inhibitor of protein kinases, to induce exit from M-phase, extracts were added with demembranated sperm and examined for SPIA by monitoring the morphological changes in sperm chromatin. The results indicated that protein synthesis during the pre-GVBD stage (60 min prior to the occurrence of GVBD at latest) is required for the establishment of SPIA.

ACTIVATION OF RAF/MEK/ERK PATHWAY DURING ACROSOME REACTION IN BOAR SPERM

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Acrosome reaction of sperm is an indispensable step for fertilization. It is well known that Ca^{2+} influx and increase in $[Ca^{2+}]_i$ play key roles in acrosome reaction, however, signaling cascade of acrosome reaction after $[Ca^{2+}]_i$ increase is still unclear. To understand the signaling cascade from $[Ca^{2+}]_i$ increase to acrosome reaction, we examined roles of ERK pathway (Raf, MEK, and ERK) in acrosome reaction of boar sperm. When the spermatozoa were incubated in IVF medium containing caffeine for 3 hours, rate of acrosome reacted sperm increased from 5.1 % to 26.7 %. Concomitantly with the acrosome reaction, phosphorylation of Raf, MEK, and ERK was observed. Treatment with MEK inhibitor, U0126, prevented sperm from acrosome reaction. Furthermore, seminal vesicle fluid, which inhibits sperm acrosome reaction, strongly decreased phosphorylation of ERK pathway. These results suggest that the activation of ERK pathway is involved in sperm acrosome reaction.

EGG-JELLY SUBSTANCES OF 122 KDA AND 90KDA MAY INVOLVE IN INDUCING SPERM ACROSOME REACTION IN THE NEWT, *CYNOPS PYRRHOGASTER*

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Sperm acrosome reaction (AR) is essential for the successful fertilization in most animals. It is induced in egg envelope by a species-specific molecule. In amphibians, it was immunologically detected in the vitelline envelope of Xenopus. On the other hand, the activity for inducing sperm AR localizes in the outermost region of egg-jelly in the newt, *Cynops pyrrhogaster*. This suggests that the AR is induced in a species-specific manner. However, little is known about the molecules involving in the induction of the AR in newts. In this study, we raised the monoclonal antibody that neutralized the induction of the sperm AR in the newt. When the conditioned medium containing that antibody was pretreated with the egg-jelly substance and the sperm were added to it, the acrosom-reacted sperm was decreased by half. The band of about 122 kDa and 90 kDa were detected in the extract from the outer region of the egg-jelly by the immunoblotting using the antibody. When the acrylamide-gel containing those bands was fragmented and sperm were put on them, the acrosome was disappeared in over 60 % of the sperm. Those molecules may have a role in the induction of the AR in the newt sperm.

AN ACROSOME REACTION IN SPERMATOZOA FROM THE AMPHIOXUS, *BRANCHIOSTOMA BELCHERI*

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Acrosome reaction in the amphioxus, *Branchiostoma belcheri*, is first reported. Intact acrosomes, in which bell-shaped acrosomal vesicle covered unstructured subacrosomal material, located in front of the nucleus. On acrosomal exocytosis, the plasma and outer acrosomal membranes fused at the sperm apex and an acrosomal process was produced. The core of the process seemed to derive from the subacrosomal material. Multi-point vesiculation that is seen in vertebrate spermatozoa, was not observed. Acrosome reaction in Branchiostomidae, members of low chordates, seemed to follow the mode known in invertebrates.

ANALYSIS OF THE TIMING MECHANISM FOR THE START OF GASTRULATION IN THE *XENOPUS* EMBRYO

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Xenopus midblastulae were continuously exposed to either aphidicolin (a DNA replication inhibitor), vinblastine (a mitosis inhibitor), DMAP (an MPF inhibitor) or urethane (local anethetic). Either embryos did start gastrulation though they ceased cell division. The timing of the start of gastrulation was, however, very much delayed in embryos exposed to DMAP or urethane as compared with control ones while it was almost the same in those exposed to aphidicolin or vinblastine as in control ones. This result implies that the start of gastrulation may be timed by cytoplasmic activities independent of the replication and division of the nucleus.

TEMPORAL AND SPATIAL DEVELOPMENT OF NERVOUS SYSTEM DURING EMBRYOGENESIS OF *NEMATOSTELLA VECTENSIS* (ANTHOZOA, CNIDARIA)

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In order to gain knowledge on the origin and evolution of nervous system, we examined development of nervous system during embryogenesis of a starlet sea anemone *Nematostella vectensis*. Anthozoa is the most primitive group in Cnidaria, whose ancestor probably obtained the first nervous system. Development of neurons was analyzed by immunostaing using antibodies specific to three distinct neuropeptide groups, RFamides, GLWamides and Hym-357(possibly YKPamides).

First RFa- and GLWa-positive sensory neurons appeared in the ectoderm of early stage-planula larva. With formation of gastrovascular cavity GLWa- and Hym-357-positive neurons were found on the inside of anterior half of planula which later developed to parynx and mesenteries. As development proceeded, these three distinct types of neurons formed a characteristic network. Further detailed analysis with pan-neuron and subtype specific markers will establish a precise sequence of neuron development in this animal. In any case, the first appearance of sensory neurons and then formation of network during embryogenesis presumably reflect the evolutionary sequence of nervous system.

THE RELATIONSHIP BETWEEN THE CORTICAL LOCALIZATION OF CDC2/CYCLIN B AND CYTOSKELETONS AT EARLY EMBRYONIC CELL CYCLES OF THE FROG, *XENOPUS LAEVIS*

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In early development of the frog, *Xenopus laevis*, an asynchronous cell cycle begins at the 13th cell cycle, which is referred to as mid-blastula transition (MBT). To clarify the molecular mechanism of the transition, we observed the distribution of cdc2/cyclin B in the blastomeres around MBT by using immunochemistry and confocal microscopy.

In the 8th cell cycle, cdc2/cyclin B was localized in the cytoplasm, in particular, in the cortical region. At the 13th cell cycle, the cortical localization of these molecules disappeared. These results suggest that the decrease of cdc2/cyclin B1 in the cortex after the 13th cell cycle might be involved in the change between the synchronous and the asynchronous divisions. To determine the involvement of cytoskeletons in these distribution, the embryos were treated with nocodazole or latrunculin A for 1 hr at 8th cell cycle. Cdc2/cyclin B was not localized in the cortex in either treatments, suggesting that actin filaments and/or microtubules participate in the cortical localization of cdc2/cyclin B at synchronous cell cycles. In addition, cyclin B1 mRNA was localized in the periphery of nucleus at the 8th cell cycle.

GAIN-OF-FUNCTION ANALYSIS OF *KRL* GENE OF THE SEA URCHIN *HEMICENTROTUS PLUCHERRIMUS*

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We performed gain-of-function analysis of *HpKrl* in the sea urchin. Fertilized eggs injected with Krl mRNA developed almost normally up to the mesenchyme blastula stage. In the injected embyos after gastrula stage, however, endomesodermal structures (expect the primary mesenchyme cells) expanded at the expence of the ectoderm. Next we constructed chimeras composed of animal cap mesomeres from a normal embryo with a mesomere from an embryo injected with Krl mRNA. In chimeric embryos, the injected mesomere not only differentiated into the foregut and secondary mesenchyme cells, but also induced midgut and hindgut from animal cap mesomeres. Thus, the chimera developmented into an almost complete pluteus larva. These observations combined with those of the loss-of-function analysis indicate that *HpKrl* is necessary and sufficicient for endomesoderm (except skeletogenic mesenchyme cells) differentiation.