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Developmental Biology

POLARIZATION OF ECTODERMAL TELOBLAST PRECURSORS (CELLS NOPQ) IN EMBRYOS OF OLIGOCHAETE ANNELID TUBIFEX Ayaki Nakamoto, Asuna Arai, Takashi Shimizu

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Ectodermal teloblasts (ectoteloblasts N, O, P and Q) of *Tubifex* are generated through an invariable sequence of cell division of a proteloblast, NOPQ, on either side of the embryo. The N teloblast is born first and located ventralmost, and the Q teloblast, which is born next, is located dorsalmost. Fates of ectoteloblasts stirctly corresponding to their birth rank and their position along the embryonic axes. In this study, we examined whether the division pattern of cells NOPQ is determined by external cues or intrinstic factors. We transplanted left NOPQ at various developmental stages to the right side of another embryo (from which right NOPQ had been deleted) and followed the divisions in the transplanted NOPQ. We found that the transplanted left NOPQ adopted a sequence of cell divisions and fates that are identical to those of the authentic right NOPQ. In contrast, when transplanted shortly before emergence of the N teloblast, left NOPQ exhibited division patterns comparable to those of the authentic left NOPQ. These suggest that NOPQ is initially plastic in terms of division pattern and fates and that it is polarized by external cues at some time after its birth.

THE ENLARGEMENT OF MALE PRONUCLEUS AND SPERM ASTER DURING SECOND MEIOSIS WERE INHIBITED IN ANDROGENETIC CLAM CORBICULA FLUMINEA AND CORBICULA SANDAI

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To examine the whether the factor regulating the second meiosis is still active in the androgenetic clam, we observed the development of sperm aster and mitotic spindle and expansion of male pronucleus during meiosis in the eggs. In bisexual *Corbocila sandai* eggs just after fertilization the cometlike shaped sperm aster disappeared. During meiosis male pronucleus remained condensed. After meiosis completed the male pronucleus started to enlarge and the biaster spindle appeared at the prophase of first mitosis. In androgenetic clam *C. fluminea* extruded the all maternall chromosomes at M-1 but showed the same pattern of sperm aster and first mitotic spindle formation. The sperm aster once formed and disappeared in the same way. After the period corresponding meiosis second the biastral spindle appeared and male pronucleus start to enlarge.

THE SPAWNING AND EARLY DEVELOPMENT OF THE JAPANESE ACORN WORM, BALANOGLOSSUS MISAKIENSIS

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Japanese acorn worm, Balanoglossus misakiensis, was collected in Masuho-ga-ura (Noto Pen., Ishikawa Pref.), and lived in aquarium for over a year. Natural spawning was induced by a shift of seawater temperature. The detail of development from fertilized egg to early tornaria larva was described.

VEG-T mRNA IS A PART OF MARGINAL CYTOPLASMIC DETERMINANTS REQUIRED FOR GASTRULATION AND MESODERM FORMATION

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When early one cell stage *Xenopus* embryo are deleted of their vegetal pole cytoplasm, the resulting embryo develops into two distinct types of axis-lacking embryos. We have designated these two types of embryos as "Gastrulating Non-axial Embryos (GNEs)" and "Permanent-Blastula type Embyos (PBEs)". PBEs did not show Veg-T expression while it expressed in the vegetal sub-surface region of GNEs. Sub-surface sub-equatorial cytoplasm from GNEs was withdrawn and injected into PBEs. This cytoplasm transplantation resulted in Veg-T translocation as shown by *in situ* hybridization. The injected PBEs gastrulated around the point of cytoplasm injection. PBEs did not express a mesodermal marker Xbra while it expressed around the GNE-cytoplasm injection point of host PBEs. Veg-T mRNA showed similar effects when injected into PBEs. Finally, the vegetally localized dorsal determinant cytoplasm (from the vegetal egg fragment) was injected into PBEs, together with the sub-surface sub-equatorial cytoplasm from GNEs. The resulting embryos developed into near normal embryos.

