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 and transcription factors. We address this problem using several model animals, including frogs, flies and ascidians, and by 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 the morphogenesis of early embryos, involving dynamic cell migration and change in embryo shape. 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 ìconvergent extension (CE)î. As convergent extension begins, cells are polarized and aligned mediolaterally, followed by the mutual intercalation of the cells that acquired planar cell polarity (PCP). In the regulation of convergent extension, several growth factor signaling pathways including Wnt/PCP pathway are implicated.

In addition to the components of the Wnt/PCP pathway, we have recently found that activin/nodal members of the TGF-β superfamily induce expression of two genes regulating cell adhesion during gastrulation: FLRT3, a putative type I transmembrane protein containing extracellular leucine-rich repeats, and the small GTPase Rnd1. Both loss- and gain-of-function analyses of FLRT3 and Rnd1 show that these proteins physically interact and modulate cadherin-mediated cell adhesion during early embryogenesis by regulating cadherinís subcellular localization. As numerous studies have linked aberrant expression of small GTPases, adhesion molecules such as cadherins and TGF-β signaling to oncogenesis and metastasis, it is intriguing that this FLRT3-Rnd1 pathway controls cell behavior and tissue morphogenesis in both embryos and adults.

More recently, we have found that a novel FGF-responsive gene encoding ankyrin repeats domain protein 5 (ANR5) has an essential role in regulating cell adhesion. In the *Xenopus* gastrula, reduced levels of xANR5 perturbed cell adhesion and tissue separation, processes that were regulated by a cadherin family protein, Paraxial Protocadherin (PAPC). We also showed that xANR5 physically interacted with PAPC and regulated PAPC-dependent morphogenetic processes and signaling pathways. Interestingly, the polarized localization of the xANR5 protein in dorsal mesodermal cells was completely disrupted by drugs that prevent intracellular calcium signaling, and mutations in xANR5ís predicted calcium-binding domains provoked its translocation into the nucleus. On the basis of these observations, we propose that the intracellular calcium signal regulates the localization of xANR5, which in turn regulates the adhesive properties of mesodermal cells during *Xenopus* gastrulation.

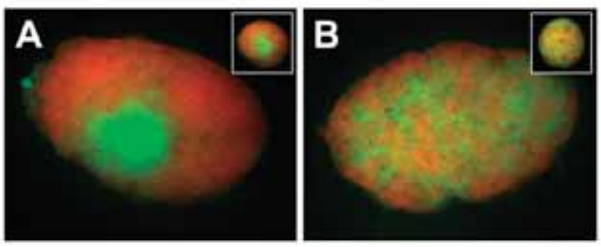

Figure 1. Cell sorting by PAPC requires ANR5 function. Expression of PAPC promotes homophilic cell-to-cell adhesion and resulting cell sorting of PAPC-expressing cells shown by red (**A**). However, depletion of ANR5 by an antisense Morpholino oligonucleotide disrupts the cell adhesion and thus PAPC-expressing cell are intermingled with non-expressing cells shown as green (**B**).

II. Protein ubiquitination involved in the regulation of gastrulation movements

Protein ubiquitination is an important mechanism to regulate the stability and/or subcellular localization of target proteins. It has previously been unknown whether or not ubiquitination is involved in the regulation of gastrulation movements in *Xenopus* embryos. We found that two distinct ubiquitination systems play crucial roles in this process. One is essential for the signal transduction

of the noncanonical Wnt pathway that regulates cell polarity and cell migration during gastrulation. We identified a novel ubiquitin ligase complex, consisting of Rab family GTPase (Rab40) and Cullin. This complex is localized in the Golgi apparatus and essential for the regulation of the localization of Dishevelled, which plays the pivotal role in the Wnt signaling pathway (Figure 2). Loss-of-function of this ubiquitin ligase resulted in the inhibition of the Wnt pathway and caused a severe gastrulation-defective phenotype in *Xenopus* embryos. We also identified the other ubiquitination system that ubiquitinates and destabilizes Paxillin, one of the focal adhesion complex components. We found that the focal adhesion plays an important role in convergent extension and its stability must be tightly regulated. Interestingly, the Wnt signaling pathway increased Paxillin ubiquitination and destabilized focal adhesions, indicating that the Wnt pathway regulates convergent extension movements through regulating the stability of focal adhesions. These findings implicate previously unidentified ubiquitin systems into the morphogenetic process during vertebrate embryogenesis.

