to a reverse bend in the more distal region, ATP induced backward sliding of the thinner bundle in about 75% of the trials. In contrast, bending in the same direction as the principal bend induced sliding without a change in the direction. These results indicate that the orientation of bending is important in the 'switching' of dynein activity in flagella.

### A NEW OPTICAL SYSTEM FOR THE DETERMINATION OF pH CHANGE IN A SEA-URCHIN SPERM CELL

Daisuke Takao, Shinji Kamimura
Department of Life Sciences, Gradiate School of Arts and Sciences, University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

For investigating the activation and regulation mechanism of flagellar motility of spermatozoa, one of the most crucial techniques is to detect the real time change of intra-cellular pH, since pH-drop is expected to represent the magnitude of flagellar activity. So far, fluorescent dyes that change the wavelength of photo emission depending on the proton ion concentration had been used. The previous methods of spectroscopic analysis, however, detected the fluorescent change of sperm suspension and it has been almost impossible to determine the local and dynamic change of pH in spermatozoa tails. In the present study, we developed a new method using a conventional microscope to detect the fluorescent change of spermatozoa. We used the fluorescent microscope equipped with a high-sensitivity photo-detecting device, to which the magnified images of specimen were projected through different fluorescence filters. Microscope images were then captured into a computer for further analysis. From the ratio of fluorescence intensity of specimen images, dynamic change of pH of a single spermatozoon could be detected.

### EFFECTS OF POTASSIUM IODIDE ON MICROTUBULE SLIDING AND DYNEIN ATPase ACTIVITY IN SEA URCHIN SPERM FLAGELLA

Izumi Nakano, Rin Fujiwara, Chikako Shingyoji
Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan

When demembranated sperm flagella of the sea urchin, *Pseudocentrotus depressus* were reactivated by 1 mM ATP in a solution containing 50 or 100 mM KI, the rate of reactivated flagella was similar to or higher than that of the flagella reactivated under 150 mM potassium acetate (KAc) conditions. The beat frequency, however, was decreased by KI: in the reactivating solution containing 150 mM KAc, the beat frequency was about 35 Hz, which decreased by about 20 and 50% in the presence of 50 and 100 mM KI, respectively. The velocity of microtubule sliding at 1 mM ATP in elastase-treated axonemes also decreased in solutions containing KI, indicating that KI inhibits the sliding activity of dynein. In the presence of KI, the ATPase activity of the axonemes, both motility-coupled and uncoupled, did not change, but the ATPase activity of 21 S dynein purified from the outer arms increased 2 to 3-fold. These results indicate that the inhibition of microtubule sliding by KI was not due to simple reduction of the dynein ATPase activity. KI possibly modifies the dynein arms to interfere with their efficient transduction of the energy of ATP hydrolysis to the force of microtubule sliding.

### PROPERTIES OF RIB72, A MAJOR COMPONENT OF PROTOFILAMENT RIBBONS IN THE AXONEME, AS STUDIED USING A RECOMBINANT PROTEIN EXPRESSED IN INSECT CELLS

Kazuho Ikeda, Keiko Morikawa, Toshiki Yagi, Masafumi Hirono, Ritsu Kamiya
Department of Biological Science, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan

Rib72 is an axonemal protein preserved in a wide range of organisms. It was first identified in *Chlamydomonas* as a protein that is lost concomitantly with the disintegration of axonemes upon protease treatment in the presence of ATP. Hence Rib72 should be important for the maintenance of the structural integrity of axonemes. It is present in a significant amount in the "protofilament ribbon", a Sarkosyl-resistant structure contained in the outer doublet microtubules. However, its true function and state of assembly in the axoneme remain unclear. To elucidate its properties, recombinant Rib72 was expressed in an insect cell expression system. It was expressed as a soluble protein. Gel overlay assays using purified protein revealed the presence of several axonemal proteins that potentially bind to Rib72. In addition, these and other experiments indicated that Rib72 can weakly interact with microtubules.

### LOCALIZATION AND FUNCTION OF GLYCOLYSIS RELATED EMZYMES IN MOUSE SPERM FLAGELLA

Chinatsu Mukai, Makoto Okuno
Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

Mammalian sperm must maintain motility for a long time to fertilize the eggs. The energy required for flagellar movement is continuously supplied by metabolic substrates. It has been supposed that ATP synthesis occurs predominantly in mitochondrial respiration and is transported to entire length of flagella. However, this is very hard to accept in mammalian sperm because of long flagella and lack of phosphocreatine shuttle.We previously reported that the energy supply by glycolysis was very important for flagellar movement. In the present study, we investigated the localization of glycolysis related enzymes in mouse sperm flagella. Immunofluorescent study revealed that some of glycolysis related enzymes were localized at sperm tail. Pyruvate kinase was detected at acrosomal region and principal piece of flagella on intact sperm. Lactate dehydrogenase was detected at principal piece of flagella on demembranated sperm. In addition, we observed that iodoacetic acid, an inhibitor of glyceraldehyde dehydrogenase, inhibited sperm motility even in the presence of respiratory substrates. We concluded that the glycolysis working at tail should supply ATP to dynein rather than respiration.

