THE SPATIAL RELATIONSHIP BETWEEN THE EGG-CUMULUS COMPLEX AND THE SITE OF FERTILIZATION IN THE OVIDUCT OF VARIOUS BATS.

T. Mōri, H. Iida. Zool. Lab. Fac. Agr., Kyushu Univ., Fukuoka

Reproductive patterns in temperate-zone hibernating bats can be divided into following two categories, the 'prolonged Graafian follicle storage' and 'delyed implantation' types. In the former (*Rhinolophus ferrumequinum*, *Myotis formosus*, *Pipistrellus abramus*, *P. endoi* etc.), the cumulus oophorus is very large, except for *R. ferrumequinum*, and in the latter (*Miniopterus schreibersii*) the cumulus oophorus is small. Poly-saccharides such as glycogen in the cumulus cells have been considered as important nutrient sources for such prolonged survival of the Graafian follicle. On the other hand, from the view point of fertilization, the behaviour of the cummulus-egg complex and its interaction with spermatozoa were reexamined. The cumulus oophorus invested the egg at ovulation, during fertilization. In the bats in which the cumulus is very large, the cumulus did not undergo pre-ovulatory mucification and expansion, and in two species with small cumulus, the mucification and expansion prominently occurred. Thus, the egg-related components tend to match the dimensions of the fertilization site.

OVULATION AND FERTILIZATION IN THE HOUSE MUSK SHREW, *SUNCUS MURINUS*.

T. Kaneko[1], H. Iida[2], J. M. Bedford[3] and T. Mōri[4]. [1,2,4]Zool. Lab., Fac. Agr., Kyusyu Univ., Fukuoka and [3]Dept. Obstet. Gyne., Cell Biol. Anat., Cornell Univ. Med., New York, USA.

In the musk shrew, *Suncus murinus*, the behaviour of the cumulus-egg complex and its interaction with spermatozoa were unusual in several respects. The cumulus oophorus was ovulated about 15.5h after mating or treatment with hCG or PMSG as a hyaluronidase-insensitive matrix-free ball of cells which remained for relatively long periods of about 14h around fertilized, and for about 24h around unfertilized eggs. As a probable function of the small number of up to about 10 or 20 spermatozoa that generally reached the oviduct ampulla from isthmic crypts, there was often a delay of up to 10h after ovulation before most eggs were penetrated. Soon after ovulation, the corona radiata retreated progressively from the zona pellucida, creating a closed perizonal space within the cumulus oophorus. All spermatozoa seen moving within the perizonal space or adhering to the zona of unfertilized eggs had shed the giant acrosome. The cumulus in *Suncus* may therefore function not only to sequester spermatozoa, but also as an essential mediator of fertilization - probably by inducing the acrosome reaction.

MECHANISM OF CALCIUM OSCILLATIONS IN ASCIDIAN EGGS

Manabu Yoshida
Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute, Wako. *Present Address:* Misaki Marine Biological Station, School of Science, University of Tokyo, Miura.

Changes in $[Ca^{2+}]_i$ are an essential factor regulating egg activation. In matured oocytes of the ascidian egg, two series of $[Ca^{2+}]_i$ transients have been observed after fertilization: transient Ca^{2+} waves just after fertilization (Series I) and $[Ca^{2+}]_i$ oscillation between first and second polar body extrusion (Series II). In this study, I investigated the mechanisms involved in elevation of $[Ca^{2+}]_i$ in the egg of the ascidian *Ciona savignyi*. Microinjection of lithium, which is a classical inhibitor of the phosphoinositide pathway before fertilization caused suppression of Series II of $[Ca^{2+}]_i$ transients, thus it is probable that $[Ca^{2+}]_i$ transients in the egg is actually induced by IP_3 production. Production of IP_3 is catalyzed by phospholipase C (PLC), and activity of PLC-β which is one of PLC subtypes is controlled by G-protein. When the egg was incubated with U73122, which is an antagonist of PLC-β, Series II of $[Ca^{2+}]_i$ transients were inhibited . The inhibition of Series II was also observed when GDP-β-S, which inhibits G-protein activation by GTP were injected into the egg before fertilization. Furthermore, microinjection of GTP-γ-S, which was non-hydrolyzable GTP analog induced $[Ca^{2+}]_i$ uptake in unfertilized eggs. These results suggest that $[Ca^{2+}]_i$ transients in the ascidian egg are caused by PLC-β-mediated IP_3 production.

