Mechanism of tail shaping morphogenesis in the embryo of the ascidian, *Halocynthia roretzi*

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To understand how tissues become shaped is a fundamental issue in the field of developmental biology. In the ascidian embryo, the boundary between the head/trunk and tail regions can be first recognized morphologically at the middle level of the embryo along the anterior-posterior (A-P) axis as a bending of the epithelial layer at the late neurula stage. This initial tail shaping morphogenesis is quite different from what is seen in other animals in a way that it does not involve cell proliferation at the posterior end of the embryos. In order to understand how the tail is formed initially in ascidian embryos, we have been investigating which morphogenetic behavior of the individual epithelial cells underlies this early tail shaping process and how the position of the epithelial bending/trunk vs. tail boundary is determined along the A-P axis.

Using the live-imaging technique, we have discovered the following phenomena. First of all, two to four rows of rectangle-shaped epithelial cells become evident and are aligned around all the circumference of the embryo with the exception of the dorsal neural tube region right before the epithelial bending formation and at the bending-forming level along the A-P axis. Secondly, the formation of the epithelial bending is accompanied by epithelial cell divisions, in which the epithelial cells of the anterior and posterior parts of the embryo divide in different orientations. Cells in the anterior part divide along the circumference of the embryo whereas those in the posterior rotate their mitotic spindles 90 degree right before they divide along the A-P axis. This results in the formation of a sharp boundary of division orientation that is located one cell row ahead of the anterior and posterior epidermis lineage boundary generated by the second cleavage after fertilization. From these observations, we have proposed a model in which cell divisions with different division orientations create the difference in the length and cell number around the circumferences of the anterior and posterior parts of the embryo, and results in the bending formation around the division orientation boundary.

In order to test this model with our loss of functional analysis, we used chemical inhibitors to block the function of dynein, which is widely known to be involved in spindle positioning in the mitotic cells. In the inhibitor-treated embryos, we successfully abrogated the 90 degree spindle rotation that we had observed in the future tail region before the cell divisions, and observed the similar division orientation pattern between the anterior and posterior parts of the embryo. As a result, these embryos showed suppression of normal epithelial bending

formation, suggesting that different division orientation is required for this epithelial morphogenesis. These results support our model that cell divisions with different division orientations play a role in shaping the tail in the ascidian embryo.