ISOLATION OF cDNA CLONES FOR mRNAs TRANSCRIBED ZYGOTICALLY DURING CLEAVAGE STAGE IN ASCIDIAN EMBRYOS.

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The ascidian larva consists of relatively small number of tissues, and the cell lineage is well described during embryogenesis. The fate of most of the blastomeres become tissue-restricted by the 110-cell stage which is just before the onset of gastrulation. During ascidian embryogenesis, primary muscle, epidermis and endoderm autonomously differentiate, and the processes are mediated by egg cytoplasmic determinants.

We are trying to isolate cDNAs for genes of which expression is directly triggered by maternal determinants during early embryogenesis of the ascidian, *Halocynthia roretzi*. We constructed cDNA library of 110-cell embryos. By differential screening using maternal mRNAs and mRNAs from 110-cell embryos, we isolated several cDNA clones for genes of which transcription starts during cleavage stage.

Hox-type genes in *Tetrahymena thermopila*

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Hox genes play a central role in axial patterning in the animal kingdom and the homeodomain is highly conserved throughout evolution. We report here the first Hox-type genes from the kingdom Protista. The 116 bp PCR fragments from the ciliated protozoan *Tetrahymena* were amplified with specific primers to the homeodomain, and were sequenced. So far we identified at least 12 kinds of genes, all of which are highly homologous to the amino acid sequences of the Hox genes. This result obliges us to reconsider the origin and evolution of Hox gene.

SELECTIVE AND LONG-TERM CULTURE OF BLACK SILKIE CHICKEN MELANOCYTES SUPPLEMENTED WITH ENDOTHELINS

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We developed a selective and long-term culture method of melanocytes of Black silkie chicken (*Gallus gallus domesticus*). Isolated neural crest cells from trunk region of 3-day embryos were separated to single cell and frozen by a routine method (10% fetal calf serum and 10% DMSO contained F-12 medium) at -80°C until using. After thawing the cells, 1-4x10^5 neural crest cells were plated in 24 well-plate with F-12 medium containing 10% fetal calf serum for one day and the medium was exchanged to F-12 medium containing 10% calf serum and one of 0-50ng/ml endothelin-1, -2, -3 or big endothelin-39 (precursor of endothelin-1). After 2 days, melanoblasts appeared and formed network-like colony. Light melanization started in these cells on 4 day-culture and then cells continued to proliferate showing clear melanization on 6-7 day-culture. A vast number of melanocyte colonies appeared in these media containing each endothelin. After one week, almost other cell types could not survive and disappeared. Also no melanoblasts and melanocytes were observed in the medium without any endothelin. In addition, melanocytes extremely proliferated and formed solid colonies overlapping each other in the cases of endothelin 1, 2, and 3. Most effective concentration of these endothelins was different. These melanocytes could be sub-cultured and maintained for 125 day. These effects of endothelins were inhibited by endothelin receptor antagonists.

CYTOPLASMIC MITOCHONDRIAL rRNA IN TETRAHYMENA CONJUGANTS

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In some metazoa, mitochondrial(mt) rRNAs located in cytoplasm are associated with germ-line differentiation. In this report, we used unicellar protozoan *Tetrahymena thermophila*, which has two different nuclei, germ-line micronucleus and the somatic macronucleus, and investigated the presence mtrRNAs in cytoplasm during the stage of nuclear differentiation as in the metazoan germ-line determination. FISH revealed that cytoplasmic mtrRNA was found in cytoplasm of conjugants, but COI RNA (cyyochrome oxidase subunit I) was not. This suggests that the possible role of cytoplasmic mtrRNA in germ-line determination is common in protozoa and metazoa.

ISOLATION OF TWO FACTORS THAT STIMULATE PROLIFERATION AND DIFFERENTIATION OF MELANOCYTES IN BUFFALO RAT LIVER CELL-CONDITIONED MEDIUM

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Buffalo rat liver cell-conditioned medium (BRL-CM) has been suggested to contain effective factor(s) on stable proliferation, differentiation and survival of cultured melanocytes of Black silkie chicken (*Gallus gallus domesticus*). However, the factor(s) and the function(s) in BRL-CM have been remained unknown. Previously, we have reported that the <10kDa fraction (FA) of BRL-CM was considerably effective for proliferation and differentiation of the melanocytes and >10kDa fraction worked to help their adhesion and survival. In this report, we prepared FA from two liter of serum-free BRL-CM and analyzed the component. For each trial of electrophoresis, 200ml of FA was concentrated by lyophilization and gel filtration. The concentrated sample was developed by Tricine-PAGE and two faint bands were detected. The electrophoretical mobilities of those two components were identical to endothelin (0.2kDa) and big endothelin (0.4kDa), respectively that have been reported to be effective factors for melanocyte differentiation. To examine whether BRL cells synthesize mRNAs encoding endothelins (ETs), amplification of cDNA using reverse transcription-polymelase chain reaction (RT-PCR) was performed. The experiments clearly showed expression of ET-1 and -2 mRNA. These results indicated that BRL-CM contained ET-1, ET-2 and their precursors as prime candidates of effective factors on the melanocytes.

ANALYSIS OF THE "FISSION ZONE" IN A FRAGMENTING EARTHWORM, *ENCHYTRAEUS JAPONENSIS*

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Nicotinic acetylcholine receptors (nAchR) are known to exist in post-synaptic region of mammalian neuromuscular junctions. By using fluorescent-labeled α-bungarotoxin, antagonist of nAchR, we showed that there were two stripes of neuromuscular junctions in the circular body wall muscle in each segment of *Enchytraeus japonensis*. One of them (A) is close to the boundary of segments, and the other (B) is in the middle of the segments. These worms undergo asexual reproduction by fragmentation and regeneration. The "fission zone" in each segment where they cut themselves spontaneously (automy) was close to stripes B. The fragments obtained by dissection were also examined. Since most of the dissected fragments had their ends at the same position as spontaneous ones, it is suggested that worms adjusted their ends by re-cutting themselves at the correct position, which is important for correct regeneration.