NATIONAL INSTITUTE FOR BASIC BIOLOGY
DEVELOPMENTAL BIOLOGY

DIVISION OF MORPHOGENESIS

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The complex morphogenesis of organisms is achieved by consecutive cell-to-cell interactions during development. Recent studies suggest that growth factors play crucial roles in controlling such intercellular communications in a variety of organisms. In addition to secretory factors that trigger intracellular signaling, transcription factors that act in the nucleus to regulate gene expression are thought to be essential for the determination of cell fates. Our main interest is to know how pattern formation and morphogenesis during development is regulated by these growth factors and transcription factors. We address this problem using several model animals, including frog, fly and ascidian, employing embryology, genetics, molecular and cellular biology, and biochemistry. In addition, we have recently introduced genomics technologies to elucidate the precise genetic programs controlling early development.

I. Molecular and cellular mechanism of vertebrate gastrulation

Gastrulation is one of the most important processes during morphogenesis of early embryo, involving dynamic cell migration and change in embryo shape. Almost all animals undergo gastrulation to form the gut. In spite of its importance, the mechanism underlying the event has just begun to be studied at molecular level. During *Xenopus* gastrulation, mesodermal cells migrate to the inside of the embryo and move on the blastocoel roof. One of the important mechanisms for this process is the cell movement called “convergent extension (CE)”. As convergent extension begins, cells are polarized and aligned mediolaterally, followed by the mutual intercalation of the polarized cells. In the regulation of convergent extension, several growth factors are implicated. Recent studies revealed that one of the Wnt signaling pathways, called Wnt/JNK (c-Jun N-terminal kinase) pathway, is shown to be important for the regulation of convergent extension. The pathway is highly conserved among species and initially found to be essential for the establishment of planar cell polarity (PCP) of *Drosophila* wing hair and often called Wnt/PCP pathway.

To clarify the molecular mechanism of gastrulation, we have been attempting to identify novel regulatory components controlling gastrulation cell movements by an expression cloning based on morphology of dorsal explant (Keller’s explant) and of embryo. One of identified genes was found to encode a member of the family of ArfGAP, GTPase activating protein (GAP) for ADP rybosylation factors (ARFs), which we named XGAP. Further functional analyses of XGAP revealed that XGAP is required for gastrulation cell movements, particularly CE and the establishment of cell polarity which is manifested by the localization of cellular protrusions such as lamellipodia. In addition, we found that XGAP is required for the proper localization of PAR proteins that are highly conserved proteins essential for establishing cell polarity in a wide range of organisms from *C. elegans* to humans. Studies on the precise molecular mechanism of PAR proteins’ regulation by XGAP are currently undertaken.

Aside from the molecular mechanism, we conducted experiments to ask how and when cell polarity is established in the cells participating in CE. To grasp cellular events triggered upon the cell polarization, we focused on microtubule (MT) formation. Based on the assumption that MT formation and direction of MT extension are closely related to cell polarity, we observed MT formation using GFP-fused EB1/3 proteins that are known to bind the plus-end of MTs. The time-lapse recording of the GFP fluorescence shows clear bidirectional extension of MTs in cells of Keller’s explant participating in CE, while MTs are radially extended in ectoderm cells. We are investigating what makes the difference in MT dynamics and how it is related to cell morphology and polarity formation.

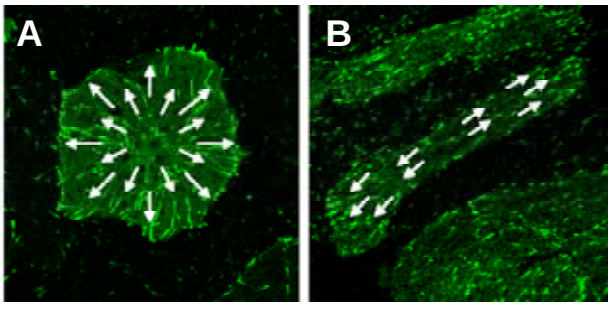

Figure 1. Microtubule dynamics in animal cap and dorsal mesoderm cells revealed by EB3 movement. In animal cap cells, the direction of EB3 movements are random (A), while the EB3 moves towards both poles of cells in dorsal mesoderm (B). Green comets: EB3-GFP, white arrows: the direction of EB3 movements.

