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Sperm Motile Mechanism in Chordates

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Activation of the sperm motility and chemotaxis toward eggs are the first communication between sperm and egg at fertilization. The nature of molecules for the phenomena derived from gametes and cell signaling mechanisms have been investigated in only non-chordate marine invertebrate species, sea urchins. Study on regulatory "*sperm motile mechanism*" in chordates has been initially focused in ascidians, which belongs to the lowest phylum of chordates (Morisawa et al., 1984) and have notochord during larval stage. Further discovery of an egg-derived factor, regulating sperm motility (Yoshida et al., 1993) in the organism opened up new insights into the investigation of signal transduction of "*sperm motile mechanism*", designating as "cell signaling for the regulation of sperm motility". Spermatozoa of the ascidians *Ciona intestinalis* and *C. savignyi* are immotile in the seawater. Spermmotility is activated around the egg (sperm activation), and then the activated sperm are attracted towards the egg (sperm chemotaxis), suggesting that factor for sperm activation and chemotaxis is released from *Ciona* egg at fertilization. The factor was named as Sperm-Activating and -Attracting Factor (SAAF) and was recently purified from egg seawater using column chromatographies, which exhibited a single peak on mass spectrometry with molecular weight of 596 that has recently identified as a novel sulfated steroid (Yoshida et al., 2003; Oishi et al., 2004). The SAAF has two functions, sperm activation and chemotaxisand causes both Ca^{2+} influx and a transient cAMP increase. Cyclic adenosine monophosphate, cAMP, and Ca^{2+} influx through voltage-dependent Ca^{2+} channel are required for sperm activation. Chemotaxis, however, does not need cAMP, and only Ca^{2+} effluxed from intracellular store in the cytoplasm and following influx of Ca^{2+} through store operated Ca^{2+} channel exists in the plasma membrane causes the phenomenon. Cell signalings underlying sperm activation and chemotaxis have recently been clarified by Morisawa and Yoshida (2005).

Sperm motility in teleost fishes is also regulated not only by the environmental ionic and osmotic changes at spawning (Morisawa and Suzuki, 1980) through contribution of cAMP (Morisawa and Okuno, 1982) but also factors derived from eggs. Spermatozoa of the Pacific herring *Clupea pallasi* are immotile in the seawater,and the motility becomes active in the vicinity of the egg or egg conditioning medium (Morisawa et al., 1992), suggesting that water soluble sperm activating factors indispensable for the successful fertilization are released from the egg. The activating-factors, Herring Sperm-Activating Proteins (HSAPs), were purified from the egg-conditioned seawater by gel filtration- and isoelectric focusing- chlomatographies. Complementary DNA (cDNA) cloning of the HSAPs revealed that the HSAPs are small secretory proteins whose molecular weight of 8 kDa which has striking homology with the Kazal-type trypsin inhibitors (Oda et al., 1998). The other activator of sperm motility has been found in USA in the same

species distributed in San Francisco Bay and designated sperm-motility initiating factor (SMIF) (Yanagimachi et al., 1992). The SMIF is a glycoprotein with molecular weight of 105 kDa and water-insoluble factor, which binds tightly to the egg. Two modes, activation of sperm by water soluble small HSAPs (Morisawa and Okuno, 1982) and chemotaxis by water insoluble large SMIF (Yanagimachi et al., 1992), may help invasion of the sperm into the eggs at fertilization in the herring: the HSAPs released from the eggs into the surrounding seawater activate the motility of spermatozoa and increase the number of spermatozoa of which meet the SMIF. Then the SMIF pasted around a micropyle, a canal that only one spermatozoon could be passing through, guides a spermatozoon towards the egg plasma membrane through the micropylar opening, resulting in completion of fertilization. A detailed analysis of the flagellar bending pattern of the spermatozoon activated with the HSAPs and the SMIF will be essential to verify this hypothesis. Cell signaling for regulatory mechanism of sperm motility by HSAPs and SMIF were investigated by collaboration with two laboratories from Japan and USA (Vines et al., 2002).

Our recent studies using gametes of human, which belongs to the highest phylum of chordates, have found that follicular fluid from human eggs contains factors with MW less than 5 kDa causing hyperactivation-like motility in the non-capacitated sperm within a short period after their contact with the sperm. Percentages of motile sperm, velocity and ALH increase, but linearity and beat/cross frequency did not. These changes in parameter of hyperactivation-like sperm behavior were seen both in the sperm donated from young volunteers and in those from men visiting infertility clinic (Morisawa and Yoshida 2005).

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