DIVISION OF MORPHOGENESIS

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The complex morphogenesis of organisms is achieved by consecutive cell-to-cell interactions of germ layers as well as tissues during development. Recent studies suggest that polypeptide growth factors (PGFs) are essential component controlling such intercellular communications in a variety of organisms. These cell communications via PGFs are regulated by a number of processes including secretion, activation, diffusion, reception by specific receptors and intracellualr signaling. In addtion to secretory factors, transcription factors which act cell-autonomously have critical roles in the determination of cell fates. Our main interest is to know how pattern formation in morphogenesis is regulated by PGFs and transcription factors during development. We address this problem using several model animals, including frog, fly, acidians and nematode with the view of extracellular and intracellular signaling, employing embryology, genetics, molecular and cellular biology and biochemistry. In addition, we are currently introducing micro/macroarray technology to understand precise genetic program controlling early development.

I. Role of Xmsx-1 as a negative regulator of head formation

Embryos are patterned along dorso-ventral (DV) axis by the action of PGFs. Signaling triggered by PGFs leads to the activation of their target genes. Several homeobox genes are induced in response to PGFs in early *Xenopus* development. In particular, Xmsx-1, an amphibian homologue of vertebrate Msx-1, is well characterized as a target gene of bone morphogenetic protein (BMP). In order to clarify molecular basis for ventalization by BMP and in vivo significance of Msx-1, we examined whether Xmsx-1 activity is required in the endogenous ventralizing pathway, using a dominant-negative form of Xmsx-1 (VP-Xmsx-1). The VP-Xmsx-1 induced a secondary body axis, complete with muscle and neural tissues, when the fusion protein was overexpressed in ventral blastomeres. Interestingly, a more potent dominat negative Xmsx-1 induced ectopic head with eyes and cement gland. These results suggest that Xmsx-1 activity is necessary for both mesoderm and ectoderm to be ventralized and that head formation may represent a default state of BMP/Msx1 activity. We are currently investigating the molecular machanism of head repression in ventral side of embryo.

II. Genetic dissection of TGF-β/BMP signaling utilizing *Drosophila* model system

Fruit fly (*Drosophila melanogaster*) is one of the ideal model organisms to elucidate certain intracellular signal transduction pathways by genetic methods. A Drosophila BMP homolog, Decapentaplegic (Dpp) plays crucial roles in a number of developmental events including patterning of the adult wing. We have established two transgenic lines that express activated Dpp/BMP receptors. These mutants exhibit stable Dpp-gain-of-function phenotypes in the wing. One of the transgenic lines, *tkv-CA::71B*, expressed constitutively activated Tkv (Dpp type-I receptor) shows ectopic wing vein formation in the entire wing blade (Fig. 1A). Another line, *ALK2::69B*, expresses activated vertebrate BMP type-I receptor and shows abnormal wing outgrowth toward anterior and posterior direction (Fig. 1B). These phenotypes are sensitive to the gene dosage of the Dpp signal component such as Mad (Fig. 1C). Combinatorial screening has been done utilizing a Deficiency-kit (minimum set of the deficiency mutant lines that covers about 70 % of the Drosophila genome) and P-element insertion lethal stocks from public stock centers. We isolated 19 dominant suppressers to either or both *tkv-CA::71B* and *ALK2::69B*. We named these mutants *Suppresser of constitutively activated Dpp signaling* (*Scad*). Alleles of punt, Mad, shn and dCrebA were found in the isolated Scad mutants. It has been reported that the function of these genes are involved in the Dpp signaling. We focused to further analyze two novel loci, Scad67 and *Scad78* at a molecular level. Candidate genes for *Scad67* and *Scad78* were isolated. *Scad67* encodes a novel putative nuclear protein of a putative Zn-finger motif with a weak homology to the PIAS family transcriptional regulators. *Scad78* encodes a homolog of vertebrate putative transcription co-factor TRAP240. Mosaic analyses of *Scad67* and *Scad78* mutants suggest that these putative transcriptional regulators are involved not only in the Dpp signal transduction but also in other signal transduction cascades.

