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The complex morphogenesis of organisms is achieved by dynamic rearrangements of tissues during embryogenesis, in which change in cellular morphology as well as orchestrated cell movements are involved. For cells to know how they should change their shape and where they should move, information called “cell polarity” is essential. How then is the cell polarity established within cells? Is it intrinsically formed within the cells or triggered by extracellular cues? Furthermore, little is known as to how coordinated and complex cell movements are controlled in time and space. We attempt to understand the mechanisms underlying these events using several model animals, including frogs, fish, mice and ascidians, taking physical parameters such as force in consideration, in addition to conventional molecular and cellular biology.

I. Biological significance of force for morphogenesis

Physical forces are a non-negligible environmental factor that can guide the morphogenesis of organisms. Such forces are generated by tissue-tissue interactions during early development where drastic tissue remodeling occurs. One good example is neural tube formation. In vertebrates, the neural tube that is the primordial organ of the central nervous system and is formed by the bending of the neural plate that is a flat sheet of neuroepithelial cells. The tissue remodeling is driven by cellular morphogenesis in which selected cells in the neural plate change their shapes from cuboidal to an elongated wedge-like shape. Recent studies have revealed that this cell shape change is controlled by cytoskeletal dynamics, namely the remodeling of F-actin and microtubules (Suzuki, M. et al. Dev. Growth Differ., 2012) . In addition, we also found that non-neural deep layer cells that underlie the non-neural ectoderm generate force to bring the two neural folds to the dorsal midline to close the tube (Morita, H. et al. Development, 2012).

Another example is the axial mesoderm, which elongates along the anterior-posterior axis during gastrulation cell movements by which rearrangement of the three germ layers is driven. The axial mesoderm is led by the anteriorly precedent tissue Leading Edge Mesoderm (LEM). When surgically isolated the LEM migrates fairly rapidly toward the predetermined anterior side, while the following axial mesoderm shows little directed tissue migration. We hypothesized that the LEM generates traction force on the following axial mesoderm. To prove the biological significance of the force generated by the LEM, we have removed the LEM, cultured to the end of gastrulation, and found that the movement of the LEM is required for the proper narrowing and elongation of the axial mesoderm (Figure 1).

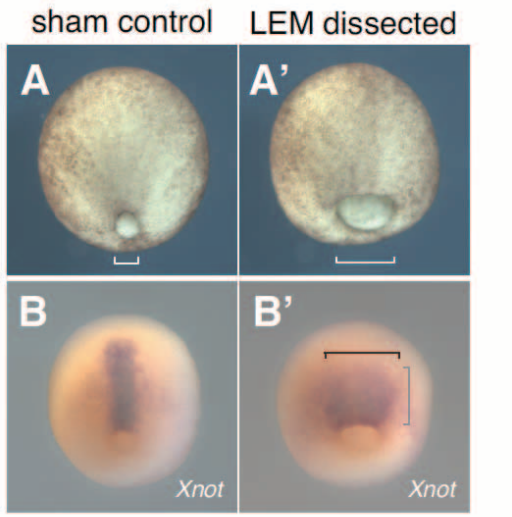

Figure 1. (A, A’) External appearance of early neurula embryos. Sham-operated embryo (A) in which the LEM was remained but not the LEM-removed (A’) embryo closed the blastopore and underwent convergent extension (B, B’, respectively)

II. The roles of PCP core components in mouse development

In epithelia, the roles of planar cell polarity (PCP) have been extensively studied, whereas in non-epithelia, they have yet to be fully understood. We are exploring the roles of PCP in the mesenchymal and neural tissues by using mouse genetics and *RNAi* knock-down technology.

Recently, we generated a hypomorphic allele of mouse *Prickle1*, one of the core PCP factors. As we have reported (Tao, H. et al. Proc. Natl. Acad. Sci., USA, 2009) *Prickle1* null/null mice die around E6.0 in gestation due to the failure of gastrulation. In contrast, *Prickle1* hypomorphic mutant mice survive to P0 in a Mendelian manner but eventually die a day after birth. Interestingly, the mutant mice also displayed abnormal morphology in some tissues where *Prickle1* is expressed. Now, we are investigating the cellular and molecular mechanisms underlying the abnormal morphology. We hope that in combination with comparative genomic analysis, these PCP mutant mice will serve as good models that can explain morphological variations of mammals.

