SINGLE MOLECULE MEASUREMENT OF ELASTICITY OF INVERTEBRATE CONNECTIN

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Invertebrate connectin is a 1960 kDa elastic protein linking the Z line to the tip of the myosin filament in the giant sarcomere of crayfish claw closer muscle. Invertebrate connectin can be extended up to 3.5 micrometers upon stretch of giant sarcomeres. There are several extensible regions in invertebrate connectin: two PEVK regions, one unique sequence region and SEK repeats. In this study, the force measurement of the single recombinant polypeptide containing biotinylated BDTC and GST tags at the N and C termini, respectively, were performed by intermolecular force microscopy (IFM), a refined atomic force microscopy (AFM) system. The force versus extension curve of invertebrate connectin-PEVK region was well fit to the wormlike chain (WLC) model. The persistence length of invertebrate connectin PEVK region was longer than the length of invertebrate connectin SEK region.

ISOLATION AND CHARACTERIZATION OF *CHLAMYDOMONAS* MUTANTS DEFICIENT IN BASAL BODY FORMATION

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Centrioles and basal bodies are formed through semiconservative duplication, a process that has been a long-standing puzzle. Our recent isolation of the *Chlamydomonas bld10* mutant totally lacking basal bodies demonstrates the feasibility of genetic approaches to the mechanism of basal body formation. Here we aimed to isolate new mutants by screening for clones showing phenotypes similar to those of *bld10*, such as defective flagellar formation and mitosis. From about 250,000 mutants generated, ten clones that fulfilled the criteria were isolated. Immunofluorescence microscopy identified five clones showing abnormalities in basal body-related cytoskeletons. Two of them (1B9-1 and 1B9-2) were genetically different from all of the known basal body mutants and the other three were new alleles of *bld2* or *bld10*. Electron microscopy revealed that 1B9-1 had multiple basal bodies in the apical region of the cell and 1B9-2 had basal bodies with triplet microtubules, which however were arranged in greatly distorted patterns. We are speculating that these mutants have defects in the regulation of basal body assembly or in the maintenance of the assembled structure.

CHICKEN ENA FAMILY PROTEINS

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We have determined the full sequences of avEna I, II, III, and N which are isolated from cDNA libraries of chicken gizzard and breast muscle. When adult chicken breast, heart, gizzard, and breast muscle were immunoblotted with anti-Mena antibody, 3 isoforms were detected. An 88-kDa isoform, avEna I, is the counterpart of the 80-kDa isoform of Mena, and a 78-kDaisoform is an avian-specific isoform derived from the avEna I gene by splicing. A 130-kDa isoform, avEna N, is the counterpart of 140-kDa neural isoform of Mena. A new isoform, avEna III, did not contain the EVH1 domain but had a part of the sequence characteristic to the neural variant of Mena in its N-terminal and contained an insert in its C-terminal EVH2 domain. The anti-Mena antibody did not cross-react with this isoform. By immunoblotting analysis of chicken organs with the antibody against the N-terminal of avEna III, the 65-kDa bands was detected in embryonic gizzard but not in adult organs.

LASP FAMILY PROTEINS OF INVERTEBRATES

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From eluates of F-actin affinity chromatography of chicken brain, we identified a novel actin-binding protein (lasp-2). Lasp-2 possesses similar domain structure to lasp-1, a previously identified actin-binding protein with LIM domain, two nebulin repeats, and SH3 domain (Terasaki et al., 2004). Searching EST / genome databases, lasp-1 and lasp-2 subfamily proteins were found in various vertebrates. Invertebrates such as ciona, sea urchin, and fruits fly have lasp family proteins with homology at the same extent to lasp-1 and lasp-2. We clones ciona lasp and revealed its actin-binding activity.

