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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. On the other hand, we also know that such cell shape change is necessary but not sufficient to cause complete neural tube closure because an embryo with an absence of these cell shape changes will still display significant neural plate bending. We reasoned that other forces to bend the neural plate and contribute to the complete tube closure are generated by tissue(s) outside of the neural plate, possibly in the non-neural ectoderm. After a series of microscopic observations and experiments using *Xenopus laevis*, we have found that non-neural deep layer cells that underlie the non-neural ectoderm migrate actively toward the dorsal side dragging the overlying superficial layer cells and bringing them to the midline (Figure 1).

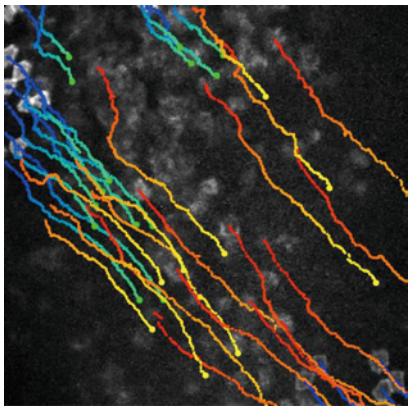

Figure 1. Tracking of cell movement of the non-neural ectoderm. Deep cells, labeled red/orange, migrate faster than superficial cells, labeled blue/green. (observation with DSLM).

This has been confirmed by the observation that disruption of deep cell migration by inhibiting the cell-substrate interaction between mesoderm and deep cells, or cell-cell interaction between deep cells and superficial cells, both of which are the basis of traction forces, caused neural tube closure defects. This study highlights the importance of physical force during complex organ formation (Figure 2).

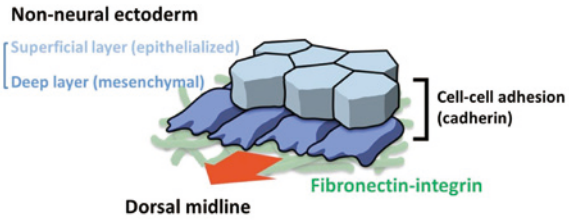

Figure 2. Model for the coordinated movement of non-neural ectoderm. Deep cells (dark blue) migrate on the fibronectin ahead of superficial cells and therefore the superficial cells are dragged toward the midline. This also causes a stretching force for passive shape change of superficial cells.

Another example is the axial mesoderm, which elongates along the anterior-posterior axis during gastrulation cell movements by which rearrangements 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

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

following axial mesoderm. To prove the biological significance of the force generated by the LEM, we have estimated the magnitude of the traction force by measuring displacement of a glass needle with a known spring constant by the anterior movement of the axial mesoderm. Furthermore, we have been examining whether the artificial application of force, for example by tissue-stretching, can cause biological outputs such as intracellular relocalization of signaling molecules or formation of cell polarity.

II. 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 show polarized morphological change and coordinated cell migration. To achieve this systematic cell movement, cell-to-cell interaction must be tightly regulated. We focus on one of the cell adhesion proteins, paraxial protocadherin (PAPC). PAPC has been shown to be involved in gastrulation cell movements during early embryogenesis. Using *Xenopus* embryos, we found that both knock down and overexpression of PAPC in the dorsal mesoderm impair mesoderm cell movement. PAPC knock down reduced cell-cell interaction and cells migrate disorderly. PAPC overexpression inhibited cell migration toward the midline. This suggests that PAPC is essential at the early stage and then must be decreased. It has been reported that the PAPC gene is first expressed in the dorsal marginal zone at the early gastrula stage and subsequently down-regulated in *Xenopus* and zebrafish. We found that PAPC is also regulated in the same way at the protein level (Figure 3). PAPC is degraded via lysosome and excluded from the plasma membrane in the axial mesoderm at the late gastrula stage. PAPC is phosphorylated by GSK3, providing a signal for poly-ubiquitination by β-TrCP. Localization of PAPC to the plasma membrane at the early gastrula stage is mediated by a novel deubiquitinating enzyme XTI3. Taken together, our findings suggest a novel mechanism of regulation of a cell adhesion protein by the ubiquitin system, which plays a crucial role in vertebrate embryogenesis.

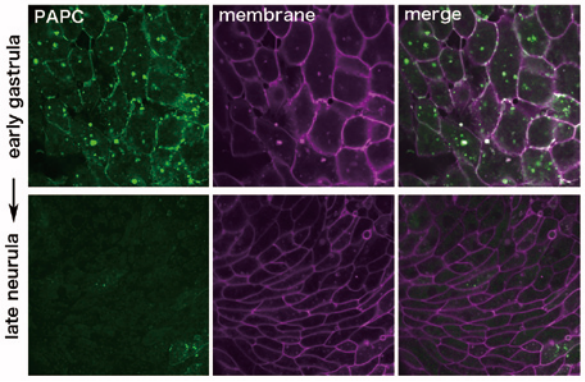

Figure 3. The dynamic regulation of PAPC stability during convergent extension. PAPC-GFP is expressed with membrane-tethered RFP in dorsal mesoderm cells. At the early gastrula stage, PAPC localized at the cell membrane and vesicles, but was drastically reduced later at the neurula stage.

