Analyses of epithelial morphogenesis during tail formation in the embryos of the ascidian, *Halocynthia roretzi*

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ABSTRACT

Epithelial morphogenesis is one of the most important processes to create tissues and organs. The mechanisms of epithelial morphogensis have been extensively studied in many model systems. However, our knowledge about the role of oriented cell division in epithelial morphogenesis has been limited to elongation processes in several tissue types. In this study, we address this issue by analyzing mechanisms and roles in tissue shaping of cell division orientation regulation during tail formation in the ascidan embryos.

One of the remarkable morphogenetic processes in ascidian development is larval tail formation. As an initial stage of the tail formation, the boudary between trunk and tail regions appears morphologically as an epithelial bending (we call it “KUBIRE”) at the late neurula stage. By live imaging of epithelial cell behaviour in this process, we found that “KUBIRE” formation was accompanied by epithelial cell divisions. We also found that cells divided in different orientations in the anterior and posterior regions of the lateral side of the embryo with a clear boundary of division orientation around the “KUBIRE”-forming region. In the anterior and the future trunk region, the epitheilal cells divided around the circumference of the embryo. In contrast, in the posterior and the future tail region, the mitotic spindles of the epithelial cells rotated about 90 degree right before cell division and the cells divided along the anterior-posterior axis. Based on these observations, we have proposed a model in which “KUBIRE” is generated by different cell division orientation.

To test this model experimentaly, we phamacologically disrupted the rotation of the spindles and observed its effect on “KUBIRE” formation. When embryos were treated with an

inhibitor called Ciliobrevin D to block the function of dynein, which is known to regulate spindle positioning in many model systems, the orientation of the cell division became abnormal. In most cases, cells in the posterior region divided in the dorso-ventral orientation. As a result, these embryos failed to form normal “KUBIRE”, supporting our model that diferent cell division orientation is involved in the epithelial morphogenesis.

In our analyses of the ventral side of the embryo, we found that the division pattern was different from the lateral side. Epithelial cells close to the ventral midline divided around the circumference of the embryo. To examine how cell division orientations are regulated differently in the lateral and ventral regions, we asked whether the tissue located underneth the ventral region contributes to it. When we ablated B7.2 cells at the 110-cell stage, which are fated to become endoderm strand cells located underneath the ventral midline of the epithelial layer at the neurula stage, the overlying epithelial cells changed their division orientations and divided along the anterior-posterior axis. This result suggests that B7.2 progenitor cells induce ventral epithelial cells not to divide along the anterior-posterior axis. This result is interesting because similar cell-cell communication has been reported in another ascidian species *Ciona intestinalis*, in which signals from endoderm strand cells induce the overlying ventral epithelial cells to become epidermal sensory neurons. In *Halocynthia*, however, epidermal sensory neurons are missing on the ventral midline, but the induction event itself seems to be retained for what might be a different function.

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