Evolution of mesoderm specification mechanism in echinoderms with insight from functional change of the *hesC* gene

Nina　Levin（筑波大学 生物学類）　　指導教員：和田　洋（筑波大学 生命環境系）

Introduction

Change in developmental processes is the driving force producing phenotypic diversity. However, the evolutionary alterations that cause phenotype change is not well understood. Developmental system drift (DSD) is a phenomenon where evolutionary processes alter genetic background without apparent change in phenotype. It is suggested that the build-up of these genetic changes over evolution can trigger phenotype change, thus allowing us to hypothesize that DSD may act as an intermediate for the rise of novel phenotype. Therefore, studying the mechanism for DSD occurrence with insight from the transition of mesoderm specification in echinoderms is beneficial for understanding fundamental evolutionary processes giving rise to phenotypic diversity.

This research utilizes starfish with an ancestral-like mesoderm regulatory pathway and the euechinoid sea urchin with a derived mesoderm regulatory pathway. The expressions of mesoderm related genes, such as *ets and pmar1*/pmar1-like gene, in both starfish and euechinoids seem to be fairly conserved, allowing specification of the mesoderm at vegetal pole of embryos during early development. However, the regulation for this specification seems to differ between these two groups. In euechinoids, the *hesC* transcription factor plays a derived regulatory role of repressing mesodermal genes (*ets*, *delta*) throughout the entire embryo except for the vegetal pole, allowing for specification of mesoderm to just the vegetal pole. Starfish *hesC*, located downstream of delta seems to function differently, not associated with mesoderm specification. This transition of *hesC* gene function to an essential upstream mesoderm regulator without significantly altering phenotypic outcome exemplifies the occurrence of DSD. Revealing the molecular alteration relevant to the evolution of *hesC* function can allow us to further understand the mechanism for evolutionary modifications leading to phenotype change.

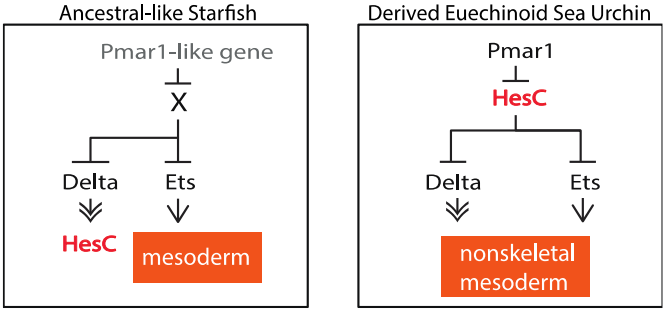

Fig. 1. Mesoderm regulatory pathway in starfish and euechinoids

Methodology

To understand if *hesC* function change is due to the difference in *hesC* protein itself, overexpression of starfish *hesC* mRNA in sea urchin fertilized eggs was performed by microinjection. At the gastrula stage (28hpf), injected embryos were fixed and skeletogenic mesenchyme cells stained using P4 antibody markers. Observation of these results can indicate if starfish *hesC* is capable of repressing sea urchin mesodermal genes (*delta*, *ets*), similarly as sea urchin *hesC*.

Preliminary results

Overexpression (OE) of starfish *hesC* in sea urchin embryos with concentrations of 50 ng/µl resulted in overall decrease in primary mesenchyme cells (Fig.2b). Concentration of 100 ng/µl resulted in overall decrease of primary mesenchyme cells and restricted archenteron growth (Fig.2c). 500 ng/µl resulted in overall deformation of the embryos (Fig. 2d). Embryos were then stained showing an overall decrease of skeletogenic mesenchyme for starfish *hesC* concentrations of 50 ng/µl (Fig. 2f). For those injected with higher concentrations, all embryos lacked any P4 staining (Fig. 2g,h). These results can suggest that starfish *hesC*, like sea urchin *hesC*, is capable of functioning as a regulatory repressor within the mesoderm regulatory pathway of sea urchins. However, further trials and experimentation must be done to confirm these results.

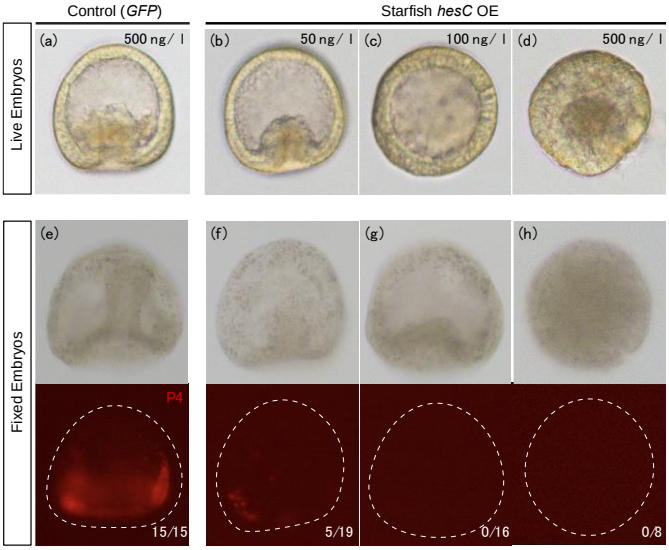

Fig. 2. Observation of sea urchin embryos injected with starfish *hesC*.

Discussion

The initial hypothesis for this research was that the change in *hesC* function was due to the modification of HesC protein itself rather than change in the binding region of multiple downstream cis elements. Ability of starfish *hesC* to act as a mesoderm regulator in sea urchins can suggest the similarity of *hesC* proteins. Thus, to understand the evolution of *hesC* function, analysing changes within downstream cis elements (such as *ets*, *delta*) and revealing possible protein partners of HesC will be some of the future aims.