GENETIC ANALYSIS OF NEW MUTATION KASUMI(*Ka*) CAUSING STEM CELL DEFICIENCY IN MICE

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The mouse strains 129/Sv-+/*ter*(+/+)(agouti) and LTXBJ(light) are susceptible to testicular teratomas and ovarian teratomas, respectively. We found a coat color mutation in their F1 female and named it as Kasumi (*Ka*). We established the *Ka* congenic strains 129/Sv-*Ka*/+ and LTXBJ-*Ka*/+ by introducing the gene to both founder strains by repeated backcrossing. The phenotypes of both congenic strains showed that males and females homozygous for *Ka* become albino with black eyes, anemic by erythrocyte deficiency and sterile by germ cell deficiency and that the *Ka* heterozygote become fertile and albino with colored patches on the head and back and black eyes. Phenotypes of some of the offspring obtained from the mating between the mice heterozygous for *Ka* and *W* (*c-Kit*) were same as those of *Ka* homozygote. Mating tests and RT-PCR analysis indicated that the *Ka* gene is different from the *Sl* (*c-Kitl*) gene. Thus, it is suggested that the *Ka* mutation may be a novel allele of *c-Kit* causing singly stem cell deficiency in gametogenesis, erythrogenesis and melanogenesis.

MATERNAL Nanos PROTEIN REPRESSES POLE CELLS APOPTOSIS IN *DROSOPHILA* EMBRYOS

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Maternal Nanos (Nos) protein is required for germline development in Drosophila. We have reported that Nos inhibits apoptosis of the germline progenitors, or pole cells during embryogenesis. However, the mechanism of how Nos inhibits apoptosis of pole cells remains to be elusive. It has been reported that Nos, along with Pumilio protein, represses translation of mRNAs with discrete RNA sequence called Nos Response Element (NRE). Here, we identified *head involution defective* (*hid*), a gene encoding apoptosis inducer, as a regulatory target for Nos. *hid* mRNA contained NRE sequence in its 3'UTR and was detectable in pole cells. Mutations in *hid* locus repressed apoptosis of pole cells lacking Nos. We further found that expression of *hid* mRNA without NRE was able to induce apoptosis in normal pole cells, while intact mRNA carrying NRE had a subtle effect. These results suggest that Nos inhibits apoptosis of pole cells by repressing *hid* activity in a NRE-dependent manner.

FUNCTIONAL ANALYSIS OF NOVEL PROTEIN KINASE INVOLVED IN APOPTOSIS OF POLE CELLS IN *DROSOPHILA* EMBRYO

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In *Drosophila*, the germline progenitors, or pole cells, are formed at the posterior pole of the blastoderm embryos. We have previously shown that pole cells lacking maternal Nanos (Nos) protein cause apoptosis during embryogenesis, suggesting that pole cells have a potential to undergo apoptosis. However, the molecular mechanism of how apoptosis is induced in pole cells has remained unclear. Here, we report that a novel protein kinase (dTAO-1) is required for apoptosis in pole cells. Maternal *dtao-1* mRNA is enriched in germ plasm and is inherited into pole cells. In the pole cells lacking Nos (nos pole cells), expression of a dominant-negative form of dTAO-1 was able to repress apoptosis, while over-expression of intact dTAO-1 enhanced it. These results show that dTAO-1 positively regulates apoptosis of pole cells in nos pole cells. As a regulatory target of dTAO-1, we identified *sickle* (*skl*), one of the genes involved in apoptosis. Its expression in pole cells was correlated well with dTAO-1 activities. These results suggest that dTAO-1 induces apoptosis in pole cells by regulating the expression of *skl*.

SCREENING FOR MATERNAL mRNAs ENCODING TRANSCRIPTION FACTORS REQUIRED FOR GERMLINE DEVELOPMENT IN *DROSOPHILA*

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In *Drosophila*, germ plasm is localized in the posterior pole region of early embryos and is partitioned into the germline progenitors, known as pole cells. Germ plasm contains maternal factors required and sufficient for germline establishment. It has been proposed that these factors may act as transcriptional activators to initiate germline-specific gene expression in pole cells. To identify these factors, we screened maternal mRNAs enriched in germ plasm and pole cells by using DNA microarray technique. We used a custom oligo-DNA microarray containing the probes for all predicted genes in *Drosophila*. Pole cells were isolated from early embryos by using fluorescence-activated cell sorting (FACS). We hybridized the microarrays with the RNA from the isolated pole cells, and identified 700 of pole cell-enriched mRNAs. Among them, we focused on 86 mRNAs encoding transcription factors. In situ hybridization analysis to locate these mRNAs in embryos and their functional analysis are now on going.

