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An Ephrin-Eph Tug and Push in Left-Right Organ Placement

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Abstract

During development, the placement of internal organs asymmetrically along the left-right axis is critical for their proper adult function. Reporting in *Developmental Cell*, Cayuso et al. (2016) demonstrate an active role of the endoderm in this process, challenging the prior view that the endoderm is passively pushed by the mesoderm.

The process of establishing left-right asymmetry occurs in three main phases (Hamada and Tam, 2014). In the first phase, bilateral symmetry is broken through a mechanically intricate process of cilia beating at the node, the organizer of the body plan. In the second phase, the asymmetry is translated into differences in gene expression in the left versus right lateral plate mesoderm. In the final phase, the differential expression of genes in left and right tissues induces changes in cell and tissue morphology, leading to asymmetrical organ placement. Signaling pathways such as Nodal and transcription factors such as PITX2 are key orchestrators of the first two steps of the left-right asymmetry establishment process. Compared to these earlier phases, the third phase, in which asymmetric gene expression is translated into morphogenesis, is poorly understood.

The little that is known about left-right organ morphogenesis is focused on the role of the lateral plate mesoderm as the driver of the process. It has been shown that asymmetric movement of the mesoderm, acting through the extracellular matrix between the endoderm and mesoderm, pushes the endoderm tube towards one side of the developing embryo (Kurpios, et al., 2008; Horne-Badovinac, et al., 2003). In this issue of *Developmental Cell*, Cayuso et al. (2016) reveal an active role for the endoderm in the positioning of the liver in zebrafish.

In zebrafish, the endoderm germ layer gives rise to the thymus, thyroid, swim bladder, liver, pancreas and gut. Cayuso et al. (2016) focused on the liver, an organ with an early and prominent loop to the left. To explore the extent to which cell shape changes and movements of the endoderm-derived hepatoblasts are important during normal hepatic budding and positioning, Cayuso et al. (2016) used time-lapse confocal microscopy and rigorous quantification to analyze cell movement and shape at different stages of hepatic development. They observed that hepatoblasts first undergo elongation along the anterior-posterior axis and then collectively migrate leftward to aggregate into the liver bud.

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Furthermore, cell movement led to the establishment of new neighbor relationships with surrounding cells (that is, they led to neighbor exchanges), a feature indicating active individual cell movement instead of cells being pushed passively as a cohort.

An obvious hypothesis stemming from active migration is a cellular behavior that probes and interprets the environment. Taking imaging one step further, the authors used mosaic genetic labeling to highlight fine cellular processes of individual endoderm or lateral plate mesoderm cells in backdrop background of unlabeled cells. In both hepatoblasts and mesodermal cells, they observed thin finger-like filopodia and flat sheet-like lamellipodia. Remarkably, the filopodial protrusions reach across multiple cells, and even across the endoderm/mesoderm boundary in both directions. This initial sensing phase transitions into the movement phase with a decrease in filopodia and an increase in lamellipodia. These observations offer a clear in vivo demonstration of an active cellular role of the hepatoblasts in liver positioning.

Dynamics of cell shape and movement are often directed by guidance cues, which attract or repel cell populations to a given location. Many of these signal-receptor pairs were first identified by their role in the nervous system, where pathfinding of long axons offers a clear, defining phenotype. A number of these molecules, including Ephrin-Eph, Slit-Robo and semaphorins, have also been found to be essential in multiple developmental contexts outside of the nervous system (Branchfield, et al., 2016; Ochsenbein, et al., 2016; Lewis, et al., 2015). While Ephrin/Eph signaling has recently been implicated in left-right pattern establishment in the zebrafish organizer, its role in later stages of left-right asymmetry had not been explored (Zhang, et al., 2016). Cayuso and colleagues (2016) found that the *EphrinB1* ligand is one of the first genes expressed in hepatic progenitors, whereas its receptor, *EphB3b*, is subsequently restricted to the lateral plate mesoderm. Inactivation of either the ligand or receptor led to defective hepatoblast positioning, without affecting liver progenitor cell number or specification. This finding identifies a bidirectional signaling interaction between developing hepatoblasts and lateral plate mesoderm cells, and corroborates the importance of both of these populations in liver positioning.

The genetic findings also represent deviations from conventional Ephrin-Eph signaling. While knockdown of Ephrin and Eph both disrupts liver positioning, *EphrinB1* morphants have defects in the formation of cellular protrusions. In contrast, *EphB3b* morphants show an increase in filopodia and randomized orientation of lamellipodia. These findings indicate that different cellular mechanisms can result in similar gross organ positioning defects. More interestingly, they suggest that Ephrin and Eph may have distinct roles outside of the obligatory ligand-receptor relationship. Indeed, the investigators show that the role of EphrinB1 in filopodia and lamellipodia formation is dependent on its PDZ domain, likely through an Eph-independent mechanism. There is also evidence of bidirectional Ephrin-Eph signaling, a unique feature compared to general ligand-receptor interactions.

The cellular and molecular findings by Cayuso et al. (2016) offer compelling evidence that left-right organ positioning is not simply driven by the lateral plate mesoderm as previously thought. Rather, it is a result of complex, long-range interactions between the endoderm and mesoderm across tissue boundary and multiple cell diameters. A number of disorders result

from defects in left-right patterning in humans, including situs inversus, in which organ placement is the mirror image of normal, and heterotaxy, in which organ placement is random. Patients with heterotaxy generally have multiple organ defects, including complex cardiovascular malfunction, which lead to significant morbidity and mortality. This study may offer insights into the basis of these disorders. More importantly, the findings raise new questions. For example, how are the complementary Ephrin and Eph expression patterns established to drive directional cell movements, and how do different organs, such as the liver and the gut, undergo distinct modes of left-right morphogenesis at different developmental times? Recent large-scale genomic sequencing of patients with left-right asymmetry defects, coupled with the validation of patient-specific variants using CRISPR-Cas9 genome editing in disease models, have and will continue to expand the list of causal mutations underlying these disorders (Duncan and Khokha, 2016; Guimier, et al., 2015). The in vivo genetic and imaging approaches employed in Cayuso et al. (2016) complement the above genomic approaches, and represent effective tools for uncovering the fundamental mechanisms of organ positioning.

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