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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 are 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. Establishment of cell polarity during vertebrate embryogenesis

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 the 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 cells that acquired planar cell polarity (PCP). In the regulation of convergent extension, several growth factor signaling pathways including Wnt/PCP pathway are implicated.

To understand how PCP is established within the cells, we have recently introduced a new method to visualize microtubule (MT) dynamics based on the assumption that cytoskeletal reorganization is one of the earliest events of the cell polarity formation. Using a GFP-fusion protein of EB3 (end-binding protein) which preferentially binds to the plus-end of growing MTs, we have been able to demonstrate that MT dynamics in non-polarized and polarized cells differ in that in polarized cells MT growth is more restricted towards the two ends of the cells even before the cells change their morphology to spindle shape. We also found that the cell polarity revealed by the MT dynamics is influenced by extracellular environments such as the notochord-somite tissue boundary and that physical contact of chordamesodermal explants with heterogenous tissues has significant impacts on the cell polarity, possibly by establishing a new tissue boundary between the two tissues. We are currently investigating what actually triggers the cell polarity formation during gastrulation.

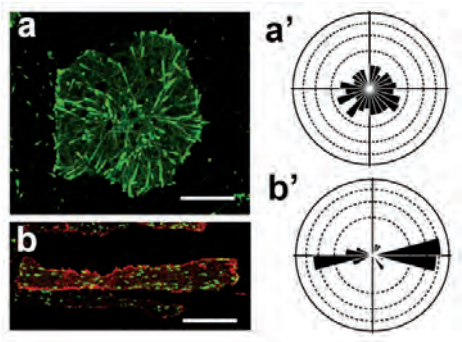

Figure 1. Tracking of EB3-GFP movements captures microtubule growth in *Xenopus* explants. In an animal cap cell (a), EB3-GFP shows radially symmetrical movements toward the rim of the cell in the rose diagram (a’). In chordamesoderm cell (b), the movements are mostly bidirectional toward both ends of the cell (b’).

Ⅱ. The involvement of protein ubiquitination systems in the noncanonical Wnt signaling pathway during gastrulation.

The noncanonical Wnt signaling pathway has been shown to play an essential role in the regulation of gastrulation movements. However, the molecular mechanisms of how Wnt signals intracellularly and how it regulates the tissue movements remain elusive. In order to clarify the Wnt signal transduction mechanism, we searched for the proteins essential for this signaling pathway, and identified a novel ubiquitin ligase complex, consisting of Rab40 GTPase and Cullin. This complex is localized in the Golgi apparatus and is essential for the regulation of the localization of Dishevelled, which plays the pivotal role in the Wnt signaling pathway. 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 another ubiquitination system essential for gastrulation, which ubiquitinates and destabilizes Paxillin, one of the focal adhesion components.

We found that the focal adhesion plays an important role in convergent extension. Paxillin stability is regulated at the focal adhesions, and the destabilization of Paxillin promotes the dynamics of focal adhesions and cell movements (Figure 2). 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 Paxillin. These findings implicated two previously-unidentified ubiquitin systems in the regulation of gastrulation movements in *Xenopus* embryos.

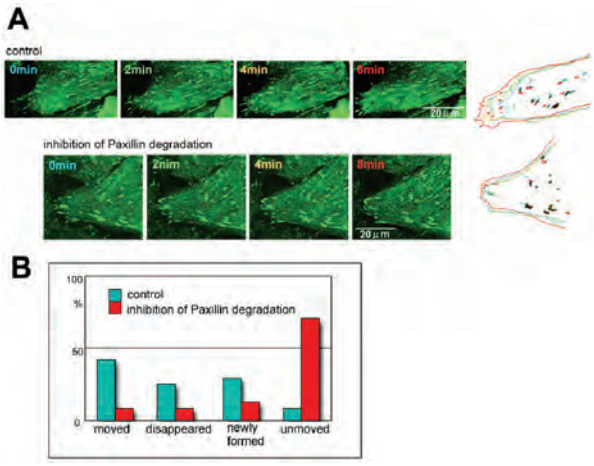

Figure 2. Paxillin degradation promotes focal adhesion dynamics and cell movement. (A) EGFP-Paxillin was expressed in the cells undergoing convergent extension. Inhibition of Paxillin degradation by antisense morpholino against XRNF185, which plays a crucial role in Paxillin degradation, decreased focal adhesion dynamics and cell movement. (B) Statistical data of focal adhesion dynamics.

