CHANGES OF RADIAL SPOKE PROTEIN LRR37 COUPLED WITH THE ACTIVATION OF SPERM MOTILITY IN *CIONA INTESTINALIS*

Yuhkoh Satouh[1,2], Tosifusa Toda[3], Hiroyuki Ide[1], Kazuo Inaba[2]
[1]Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-8578, Japan., [2]Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka 415-0025, Japan. and [3]Proteomics Collaboration Center, Tokyo Metropolitan Institute of Gerontology, Sakaecho 35-2, Itabashiku, Tokyo 173-0015, Tokyo.

Flagellar radial spoke is the protein complex playing key roles in the regulation of dynein activity. We have recently identified a 37 kDa leucine rich repeat protein, LRR37, as a component of flagellar radial spoke, and shown that it is likely to be localized at the head compartment of T-shaped spoke structure. In order to elucidate the roles of LRR37 in flagellar motility, axonemes were extracted with a low ionic strength solution and immunoprecipitated by anti-LRR37 antibody. Gel electrophoresis revealed two proteins in the immunoprecipitate with molecular weights of ~100kDa and ~50kDa, along with LRR37, suggesting that these proteins form a complex in the radial spoke structure. On the other hand, two-dimensional gel electrophoresis of *Ciona* sperm proteins before and after motility activation by sperm-activating and attracting factor, SAAF, revealed that LRR37 remarkably shift to more basic isoelectric point after motility activation, possibly due to the dephoshorylation. It is possible that interaction of LRR37 with the other axonemal proteins changes by dephosphorylation, resulting in the activation of dynein-driven axonemal movement.

IDENTIFICATION AND MOLECULAR CLONING OF RADIAL SPOKE PROTEIN 4 AND 6 AS CA2+/CALMODULIN-ASSOCIATING PROTEINS IN *TETRAHYMENA* CILIA

Hironori Ueno, Osamu Numata
Institute of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Cilia and flagella in various eukaryotic organisms possess essentially the same fine structure comprised of nine doublet microtubules (MTs) surrounding central pair MTs. Changes of the waveform of cilia and flagella in various eukaryotic organisms are known to been occurred by an increase in the Ca^{2+} concentration. Calmodulin (CaM) localizes a ciliary and flagellar axoneme. However, the function of CaM in cilia or flagella has not been well understood.In this study, to examine the pathway of Ca^{2+}/CaM signaling in cilia, we identified target proteins of Ca^{2+}/CaM in *Tetrahymena* cilia by Ca^{2+}/CaM-affinity column. The crude dynein fraction of cilia included outer/inner arm dynein, radial spoke and central pair structure. We detected 120kDa, 116kDa, 66kDa, 62kDa, 33kDa, 30kDa Ca^{2+}/CaM-binding protein in this fraction, separated 66kDa and 62kDa Ca^{2+}/CaM-binding proteins with 2D-SDS-PAGE and analyzed those amino acid sequences. The results suggested that the 66kDa and 62kDa Ca^{2+}/CaM-binding protein were related to *Chlamydomonas* RSP4 and 6, respectively. These compose the radial spoke head, which is known to be regulated the flagellar bending pattern in *Chlamydomonas* and sea urchin sperm.

FLAGELLAR MOTILITY ACTIVATION MEDIATED BY THE INCREASE IN INTRACELLULAR pH AND THE RELATED PROTEIN PHOSPHORYLATION OF FLAGELLA IN STARFISH SPERMATOZOA

Ayako Nakajima[1], Masaya Morita[2], Akihiro Takemura[2], Makoto Okuno[1]
[1]Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Meguro-ku, Tokyo 153-0041, Japan and [2]Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyu, 3422 Sesoko, Motobu, Okinawa 905-0227, Japan

Starfish testicular spermatozoa show low motility when they are suspended in seawater. It has been reported that they are activated by histidine, but the molecular mechanism is not well known. We showed NH_4Cl, which increases intracellular pH, was also effective in activating starfish testicular spermatozoa (Zool. Sci., Suppl.2003). In the present study, we confirmed the increase in intracellular pH ($[pH]_i$) during motility activation by NH_4Cl using fluorescent pH indicator, SNARF-1 AM. In addition, we found that histidine increased $[pH]_i$ accompanying with the motility activation in starfish (*Asterina pectinifera*) in dependence on extracellular Na^+. Effects of the increase in [pH]i was also confirmed by Triton model. Testicular spermatozoa were demembranated before and after activation, then diluted with reactivation solutions of various pH. The reactivation rate increased in a pH-dependent manner. Spermatozoa demembranated after activation showed high reactivation rate in lower pH than spermatozoa demembranated before activation. Furthermore, protein phosphorylations in demembranated flagella during motility activation mediated by increase in $[pH]_i$ were detected using $[^{32}P]$ATP.

