

RPNVELCRD, which is conserved in all tektin family members. The tektin-like protein transcript of 1.7 k base length was detected in rat testis by Northern blot analysis. RT-PCR analysis showed that the gene was highly expressed in testis and its expression level was developmentally increased. In situ hybridization analysis revealed that mRNA of tektin like protein was found to be present in haploid spermatids of adult rat testis.

THE UNIQUE MITOTIC APPARATUS AND DISTRIBUTION OF γ -TUBULIN IN SPERMATOCYTES OF THE SILKWORM, *BOMBYX MORI* (LEPIDOPTERA)

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A characteristic feature of the silkworm spermatocytes is that they are furnished with axonemes from prophase I. The microtubule (MT) cytoskeleton of the mitotic apparatus was stained by immunofluorescence using antibodies against α - and γ -tubulin. In this study, special attention was paid to the features of cell division in spermatocytes and in spermatogonia that do not bear axonemes. In metaphase spermatocytes, the asters with axonemes were separated from the spindle pole. In early anaphase, chromosomes reached the spindle poles, and then they moved toward the aster on the elongating spindle. Many of the overlap-MTs appeared newly in the interzonal region. After reaching the astral center, the spindle continued to elongate and the chromosome groups were separated further. On the other hands, spermatogonia formed a usual mitotic apparatus and chromosome separation proceeded as usual. In dividing spermatogonia and spermatocytes, γ -tubulin was located on the centrosomes. In anaphase spermatocytes, faint signals were observed in the spindle region and it seems possible that this type of γ -tubulin is involved in the nucleation of overlap-MTs.

THE EXPRESSION AND LOCALIZATION OF COHESIN SUBUNITS IN THE GONADS OF MEDAKA, *ORYZIAS LATIPES*

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Until the onset of anaphase, sister chromatids are bound to each other by a multi-subunit protein complex called cohesin. Since chromosomes in meiosis behave differently from those in mitosis, the cohesion and separation of homologous chromosomes and sister chromatids in meiosis are regulated by meiosis-specific cohesin subunits. However the information on cohesin, especially in meiosis-specific cohesin, has been poor except for mammals. For the better understanding of the characteristics and roles of cohesin, we need to accumulate further knowledge regarding the nature of cohesin in various animals. Using the medaka fish, *Oryzias latipes*, as an experimental model, we examined the expression and localization of cohesin subunits in the testis and ovary. We found many differences between medaka and mammals. Our findings suggest that although the basic mechanisms are ubiquitous in all eukaryotes, the fine roles of each cohesin subunit in assuring the meiosis-specific chromosome behaviors exhibit considerable variations according to different species.

HEAT SHOCK PROTEIN DNAJ HOMOLOGUE IS ASSOCIATED WITH IBA1

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An ionized calcium binding adaptor-molecule 1 (Iba1) is expressed in microglia and macrophages of the central nerve tissues. Iba1 has the actin-bundling activity and participates in membrane ruffling and phagocytosis in activated microglia. In testis, Iba1 protein is expressed in the cytoplasm of elongated spermatids, suggesting that it might be involved in the final stage of spermiogenesis. To look for the proteins interacting with Iba1, we screened a mouse testis cDNA library by a yeast two-hybrid system and obtained several clones including DnaJ homologues that are involved in assembly and disassembly of protein complexes, nascent protein folding, and prevention of protein aggregation. To examine the physical interaction between Iba1 and DnaJ homologues, we carried out immunoprecipitation assays using transfected COS-7 cells as well as in vitro binding assays using GST-fused proteins. The outcome of the assay suggested the physiological interaction between Iba1 and DnaJ homologue in both assays.

