

The aim of this laboratory is to research reproductive hormones in invertebrates and to analyze the mechanisms by which they work. The comparisons of such molecules and mechanisms in various species are expected to provide insights into the evolution of reproductive hormone systems.

### I. Gonadotropins in the starfish, *Asterina pectinifera*

Gonadotropins play important regulatory roles in reproduction in both vertebrates and invertebrates. The vertebrate gonadotropins, LH and FSH are structurally and functionally conserved across various species, whereas no such molecule has been identified in invertebrates. The insect parsin hormones are assumed to be the physiological counterpart of LH and FSH in mammals. Some gonadotropic hormones, such as the egg development neurosecretory hormone of the mosquito, the egg-laying hormone of the sea hare, and the androgenic gland hormone of the terrestrial isopod, have been found in invertebrate species. More recently, an insulin-like peptide was reported to be responsible for the regulation of egg maturation in the mosquito, *Aedes aegypti*, demonstrating the involvement of insulin signaling in egg maturation among invertebrates.

The gonad-stimulating substance (GSS) of an echinoderm, the starfish, was the very first gonadotropin to be identified in invertebrates. GSS mediates oocyte maturation in starfish by acting on the ovary to produce the maturation-inducing hormone (MIH), 1-methyladenine, which in turn induces the maturation of the oocytes. In this sense, GSS is functionally identical to vertebrate LH, especially piscine and amphibian LHs, acting on the ovarian follicle cells to produce MIH to induce the final maturation or meiotic resumption of the oocyte. Considering the functional similarity that GSS shares with vertebrate LH, it is very important from an evolutionary point of view to know the chemical and molecular structure of GSS. We cloned the gene encoding GSS referred to amino acid sequence of purified GSS from radial nerves of the starfish, *Asterina pectinifera*. Interestingly, phylogenetic analyses revealed that it belonged to the insulin/insulin-like growth factor (IGF)/relaxin superfamily and, more precisely, to the subclass of relaxin/insulin-like peptides (Figure 1).

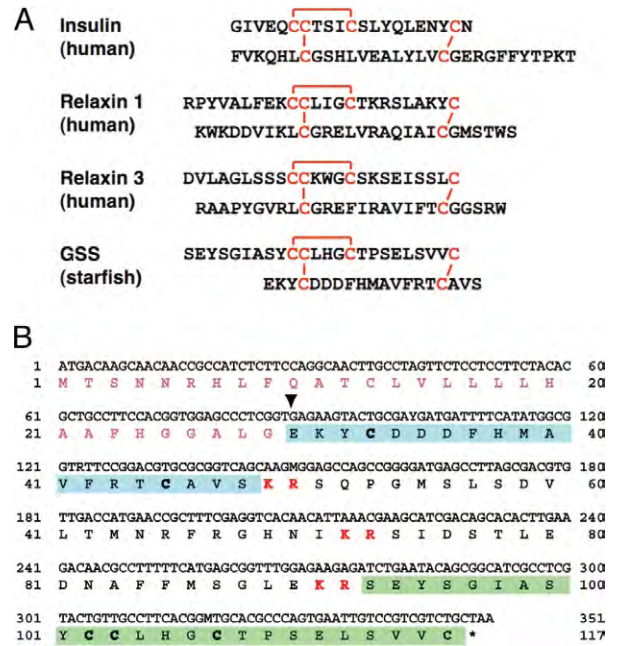


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of A and B chains are shown in green and blue boxes, respectively. Characters shown in red boldface indicate basic dipeptides that are the sites of proteolytic cleavage. Inverted triangle shows the deduced cleavage site of the signal peptide.

### II. Search for reproductive hormones in invertebrates

In a collaborative effort with Prof. Yoshikuni's Laboratory of Kyushu Univ. and Dr. Yamano and Dr. Awaji of the National Research Institute of Aquaculture, Fisheries Research Agency (NRIA), we are searching for reproductive hormones in invertebrates; sea urchin, sea cucumber, oyster, and shrimp. While the collaborators are partially purifying physiological materials which induce egg maturation from nerve extracts and analyzing them with a tandem mass spectrometer, we are creating and analyzing EST libraries from nerve tissues and developing a database of the mass analysis performed in this laboratory.