IDENTIFICATION OF A NOVEL ACTIN-RELATED PROTEIN IN *TETRAHYMENA* CILIA

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Actin is an ancient cytoskeletal protein, which plays many essential roles in cell motility. In eukaryotes, it forms a highly conservative gene family, while it also forms an actin super family with various kinds of actin-related proteins (Arps). The ciliate *Tetrahymena* has a unique conventional actin. Data from the TIGR *Tetrahymena* genome project and our own research suggests the existence of twelve actin-like sequences: four conventional actins, two of Arp4, and one each of Arp1, Arp2, Arp3, Arp5, Arp6, and tArp. tArp is a novel actin-related protein, which is not included in any Arp subfamily. Unlike other known Arps, tArp localizes in cilia, and its expression is upregulated after deciliation. To see the precise localization of tArp, cilia were fractionated and analyzed using specific antibodies. Our latest findings show that tArp was mainly observed in the "outer doublet" fraction, while actin was found in the "crude dynein" fraction. In immunoelectron microscopy, most of the gold particles were found either at the outer doublet or at the central pair-microtubules. These results suggest that tArp has a unique function in the formation and maintenance of cilia.

PROTEIN DIFFERENTIAL DISPLAY FOR SPERM HYPERACTIVATION BY MEANS OF SELDI PROTEIN CHIP SYSTEM®

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Hyperactivation is one of important event in mammalian sperm and occurs at sperm flagellum after acrosomal reaction. From many studies, it is accepted that hyperactivation is regulated with protein modification, such as phosphorylation, fragmentation and etc... So, we examined protein modification associated with sperm hyperactivation using SELDI protein chip system® in the present study. SELDI protein chip system is new technology to analyze proteins and consists of protein chip and time of flight mass spectrometry (TOF MS).Using ion exchange chips, we detected protein fragmentation during hyperactivation. In several experiments, we found many fragments of sperm proteins. Those fragments time dependently appeared during hyperactivation. Moreover, we detected phosphorylation during hyperactivation using immobilized metal affinity capture (IMAC) gallium (III). From the experiment, we found many phosphorylations and dephosphorylations of sperm proteins. Those proteins were time dependently phosphorylated or dephosphorylated during hyperactivation.

K^+ INDEPENDENT MOTILITY INITIATION IN CHUM SALMON SPERM TREATED WITH ORGANIC ALCOHOL, GLYCEROL

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Sperm of salmonid fishes are quiescent in the presence of extracellular K^+, and motility initiation occurs when sperm are suspended in K^+-free medium. In this study, glycerol treatment in the presence of K^+ on intact sperm induced the initiation of motility by diluting in isotonic to hypotonic solution even in the presence of large amount of K^+. Other organic alcohol erythritol also showed the same effect but ethylene glycol did not. Furthermore, this glycerol-treated sperm showed motility without ATP and cAMP. CCCP suppressed motility of glycerol-treated sperm, suggesting that ATP synthesis occurred in glycerol-treated sperm. The amount of intracellular cAMP did not increase in glycerol-treated sperm on motility activation, but a PKA inhibitor, H-89, inhibited glycerol-treated sperm motility. Furthermore, phosphorylation of protein associated with motility initiation also occurred in glycerol treated-sperm, suggesting that the glycerol treatment induces activation of PKA without the increase in cAMP. Therefore, it is likely that glycerol induces phosphorylation for motility initiation bypassing the increase in cAMP as a result of the decrease in extracellular K^+.

EFFECTS OF SEMENOGELIN AND ZINC ON THE MOTILITY OF HUMAN SPERM

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Motility of human spermatozoa is suppressed at ejaculation by coagulum formed by semenogelin (Sg) and zinc, which are derived from seminal vesicle and prostate, respectively. After the ejaculation the coagulum is liquefied, and the sperm motility is gradually recovered. In the present study, it was shown that sperm motility was completely suppressed by semenogelin at the concentration of 15mg/ml. The motility was recovered by washing with percoll. On the other hand, sperm was agglutinated by Sg at the concentration of 2.5 mg/ml in the presence of zinc, and sperm motility was suppressed. However the motility was not recovered by washing with percoll.