II. Roles of membrane trafficking in the regulation of gastrulation movements

Membrane trafficking has been implicated in signal transduction, cell polarity control and cell movements. To investigate the role of membrane trafficking in the regulation of gastrulation movements in *Xenopus* embryos, we focused on Rab GTPases, key regulators for many steps of membrane trafficking. We searched for Rab GTPases that function in gastrulation by overexpressing dominant-negative Rab mutants and identified four Rabs that inhibited gastrulation movements. Loss-of-function of one of these Rabs by the specific morpholino oligonucleotide (Mo) also perturbed normal gastrulation, suggesting that it plays an essential role in this process. Interestingly, the Rab Mo inhibited the non-canonical Wnt pathway which has been implicated in the regulation of convergent extension movements. In the Mo-injected cells, the Wnt ligand and its receptor Frizzled were localized normally, but they were not able to activate the downstream signal in the cells. Dishevelled, which plays the pivotal role in the Wnt signal pathway, is known to be translocated from the cytoplasm to the plasma membrane in response to the Wnt signal activation. The Rab Mo inhibited its translocation, suggesting that membrane trafficking might be important for signaling from Wnt/Frizzled to Dishevelled in this pathway.

III. Notochord-derived fibrinogen-like protein regulates a long-range patterning of nervous system in chordate embryos

The dorsal nervous system (CNS) and the notochord underneath CNS are two major organs characteristic to chordate body plans. Experimental embryology demonstrated that the notochord play a critical role in the patterning of CNS during vertebrate embryogenesis, but little is known about genes or molecules involved in this interaction. In vertebrates, a T-box gene, *Brachyury* (*Bra*), plays a pivotal role in the formation of notochord. This is the case of urochordate ascidian; *Bra* is expressed exclusively in primodial notochord cells and its role is essential for the notochord cell differentiation. We have already isolated nearly 40 genes that are direct or indirect targets of *Ci-Bra* of *Ciona intestinalis*. An ascidian homolog (Ci-scale) of the *Drosophila Scabrous* gene encodes a fibrinogen-like protein is specifically expressed in notochord cells, its protein product is not refined to notochord but distributed underneath the CNS with fibril-like protrusions or vesicles. Knockdown of Ci-Scale function resulted in failure of convergent extention of notochord cells and differentiation of neuronal cells and axon guidance. A proper distribution of Ci-Scale proteins is dependent on Notch signaling delivered by the CNS. These results suggest that cooperative function of Ci-Scale and Notch is essential for the patterning of CNS and axon guidance in chordate embryos.