### LOCALIZATIONS OF PHOSPHO-PROTEINS OF EURYHALINE TILAPIA (*OREOCHROMISS MOSSAMBICUS*) SPERM FLAGELLA

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

Euryhaline tilapia (*Oreochromiss mossambicus*) can acclimate from freshwater to seawater. They can also reproduce in this wide osmotic environment. Freshwater-acclimated tilapia (FWT) and seawater-acclimated tilapia (SWT) showed different motility manner. It is likely that motility regulatory mechanisms of flagella of FWT and SWT sperm are modulated. In this study, we detected protein phosphorylation of serine residue(s) and nitration of tyrosine residue(s) of flagellar protein upon motility activation. FWT sperm flagella, 66, 48, 45, 32 and 27 kDa proteins were phosphorylated and nitrated, by contrast, those of SWT sperm were dephosphorylated and denitrated. In 0.6 M NaCl-extracted fraction of FWT and SWT, which contains outer dynein arm, 32 and 27 kDa proteins were phosphorylated and nitrated. In FWT sperm, the remnant of the axoneme, 66, 48, 45, 32 and 27 kDa proteins were phosphorylated and nitrated. By contrast, those proteins of SWT sperm were dephosphorylated and denitrated. It is possible that outer dynein arm-dependent acceleration of flagellar motility is in common with FWT and SWT but regulatory mechanisms of proteins attached tightly to the axoneme are modulated.

### FUNCTIONAL ANALYSIS OF GENE PRODUCTS EXPRESSED HIGHLY AND SPECIFICALLY IN TESTIS IN THE ASCIDAIN *CIONA INTESTINALIS*

Aru Konno[1,2], Nori Satoh[3,4], Hiroyuki Ide[1], Kazuo Inaba[2,4]
[1]Graduate School of Science, Tohoku University Aoba-ku, Miyagi 980-8577, Japan, [2]Shimoda Marine Research Center, University of Tsukuba, Shizuoka 415-0025, Japan, [3]Graduate School of Science, Kyoto University, and [4]CREST, JST

Extensive EST analysis has made it possible to identify a number of genes that are specifically expressed in a certain tissue. We have recently carried out EST analysis of testis-expressed genes in testis of the ascidian *Ciona intestinalis* and revealed the presence of a set of genes with less information on their functions (class DI and DII). Here we report the functional analysis of the six gene products, named DI/II000, 001, 002, 003, 004 and 007, that are highly and specifically expressed in *Ciona* testis. Western blotting with antibodies against thioredoxin-fusion proteins revealed that all these gene products are present in sperm, indicating that they are the components of sperm. Immunofluorescent microscopy with each antibody showed localization to a specific sperm compartment: head (002, 004), mitochondria (003, 007) and flagella (000, 001, 007). Three of them (000, 003, 007) were extracted from sperm by Triton X-100, whereas the other (001, 002, 004) were retained in the residues. These results suggest that these DI/II class of gene products are involved in specific function of sperm.

### 14-3-3 PROTEIN IS INVOLVED IN THE ACTIVATION OF SPERM MOTILITY IN THE ASCIDIAN *CIONA INTESTINALIS*

Akiko Hozumi[1,2], Tosifusa Toda[3], Hiroyuki Ide[1], Kazuo Inaba[2]
[1]Graduate School of Life Scieneces, Tohoku University, Miyagi 980-8578, Japan, [2]Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka 415-0025, Japan and [3]TMIG Proteomics Collaboration Center, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan

We have identified twelve proteins that change the molecular mass or isoelectric points at activation of sperm motility in *Ciona intestinalis*. We have prepared a polyclonal antibody against one of the twelve proteins, 14-3-3 protein, which generally functions in signal transduction by binding to target proteins. Immunoblotting with the antibody revealed that 14-3-3 protein was exclusively extracted from sperm by Triton X-100. Immunofluorescent microscopy showed that 14-3-3 protein is localized in both head and tail of sperm. The sperm extract with Triton X-100 was separated by HQ anion exchange chromatography, followed by Superose 6 gel filtration, and a fraction rich in 14-3-3 protein was obtained. A set of proteins with the molecular masses of 64, 62, 55 and 31 kDa were coeluted with the 14-3-3 protein, suggesting that they forms a complex with 14-3-3 protein. These proteins were subjected to tryptic digestion, followed by MALDI-TOF/MS. MS search against our local database (MSCITS) by PerMS resulted in the identification of these proteins.