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

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ABSTRACT

Morphogenesis is one of the important processes in animal development. To generate a new morphological structure or a new tissue, embryonic cells need to behave properly at right timing and right place. One of the remarkable morphognesis duirng ascidian development is the tail formation. In the initial stage of tail formation (i.e., late neurula stage), the boundary between trunk and tail regions is morphologically appeared as small waist (in japanese "Kubire") at the specific position along anterior-posterior axis. Tail region then undergoes extensive elongation along the anterior-posterior axis. At the tadpole larval stage, the length of tail is 4-5 times longer than that of the trunk region. The series of morphogenetic events duirng ascidian tail formation are unique compared to other animals such as vertebrates, annelids, and insects. Therefore, to understand the mechansims of tail formation of ascidian embryos would provide a novel principle about the mechanisms of morphogenesis.

In this study, we focus on the initail stage of tail shaping – the "Kubire" formation in the embryos of *Halocynthia roretzi*. We observed that "Kubire" was formed along with the cell division of epithelium. We also found that epithelial cells divided along different orientation, and there was a clear boundary between these oriented cell divisions. In the trunk region, cells divided around circumference of the embryo. In contralst, cells divided along anterior-posterior axis in the tail region. Our current hypothesis is that "Kubire" is generated by the different orientation of cell division, which occur on either side of a clear boundary in the embryo.

To test this hypothesis experimentaly, we phamacologically disrupted the orientation of the cell division of epithelium and observed "Kubire" formation of the embryos. When embryos were treated with a dynein inhibitor Ciliobrevin D, which is known to block spindle rotation and thus disrupt spindle orientation in many model systems, the orientation of the cell division was abnormal. Furthermore, these embryos failed to form "Kubire". This observation supports our hypothesis that cell division along different orientations generates a new morphological structure.

An important condition which makes the hypothesis valid is that circumference of the embryo needs to be closed. "Kubire" is formed at the late neurula stage when neural tube is almost closed. Interestingly, it has been known that neural tube is not closed in the embryos which are incubated without egg membrane. It is also known that these embryos do not form "Kubire". Therefore we currenly chareacterize the division pattern of epithelium cells of the embryos whose neural tube is not closed in the absence of egg membrane. We will also describe the orientation of cell division in the ventral epithelium during the "Kubire" formation.

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Regulation of germline gene expression during ascidian embryogenesis

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Germline is specialized lineage as it is the only lineage that transmits genetic information to the next generation. In many animals, germline is separated from somatic line in early embryogenesis. Transcriptional repression in early embryonic germline is important for the separation from somatic line. As embryogenesis progresses, germline transcription is de-repressed and zygotic transcription for germ cell formation, but not for somatic tissue formation, is started. The mechanisms by which germline transcription is regulated during embryogenesis are not much known.

Ascidian's germline cells inherit the centrosome-attracting body (CAB), which is morphologically similar to germ plasm. They also inherit maternal mRNAs known as *Postplasmic/PEM* RNAs which localize in the CAB. PEM, a member of *Postplasmic/PEM* RNAs represses transcription in the germline globally and contributes to the separation from somatic line. However, functions of other *Postplasmic / PEM* RNAs in the germline formation have not been analyzed. Therefore, using the ascidian, *Halocynthia roretzi*, we studied functions of the RNAs and focused on their roles in germline transcriptional repression. Here, we analyzed three *Postplasmic/PEM* RNAs: *POPK-1*; *PEM*; *ZF-1*.

Our results suggest that 1) POPK-1 contributes to repress germline transcription indirectly by regulating the proper CAB formation, which ensures that the sufficient amount of *PEM* mRNA is localized to the CAB and translated for transcriptional repression, 2) decreasing PEM protein level is necessary and sufficient for the de-repression of germline gene expression, indicating that PEM not only suppresses somatic gene expression, but also controls the timing of germline zygotic transcription, 3) ZF-1 accelerates zygotic gene expression in the germline by decreasing the PEM protein level.