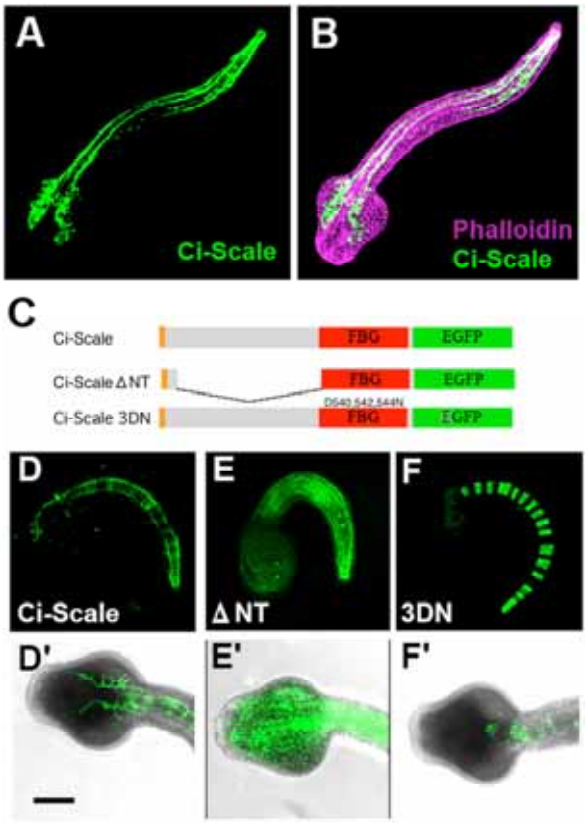

Figure 2. Distribution of Ci-Scale protein in *Ciona intestinalis* embryos. Staining of tailbud stage embryos with an anti-Ci-Scale antibody shown in green and staining with phalloidin in magenta (A, B). Fusion gene constructs tagged with EGFP to examine the role of Ci-Scale components in its extracellular distribution. dNT mutation lacks the N-terminal half, and 3DN was a mutation in which the three conserved D residues in the DXDXD motif are changed to N residues (C). Distribution of EGFP in embryos electropolated with (D) Ci-Scale (control), (E) dNT or (F) 3DN; side view of the tailbud embryos, and (D'-F') dorsal view of the head region of embryos. EGFP in a dNT mutated embryo was seen disused distribution while 3DNmutant was seen only in the cytoplasm of notochord cells, suggesting the NT domain and the FBG domain, especially the DXDXD motif, is necessary for its proper distribution. Scale bar, 50 μm.

IV. Trithorax group components *tonalli* and vertebrate homolog TONAS proteins participate roles in protein SUMO modification and epigenetic regulation

Drosophila tonalli (*tna*) mutant is a previously reported mutant that is involved in epigenetic regulation. The *tna* mutant phenotype mimics the homeotic loss-of-function phenotype and this mutant shows striking genetic interaction with the mutants of *trithorax group* genes. We isolated two mutant alleles of *tna* as a putative downstream target of the DPP (corresponding to vertebrate BMP) signaling. We also isolated vertebrate homologues of *tna* and named as *tonalli* related SP-RING finger protein, TONAS-1 and TONAS-2. The role of

Tna/TONAS proteins in the epigenetic regulation and also in Trithorax group function is totally unknown. The most characteristic feature of Tna/TONAS proteins is the existence of a single SP-RING finger motif in the middle. The SP-RING motif was originally found in the PIAS family SUMO-E3 ligase proteins. We have addressed TONAS SUMO E3 ligase activity by in vitro experiments as well as cultured cell over-expression study. TONAS itself is a good substrate for SUMO-2/3 modification and the SP-RING motif is essential for this activity. Other SUMOylation targets of TONAS proteins are currently unknown. We isolated TONAS binding partners from FLAG-TONAS expressing HEK293T cell-extract by protein affinity purification and LC-MS-MS analysis. Some of the candidate interactors are known to be substrates of SUMO-modification. We will confirm whether these proteins are specific TONAS SUMOylation targets by further analysis.

V. *Xenopus* functional genomics (Xenomics)

Using *Xenopus laevis* as a model, we have been attempting to reveal the complex regulatory network controlling the morphological processes in early embryogenesis through the clarification of gene function and their interactions in the system.

We constructed the comprehensive database XDB3 that stores EST sequences, assembled sequences, full insert sequences and WISH (whole-mount *in situ* hybridization) data with the descriptions of predicted gene function. We added a new browsing method of WISH data as 3D animation model, a method developed under collaboration with Drs. P. Vize and V. Gerth (University of Calgary).

We have also created several transgenic *X. laevis* lines that are labeled with fluorescent proteins (EGFP, Venus, or RFP) localized in subcellular components such as nucleus, nuclear membrane, mitochondria, Golgi, and ER. These frogs will be very powerful tools for the functional analysis of genes in the post genome era.

Figure 3. Xenopus Data Base 3 (http://xenopus.nibb.ac.jp).

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Original papers

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