III. Cellular morphogenesis during development

For the morphogenesis of organs, cellular morphogenesis as well as cellular behaviors play critical roles, as discussed above for neural tube formation of *Xenopus laevis*. Unlike *Xenopus* and amniote such as chicks and mammals, however, some of the teleost fish display a unique way of forming the neural tube in that rather than bending of a neuroepithelial sheet, they adopt drastic cell rearrangement of neural cell progenitors. During zebrafish neurulation, for example, highly motile cells converge to the midline, elongate their shape mediolaterally, and interdigitate with contralateral cells to form the neural rod in a highly coordinated manner. To date, although non-canonical, the Wnt/PCP pathway and several cadherins have been reported to function in this process, how the cytoskeleton controls these cellular behaviors is poorly understood.

We analyzed non-muscle myosin II (NMII) activity focusing on its regulatory light chain (MRLC) during zebrafish neurulation. Our analysis revealed that activated MRLCs were enriched in the cortex of highly migratory neural cells, suggesting that NMII functions in zebrafish neurulation. Consistent with this, inhibition of NMII suppressed cell elongation and interdigitation. Then we performed live-imaging analysis with GFP-tagged MRLC and found that its mutants that mimic the active forms were accumulated in the cell cortex as foci. These foci were dynamically formed and coalesced during cell elongation and interdigitation. We further found that these positioned proximal to cell protrusion and partially colocalized with adherens junction and tight junction proteins. These data suggest that NMII containing phosphorylated MRLC controls local cell-cell adhesion as intercellular linkage, which positively regulates highly coordinated cell shape changes in zebrafish neurulation.

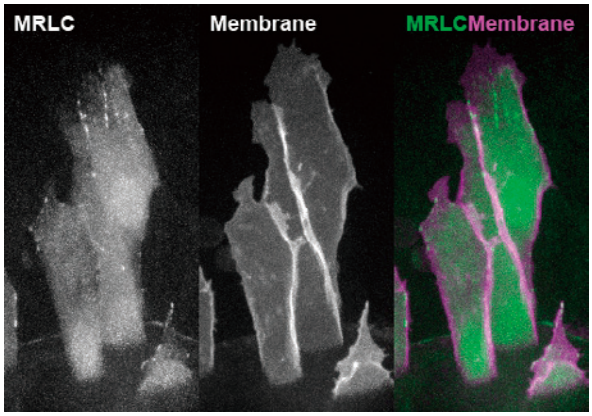

Figure 4. By injecting in vitro transcribed mRNA, we observed active MRLC-GFP foci at the single cell level. During neural cells undergoing convergence and cell elongation processes, these foci were dynamically formed in the cell cortex and coalesced, sometimes resulting filamentous structures, which seems more stable than cell protrusive activity in the distal region. (Left) MRLC-GFP, (middle) membrane-RFP, (right) merge images of elongating neural cell to medial side of the neural plate.

IV. Notochord and evolution of chordates

The early embryogenesis of amphioxus, up to the late neurula stage, provides us with useful suggestions about chordate origins and evolution. Its cleavage pattern, resulting in the formation of hollow blastula, resembles those of sea cucumbers (echinoderms) and acorn worms (hemichordates). In contrast to the mode of non-chordate deuterostome gastrulation, in which the archenteron invaginates into a wide blastocoelic space and causing the embryo to become cup-shaped, with a deepened archenteron. This mode of gastrulation is seen in ascidian embryos as well. By the late gastrula stage, the embryo has become ovoid and slightly flattened, and the neural plate is formed from the flattened dorsal side of the embryo. As in vertebrates, neurulation begins with enclosure of the neural plate.

During the period of neural tube formation, the notochord develops from the adjacent chordamesodermal plate that constitutes the roof of the archenteron (Figure 5). Specifically, the notochord is formed by pounching off from the archenteron. Interestingly, *Brachyury* is expressed not only in the region where the notochord pouches off, but also in the region where the somite pouches off. Furthermore, the amphioxus notochord has properties of muscle tissue. The morphogenetic movement of the notochord (and somites) in cephalochordate embryos looks like a continuation of the archenteron invagination. In other words, amphioxus might have recruited the secondary *Brachyury* expression for this second invagination-like morphogenetic movement.

By investigating the cellular morphogenesis and remodeling of tissues underlying amphioxus development and by gaining a comparative developmental biology view of the events, specifically relevant to notochord development, we aim to obtain new insights into the evolutionary changes that took place in a branch of the bilaterian lineage and gave rise to the chordates more than 550 million years ago.

Figure 5. Amphioxus embryos (Branchiostoma floridae). Side views. Anterior at left. (A) Mid-neurula stage embryo. (B) Early larval stage embryo. The notochord runs through the dorsal side by reaching to the anterior tip of the body. Embryos were stained with Alexa 488 phalloidine (green) and CellMask (red and magenta).

Publication List

〔Original papers〕

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