PLANARIAN VASA-RELATED GENES EXPRESSED IN THE STEM CELLS

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In order to characterize the totipotent stem cells participating in planarian regeneration, we focused on *vasa*-related genes expressed in a germline-specific manner, because of morphological similarity between chromatoid bodies in the cytoplasm of planarian stem cells and germplasm of various animals. In addition to *vasa*-like genes *vlgA* and *B* which were isolated previously, *vlgC* and *Plvas1* were isolated from the planarian *Dugesia japonica*. Histochemical analyses with antisera against PLVAS1 and VLG-A revealed that PLVAS1 was present in cytoplasm of germ and somatic stem cells. In contrast, VLG-A was not detected in the stem cells of sexually immature planarians in spite of the presence of the mRNA. This suggests that PLVAS1 is a component of the chromatoid body.

CULTURE AND CELLULAR CHARACTERIZATION OF PLANARIAN STEM CELL

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Planarian is well known as a model animal for regenerative organism, and the high regenerative capability is supported by the stem cells. To understand the planarian stem cell system it is indispensable to isolate and cultivate the stem cells *in vitro* conditions. Recently, we have succeeded in identification of planarian putative stem cell fraction, X1 and X2, by a fluorescence activated cell sorter. These fractions are specifically eliminated by X-ray irradiation. Recent studies using single-cell PCR analyses indicated that X1 fraction contains proliferating stem cell or stem cell-derived cells, and X2 fraction may conditions stem cells in G0/G1 state. Here, we have tried to investigate the self-renewal capacity of these cells in *in vitro* culture conditions, and to introduce foreign genes and marker proteins into them.

RETROTRANSPOSON EXPRESSION ON THE NO-REGENERATING TAIL FRAGMENT IN THE PLANARIAN *BDELLOCEPHALA BRUNNE*

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The head regenerative ability of planarian differs by genus *Bdellocephala* and genus *Dugesia*. A tail fragment of *B. brunnea* can not regenerate a new head in the case of having been cut at the rear level from the base of pharynx. In order to investigate the difference in this regenerative capability, total RNA was extracted from each piece of *B. brunnea*, and the gene was discovered by comparison with the expression pattern using the differential display method. The gene showed strong expression in the 1st day tail fragment cut at the rear level from the pharynx base. The gene expressions of each worm in the different regeneration period (1 day, 2 day and 3 day) and cutting levels (front or rear from the pharynx base) were analyzed by RT-PCR. Consequently, although the gene expression of the tail fragment cut at the rear level from the pharynx base was seen strongly, the expression was not detected in the head fragments in all regeneration period. Moreover, the expression of the similar grade was detected in both head and tail fragments cut at the front level from the pharynx base. As the result of identifying this gene by the 3'RACE and the SLIC method, it was a retrotransposon gene.

THE MAPK DEPENDENT PERIOD OF STARFISH EGG APOPTOSIS

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Fully grown starfish oocytes are arrested at prophase of meiosis I. The hormonal stimulation of 1-methyladenine (1-MA) induces meiosis reinitiation. In the absence of sperm, oocytes complete both meiotic divisions to yield haploid interphase-arrested eggs. In these eggs, spontaneous and synchronous activation of caspase-3 occurs 9-12h after 1-MA stimulation. Then, caspase-dependent membrane blebbing and apoptotic body formation occur, indicating that mature eggs undergo apoptosis if not fertilized. MAP kinase activity increases 30 min after 1-MA treatment and is maintained for several hours, then decreases immediately before the onset of blebbing. It is known that persistent activation of MAPK is important for caspase-3 activation, since apoptosis is blocked when eggs were treated with a MAPK kinase (MEK) inhibitor 3h before the onset of blebbing. The MEK inhibitor, however, did not block apoptosis when applied 1h before induction of apoptosis. Thus, for induction of apoptosis, MAPK should be activated for a definite period, called the MAPK dependent period.

