Expression analysis of key genes for germ layer specification in mollusc: insight into the evolution of spiralian development

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Introduction

Spiralia is known as an animal clade which mainly include mollusks, annelids, platyhelminths, etc. Unique characteristics of Spiralia include its spiral cleavage during early development and its early cell fate segregation along animal-vegetal axis. (Lambert 2010). However, it remains unclear about the gene regulatory network (GRN) for cell fate segregation and germ layer specification. Recent studies suggest that the insertion of novel TALE transcription factor, Spiralian-TALE (SPILE), into GRN of early development may have contributed to the evolution of the cell fate separation mechanism (Morino et al. 2017)

Despite the previous research, it is still unclear how the SPILE genes could have been inserted in early developmental GRN without disrupting the downstream development. As an initial step to solve it, this study aimed to verify whether the expression sites of key transcription factors for germ layer specification were affected by the insertion of the SPILE gene. Since Echinodermata (sea urchin)'s mesoderm genes in GRN were best understood (Peter. et al 2012)). It would be a good comparison to mollusks. I chose five genes (*alx, erg, ets-1, hex, tgif*) which were crucial in mesoderm specification of sea urchin.　In this study, I conducted *in situ* hybridization of the five genes, using *Nipponacmea fuscoviridis* (Nf) embryo samples to observe the expression patterns of those genes. In addition, I did comparative studies of results in limpets with other non-spiralian animals to test my question.

Material & Method

I collected samples of *Nipponacmea fuscoviridis* and fixed their embryos in several developmental stages in germ layer differentiation occur (4 and 5 hpf, gastrula; 7 hpf, early trochophore; and 10hpf, trochophore). After that, I performed *in situ* hybridization according to the method described in Morino et al., 2017.

Results

From my observation, there is no clear signal of gene *hex*, so I will discuss the genes except *hex*. *alx* expressed in only apical ectoderm in gastrula and early trochophore. In trochophore stage, the expression of *alx* was detected in ectomesoderm. For *erg*, consistent expression in apical ectoderm was observed from gastrula to trochophore. For *ets-1*, from gastrula to trochophore stages, the expression in apical ectoderm and posterior epidermis were observed. Since the expression were shown in epidermis, I judged the posterior expression site was in ectoderm. For *tgif*, from gastrula to early trochophore, expression in apical ectoderm and posterior epidermis region were observed, which might be ectoderm. In trochophore stage, there was no expression detected in apical region, but bilateral and posterior expression in epidermis was still detected.

Discussion

In sea urchin, all five genes were expressed in mesodermal cells. In cnidaria, although there are no data of gene *tgif*, three of the genes (*erg, ets-1, hex*) were found to be expressed in endomesoderm region. In limpet, only one gene, *alx* was shown expression in mesoderm.

Through those comparisons, there were obvious differences in the expressions of those genes, which suggested that there is change in expression patterns of spiralian lineages. From those results, it might imply that the insertion of novel gene SPILE had effect on the expression patterns of downstream genes in spiralian's GRN. However, further research like expression patterns of other downstream transcription factors and SPILE gene function analysis are required to reveal evolutionary history of early developmental GRN in spiralian development.

References

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