CLONING OF cDNAS WHICH ARE EXPRESSED DIFFERENTLY IN AMPUTATED MUSCLE DURING URODELE LIMB REGENERATION.

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To study the molecular mechanism of dedifferentiation of myotyubes during blastema formation, we attempted the cloning of cDNAs which are expressed differently in the amputated muscle during limb regeneration. Forelimbs of Japanese newt, *Cynops pyrrhogaster*, were amputated and muscles near the amputated site were collected at various periods. Differential display method was applied to analyze profile of the gene expression of the amputated muscle. The cDNAs which showed different expression pattern were amplified. Then the cDNA fragments were used as probes for library screening. Several cDNAs were cloned from libraries of normal limbs, 2 days after amputation and early blastema. These cDNAs showed a unique expression pattern during regeneration. For example, one gene was not expressed in normal muscle but was up-regulated at 1 day post amputation. The expression of another gene was down-regulated during regeneration. These genes will be useful molecular markers for studying the limb regeneration.

SPECIFICATION OF PIGMENT CELL LINEAGE AND CELL INTERACTIONS IN EMBRYOS OF THE SEA URCHIN, *HEMICENTROTUS PULCHERRIMUS*

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Founder blastomeres of pigment cell lineage in sea urchin embryos was suggested to be established through cell interactions during mid-to-late blastula stage. To reveal the mechanism involved in the specification of pigment cell lineage, embryos were treated with LiCl, and the number of differentiated pigment cells were examined. Treatment with LiCl before hatching drastically increased the number of pigment cells, while the treatment after haching had no effect. The sizes of differentiated pigment cells in the treated larvae were almost same as those in control larvae, suggesting that the increase in the number of pigment cells is due to the increase in the number of founder blastomeres. This was ascertained by Aphidicolin treatement from the stage when the founder blastomeres seemed to have been determined. Interestingly, the effect of LiCl treatment was weakend by Ca^{2+}-free ASW treatment. In addition, it was elucidated that the amounts of negative cell surface charges in LiCl-treated embryos were smaller than in control embryos, implying the increase in the adhesiveness among blastomeres in LiCl-treated embryos and the involvement of cell interactions in the specification process.

SCLEROTOMAL DISTRIBUTION OF CHONDROITIN SULFATE IS ESSENTIAL FOR THE RESTRICTION OF MIGRATORY REGIONS OF NEURAL CREST CELLS

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We have previously reported that migration patterns of mouse trunk neural crest cells were closely correlated with distribution patterns of sclerotomal chondroitin sulfate proteoglycans (CS-PG) and both patterns disappeared in embryos treated with the inhibitors of sulfated proteoglycan biosynthesis. We have achieved the notion that mouse trunk neural crest migration patterns are generated by systematic changes of the sclerotomal distribution of CS-PG. To confirm this notion, we examined the distribution of other sulfated proteoglycans and analyzed the organization of crest-derived dorsal root ganglia (DRG) in treated embryos. Distribution patterns of sulfated proteoglycans except for CS-PG were irrelevant with the migration patterns. Furthermore, abnormal DRG segmentation as adjacent DRG conbinated was found in treated embryos. We conclude that systematic changes of the sclerotomal distribution of CS-PG are a key requisite for the restriction of migratory regions of trunk neural crest cells and this restriction affects segmented patterns of DRG in mouse embryos.

