encompasses ~1/3 of the catalytic domain, a single point mutation can have equally dramatic effects, with the R403Q α-MHC mutation in a mouse model of familial hypertrophic cardiomyopathy leading to a profound gain of function in ATPase activity, actin filament sliding velocity, and force generation (38).

We are only beginning to understand the functional consequences of cardiac myosin isoform shifts in disease. In mice, a genetically induced shift from α- to β-MHC has detrimental effects under severe cardiovascular stress (9). Vice versa, a transgenic shift in the rabbit ventricle from β- to α-MHC protects the hearts against pacing-induced failure (39). These findings indicate that modulation of MHC functionality towards more α-like properties might become a future therapeutic avenue for heart failure patients. Whether this can be achieved by targeting small single areas of the MHC molecule, or whether more than one region needs to be structurally altered is unknown. However, single point mutations such as R403Q have taught us that individual amino acid substitutions can radically alter MHC function, implying that such a therapeutic strategy could become feasible. Given the regional preference of one MHC isoform over the other, such strategies would possibly not need to affect a global change in MHC functionality, but restricted alterations in selected areas may be sufficient to improve cardiac function.
### REFERENCES


FOOTNOTES

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1The abbreviations used are: MHC, myosin heavy chain; ATPase, adenosine triphosphatase; TG, transgenic; LV, left ventricle; ABC, α/β-chimera; PTU, propylthiouracil; WT, wild type.

2M. Krenz and J. Robbins, unpublished observations (2006)


4MMDB # 5275, PDB # 2MYS

5During the review process and in response to the request for force measurements, we could no longer generate the 70% replacement ABC mice due to the irretrievable loss of the α-MHC knockout mouse line needed for cross-breeding. Thus, skinned fiber measurements were performed with lower level (i.e. 43%) expressing ABC-mice.
FIGURE LEGENDS

FIGURE 1. Cardiomyocytes expressing β-MHC are found in distinct regions of the adult mouse heart. All sections are from 5-9 month-old FVB/N mice. (A, B) - Sections cut tangentially, approximately 200 µm apart through the heart base reveal a ring of β-MHC-positive (green fluorescent) cells. (C – E) - β-MHC-containing cardiomyocytes (green) are interspersed among the α-MHC-positive cells (red) at the base of the heart. The majority of β-MHC positive cells are facing the lumen of the LV and the atrio-ventricular junction area. (C) - posterior base of the heart; (D) - interventricular septum; (E) - LV free wall. (F – H) - β-MHC-containing cells in the papillary muscles. In F and G, sagittal sections reveal β-MHC-positive cells at both anchorage sites; that is, at the tip - F, H (arrowhead) and at the origin - G (arrow). (I - K) - β-MHC-positive cells in the area where the right ventricle, interventricular septum, and apex merge. Panels I - J show that the β-MHC-expressing cells form a sub-endocardial lining (arrowhead) as well as small clusters in the septum (arrow). Panel K shows that they are also interspersed among α-MHC-positive cells in the apex. (L) - This view of a posterior portion of a mouse heart schematically shows the distribution of β-MHC-positive cells. The larger filled circles represent major groups of cells, while smaller circles represent single cardiomyocytes or small groups of cells. LA; left atrium, RA; right atrium, LV; left ventricle, RV; right ventricle, SP; interventricular septum, AP; apex, pPAP; posterior papillary muscle, aPAP; anterior papillary muscle.

FIGURE 2. Presumed structure of the ABC protein. (A) Schematic of the ABC construct. α-MHC cDNA was modified to contain the β-MHC sequence from amino acid residue 417 – 682 and inserted into the α-MHC promoter cassette. (B) 3D-view of the myosin head (from x-ray crystallography data of chicken S1 (25) using Cn3D software (23)). Residues remaining in α-MHC protein sequence in grey, the whole domain switched to β-MHC in the ABC construct marked in red with the 6 non-conservative substitutions highlighted in green, nucleotide in blue. (C) posterior view of B.