FUNCTIONAL CHARACTERISTICS OF TROPONIN IN AMPHIOXUS STRIATED MUSCLE

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Troponin is a protein characteristic of striated muscles in vertebrates. It is an inhibitor for muscle contraction or actin-myosin interaction and the inhibition is removed by Ca^{++}. We previously discovered troponin in the smooth muscle of ascidian, one of protochordates, and observed that it functions as a Ca^{++}-dependent accelerator of muscle contraction. The distinct properties of ascidian troponin were attributed to the characteristics of troponin I (TnI) and T (TnT). In this study, we were interested in whether troponin in protochordate striated muscles functions as an inhibitor for muscle contraction or as a Ca^{++}-dependent accelerator. We prepared recombinant TnI and TnT of Amphioxus, *Branchiostoma belcheri* and combined them with chicken troponin C. The complex markedly inhibited actomyosin-ATPase in the absence of Ca^{++} but the inhibition was eliminated by Ca^{++} just as in the case of chicken troponin. Amphioxus TnI and TnT shared the common properties with chicken counterparts. These results show that the Amphioxus striated muscle troponin functionally resembles troponin in vertebrate striated muscle.

EFFECTS OF BTS ON MYOFIBRIL ASSEMBLY IN CHICKEN SKELETAL MUSCLE CELLS IN CULTURE

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Most of actin filaments align around myosin filaments with correct polarity and hexagonal arrangement parallel to myosin filaments at the early stage of myofibrillogenesis. An inhibitor for actin-myosin interaction, BDM, although not highly specific for myosin, suppressed myofibril formation in muscle cells in culture. These observations suggested that actin-myosin interaction is important in myofibrillogenesis. In this study, we examined further the role of actin-myosin interaction in myofibril assembly by applying a more specific inhibitor of myosin ATPase, BTS, for primary cultures of chick embryonic skeletal muscle. 20É M BTS was added to myotubes at 2-day of culture and myofibril assembly was examined by immunofluoresence microscopy using several antibodies to myofibrillar proteins. The results show that myotubes in a BTS-containing culture medium failed to form myofibrils, namely assembly of myosin and actin into cross-striated structures. However, when BTS was removed from the medium, myofibrils were formed 2 days later. These results indicate that actin-myosin interaction plays a critical role in the early process of myofibrillogenesis.

RELATION BETWEEN FUNCTION AND CRYSTALLINITY OF SARCOMERIC STRUCTURE IN INSECT FLIGHT MUSCLES

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Conventional X-ray diffraction patterns as well as end-on microdiffraction patterns were recorded from the flight muscles of a variety of insects, to examine the extent of crystallinity of sarcomeric structure. The layer line reflections from the asynchronous flight muscles from a Hymenoptera (bumblebee) and a Heteropteran (waterbug) were strongly sampled at positions indexable to a hexagonal lattice, indicating a high degree of crystallinity. The asynchronous flight muscles from a Coleopteran (beetle) showed poorer crystallinity. The synchronous flight muscle from a Homopteran (cicada) showed some extent of crystallinity, indicating that the contractile proteins are better ordered than in other insects with synchronous flight muscles. These results suggest that, although the difference in the function (asynchronous or synchronous) of insect flight muscles is discrete, their variation in the crystallinity of sarcomeric structure is continuous.

REQUIREMENT OF GOLGI GDP-FUCOSE TRANSPORTER (GFR) FOR NOTCH SIGNALING IN *DROSOPHILA*

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Congenital Disorder of Glycosylation IIc (CDG IIc) is a recessive syndrome characterized by slowed growth, mental retardation, and severe immunodeficiency. Recently, the gene responsible for CDG IIc was found to encode a GDP-fucose transporter. Here, we investigated the possible cause of the developmental defects associated with CDG IIc using a *Drosophila* model. Biochemically, we demonstrated that a *Drosophila* homolog of the GDP-fucose transporter, the Golgi GDP-fucose transporter (Gfr), transports GDP-fucose *in vitro*. To understand the function of the *Gfr* gene, we generated null mutants of *Gfr* in *Drosophila*. Our phenotype analyses revealed that Notch signaling was