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Transgenic Ascidians

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Foreword

The ascidian *Ciona* provides one of the best experimental systems in developmental biology, developmental genomics, and evolutionary developmental biology. We ascidian developmental biologists are justifiably proud of the fact that we established the *Ciona* system.

In traditional embryology, ascidians were recognized as organisms exhibiting a "mosaic-mode of embryogenesis", in contrast to the regulatory mode of embryogenesis seen, for example, in sea urchins. A detailed description of embryonic cell lineage by Edwin G. Conklin revealed that the mosaic-mode of embryogenesis is determined by determinate cleavage of the embryo and a robust pattern of differentiation and morphogenesis. Maternal factors and cellular communications that play essential roles in gastrulation, neurulation, tailbud-embryo formation, and tadpole-type larval development work together under the harmonious, but rigid control of a gene regulatory network. In addition, newly hatched larvae comprise only about 2600 cells, including 40 notochord cells and 38 muscle cells. Thus, ascidians are considered embryonically simple compared to vertebrates and other deuterostomes, such as sea urchins. In other words, ascidians represent a system to challenge the most basic question of developmental biology: How does a complex, multicellular, metazoan body arise from a single cell, the fertilized egg?

Decoding of the draft genome of *Ciona intestinalis* in 2002 identified almost all of the developmentally relevant genes in its genome, which represent the basic set of gene components in chordates prior to the two rounds of genome duplication that occurred in the vertebrate lineage. The absence of redundancy in ascidian regulatory gene functions makes it easier to ascertain developmental roles of individual genes. In association with the genome sequencing project, a cDNA sequencing project was also carried out, providing a great quantity of information on expression profiles of regulatory genes. Many whole-mount in situ hybridization studies reveal distinct gene expression profiles in embryos at the single-cell level. Furthermore, results obtained from embryological research provide straightforward insights into chordate evolution as well as the origin of vertebrates, because ascidians are the closest relatives to vertebrates.

In this research environment, it is desirable to introduce new, state-of-the-art techniques into the *Ciona* system, with the most valuable at this time being "transgenic" techniques. These include microinjection and/or electroporation of exogenous DNA to reveal mechanisms involved in gene regulatory networks, germ-line transgenesis to create various marker lines for

studies of gene regulation, and TALEN-based and CRISPR/Cas9-based knockout lines to facilitate studies of gene function.

This book, edited by Prof. Yasunori Sasakura at the University of Tsukuba, constitutes a very timely discussion of recent advances in this field. In addition, most authors of this book are new or mid-career researchers. Indeed, we owe them further development of the ascidian systems. Lastly, I wish to express once again my conviction that *Ciona* is the most promising system to explore molecular and genetic mechanisms involved in animal embryogenesis at the single-cell level, the tissue and organ level, and the individual level.

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Preface

Undoubtedly, transgenic technologies are inevitable for studying molecular functions during development. In several model ascidian species, creation of transgenic animals has been routinely performed, and the methods are easily retrieved from many articles and from some book chapters. However, the details of the methods have not always been provided in the literature. On the one hand, microinjection is basically a simple method for introducing exogenous DNAs without the requirement of an expensive machine. At the same time, because microinjection can be a relatively difficult process, many laboratories have their own practical tips that are suitable for their study of species. These tips are usually not seen in original articles whose main purpose is describing development mechanisms. To solve the issue, this book has been purposed to gather and describe the devices that have arisen from our own great interest and enthusiasm, and that are not usually seen or known as major methods for performing transgenesis in ascidians.

I hope this book will be useful for all researchers, including beginners in tunicate research who wish to introduce a tunicate in their laboratories and also specialists of tunicates who wish to find a clue to improve their methods.

Shimoda, Japan — Yasunori Sasakura

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