Ⅲ. *Brachyury*-downstream notochord genes and convergent extension in *Ciona intestinalis* embryos

Formation of the chordate body is accomplished by a complex set of morphogenetic movements including the convergent extension of notochord cells. In the ascidian *Ciona intestinalis, Brachyury* plays a key role in the formation of the notochord, and more than thirty *Bra*-downstream notochord genes have been identified. In the present study, we examined the effects of functional suppression of nine *Bra*-downstream notochord genes, which include *Ci-PTP*, *Ci-ACL*, *Ci-prickle*, *Ci-netrin*, *Ci-trop*, *Ci-Noto3*, *Ci-ASAK*, *Ci-ERM* and *Ci-pellino*. When the function of the first two genes (*Ci-PTP* and *Ci-ACL*) was suppressed with specific morpholinos, the notochord cells failed to converge, while functional suppression of *Ci-prickle* resulted in a failure of intercalation, and therefore the cells in these three types of embryo remained in the mid-dorsal region of the embryo (Fig. 3). Functional suppression of the next four genes (*Ci-netrin*, *Ci-trop*, *Ci-Noto3* and *Ci-ASAK*) resulted in the partial defect of intercalation, and the notochord did not align in a single row. In addition, when the function of the last two genes (*Ci-ERM* and *Ci-pellino*) was suppressed, notochord cells failed to elongate in the embryo, even though convergence/extension took place normally. These results indicate that many *Bra*-downstream notochord genes are involved in convergence/extension of the embryo.