THE ROLE OF MAP-KINASE ON MEIOTIC METAPHASE-I(META-I) ARREST IN THE UNFERTILIZED EGG OF ASCIDIAN (*PHALLUSIA NIGRA*)

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In the unfertilized egg, meiotic division is arrested at various steps in species specific manner. It is well known that Mos-MAP-kinase cascade play a crucial role on the arrest at meiotic metaphase II in the unfertilized egg of vertebrates. But, concerning the arrest mechanism at meiotic metaphase I (Meta-I) is little known. We investigated the role of MAP-kinase on the arrest at Meta-I in the unfertilized egg of an ascidian (*Phallusia nigra*). When the unfertilized egg was treated with U0126, MAP-kinase kinase-inhibitor, activity of MAP-kinase decreased soon after the treatment, then 20 min after its meiotic division reinitiated from Meta-I in accordance with changes in MPF activity. First polar body-like protrusion which was often larger than normal one and nuclear division were observed, although its cytokinesis was not completed in most case. The chromosomal decondensation, which occurs after the second meiotic division in the normal fertilization, was observed soon after the first meiotic division. These facts suggest that MAP-kinase preserve the state at Meta-I in the unfertilized egg.

INDUCTION AND INHIBITION OF OOCYTE MATURATION BY EDCs IN ZEBRAFISH

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We previously described the induction of fish oocyte maturation by an endocrine-disrupting chemical (EDC), diethylstilbestrol (DES), a nonsteroidal estrogen. In this study, stimulatory and inhibitory effects of EDCs on oocyte maturation were examined in zebrafish. Among tested agents, tamoxifen (TAM) and its metabolite 4-hydroxytamoxifen (4-OHT) showed inducing activity as DES. The time course of the change in germinal vesicle breakdown and an intracellular molecular event induced by TAM and 4-OHT were indistinguishable from those induced by a natural maturation-inducing hormone (MIH): 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20 β -DHP) as DES-induced maturation. On the other hand, pentachlorophenol (PCP) represented a potent inhibitory activity on 17,20 β -DHP-induced oocyte maturation. These results suggest that EDCs may act as agonist or antagonist on induction of fish oocyte maturation. Furthermore possible interacting residue of stilbene with MIH receptor is discussed based on comparison of low-energy conformation of 17,20 β -DHP and DES and relative potency of steroids related to 17,20 β -DHP to induce maturation.

EXPRESSION OF NEUREGULIN IN THE TESTIS OF *XENOPUS LAEVIS*

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Neuregulin is a member of epidermal growth factor family and signals through erbB receptor. It has been revealed in mouse and *Xenopus laevis* that neuregulin is expressed in brain, heart, and etc and plays an important role in regulation of cell proliferation and differentiation. Neuregulin has multiple isoforms including secreted and transmembrane type in mammals, which are generated by alternative splicing of a single gene. It has been so far reported that *Xenopus laevis* has at least 2 isoforms with cysteine-rich domain and Immunoglobulin-like domain at the amino-terminal region. Little is known, however, about expression and function of neuregulin in testis. To investigate their expressions in the testis of *Xenopus laevis*, we cloned complementary DNAs encoding 2 isoforms of neuregulin from the testes. Analyses by *in situ* hybridization showed expression of the messenger RNA for both isoforms in Sertoli cells of the testes, suggesting that neuregulin is involved in spermatogenesis. Now, we examine the expression of each isoform in various spermatogenic stages of the testes of *Xenopus laevis* using *in situ* hybridization with specific probes.

IDENTIFICATION OF α -SUBUNITS OF *XENOPUS* 20S PROTEASOME AND ANALYSIS OF CHANGES DURING THE MEIOTIC CELL CYCLE

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The proteasome is a large, multi-subunit protease which regulates proteins through the ubiquitin-dependent proteolytic system in eukaryotic cells. To investigate the regulatory mechanism for the 26S proteasome in oocyte maturation and fertilization, we prepared polyclonal antibodies for five species of α -subunits. 2-D PAGE and immunoblot analysis of the 26S proteasome prepared from immature and mature oocytes was performed. With these antibodies and previous prepared monoclonal antibodies, we detected each subunit. All subunits except $\alpha 7$ were detected as two or more spots in immature. A difference in the spots between the 26S proteasome from immature and mature oocytes was detected in the blots of subunits $\alpha 2$ and $\alpha 4$. These results suggest several species of α -type subunits are produced by post-translational modification and modifications for two subunits ($\alpha 2$ and $\alpha 4$) change meiotic cell cycle-dependently.