TISSUE MORPHODYNAMICS SHAPING THE EARLY MOUSE EMBRYO

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Abstract

Generation of the elongated vertebrate body plan from the initially radially symmetrical embryo requires comprehensive changes to tissue form. These shape changes are generated by specific underlying cell behaviors, coordinated in time and space. Major principles and also specifics are emerging, from studies in many model systems, of the cell and physical biology of how region-specific cell behaviors produce regional tissue morphogenesis, and how these, in turn, are integrated at the level of the embryo. New technical approaches have made it possible more recently, to examine the morphogenesis of the mouse embryo in depth, and to elucidate the underlying cellular mechanisms. This review focuses on recent advances in understanding the cellular basis for the early fundamental events that establish the basic form of the embryo.

Key words

Gastrulation

Axial elongation

Morphogenesis

Convergent extension

Planar cell polarity

Mouse

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1. Introduction

Creating an elongated embryo with a distinct head and tail from a spherical ball of cells requires extensive tissue morphogenesis. The changes in tissue shape are driven by specific, coordinated behaviors of the cells that make them up, and these behaviors lead not only to changes in tissue shape, but also to specification and differentiation. Cell function in tissue shape change has been thought about for some time and in some depth. In seminal work, Gustafson and Wolpert [1, 2] noted that, in theory, differential adhesions of lateral and basal surfaces of cells in sheets to one another or to surfaces of extracellular matrix could drive cell shape changes. In turn, these changes applied to a cuboidal cell, and integrated across cell sheets, could cause change in tissue shape. For example, cell thinning or flattening causes spreading of the sheet, cell columnarization or palisading causes shrinkage or contraction of the sheet, and

cell wedging causes bending of the tissue. Others, such as Holtfreter [3-5], argued that internally driven, cell autonomous, changes in cell shape were also important in behavior of cell sheets, and locally autonomous, if not cell autonomous, changes in cell shape generate endogenous forces that reshape the neural plate [6, 7]. Current work has built on these pioneering studies, providing evidence for actomyosin-based apical constriction driving bending of cell sheets (invaginations) [8-10], as well as apical-basal shortening with apical constriction [11]. Cell division, even without growth (increase in cell volume), as is the case in many yolky embryos, such as many amphibians [4], can contribute to tissue shape change by being oriented. Cell division with growth has long been thought to drive the folding movements of embryos [12-14], a particularly significant mechanism in embryos that show dramatic growth in early development, including birds and mammals [14, 15]. Intercalation of cells along an axis within the plane of a multilayered tissue can produce a narrower, longer tissue (often called convergent extension, or CE), and cell intercalation normal to the plane of a multilayered tissue (radial intercalation) can thin the tissue [16, 17]. Finally, movements and associations of cells may be regulated by cell-cell recognition and selective affinity [18], which is the notion that cells recognize one another on contact as similar or different, and that they can show either a positive or negative affinity, seen as a high or low probability of maintaining contact.

1.1. Passivity vs. activity

In all cases of tissue shape change, the question arises, is it endogenously driven and active, or is it a passive response to external forces? Many large morphogenic processes involve both active and passive phases, and it is only with detailed analysis of the relationship of cell behavioral changes and mechanical interactions of tissues can this be determined [19]. Only in a very few cases do we know where and how the forces are being generated, and this is particularly true in mammals. Recent advances in live imaging techniques [20-23] have made it possible to more fully analyze the morphogenesis of the mouse embryo and to elucidate the underlying cellular mechanisms. This review

summarizes our current knowledge of the cell behaviors that generate the basic form of the mouse embryo, and identifies areas where further insight is needed.

2. Formation and migration of the distal visceral endoderm (DVE) to become anterior visceral endoderm (AVE).

One of the critical morphogenetic events that shapes the early mouse embryo is formation of the DVE at the distal tip of the embryo and its subsequent migration proximally to a position near the boundary with the extraembryonic tissues, to become the AVE ([24-26], reviewed [27]). How the AVE is formed is particularly important because it is a key signaling tissue that regulates establishment of the embryonic anterior/posterior (A/P) axis through inhibitory effects on Nodal signaling [28-30]. Cells of the AVE produce Cerberus-like 1 (*Cer1*) and Lefty 1 (*Lefty1*), inhibiting Nodal signaling anteriorly, inducing differentiation of ectoderm in the underlying epiblast, and restricting the position of the primitive streak to the posterior side ([29, 30], reviewed [27, 31]).