ROLE OF ZINC ION ON THE INITIATION OF SPERM MOTILITY IN STARFISH

Masaaki Morisawa[1], Kensuke Komatsu[3], Tatsuru Togo[1], Sachiko Morisawa[2], Kazuya Kikuchi[3], Tetsuo Nagano[3]
[1]Misaki Marine Biological Station, The University of Tokyo, Miura, kanagawa 238-0225, Japan, [2]Department of Anatomy, St Mariannna University School of Medicine, Kawasaki, Kanagawa 216-8511, Japan and [3]Department of Pharmacology, Graduate School of Science, The University of Tokyo, Tokyo 113-8691, Japan

Spermatozoa of the starfish *Asterina pectinifera* are immotile in seawater. When sperm were suspended in the artificial seawater containing zinc ion specific fluorescence dye, ZnAF-2DA, zinc ion specific fuorescence was observed in the midpiece region of sperm. Spermatozoa, which are immotile in the seawater became motile upon addition of zinc ion specific chelating agent, TPEN. These suggest that removal of zinc ions from the midpiece region of sperm participate in the initiation of sperm motility in starfish.

Ca^{2+}-DEPENDENT REGULATION OF CILIARY REVERSAL-COUPLED VOLTAGE-GATED Ca^{2+} CHANNELS BY *PARAMECIUM* Ca^{2+}-BINDING EF-HAND PROTEIN

Kohsuke Gonda, Mihoko Takahashi
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Paramecium swims backwards by reverse of ciliary beat direction, when the front end of *Paramecium* is mechanically stimulated or exposed to certain chemicals. The reverse of ciliary beat direction is dependent on the change of the internal Ca^{2+} concentration in cilia. It is electrophysiologically clarified that the entry of Ca^{2+} into the cilia are controlled by voltage-gated Ca^{2+} channels on ciliary membrane. However, the primary structure and regulatory mechanisms of the voltage-gated Ca^{2+} channels are not yet fully understood. One of the *P. caudatum* mutants, *cnrC*, has a defect of the Ca^{2+} channel function. We found that *cnrC*$^+$ gene product is a *Paramecium* new Ca^{2+}-binding EF-hand protein, which belongs to the calmodulin superfamily, and the protein is essential for the Ca^{2+} channel activity. To know whether the Ca^{2+}-binding protein controls the Ca^{2+} channels in a Ca^{2+}-dependent manner, we examined the transformation ability of *cnrC*$^+$ gene that had mutations in the Ca^{2+} sensitivity of EF-hand, and then obtained the results showing their Ca^{2+}-dependency. We will discuss how the Ca^{2+}-binding protein controls the ciliary reversal-coupled voltage-gated Ca^{2+} channels.

TWITCHIN OF STRIATED ADDUCTOR MUSCLES OF BIVALVES REGULATES 'CATCH' INTERACTION BETWEEN ACTIN AND MYOSIN FILAMENTS

Yasutaka Tsutsui[1], Maki Yoshio[2], Kazuhiro Oiwa[1,2], Akira Yamada[2]
[1]Department of Life Science, Graduate School of Science, University of Hyogo, 3-2-1 Kouto, Kamigori, Ako-gun, Hyogo 678-1297, Japan and [2]Kansai Advenced Research Center, 588-2 Iwaoka, Nishi-ku, Kobe 651-2492, Japan

The titin/connectin family comprises a number of proteins which play important roles in the structure or the regulation of the actin and myosin cytoskeleton. It includes titin/connectin, projectin and twitchin, of which functions and their molecular mechanisms are not fully understood. We isolated myosin and twitchin from smooth (catch) and striated (non-catch) adductor muscles of an oyster (*Crassostrea gigas*), and striated adductor muscle of a scallop (*Mimachlamys nobilis*). The NTCB cleavage of the oyster striated and smooth muscle twitchins produced almost identical cleavage patterns indicating that their primary structures are identical or very similar to each other. By using the *in vitro* catch assay (Yamada *et al.* (2001) PNAS. 98, 6635-6640), we found that the striated muscle twitchin induced and released the 'catch' binding of actin to striated muscle myosin filaments through its dephosphorylation and phosphorylation respectively, as well as the smooth muscle twitchin and myosin did. The results suggest that similar to the catch muscle twitchin, the twitchin of molluscan striated muscle has a regulatory property that controls interaction between actin and myosin filaments.

CA REGULATION MECHANSIMS AND BIOCHEMCAL PROPERTIES OF INVERTEBRATE BIVALVE SHELL MUSCLE AND THE PROTEINS

Yoichi Yazawa
Department of Health Science, Hokkaido University of Education Asahikawa, Hokkaido 070-8621

Many investigations of scallop adductor muscle have been reported but that of other bivalve shell muscles were a little.

I have studied about Ca regutation mechanism and biochemical properties of three species of bivalve shell muscles, Patinopecten yessoensis(scallop), Crassostrea gigas (oyster), and Spisula sachalinensis. In this study, I found new type of Ca regulation mechanism of muscle contraction and new biochemical properties of each muscle proteins.