Daam2 with specific small hairpin RNA, the authors demonstrated that Daam2 represents a key mediator of Pitx2 signaling and is indispensable for LR asymmetry in the DM.

It has previously been shown that asymmetric changes in the cell architecture in the DM are partially dependent on the exclusive left-side expression of the cell adhesion protein N-cadherin (Kurpios et al., 2008; Plageman et al., 2011). To uncover the role of Daam2 in this process, the authors inhibited Daam2 activity in the left DM, which resulted in perturbed intercellular N-cadherin-mediated adhesion. Conversely, the introduction of CA-Daam2 into the right DM produced an accumulation of both N-cadherin and α-catenin, as well as lengthening of the cell-cell junctions. This suggests the intriguing possibility that Daam2 may play a role in stabilizing N-cadherin-based junctions. Indeed, the authors demonstrated not only that Daam2 partially colocalizes with α-catenin at cell borders but also that it forms a protein complex with α-catenin and N-cadherin. Although it still remains to be determined whether this interaction is direct or requires additional components, this finding is nevertheless very important as it provides new insights into the mechanism of Daam2 action.

Thus, the work by Welsh et al. (2013) uncovers a connection between two major conserved signaling pathways, Pitx2 and noncanonical Wnt, in the context of LR asymmetry establishment in the developing embryonic gut. The findings presented in this study will help to clarify the molecular mechanisms of midgut malrotations that usually occur in early embryonic development and lead to devastating gut disorders. Future studies will surely focus on whether this connection between Pitx2 and noncanonical Wnt signaling represents a general mechanism that governs polarization and LR asymmetry in other internal organs.

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Forces in Epithelial Origami

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Recent efforts examining forces during morphogenesis suggest a role for mechanical crosstalk between epithelium and mesenchyme in tissue patterning. Reporting in *Science*, Shyer et al. (2013) show that differences between the mechanical properties of the developing intestinal epithelium and surrounding smooth muscle fold the epithelium into villi via mucosal buckling.

Most biological vessels (airways and gut mucosa, for example) are comprised of an inner layer of epithelium surrounded by one or more layers of smooth muscle. In the adult, the intestinal epithelium is folded into either ridges or finger-like protrusions called villi, depending on the species, thus increasing the surface area available for nutrient absorption. The genesis of villi in the embryonic intestinal epithelium is a striking example of geometric changes in form during tissue morphogenesis. In chicken and human, the endodermally derived epithelial layer of the duodenum is initially smooth before forming up to eight ridges directed longitudinally along the length of the tube. These fold into a regular herringbone-like "zigzag" pattern before becoming indented to form the villi (Figure 1). The entire process of epithelial folding takes 9 days in the chicken embryo (embryonic day [E] E7 to E16) and 5 weeks in the human fetus (week 9 to week 14).

In an early study of intestinal villus development, the husband-and-wife team of Alfred and Jane Coulombre reported that these steps of epithelial folding coincided with dramatic morphogenetic changes in the surrounding mesenchyme, with smooth muscle consecutively forming three layers aligned in circumferential, external longitudinal, and internal longitudinal patterns (Coulombre and Coulombre, 1958). Inspired by the seminal work of D'Arcy Thompson (Thompson, 1917), the Coulombres hypothesized that morphogenesis of the duodenal villi is driven by mechanical forces acting on the mucosa resulting from contraction of developing smooth muscle and expansion of the epithelium