1.1. DVE formation: Potential uterine influence

A very novel cellular mechanism for the formation of the DVE has been proposed [32], that implicates mechanical constraint by the uterus as a morphogenic force. Circumferential restriction of the early embryo (E5.0) in in vitro culture, mimicking the compression forces of the decidua on the growing embryo in vivo, leads to preferential proximal-distal elongation of the embryo. The mechanical force imposed on the distal tip results in basement membrane breaches that allow extrusion of a small number of epiblast cells into the visceral endoderm (VE) layer, where they become the *Cer1*-positive cells that form the DVE. However, a recent report [33] calls this mechanism into question, demonstrating DVE formation and migration in embryos cultured in vitro without any physical restraint. Technical advances in in vitro culture of mouse embryos through the implantation stage should facilitate a more thorough analysis of this event to resolve these conflicting findings [34].

2.2. Migration and intercalation in the AVE

Once established, the DVE undergoes a very unusual migration [24, 25, 35], forming very long basal protrusions that extend in the direction of migration [25, 36]. At the same time, DVE cells maintain their epithelial characteristics, and exchange neighbors along the migration pathway [35, 37]. This behavior not only characterizes the leading DVE cells, but also surrounding cells that will contribute to the AVE [38], establishing this as a coordinated, or collective, cell migration. Genetic studies have identified roles for actin regulators Rac1 and the WAVE complex as well as PTEN in this migration [35, 39, 40], consistent with the active, directed protrusive behavior. The factors that regulate the directionality and extent of migration are currently unknown, but may emanate from the extraembryonic ectoderm (ExE). Regulation of DVE formation relies on repressive factors produced by the ExE [41], and the AVE cells shift from anteriorly- to laterally-directed migration when they reach the boundary with the DE overlying the ExE [25, 35-37] suggesting a regulatory activity associated with this region. The AVE thus represents a very interesting model system for studying coordinated epithelial migration in an epithelium.

3. Gastrulation movements

3.1. Comparative overall cell movements

Formation of the AVE restricts Nodal signaling to the prospective posterior of the embryo, where it induces formation of the primitive streak and the onset of gastrulation [29], reviewed [27, 42, 43]. The primitive streak is a region of epithelial-mesenchymal transition (EMT) where epiblast cells ingress to generate a new population of mesenchymal cells that give rise to mesoderm and definitive endoderm (DE). The cellular mechanisms underlying this critical morphogenetic event have been most thoroughly studied in chick [44-47] and rabbit embryos [48-52], and much less is known about gastrulation in the mouse [21, 53-57]. The chick and rabbit, which are relatively large, flat disc-shaped embryos at the time of gastrulation, exhibit large-scale movements of cells toward the midline. In the chick, epiblast cells located far laterally in the blastoderm move posteriorly in

circular whorls on either side of the nascent primitive streak, and when they meet at the posterior midline, they turn anteriorly together to form the streak, a movement known as “polonaise movements” [46, 58]. Movements in the rabbit epiblast describe L- and U-shaped patterns from peripheral toward the primitive streak [51]. In both cases, planar intercalation of precursor cells leads to elongation of the streak structure, followed by ingression and migration of mesendoderm [47, 51].

By contrast, in the mouse embryo there are no large-scale movements of epiblast cells, and cell intercalation does not appear to play a role in formation or elongation of the streak [53, 54]. The mouse primitive streak forms by progressive initiation of EMT in the posterior epiblast, beginning at the proximal posterior boundary with the ExE and extending to nearly the distal tip [53]. There is no obvious active migration toward the primitive streak, or cell rearrangement in the epiblast, suggesting that ingression of epiblast cells at the streak provides the force propelling new cells to the streak region [53]. The differences observed between the mouse, chick and rabbit systems may stem from the vast differences in size and morphology of the embryos; chick and rabbit embryos are many times larger than the E6.75 mouse embryo at the start of gastrulation, and are flat, while the mouse embryo is cup-shaped. In addition, while the mesoderm is specified at the embryonic/extraembryonic border in the chick [59], it lies adjacent to the primitive streak in the mouse [60-62] and so does not have to translocate great distances to undergo ingression at the proper place.

