

Research Highlight in Developmental Biology

The Cell Biology of Separation—During the Most Important Event of Life

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In an attempt to convince a clinician of the importance of studying early development, the British developmental biologist Lewis Wolpert notably remarked that it is not birth, marriage, or death, but gastrulation which is truly the most important event in your life (Wolpert, 1991). The importance, and wonder, of gastrulation – a process that turns a spherical ball of early embryonic cells into a torus – rests in its outcome. It is during gastrulation that the body plan of animal embryos along with the three germ layers is laid down. Gastrulation also serves as the focal event for the morphogenetic changes that precede the emergence of internal structures during development.

The hallmark process of gastrulation is emboly—internalization of the presumptive mesoderm cells (Solnica-Krezel and Sepich, 2012). Two recent publications (Mason et al., 2013; Nakaya et al., 2013) highlight the cell biology of mesoderm separation during emboly. These studies provide critical insights into the interplay between cell polarity and the molecular stabilizers of cell cytoskeleton that enables the separation of mesoderm during gastrulation.

Gastrulation in the amniote embryo is marked by ingression – the internalization of prospective mesodermal cells – at the transient

midline furrow called the primitive streak (Figure 1). The morphological change that enables cell ingression is the epithelial-to-mesenchymal transition (EMT) of the ectoderm (epiblast) cells. In the study by Nakaya *et al.*, published in the *Journal of Cell Biology*, the authors investigated the role of basal microtubule anchoring proteins in the stabilization of the epiblast-basement membrane interaction, and the facilitation of EMT upon disengagement of the stabilizing mechanism.

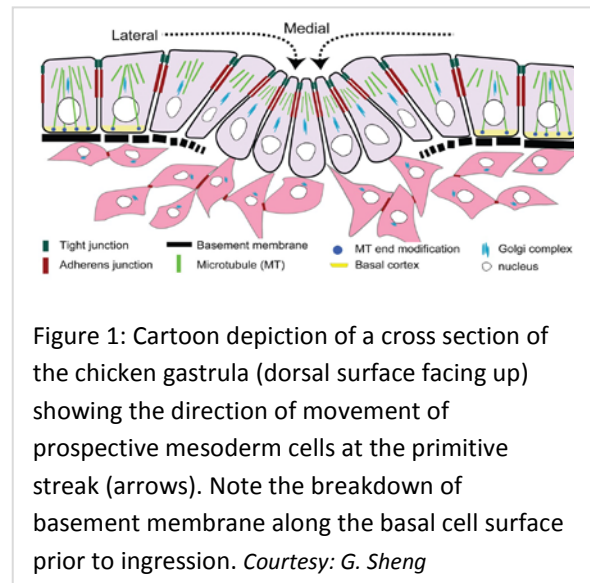


Figure 1: Cartoon depiction of a cross section of the chicken gastrula (dorsal surface facing up) showing the direction of movement of prospective mesoderm cells at the primitive streak (arrows). Note the breakdown of basement membrane along the basal cell surface prior to ingression. *Courtesy: G. Sheng*

Nakaya *et al.* looked at the role of CLASPs (CLIP-associated proteins) and dystroglycan mediated microtubule anchoring to the basal cell cortex during chicken gastrulation. Sequence alignment analysis of the chicken *CLASP1* and *CLASP2* revealed two human counterparts with microtubule binding domains. The mRNA expression patterns were verified for their restricted localization to the epiblast cells prior to gastrulation EMT.

Electroporation of constructs expressing enhanced GFP tagged human CLASPs into the epiblast cells prevented the breakdown of basement membrane (BM), the extracellular matrix anchor, suggesting that the CLASPs function to maintain BM integrity in gastrulating embryos. In addition, introduction of mutant CLASPs lacking their ability to bind to the microtubule plus end as well as to the basal cell cortex resulted in BM breakdown. Furthermore, potent breakdown of epiblast-BM interaction was observed when an antisense morpholino to CLASP1 was used to knockdown the endogenous protein. Nakaya *et al.* also verified that the endogenous CLASPs were localized to the epiblast basal membrane. These data suggested that the maintenance of epiblast-BM interaction requires CLASPs, and their down-regulation promotes gastrulation EMT.

