MECHANISMS UNDERLYING CESSATION OF SPERM ATTRACTION AND PROGRESSION OF CELL CYCLE IN FERTILIZED EGGS OF THE HYDROZOAN JELLYFISH

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It is known that hydrozoan eggs lose the ability to attract sperm and initiate cell cycle after fertilization. We have investigated the mechanisms underlying the two post-fertilization events in the hydrozoan jellyfish *Clytia*. When an unfertilized *Clytia* egg was cut in the equatorial plane, both the fragment that contains a female pronucleus (animal fragment) and the opposite fragment (vegetal fragment) attracted sperm following insemination. The animal fragment accepted a sperm, lost the ability for sperm attraction, and underwent normal cleavage subsequently. In contrast, the vegetal fragment kept attracting sperm without being fertilized. The animal and vegetal fragments both ceased sperm attraction when treated with the calcium ionophore A23187 or the MEK inhibitor U0126. Following the cessation of sperm attraction, the animal fragments resulted in pseudocleavage, whereas the vegetal fragments did not. These results suggest that the two post-fertilization events are regulated by a Ca^{2+} increase and MAPK inactivation in *Clytia* eggs. Moreover, there is a possibility that the targeted sites and downstream pathways for Ca^{2+}/MAPK are different in the two events.

SPERM ATTRACTING ACTIVITY IN FERTILIZED EGGS OF THE ASCIDIAN *CIONA INTESTINALIS*

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Sperm chemotaxis toward unfertilized eggs is observed in many animals, and the eggs of some animals look to cease sperm attraction after fertilization. In this study, we examined sperm-attracting activity of the egg after fertilization in the ascidian Ciona intestinalis. When we observed sperm behavior after fertilization, sperm stopped showing chemotactic behavior toward the egg on 120 sec after the egg deformation. When same volumes of the fertilized-egg seawater (fESW) and 500 nM SAAF, the chemoattractant of the Ciona sperm were mixed and incubated 1 hr, SAAF activity in the mixture was decreased, whereas artifitial seawater and SAAF mixture had the activity of the sperm chemoattractant. When the fESW was pre-treated with 0.01% actinase E and mixed with SAAF, the mixture had the SAAF activity. Furthermore, when the fESW was boiled at 100°C for 30 min and mixed with SAAF, SAAF activity was decreased. These results suggest that the ascidian egg releases some heat-stable enzymes which digests SAAF after fertilization, and induces the cessation of sperm attraction.

YEAST TWO-HYBRID SCREENING FOR MOLECULES THAT INTERACT WITH ASCIDIAN SPERM RECEPTOR HrVC70

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It is known that HrVC70, a major component of the vitelline coat, plays multiple roles during fertilization in the ascidian *Halocynthia roretzi*. This molecule functions as a sperm receptor on the egg envelop, which appears to be involved in self-sterility, species-specificity, and speciation in ascidians. In particular, self-sterility in *H. roretzi* is very strict, suggesting that several sperm-derived proteins may be involved in the achievement of gamete interaction mediated by HrVC70. In addition, it is also proposed that HrVC70 is degraded by sperm 'extracellular' ubiquitin-proteasome system, which allows sperm penetration of the vitelline coat. This suggests that ubiquitin ligase E3 may also interact with HrVC70.

To gain insight on molecular basis of the biological functions of HrVC70, we attempted to explore gamete proteins that are capable of interacting with HrVC70. By yeast two-hybrid screening of a gonad cDNA library using the entire HrVC70 as the bait, several clones that encode extracellular proteins were isolated. In this report, we discuss their possible functions in fertilization.

IDENTIFICATION OF SPERM LIGAND HUURABIN FOR SPERM RECEPTOR HrVC70 IN THE ASCIDIAN *HALOCYNTHIA RORETZI*

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Ascidians are hermaphrodite, releasing sperm and eggs simultaneously. But, many ascidians, including *Halocynthia roretzi* and *Ciona intestinalis*, are self-sterile, due to the occurrence of self/nonself-recognition system between sperm and the vitelline coat of the eggs. We previously reported that a 70-kDa vitelline coat protein HrVC70, which is a highly polymorphic protein made up of 12 EGF-like repeats, is a candidate self/nonself recogntion molecule during fertilization. However, a sperm ligand for HrVC70 has not yet been identified. Here, we show that a 35-kDa glycoprotein localized on the sperm membrane RAFT is able to bind to HrVC70 on the basis of Far Western blot analysis. In order to obtain insight on the molecular structure of HrVC70, cDNA cloning was carried out. The sequence data showed that this molecule is a novel protein having a signal for GPI-anchored protein and two SCP motifs. Thus, we designated this molecule as HrUrabin, a unique RAFT-derived HrVC70-binding protein. We also discuss about the molecular structures, polymorphisms, and possible roles in fertilization of HrUrabin.