3.2. Mechanism of streak formation.

Formation of the primitive streak is defined operationally as induction of competence for EMT in the posterior epiblast. The process of primitive streak EMT has three distinct phases; basement membrane loss, ingression, and migration, with specification of mesendoderm fate accompanying ingression and migration. A signaling network comprised of BMP4 from the ExE and Nodal and Wnt3 from the embryonic region is required for streak formation (reviewed [31]),

because mutants lacking BMP4, Nodal, or Wnt3 do not form a streak and fail to specify mesendoderm.

3.3. Mechanisms of EMT at the streak.

Basement membrane loss is required but not sufficient to trigger EMT and ingression of cells from the epiblast [53]. Studies in the chick have demonstrated that the basement membrane is maintained by basal RhoA activity and stabilized basal microtubules in the epiblast cells of the primitive streak [63, 64]. Inhibition of RhoA leads to basement membrane loss and down-regulation of integrin $\alpha6\beta1$, and destabilization of microtubules with nocodazole has a similar effect [63, 64]. These findings are consistent with data showing that constitutive activation of RhoA in the epiblast of the mouse [65] causes failure of ingression and migration and leads to cell accumulation at the primitive streak.

Ingression involves specific changes in cell shape and in cell-cell adhesion [48, 53, 63, 64, 66]. The cells begin by undergoing apical constriction to become flask-shaped, reduce expression of components of the tight and adherens junctions (e.g. occludin, E-cadherin) and of the apical membrane domain (aPKC) [53, 64]. As an ingressing cell constricts, the surrounding cells are brought together, and as the cell leaves the epithelium a rosette is formed by the surrounding cells [67]. Note that these rosettes are formed by contraction of the apex of a single cell, rather than contraction of the connected boundaries of a number of cells, as in epithelial cell intercalation ([68] discussed in Section 4.2.2), and they represent very different processes beyond the fact that both involve a myosin mediated contraction.

3.4. Dynamic changes in E-cadherin regulate ingression and migration.

The adhesion molecule E-cadherin acts as a trigger for EMT in epiblast cells. Inhibiting cell adhesion in epiblast explants with a function-blocking E-cadherin antibody causes conversion to a mesenchymal phenotype, with a concomitant switch in intermediate filament expression from cytokeratin to vimentin [69].

Conversely, retention of E-cadherin is associated with failure of ingression and/or migration. FGF8 and NIK/Map4k4 signaling pathways control E-cadherin expression in parallel [70-73]. FGF8 induces expression of the transcription factor Snail [72], which represses transcription of *cdh1* [72, 74, 75]. However, transcriptional repression of the E-cadherin gene alone is not sufficient to alter cell-cell adhesion, as seen in embryos mutant for p38 interacting protein (p38IP) [73], where epiblast cells continue to express E-cadherin protein despite having normal levels of FGF8 and Snail [73]. Analysis of p38IP mutants demonstrated that activation of p38 by NIK/Map4K4 through p38IP leads to downregulation of the E-cadherin protein, which, together with the transcriptional repression by Snail, rapidly eliminates E-cadherin and facilitates mesendoderm migration [73]. Interestingly, evidence for yet another level of E-cadherin regulation during ingression comes from embryos lacking Dapper Antagonist of Beta-catenin 1 (Dact1; [76]). Dact1 binds to Dishevelled (Dvl) and modulates both canonical Wnt/Beta catenin and Wnt/planar cell polarity (PCP) signaling pathways [77-80]. In the primitive streak, Dact1 regulates Van Gogh-like 2 (Vangl2) post-translationally to reduce E-cadherin in the membrane of epiblast cells [76]. Streak epiblast cells lacking Dact1 maintain E-cadherin localization in the membrane, and ingression fails, particularly in the later, posterior streak, a failure that is ameliorated by reducing Vangl2 levels [76]. Altogether, it appears that destabilization of E-cadherin at the membrane permits ingression, and inhibition of transcription of E-cadherin mRNA, and degradation of existing E-cadherin protein permit migration.