LOCALIZATION OF THE TIMING SYSTEM FOR THE ONSET OF GASTRULATION IN XENOPUS EMBRYOS

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Fertilized Xenopus eggs were deprived of the female nucleus and a small portion of the egg was protruded through a tiny hole of the fertilization membrane made at the sperm entry point. After having undergone 3-4 cycles of nuclear divisions there, the male nucleus moves into the main part of the egg within the fertilization membrane and induces cycles of cell divisions and development there. Gastrulation starts at a certain time after the first cell division in the main part, but not the extruded portion, of these eggs. The main part undergoes the same number of cycles of the portion while the protruded portion undergoes cycles of cell divisions. These results suggest that the onset of gastrulation is timed by interactive activities between the nucleus and cytoplasm.

ISOLATION AND ROLES IN CELL-CELL INTERACTION OF THE MEMBRANE MICRODOMAINS (LIPID RAFTS) OF DEVELOPING EMBRYOS OF MEDAKA

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Recently it has been shown that membrane microdomains (lipid rafts) are present in the plasma membrane preparation of various animal cells. The rafts are recognized as a hot spot for signal transduction and contain transducer molecules. The rafts are also characterized by enrichment of glycosphingolipids, which may give a molecular base for carbohydrate-mediated interactions. We have recently proposed a hypothesis that the rafts are involved in the carbohydrate-mediated cell adhesion and the subsequent signal transduction. To test this hypothesis in early embryogenesis, we have begun to study on the rafts in developing embryos (medaka fish. First, we demonstrated that the rafts from medaka embryos (early blastula to early gastrula) contain glycolipids and glycoproteins having the Le* antigen which is known to mediate compaction in mouse embryogenesis. Secondly, we showed that the rafts bind the neoglycolipid and the neoglycopeptide having Le* antigen. We also showed that the rafts bind each other in a Ca²⁺-dependent manner. Finally, we found that the rafts contain transducer proteins and a cell adhesion molecule.

sva53, A MATERNAL GENE REQUIRED FOR MEIOSIS

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In Drosophila, maternal factors sufficient for germline development are localized in germ plasm and are partitioned into the germline progenitors, or pole cells. It has been believed that these factors may ultimately trigger germline-specific events, such as meiosis. We have isolated an X-linked maternal mutation, sva53 that affects meiosis. Pole cells which were formed in the embryos derived from sva53 homozygous germline clone (sva53 pole cells) were able to develop into the ocytes, but they failed to execute meiosis. We also found that the germline-specific expression of vasa gene was severely affected in sva53 pole cells. These results indicate that the maternal factor encoded by sva53 gene is essential for meiosis, as well as for germline-specific gene expression. In order to identify sva53 gene, we mapped sva53 mutation to 200kb-region of 11C by using duplications and deficiencies.

THE EFFECT OF MITOCHONDRIA-TYPE TRANSLATIONAL INHIBITORS ON POLE CELL FORMATION IN DROSOPHILA

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Mitochondrial large ribosomal RNA is transported out of mitochondria to polar granules and functions to form pole cells. We have previously reported that mitochondrial small ribosomal RNA and mitochondrial ribosomal proteins (L7/L12, S12) also localized on the surface of the polar granules, indicating that there are mitochondria-type ribosomes on the surface of the polar granules. These findings lead us to speculate that pole-cell-forming factor(s) is produced by these mitochondria-type ribosomes. To address this issue, we examined the effect of mitochondria-type translational inhibitors (chloramphenicol, kasugamycin and puromycin) on pole cell formation. We found that treatments of cleavage embryos with the inhibitors significantly impaired their ability to form pole cells. In contrast, these inhibitors did not affect mitochondrial function detected by Rhodamine 123 at the concentration we used. These results support the idea that mRNA(s) encoding pole cell-forming factor (s) is translated by mitochondria-type ribosomes on the surface of the polar granules.