The timing of dorsal axis induction by transplanted D1 cells in *Xenopus* embryo

T.Nagano, Y.Kobayakawa[1] and M.Sakai[2].

[1]Dep. of Biol., Fac. of Sci., Kyushu-Univ., Fukuoka. [2]Dep. of Chem. and Biosci., Fac. of Sci., Kagoshima-Univ., Kagoshima.

In *Xenopus* embryo, dorsovegetal cells (D1-cells) of the 32-cell stage are known to produce the 2nd dorsal axis structures when they are transplanted into the ventral side of recipients. The D1-cells are thought to induce the dorsal structures in the recipients' tissues because progenies of the transplanted D1-cells contribute only to the endodermal structures but not to the dorsal structures. The timing of the dorsal axis induction, however, is still unknown.

In order to know the timing of the dorsal axis induction by the transplanted D1-cells, following experiments were done. Two D1 cells were transplanted into D4 position of each recipient and neighboring parts located on the animal side of the transplanted D1-cells were explanted at the 256-cell stage, 1024-cell stage and the onset of gastrulation. The explanted parts were progenies of A3,A4,B3,B4,C3,C4 cells at the 32-cell stage embryo, but did not include progenies of the transplanted D1-cells. Explants were isolated at the gastrulation stage always formed dorsal structures (somites) but those from the 256- and 1024-cell stage had no dorsal structure. These results suggest that the induction of dorsal axis structures by the D1-cells occur after the 1024-cell stage (maybe after the MBT) or need longer contact period.

IDENTIFICATION OF GELSOLIN-POSITIVE CELLS IN ASCIDIAN TADPOLE LARVA AS EPIDERMAL NEURONS.

Y. Ohtsuka[1], Y. Okamura[1] and T. Obinata[2]. [1]National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology M.I.T.I., Tsukuba, [2]Department of Biology, Faculty of Science, Chiba University, Yayoi-cho, Chiba

We previously reported that gelsolin, an actin filament severing and capping protein, was detectable in the cells within epidermis during early embryogenesis. These cells possessed cilia which extended into larval tunic. We assumed that they are epidermal sensory neurons as judged by morphological characteristics TuNaI is neuron-specific voltage-gated sodium channel, and its expression in tadpole was very similar to that of gelsolin. In this study, to confirm that the gelsolin-positive cells are epidermal sensory neurons, we performed two-color *in situ* hybridization using both gelsolin- and TuNaI-specific riboprobes. Both signals were detected in the same cells within epidermis, whereas the signal of troponin T, a marker for larval tail muscle, was detected in a pattern distinct from the gelsolin-signal under the same condition. These results indicate that the gelsolin-positive cells in epidermis are epidermal neurons, and that transcription of gelsolin gene scarcely occurs in the larval muscle, although gelsolin was abundant in the adult body wall muscle.

SPATIOTEMPORAL EXPRESSION OF BULLFROG α1(I) AND α2(I) COLLAGEN GENES IN INTESTINE DURING METAMORPHOSIS.

○R. Utoh, K. Asahina, M. Obara, and K. Yoshizato
Dev. Biol. Lab., Dept. of Biol. Sci., Fac. of Sci., Hiroshima Univ., Higashihiroshima

The intestine of anuran tadpole drastically transforms its structure and function in a thyroid hormone (TH)-dependent manner during metamorphosis, which requires the spatiotemporally regulated epithelio-mesenchymal interactions.The present study attempted to characterize the development of the intestinal mesenchyme during metamorphosis at the gene level, utilizing cDNAs of type I collagen as a probe. The full-length of α2(I) chain of bullfrog had been previously cloned (Asahina *et al* 1997) and that of α1(I) was cloned in the present study by RT-PCR, the sequence of which was found to be highly homologous to that of mammalian α1(I) collagen gene. We investigated the expression pattern of these α1(I) and α2(I) collagen genes during metamorphosis. Expression of α1(I) collagen mRNA was drastically up-regulated at the climax period of spontaneous metamorphosis. This expression pattern was precociously mimicked by TH-treated animals. Expression of α1(I) and α2(I) collagen mRNA were localized in mesenchymal fibroblasts of the intestine. The increased expression of these genes at the climax stage was correlated well with the conversion of the larval mesenchyme to more thick and dense connective tissues of the adult intestine. These results strongly suggest that bullfrog α1(I) and α2(I) collagen genes are spatiotemporally regulated by TH during the intestine remodeling.