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EXPRESSION OF COFILIN IN AVIAN AND MAMMALIAN MUSCLE TISSUES.

N. Minami[1], K. Yabusaki[1], H. Abe[1], T. Totsuka[2] and T. Obinata[1]. [1]Dept. of Biol., Fac. of Sci., Chiba Univ., Chiba, [2]Dept. of Physiol., Aichi Pref. Colony, Kasugai.

Cofilin is an actin-binding protein which was discovered in mammalian non-muscle tissues. It is involved in the regulation of actin assembly in embryonic skeletal muscle. It was described that cofilin is absent from skeletal muscle (Yonezawa et al., 1987), but recently, cofilin was detected in adult chicken muscles. In this study, we re-examined the distribution of cofilin in mammalian and avian muscles by immunoblotting with the antibody to chicken cofilin (MAB-22). When tissue lysates were displayed on SDS-PAGE and reacted with MAB-22, cofilin was detected in smooth and skeletal muscles of chicken and mammals and in non-muscle tissues, as well. The cofilin spots of mammalian brain and skeletal muscle did not co-migrate on 2D-PAGE suggesting both may be different isoforms. Cofilin was enriched in slow fibers both in mammals and chicken. In dystrophic (dy) mouse muscle, the amount of cofilin increased significantly. When the cultures of mouse skeletal muscle cells were treated with 10% DMSO, actin-cofilin-rods were formed in nuclei. From these results, we conclude that cofilin is expressed in both chicken and mammalian skeletal muscle cells.

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CHANGES IN MYOSIN AND TROPONIN T (TNT) ISOFORMS DURING METAMORPHOSIS OF FLOUNDER

T. Obinata[1], Y. Itoh[1], H. Takano-Ohmuro[2], K. Yamano[3], Y. Inui[3]. [1]Dept. of Biol., Chiba Univ., Chiba., [2]Tokyo Metro. Inst. Med. Sci., Tokyo, [3]Natl. Res. Inst. Aquaculture, Mie.

It is well known that many isoforms exist in myofibrillar proteins of avian and mammalian skeletal muscles and their expression changes during development, but little is known as to the isoforms in fish. Here, we report that myosin and troponin T (TNT) isoforms in flounder skeletal muscle change markedly during metamorphosis. By PPi-PAGE, a single myosin isozyme in adult but three bands in larva were detected. The relative proportion of the three isozymes varied with the progression of metamorphosis. In accord with this change, changes in myosin heavy (MHC) and light chains (MLC) were detected; MHCs in larva and adult differed in size and peptide map. Larva and adult contained different regulatory MLCs. A major catalytic MLC did not change during development, although a minor larva-specific MLC was detected. TNT variants with approximate Mr of 42 kDa and 34 kDa, respectively, were identified in adult with anti-TNT antibody (NT-302); the former is a basic protein as TNTs in the other animals, but the latter is an acidic protein. The amount of the higher Mr-TNT variant decreased during development.

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MYOSIN HEAVY CHAIN ISOFORMS IN ADULT AND EMBRYONIC CHICKEN GIZZARD SMOOTH MUSCLES AND POSSIBLE POST-TRANSLATIONAL MODIFICATIONS ON THE ISOFORMS.

H. Takano-Ohmuro[1], Y. Kasuya[2], Y. Katsuragawa M. Yanagisawa[2], Y. Okamoto[3], T. Masaki[2]. [1]The Tokyo Metro. Inst. Med. Sci., Tokyo, [2]Inst. Basic Med. Sci., Univ. of Tsukuba, Ibaraki 305, [3]Juntendo Univ. Tokyo 113.

It has been reported that there are two myosin heavy chains (MHCs) in various adult vertebrate smooth muscles (SMs) : molecular weights of these proteins on SDS PAGE are 204 KDa (MHC_1) and 200 KDa (MHC_2). Yanagisawa et al. (1987) showed that the gizzard (G) SM MHCs were encoded by a single-copy gene, while Nagai et al. (1989) reported that two MHCs in rabbit uterus were generated by different mRNAs which are made from respective cDNA.

We found that embryonic GSM MHCs were immunochemically different from adult GSM MHCs. However, the reactivites of embryonic GSM MHCs became simillar to that of the adult MHCs after incubation with an adult GSM cytosol fraction which did not contain any myosin. S1 nuclease mapping with fragments of embryonic G MHC cDNA as probes revealed that MHC mRNAs in adult and embryonic GSMs appeared to be identical. Furthermore, chemically methylated MHC_2 was co-migrated with MHC_1 on SDS PAGE.

These results strongly suggest that MHC isoforms in chicken GSMs are produced due to post-translational modifications.

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SCAFFOLDING STRUCURE OF MYOFIBRILS: DESMIN NET AND EFFECT OF PHOSPHORYLATION.

M. Kuroda[1], A. Matsuno[1] and H. Ohmuro[2]. [1]Dept. of Biol., Fac. of Sci., Shimane Univ., Matsue and [2]Tokyo Metropol. Inst. for Med. Sci., Tokyo

Extraction of strated muscle with dilute lactic acid gave scaffolding structure of myofibrils, in which Z-disks were arrayed as a ladder-like orientation. Even after extraction of major body of contractile proteins by the acid, Z-disks of the scaffolding structure did not leave apart but retained its original disposition. Electron microscopy of this scaffolding structure networks of short 100-nm filaments interconnected adjacent Z-disks. Immunofluoresence and immunoelectron microscopy with anti-desmin indicated that desmin existed not only at Z-disks but also in the longitudinal spaces between adjacent myofibrils. From these studies, we concluded that network of short desmin filaments constructed basic unit in the scaffolding structure. Phosphorylation by either A- or C-kinase induced drastic changes in the scaffolding strcture. Mushroom-like projections periodically emerged from Z-disks. The mushroom with diameter of 180 nm in its head portion attached to Z-disk through a stem of 38-nm wide. We speculated that disintegration of the desmin net by the phosphorylation resulted in clumping of desmin and other components of scaffoldng structure.