3.5. *Dynamics of DE formation.*

Once they have left the epiblast epithelium and depending on their position within the streak [62, 81], mesoderm cells will migrate in one of two ways: proximally to form extraembryonic mesoderm [82], or laterally and anteriorly between the epiblast and the overlying VE to form “wings” of mesoderm [81]. The cells migrate directionally and individually [53, 54, 56] until they reach the anterior. Cells that are fated to become DE, on the other hand, first exit the primitive

streak to migrate anteriorly and laterally with the mesoderm [83], and then undergo a mesenchymal to epithelial transition (MET) to intercalate into the VE [83, 84]. Intercalation occurs at the level of single cells, generating a salt and pepper mix of cells of VE and of DE lineage in the final layer of endoderm ([83, 84]; reviewed [85]). The transcription factors FoxA2 and Sox17 are required in complementary ways for the prospective DE of the foregut and mid-/hind-gut respectively, to intercalate into the VE [83, 85]. Prospective DE cells lacking FoxA2 can intercalate into the VE, but fail to express the tight junction protein claudin 4, or to localize E-cadherin and ZO-1 properly, and are subsequently excluded from the epithelial layer [86]. This implicates changes in apico-basal polarity and junctional adhesion proteins as part of the cellular mechanism for the MET.

4. Elongation of the body plan: Axial and paraxial morphogenesis.

The initial stages of gastrulation are characterized by extensive ingression and migration of mesendoderm, which place the definitive germ layers in their proper positions and are accompanied by significant changes in overall size, but little change in overall shape of the embryo [87]. However, this is followed by very dramatic changes in tissue shape as the embryonic axis undergoes morphogenesis (Fig. 1). In this phase, the anterior portion of the axis becomes concave, folding perpendicular to the A/P axis of the embryo to form the head folds. The axial and paraxial mesoderm and the overlying neural ectoderm of the trunk region narrow and lengthen extensively, and the neural ectoderm and the gut endoderm fold along the A/P axis of the embryo to form the neural tube and the gut tube, respectively (Movie 1.).

4.1. Cranial morphogenesis: Formation of the head folds.

Axial morphogenesis begins with thickening and flattening of the anterior half of the embryo to form the neural plate [87], followed by infolding and expansion in a concave shape to form the head folds on either side of the neural groove (Fig. 1). Expansion of the head folds proximally and anteriorly helps rotate the cardiac

mesoderm distally, placing it posterior and ventral to the future cranial region (Movie 1; reviewed [88]). In addition, this rotation and folding creates the foregut pocket, the anterior opening of the future gut tube (Fig. 1). This represents an additional function of the AVE in specifying axial development, as BMP2 produced by the AVE signals to the anterior epiblast to initiate this morphogenesis [89]. Interestingly, a similar function in inducing neural head fold formation is seen for the anterior endoderm in the chick embryo [90].

Several genetic mutants, in addition to BMP2, affect head fold formation, namely *Flrt3* (fibronectin leucine-rich transmembrane protein 3) [91, 92], the transcription factor *Gata4* [93, 94], *Hgs/Hrs* (HGF related tyrosine kinase substrate; [95]), and the proprotein convertase *Furin* [96], however it is completely uncharacterized at the cellular level. There are very important tissue-level changes occurring that must involve cell behaviors that generate specific forces. The first is the infolding of the epiblast. The action of BMP2 may play a role in driving cell shape changes in the neighboring epiblast and endoderm to generate this tissue change [89]. These are likely complementary shape changes on the part of the endoderm and the epiblast: for example, apical constriction for the endoderm, and basal constriction/apical expansion for the neural plate [97, 98]. The increase in length of the cranial region likely drives neural fold expansion by CE. The inflection points just posterior to the AVE and at approximately the posterior hindbrain boundary, where the curvature of the embryo changes from concave to convex (Fig. 1, Movie 1) appear to be critical elements of the infolding process. These regions may act as stabilizing points against which CE of the cranial region exerts force to expand the head folds. Needless to say, these events deserve much more in depth analysis to characterize the underlying cell behaviors.