We have also been interested in the function of a MAPKKK class protein kinase, TGF-β activated

kinase1 (TAK1). TAK1 was originally isolated as a downstream signal component of TGF-β and BMP receptors. TAK1 function was extensively studied in cultured cells, however, the in vivo function of TAK1 has not yet been fully understood. To dissect in vivo function of TAK1, we generated transgenic flies which express an activated form and a dominant negative form of TAK1s (Drosophila and mouse TAK1s) in the fly visual system. Ectopic activation of TAK1 signaling led to ectopic apoptosis and resulted in small eye phenotype. Genetic and biochemical analyses indicated that the JNK signaling pathway is specifically activated by TAK1. Loss-of-function analysis using dominant negative TAK1s suggested that TAK1 participates in cell movement, cell shape control and apoptosis in *Drosophila*. Recently candidates of *dTAK1* mutants were isolated. Phenotypic analysis of these mutants is ongoing.

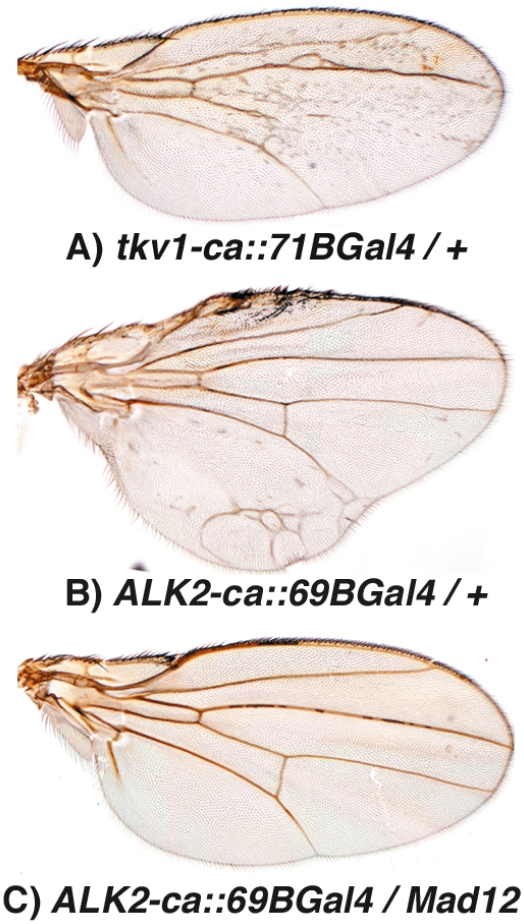

Figure 1. The wing phenotypes genetic interaction utilized for the screening. Expression of activated Dpp/BMP type-I receptors are resulted in the stable wing phenotypes (A, B). These phenotype is sensitive to the dosage of Dpp signal comonent *Mad* (C).

III. Brachyury downstream notochord differentiation in the ascidian embryo

Ascidians, urochordates, are one of the three chordate groups, and the ascidian tadpole is thought to represent the most simplified and primitive chordate body plan. It contains a notochord, which is a defining characteristic of chordate embryo composed of only 40 cells. To understand the morphogenesis in this simple system, we have focused on a gene, *Brachyury*, which is known to play an important role in the notochord development. In ascidian, *Brachyury* is expressed exclusively in the notochord and the misexpression of the *Brachyury* gene (*Ci-Bra*) of *Ciona intestinalis* is sufficient to transform endoderm into notochord. This gene encodes a sequence-specific activator that contains a T-box DNA-binding domain, and in vertebrates, it is initially expressed throughout the presumptive mesoderm and gradually restricted to the developing notochord and tailbud. The phenotype of the *Brachyury* mutants in mice and zebrafish revealed that this gene is essential for notochord differentiation. Our goal is to elucidate the down stream pathway of this important gene in ascidian in order to set the stage for understanding not only the formation and function of the notochord but how this important structure has evolved. We conducted the subtractive hybridization screens to identify potential *Brachyury* target genes that are induced upon *Ci-Bra* overexpression. Out of 501 independent cDNA clones that were induced cDNAs, 38 were specifically expressed in notochord cells (Fig. 2). We characterized 20 of them by determining the complete nucleotide sequences and *in situ* hybridization analyses which show the spatial and temporal expression patterns of the cDNAs. These potential *Ci-Bra* downstream genes appear to encode a broad spectrum of divergent proteins associated with notochord formation and function.