FIGURE 3. Protein expression profiles of ABC mouse hearts. (A) Western blots of myofibrillar proteins derived from WT, PTU-treated or TG mice carrying the chimeric myosin using the loop 2 β-MHC antibody. Replacement levels were 43% in line 104 and negligible in all other lines. (B) After breeding line 104 to mice carrying a deletion of one allele of α-MHC, protein replacement was 70%. (C) RNA dot blots to determine expression levels of α-skeletal actin and GAPDH as a loading control. (D) 2D-electrophoresis to show light chain phosphorylation status and tropomyosin and troponin T isoforms. (E) Expression of TnT isoforms in WT, ABC, β-MHC transgenic, and PTU-treated hearts compared to embryonic heart tissue. (F) Expression of tropomyosin isoforms in WT, ABC, β-MHC transgenic, and PTU-treated hearts compared to skeletal muscle tissue. quad, RNA or protein extracted from quadriceps muscle; β, β-MHC; emb, protein from WT ventricular tissue taken at embryonic day 14; α-sk., α-skeletal; TnT, troponin T; TM, tropomyosin; RLC2v, regulatory light chain 2v; ELC1v, essential light chain 1v.

FIGURE 4. ATPase activity of the chimeric myosin. (A) Ca2+-stimulated Mg2+-ATPase activities were determined in myofibrillar preparations from mouse heart tissues, n=4-5 in all groups. *, p<0.05 versus WT; #, p<0.05 versus PTU. (B), actin-activated ATPase activity (n=5 in all groups) and (C) Ca2+-activated ATPase activity (in the absence of actin) of the different purified myosins, n=5 in all groups. WT, wild type; ABC, α-/β-chimera; β-MHC, 70% β-MHC / 30% α-MHC extracted from transgenic mouse hearts; PTU, 95% β-MHC extracted from hypothyroid mice. *, p<0.05 versus WT; #, p<0.05 versus ABC.

FIGURE 5. Mechanics of ABC myosin. (A) In vitro actin filament motility assay. The bars indicate velocities of actin filaments (mean ± s.e.) translocated by the respective myosin heavy chains. WT, wild type; ABC, α-/β-chimera with 70% protein replacement; β-MHC, purified MHC from transgenic mouse.
with 70% β-myosin expression level; 30α/70β, mixture of purified myosins from WT / PTU hearts; 30%α, motility assay performed with only 30% of the α-MHC on the surface; PTU, 95% β-MHC extracted from hypothyroid mice. N=6-11 for WT, ABC, and β-MHC groups, n=4 for PTU, n=2-3 for 30α/70β and 30%α groups. *, p<0.05 versus WT; #, p<0.05 versus ABC. (B) In vitro motility mixture assay for mouse α- and β-MHC. Shown is the relationship between the actin filament sliding velocity and % of mouse a-MHC with respect to the myosin mixture that was applied to the motility surface. (C) Calcium-dependency of isometric force in skinned left ventricular papillary fibers. In contrast to the data shown in Figs. 2-5A, the ABC skinned fibers contained only 43% chimeric protein (therefore labeled low-level ABC). All values are expressed as mean ± s.e. Hill coefficients were 4.5 ± 0.2, 5.0 ± 0.2, and 4.3 ± 0.2 for WT, ABC, and β-MHC fibers, respectively. pCa_{50} was 5.84 ± 0.02, 5.85 ± 0.01, and 5.85 ± 0.01 for WT, ABC, and β-MHC fibers, respectively. N=5 in all groups.
Figure 3
Figure 5

A

Velocity (μm/s)

WT  ABC  β-MHC  30% wild 30%α  PTU

B

Percent β-Cardiac Myosin

Percent α-Cardiac Myosin

Velocity (μm/s)

C

Relative Force (%)

pCa

WT  low-level ABC  β-MHC