Next, the authors examined the CLASPs binding partners, LL5 α and LL5 β . Overexpression of LL5s recapitulated the phenotype of overexpression of CLASPs, suggesting their agonistic roles in gastrulation EMT. Moreover, the LL5s also localized to the epiblast basal cortex, and their knockdown led to premature BM breakdown, thus paralleling the results of CLASP knockdown experiments. Meanwhile, when cortical microtubule stability was compromised by nocodazole treatment of the embryos, the localization of CLASPs was severely affected in contrast to the mild effects on LL5s. These experiments suggested that although CLASPs and LL5s function as stabilizers of epiblast-BM interaction, the cortical positioning of LL5s is microtubule-independent. Interestingly, however, live imaging experiments revealed that the basal MT stability in epiblasts undergoing EMT is dependent on both CLASPs and LL5s. These results suggested that the epiblast basal cortical interaction of microtubules with CLASPs and LL5s was critical for epiblast anchoring to the BM.

Bioinformatics data mining led the authors to consider basal membrane localized

dystroglycans (DGs) as potential mediators of epiblast-BM interactions at the plasma membrane. DG knockdown cells phenocopied CLASPs/LL5s knockdowns, and the localization and expression profile of DG was strongly suggestive of its role in gastrulation EMT. Finally, the reduction in basal β -DG expression in CLASP knockdown cells indicated their regulation by CLASPs. Evidence for a physical interaction between the CLASPs and DG in epiblast cells undergoing gastrulation EMT came from immunoprecipitation studies and proximity ligation analysis.

From these results, Nakaya *et al.* arrive at a working model of gastrulation EMT in which cell stabilization is mediated by the basal cortical anchoring of microtubules by the interaction between CLASPs and LL5. While CLASPs anchor microtubules to the basal cortex, LL5s localize to the basal cortex independent of MT. CLASPs, in addition, also bind DG at the plasma membrane, and hence indirectly interact with the BM. When the positive interaction loop is compromised, EMT is facilitated and cell separation occurs. Important questions remain on the nature of the role of integrins in this model, and the identity of the developmental signals that break the positive feedback interactions between the epiblast basal cortex and BM during gastrulation EMT.

Unlike the chicken embryo, the separation of mesoderm during gastrulation in the *Drosophila* embryo is accomplished by invagination—the internal folding of the presumptive mesoderm as a continuous sheet—at the transient midline furrow called the ventral furrow (Figure 2). The morphological change that initiates invagination of a cell sheet is apical constriction (AC). AC promotes tissue bending through a cell shape transformation from columnar to a wedge shape. The cell shape change in the *Drosophila* presumptive mesoderm occurs in a pulsed or ratchet-like manner. Induction of AC has been attributed to the expression of two transcription factors: Twist and Snail. Initiation of AC pulses requires Snail, while Twist is

required to stabilize the incremental cell shape changes between the pulses. At the effector level, AC pulses of the ventral furrow cells result from the activity of the network comprising apical F-actin and non-muscle myosin II (Myo-II) accompanied by the remodeling of peripheral adherens junctions (AJs). In their work, published in *Nature Cell Biology*, Mason *et al.* propose a role for the apical domain polarization of actin-myosin activity, acting downstream of Twist target-induced Rho1 GTPase activity, in stabilizing the cell shape transformations during AC.

