EFFECTS OF THE INHIBITORS OF APOPTOSIS ON MORPHOGENESIS OF SEA URCHIN EMBRYO

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In the embryo of the sea urchin *Hemicentrotus pulcherrimus* , apoptosis-like cells were found at the blastula stage and gastrula stage by the Tunel method. About 20 apoptosis-like cells localized uniformly at the blastra stage. At the gastrula stage, about 10 apoptosis-like cells were found near the blastopore and apical taft regions of the embryo. On the other hand, 5-bromodeoxyuridine (BrdU) and Zn are known inhibitors of apoptosis. We investigated the effects of these reagents on the development of sea urchin embryo to clarify the cellular mechanism of morphogenesis. Archenteron formation was inhibited by the treatment of BrdU and Zn. After prism stage, the diameter of the embryo was shorter than that of the normal embryo and morphology of blastopore and gut were abnormal by the treatment of these reagents. Detection of apoptosis-like cells was mainly performed using Cardio TACS *in situ* Apoptosis Detection kit (Trevigen). The number of apoptosis-like cells of BrdU or Zn treated embryo was small compared with the control ones in the average level. It seems that these results indicate apoptosis occurs just after hatch out and may contribute morphogenesis especially to archenteron and spicule formation.

DOES THE REJUVENATION OF THE MATERNAL MACRONUCLEUS OCCUR AFTER CONJUGATION OF *Paramecium caudatum*?

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Each cell has a macronucleus and in *Paramecium caudatum*. When conjugation was induced between cells, the new macronuclei (anlagen) developed and the prezygotic macronucleus fragmented into about 50 pieces. Therefore, the exconjugant cell had two kinds of macronuclei, that is, the fragmented prezygotic macronucleus and newly developed macronuclear anlagen. The macronuclear fragments remained at least for 7 cell cycles after conjugation but degenerated later on. The exconjugant cells which had macronuclear anlagen did not show mating reactivity (immaturity) for about 50 cell cycles after conjugation. When macronuclear anlagen are removed at 3th or 4th cell cycles, the prezygotic macronuclei regenerated (MR). These MRed cells showed mating reactivity (no immaturity). When the macronuclear anlagen were removed at 5th or 6th cell cycles after conjugation, however, MRed cells did not show mating reactivity. The result suggests a possibility that the macronuclear fragments rejuvenated in terms of mating reactivity, though it was not ruled out that the protein immaturine produced by the macronuclear anlagen remained in the cytoplasm or macronuclei of the MRed cell for a long time.

NEUROTRANSMITTERS AND ADRENERGIC RECEPTOR PLAY A ROLE IN THE METAMORPHOSIS OF ASCIDIAN LARVAE

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Tadpole larvae of ascidians become settled on the substratum at the beginning of metamorphosis, then, the tail is resorbed within few hours. The tail resorption is induced by many external and internal factors, and the nervous system is considered to play a role in initiation of metamorphosis, although mechanisms underlying the process have been scarcely known.

Treatment of swimming larvae of the ascidian *Ciona savignyi* with noradrenalin and adrenalin strongly promoted tail resorption of the larvae. Isoproterenol, beta-adrenergic receptor agonist, was more effective on induction of tail resorption than noradrenalin and adrenalin. Propranolol, an antagonist of beta-adrenergic receptor, and metoprolol, the $beta_1$-type receptor antagonist, inhibited the effect of noradrenalin. On the other hand, phentolamine, the alpha-type receptor antagonist, and butoxamine, the $beta_2$-type receptor antagonist, had no inhibitory effect. Receptor of the neurotransmitter stained with anti $beta_1$-adrenergic receptor antibodies was localized on the adhesive papillae and neuron-like filaments that located along the brain vesicle to the end of the tail. The receptor protein was initially detected in the newly hatched larvae, and its expression increased with time proceeds in the competent larvae by the early postlarval stage that initiates metamorphosis.

These results suggest that interaction between neurotransmitters, noradrenalin and adrenalin, and the $beta_1$-adrenergic receptor mediates the process of metamorphosis in the ascidian.

THE CHANGE IN SHELL MEMBRANES DURING THE DEVELOPMENT OF QUAIL EMBRYOS.

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The shell membrane of an avian egg acts as a bag enclosing the albumen and water. At its interface with the albumen, the shell membrane is demarkated by a smooth layer of homogeneous dense material called the limiting membrane. The present study aimed to show the change in the limiting membrane during the development of quail embryos which are grown with both natural and artificial rotation. Sixty-three percent of the embryos were born when the eggs were incubated at 39 °C and 60% humidity with automatic rotation around the axis of blunt-sharp ends and with their equatorial side down, whereas the birth rate decreased to 24% when the eggs were incubated without rotation. The width of the limiting membrane at the equatorial region of rotated eggs gradually decreased from 77 nm on days 0-3 of incubation to 35 nm on day 10 and thereafter. Conversely, water permeability, measured by evaporation through the limiting membrane, increased from 4-5 $nl/mm^2 \cdot min$ on days 0-3 to 10 $nl/mm^2 \cdot min$ on day 10 and thereafter. In stationary eggs, the decrease in the width of the limiting membrane at the lower side of egg delayed till day 8 of incubation. The water permeability of the membrane in this case was 60% of that of the membrane at the upper side of eggs on day 8 of incubation. It was also shown that the albumen hinders water permeation through the membrane. These results show that (1) the limiting membrane is made thin during the development at whole surface with rotation, possibly through digestion with still unknown agents, and (2) this thinning accelerates, besides the respiration, the water permeation through it, enabling the movement of calcium from eggshell to embryo.

DISTRIBUTION OF MYOFIBROBLASTS AND HEPATIC STELLATE CELLS IN THE LIBER IN TADPOLES (*Rhacophorus owstoni*)

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The cytoskeletal components of the hepatic stellate cells (HSCs) and myo- fibroblasts were identified by immunohistochemistry for glial fibrillary acidic protein (GFAP) and alfa-smooth muscle actin (SM-actin) . At stage 25, bile canaliculi was slightly observed in the between hepatocytes in immature hepatic lobules. At stage 33, a high number of myofibroblasts were observed in the perisinusoidal spaces, but these number decreased from stage 34 onward. At stage 34, the sinusoidal structure was completely formed in hepatic lobules. At stage 40 about the same density of GFAP containing HSCs was found in tadpole livers as was found in adult frog livers. These findings suggest that SM-actin-positive myofibroblasts produce extracellular matrix proteins in sinusoidal spaces in developmental livers and these myofibroblasts are transformed into GFAP-positive HSCs.

HISTOCHEMICAL CHANGE WITH METAMORPHOSIS IN THE SMOOTH MUSCLES OF THE ALIMENTARY CANAL IN TADPOLES (*Rhacophorus owstoni*)

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We used immunohistochemistry, with alfa-smooth muscle actin antibody, to study the musculature of the alimentary tract in tadpoles of the treefrog, *Rhacophorus owstoni*. Before metamorphosis the smooth muscle fibers, which cause peristaltic movements in the gut, stain markedly brown with this procedure and are arranged in two layers that are longitudinal and circular in orientation. We found no distinct difference in staining among the stomach, small or large intestine before metamorphosis. However by the end of the metamorphosis the same procedure yielded weaker brown staining of the smooth muscle. There was concomitant increase in the number of muscle fibers in both layers during metamorphosis. The histochemistry suggests structural change in the cytology of the smooth muscle fibers of the alimentary tract, as well as proliferation of fibers, with the metamorphosis.