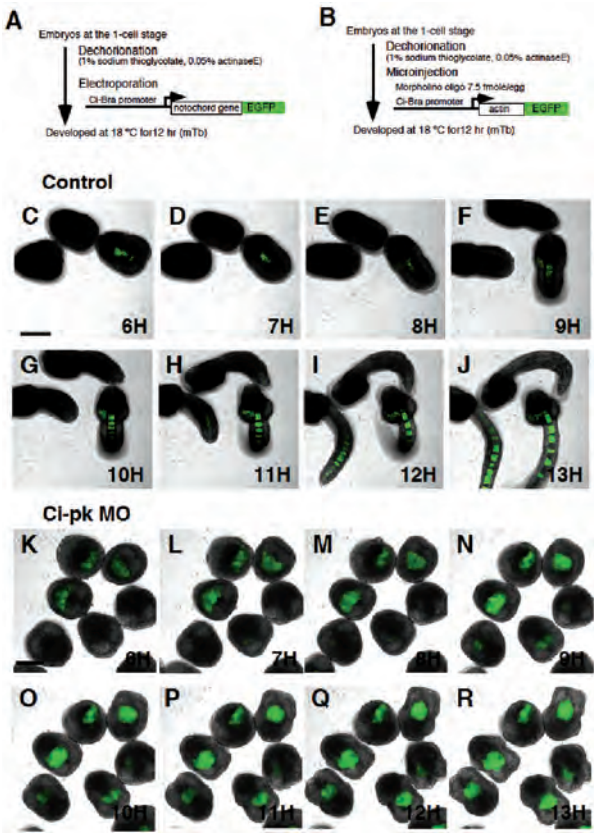

Figure 3. Examination of intracellular localization and function of proteins encoded by *Ci-Bra*-downstream notochord genes. (A) Experimental procedure. Fertilized eggs were dechorionated and electroporated with *Ci-Bra(promoter)/Bra*-downstream notochord gene:EGFP constructs. Manipulated embryos were allowed to develop at 18 ℃ for 12 hrs (to the mid tailbud stage). (B) Experimental procedure. Fertilized eggs were dechorionated and microinjected with specific morpholino oligos against *Ci-Bra* downstream genes together with the *Ci-Bra(promoter)*/actin:EGFP construct. Manipulated embryos were allowed to develop at 18 ℃ for 12 hrs (to the mid tailbud stage). (C-J) Control embryos at (C) 6, (D) 7, (E) 8, (F) 9, (G) 10, (H) 11, (I) 12, and (J) 13 hrs after fertilization. Notochord cells with actin:EGFP expression show convergence (C, D), intercalation (D-F), extension (F-H) and elongation (H-J). Scale bar, 100 μm. (K-R) Functional suppression of *Ci-pk* results in the failure of intercalation and extension of notochord cells, which are evident with actin:EGFP expression. Embryos at (K) 6, (L) 7, (M) 8, (N) 9, (O) 10, (P) 11, (Q) 12, and (R) 13 hrs after fertilization are shown to compare with normal embryos (C -J). Scale bar, 100 μm.

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

Translational regulation takes a major role in *Drosophila* body patterning. One of the eIF4G family proteins NAT1/p97/DAP5 has been identified and analyzed mainly in vertebrate culture cells. NAT1 has a structural similarity to 2/3 of eIF4G-C-terminal region. Since NAT1 deletes a binding motif to eIF4E, it has been hypothesized that this

protein participates in CAP-independent translation or plays an antagonistic role in translation. To elucidate *in vivo* function of *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 allele corresponding to the *dNAT1* locus. One of the P-element insertion lines $dNAT1^{GS1}$ shows severe embryonic lethality with abnormal germband extension defect. The lethality and morphological phenotype were completely rescued by introduction of the 12 kb *dNAT1* genomic DNA fragment by germ line transformation. We also found some similarity in the phenotype between *dNAT1* hypomorphic mutant and mutants in ecdysone signaling cascade. Both mutants show lethality at late 3rd instar larva to pupal stage with various metamorphosis defects. We found that one of the ecdysone target genes E74A is not properly translated in the *dNAT1* mutant animals. E74A is one of the rare examples of the genes whose translation is started from non-AUG start codon. E74A is translated from CUG start condon. Our analysis strongly suggested that dNAT1 participates in translation initiation from CUG start condon. We also speculate that dNAT1 also regulates other target(s) translation in early embryogenesis, since *dNAT1* null mutant shows early lethality. We would like to elucidate the general importance of NAT1 mediated non-AUG translation initiation in *Drosophila* development as well as vertebrate development.

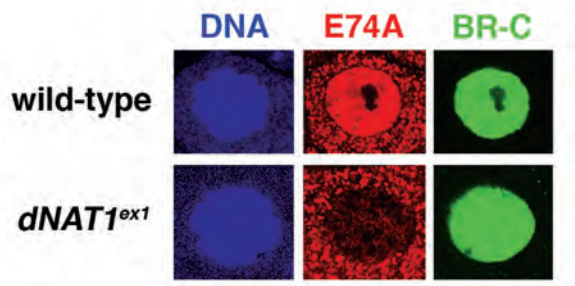

Figure 4. Salivary gland cells of wild-type and *dNAT1* mutant were stained with antibodies against E74A (red) BR-C (green) as well as DNA-staining dye (blue). In a *dNAT1* mutant cell, nuclear signal of E74A is completely missing while another nuclear marker BR-C is expressed normally.

Publication List

〔Original papers〕

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- Iioka, H., Iemura, S., Natsume, T. and Kinoshita, N. (2007). Wnt signalling regulates paxillin ubiquitination essential for mesodermal cell motility. Nat. Cell Biol. *9*, 813-821.
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- Yoshikane, N., Nakamura, N., Ueda, R., Ueno, N., Yamanaka, S., Nakamura, M. (2007). Drosophila NAT1, a homolog of the vertebrate translational regulator NAT1/DAP5/p97, is required for embryonic germband extension and metamorphosis. Dev. Growth Differ. *49*, 623-634.