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Recent Research Topics in Reproduction and Innate Immune System of Bivalve Mollusks

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Summary

Bivalve species is an important fishery product in the industry of aquaculture in Asia, Oceania, Europe, and North and South America. An advanced technology for artificial seed (juvenile) production, control of reproduction and growth in a healthy condition is necessary to achieve an efficient production in bivalve aquaculture. We focus on 1) a signal transduction of neurohormone and sex hormone via receptor mechanism in relation to reproduction and endocrine disruption and 2) a cellular and humoral defense mechanism as an innate immune system associated with non-self recognition and elimination to aim at the above-mentioned goal.

Gonial Mitosis and Oogenesis

The pattern of proliferation of gonial cells was divided into two phases: phase I; oogonia and spermatogonia slowly proliferate through the growing stage: phase II; oogonia develop into oocytes and spermatogonia start to proliferate rapidly from the mature stage through the spawning stage. The neurons detected with anti-mammalian GnRH antibody were distributed in the central nervous system (CNS) of both sexes. The neuropeptide fraction from the CNS and blood cell of both sexes promoted proliferation of spermatogonia in the *in vitro* culture of the testicular tissue as well as mGnRH, which was abolished by the mGnRH antagonists and anti-mGnRH antibody, suggesting that the spermatogonial proliferation is regulated by GnRH-like peptide. These findings suggested that the spermatogonial proliferation at phase II in the scallop was under the neuroendocrine control by GnRH.

The accumulated vitellin protein in the oocyte is an essential material for energy production and development of larval tissue until the onset of feeding. However, little information has been available concerning the vitellogenin (Vtg)

synthesis and endocrine control of vitellogenesis in molluscs. The scallop Vtg mRNA signal was detected in the auxiliary cell closely associated with growing oocytes, suggesting that the Vtg synthesis occurs through heterosynthetic pathway without mediation through the blood flow but occurs *de novo* in the ovary. A novel neuropeptide, vitellogenesis promoting factor (VPF), from the CNS strongly promotes Vtg protein synthesis, while the transcription of Vtg mRNA is promoted by estradiol-17β (E_2) via estrogen receptor (ER). It was suggested that vitellogenesis in bivalve mollusks is under the control of E_2 at the transcriptional level and VPF from the CNS at the translational level.

Vitellogenesis controlled by these signal transductions is supposed to be influenced by endocrine disrupting chemicals (EDC). We conducted an analysis for effect of EDC on the gene expressions of *Vtg* and *ER* as biomarkers in blue mussel. No significant change in the expression of either the *Vtg* or *ER* gene was recorded in the E_2-exposed mussel *in vivo*, suggesting that a regulatory mechanism exists that is able to maintain constant level of free E_2 by converting excess E_2 into esterified product which may have reduced affinity for ER.

Oocyte Maturation and Spawning

Serotonin (5-hydroxytriptamine; 5-HT) is a major neurotransmitter that triggers oocyte maturation and sequential spawning in bivalve mollusks. However, ineffectuality and/or individual variation of exogenous 5-HT-induced spawning *in vivo*, leading to unstable artificial induction of spawning and seed production, which is the bottleneck of aquaculture industry in bivalves, have been reported. An existence of inhibitor to regulate 5-HT function was expected. Recently we demonstrated a new pathway from the central nervous system (CNS) to regulate oocyte maturation. We found a novel neuroprotein with a molecular mass of 60 kDa, named oocyte maturation arresting factor (OMAF), which inhibits a reinitiation of oocyte meiosis induced by 5-HT. The OMAF, which is a universal substance for bivalve species, is transported from the CNS to the ovary through blood flow and prohibit 5-HT-induced oocyte maturation due to the interference of extracellular Ca^{2+} influx into oocytes, eventually resulting in the inhibition of spawning. On the other hand, it seems that PGF_{2a} inhibits 5-HT-induced transport of mature eggs through the gonoduct, which is resulted from an inhibition of the cilioexcitatory activity of 5-HT in the ciliated epitherium of the gonoduct. We concluded that oocyte maturation and subsequent egg release triggered by 5-HT are suppressively controlled by OMAF and PGF_2, respectively.

Critical Components in Humoral Defense Mechanisms : PRPs and Lysozymes

In order to combat infection by bacteria, fungi and parasites, bivalve molluscs rely on innate immunity comprised of non-specific phagocytosis by hemocytes and humoral defense factors. For a principle of humoral defense mechanisms, typical microbial pathogen-associated molecular patters (PAMPs) are recognized and distinguished by multiple pattern recognition proteins (PRPs), such as lectins and peptidoglycan recognition proteins (PGRPs), and subsequently various immune reactions are induced.

Carbohydrate specific binding proteins, lectins, are known to recognize non-self molecules and act as opsonin for phagocytosis, and thus considered as an important PRPs. So far, identification of C-type lectins, galectins as well as lectin cDNA sequences and their tissue expression analysis have been conducted in various species of bivalve mollusks. Our previous works demonstrated that MCL-4, a novel isoform lectin purified from the plasma of the Manila clam, *Ruditapes philippinarum*, significantly facilitates *Vibrio* cells phagocytosed by clam hemocytes, in addition to bacteriostatic ability. We also discovered presence of multiple PGRPs in the Pacific oyster, *Crassostrea gigas*, thus, mechanisms to recognize PAMPs appear to be crucially important for bivalve mollusks.

Lysozymes (EC 3.2.1.17), cleaving the glycosidic bond of bacterial cell walls in a great variety of organisms, are considered as one of the represent humoral defense factors in bivalve molluscs. Our recent molecular-based works demonstrated that the Pacific oyster possesses three types of lysozymes, CGL-1, CGL-2 and CGL-3, and each of them has different biochemical properties and different tissue expression. CGL-1, identified from the hemocytes, is expressed in multiple tissues, especially mantle, hemocytes, and digestive gland, and recombinant CGL-1 showed great activity in relatively high salt concentrations and pH. Based on these characteristics, it is considered that CGL-1 is better suited to function in the mantle tissues and the hemolymph, where pH and salt concentrations are usually high, for self-defense, although its expression in the digestive diverticula may indicate an additional function, digestion. Another lysozyme, CGL-2, is expressed in the digestive cells of the digestive tubule, and its isoelectric point is lower than the other lysozymes. Moreover, the number of arginine residues and protease cutting sites in CGL-2 are fewer, suggesting that CGL-2 has an increased resistance to proteolytic digestive enzymes, and function for digestion. The third lysozyme, CGL-3 is mainly synthesized in the mantle but less in the digestive diverticula, and the CGL-3 seems to be suited in higher pH condition. These properties suggest that the predicted function of CGL-3 is self-defense rather than digestive enzyme.

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