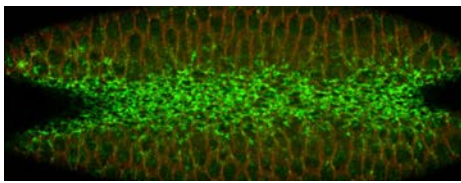


Figure 2: Ventral view of a gastrulating *Drosophila* embryo (anterior facing left). The ventral furrow runs across the length of the embryo along the anterior-posterior axis.
 Courtesy: A.C. Martin

Mason *et al.* began by identifying foci of medioapical Rho-kinase (Rok) and Myo-II localization, in addition to the circumferential localization of E-cadherin (AJs component) in the ventral furrow cells, a pattern they term radial cell polarity (RCP). Then, they looked at the localization of Rho1, and its effector formin Diaphanous (Dia), both acting downstream of Twist targets. They determined that Rho1 localization partially overlapped with Rok foci, but also extended to the domain of adherens junctions. A pattern similar to Rho1 localization was also observed with Dia, its effector that mediates unbranched actin polymerization. These observations implied that Rho1, Rok, and Dia are differentially localized in the gastrulating ventral furrow cells, and the components of Twist pathway are spatially regulated during AC.

Using time-lapse imaging in the presence of a Rok inhibitor, the authors disrupted Myo-II RCP. Interestingly, Myo-II RCP was found to be dispensable for medioapical F-actin assembly. However, Myo-II RCP was required to condense the medioapical F-actin cables at the apical domain. When embryos were injected with cytochalasin D to inhibit actin polymerization, the authors noticed that despite the accumulation of Myo-II and the presence of AC, the medioapical contraction pulses and the F-actin mediated connections between the contractile medioapical domain and adherens junctions were compromised. Their analysis of germline clones using the partial loss-of-function *dia^M* allele with mild pre-gastrulation defects, together with cytochalasin D injections, revealed that Dia-mediated F-actin assembly is indispensable for RCP of E-cadherin in ventral furrow cells. The latter results allowed the authors to propose that Dia-mediated F-actin assembly could facilitate the spatial segregation of the medioapical contractile network and the adherens junction assembly into distinct apical domains while simultaneously linking them via F-actin cables.

Finally, Mason *et al.* analyzed the medioapical actin network in the *twist* mutants, and noticed that the medioapical F-actin network was defective. Meanwhile, the junctional F-actin cables were unaffected in *twist* mutants. In addition, the authors also observed a reversal of Rok and Myo-II RCP in *twist* mutant embryos. Remarkably, instead of medioapical localization, Rok demonstrated junctional localization at three-way vertices of ventral furrow presumptive mesoderm cells. Myo-II enrichment at the junctional vertices followed Rok localization in *twist* mutants. Collectively, these results allowed Mason *et al.* to propose that Twist polarizes Rho1 signaling in the plane of the apical cell surface, leading to the polarization of the effectors of AC—Rok and Myo-II— to the medioapical domain.

The results obtained by Nakaya *et al.* and Mason *et al.* underscore the importance of

spatial segregation/polarization of molecular mediators of morphogenesis in the effector cells. Together, they also demonstrate the relative importance of basal and apical cell cortices in the internalization of presumptive mesodermal cells depending on the morphogenetic paths those cells take during gastrulation. In the chick embryo, where gastrulation requires the “break-up” or “loosening” of the presumptive mesoderm cells from the epiblast layer prior to EMT and ingression, the basal cortical microtubule destabilization leads to the separation of the mesoderm. Meanwhile, in the *Drosophila* embryo, where gastrulation requires the “bending” or “folding” of the presumptive mesoderm cell sheet prior to invagination, the apical cortical F-actin and Myo-II stabilization, and junctional remodeling by Twist signaling, leads to the separation of the mesoderm. In both cases, the effector molecules for separating the presumptive mesoderm during gastrulation act within the spatially segregated domains of internalizing cells.

Although these studies provide some interesting insights into the cell biological mechanisms that make gastrulation possible, the most important event in our life still remains open to more interesting questions for investigations. For example, determining the signals that prompt disengagement of the basal cortical microtubule-CLASPs-DG complex is critical to understanding the mechanism by which EMT is initiated during amniote gastrulation. Also, the roles of LL5s and integrins in destabilizing the cell-BM attachments during gastrulation await further exploration. In *Drosophila*, as the authors acknowledge, investigation of the mechanism by which RCP is established during AC will be critical for our understanding of how a common set of cytoskeletal and signaling proteins are recruited to generate a variety of cellular forms during development.

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