IS A SPINAL CORD REQUIRED FOR *XENOPUS* TAIL REGENERATION?

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It is well known that the nervous tissue is required for urodele limb and tail regeneration, although tail regeneration of anuran larva is suggested to be independent of the spinal cord. A regeneration of the larval tail in *Xenopus* laevis is a rapid process and is a suitable model system for molecular analysis of organ regeneration. To reveal whether the spinal cord is required for the tail regeneration in *Xenopus*, we attempted to remove the spinal cord from the larval tail. After amputation of the distal half of the st.48 tadpole tail, the remaining tail was incised from dorsal side to cut the spinal cord. The spinal cord in the remaining tail was then removed by pulling a proximal cut end from the incision. Immunostaining for nervous cells revealed complete absence of spinal cord in the operated tail. The spinal cord-ablated tadpole regenerated a tail with a notochord, muscle tissue and fins but without a spinal cord. The regenerated tail was shorter than a control tail and wavy. It was thought that the abnormal morphology was ascribed to a slim and wavy notochord in the regenerated tail.

PROXIMODISTAL AXIS FORMATION DURING *XENOPUS* LIMB REGENERATION

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Xenopus laevis can regenerate an amputated limb completely at early limb bud stages of tadpoles, but the capacity gradually declines as metamorphosis progresses. Limbs in a froglet, a young adult frog, do not regenerate any well-patterned limb structures but form a hypomorphic structure, a so-called spike, after amputation. It is unclear whether the specification of positional identities along the proximodistal (PD) axis in the regenerating blastema of the froglet occurs as in the developing and regenerating limb bud in the tadpoles. We examined that expression of marker genes for the PD axis formation, Hoxa11 and a13, in the regenerating blastema of tadpoles and froglets, with comparing to the developing limb bud. We show that the regenerating tadpole blastema has the same expression pattern as the developing limb bud, whereas reprograming of these gene epxrssions in the froglet blastema is incomplete. Taken together with a sorting-out experiment in vitro and transplantation experiments in vivo, we suggest that the incomplete reprograming of positional identities along the PD axis is one of causes that froglet forms only the spike.

HISTOLOGICAL PROFILING FOR HEAD AND TAIL DETERMINATION IN REGENERATING *ENCHYTRAEUS JAPONENSIS*

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The Enchytraeida Oligochaeta *Enchytraeus japonensis* autotomizes at the specific position in each segment. Each autotomized fragment regenerates a head anteriorly and a tail posteriorly without exception. However, when worms are amputated artificially, a head is occasionally formed posteriorly in addition to the normal anterior head, resulting in a bipolar worm. This phenomenon prompted us to conduct a series of histological observation to clarify how the head and the tail are determined during fragmentation and regeneration. We have made microscopical observation on external groove, nervous system and muscle structure around the wound caused either by autotomy or amputation. Also was made an extensive examination on wound closure and blastema formation by scanning electron microscopy (SEM), fluorescent conjugated tissue specific antagonists, BrdU, *in situ* hybridization.

POSSIBILITY OF MOUSE LIMB REGENERATION BY SUPPLYING LIMB BUD CELLS

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Mammals have a low potency of limb regeneration when compared to amphibians. One explanation for the low potency is deficiency of cells for regenerating amputated limbs in mammals. Amphibians can use degenerating cells as a blastema, but mammals have few such cells. We report that mouse limb bud cells, grafted to amputated neonatal mouse limbs, can grow and differentiate into bone, cartilage and soft tissues. Furthermore, the grafted limb bud cells can form digits-like structures that seemed to compensate amputated digits. Results suggest that the limb bud cells can act as a source of regenerating skeletal pattern in amputated limb, and the environmental tissues of the stump allow the differentiation of grafted embryonic cells.

