ON THE EGG CYTOPLASMIC FACTORS RESPONSIBLE FOR THE MUSCLE CELL DIFFERENTIATION OF ASCIDIAN CIONA SAVIGNYI: EGG FRAGMENTS PRODUCED BY CENTRIFUGAL FORCE.

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Dechorionated unfertilized eggs of C. savignyi were incubated for 10 min in the sea water that contains 10 ug/ml cytochalasin B and then centrifuged for 20 min at 1500xg. These treatments separated eggs into several types of fragments, large red fragments (-60% egg volume), small gray ones (-10% egg volume), and small transparent ones. Red fragments contained egg chromosomes. If the fragments were inseminated, they developed into the so-called permanent blastulae, in which epidermal differentiation was evident but muscle cell differentiation was not detected. Gray and transparent fragments never cleaved even if they were inseminated. However, if red fragments were inseminated after the fusion with gray fragments they developed into the embryos in which muscle cell differentiation was conspicuous. The embryos derived from these fused fragments sometimes appeared as normal tadpole larvae. These results suggest that muscle determinant in the eggs was mainly partitioned into gray fragments and not into red fragments.

LOCALIZATION PATTERNS OF CYTOSKELETAL COMPONENTS AND MYOPLASMIN-C1 DURING EARLY DEVELOPMENT OF THE ASCIDIAN EMBRYO.

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The myoplasm of the ascidian egg is believed to contain muscle determinants, and is distributed into muscle precursor blastomeres through the ooplasmic segregation. The segregation consists of two phases, each mediated by different systems, the first by microfilaments and the second by microtubles (Sawada & Schatten, 1989).

Myoplasmin-C1 is one of the myoplasmic components which are thought to play an important role in the muscle cell differentiation. In order to examine the interaction of myoplasmin-C1 and other cytoskeletal proteins, we immunologically detected tubulin, actin and p58 (resembles to the porcine neurofilament 160; Swalla *et al.*, 1991) with myoplasmin-C1 on the same sections. During the first phase of the segregation, the distribution of myoplasmin-C1 was closely related to that of the actin rather than the tubulin. On the second phase, myoplasmin-C1 migrated posteriorly together with the sperm aster. As far as we examined, myoplasmin-C1 and p58 segregated to the same area of the egg during ooplasmic segregation. These results implicate the image of the myoplasm which is composed of the intricate complex of the cytoskeletal components; these components dynamically move, rise and fall, associate and disassociate with each other.

AN ATTEMPT TO ISOLATE cDNA CLONES SPECIFIC FOR B4.1 BLASTOMERES OF THE ASCIDIAN EMBRYO

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During ascidian embryo genesis, differentiation of primary lineage muscle cells, which are derived from B4.1 blastomeres of the 8-cell embryo, seems to be controlled by factors localized in the egg cytoplasm. To analyze mRNAs specific for B4.1 blastomeres, we collected B4.1 blastomeres and blastomeres of the animal hemisphere (a4.2+b4.2) from 8-cell stage embryos of the ascidian *Halocynthia roretzi*, and we made each cDNA library. Then we made a subtracted cDNA library, and by differential screening we isolated some cDNA clones which are present in the B4.1-cDNA library but not in the animal hemisphere-cDNA library. Now, we are continuing farther analysis of these clones.

ANALYSIS OF THE EXPRESSION OF THE MYOGENIC bHLH PROTEIN GENE IN THE ASCIDIAN *Halocynthia roretzi*.

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To address the question whether MyoD1-like factors play pivotal roles in muscle differentiation in *H.roretzi* embryo, we isolated by PCR method two cDNA clones and one genomic clone encoding a protein which belongs to the myogenic bHLH protein family. The difference between cDNA clones occur in the poly(A) sites. The gene consists of four exons. In the 5' upstream region four CANNTG motifs (or E-boxes) are clusterd around -550. They may be target sites of its autoregulation. This protein consists of 435 amino acid residues and its bHLH domain is well conserved.

RT-PCR analysis has demonstrated that the expression of this gene begins at 64-cell stage and continues until swimming larva stage. In adult, body wall muscle and heart express this gene, while liver, gill and intestine don't, although none of the members of the myogenic bHLH protein gene was expressed in the heart of mammals. These results suggest that a MyoD1-like factor may be involved in ascidian muscle differentiation.