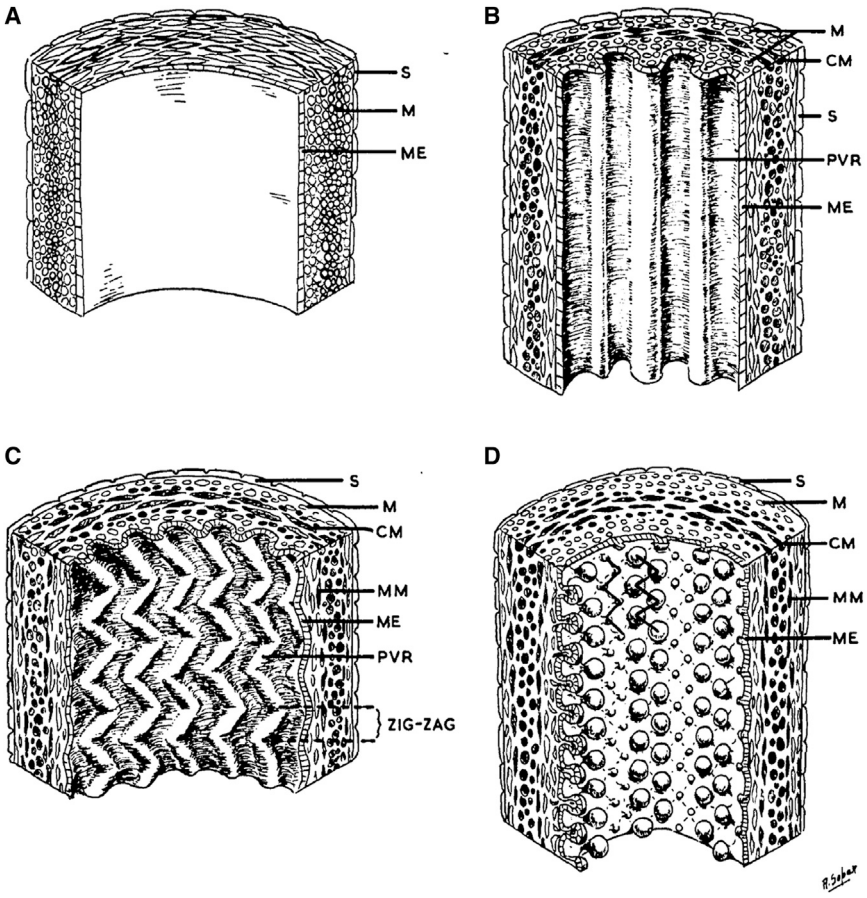

Figure 1. Folding of the Mucosal Epithelium in the Embryonic Duodenum
The initially smooth epithelium (A) first folds into longitudinal grooves known as previllous ridges (B), which then bend into a parallel array of zigzags (C), which fold inward into villi (D). ME, mucosal epithelium; M, mesenchyme; S, serosal epithelium; CM, circumferentially oriented smooth muscle; PVR, previllous ridge; MM, longitudinally oriented smooth muscle. Reproduced with permission from *Development* (Coulombre and Coulombre, 1958), http://dev.biologists.org/content/6/3/403.long.

via growth. This proposed mechanism would have been consistent with the buckling instability of vessels observed in the adult: when the airways are constricted by contraction of the pulmonary smooth muscle, longitudinal folds emerge along the luminal surface in a characteristic pattern that depends on the relative geometric (diameter, thickness) and mechanical (stiffness) properties of the epithelium (Hrousis et al., 2002). Nonetheless, subsequent studies showed that villus formation was impervious to surgical removal of portions of the smooth muscle layer, which interfered with muscular contraction (Burgess, 1975). The buckling epithelium hypothesis was thus abandoned in favor of molecular pursuits.

Whereas smooth muscle contraction may not contribute to morphogenesis of intestinal villi, crosstalk between the developing epithelium and its surrounding mesenchyme is absolutely essential (McLin et al., 2009). Experiments using transgenic and knockout mice have revealed that the intestinal epithelium signals to its mesenchyme via sonic hedgehog (SHH) and platelet-derived growth factor (PDGF). This stimulates signaling through forkhead transcription factors including FoxF1 and FoxF2, which in turn regulate synthesis of Wnts and bone morphogenetic protein (BMP). In mice, BMPs are expressed by clusters of mesenchymal cells, which have been proposed to instruct adjacent epithelial cells to form a periodic pattern of villi (Karlsson et al., 2000) in a mechanism similar to that which forms periodic patterns of hair and feathers in the skin. According to this proposed mechanism, the folding of the intestinal epithelium would thus be a mirror image of the pattern of BMP expression in the mesenchyme. Species-specific differences in mucosal folding (ridges in salamanders and honeycomb patterns in snakes, for example) would necessarily require similar differences in the pattern of mesenchymal expression of BMPs. To date, however, no such patterns have been reported.

Although the catalog of molecules that regulate morphogenesis has grown, our understanding of how these signals direct changes in tissue form remains incomplete. The past 10 years have thus seen a resurgent interest in the ideas of Thompson, Wilhelm His, and other early developmental biologists and renewed efforts to investigate the mechanical mechanisms that underlie tissue morphogenesis (Nelson and Gleghorn, 2012). These studies have identified some of the mechanical forces required for tissues to fold themselves, including myosin-mediated line tensions that drive intercalation during germband extension in *Drosophila* and apical constrictions that induce epithelial invagination during gastrulation, optic cup formation, and avian airway branching.