4.2. Neural, axial/paraxial elongation: formation of the spinal cord, notochord, and somitic array.

The body axis is formed through anterior-posterior elongation of the posterior neural plate and the associated axial and paraxial mesoderm, which lays the foundations for the spinal cord and vertebral column. Lengthening and narrowing of these tissues is driven, in all chordates, by convergent extension (CE), a kinematic description of tissue narrowing (convergence) with commensurate elongation (extension). CE can occur as a passive stretching of tissue due to external tensile forces, or, of more interest here, as an active, force-producing process in which cells become polarized transverse to the axis of elongation and actively intercalate between one another, acting as wedges to force either neighbors apart to form a narrower, longer tissue (reviewed [17]).

Intercalation of *mesenchymal* (non-epithelial) cells occurs as cell protrusive activity [99], contraction of the associated actomyosin cytoskeleton [100, 101], and cell traction on neighbors all become bi-polarized in the mediolateral direction, and are collectively known as mediolateral intercalation behavior (MIB). MIB generates tensile forces that result in cell elongation, alignment, and wedging between one another along the mediolateral axis to form a narrower, longer array [102]. The mesenchymal mode of cell intercalation was originally defined in the mesodermal tissue of the frog [103, 104] and fish [105-107], and since then in many systems [17].

Epithelial tissues also undergo CE, and two major cellular mechanisms have been established for this. Analogous to the MIB of mesenchymal CE, cells in the *C. elegans* epidermis intercalate by insertion of basolateral protrusions between neighboring cells followed by “plowing” of the remainder of the cell through the adherens junctions as it moves forward [108-110]. In contrast, in the *Drosophila* germ band, cells intercalate through polarized apical junctional boundary shortening without basal protrusive activity, resulting in movement of cells between one another and into contact with new neighbors, either in pairs (the T1/T2/T3 mode) [111] or in groups (the rosette mode) [68]. The cells then “resolve” into narrower, longer arrays by exchange of neighbors. In addition to

polarized cell intercalation, polarized epithelial cell shape change has also been implicated in convergence, though not much extension, and neural plate bending in the chick embryo [112].

4.2.1. Planar cell polarity regulation of convergent extension.

A major advance in understanding the genetic and molecular mechanisms of these polarized cell behaviors in vertebrate tissues was the discovery that components of the planar cell polarity (PCP) signaling pathway, which specifies polarity within the plane of the tissue in *Drosophila* [113-116] are also essential for the polarized cell behaviors underlying CE in vertebrates [117-121]. The non-canonical Wnt/PCP pathway was identified and characterized in vertebrates on the basis that it polarizes cell behaviors and cell structure in vertebrates, and shares many components with the *Drosophila* pathway (reviewed in [122-124]). It consists of Frizzled (Fz) receptors and proteins homologous to the *Drosophila* Van Gogh, Starry night/Flamingo, Dishevelled, Prickle, and Diego, and, in addition, Wnt5a and Wnt11 ligands for Fz, whereas in *Drosophila* no Wnt ligand has been identified thus far [125]. In frogs [118, 119] and fish [117], perturbation of this pathway results in failure of MIB in the presumptive mesoderm and neural plate [126-129].

In mice, mutations of Wnt/PCP pathway genes, including Van Gogh-like (*Vangl1/Vangl2*) [130-133], Flamingo (*Celsr1*) [134], Frizzled (*Fz3/Fz6*) [135], and Dishevelled (*Dvl1/2*) [136, 137] yield mice with short body axes and an open neural tube posterior to the hind brain, a phenotype known as craniorachischisis (CRN) (Fig. 2). In addition, each of these mutations also affects the polarized organization of hair cells in the inner ear, as well as CE of the cochlea (reviewed in [138]). Mutations in two other genes, *Protein tyrosine kinase 7* (*Ptk7*) and *Scribble1* (*Scrib*) have similar defects and interact genetically with *Vangl2* and *Celsr1* [132, 139, 140]. The combined phenotype of CRN and hair cell polarity defects has become the hallmark of Wnt/PCP disruption in mammals.

