SUMMARY OF Ph.D. DISSERTATION

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Title

Study on species-specificity of fertilization in starfish

Abstract

Fertilization is composed of temporally and spatially regulated multiple primary processes, i.e. sperm-egg interactions and occurs species-specifically to maintain species. In starfish, a marine invertebrate reproducing with external fertilization, the species-specificity in fertilization must be important not only for effective fertilization but also for avoiding cross-species fertilization. The molecules involved in the two primary processes, sperm activation/attraction and acrosome reaction in starfish have been identified. In *Asterias amurensis*, asterosap (asteroidal sperm-activating peptide), a group of peptides with 34 amino acids from egg jelly layer binds to membrane-bound guanylate cyclase in sperm to activate/attract sperm. Also in *A. amurensis*, sperm acrosome reaction is induced by the concerted action of 3 components of egg jelly, ARIS (Acrosome Reaction-Inducing Substance), Co-ARIS (cofactor of ARIS) and asterosap. ARIS is a sulfated proteoglycan-like molecule which plays a central role in acrosome reaction although it alone has no activity in normal sea water. Co-ARIS is a group of sulfated steroid saponins. Both Co-ARIS and asterosap assist ARIS in acrosome reaction. In this study, I investigated the species-specificity of fertilization, sperm activation/attraction and acrosome reaction, their involvement in the speciation, and the signaling mechanism of sperm activation/attraction.

In chapter 1, I summarized the knowledge about the molecules involved in sperm activation/attraction and acrosome reaction in starfish, and their mechanism of action. I also summarized the specificity of fertilization and acrosome reaction in starfish and pointed its importance.

In chapter 2, I tried to verify a hypothesis that the specificity of acrosome reaction establishes species-specificity of fertilization. It was demonstrated that fertilization evaluated by the formation of fertilization membrane was species-specific, if not fully, while induction of acrosome reaction by egg jelly was subfamily-specific. This suggested other process(es) involved in the establishment of species-specific fertilization. On the other hand, an immunoassay (ELISA) found the correlation between an active glycan structure of ARIS and the subfamily-specificity in acrosome reaction.

In chapter 3, I demonstrated the superorder-specificity in sperm activation by asterosap. To compare primary sequences among species, I cloned asterosap and its receptor from 5 species in the same order sharing asterosap activity. Both asterosap and its receptor sequences shared high homology among these species. The molecular evolutionary analysis estimated that they have not been a direct cause of speciation. On the other hand, a model for binding and activation of asterosap-receptor shared in the same order was proposed based on their sequences and structural modeling. This model indicated the similar mechanism of binding and activation in mammalian atrial natriuretic peptide and its receptor.

In chapter 4, in order to search candidate proteins involved in sperm signal transduction, the proteome of sperm head and tail fraction was analyzed using 1D-PAGE-LC/MS/MS. In each fraction, 50-100 proteins were identified. Among them asterosap receptor was a major tail protein in all species examined, *A. amurensis*, *A. forbesi* and *Asterina pectinifera*. The position of identified peptide fragments indicated that these asterosap receptors shared their function. Other signaling proteins identified not only supported an existing hypothesis about sperm signaling, but also suggested several signaling processes novel in starfish.

In chapter 5, I generalized this study and described possible expansion of this study.