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Cellular dynamics in the early mouse embryo: from axis formation to gastrulation

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Summary

Coordinated cell movements and reciprocal tissue interactions direct the formation of the definitive germ layers and the elaboration of the major axes of the mouse embryo. Genetic and embryological studies have defined the major molecular pathways that mediate these morphogenetic processes and provided “snapshots” of the morphogenetic program. However it is increasingly clear that this foundation needs to be validated, and can be significantly refined and extended using live imaging approaches. *In situ* visualization of these processes in living specimens is a major goal, as it provides unprecedented detail of the individual cellular behaviors, which translate into the large-scale tissue rearrangements that shape the embryo.

Keywords

mouse embryo; morphogenesis; axis formation; gastrulation; epiblast; mesoderm; endoderm; cell dynamics; migration; intercalation; ingression; egression; EMT; MET; imaging

Introduction

A major challenge in developmental biology is the elucidation of the cellular behaviors driving morphogenesis and the molecular signals that control them. Genetic studies have uncovered key signaling pathways regulating cell communication and differentiation, and have revealed the basic networks of genes controlling early mouse development. Many of the basic morphogenetic mechanisms, such as collective cell migration or cell intercalation, are reiteratively used and evolutionarily conserved. Even so, we still possess only a rudimentary understanding of the cellular dynamics driving the morphogenesis of the mouse.

Deciphering a morphogenetic program is a complex problem to tackle, being multidimensional and involving both interactions between cells, as well as with substrata. Microscopy has been an essential tool in developmental biology for studying morphogenesis and determining the normal course of events, then contrasting them with the results of experimental perturbations. Given the complexity of the system, our understanding of these processes in the mouse has been lagging, when compared to other more experimentally-tractable models. However, the increasing availability of mutants, live imaging reporters, improvements in *ex utero* cultures, along with advances in optical imaging modalities and

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data analysis tools have come together to provide valuable insights into morphogenesis and consequently, our understanding of these processes in the mouse is slowly catching up.

In this review, we discuss our current understanding of some of the ordinal cell movements that coordinate the morphogenesis of the early postimplantation embryo.

Tissue architecture of the late blastocyst

After implantation into the maternal uterus, the mouse embryo undergoes a transformation in size and shape, as the rudimentary features of the basic body plan emerge (Figure 1). As with all mammals, the initial stages of mouse development involve the allocation of extraembryonic lineages, which have long been recognized as critical for maternal-fetal communication and nutrient exchange. However, genetic and embryological studies have shown that extraembryonic tissues also play a pivotal role in patterning the embryo (reviewed in [1]), and, as recent data suggest, they may even contribute cells to fetal tissues, thereby challenging the concept of strict lineage segregation (Figure 2, [2]). At preimplantation stages, the emergence of an extraembryonic fate is coincident with cell polarization, establishment of cell-cell (tight and adherens) junctions and acquisition of an epithelial state.

The implanting (late) blastocyst stage embryo (E4.5, Figure 1) comprises three lineages: trophoblast (TE), primitive endoderm (PrE), and epiblast (EPI). TE will give rise to trophoblastic tissues, including the chorion, ectoplacental cone and the extraembryonic ectoderm. PrE will primarily generate the endoderm of the visceral and parietal yolk sacs. The pluripotent EPI will give rise to the embryo-proper which generates the tissues of the adult mouse, as well as the fetal portion of the placenta (Figure 2). TE and PrE encapsulate the EPI, an arrangement characteristic to all mammals. This juxtaposition of tissues is critical for early patterning, and even though the embryo grows in size, this close association is maintained until the onset of gastrulation almost two days later.

PD polarity at early postimplantation results in DVE cell specification

Shortly after implantation the embryo elongates to form the radially symmetric egg cylinder comprising a bilaminar cup shaped epithelial structure with a defined proximal-distal (P-D) axis (E5.5, Figure 1). The PrE-derived visceral endoderm (VE) encapsulates the EPI distally and the extraembryonic ectoderm (ExE) proximally. The VE and EPI/ExE epithelia exhibit reversed apical-basal polarity, with the basement membrane (BM) at their common basal interface. The VE has been subdivided into two domains: proximally (where it is referred to as exVE) it comprises a cuboidal epithelium exhibiting a regular hexagonal packing of cells, and distally (emVE) a squamous epithelium with a more disorganized arrangement of cells [3,4].