Importantly, mutations in the human genes *VANGL1*, *VANGL2*, *DVL2*, *CELSR1*, *PK1*, and *SCRIB1* are also associated with neural tube defects [141-146]. Within the chordates, the function of this pathway in regulating CE is conserved in ascidians [147], fish [117, 148], frogs [118, 149-152] and mice, and has provided a way to analyze the cellular and molecular bases of neural tube defects in humans [123, 143, 144, 146, 153, 154].

4.2.2. CE of mouse paraxial mesoderm.

This mesenchymal mode of CE through MIB characterizes the behavior of the mouse paraxial mesoderm (Movie 2; [155]). It is induced as these cells leave the primitive streak by multipolar, anteriorly directed migration, and then elongate and orient their long axis towards the midline of the embryo [155]. As they progress anteriorly in the presomitic files, they further elongate and polarize their protrusive activity to the medial and lateral ends [155]. As they progress from multipolar to bipolar protrusive activity, the cells undergo a transition from directed migration, first to a zig-zag movement that leads to very promiscuous and inefficient neighbor exchange, and then to a very conservative, efficient MIB that leads to significant change in tissue shape. The convergence of presomitic mesoderm through MIB leads to extension, but also to thickening through radial cell movement, so overall the paraxial mesoderm becomes narrower, longer, and thicker. The somites are thus relatively cuboidal in shape.

4.2.3. Neural convergent extension in the mouse.

The neural plate of the mouse embryo is a pseudo-stratified epithelium, which is a single cell layer at the time that axial elongation occurs. The trunk neural plate undergoes CE through mediolateral intercalation (Movie 3), and, similar to the germ band of *Drosophila*, the neural epithelial cells undergo apical junctional neighbor exchange through both T1-T2-T3 rearrangements and rosette formation and resolution [20], as well as single-cell intercalation, a mode not seen in the *Drosophila* germ band. There is also substantial cell growth and proliferation

within the neural plate, which contributes to CE indirectly by increasing the tissue volume.

Mouse neural cells are also elongated basally along the mediolateral axis of the embryo, and exhibit MIB [20]. Notably, the apical junctional intercalation and the polarized basal MIB lead the cell in the intercalation process with equal frequency, suggesting that both actively contribute to the process [20]. Thus the mouse neural plate is an example in which the junctional rearrangement models and basal MIB models co-exist, which raises the question of what role the vertebrate, non-canonical PCP pathway has in each. Neural CE does not drive thickening of the tissue by radial cell movement such as is seen in the paraxial mesoderm, but the cells elongate in the apical-basal dimension and constrict their apical area, becoming somewhat cone-shaped [20] a shape change nearly universally associated with bending of epithelial sheets [102].

4.2.4. Genetic regulation of specific cell behaviors underlying cell intercalation.

Mutations in a number of genes result in failure of axis elongation and/or CRN, but few have been characterized in terms of what they do to the underlying cell behavior, a critical step in establishing a mechanism by which genes affect tissue morphodynamics.

To date, the effect of disrupting Wnt/PCP signaling components on mesodermal cell behavior has been extensively analyzed only in the *Ptk7* mutant [155]. Analysis of the *Ptk7* mutant established that the key event is the signal triggering elongation and orientation of prospective somitic mesoderm as they leave the streak. The nature of this signal unknown, but clearly involves the function of Ptk7. In the absence of Ptk7 the paraxial mesoderm cells fail to elongate, orient, or convert to bipolar protrusive activity, but continue their multipolar, anteriorly directed migration. As a result, in the absence of CE the presomitic tissue is very short in the anterior-posterior dimension, and very wide in the mediolateral

dimension, leading to somites that are rectangular rather than cuboidal [140, 155]. Surprisingly, the loss of convergence also uncovered a previously undetected radial intercalation behavior in the pre-somitic mesoderm [155]. In the *Ptk7* mutant, active radial intercalation of the presomitic mesoderm thins the tissue and increases its area, thus adding to the mediolateral extent of each somite.