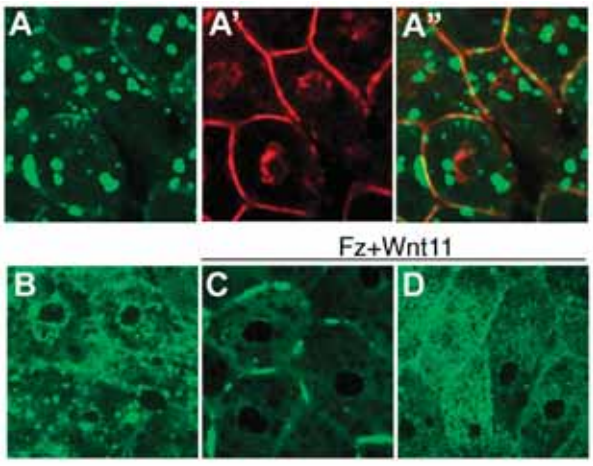

Figure 2. Localization and function of Rab40/Cullin ubiquitin ligase. **(A)** localization of Rab40-GFP in the *Xenopus* embryonic ectodermal cells. **(A')** the plasma membrane and the nuclei. **(A")** merged image. **(B, C and D)** Localization of Dishevelled-GFP **(B)** It is localized in the cytoplasmic vesicular structures without Wnt signaling, (**C**) Coexpression of Frizzled and Wnt11 activates Wnt signaling and translocates Dishevelled to the plasma membrane, (**D**) Coinjection of Rab40 antisense morpholono oligo inhibits the translocation of Dishevelled in response to Wnt signaling

III. Involvement of a fibrinogen-like protein in notochord-dependent dorsal patterning of the nervous system in *Ciona intestinalis* 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-fibrn*) of the *Drosophila Scabrous* gene is one of the downstream target gene of *Brachyury*. While the gene (*Ci-fibrn*) 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 (Figure 3). Knockdown of Ci-fibrn function resulted in failure of convergent extention of notochord cells and differentiation of neuronal cells and axon guidance. Correct distribution of Ci-fibrn protein is dependent on the Notch signal delivered by the overlying CNS. Disturbance of Ci-fibrn distribution caused ventral positioning of neuronal cells and abnormal track of axon extension. Therefore, it is highly likely that the interaction of the notochord-based fibrinogen-like protein and neural tube-based Notch signal is essential for the dorsal patterning of the nervous system or for the establishment of the chordate body plan.

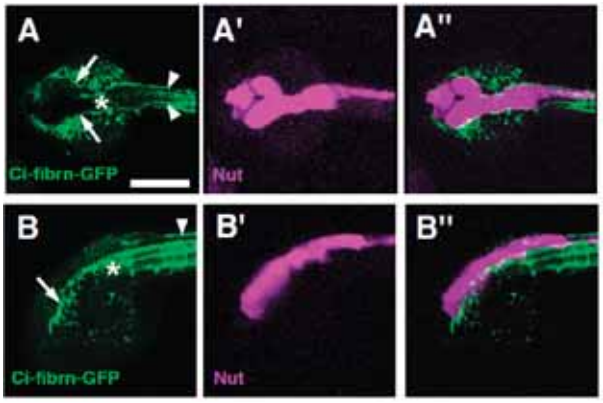

Figure 3. Expression and distribution of Ci-fibrn into anterior and dorsal regions of the trunk, surrounding the CNS, at the late tailbud stage in (**A**) dorsal and **(B)** lateral view. (**A, B**) Expression of GFP reporters in an embryo injected with *Ci-Bra*(notchord promoter):fibrn:EGFP (green), **(A', B')** *Ci-Nut*(CNS promoter):RFP (magenta), and **(A", B")** merged photomicrograph. Arrows indicate the border of the sensory vesicle; the asterisk indicates the anterior border of the notochord; and arrowheads indicate Ci-fibrn underneath elongated axons. Scale bar, 50 μm.

