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Developmental Genetics in the Ascidian *Ciona intestinalis*

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The ascidian *Ciona intestinalis* provides an appealingly simple experimental system for the investigation of the molecular mechanisms underlying cell-fate specification during development. The draft genome sequences and a large EST/cDNA databases supports the study of gene functions. However, forward genetics, as exemplified by germline transgenesis and creation of mutants, has not been developed in the marine invertebrates. Our recent research focused on the establishment of such genetic approaches in *Ciona*. Currently, creation of stable transgenic lines, enhancer trappings and insertional mutagenesis have been successfully introduced in *Ciona* with *Minos* transposon.

Germline transgenesis of *Ciona intestinalis* with *Minos* transposon

Minos is a member of Tc1/*mariner* superfamily transposon derived from an insect, *Drosophila hydei*. *Minos* has the high activity for germline transformation in *Ciona intestinalis*. When *Minos* transposon DNA is introduced in *Ciona* with its transposase enzymes, the transposon is excised from DNA and transposed in *Ciona* genome. As much as 37% of *Minos*-injected *C. intestinalis* transmitted transposon insertions to the subsequent generation. *Minos* can also be applicable to enhancer trappings. By these techniques, many useful transgenic lines which show tissue-specific reporter gene expression have been generated. These marker lines provide useful resource for future studies of gene expression and functions.

Insertional mutagenesis revealed the function of ascidian cellulose

Cellulose biosynthesis is a major characteristic of tunicates. The genome project of *Ciona intestinalis* revealed that *Ciona* has a gene encoding cellulose synthase *Ci-CesA*. Further study has suggested that *Ci-CesA* was acquired in ascidians by lateral gene transfer from bacteria. The functions of animal cellulose have been uncovered by insertional mutagenesis with *Minos*.

We have successfully created several insertional mutants by *Minos* system. One mutant named *swimming juvenile* (*sj*) is the mutant of *Ci-CesA*. *sj* showed defects in the cellulose biosynthesis and tunic formation. In addition, *sj* showed abnormal metamorphosis (the most initial step of settlement) and severe defects in the ability of settlement. These phenotypes suggest that cellulose biosynthesis may have been closely related to the acquisition of the life style of settlement during the evolution of ascidians.

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