INHIBITION OF THE HEAD STRUCTURE DEVELOPMNT BY EXPOSURE TO RETINOIC ACID AT GASTRULATION STAGES DURING EMBRYOGENESIS OF THE ICE GOBY (SHIRO-UO), *Leucopsarion petersii.*

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The most advanced and largest group of the teleosts is Perciformes which include very diverse fish, but their embryogenesis has been rarely examined. The ice goby (shiro-uo in Japanese), Leucopsarion petersii , belongs to this Perciformes group. It is one member of gobies and inhabiting along the coast, and they enter the river for spawning in coastal streams in spring. We previously reported its transparent eggs and embryos and main stages of embryonic development to illustrate its usefulness as experimental material (*Zool. Sci.* 14, 443-448, 1997). Recent studies have reported that exposure of vertebrate embryos to retinoic acid at early embryonic stages inhibits development of the anterior axis structures. We examined effects of retinoic acid on embryogenesis of ice goby. Early embryos obtained by artificial fertilization were allowed to develop to the early gastrula stage. They were exposed to 10^{-5} to 10^{-7} M retinoic acid for 60 min during early, middle or late stages of epiboly. Examination of developed embryos after one week showed various degrees of abnormality. The lowest concentration (10^{-7} M) caused formation of the smaller head structures, while higher concentrations (10^{-5} - 10^{-6} M) caused more pronounced anterior inhibition such as complete deletion of the head region. In addition, elongation of the tail was also suppressed. Such affected embryos, however, showed high rates of survival up to the hatching stage, and may provide adequate material to analyze how retinoic acid causes abnormality of axial structure in vertebrates.

ESTABLISHMENT OF PIGMENT CELL LINEAGE IN EMBRYOS OF THE SEA URCHIN, *HEMICENTROTUS PULCHERRIMUS.*

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To know the timing of the specification of pigment cell lineage, sea urchin embryos were treated with aphidicolin from various stages of development, expecting that some sign of pigment cell differentiation would be observed, in spite of the inhihibition of DNA synthesis and cell divisions. Surprisingly, the cells containing pigment granules appeared in the embryos treated even from pre-hatching stages. The number of such cells in the embryos treated from 10 hr of development ranged from 10 to 16 in most cases. This number is about one forth of the number of pigment cells observed in control pluteus larvae. On the contrary, the averaged volume of those cells was four times larger than that of control larvae. These findings indicate that the founder blastomeres of pigment cells are determined by 10 hr of development, and that they divide two times before they differentiate into pigment cells. Aphidicolin treatment also clarified the timing of divisions of pigment founder blastomeres; they divide once before the onset of gastrulation (between 10-16 hr), and once more during gastrulation (between 16-24 hr). After their invasion into the ectodermal layer, further division seemes to occur in a small portion of pigment precursor cells.

REMOVAL OF THE UPPER TIER BLASTOMERES AT 8-CELL STAGE CAUSES DEFECTIVE EMBRYONIC AXIS FORMATION IN EMBRYOS OF THE ICE GOBY (SHIRO-UO), *Leucopsarion petersii.*

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The ice goby, Leucopsarion petersii, belongs to the most advanced and largest group of the teleosts, Perciformes group. We previously reported its embryonic development to illustrate the usefulness as experimental material (Zool. Sci. 14, 443-448, 1997). During the initial steps of the embryogenesis, the first two cleavages produce 4 blastomeres on the yolk surface. The third cleavage, however, is horizontal in all blastomeres, and it produces two tiers of blastomeres. Such cleavage pattern is similar to that in the echinoderm and amphibian embryos, but it is different from other fish embryos, in which the early cleavages produce blastomeres in one layer on the yolk surface up to 16 or 32-cell stages. Also, the chorion of the ice goby eggs can be easily removed with forceps, thus allowing manipulation of early embryos. We took advantage of such unique cleavage pattern and examined effects of the removal of upper tier blastomeres at 8- or 16-cell stages on the following development. We found that mechanical removal of 3 out of 4 upper blastomeres at 8-cell stage allowed almost normal development, but removal of all the upper blastomeres drastically inhibited embryogenesis. The cleavage continued almost normally, and epiboly started and proceeded so that the blastoderm layer almost engulfed the yolk mass. From the late epiboly stage, however, formation of the embryonic shield was defective and no prominent axial structures developed. Such results may suggest that the animal pole blastomeres in teleost embryos have an important role in development of the embryonic axis, in a similar manner as echinoderm or amphibian embryos.