

Z305 The Regulation of early embryonic morphogenesis by the cytoplasmic factor(s) in mouse

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Early embryonic development is characterized by a variety of morphogenetic processes and the stage specific activation of the embryonic genome. It was suggested that the time schedule of these events is regulated by different programmes including cytoplasmic clock mechanisms, counting of DNA replication cycles and the quantitative nucleocytoplasmic(or DNA/cytoplasmic) ratio. This study was performed to investigate whether morphogenesis was regulated by the cytoplasmic content(nucleocytoplasmic ratio) or concentration of cytoplasmic factor-dependent manner in preimplantation mouse embryo. The fertilized mouse oocytes were removed a half(HP group) and a third cytoplasm(TP group) by micromanipulation, and their embryonic development and morphogenesis were compared with the sham-operated oocytes(SP group). A half fertilized oocytes with both pronuclei were reconstructed with the half enucleated cytoplasm of fertilized oocytes(P+P group), 2-cell(P+2 group) and 4-cell stage embryo (P+4 group) by electrofusion. The embryonic development and morphogenesis of these reconstructive embryos were examined during cultivation. Cell numbers were counted at the time of early signs of compaction and blastocoel formation. Also, in these groups, the expression of ZO-1 α isoform was analyzed by RT-PCR. Embryonic development and gene expression of ZO-1 α isoform of HP and TP groups were not different when compared with SP group. Meanwhile, the compaction and blastocoel formation was accelerated in time and cell stage. However, embryonic development, compaction in time and cell stage, and stage-specific gene expression of ZO-1 α isoform in P+2 and P+4 groups was accelerated than the that in P+P group. From these results, cytoplasmic content was not affected to this stage specific gene expression, but facilitated morphogenesis of embryo. Also, addition of cytoplasm from advanced stage embryo, although unchanged of nucleocytoplasmic ratio, accelerated gene expression and morphogenesis of embryo. Therefore, we may suggest that early embryonic morphogenesis is regulated by the cytoplasmic factor(s) and its concentration-dependent manner.

Z306 Histochemical Distribution and Some Properties of Embryonic Alkaline Phosphatase in the Earthworm, *Eisenia andrei*

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It is well known that in some animal species, embryonic or fetal form of intestinal alkaline phosphatase(ALP, orthophosphomonoester phosphohydrolase, EC 3.1.3.1) is different from the adult form. In the earthworm, recent studies have revealed that developing embryos of the earthworm have single form of ALP which displays a different electrophoretic mobility from that of the mature worm, suggesting that the embryonic form of intestinal ALP, if present, should be different from adult form. Therefore, in present study, the histochemical distribution of embryonic ALP and some properties such as molecular weight, isoelectric point and substrate specificity were investigated. Almost all of the ALP activity was expressed in the lumen of the archenterone and the lining of the archenterone-forming cells were stained very densely. The histochemical distribution of embryonic ALP clearly indicates that the embryonic ALP of the earthworm was secreted from archenterone-forming cells into the lumen of the archenterone. In addition, this result confirms that embryonic form of intestinal ALP is different from adult forms. The molecular weights, determined by gel filtration chromatography and SDS-PAGE, appeared to be approximately 330 and 152 kDa, respectively, indicating that embryonic ALP consists of two subunits. The isoelectric point was estimated at 4.0. Compared with adult forms, the substrate specificity of embryonic ALP was discussed.