SUGAR CHAIN STRUCTURE OF SPERM RECEPTOR HrVC70 ON THE VITELLINE COAT IN THE ASCIDIAN *HALOCYNTHIA RORETZI*

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Ascidians are hermaphrodite, releasing sperm and eggs simultaneously, but self-fertilization of *Halocynthia roretzi* is strictly blocked by self/nonself-recognition system in gamete interaction. We previously reported that a 70-kDa vitelline-coat sperm receptor HrVC70, which is a highly polymorphic protein made up of 12 EGF-like repeats, is a candidate allorecognition molecule during fertilization. In EGF-like repeats-containing molecules such as Notch, protein *O*-fucosylation is essential for Notch signaling. Furthermore, it is reported that sperm α-L-fucosidase is responsible for sperm binding to the vitelline coat of *H. roretzi*. Although there are one potential *O*-glucosylation site and five potential *O*-fucosylation sites in HrVC70, it is not known whether these potential sites are really glycosylated. By LC/MS analysis of tryptic fragments of HrVC70, we revealed that Thr-342 is fucosylated almost completely and that Ser-558 is not modified by fucosylation. The other potential fucosylation sites were found to be partially modified. Thr-64, a potential glucosylation site, was not glucosylated. Roles in fertilization of sugar moiety in HrVC70 are also discussed.

LOCALIZATION OF CARBOHYDRATE MOIETIES IN EGG-JELLY OF *XENOPUS TROPICALIS* AND THEIR SECRETION IN OVIDUCT

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Amphibian egg-jelly is composed of several sublayers that are formed in posterior parts of oviduct, *pars convoluta* and uterus. We have found that the outermost sublayer contained some active substances for sperm-egg interactions in the newt, *Cynops pyrrhogaster*, and contributed to achieve internal fertilization. It was indicated that the outermost sublayer was formed by secretion in uterus. In the present study, we used *X. tropicalis*, as a representative of species that undergo external fertilization, and examined the distributions of carbohydrate moieties in egg-jelly and oviduct using ten kinds of FITC-conjugated lectins. Egg-jelly of *X. tropicalis* was composed of three sublayers and each lectin showed the specific pattern of binding in the sublayers. It also stained secretory cells in the corresponding regions of *pars convoluta*, suggesting that the egg-jelly substances were added by turns in oviduct when an ovulated egg passes through it as in many other amphibians. Interestingly, little sublstance was found to be added to the surface of egg-jelly in uterus of *X. tropicalis*. Secretion in uterus may be specific for achieving fertilization internally in amphibian species.

CHANGES IN ACTIVITY OF CELL CYCLE RELATED KINASES DURING THE MEIOSIS INDUCED BY Ca^{2+} IONOPHORE IN THE EGG OF ASCIDIAN

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Two series of $[Ca^{2+}]$ transients appear in accordance with the meiotic processes at the fertilization in the oocyte of ascidian. To investigate role of these $[Ca^{2+}]$ transients on the meiosis, in unfertilized oocyte treated with Ca^{2+} ionophore, ionomycin, the changes in activities of both MAPK and MPF (H1K) related to cell cycle and the change in morphology of nucleus were observed. After the pulse treatment of the unfertilized oocyte with ionomycin, its meiosis progressed from meiotic metaphase I (Meta-I) to meiotic metephase II (Meta-II). In the oocyte, the activity of MAPK increased transiently, then it was kept at certain level. On the other hand, the activity of H1K disappeared once and increased transiently and was kept at certain level. Although the meiosis was arrested at Meta-II after above treatment, re-treatment of the oocyte with ionomycin restart its meiosis. In the oocyte, both activity of MAPK and H1K disappeared, then the actiyity of H1K began cyclic changes. These results suggest that 1st series of $[Ca^{2+}]$ transients at fertilization drive the meiosis from Meta-I to Meta-II and that 2nd series of $[Ca^{2+}]$ transients drive the meiosis from Meta-II and exit its meiosis.