

Chamber specific expression of Myosin heavy chain 7b in the heart of vertebrates

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In extant vertebrates, myosin heavy chain (MyHC) 6 and 7 are the main isoforms of atrial and ventricular myocardium respectively, whereas MyHC7b has been proposed to be an ancient cardiac isoform only expressed during embryonic development in modern species. The MYHC7b DNA sequence has an intronic miRNA thought to be a regulator of slow-twitch versus fast-twitch muscle differentiation.

The pan-MyHC antibodies MF20 and A4.1025 are commonly used to highlight the myocardium in histomorphological studies. In preliminary immunohistochemical studies of the heart of the lesser spotted dogfish (*Scyliorhinus canicula*; Chondrichthyes), we have observed that while MF20 labels homogeneously all the myocardium, A4.1025 labels the inflow cardiac segments (sinus venosus and atrium) but not the outflow segments (ventricle and conus arteriosus). In order to interpret these results, we have performed western and slot blots from samples of dogfish and hamster (*Mesocricetus auratus*) hearts, HPLC-ESI-MS/MS from dogfish samples, and immunohistochemistry in hearts of representative species of vertebrates, namely elasmobranchs, polypteriforms, acipenseriforms, teleosts and mammals, using MF20 and A4.1025 antibodies.

Western and slot blot results confirmed the specificity of MF20 and A4.1025 for MyHC in dogfish, as well as their differential reactivity against different myocardial segments. HPLC-ESI-MS/MS using protein databases from *Callorhynchus milii* (Chondrichthyes) and Chordata revealed the presence of MyHC6 and 7 in all the dogfish myocardial segments, and of MyHC7b only in the outflow segments. Immunohistochemistry showed that while MF20 signals were homogeneous in all the myocardial segments of all the species studied, A4.1025 signals were restricted to the inflow myocardial segments in elasmobranchs, homogeneous in teleosts and acipenseriforms, and low in the ventricle of polypteriforms.

From our results it can be inferred that the A4.1025 antibody, as opposed to MF20, has a low affinity for MyHC7b, at least in the dogfish. In addition, we show that MyHC distribution in the cardiac chambers has changed during the evolution of gnathostomes. We raise the hypothesis that in early diverged groups of gnathostomes, MyHC6 and 7 were distributed in all the myocardial segments, whereas MyHC7b was specific for the outflow myocardium. In derived gnathostomes, MyHC6 and 7 became mainly restricted to the atrium and ventricle respectively, whereas MyHC7b disappeared from the heart, acquiring a regulatory role in cardiac and extracardiac myogenesis through its intronic miRNA.

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