The Zoological Society of Japan

CLONING OF THE Na^+/H^+ EXCHANGER FROM STARFISH OVARIES

Kaori Harada, Kazuyoshi Chiba
Department of Biology, Ochanomizu University, Bunkyo-ku, Tokyo 112-8610, Japan

Reinitiation of meiosis in oocytes usually occurs as a two-step process during which release from the prophase block is followed by an arrest in metaphase of the first or second meiotic division (metaphase I [MI] or metaphase II [MII]). The mechanism of MI arrest in meiosis is poorly understood, although it is a widely observed phenomenon in invertebrates. The blockage of fully grown starfish oocytes in prophase of meiosis I is released by the hormone 1-methyladenine (1-MA). It has been believed that meiosis of starfish oocytes proceeds completely without MI or MII arrest, even when fertilization does not occur. We have showed that MI arrest of starfish oocytes occurs in the ovary after germinal vesicle breakdown. This arrest is maintained by the blockage of an increase of intracellular pH in the ovary before spawning. Immediately after spawning, activation of Na^+/H^+ exchanger (NHE) via the heterotrimeric G protein coupling to 1-MA receptor leads to an intracellular pH increase that can overcome the MI arrest.

In this study, we isolated the cDNA fragments encoding NHE from starfish ovaries by RT-PCR analysis, and found that it shows high identity to mammalian NHE.

MOLECULAR CLONING OF REPRODUCTIVE ORGAN SPECIFIC GENE FROM *EISENIA FETIDA*

Tomoharu Suzuki, Makoto Honda, Seiji Matsumoto, Shinobu Gamou
Department of Environment and Life Sciences, Kyorin University School of Health Sciences, 476 Miyashita-cho, Hachioji, Tokyo 192-8508, JAPAN

Earthworms are generally hermaphroditic and have testes and ovaries in separate segments. In order to understand gonad-morphogenesis and reproduction mechanism, we employed the molecular approach and isolated the gene that expresses specifically in reproductive organ in common earthworm, *Eisenia fetida*. The isolated gene shows approximately 60% homology to *vcp* (valosin containing protein) or *ter* (transitional endoplasmic reticulum ATPase), those belong to AAA (ATPase-Associated with different cellular Activities) family and suggest to take roles in Golgi membrane fusion, endoplasmic reticulum-associated protein degradation, and germ cell development. The AAA consensus motif was well conserved in the gene isolated from the earthworms. Reverse transcription-polymerase chain reaction (RT-PCR) analyses revealed that this gene expresses in anterior segments of mature worms where gonads are localized, whereas no signals was detected in immature worms without clitella. *In situ* hybridization clearly demonstrated the gene expression in seminal vesicle where spermatogenesis takes place. These findings together suggest that *E. fetida vcp* homolog participates in spermatogenesis.

OOGENESIS OF *MILNESIUM TARDIGRADUM*

Atsushi C. Suzuki
Department of Biology, Keio University, Hiyoshi 4-1-1, Yokohama 223-8521, Japan

A parthenogenetic strain of *Milnesium tardigradum* has been maintained since autumn 2000. These animals were fed on a monogonont rotifer and grew into mature adults at the 3rd-instar stage. Egg laying accompanied the moulting process and the eggs were laid in the space between the old and new cuticles. The egg-laying / moulting intervals of adult animals were around 6-10 days. The life history of *M. tardigradum* under the rearing environment included up to seven periods of moult or five times of egg laying. The number of eggs in a clutch ranged from 1-12 eggs/clutch. The possible factor caused the difference of the clutch size is the nutritional condition of the mother. The relationship of oocytes and other ovarian cells should be clarified first to ask the question how the number of eggs is decided. Specimens from immature larvae and adults with various stages of ovaries were fixed in glutaraldehyde and embedded in Epon. Semithin and ultrathin sections of these specimens were observed by light microscopy and transmission electron microscopy, respectively, and fine structures of the ovarian cells were investigated.

THE BEHAVIOR OF γ-TUBULIN DURING SPERMATOGENESIS OF THE GRASSHOPPER, *CHORTOPHAGA VIRIDIFASCIATA*

Yumi Matsuda, Naoko Yamashiki
Biol. Lab., Rakuno Gakuen Univ., Ebetsu

γ-tubulin is known to have functions for organizing microtubules in cell division. We observed the distribution of γ-tubulin during spermatogenesis from the gonial stage to sperm maturation by immunostaining method with use of mouse monoclonal anti γ-tubulin. At the same time, special attention was paid to the transformation of microtubule assembly. In cell division of spermatogonia and spermatocytes, a spot of γ-tubulin was located in the astral center, and then, in the spindle pole. It seems that the location of γ-tubulin is coincided with that of centrosome. In spermatids, the spot disappeared just after meiosis, and then a new cluster of γ-tubulin appeared and gradually concentrated on the base of flagellum. When the manchette that consists of microtubules, appeared around the nucleus, the other cluster of γ-tubulin appeared in the opposite side of the nucleus. By the time manchette disappeared, γ-tubulin could not observe anymore. It is found that γ-tubulin and centrosome behave in the same way during meiosis, and thereafter, γ-tubulin changes the distribution along with the transformation of microtubule assembly during spermiogenesis.