IV. Functional and genetical study of epigenetic regulator *tonalli* and a putative translation regulator *dNAT1* in *Drosophila* body patterning

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 confirmed that most of Hox genes expression is significantly reduced in the *tna* loss-of-function mutant. *tna* encodes 1109 amino-acid protein containing single SP-RING motif, which is thought to be involved in protein SUMO conjugation process. To visualize TNA protein we generate specific antibody to TNA. One of the antibodies to TNA specifically detects TNA protein in vitro and in vivo. TNA protein predominantly localized in the nucleus and we also observed TNA localization at substantial level in the cytoplasm. TNA localizes specific chromosomal region of the salivary gland chromosomes. This result is consistent with the hypothesis by which TNA participates in an essential role in the TRX-group function.

Translational regulation also participates in major roles the early patterning of *Drosophila* embryo. One of the eIF4G family proteins NAT1/p97/DAP5 has been identified as a novel translational repressor. To elucidate in vivo function of the *NAT1* we isolated *Drosophila NAT1* (*dNAT1*) mutant by reverse-genetical approach. We isolated four transposon insertion mutants as well as a 1.4 kb deletion alleles corresponding to the *dNAT1* locus. One of the P-element insertion lines $dNAT1^{GS1}$ shows severe embryonic lethality with abnormal germband extension defect. This lethality and morphological phenotype were completely rescued by introduction of the 12 kb *dNAT1* genomic DNA fragment by germ line transformation. Expression of some of the segement polarity genes were apparently abnormal in the $dNAT1^{GS1}$ mutant (Figure 4). We are currently trying to identify the molecule(s) that is regulated by dNAT1 at a level of tranlational regulation.

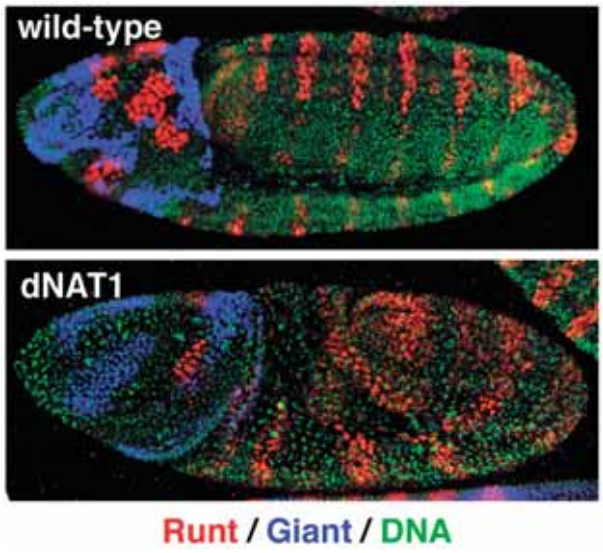

Figure 4. *Drosophila* stage-11 embryo stained with antibody to Runt (red), Giant (blue) and DNA dye (green). Embryo shows in anterior left and dorsal up. Germband is fully extended at this stage in the wild-type animals. $dNAT1^{GS1}$ mutant animal shows defective germband extension and abnormal expression in some of the segmentation marker proteins.

Publication List:

Original papers

Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T., and Takada, S. (2006). Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. Dev. Cell. *11*, 791-801.

Hyodo-Miura, J., Yamamoto, T.S., Hyodo, A.C., Iemura, S., Kusakabe, M., Nishida, E., Natsume, T., and Ueno, N. (2006). XGAP, an ArfGAP, is required for polarized localization of PAR proteins and cell polarity in Xenopus gastrulation. Dev. Cell. *11*, 69-79.

Waldner, C., Sakamaki, K., Ueno, N., Turan, G., and Ryffel, G.U. (2006). Transgenic Xenopus laevis strain expressing cre recombinase in muscle cells. Dev. Dyn. *235*, 2220-2228.

Kominami, K., Takagi, C., Kurata, T., Kitayama, A., Nozaki, M., Sawasaki, T., Kuida, K., Endo, Y., Manabe, N., Ueno, N., and Sakamaki, K. (2006). The initiator caspase, caspase-10beta, and the BH-3-only molecule, Bid, demonstrate evolutionary conservation in Xenopus of their pro-apoptoticactivities in the extrinsic and intrinsic pathways. Genes Cells. *11*, 701-717.