It is against this backdrop that the forces driving morphogenesis of the intestinal villus were recently revealed by the work of Shyer et al. (2013), published in *Science*. In an elegant combination of classical embryology and computational modeling, the authors show that each step of epithelial morphogenesis, from the formation of longitudinal ridges to zigzags and to villi can be explained simply by the mechanical forces acting on the epithelium as it grows within a stiffening tube of developing smooth muscle, similar to the mechanism first proposed 55 years ago by the Coulombres. Importantly, and where the earlier hypothesis was incorrect, smooth muscle contraction is not required for the embryonic intestinal epithelium to fold. Differentiation of the three layers of smooth muscle provides a sufficiently stiffened barrier to expansion of the growing epithelium and thus causes the epithelium to buckle inward. Somewhat contrary to the dogma of epithelial-mesenchymal crosstalk in developmental biology, Shyer et al. (2013) also show that the smooth muscle layers are only required mechanically: culturing the initially flat mucosal epithelium within a stiffer sheath of silk fibers in

the absence of smooth muscle was sufficient to constrain the tissue and induced the sequential folding observed in vivo.

Folding of the intestinal epithelium can thus be described as a buckling instability. Importantly, the details of this process can explain the different mucosal morphologies observed across species. The *Xenopus* intestine lacks the second longitudinally oriented smooth muscle layer, and the epithelium correspondingly stops its morphogenesis at the zigzag folding pattern; no zigzag pattern of BMPs is needed. In mice, the intestinal mucosa forms villi directly from the flat epithelial surface, a phenomenon that the computational model of Shyer et al. (2013) suggests is a result of the relatively fast pace of smooth muscle differentiation in this species (3 days, from E11.5 to E14.5). The intermediate and final geometries obtained by the intestinal mucosa thus depend on its rate of growth, its geometry, and the mechanical properties (and their rate of change compared to those of the epithelium) of the surrounding smooth muscle.

In light of these results, our basic understanding of epithelial-mesenchymal crosstalk in the developing intestine should be reexamined. Past analyses of the roles of SHH, PDGF, Wnts, and BMPs were based on manipulations that may have compromised the simultaneous differentiation and/or alignment of smooth muscle. Although Shyer et al. (2013) suggest that the effects of smooth muscle on the folding epithelium are purely mechanical and that the epithelium folds passively, it is possible that some of the molecular signals secreted by the mesenchyme induce a parallel active folding by the epithelium in the form of apical or basal actomyosin constrictions. More controlled inducible genetic manipulations, perhaps combined with computational modeling of tissue mechanics, should shed light on this process.

More broadly, communication between an epithelium and its surrounding mesenchyme is critical for the morphogenesis of most tissues. Epithelial morphogenesis occurs at the same time as smooth muscle differentiation during the development of many organs, including the lung and prostate. While the molecular signals derived from the mesenchyme are well appreciated, recent efforts have uncovered some of the active forces in the epithelium that permit it to fold. It is thus tempting to speculate a similar role for smooth-muscle-induced buckling in these organs as well, for "we have little reason to doubt, and no just cause to disbelieve, that the whole configuration… is accurately determined by simple physical laws" (Thompson, 1917).

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Polycomb Group Protein Bodybuilding: Working Out the Routines

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Polycomb group (PcG) proteins regulate gene expression by modifying chemical and structural properties of chromatin. Isono et al. (2013) now report in *Developmental Cell* a polymerization-dependent mechanism used by PcG proteins to form higher-order chromatin structures, referred to as Polycomb bodies, and demonstrate its necessity for gene silencing.

Polycomb group (PcG) proteins are required for the maintenance of cell-type-specific gene expression and, thereby, play a major role in the formation of cell type diversity observed in animals and plants. PcG-mediated gene regulation requires the localization of multimeric protein complexes, such as the Polycomb repressive complexes (PRC1 and PRC2), to specific chromatin targets, where they catalyze particular chemical modifications at nucleosomes such as trimethylation of lysine residue 27 in histone 3 (H3K27me3) (Simon and Kingston, 2013). These modifications in turn are thought to block gene expression by interfering with transcriptional elongation or by causing compaction of the chromatin fiber (Simon and Kingston, 2013). A detailed understanding of the PcG-mediated link between chromatin