IMPAIRED MEIOTIC CHROMOSOME SYNAPSIS/RECOMBINATION IN THE NEWT TESTES AT LOW TEMPERATURES

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In the newt testis, spermatocytes never appeared in the winter month because spermatogonia in penultimate stage induce apoptosis (cessation of spermatocytogenesis). This phenomenon indicates that the passage of this regulatory point and the appearance of spermatocytes under low temperature are unfavorable for newt spermatogenesis. In this study, we investigated the biological significance of cessation of spermatocytogenesis. Transfer of newts to low temperature (8, 12 and 15C) impaired normal synapsis/recombination and induces defects of spermtogenesis. In this condition, downregulation of DMC1 protein that is involved in meiotic pairing in eukaryotes occurred. Eggs inseminated by spermatozoa derived from the newt kept at 15C has a developmental restriction even though spermatozoa had a capacity of fertilization as well as from the one kept at 22C. These results that cessation of spermatocytogenesis is a tool to prevent from the production of spermatozoa disadvantageous for survival of the species.

THE ROLE OF M-COFILIN DURING MOUSE SPERMATOGENESIS

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Cofilin (CF) is an actin regulatory protein that plays a critical role in actin filament dynamics in a variety of cells. Two cofilin isoforms, M-cofilin (M-CF) and NM-cofilin (NM-CF) encoded by different genes, exist in mammals; in the adult, the former is predominantly expressed in muscle tissues and testis, while the latter is distributed in various non-muscle tissues (Ono et al., 1994). In this study, to clarify the role of M-CF *in vivo*, we generated chimeric mouse with M-CF deficient ES cells, and analyzed histologically the effect of M-CF deficiency in mouse tissues. In the testis of chimeric mouse, morphologically abnormal spermatogenic cells were detectable and sperms were diminished in the regions where M-CF null cells were concentrated in the semineferous tubules. These results suggest that M-CF plays an important role during spermatogenesis.

STRUCTURE AND SITE-SPECIFIC EXPRESSION OF YOLK PROTEINS IN THE TERRESTRIAL ISOPOD, *Armadillidium vulgare*

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Vitellins were purified separately from ovaries and eggs of the isopod, Armadillidium vulgare. Ovarian vitellin consisted of at least six proteins with relative molecular masses of 205 kDa, 200 kDa, 185 kDa, 180 kDa, 122 kDa, and 112 kDa. The larger four proteins disappeared in eggs within a week after oviposition and a 59 kDa protein newly appeared. The amino-terminal amino acid sequences of these vitellin proteins except for the ovarian 112 kDa, and egg 112 kDa and 59 kDa proteins were identical, and showed considerable similarity to those of known vitellogenins from other animals. Similar patterns of tryptic peptide maps of the 122 kDa and 112 kDa proteins from eggs on reversed-phase HPLC and sequence identity of two randomly selected peaks with the same retention times indicated that they shared most part of the sequence. PCR-assisted cloning of the 5' region of a cDNA (591 bp) encoding vitellogenin revealed the presence of a signal peptide consisting of 16 amino acid residues and clarified the structural relationship of the

protein components except for the ovarian 112 kDa and the egg 59 kDa proteins. Northern blot analysis revealed that fat body is the main vitellogenin producing organ.

GOLDFISH GV LAMIN B3: SITE-SPECIFIC PHOSPHORYLATION AND ITS POSSIBLE ROLE DURING OOCYTE MATURATION

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A germinal vesicle (GV) is a huge, highly specialized nucleus in goldfish and *Xenopus laevis* oocytes. A GV nuclear membrane is supported with a meshwork structure consisting of oocyte-type nuclear lamin B3. We constructed N-termianl mutated recombinant goldfish lamin B3 (GFLB3) and expressed it in *E.coli*. Microinjection of these purified mutant lamin proteins into *Xenopus laevis* immature oocytes revealed that the N-terminal head domain was important for nuclear lamin transport and lamina assembly *in vivo*. Mitotic and meiotic lamina disassembly is accompanied by phosphorylation of a serine residue (S28 for GFLB3) within a conserved motif (ser-pro-thr-arg-ile/leu) of the N-terminal head domain. In this study, we raised specific anti-GFLB3 monoclonal antibodies against the phosphorylated or dephoshorylated forms of the motif. Western blotting analysis using these antibodies showed that a portion of GFLB3 was already phosphorylated in GV-stage oocytes. The ratio of phosphorylated/dephosphorylated forms increased with lamina disassembly during oocyte maturation. Based on these results, we propose a model that lamin assembly/disassembly is regulated by conformational change in N-terminal head domain *in vivo*.

ORGAN CULTURE OF THE UNDIFFERENTIATED GONAD IN TILAPIA

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Gonadal sex differentiation in fish has been studied mainly through morphological and manipulative methods. To clarify the basic mechanism of gonadal sex differentiation in vertebrates, we utilize the tilapia *Oreochromis niloticus* as a model. In tilapia, gonads are formed 3–4 days after hatching (dah). Although morphological differences between ovaries and testes are distinguishable at 20–25dah, the exact mechanism of ovarian formation during gonadal sex differentiation remains unclear. We have established a system to supply all male or female populations and have developed an aromatase antibody to detect steroid producing cells. First, we examined the differentiation of the steroid producing cells in vivo using the anti-aromatase antibody. Aromatase immunoreactive cells first appear in gonads of female tilapia 7–10dah. These immunoreactive cells gradually increase in number with the development of gonads. To further examine the mechanism of gonadal sex differentiation, we developed an organ culture system for tilapia gonads. Undifferentiated gonads (5–10dah) were cultured for 10–15 days (equivalent to 20dah). All cultured gonads showed an increase in both somatic and germ cell numbers. The general condition and growth rates of cells isolated on different days (5–10dah) are similar. To assess the differentiation of the cultured gonads, we used immnohistochemistry with the anti-aromatase antibody. Unfortunately, aromatase-positive cells were undetectable after long culture. To check the immumoreactivity of steroid producing cells under organ culture conditions, gonads of females (10, 17–26, 122dah) were cultured for 3days. Aromatase-positive cells were detectable in gonads collected at 18–122dah. This organ culture provides a useful system for analysis of gonadal sex differentiation.

DEGRADATION OF THE CYCLIN B IN A CELL-FREE PREPARATION OF STARFISH OOCYTE

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In a cell-free preparation of starfish (*Asterina pectinifera*) oocyte at metaphase I, cyclin B was stable for 40 min, and then degraded by the proteasome. When the pH value of the preparation was changed from 7.0 to 7.5, cyclin B was rapidly degraded. Thus, cyclin B degradation was likely to be promoted at higher pH value. Indeed, following fertilization, the intracellular pH rose from 7.0 to 7.3, resulting in the shortening of the duration of metaphase I or acceleration of cyclin B degradation.