RECONSTITUTION OF SPERM NUCLEI OF ZEBRAFISH [*DANIO RERIO*] IN *XENOPUS* EGG EXTRACTS

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Incubation of plasmids and sperm nucleus in *Xenopus* egg extract have been shown to induce reconstitution of sperm nucleus which incorporate plasmids during formation of a new nuclear envelope around the dispersed chromatin. Transgenic frogs which express integrated gene nonmosaically were generated with a high frequency by transplantation of these nucleus into unfertilized eggs. The experiments reported here were carried out for a purpose which we apply this way to generate transgenic zebrafish. When lysolecithin-treated sperm from zebrafish were incubated in *Xenopus* eggs, we were able to observe a series of changes in sperm nuclear morphology periodically. This result showed that there is a possibility of generation of transgenic zebrafish in a similar way as *Xenopus*.

A SCREEN FOR MATERNAL MUTATIONS AFFECTING GERM-LINE DEVELOPMENT IN DROSOPHILA.

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In *Drosophila*, maternal factors sufficient for germ-line development is localized in germ plasm and are partitioned into the germ-line progenitors, or pole cells. There are still many uncharacterized maternal genes that functions in germ-line development. These genes may have an additional role in a subset of somatic cells, making them difficult to be identified in screens for grandchild-less mutations. One way to overcome this ploblem is to generate germ-line clones using FLP-FRT method. In this study, we screened a collection of X-linked lethal mutations by using the FLP-FRT method. We have identified several mutations showing grandchild-less phenotype. Further analysis is needed to test the possibility that these genes encode germ plasm components required for germ-line development.

A SCREEN FOR RNA MOLECULES LOCALIZED IN POLAR PLASM OF DROSOPHILA EMBRYOS.

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In *Drosophila*, maternal factors sufficient for germ-line formation are localized in the posterior pole region of egg cytoplasm, or polar plasm. Genetic and molecular screens have identified several molecules that are localized in polar plasm and are required for germ-line development. However, it has been suggested that additional factor(s) is needed to form functional germ line. We have been performing a screen for RNA molecules localized in polar plasm with the expectation of finding factors for germ-line formation. We constructed a cDNA library from poly(A)+RNA extracted from polar plasm. Random clones were screened by *in situ* hybridization to early embryos. From approximately 3000 cDNA clones, we obtained 109 that hybridized with localized RNAs. Sequencing analysis of these cDNA clones has revealed that only 4 clones encode novel RNA species. The rest of the clones were of the RNA molecules known to be localized in polar plasm, such as mitochondrial large rRNA, *Pgc* RNA, *germ cell-less* and *cyclin B* mRNAs. Further analysis will be carried out to determine the localization of the 4 novel RNAs during embryogenesis and their role in germ-line formation.

CLONING OF *XENOPUS* HOMEOBOX GENE FROM METAMORPHOSING TADPOLES.

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DNA fragments were cloned from a cDNA library of thyroid hormone treated tadpoles of african clawed frog (*Xenopus laevis*) by PCR using the primers for amplifying the consensus region of homeobox genes. The similarity test indicated that one of them, named clone/d, contained a homeobox. This is the first suggestion that a homeobox gene plays some roles during anuran metamorphosis.

For further characterization, a set of specific primers for the clone/d were designed and subjected to PCR cloning technique. As a result, both of 5'- and 3'-flanking regions of clone/d were isolated from the same library. The whole nucleotide sequence of the cDNA fragments which contain the complete ORF coding a homeobox gene was determined by combining sequences of these cDNA fragments. The similarity test suggests it resembles *Xenopus* Hoxb-5. The temporal expression pattern of *Xenopus* Hoxb-5 has been reported during development up to the swimming tadpole, in which the gene begins its expression at the mid-gastrula and peaks at the late neurula or tailbud stage (Fritz *et al.* and Harvey *et al.*).

We are trying to analyze the temporal and the spatial expression pattern of this clone during the period from early embryo to metamorphosing tadpole.

Ref. Fritz et al. (1988) Nucleic Acids Res. 16 (4), p.1453-1469
Harvey et al. (1986) EMBO J. 5 (6), p.1237-1244

DEGRADATION OF GERM-PLASM COMPONENTS IN DROSOPHILA EMBRYOS WITHOUT POLE-CELL FORMATION

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In *Drosophila*, the factors required for germ-line formation are localized in germ plasm, and are inherited into the germ-line progenitors, or pole cells. We have previously shown that the reduction of mitochondrial large ribosomal RNA (mtlrRNA) by injecting anti-mtlrRNA ribozymes into the cleavage embryos causes their failure to form pole cells. Here, we found that in such embryos, germ plasm components, such as Vasa protein were degraded rapidly at the syncytial blastodermal stage. In contrast, Vasa was normally maintained in pole cells of the control embryos injected with distilled water. Furthermore, a similar situation was observed in mutant embryos [gs(1)N26]. Females homozygous for N26 produce embryos with germ plasm, but the embryos fail to form pole cells due to delayed migration of cleavage nuclei into germ plasm. These results suggest that polar plasm components are stably maintained, only when they are sequestered into pole cells. This mechanism may enable only pole cells to develop as functional germ line.

THE ANALYSIS OF GASTRULA FORMATION BY USING NOCODAZOLE WHICH INHIBITS BLASTOPORE FORMATION.

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The lower blastoporal lip which consists of endodermal epithelium and blastopore including the bottle cells are important for gastrula movement in amphibian embryogenesis. For the analysis of gastrula movement, the *Cynops* blastrula embryo was treated with nocodazole which depolymerizes the microtube and inhibits cell movement. In the nocodazole-treated embryo, the blastopore formation was inhibited and then the axis was not formed. The embryo could not develop into the normal gastrula. However, even in the embryo, the organizer-related genes was expressed and the blastoporal lip had mesoderm-inducing capacity and induced the secondary embryo. The bottle cells of the nocodazole-treated gastrula could not form normal blastopore and the involution at the dorsal marginal zone was disturned. Therefore, we can condude that the involution of the dorsal marginal zone was essential for determination of the axial mesoderm.