論文 / 著書情報
Article / Book Information

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Title(English)	
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種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 生命理工学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ 理学 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

During the process of evolution, multicellular organisms have acquired the ability to regenerate damaged tissues and organs, which enabled them to survive for long periods as individual organisms. Although our understanding of the regeneration mechanism has advanced in the past decades, an important question of how tissues sense injuries and initiate the regeneration program remains largely unexplored. Research into this question will contribute to human regenerative medicine.

There are significant differences in regenerative ability among species and tissues. For example, mammals, including humans have relatively poor regenerative ability, while fish and amphibians have remarkable regenerative ability in various tissues. Fins of zebrafish have been used as a good model for exploring the regeneration mechanism. Recent research in this field has shown that a group of genes that may play important roles in regeneration is induced during regeneration. It is thought that enhancer elements in response to injuries may be involved in regulating the expression of these genes. Indeed, several studies have recently identified regeneration-response enhancers (RREs). Interestingly, it was shown that an identified RRE works both in the regeneration of the caudal fin and heart of the zebrafish. Furthermore, the RRE was also activated by finger amputation in mice.

In the preceding study in our group, several RREs have been identified in the zebrafish *fibronectin1b (fn1b)* promoter. The study demonstrated that RREs commonly contain two transcription factor (TF) binding motifs: E-box, a motif for bHLH TFs including Tcf/Lef, and TRE, a motif for bZIP TFs including Jun/Fos. Also, the study has shown that the combination of these TF binding motifs displays the RRE activity. Thus, the entity of RREs has been revealed; however, the pathways from injuries to RRE activation are still an open question. In this study, I aimed to explore the pathways and regulation mechanisms from injury to RRE response.

By using the RRE transgenic (Tg) lines that show the EGFP expression by RRE activation, firstly I investigated the upstream signals for RRE activation. The Jun N-terminal kinase (JNK) is known to phosphorylate the Jun to regulate its transcriptional activity. I found that JNK inhibitor treatment impaired amputation-induced RRE response and fin regeneration. Further, I tested the inhibition of the E-box-mediated signal by expressing the dominant-negative Lef1 (dnLef1), which lacks the β-catenin binding domain and suppresses the activator function of bHLHs including the Tcf/Lef. The dnLef1 expression also impaired RRE response and fin regeneration. These results indicated that the cooperative action of bHLH-E-box and Jun/Fos-TRE signals are required for the transcriptional response of RREs and fin regeneration.

Secondly, I verified whether RRE can be activated by injuries in various zebrafish tissues, as zebrafish can regenerate a variety of tissues besides the caudal fin. I tested RRE responses to pectoral fin amputation, scale removal, and partial heart ventricle resection and observed that EGFP expression by RRE response was caused in all cases. Furthermore, I introduced the RRE reporter construct into *Xenopus laevis* eggs and showed that RRE response was induced by the limb bud amputation of *Xenopus* tadpole. These results indicated that the identified RREs are activated during regeneration regardless of tissues or species.

Thirdly, to further explore the cues required for RRE activation, I investigated the RRE activation in various injury models. Though the RRE response was not caused by injuries such as skin scratches or inter-ray incisions, the response was induced by fin puncture. Intriguingly, RRE response was induced both on the proximal and distal sides of the punctured hole, whereas the expression of the blastema marker, *msxc*, was only induced on the proximal side. These observations suggested that RRE is activated by an injury signal and is independent of blastema formation.

To further verify the relation between RRE response and blastema induction, I developed a new injury model that does not cause severe tissue damage. I established a new injury method, mild cryoinjury, in which the local fin area was injured using a cooled needle. By this model, I showed that RRE activation was induced, but it did not cause the expression of the blastema marker, *msxc*. Together, these results suggest that the RRE response was induced by an injury signal, and that RRE activation is not necessarily accompanied by the blastema formation. The cryoinjury model and RRE Tg may provide an excellent platform for exploring the injury signal that is required for RRE activation.