Two mutant mouse strains that exhibit CRN have been analyzed for effects on neural CE, and have illuminated some of the critical mechanisms. Embryos lacking *Ptk7* exhibit almost complete failure of neural CE, while *Lp* mutant embryos have significantly decreased CE but do elongate to a greater extent than the *Ptk7* mutant [20]. Interestingly, the two mutants have completely different effects on cell behavior. Neural cells in the *Lp* mutant are mediolaterally elongated basally and exhibit normally polarized basolateral protrusive activity and normal polarity of apical junctional rearrangement and neighbor exchange. However, they rearrange only half as often as the wild-type, and the effect appears to be localized to the apical region. In contrast, in the *PTK7* mutant embryo the cells rearrange with normal frequency but with randomized direction. In addition, the basal ends are not elongated along the mediolateral axis, and the basolateral protrusions are randomly distributed.

These two mutant lines illustrate that neural CE requires both polarized intercalation and also a frequency of cell rearrangement sufficient to generate normal elongation. In addition, the basal and apical ends of neural cells must cooperate to promote cell rearrangement in this system, as the normal basal protrusive activity in the *Lp* mutant cells was not sufficient to promote normal frequency of cell rearrangement. This cooperative behavior is in contrast to what is seen in the *Drosophila* germ band, where apical rearrangement appears to be the primary driving mechanism [68, 111], and to what is seen in the *C. elegans* epidermis, where MIB is the primary driver [108, 110].

The fact that the *Lp* mutation did not affect the polarity of either protrusive activity or cell intercalation was surprising, because Vang in *Drosophila* and Vangl in frog, fish, and mouse have been shown to affect cell and tissue polarity [132, 148, 151, 152, 156]. The lack of effect on polarity of neural CE may reflect compensatory activity of Vangl1 in specifying polarity [133], or it may be that the core Wnt/PCP signaling complexes are not required for polarity in the neural epithelium in the same way as in other epithelia. The effect that the *Lp* mutation has on apical junctional rearrangement, for example, may indicate a role for Vangl2 in regulating cadherin dynamics, similar to what is seen in the primitive streak [76]. More in-depth analysis of cell behavior in embryos lacking PCP components is needed to clarify the function of PCP signaling in CE.

4.2.5. Competition of forces shaping the axis.

Analysis of mesodermal and neural CE illustrates a general principle of how cell behaviors generate competing forces that lead to the final tissue form. There are three distinct forces at work in the mouse axis. The first is proliferation and growth, which is not polarized and thus generates tissue expansion in all dimensions (A/P, mediolateral, dorsal-ventral). The second is a mediolateral convergence force generated by MIB, which restrains lateral tissue expansion, channeling it instead along either the A/P axis (extension) or along the dorsal-ventral axis (radial de-intercalation or thickening) (Fig. 2). Finally, there is a radial intercalation force in the paraxial mesoderm that restrains tissue expansion in the dorsal-ventral dimension, channeling it instead along either the A/P axis (extension) or the mediolateral axis (thinning) (Fig. 2). The epithelial nature of the neural tissue restrains expansion in the dorsal-ventral dimension, leading to apical-basal lengthening in response to mediolateral convergence force. The balance of the mediolateral convergence and radial intercalation forces in the context of constant growth pressure and a consistent A/P resistance to extension determines the ultimate shape of the tissue: more mediolateral convergence force and less radial intercalation force give both elongation and thickening, while

more radial intercalation force and less mediolateral convergence force leads to extension and tissue thinning. The mouse model also provides a novel insight into how cryptic cell behaviors can help shape a tissue, as an underlying (unseen) cellular process may be at work countering tissue movements occurring in an opposite direction. During normal development, the pre-somitic mesoderm thickens substantially due to cells leaving the planar surface, the opposite of what is expected from radial intercalation. But in *Ptk7* mutants, the driving convergence force is deleted, revealing the unopposed and very active radial intercalation that dramatically thins the pre-somitic mesoderm. Thus, during normal development the radial intercalation force cannot be detected because it is not as great as the thickening generated by the mediolateral convergence force, but it acts to reduce thickening and promote tissue extension.

The mouse embryo provides clear alternative examples of how these competing forces shape a tissue. In the paraxial mesoderm, a relatively greater mediolateral convergence force pairs with proliferation to generate both extension and thickening, whereas a relatively smaller mediolateral convergence force pairs with proliferation in the neural plate to generate extension alone [20, 155]. At the level of the embryo the balance between the overall extension of each of these tissues gives rise to the shape of the axis, and failure in one tissue is likely to lead to axial defects in the other. Indeed, mutants that affect paraxial mesoderm morphogenesis (e.g. $\alpha 5$ integrin [157] or fibronectin [158]) lead to kinking and waviness in the neural tube, which may represent unbalanced extension of the neural tissue in the absence of appropriate extension of the mesoderm.

