Thesis Summary

Targeted mutagenesis using CRISPR-Cas9 in sea urchin, *Hemicentrotus pulcherrimus*
(バフンウニにおける CRISPR-Cas9 を用いた標的遺伝子への変異導入)

Name　LIU DAMING

1. Background and purpose of the research

Sea urchins are used as a model organism for research on developmental biology and gene regulatory networks during early development. Gene knockdown by microinjection of morpholino antisense oligonucleotide (MASO) has been used to analyze gene function in early sea urchin embryos. However, as the effect of MASO is not long lasting, it is impossible to perturb genes expressed during late development by MASO. Recent advances in genome editing technologies have enabled gene modification in various organisms. Genome editing in the sea urchin *Hemicentrotus pulcherrimus* using zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) has been reported previously; however, the efficiencies of these technologies were not satisfactory. A higher mutagenesis efficiency is indispensable for detailed analysis of gene functions of *H. pulcherrimus*. Furthermore, the effect of genome editing in adult sea urchin after metamorphosis has never been studied.

In this thesis, to establish an efficient gene knockout method, I employed CRISPR-Cas9 system in *H. pulcherrimus*.

2. Establishment of an efficient gene knockout method in *H. pulcherrimus* using CRISPR-Cas9

To validate the feasibility of genome editing with CRISPR-Cas9 in the sea urchin *H. pulcherrimus*, I targeted *HpNodal* gene that has the pivotal role in the dorso-ventral pattern formation in sea urchin embryos. Efficient mutagenesis was detected within 24 hr post fertilization (hpf) by microinjection of *SpCas9* mRNA and sgRNA and radialized phenotype was observed at the prism stage (Fig.1), indicating that the CRISPR-Cas9 can be used in *H. pulcherrimus* to efficiently induce mutations.

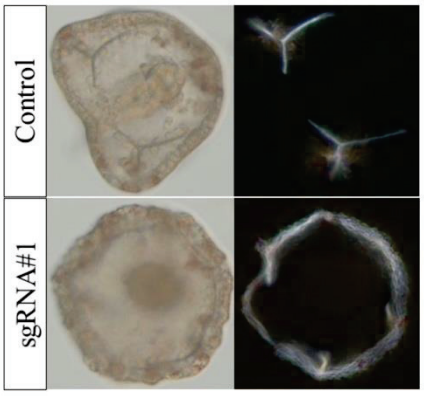

Fig.1 Phenotype of *HpNodal* knockout embryo

3. Establishment of knockout adult sea urchins by using CRISPR-Cas9

Pigmentation-related *Pks1* homolog of *H. pulcherrimus* (*HpPks1*) was targeted using the CRISPR-Cas9-mediated knockout method. I prepared 3 sgRNAs targeting 2nd coding exon of *HpPks1* gene. Highly efficient mutagenesis was achieved by co-injection of sgRNA with *SpCas9* mRNA, and one of the sgRNAs yielded 100% mutagenesis efficiency (Fig2a and b). The mutagenesis was first detected at 6 hpf and reached a maximum level at 12 hpf. However, no off-target effect was detected, suggesting that CRISPR - Cas9 - mediated mutagenesis targeting *HpPks1* gene in this study was highly specific to the target site. In addition, when I analyzed the phenotype of the SpCas9/sgRNA - injected embryos, the albino phenotype was observed. SpCas9/sgRNA - injected pluteus larvae showed no pigmentation (Fig2c, top), and this albino phenotype was maintained in juvenile sea urchins after metamorphosis (Fig2c, middle), and the knockout sea urchins survived for at least one year and grew to albino adult sea urchins (Fig2c, bottom). This albino phenotype was observed in the entire body even in skeletons. Furthermore, I revealed that the embryonic albino phenotype was not due to loss of secondary mesenchyme cells but simply due to disruption of pigmentation enzyme. These findings suggest that knockout adult sea urchins were successfully established and the CRISPR-Cas9 is a feasible method for analyzing gene functions from late developmental to adult stage.

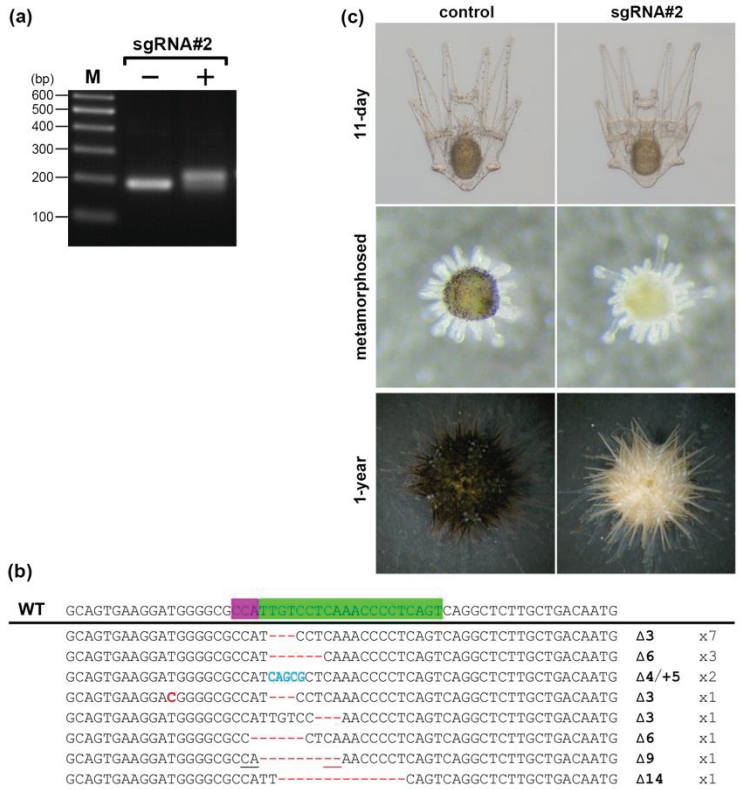

Fig.2 CRISPR-Cas9-mediated knockout of *HpPks1* gene.

(a) Heteroduplex mobility shift assay.

(b) Sequencing analysis.

(c) Albino phenotype observed in SpCas9/sgRNA-injected larvae and adult sea urchins.