Establishment of Body Axis during Sea Urchin Embryogenesis

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Establishment of Body Axis during Sea Urchin Embryogenesis

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Abstract

Most of the multicellular organisms develop from one fertilized egg and establish a three-dimensional body by precisely specifying the body axes during embryogenesis. To understand how the body axes are accurately specified and how the established body axes are utilized for morphogenesis is one of the central questions in developmental biology. In this dissertation, I have challenged to answer these two questions by analyzing the developmental process of the sea urchin *Hemicentrotus pulcherrimus*, which is one of the model organisms for developmental biology in East Asia, with embryological and molecular biological approaches. The dissertation consists of two sections to direct each of two questions:

1) Initial analysis to understand the molecular mechanisms of regulative development: It is well known that the early animal embryos of some species have a remarkable ability for regulative development, by which their halved embryos can develop into a complete individual. Especially, the embryos of mammals and echinoderms, including the sea urchins, have strong ability for regulative development, and this phenomenon has been reported over 100 years ago. However, the details of the developmental process and the mechanisms of the body axes specification in the halved embryos remained largely unknown. The terminal goal of this study is to reveal the complete molecular mechanisms of regulative development, but because almost all fundamental information for the regulative development is lacking, I initially challenged to describe the details of the developmental process of *H. pulcherrimus* blastomere, which is halved at the 2-cell stage. Interestingly, the isolated one blastomere of 2-cell stage embryo grew like a plate at the beginning, and then the edge of the plate curled up into a cup shape. The cup-shaped embryos became gradually rounded, like the closing of the mouth of a drawstring bag, then became completely sphere shape, and finally showed the blastula shape. Along with the characteristic morphological

changes in halved embryos, the gene expression patterns of axis formation factors have been dramatically changed compared to normal embryos; those were temporally abnormal at the beginning and finally became normal at the blastula stage. The dynamic developmental process of the halved embryos described in this section shows how the body axis information is regulated and established. These results are crucial to discuss how the halved embryos acquire the accurate body axis information, and whether they use the molecular mechanisms underlying the normal embryogenesis and/or mechanisms unique to halved embryos, during the regulative development.

2) TGF-ß signaling pathway regulates the correct gut bending in the sea urchin embryo: Gastrulation is an essential event to form the through-gut for multicellular organisms. For forming the functional digestive tract, the archenteron of echinoderm larvae, including sea urchins, invaginates and bends toward the ventral ectoderm along the established dorsoventral axis, and this bending behavior leads the archenteron tip to the correct position. Even though the gut bending is important for the formation of a functional through-gut, how it bends has not been recognized as an important biological question. The purpose of the second section is to reveal the mechanism that accurately regulates the gut bending along the established dorsal-ventral axis in sea urchin embryos. During the embryogenesis of the sea urchin embryos, a member of TGF-ß family, Nodal, regulates the dorsal-ventral axis formation as the top of the molecular hierarchy, and also specifies the ventral ectoderm. At first, I tried to knockdown Nodal, but the dorsoventral axis in the morphants was completely lost and the archenteron elongated straightly to the animal pole. Therefore, complete lack of Nodal function was not suitable for analyzing the role of Nodal in the process of gut bending. Thus, after dorsal-ventral axis is specified on the ectoderm, I interfered the function of TGF-ß receptor, alk4/5/7, by employing a specific inhibitor, SB431542 in both *H. pulcherrimus* and *T. reevesii*. As a result, SB431542-treated embryos showed weaker gut bending to the ventral ectoderm than that of control embryos. Because similar results were

obtained in both sea urchin species, it was suggested that TGF-ß signaling is required for the accurate gut bending during sea urchin gastrulation commonly among Echinoidea. In addition, even in the chimeric embryos, in which only the archenteron cannot directly receive TGF-ß signal, the gut bending was significantly weaker than that in the control chimeras, indicating that the reception of TGF-ß signals on the gut is required for proper gut bending. Furthermore, the downstream of Alk4/5/7, Smad2/3, function at the ventral side of the tip of the archenteron, indicating that the TGF-ß signal was surely received at this position. Altogether, it was clarified that the directly received TGF-ß signals at the tip of the archenteron regulates the correct gut bending along the established dorsoventral axis.

In the first section, by halving the early embryos to promote unusual development, I suggested that modification of the anterior-posterior and dorsal-ventral axis is basis of regulative development and revealed the part of the mechanism that definitely builds firm body axes in sea urchin embryos. In the second section, by focusing on the gut bending, I clarified a part of the mechanism how the established body axis is reflected in morphogenesis. Taken together, this research provides a milestone for a comprehensive understanding of the body formation of sea urchin embryos, from the specification of the body axes to the process of forming a three-dimensional body based on the established body axes.