IV. TGF-β family in nematode

We have previously shown that CET-1/DBL-1, a member of TGF-β superfamily regulates body length in *C. elegans*. To understand molecular mechanism of body length regulation by the secreted factor, we aimed to identify target genes regulated by CET-1/DBL-1 signaling using a cDNA-based array analysis followed by functional analysis with double stranded RNAi. We identified yk298h6 to be one of genes suppressed in CET-1/DBL-1 overexpressing worm with a shortened body length. Disruption of yk298h6 resulted in long worm, suggesting that it encodes a negative regulator of body length.

yk298h was then mapped to, and shown to be identical with, *lon-1*, a known gene that affects body length. LON-1 encodes a protein with a motif sequence that is conserved from plants to human. Expression studies confirm that LON-1 is repressed CET-1/DBL-1 suggesting that LON-1 is a novel downstream component of the *C. elegans* TGF-β.

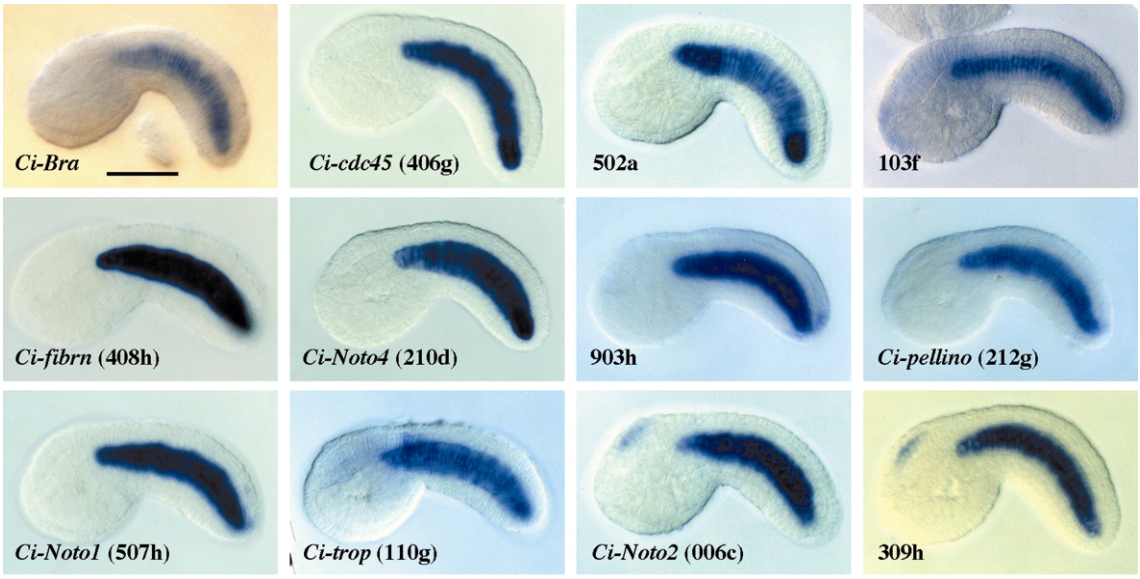

Figure 2. Expression of *Ci-Bra* and its downstream notochord genes in *C. intestinalis* tailbud embryos revealed by whole-mount *in situ* hybridization. Scale bar, 100 μm.

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