INDUCTION OF APOPTOSIS IN MOUSE BLASTOCYST CAUSES DEVELOPMENTAL ARREST AND RETARDATION

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We have reported that apoptosis induced with extra glucose *in vitro* in mouse blastocyst have reduced by the addition of the glutathione. Furthermore inhibition of glutathione synthesis by BSO increased the number of apoptotic cells. In the present experiment, the effect of increased number of apoptosis on the later development was observed at day 12.5 of gestation. Mice used were ICR strain. Eight cell-stage embryos were collected from the oviducts and cultured in M16 with 10 mg/ml glucose or in M16 with 0.1 mM GSH. After cultivation, the embryos were injected into the uteri of pseudo-pregnant mothers. At day 12.5 of gestation, embryos were removed from uteri into Hanks solution and checked their external appearances under a dissecting microscope. There was no different in the ratio of implantation between both groups. However, the resorption of embryos increased in the apoptosis induced group. The both body weigh and crown-rump length were varied in the apoptosis induced group. These results show that glutathione has anti-oxidative role in mouse preimplantation development and induction of apoptosis has affected the development after implantation.

THE GENETIC CONTROL OF ENVIRONMENTALLY INDUCED APOPTOSIS DURING *DROSOPHILA* OOGENESIS

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Many organisms have mechanisms to relate egg production to their environment. In *Drosophila* the link between the environment and the genetic control of reproduction is achieved in part by specific developmental choices being made by individual egg chambers. At a key control point prior to yolk uptake egg changes decide to arrest, to proceed with vitellogenesis, or to undergo apoptosis. We study the hormonal and genetic basis of how egg development is linked to environment. Both JH and ecdysone play key roles in mediating this response. We show that diap 1, and E75A are expressed at stages 8 and 9 of oogenesis when this developmental choice is executed. BR-C and E74 show differential expression patterns in previtellogenic oocytes dependent upon whether food is available or not. The apoptosis initiated at stages 8 and 9 in starved flies is suppressed by JHA and conversely, 20-hydroxyecdysone (20E) induces apoptosis in fed flies. These hormones affect the spatial and temporal expression of these regulatory genes duing previtellogenic stages. We propose that to complete apoptosis needs an apoptosis commitment, a preparation and an execution phases initiated at stages 8 and 9.

ECDYSTEROID-INDUCIBLE GENES WITH NO ORF IN THE PROGRAMMED CELL DEATH OF THE ANTERIOR SILK GLAND OF THE SILKWORM, *BOMBYX MORI*

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Anterior silk gland of the silkworm, *Bombyx mori*, undergoes programmed cell death (PCD) during pupal metamorphosis, and the PCD is triggered by 20-hydroxyecdysone (20E) *in vivo* and *in vitro*. We previously obtained seven novel 20E-inducible genes that might be involved in the PCD. Two of those genes, EC08 and EN78 possessed no ORF, were induced by 20E in a dose-dependent manner, and a protein synthesis inhibitor did not suppress the 20E-induced expressions. These results indicate that both genes are classified as early genes that are directly induced by 20E. Temporal expression profiles of those genes in the anterior silk gland during 4th and 5th stadium were different to each other. EC08 RNA appeared only at the end of 5th stadium. EN78 expression increased at the end of 4th stadium and disappeared until 6th day of 5th stadium, after which it increased again. Thus EC08 and EN78 expression may be under different regulating mechanisms though they are induced by 20E *in vitro*.

EARLY AND EARLY-LATE GENE EXPRESSIONS IN THE ANTERIOR SILK GLAND OF THE SILKWORM, *BOMBYX MORI* DURING PROGRAMMED CELL DEATH

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Silk gland is a larval specific tissue and begins to degenerate during larval-pupal metamorphosis. The programmed cell death (PCD) is triggered by 20-hydroxyecdyson (20E), but little is known about the molecular mechanisms of the 20E-triggered PCD. We analyzed the expression patterns of early genes (*EcR A, EcR B1, E75 A* and *E75 B*) and an early-late gene (*BHR 3*) in the anterior silk gland *in vitro* and *in vivo*. *E75 A* and *BHR 3* were directly induced by 20E *in vitro* but *E75B* was not. In *in vivo* conditions, *E75 A* and *BHR 3* expressions increased during metamorphosis, while *E75 B* was undetectable. Thus, we suggest that *E75 A* and *BHR 3* are involved in the PCD, but not *E75 B*. *E75 A* expression increased greatly one day after gut purge (G1) and disappeared on the following day. *BHR 3* expression was at considerable levels through day 2 (G2) and day 3 (G3). The difference between early and early-late gene expression was confirmed by their dose-responses to 20E in