

MITOTIC ASTERS ARE NOT INDISPENSABLE FOR CYTOKINESIS IN THE EGG OF *XENOPUS LAEVIS*.

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We reported previously that *Xenopus* eggs stopped cleaving when cultured in a medium containing 150 mM or higher concentrations of urethane. We ascribed the cleavage arrest of the eggs to the inhibition of mitotic asters because they did not contain asters but the spindle. This time we have cultured eggs in a medium containing 75mM-120mM urethane and have found that although the eggs undergo cleavages rather normally, they do not contain any asters at any time of the cell cycle. This implies that mitotic asters are not indispensable for cytokinesis in *Xenopus* eggs.

ORIGIN OF PRECHORDAL PLATE ENDOMESODERM AND ITS ROLE ON THE ANTERO-POSTERIOR REGIONAL SPECIFICATION OF THE *CYNOPS* EMBRYO

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In *Cynops* gastrula, prospective materials of the pre-notochordal endomesoderm (PEM) and notochord (PN) were entirely located as a distinct domains on the egg surface. During gastrulation, these domains invaginated and formed archenteron roof (AR). PEM, especially the prechordal plate (PCP), was thought to act as a source of neural activation. However, it was not clear where is the PCP, and what is the function of the PCP. To clarify origin and function of the PCP, we previously determined the junction between PN and PEM of *Cynops* gastrulae. In this report, using *in situ* hybridization and RT-PCR, we examined *gsc* and *bra* expression of the PEM and PN. The results showed that *gsc* is expressed in the PEM before the onset of gastrulation. After invagination, *gsc* expression gradually restricted to the junctional region between PEM and PN. On the other hand, *bra* expression was not detected at the early gastrulae, but was induced in the PN at mid gastrula onward. These data indicated that PCP was established at the junction between PEM and PN during gastrulation, and that spatio-temporal relationship between PCP and PN determine the antero-posterior regional specification of the AR.

ISOLATION AND EXPRESSION ANALYSIS OF *TUBIFEX* HOMOLOGUES OF *DORSAL* AND *DPP*

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It has been widely known that dorsoventral construction is morphologically inverted between arthropods and chordates. Several genes involved in dorsoventral specification are conserved and expressed invertedly between these animals, providing molecular evidence for axis inversion. Oligochaete annelid *Tubifex* specifies the dorsoventral axis during embryogenesis and belongs to protostomes as arthropods do. Unlike arthropods, however, *Tubifex* belongs to Lophotrochozoa but not to Ecdysozoa. To gain an insight into the evolution of developmental programs, we have cloned homologues of *Drosophila* genes *dorsal* and *decapentaplegic (dpp)*, both of which are well known to contribute to establishment of the dorsoventral axis. Our expression analyses using whole-mount *in situ* hybridization have shown that *dorsal* homologues are expressed in micromeres specifically and that *dpp* homologues are expressed as early as one cell stage albeit later restriction to micromeres. These findings indicate that these homologues are unlikely to play a role in dorsoventral axis formation, but raise the possibility that these genes might have a role in cell-type specification during *Tubifex* embryogenesis.

ANALYSIS OF *HAIRY* HOMOLOGUE IN OLIGOCHAETE ANNALID *TUBIFEX*

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The segmentation gene *hairy* has been known to be conserved in a variety of animals. This gene is involved in segmentation processes in chick as well as in *Drosophila*, though segmentation patterns and mechanism are different between these animals. These facts suggest that *hairy* is conserved and plays a role in segmentation in another segmented animals Annelids. In order to examine this possibility, we cloned *hairy* homologue in *Tubifex* and analyzed the expression pattern by RT-PCR and whole-mount *in situ* hybridization. *Tubifex hairy* transcripts are detected by RT-PCR in embryos undergoing teloblastogenesis. Embryo either younger or older than this stage do not appear to express this gene at detectable level. Spatial distribution of *hairy* transcripts in stage12 embryos is confined to a set of cells including teloblasts. In light of these results, we have discussed the possibility that *hairy* might play a role in segmentation in *Tubifex*.