LOCALIZATION OF PHOSPHORYLATED REGULATORY LIGHT CHAIN OF MYOSIN II DURING CYTOKINESIS

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Phosphorylation of regulatory light chain of myosin II (MRLC) is considered to regulate the cellular function in several types of cells, including smooth muscle cells and cultured nonmuscle cells. Phosphorylation of MRLC controls the actin-activated MgATPase activity of myosin II and the assembly of myosin II in vitro and in vivo. It is well known that phosphorylated MRLC localizes at the constriction area (clearage furrow) of dividing cells. Here, to elucidate the mechanism of constriction of cleavage furrows, we investigated roles of phosphorylated MRLC in cytokinesis, using HeLa cells transfected with non- or pseudo-phosphorylated MRLC. Our results showed that HeLa cells transfected with pseudo- and non-phosphorylated MRLC constricted faster and slower than did control cells, respectively, suggesting that the phosphorylation of myosin II plays important roles during cytokinesis.

TETRAHYMENA ELONGATION FACTOR-1α (EF-1α) BUNDLDES F-ACTIN BY FOEMING A DIMER

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In Tetrahymena, EF-1α has been reported to localize at the division furrow and has the character to bundle filamentous actin (F-actin). It has been reported that the binding ratio of EF-1α and actin is approximately 1:1. With Ca^{2+}/calmodulin (CaM), EF-1α cannot bundle F-actin even though it still keeps binding F-actin.In this study, using gel filtration column chromatography, we examined the native molecular weight of purified EF-1α and revealed that EF-1 α itself exist as monomer, dimer. In addition, with chemical cross-linker (DSS), we found that EF-1α forms dimer. When Ca^{2+}/CaM was added to EF-1α monomer increased, while dimer decrease, suggesting that Ca^{2+}/CaM alters EF-1α dimer into monomer and inhibits bundle formation of F-actin. Next we separated EF-1α monomer and EF-1α dimer using gel filtration column, and examined interaction between F-actin and EF-1α monomer or EF-1α dimer using low-speed cosedimentation assay and electron microscopy. EF-1α dimer coprecipitated with F-actin and bundled F-actin but EF-1α monomer did not.

PHOSPHOPROTEOME FOR MAMMALIAN SPERM CAPACITATION

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It has been accepted that mammalian sperm capacitation is regulated by protein phosphorylation and dephosphorylation. In many studies, it has been demonstrated that AKAP (A-kinase Anchoring protein), which is a major component of fibrous sheath of sperm flagellum, was phosphorylated at tyrosine residues associating with mammalian sperm capacitation. However, it is not clear the regulatory mechanism and the signal transduction for sperm capacitation. We tried to detect protein phosphorylations and dephosphorylations associated with sperm capacitation. In order to detect comprehensively protein phosphorylation and dephosphorylation, sperm proteins were extracted using the urea-thiourea solution which dissolved all sperm components except for nuclear. In hamster spermatozoa, 50k-Da ~ 100k-Da proteins were phosphorylated at tyrosine residues during capacitation. Moreover, 10k-Da ~ 80kDa proteins were phosphorylated at serine residues during capacitaiton. Several of serine phosphorylation were phosphorylated by A-kinase. Approximately 80k-Da, 70k-Da and 60k-Da proteins were phosphorylated and dephosphorylated at threonine residues during capacitation.

DEVELOPMENT OF AUTOMATED SPERM MOTILITY ANALYSIS SYSTEM AND ITS APPRICATION TO STARFISH AND BULL SPERMATOZOA

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The percentage of motile mammalian spermatozoa is not so high. This is mainly because the semen contains a higher percentage of abnormal spermatozoa. The abnormality in the intracellular environment in which the axoneme functions is important for an understanding of the mechanism of fertilization and of the mechanism regulating the flagellar movement of spermatozoa. To determine the movement characteristics of the spermatozoa objectively and quickly, an automated sperm motility analysis system based on the particle image velocimetry has been developed. Using this system, we found that almost 100% of starfish spermatozoa were motile, whereas the mean percentage of motile bull spermatozoa varied widely from animal to animal.