NOVELTIES OF SPERMATOZOA AND SPERMATOGENESIS IN JAPANESE INSECTIVOROUS MAMMALS (LIPOTYPHLA)

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In insectivorous mammals, the testes are in a abdominal or inguinal position, at a temperature close to that of the body. The aim of the present study was to investigate with light and electron microscopes the comparative morphology of spermatozoa and spermatogenesis in Japanese insectivore species belonging to two families, Soricidae and Talpidae. There was a gap in the sperm morphology between the families Soricidae and Talpidae.

DIFFERENCE BETWEEN GERMLINE AND SOMATIC MITOCHONDRIA IN *XENOPUS LAEVIS*

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The germ plasm is a particular cytoplasm localized in the egg and inherited in the germ line cell of some animal species including *Xenopus laevis* and usually contains a number of mitochondria. Mitochondrial ribosomal RNA in the germ plasm plays an essential role in gonogenesis. It is expected that mitochondria are intimately related to the gonogenesis. It is known that mitochondria in different tissues contain specific compornents. Our question is whether or not mitochondria in the germ plasm (germline mitochondria: GM) are different from those in the other area of the oocyte (somatic mitochondria: SM).

Germ plasm fraction was isolated from stageI oocytes, and used as GM rich preparation without further purification. For SM rich preparation, we isolated mitochondria from stageVI oocytes, in which a few germline mitochondria are present. We compared GM with SM using a SDS-PAGE, and found that the amount of ATP synthase β, δ and B subunits of GM is lower than those of SM.

To confirm these difference between GM and SM in the single oocyte, we are making antibodies against ATP synthase β, δ and B subunits.

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL NEUREGULIN ISOFORM IN THE TESTIS OF *XENOPUS LAEVIS*

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Neuregulin (NRG), a member of epidermal growth factor (EGF) family, signals cell proliferation and differentiation thorough ErbB receptor in brain, heart, and etc. Alternative splicing of a single NRG gene generates many isoforms in mammals and at least 2 isoforms with cysteine-rich domain (CRD) and immunoglobulin-like domain (IgD) at the N-terminal region, respectively, in *Xenopus laevis*. However, little is known about its expression in the testis and function in spermatogenesis. To investigate the potential role of NRG, a specific primer-based RT-PCR approach was used to isolate the cDNAs from the testis of *Xenopus laevis*. Among the 3 cDNA clones isolated, 2 clones were the same as reported, but one was unique. Subsequent isolation and sequencing of the full-length cDNA confirmed that it encoded a novel 80-kDa protein containing IgD and b2 type EGF like domain at the N-terminal region. RT-PCR analyses showed that this isoform was predominantly expressed in the testis. Now we examine its effect on cell proliferation. These findings suggest that this novel isoform may be critically involved in cell proliferation and signaling during spermatogenesis of *Xenopus laevis*.

THE ROLES OF TWO-STEP Ca^{2+} RISES IN FERTILIZED OOCYTES OF THE POLYCHAETE *PSEUDOPOTAMILLA OCCELATA*

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We have examined the pattern and the roles of intracellular Ca^{2+} rises at fertilization in polychaete oocytes. Shortly after a fertilizing sperm had bound to the vitelline membrane (VM) of oocyte, a non-propagating Ca^{2+} rise took place in the localized cytoplasm just beneath the sperm-binding site on VM. Subsequently, the cortex around the site of Ca^{2+} rise protruded gradually and the plasma membrane of this portion eventually touched to VM 30-40s later. The oocyte with a protrusion allowed the sperm to penetrate VM and then exhibited Ca^{2+} influx from the entire cortex. The oocyte reverted to the original spherical shape following the Ca^{2+} influx and underwent polar body formation (PBF). When an oocyte was inseminated in the presence of D-600, a blocker of voltage-gated Ca^{2+} channels, it showed a localized Ca^{2+} rise and a cortical protrusion, but failed to display a Ca^{2+} influx or PBF. Conversely, excess K^+ seawater induced Ca^{2+} influx and PBF. These results suggest the different roles of two-step Ca^{2+} rises: an initial localized Ca^{2+} rise for a cortical protrusion that permits sperm penetration and a subsequent Ca^{2+} influx for meiosis reinitiation leading to PBF.