EXPRESSION ANALYSIS OF *VASA* IN EMBRYOS OF OLIGOCHAETE ANNELID *TUBIFEX*

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In the oligochaete annelid *Tubifex*, stem cells called teloblasts produce segmental founder cells called blast cells, which contribute to formation of segments in a lineage-specific manner. It is well known that segments individually have distinct identities. For example, segments 10 and 11 are characterized by the presence of primordial germ cells (PGCs) and thus called genital segments. During the course of searching for an appropriate marker for PGCs in *Tubifex*, we found that *vasa* gene is one of the promising candidates. In this study, we have characterized expression patterns of *vasa* in *Tubifex* embryos by whole-mount *in situ* hybridization with DIG-labeled RNA probe. During early cleavage stages, *vasa* transcripts are found to colocalize with pole plasms and to be segregated to teloblast precursor cells. In later embryos undergoing gastrulation, *vasa* transcripts become confined to single cells located in the mesodermal germ band. As development proceeds, *vasa*-expressing cells gradually decrease in number; finally they are detectable only in segments 10 and 11, but not in other segments. We have discussed the possibility that these *vasa*-expressing cells are PGCs in *Tubifex*.

CHANGES OF TROPOMYOSIN ISOFORMS DURING POSTEMBRYONIC DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

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Two tropomyosin (TM) genes, *TmI* and *TmII*, are present in *Drosophila melanogaster*. Five tropomyosin isoforms, Ifm-TmI, Scm-TmI, mTm-TmII, 33-TmII and 34-TmII, are expressed in the *Drosophila* muscle from these genes by the alternative splicing mechanism, and two or three TM isoforms are present in each of *Drosophila* muscles. It is also known that Scm-TmI and mTm-TmII are contained in the larval muscles. To investigate TM isoforms during postembryonic development of *Drosophila*, we compared TM extracts prepared from *Drosophila* pupae with the extract from larvae. *Drosophila* embryos raised at 20°C eclosed on the fifth day after pupation, so we prepared pupa extracts at every 24 hrs for 4 days. Scm-TmI and mTm-TmII could be recognized in the larval muscles, and two novel TM isoforms in addition to Scm-TmI and mTm-TmII were recognized in pupae. Relative amounts of the pupa-specific TM isoforms to those of Scm-TmI and mTm-TmII were gradually increased and then decreased during the pupal stage, and pupa-specific TM isoforms disappeared in the extract from 4 day-pupa in which indirect flight muscle-specific isoform, Ifm-TmI, was recognized.

A MAMMALIAN HOMOLOG OF THE *DROSOPHILA* DISCS LARGE TUMOR SUPPRESSOR PROTEIN IS INVOLVED IN THE DEVELOPMENT OF MOUSE KIDNEY

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DLG(Discs Large) is a *Drosophila* tumor suppressor protein and involved in cellular proliferation, polarization, and adhesion in invertebrate epithelia. In order to clarify the function of DLG homolog in vertebrate, we surveyed the expression pattern of a mammalian homolog of DLG protein in the normal mouse kidney in development and observed the DLG KO mouse kidney. In the normal mouse kidney, DLG expression was observed in basolateral region of the collecting duct and basal region of the proximal and distal tubules. In the fetal metanephros, DLG was expressed in the ureteric bud and developing urinary tubules, while the expression level was low in immature mesenchymal cells. Kidneys of DLG KO mice were smaller than those of wild type mice at birth. These results suggest that DLG is involved in the kidney development in mice, particularly in regulating the epithelial functions.

MECHANISMS OF ARCHENTERON FORMATION IN STARFISH EMBRYOGENESIS: DETERMINATION OF VEGETAL POLE CELLS BY WNT/ β -CATENIN PATHWAY.Kyoji Miyawaki¹, Lei Pan¹, Jie Chen¹, Shuang-yan Gao¹, Kazuhiro Shigemoto², Kyoko Saito¹, Takehiro Terashita¹, Katsumi Mominoki³, Shouichiro Saito¹, Naoto Kobayashi¹, Seiji Matsuda¹

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Previously we reported that the wnt/ β -catenin pathway seemed to induce the differentiation of presumptive archenteron cells during starfish embryogenesis (Miyawaki et al., 2003, Develop Growth & Differ 45: 121-8). To more directly confirm the determination of archenteron cells was induced by wnt/ β -catenin pathway, we