III. Regulation of cell adhesion by the ubiquitin system during gastrulation

During gastrulation, dorsal mesoderm cells migrate toward the midline and align along the anteroposterior axis to form the notochord. In this process, cells change their shape and migrate in a highly coordinated manner. To achieve this systematic cell movement, cell-to-cell interaction must be tightly regulated. The cell adhesion molecule cadherin plays a crucial role in this regulatory system. It is known that of the cadherin family members, paraxial protocadherin (PAPC) is an important molecule that regulates cell adhesion in mesoderm cells. We have found that the localization and the stability of PAPC protein is regulated by the ubiquitin system. The inhibition of E3 ubiquitin ligase SCF/β-TrCP blocks its plasma membrane localization. The inhibition of PAPC ubiquitination by the dominant-negative ubiquitin protein also impaired the plasma membrane localization and weakened cell adhesion of mesoderm cells (Figure 2). Furthermore, this ubiquitin system regulates the localization of another cell adhesion molecule, C-cadherin. Our findings uncovered a novel mechanism of regulation of cell adhesion proteins by the ubiquitin system, which plays a crucial role in the actively-migrating mesoderm cells.

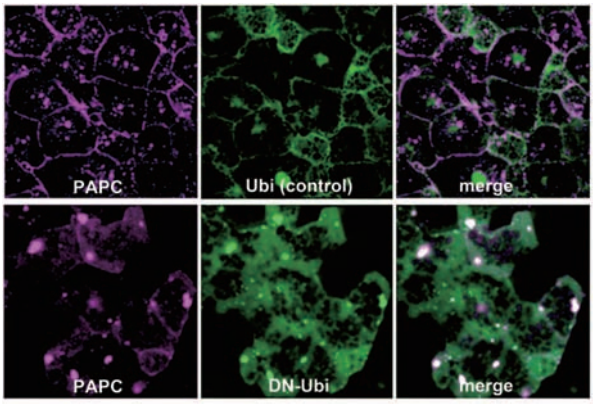

Figure 2. The ubiquitin system regulates PAPC localization and cell adhesion. In the normal mesoderm cells, PAPC localizes at the plasma membrane and cytoplasmic vesicles. Dominant-negative ubiquitin (DN-Ubi), which inhibits polyubiquitination, impairs PAPC localization to the plasma membrane and weakens cell adhesion.

IV. Cellular morphogenesis during neural tube closure

For the morphogenesis of organs, cellular morphogenesis as well as cellular behaviors plays critical roles. During early development of the central nervous system (CNS) in vertebrates, the neuroepithelial cells undergo a typical shape change, called apical constriction (AC), the cumulative action of which cause the neural plate to bend to form the neural tube. In AC, cell apices are contracted and stabilized, causing cells to adopt wedge-like shapes from columnar ones. Recent studies have revealed that AC is controlled by cytoskeletal dynamics, namely the remodeling of F-actin, and non-muscle myosin II activity, yet how AC is dynamically controlled in time and space is not fully understood.

Calcium ions act as second messengers, triggered by both extra- and intracellular cues. The level of cytoplasmic calcium is increased by its influx from either extracellular space or intracellular storage areas such as the endoplasmic reticulum (ER). We found that inhibition of calcium influx delayed neural tube closure (NTC), suggesting that calcium signaling plays an important role(s) in AC. Long-term time-lapse imaging with calcium indicators revealed the dynamic calcium transients throughout NTC. Occurrence of the transients correlated with AC at the cellular level and with the speed of NTC at tissue level. Furthermore, the forced increase of cytoplasmic calcium by caged-compounds caused cell shape change similar to AC. These suggest that calcium is a positive regulator of AC and accelerates NTC to achieve the correct formation of the hollow structure of the primitive CNS.