REGENERATION OF VASCULAR SYSTEM IN *E. JAPONENSIS* ANTERIOR REGION

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E. japonensis reproduces in asexual fashion: a full-grown worm fragments its body and then regenerates its head and tail regions. To analyze the processes of regeneration, we carried out fluorescent dye staining of the vascular structures and TUNEL assay in the regenerating anterior region. We observed whole-mount worms by confocal laser scanning microscopy (CLSM). The dorsal and ventral vessels were once amputated in the fragmentation process. Both vessels were re-connected and branched at the early stage of regeneration. Then, the connected vessels appeared to be extended and dilated in the growing anterior region, where new seven segments were reconstructed. From TUNEL assay, we detected apoptotic cells adjacent to the vascular structures. Thus, the vascular system of *E. japonensis* would crucially participate in the regeneration of anterior region, possibly through the induction of apoptosis.

BLEBBING AND APOPTOTIC BODY FORMATION IN STARFISH EGGS

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The hormonal stimulation of 1-methyladenine (1-MA) induces meiosis reinitiation in starfish oocytes. Without fertilization, spontaneous and synchronous activation of caspase-3 occurs about 10 h after 1-MA stimulation. When we treated eggs with proteasome inhibitor MG-115 (Z-LL-Nva-CHO), both MAPK inactivation and occurrence of blebbing were significantly delayed in these eggs. These results suggest that the proteasome pathway may contribute to apoptosis of starfish eggs. And, when we treated eggs with p38MAPK inhibitor SB203580, apoptotic body formation was inhibited. These results suggest that p38MAPK is involved in apoptotic body formation.

EXPRESSION PROFILES OF EARLY AND EARLY-LATE GENES IN THE STEROID-INDUCED PROGRAMMED CELL DEATH IN THE ANTERIOR SILK GLANDS OF *BOMBYX MORI*

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Silk gland of the silkworm, *Bombyx mori*, is the larval specific tissue and begins to degenerate during larval-pupal metamorphosis. Although 20-hydroxyecdyson (20E) triggers the programmed cell death (PCD), little is known about its molecular mechanisms. Therefore, we analyzed the expression profiles of four early genes (ecdysone receptor (EcR), E75, BR-C and FTZ-F1) and an early-late gene (BHR 3) in the anterior silk glands *in vitro* and *in vivo*. Expressions of E75 A and BHR 3 were induced within 1 h and between 4 and 8 h after 20E challenge, respectively, and CHX did not affect both expressions, indicating that E75 A and BHR 3 were stimulated directly by 20E. By contrast, E75 B was not up-regulated by 20E *in vitro* and *in vivo*, indicating that E75 B is not involved in the 20E-induced PCD. Other genes were expressed without 20E, and 20E exhibited little effects on these expressions. These results indicate that E75 A and BHR 3 are involved in the genomic action of 20E on the PCD.

NON-STEROID SIGNAL CASCADE IN CELL DEATH OF THE ANTERIOR SILK GLANDS OF *BOMBYX MORI*

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20-hydroxyecdyson (20E) induces the programmed cell death (PCD) in the anterior silk gland of *Bombyx mori in vivo*, as well as *in vitro*. In addition, protein synthesis inhibitors (PSI), such as CHX, induced cell death *in vitro*. The morphology of cells affected by PSI exhibited only slight cell shrinkage but no apoptotic body formation, which was different from that in 20E induced PCD, DAPI signals were scattered over the branches of thread-like nucleus, an indication that nuclear fragmentation occurred, but nuclear condensation did not. In addition, DNA fragmentation occurred in those cells. PSI-induced cell death was inhibited by PKC and caspase-3 inhibitors, similarly to 20E induced PCD. PSI inhibited more than 90% *de novo* protein synthesis. These suggest that PSI action is non-genomic. When day 6 last inatar larval glands underwent the PCD in response to 20E, while day 5 glands did not do so. PSI was capable of inducing the cell death in day 6 glands, but day 5 ones not. Thus, the time when the glands became competent to respond to PSI appears to be the same as that for 20E. Taken together, the present results indicate that PSI and 20E share partly the same signaling pathway.