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INTERACTION OF CHICKEN DIAPHANOUS PROTEIN WITH ACTIN

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Diaphanous family proteins are known to involve in various actin-mediated cellular processes. All members contain a Rho-binding domain, a proline-rich formin-homology domain 1 (FH1), two coiled-coil domains, FH2, and FH3. Recently, the FH1 and 2 of yeast diaphanous (Bni1p) was found to nucleate actin filaments in vitro. Immunoblot analysis with anti-cDia antibody showed the relatively stronger reaction with the 145kDa band in brain than in other organs. cDia in the low-salt alkaline extract of chicken brain bound to an F-actin affinity column. Full-length cDia carrying a histidine tag was expressed in an insect cell line (High Five) and purified with a Ni-NTA agarose column. G-actin was polymerized in the presence of the recombinant cDia protein and ultra-centrifuged at 50,000 rpm. cDia was co-precipitated with F-actin. Then the polymerizing process was monitored with the electron microscope. cDia seemed to accelerate the actin polymerization. Large bundles of F-actin were observed in the presence of cDia at the molar ratio of 10:1.

CALMODULIN IN THE CILIARY CONTROL OF *PARAMECIUM CAUDATUM*

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The ciliary movement is controlled by the change in intraciliary concentration of second messengers in response to the extra-cellular stimuli. Especially, an increase in intraciliary Ca^{2+} ion induces ciliary reversal. Many reports have indicated that eukaryotic flagella and cilia contain some Ca^{2+} binding proteins such as calmodulin. However, the relevance of these Ca^{2+} binding proteins to the ciliary reversal is still unknown. We tried to find out the involvement of calmodulin in the ciliary reversal. We have found that trypsin treatment removes the ciliary response to Ca^{2+} in the cell model of *Paramecium*. The resulted insensitivity to Ca^{2+} may be due to a degradation of a Ca^{2+} receptor protein such as calmodulin. We found a lot of calmodulin like protein in deciliation supernatant and a little in ciliary axonemes using SDS-PAGE. The protein was identified as calmodulin using anti calmodulin antibody. The calmodulin in deciliation sup was degraded by trypsin showing a similar time course as of disappear of the ciliary response. The degradation of calmodulin within axonemes, however, was not conspicuous. The identification of the key Ca^{2+} binding protein remains to be elucidated.

PROTEIN PHOSPHORYLATION DURING MOTILITY ACTIVATION IN STARFISH (*ACANTHASTER PLANCI*) SPERMATOZOA

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Starfish testicular spermatozoa are known to show low motility when they are suspended in seawater. It has been reported that they were activated by histidine (His). EDDA, specific chelator of Zn^{2+}, was also effective in activating starfish testicular spermatozoa, and the release of Zn^{2+} is thought the major factor in activation by His, but the molecular mechanism is not well known. In this study, we examined the protein phosphorylation of sperm flagella during motility activation. Testicular sperm were activated by (1)0.5M Choline chloride, (2) (1) + 10mM His, (3) (1) + 0.1mM EDDA, and (4) (1) + 20mM NH_4Cl. When spermatozoa were suspended in the solution(1), they showed low motility. In the solution(2) and (3), their motility was activated in a few minutes, while in the solution(4), they immediately became motile. Several proteins were found to be phosphorylated after motility activation (260kDa, 45kDa, 29-34kDa), and the phosphorylation of 45kDa protein was stronger when sperm were activated by NH_4Cl. The 75kDa protein was phosphorylated only when activated by His. The dephosphorylation of 130kDa protein, which was extracted with 0.6M NaCl was also observed after motility activation.