5. Conclusions

By analyzing the morphogenesis of the early mouse embryo in greater cellular detail we have shown that many cellular behaviors identified in other model systems, both vertebrate and invertebrate, contribute to tissue morphodynamics in mammals. In particular, the mesenchymal MIB seen in frogs and fish axial and

paraxial mesoderm [16, 17] characterizes mouse mesodermal cells, and both the apical junctional shortening and basal protrusive activity model of *Drosophila* and *C. elegans*, respectively, describe the behavior of mouse neural cells. However, there are clear differences in the repertoire of cell behaviors that mouse cells use to drive CE from those described in other systems, and distinct differences in the regulation of these behaviors by PCP signaling. These may relate to the differences in the context in which these cell behaviors act, in particular the mechanical force landscape in which gastrulation and axial elongation occur in the different embryos, as well as the significant contribution that growth makes to morphogenesis in the mouse embryo compared to frog, fish, and fly embryos. In this context it is interesting that changes in hydrostatic pressure within the amniotic cavity can regulate CE in mouse axial mesoderm [159]. Likewise, given the substantial difference in morphological contexts for cranial and trunk neural tube morphogenesis, it is intriguing that mouse mutants that give rise to cranial neural tube dysgenesis do not affect trunk neural tube morphogenesis and vice-versa [160]. At the moment the mechanical aspects of mouse development remain to be identified; we do not know the full range of forces that are acting at the tissue and embryo level nor how they are related to the cell behaviors that we have identified, and those yet to be characterized. It will be important not only to further explore the cellular behavior that underlie mouse morphogenesis, but also to develop methods for characterizing the mechanical forces that the cells both sense and generate to effect changes at the tissue level.

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FIGURE LEGENDS

Figure 1. Stages of axial morphogenesis. Anterior is to the left in all images. Asterisks mark the position of the PNC, arrowheads indicate the position of the forming foregut pocket, and the small arrows indicate the inflection points defining the headfold region. A. Embryo at E7.5, before the major tissue shape changes. AVE, anterior visceral endoderm. B. Embryo at E7.75, showing neural plate (np) formation. C. The same embryo shown in B, 3 hours later. The neural plate has begun the infolding process to form the head folds. D. A different embryo with head folds formed. E. The same embryo as in D, 10 hours later. The head folds have fully rotated, and the heart (h) is now in its correct position posterior and ventral to the head folds. The change in distance between the bottom arrow and the asterisk in D and E indicates the progression of axial elongation. The images in D and E are from Movie 1.

Movie 1. Axial morphogenesis. Timelapse movie of development from the early headfold stage to 4 somites. This movie illustrates both the rotation of the heart primordium (H) into its correct position by the elevation of the head folds (HF), and the elongation of the axis concomitant with formation of the somites (S). The location of the primitive streak is indicated (PS). Anterior is to the left.

Movie 2. Mesoderm CE. Laser scanning confocal timelapse movie of CE in the paraxial mesoderm in an embryo of the EGFP-x strain. The posterior notochord (PNC), primitive streak (ps), and paraxial mesoderm (pm) are indicated.

Movie 3. Neural CE. Laser scanning confocal movie of CE in the neural plate in an embryo of the mT/mG strain. Neural plate (np), posterior notochord (PNC) and somites (s) are indicated.

Figure 2. Competition of cell intercalation forces determines tissue shape. Mediolateral convergence (green arrows and protrusions) leads to tissue

extension (dark blue arrows) and thickening (black arrows). Radial intercalation (magenta arrows and protrusions) counters the thickening, enhancing tissue extension. The relative magnitude of these two forces determines the ultimate shape of the tissue. An innate resistance of the tissue to extend (light blue arrows) is assumed.

Figure 1

FIGURES

Figure 2

Mediolateral convergence tends to produce radial de-intercalation or thickening.

Radial intercalation restores thinning

Diagram of forces acting on the tissue