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LEFT-RIGHT ASYMMETRY IN DISTRIBUTION OF SMALL MICROMERE DERIVATIVES BETWEEN COELOMIC POUCHES OF SEA URCHIN EMBRYOS.

H. Nishigori and S. Amemiya Dept. of Biol. Sci., Grad. Sch. of Sci., Univ. of Tokyo, Hongo, Tokyo 113

Sea urchin eggs divide equally in first three cleavages to form eight blastomeres of equal size. Vegetal blastomeres devide unequally at fourth cleavage to form four micomeres in vegetal pole. The micromeres divide unequally again at the next cleavage to form four small micromeres in the vegetal pole. The small micromeres are located on the tip of invaginating archenteron and incorporated into a pair of coelomic pouches formed left and right sides of the archenteron tip. According to the mode of distribution of the small micromere derivatives into the coelomic pouches, the sea urchin species were classified into two different groups. In the first group which is refered to as "5-3 type", the small micromere derivatives distributed into the left and right coelomic pouches in the ratio about 5 : 3. In the second group refered to as "8-0 type", they did in the ratio 8 : 0. In the present study, the mode of distribution of the small micromere derivatives into the coelomic pouches in the hybrid embryos between the species of "5-3 type" and "8-0 type" was examined. The results indicated that the distribution of the small micromere derivatives into the coelomic pouches is under the control of nuclear activity after fertilization.

THE EARLY SEXUAL DIFFERENTIATION OF GERM CELLS IN THE MEDAKA, *ORYZIAS LATIPES*

N. Saito and N. Shibata, Dept. of Biol., Fac. of Sci., Shinshu Univ., Matsumoto.

In medaka, there have been several reports that the numbers of germ cells are different between male and female at hatch, considered as the primary morphological indication of sex differentiation. In the present study, germ cell kinetics in embryos were investigated using inbred strain HdrR to reveal earlier sex-linked differences.

After st. 38 (2 days before hatch), labeling index of germ cells in female after 24 hr incorporation of BrdU became twice as high as that in male. Furthermore, at st. 36, number of germ cells was slightly different between male and female. These results suggest that sexual differentiation of germ cells already begins around the time of gonadal anlage formation. In addition, no mitotic germ cells were observed in all males during hatch 1 day to 4 day whereas oocytes at zygotene stage appeared in all females at hatch 1 day. Thus HdrR strain is the good material to investigate sexual differentiation process without individual differences.

NECESSITY OF CARTILAGE TISSUE FOR PROGRAMMED CELL DEATH IN DEVELOPING CHICK LEG BUDS.

Minoru OMI, Mika SATO-MAEDA, Yoriko SHIRAKASHI and Hiroyuki IDE Biol. Inst., Tohoku Univ., Sendai.

A massive cell death occurs in the interdigital region of chick leg buds. Previously, we reported that inhibition of interdigital cell death occurred when interdigital zone was separated from neighboring digit cartilage. Here, we examined cell fate in the prospective interdigital zone and digit region using DiI. The labeled cells remained as a narrow band in the digit region, but expanded in the interdigital zone and moved to the digit region at the proximal level. In monolayer culture of the leg bud cells, the cells migrated into the high-cell density area, and many TUNEL-positive cell death occurred between cartilage nodules. These results suggest that developing cartilage tissue tends to pull neighboring cells into the chondrogenic region, and decreasing of cell density between the chondrogenic region may induce the cell death.

GONADAL DEVELOPMENT IN AN ASEXUALLY FRAGMENTING EARTHWORM, *ENCHYTRAEUS JAPONENSIS*

J. Kutsuna[1], F. Kobari[1], M. Myohara[2] and S. Tochinai[1]. [1]Div. Biol. Sci., Grad. Sch. Sci., Hokkaido Univ., Sapporo and [2]Natl. Inst. Sericult. and Entomol. Sci., Tsukuba.

An enchytraeid earthworm, *Encytraeus japonensis*, usually propagates asexually by autotomizing a fully grown body. However, it was demonstrated that under special conditions they display a sexual reproduction (Myohara and Tochinai, 1997). This phenomenon gives us an opportunity to solve a very interesting question as to the origin of germ cells in a regenerating asexual fragment.

Careful histological examinations revealed that in the newly regenerated anterior region, testis formation was always observed with actively dividing spermatogonia. However, it will not develop further under normal conditions. On the other hand, if these "asexual" worms were put in sexual reproduction promoting conditions, spermatogenesis resumed promptly. Within a few days, mature sperms were observed in well-developed seminal vesicles, along with the ovarian development associated with s set of accessory organs. Eggs were laid in a transparent cocoon within 2 weeks. Although in the previous experiments we were unable to find any gonadal traces in asexually reproducing worms, it was demonstrated from the present investigation that the gonadal development always occurred in the head region independently from the asexual fragmentation. It suggests in turn that the rudimental gonads must be preserved in the head region of asexually reproducing worms in a cryptic form.

CHANGES OF CHONDROGENIC PATTERNS AT THE BOUNDARY BETWEEN MESENCHYMAL CELLS AT DIFFERENT DEVELOPMENTAL STAGES OF CHICK LIMB BUDS.

Mika Sato-Maeda, and Hiroyuki Ide.
Biol. Inst., Tohoku University, Sendai, Japan.

Mesenchymal cells of the progress zone (PZ-cells) in chick limb buds at different developmental stages segregate one from the other in mixed cell cultures, which showed they have different cell affinity. To know possible roles of such differences on chondrogenic patterns, we juxtaposed two heterotypic leg PZ-cells populations *in vitro* and *in vivo*. In the adjoining monolayer cell cultures,two new chondrogenic patterns along the boundary were produced by 4-day: aggregates of chondrocytes formed by st.20/21 PZ-cells and a chondrocyte-free band formed by those at st.25 /26. While, in the recombinant limbs, discontinuity of cartilage pattern along the proximodistal axis was observed around the boundary between the two heterotypic re-aggregated cells. The results as well as those of in situ hybridization suggest that the different cell affinity might have a role in the segmentation of cartilage patterns at a point along the proximal-distal axis.

THYROID HORMONE-INDEPENDENT TRANSITION OF THE GLOBIN GENE EXPRESSION IN *HYNOBIUS RETARDATUS*.

M. Yamaguchi[1], H. Takahashi[2] and M. Wakahara[1]
[1]Div. Biol. Sci., Grad. Sch. Sci., Hokkaido Univ., Sapporo 060-0810, [2]Dept. Zool., Grad. Sch. Sci., Kyoto Univ., Kyoto 606-8502

In order to know the molecular mechanism of globin transition in *H. retardatus*, larval and adult globin cDNAs were cloned, and their expression was analyzed in normally developing animals, metamorphosis-arrested larvae, and phenylhydrazine-treated larvae. Adult globin genes were expressed in peripheral erythroid cells at 20 days after hatching, much earlier than the initiation of their morphological metamorphosis. Furthermore, the transition of globin gene expression in metamorphosis-arrested larvae was almost identical to that in the normal controls, suggesting that the transition occurs independently of thyroid hormones. It was also demonstrated that single erythroid cells expressed concurrently larval and adult globin genes, showing the transition occurred within single erythroid cell population.

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