ANNUAL REPRODUCTIVE CYCLE AND SPERMATOGENIC PROCESS IN MALE MOSQUITOFISH, *GAMBUSIA AFFINIS*

Yasunori Koya, Ayako Iwase
Department of Biology, Faculty of Education, Gifu University, Gifu 501-1193, Japan

To clarify the annual reproductive cycle of wild male mosquitofish in central Japan, changes in testicular histology were investigated. The reproductive cycle in Mie Prefecture was divided into the following two periods: 1) Spermatogenic period (May to October). Transition from spermatogonia to spermatocytes occurs, and fish undergo active spermatogenesis. 2) Resting period (October to April). Transition from spermatogonia to spermatocytes ceases, and progress only from residual spermatocytes to sperm balls occurs. From observations of spermatozoa in the ovary, it is suggested that copulation was underwent from February to October. To clarify the spermatogenic process, 5-bromo-2'-deoxyuridine (BrdU) was injected intraperitoneally and incorporated into cells synthesizing DNA (e.g., spermatogonia). Labeled cells were detected immunohistochemically using anti-BrdU antibody. Incorporated BrdU were detected in spermatocytes 1 day, in spermatids 5 days, in spermatozoa 10 days, and in sperm balls 20 days after injection, respectively, suggesting that about 20 days are needed for spermatogenesis in the condition of the present study (25°C, 16L8D) in mosquitofish.

CASPASE INHIBITORS BLOCKED THE PROGRESS OF SOME EVENTS IN SPERMIOGENESIS OF THE MEDAKA, *ORYZIAS LATIPES.*

Takahiro Sasaki, Eriko Takayama-Watanabe, Akihiko Watanabe, Kazuo Onitake
Department of Biology, Faculty of Science, Yamagata University, Yamagata 990-8560, Japan

Spermiogenesis is the final stage of spermatogenesis in which several events containing nuclear condensation, flagellum formation and formation of cytoplasmic lobe, take place in spermatids. The mechanism for regulating those events is not known well in vertebrates. The medaka fish, *Oryzias latipes*, suited for the study because primary spermatocytes develop through meiosis and spermiogenesis to fertilizable sperm in cell culture. We have already reported that a universal inhibitor for caspases inhibited the formation of cytoplasmic lobe in spermatids of the medaka fish. Caspases are known as second messengers in the intracellular signaling cascade for apoptosis. In this study, we estimated the involvement of caspases in regulating the other event of spermiogenesis. When 10mM Ac-WEHD-CHO, an inhibitor for caspase-I was added to the media that the differentiating spermatids were cultured, the nuclei of those cells were dissoluble by the DNase I digestion. Whereas, the nuclei were not dissolved in spermatids cultured with the inhibitor for the other caspases. This result suggests that the caspase-I involves in the progress of nuclear condensation in the spermiogenesis of *O. latipes*.

DEVELOPMENT OF PRIMORDIAL GERM CELLS (PGCS) IN THE JAPANESE NEWT *CYNOPS PYRRHOGASTER*

Yoichiro Tamori, Masami Wakahara
Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, JAPAN

Mechanisms of PGC development are being clarified in some typical model organisms. In anuran amphibians, PGCs are believed to form preformistically by a presence of the germ plasm in oocytes and fertilized eggs, like in *Drosophila*. Contrary to this, in urodelan amphibians it is generally accepted that there are no germ plasm in their oocytes and eggs, so we expect that PGCs are epigenetically induced in the lateral plate mesoderm during the embryogenesis as in mammals. In order to analyze the developmental and molecular mechanisms of the PGC formation in the newt, *Cynops pyrrhogaster*, we isolated at first cDNA clones of *Cynops Dazl* (*Cydazl*) and *Cynops Vasa* (*Cyvasa*)genes from adult gonads. We analyzed the spatio-temporal expression of the *Cyvasa* and *Cydazl* genes during the normal embryogenesis using RT-PCR and *in situ* hybridization. In addition, we made explants consisted of the animal cap ectoderm and vegetal endoderm pieces from late blastula embryos to confirm whether the *Cynops* PGCs can be induced in animal cap ectoderm *in vitro*. It is expected that either *Cyvasa* or *Cydazl*, or both are used as specific molecular marker for the PGC formation *in vivo* as well as *in vitro*.

IDENTIFICATION OF THREE *XENOPUS* PROTEASOME α-SUBUNITS AND ANALYSIS OF MODIFICATIONS DURING MEIOTIC CELL CYCLE

Yuka Wakata[1], Mika Tokumoto[1,2], Ryo Horiguchi[1,2], Katsutoshi Ishikawa[1], Yoshitaka Nagahama[2,3], Toshinobu Tokumoto[1,2]
[1]Department of Biology and Geosciences, Faculty of Science, Shizuoka University, Ohya 836 Shizuoka 422-8529, Japan, [2]CREST Research Project, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan and [3]National Institute for Basic Biology, Okazaki 444-8585, Japan

The proteasomes are large, multi-subunit proteases which have been shown to be involved in the regulation of various biological functions in eukaryotic cells

NII-Electronic Library Service