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INTRODUCTION

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Genomewide surveys of developmentally relevant genes in *Ciona intestinalis*

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Ascidians belong to the subphylum Urochordata, the earliest branch in the phylum Chordata. Ascidian larvae represent a prototype of the chordate body plan: the larval tail contains an axial notochord flanked dorsally by the nerve cord, ventrally by the endodermal strand and bilaterally by muscle cells (Satoh 1994, 2003; Corbo et al. 2001). Compared to the genomes of vertebrates, the ascidian genome is compact and simple. The haploid genome of *Ciona intestinalis* was previously estimated to be ~160 Mbp in size and to contain 15,500 genes (Simmen et al. 1998). Indeed, the *C. intestinalis* draft genome sequence we determined showed that this estimate was quite accurate; its 153~159 Mbp genome is estimated to contain 15,852 protein-coding genes (Dehal et al. 2002). The ascidian genome contains a basic set of genes for the chordate body plan with less genetic redundancy than the genomes of vertebrates; in other words, the ascidian genome is thought to retain the original form that existed before genome duplications occurred during vertebrate evolution (Holland et al. 1994; Dehal et al. 2002). Knowledge about the *C. intestinalis* genome should therefore provide us insight into the origin and evolution of chordates.

Ascidians also provide an appealingly simple experimental system for investigating the molecular mechanisms that underlie cell-fate specification, morphogenesis, and metamorphosis during development. First, the fertilized egg develops rather quickly, through bilaterally symmetrical cleavage, gastrulation, neurulation, and tail-bud embryo formation, into a tadpole larva. The ascidian tadpole is composed of only ~ 2,600 cells, which constitute a small number of organs and tissues including epidermis, central nervous system, endoderm and mesenchyme in the trunk, and notochord and muscle in the tail. Second, extensive information on the cell lineage of most major organs of the larva (Conklin 1905; Nishida 1987) as well as adult organs and tissues (Hirano and Nishida 1997) is available. Third, the early embryonic cells with definite developmental fates are large and easy to manipulate (Nishida 2002). Fourth, the embryonic cells also permit the detailed visualization of differential gene expression during development (Satou et al. 2002a). In situ hybridization analysis shows that signals for zygotic expression of developmental genes is first detectable in the nuclei of certain blastomeres, permitting us to distinguish which cells, as well as when, begin the expression of the gene.

Fifth, novel functions of developmentally relevant genes can be determined by misexpressing or overexpressing a variety of regulatory genes that encode transcription factors or signaling molecules or by the functional suppression of genes with morpholino antisense oligonucleotides (Satou et al. 2001). One such study focused on the endodermal development of ascidian embryos, which is controlled by maternal β-catenin via what appears to be one of the highly conserved mechanisms among metazoans (Imai et al. 2000). β-Catenin triggers the expression of its downstream genes, including *Lhx3*, *Fgf9/16/20*, *FoxD* and *ZicL* (Satou et al. 2001). Knockdown studies of these genes revealed that *Lhx3*, which is expressed in the presumptive endoderm, plays an essential role in the differentiation of the endoderm (Satou et al. 2001), that *Fgf9/16/20* is essential for induction of the mesenchyme by the endoderm precursors (Imai et al. 2002a), and that *FoxD* and *ZicL* are essential for specification of the notochord, which is also induced by the endoderm precursors (Imai et al. 2002b, c). Although those experiments were done using a closely related species of ascidian, *Ciona savignyi*, the findings should also hold true for *C. intestinalis*. There are at least 2, 3, 4 and 5 vertebrate orthologues for *Ciona Lhx*-3, *Fgf9/16/20*, *FoxD* and *ZicL*, respectively. As these findings indicate, the ascidian is one of the ideal model systems for understanding vertebrate development, be-

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cause of the low redundancy of related genes. Sixth, transgenic DNAs can be introduced into fertilized eggs using simple electroporation methods that permit the simultaneous transformation of hundreds, even thousands, of synchronously developing embryos, providing a special advantage in exploring *cis*-regulatory systems governing specific gene expression (Corbo et al. 1997). Genomewide promoter analysis of developmentally regulated genes may be achieved by this method (Harafuji et al. 2002). Seventh, embryogenesis of the most studied ascidians *C. intestinalis* and *C. savignyi* is rapid and the entire life cycle takes less than 3 months, facilitating mutagenesis and mutant screening (Nakatani et al. 1999).

Under such research circumstances, it is necessary to know whether or not the genes of interest exist in the ascidian genome, and the number of copies of these genes. In the following studies of this issue of *Development, Genes and Evolution*, we have surveyed developmentally relevant genes against the draft genome sequence of *C. intestinalis*. These include (I) genes for bHLH transcription factors, (II) genes for homeobox transcription factors, (III) genes for Fox, ETS, nuclear receptors and NFκB, (IV) genes for HMG transcriptional regulators, bZip and GATA/Gli/Zic/Snail, (V) genes for receptor tyrosine kinase pathway and Notch signaling pathway, (VI) genes for Wnt, TGFβ, hedgehog and JAK/STAT signaling pathways, (VII) genes involved in the regulation of cell polarity and actin dynamics, (VIII) genes for PI3K signaling and cell cycle, (IX) genes for muscle structural proteins, and (X) genes for cell junctions and extracellular matrix. The results provide molecular information for further investigation of complex developmental processes, and an insight into the chordate evolution as well.

To analyze the function of a gene, it is necessary to clone the corresponding cDNA. To address this need, we made a cDNA collection (the current version consists of so-called release 1 and release-1 supplements) that contains a set of ~17,800 non-redundant cDNAs clones (Satou et al. 2002b). In the following studies, we also have checked whether or not the corresponding cDNA is in the cDNA collection. Actually, about 85% of previously identified genes were found in our cDNA collection. Thus, at present, ascidians are among the experimental animals with the best-characterized molecular background. With the cDNA resources described above and the complete genomic sequence information, the ascidian experimental system will be able to contribute to our understanding of the origins of chordates, as indicated, for example, by the following studies.

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