MODULATION OF PROTEIN EXPRESSION IN SPERMATOZOA OF EURYHALINE TILAPIA (*OREOCHROMIS MOSSAMBICUS*) DURING OSMOTIC ACCLIMATION

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Tilapia can acclimate and reproduce from in freshwater to seawater. We have been studying on the changes in regulatory mechanism of sperm motility during the acclimation. In the present study, we analyzed total protein of FWT (fresh water acclimated tilapia) and SWT (fresh water acclimated tilapia) sperm by means of 2D electrophoresis. We found that expression of 18kDa protein was different between FWT and SWT sperm. Larger amount of 18kDa was expressed in SWT sperm than in FWT sperm. Amino acid sequence of N-terminus of 18kDa revieled homology to Cu/Zn superoxide dismutase (SOD), a scavenger of radical O_2^-. Peroxinitrate ($ONOO^-$) produced from nitric oxide (NO) and superoxide(O_2^-) reacts to tyrosine residues, called nitration. 41kDa protein in FWT sperm was nitrated in hypotonic condition. By contrast, in Ca^{2+} present hypertonic condition, 41 kDa in SWT not FWT sperm was denitrated. Since 41kDa is a key protein of the motility activation cascade it is likely that free radical affects sperm motility. Furthermore, it is likely also that the modulation in expression of 18kDa protein affects the production of peroxinutrate, resulting in modification of motility activation cascades.

THE IMAGE ANALYSIS OF SPERM MOVEMENT MECHANISM OF THE *PROSOPOCOILUS INCLINATUS*

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Sperm dissected from the male vesicula seminalis of *Prosopocoilus inclinatus* were examined their movements with a CCD video camera under the microscope. Furthermore the cross sections of flagella axoneme and whole sperm negatively stained were observed by electron microscope. When the spiral motion transmits from the baseal body of the flagellum, the characteristics were obtained such as periodic oscillation, rotation, directivity of the sperm flagellum. It was considered that the relationship between the whole sperm shape and ultrastructure of the flagella axoneme.

ELECTROMAGNETIC FIELD EFFECTS ON REPRODUCTIVE CELL(10) MOVIE DATA ACQUISITION OF SPERM FLAGELLA UNDER SUPER-IMPOSED MAGNETIC FIELD

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In the series of our work, we have been trying to establish the monitoring scheme to the real time response of the reproductive cell against the superimposed electric and magnetic field. In this presentation, we introduce the quantitativeimage processing technique on the insect sperm under superimposedmagnetic field simulating the maximum allowable magnetic field of"Japanese Linear Chuo Express" : 2 mTesla.The measurement is done with the conventional non-interlace CCD camera as well as 8000 frame /sec high speed camera for aliasing check.

SOLUBILIZATION OF SPERM FLAGELLAR PROTEINS FOR PROTEOME ANALYSIS

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We have studied on protein phosphorylation pathway associated with the motility of sperm. For precise analyses, we comprehensively analyzed protein phosphorylation associated with sperm motility. In order to examine protein phosphorylation comprehensively, we investigated about solubilization of all sperm flagellar proteins using salmonid sperm and hamster sperm in the present experiment. As for salmonid sperm, sperm flagellum dissolved in a 5 M urea solution containing 5 M urea and 10% 2-ME. On the other hand, flagellar structures except to fibrous sheath of hamster sperm flagellum dissolved in a 7 M urea solution containing 7 M urea and 10% 2-ME. Fibrous sheath dissolved in a urea-thiourea solution containing 5 M urea, 1 M thiourea, 2% NP-40 and 10% 2-mercaptoethanol. In both salmonid sperm and hamster sperm, sperm head was removed by centrifugation. Flageller proteins dissolved in a urea solution were analyzed with2D-electrophoresis consisted of the IEF with 5 M urea and the SDS-PAGE. Fibrous sheath proteins dissolved in a urea-thiourea solution were analyzed with 2D-electrophoresis consisted of the IEF with 5 M urea and 1 M thiourea and the SDS-PAGE.

ELECTROPHORETIC STUDIES ON A CONNECTIN /TITIN-RELATED PROTEINS IN MUSCLE CELLS OF THE FRESH WATER PLANARIANS

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Connectin/titin-related proteins have been found in many animal species' muscle cells. In Platyhelminthes, we have been shown that 540-800 kDa connectin/titin related proteins exist in muscle cells of the Japanese freshwater planarians, *Dugesia japonica*, *Dugesia ryukyuensis*, *Polycelis auriculata*, and *Bdellocephala brunnea*, but

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