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Structure of the Human Cardiac Muscle Myosin Filament

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Human cardiac muscle myosin filaments are assemblies of myosin molecules and accessory proteins. Myosin molecules consist of two head domains and a rod-like coiled-coil domain. Each head domain possesses the ATPase activity necessary for contraction. Cardiac myosin filaments are bipolar with the myosin tails packing together and the heads adopting a 3-stranded quasi-helical arrangement. A detailed knowledge of the structure of human cardiac myosin filaments in the normal relaxed state is likely to be important in understanding how mutations give rise to various cardiomyopathies. To address this issue we have successfully developed a method to isolate myosin filaments from human cardiac muscle that preserves the highly ordered pseudo-helical structure of the relaxed filaments, thus making them amenable to analysis by electron microscopy and single particle image methods. From such samples we have produced a 3D reconstruction of the C-zone of the myosin filament at ~28 Å resolution which allows the detailed docking of myosin heads.

Myosin filaments were obtained from the ventricular muscle of donor human hearts, which had been frozen and stored in liquid nitrogen. Small pieces of muscle were incubated in relaxing solution and treated with elastase to release the myosin filaments which were harvested by centrifugation. Samples were applied to holey Formvar EM grids coated with a thin carbon film and negatively stained with 1% (w/v) uranyl acetate and 0.025% glycerol. Images were recorded with an FEI Tecnai TF20 electron microscope at an accelerating voltage of 200kV, in low dose mode with an exposure of ~100 e^{-} $Å^{-2}$ and a nominal magnification of x29,000 using a Tietz F415 CCD camera giving rise to a calibrated sampling of 5.86 Å at the specimen level. Half filament images were rotationally aligned parallel to the image y-axis and dimensionally rescaled with reference to the 6^{th} order of the 429 Å repeat in their power spectra. The C-zone of each half filament was windowed into 7 segments 950 Å in length centered on a 429 Å repeat. The segments were subjected to three-dimensional analysis exploiting the C3 symmetry of the myosin filament using a single particle approach implemented with Imagic [1] and locally developed software.

Incoherent averaging of the amplitude spectra of the filament segments (Figure 1A) shows good recovery of detail with the characteristic layer-line pattern arising from the quasi-helical myosin head distribution together with meridional reflections extending to ~36 Å (the 12^{th} order of the 429 Å repeat). The three-dimensional reconstruction calculated from the segments reveals a 429 Å repeating unit containing three distinct sets of densities on the outer surface of the filament (Figure 1B). These correspond to the three crowns of myosin heads, labeled 1, 2 & 3, the locations of which correspond to that previously described for vertebrate skeletal and cardiac muscle [2, 3]. Each crown is characterized by features with a close resemblance to the myosin head pairs identified in smooth muscle myosin [4]

and in tarantula myosin filaments [5]. Accordingly, atomic models of myosin head pairs derived from the tarantula structure were fitted into each crown using the docking program Uro [6] (Figure 1C). The close agreement between the models and the fitted density supports the proposal that the myosin heads of relaxed vertebrate cardiac myosin filaments adopt this configuration [7]. The location and orientation of the docked head pairs allows the pseudo-helical myosin head arrangement within the C-zone of the myosin filament to be defined (Figure 1D) as well as allowing the identification of the intermolecular interactions between the head pairs on different crowns which stabilize the relaxed conformation of human cardiac myosin filaments [8].

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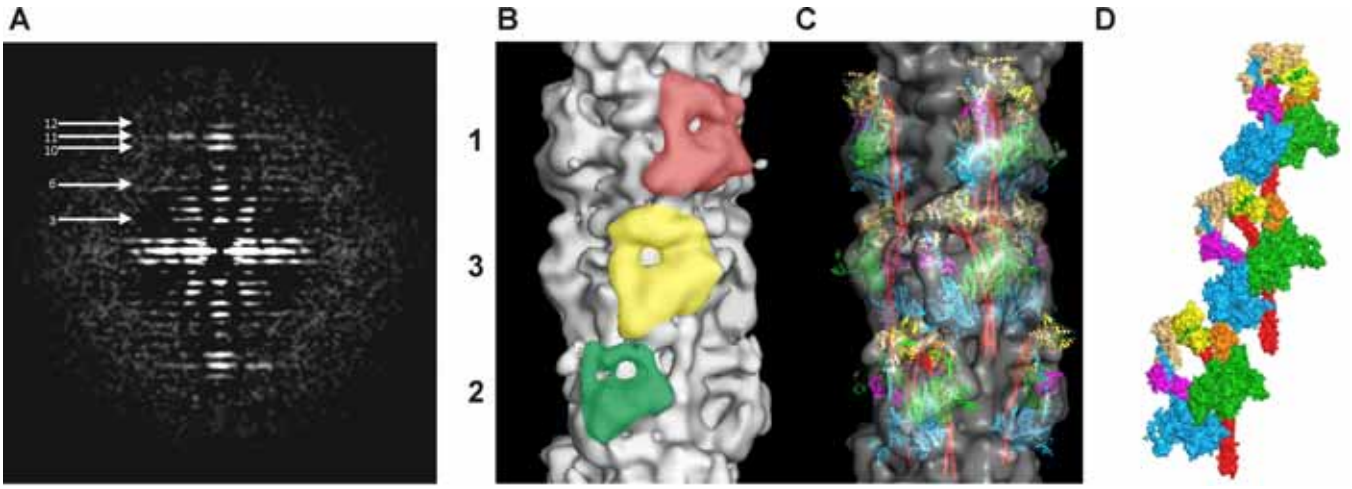

Figure 1. Structural analysis of the human cardiac myosin filament. **A.** Incoherently average of amplitude spectra from images of filament segments. Orders of the 429 Å repeat are marked. **B.** Surface view of the three-dimensional reconstruction of the cardiac myosin filament. Individual crowns are numbered and head pairs on each crown are colored red, yellow and green. **C.** Myosin head pairs docked into the three-dimensional reconstruction. **D.** Docked myosin head pairs shown as molecular surface.

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