Studies in *Drosophila* have revealed that the arrangement of cells in proliferating epithelia can serve as predictors of their behaviors [5]. The distribution of polygonal cell types within an epithelium was shown not to result from cell packing, but was instead shown to be a direct consequence of cell proliferation. These observations might imply that proliferation-dependent cell patterns underlie morphogenesis throughout metazoa. It will therefore be intriguing to determine if this is the case in the mouse visceral endoderm epithelium where clear geometric subdivisions exist.

Genetic studies demonstrate that Nodal/Smad and Wnt/ β -catenin signaling is required to generate and maintain P-D regionalization within the conceptus (reviewed in [1]). The A-P axis is established through a sequence of events initiated by the specification of the distal visceral endoderm (DVE), a group of cells at the distal tip of the egg cylinder around E5.5

[6,7]. DVE cells emerge as morphologically distinct from their VE neighbors [8–11]. Given the difficulty in recovering implanting embryos, it remains to be determined how this P-D regionalization might relate back to any asymmetries present in the blastocyst. The existence of earlier asymmetries is supported by the recent discovery of asymmetric expression of *Lefty1* in implanting blastocysts [12].

P-D axis rotation: cell movements within the VE epithelium break radial symmetry and define the A-P axis

Once specified, visceral endoderm cells at the distal tip of the embryo migrate proximally toward the embryonic-extraembryonic junction (the region where EPI and ExE meet) to form the anterior visceral endoderm (AVE). This proximal-ward cell migration occurs rapidly and halts once cells reach the boundary (E5.75, Figure 1). Thus the unilateral repositioning of cells within the emVE rotates the proximal-distal (P-D) axis thereby converting it to an A-P axis. Embryological and genetic studies confirm that rotation of the P-D axis is essential for A-P axis specification (reviewed in [1]). Even so, remarkably little is known about the signals or cellular mechanisms that direct this dynamic collective cellular behavior taking place within the VE epithelium.

Compelling live imaging studies reveal that DVE cells actively migrate. Cells extend filopodia in the direction of movement, suggesting they may actively migrate towards an unidentified chemoattractant, possibly regulated by Wnt signaling, as cells are displaced away from a region of high to one of low Wnt activity [13]. Alternatively, instructive cues from the extracellular matrix (ECM) may facilitate or direct cell migration. Genetic studies support the imaging data, revealing that this migration is mediated by the WAVE complex of cytoskeletal regulators involved in remodeling the actin cytoskeleton in response to extracellular signals [14].

Cells need to gain traction to move. VE cells may gain the traction they need for this movement through interactions with either the underlying ECM, or with their neighbors by modulating cortical tension exerted by the actin-myosin network and cell-cell adhesion. Alternatively, they may polarize and use cellular protrusions to generate traction. It has also been suggested that planar cell polarity (PCP) dependent cellular rearrangements may facilitate collective cell migration or reorganization within the VE [15,16].

The behavior of surrounding cells both within the visceral endoderm and in the adjacent epiblast is currently unclear. The exVE and emVE do not appear to correspond to distinct cellular compartments as fate mapping studies have revealed “polonaise” movements within the VE that traverse their boundary, suggesting that their subdivision may be non-cell autonomously regulated by the adjacent ExE and EPI respectively [4]. It will therefore be important to determine whether compensatory, active or passive, rearrangement of cells within the VE epithelium occurs to accommodate for the displacement of DVE cells. It is also unclear how the boundary between the exVE and emVE territories is established [3], and how migrating cells respect it. Though live imaging of cell behaviors other than in AVE migration have yet to be reported, data from fate mapping studies suggest minimal long-range or directional cell rearrangement taking place within the ExE and EPI prior to the onset of gastrulation which occurs almost a day later.

The emergence of the primitive streak

Rotation of the P-D axis is crucial for setting up the A-P axis by establishing a gradient of Nodal and Wnt signaling which confines the site of primitive streak formation. Surprisingly, prior to the onset of streak formation, the newly established A-P axis changes its orientation

of bilateral symmetry, as the embryo remodels its shape [17,18]. Studies in pre-gastrula stage embryos revealed the A-P axis of the embryo shifts, resulting in it becoming aligned with the long traverse axis at gastrula stages [17,18]. This progression has been proposed to be independent of cell movement and could, in principle, be achieved through modulation of the apical-basal height of the columnar epithelial epiblast.

