Thesis Summary

Origin of the chordate dorsal structure

(脊索動物に特異的な背側構造の起源)

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Among the features of the chordate body, dorsal structures such as the notochord and the hollow nerve cord are most conspicuous. Specification and formation of these structures has been intensively studied in vertebrates and ascidian embryos. However, the present understanding does not explain how chordates acquired dorsal structures. The purpose of my study is thus to unravel what is the initial trigger to set up the dorsal specification and how the initial setting gives rise to the dorsal structures in extant amphioxus, as well as how the amphioxus pattern gives insights into the origin of chordate body plan.

As sperm entry point affects the dorso-ventral axis specification in ascidians and anamniote vertebrates, I observed the sperm entry point and probable trajectory of male pronucleus by fixing eggs soon and five minutes after insemination. I found that sperm entry point was not restricted to the animal hemisphere but could occur anywhere on amphioxus egg in contrast to ascidian and frog eggs. It means that the sperm entry point does not affect embryonic axis determination in amphioxus embryos. This is the same as the case of sea urchin eggs.

In sea urchin embryos, zygotic Nodal signaling activates *lefty* gene on the ventral (oral) side, and then *nodal-lefty* expression domain is refined. The Nodal signaling restricted in this domain subsequently activates genes that promote the oral specification. As amphioxus *lefty* has been reported to be expressed in blastulae as seen in sea urchin embryos, it is essential to understand whether *lefty* is controlled by maternal Nodal. I demonstrated that asymmetrically distributed maternal *nodal* mRNA triggered an asymmetrical patch of zygotic *lefty* expression at the blastula stage across a circular *wnt8* expression along the equator. Pharmacological perturbations with a Nodal signaling inhibitor (SB505124) confirmed that maternal Nodal signaling directly regulated the asymmetrical expression of zygotic *lefty* gene in amphioxus embryos. Subsequently zygotic *nodal* was activated within the *lefty* expression domain at the late blastula stage. Amphioxus embryos start gastrulation flattening the vegetal hemisphere as in sea urchin embryos, but wider than the latter expanding to the equatorial region. The invagination process occurred at the equator where *wnt8* was expressed. In this gastrulation, the *lefty-nodal* co-expression domain was located on one side of the blastopore and activated dorsal specific genes that promote the formation of the dorsal structures. The location and function of the *lefty-nodal* expression domain is thus comparable to the vertebrate organizing center.

To elucidate the mechanism causing the initial asymmetrical distribution of maternal *nodal*

mRNA, I examined interactions between maternal *nodal* mRNA and cortical cytoskeletons and found that the asymmetrical distribution of *nodal* mRNA was apparently regulated by cortical cytoskeleton remodeling after sperm entry. Specifically, active Arp2/3 complex, which acts in reorganization of F-actin and microtubules, was co-localized with asymmetrically distributed *nodal* mRNA. When active Arp2/3 complex was blocked with an inhibitor CK666, blastulae were deformed and abnormally co-localized active Arp2/3 and *nodal*/*lefty* expressing cells.

Further, to examine whether the Nodal signaling in the *lefty-nodal* co-expression domain activates dorsal specific genes such as *goosecoid*, *chordin*, *not-like*, and *brachyry1* as seen in the oral ectoderm in sea urchin embryos, the Nodal signaling was blocked with SB505124. Treated embryos failed to express these genes and lacked the notochord and the neural plate beneath the epidermis in neurulae. This experiment confirms that the dorsal specific genes are directly or indirectly regulated by Nodal signaling as in the case of sea urchin oral ectoderm. Treated embryos also showed a small blastopore and resultant small primitive gut that was separated from the outer layer (ectoderm). The small blastopore and primitive gut are similar to those of echinoderm and hemichordate embryos.

Bmp2/4 and Chordin, which are the marker molecules for dorso-ventral polarity, are co-expressed by Nodal signaling on the ventral side of sea urchin embryos. However, Bmp2/4 is translocated and works on the dorsal side. Notably, I found that *bmp2/4* and *chordin* were co-expressed on the dorsal side in amphioxus embryos, and Bmp signaling detected with pSmad1/5/8 was activated on the ventral side of embryos. This distribution pattern is comparable to those of sea urchin embryos but is inversed in dorso-ventral polarity.

Although adult sea urchin body displays the penta-radial symmetry, sea urchin embryos develop as a bilaterally symmetrical body passing through cleavage and blastula stages similar to those of amphioxus. Here I showed a multiple lines of similarity between amphioxus and sea urchin embryos in molecular mechanisms underlying regional specification. In amphioxus embryos, the asymmetrical *lefty-nodal* co-expression domain, which is comparable to the sea urchin oral domain, is located on one side of the blastopore. The *lefty-nodal* co-expression domain promotes dorsal specification like the organizing center of vertebrates. The present findings support the hypothesis that dorso-ventral inversion occurred in the last common ancestor of chordates. Considering shared characters between outgroup echinoderm lineage and chordate amphioxus, developmental pattern found in amphioxus embryos is regarded as ancestral. I propose that extant amphioxus retains evidence of the dorso-ventral inversion in its development. The last common ancestor of deuterostomes may have developed passing through a coeloblastula stage, and in the chordate lineage the blastopore margin expanded toward the equator, which modified the ancestral oral region to be located on one side of the blastopore margin, from which chordate dorsal structures were newly formed.