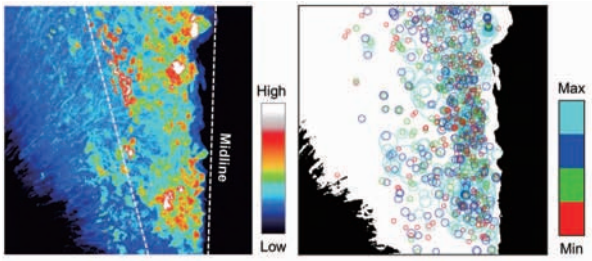

Figure 3. By injecting in vitro transcribed mRNA, we observed the intracellular calcium patterns during *Xenopus* neural tube closure. During neural cells undergoing apical constriction, calcium transients at single cell level dynamically occurred, sometimes resulting in multicellular propagation. (Left) Frequency of calcium transients visualized by pseudocolor, (right) Time-projection of calcium patterns by colored circle representing area of the transients.

V. Notochord and evolution of chordates

Gastrulation is a morphogenetic movement that is essential for the formation of two- or three-germ-layered embryos. *Brachyury* is transiently expressed in the blastopore region, where it confers on cells the ability to undergo invagination. This process is involved in the formation of the archenteron in all metazoans. This is a “primary” function of *Brachyury*. During the evolution of chordates, they gained an additional expression domain at the dorsal midline region of the blastopore. In the new expression domain, *Brachyury* served its “secondary” function, recruiting another set of target genes to form a dorsal axial organ, the notochord (Figure 4). In order to better understand the molecular mechanisms underlying the origin of the notochord during chordate evolution, we are currently investigating to compare the *Brachyury* gene regulatory networks of hemichordates (a nonchordate deuterostome closest to chordates) and cephalochordates (the most basal chordate).

VI. Epigenetic modification in the invertebrate genome

Epigenetic modifications, such as DNA methylation and histone modification, alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. These processes are crucial to normal development and

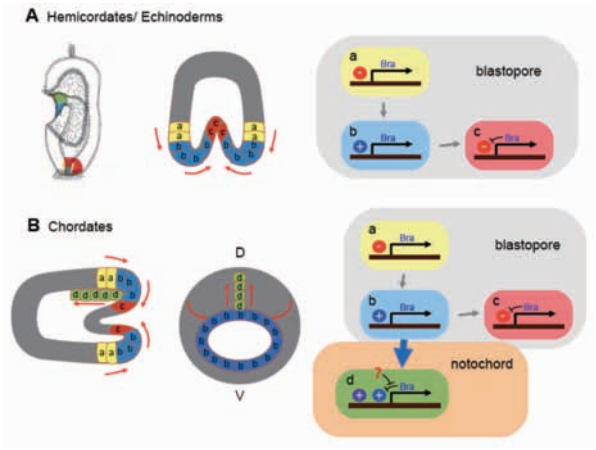

Figure 4. A schematic representation of the innovation of secondary *Brachyury* function that leads the notochord formation during chordate evolution. (A) *Brachyury* appears to be expressed continuously in the blastopore, but engaged cells never continue expressing the gene. (B) The expression of *Brachyury* in the cells fated to form the notochord is not transient, but instead continues till the process of notochord differentiation progresses to some extent.

differentiation of distinct cell lineages in mammals. In invertebrates, gene coding regions are the primary targets of DNA methylation, but the role of DNA methylation in actively transcribed genes is unknown. We investigated the tissue variability of both the global levels and distribution of 5mC in the seasquirt *Ciona intestinalis*. We found that global 5mC content of early developmental embryos is high, but is strikingly reduced in body wall tissues. We chose sperm and adult muscle cells, with high and reduced levels of global 5mC respectively, for genome-wide analysis of 5mC targets. By means of CXXC-affinity purification followed by deep sequencing (CAP-seq), and genome-wide bisulfite sequencing (BS-seq), we designated body-methylated and unmethylated genes in each tissue. Surprisingly, body-methylated and unmethylated gene groups are identical in the sperm and muscle cells. We conclude that gene body methylation is not a direct regulator of tissue specific gene expression in *C. intestinalis*. Instead, methylated genes are often stably or maternally expressed. Moreover, we demonstrate that transgenes can be modified by gene body methylation, when their expression is driven by promoters of endogenously body-methylated genes. Our findings reveal constant targeting of gene body methylation irrespective of cell types, and they emphasize a correlation between gene body methylation and ubiquitously expressed genes.

Publication List

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