The primitive streak is the first overt morphologically distinct structure that breaks the bilateral symmetry of the embryo. The primitive streak (PS) forms within the epiblast at the extraembryonic-embryonic boundary diagonally to the AVE and in doing so defines the future posterior of the embryo and the site of gastrulation (E6.5, Figure 1). During gastrulation a single epithelial cell layer, the EPI, generates two others: the mesoderm, which is mesenchymal, and the definitive endoderm, which is epithelial. The formation of the primitive streak and the subsequent morphogenesis of the mesodermal and endodermal tissue layers involve complex cellular dynamics. Key molecular players important for PS formation have been identified and shown to be conserved among vertebrates. However, little is known about stereotypical epiblast cell behaviors that are coincident with streak formation in the mouse.

Several models suggest that Nodal/Smad and Wnt/ β -catenin signals from extra embryonic tissues, the ExE and posterior emVE, are involved in the induction of the primitive streak [19,20] [21]. Evidence for a role of the PVE comes from studies of *Wnt3* mutants, which lack a streak and mesoderm and die by E8.5. The observation of restricted *Wnt3* expression in the PVE as early as E5.5, almost 24 hours before streak formation suggests that signals from the PVE may have an inductive role in streak formation [22].

Recent live imaging studies in chick have identified “polonaise” movements and medio-lateral cell intercalation within the epiblast at the site of primitive streak formation. These cellular behaviors are regulated by Fgf and Wnt/PCP signaling [23]. Albeit attractive to speculate that this is a conserved mechanism for streak formation in amniotes, the existence of such behaviors as well as their molecular regulation still need to be confirmed in the mouse. Such studies will require the characterization of large-scale epiblast cell flows, precise velocities and trajectories coincident with streak formation.

Cell ingression and EMT at the primitive streak

Columnar epithelial cells of the EPI exhibit defined apical-basal polarity and lie in close contact with the basement membrane, which is likely to be critical for maintaining their epithelial polarity. At the streak cells ingress by elongating and acquiring a bottle-shaped morphology, concomitantly losing apical-basal polarity, downregulating junctional proteins, and downregulating or dissolving basement membrane proteins thereby transitioning to loose mesenchyme (epithelial-to-mesenchymal transition, EMT). These nascent mesodermal cells migrate away from the streak as mesodermal wings, which circumnavigate the space between the EPI and VE, and in doing so form a new mesenchymal cell layer.

Several signaling pathways, including Fgf and Wnt, are required for EMT at the streak. Mutants in components of the Fgf signaling pathway including fibroblast growth factor 8 (Fgf8) and Fgf receptor 1 (Fgfr1) are able to undergo or initiate EMT, but cells either fail to migrate away or are unable to maintain the mesenchymal state resulting in their accumulation in the vicinity of the streak [24,25]. An absolute requirement for undergoing EMT is downregulation of junctional proteins and cell adhesion molecules such as E-cadherin. Failure to do so, as in mutants for the Zn finger transcription factor Snail1, results in ingressing cells, which retain apical-basal polarity, as they migrate away [26,27]. E-Cadherin expression is also regulated by the T-box transcription factor Eomes, as EPI ablation of Eomes results in partial downregulation of E-cadherin and EMT arrest [28].

Since little is known about cues directing cell migration *in situ* in mouse embryos, precisely what causes the changes in cell shape and behavior during EPI cell ingression is still an open question. A study in the chick has identified regionally-distinct variations in the cytoskeleton of EPI cells. A radial organization of microtubules was stereotypical, however, cells close to the streak appeared to acquire a more polarized organization where they existed in multicellular rosette-like structures. Since rosettes are a thermodynamically unfavorable structures their resolution may provide a driving force for cell ingression [29].

In addition to cell-cell interactions, the extracellular matrix likely plays a crucial role in cell migration away from the streak by providing a substrate for the generation of traction forces [30–32]. The dynamics of cell-matrix interactions and ECM remodeling and assembly are not well documented. ECM could primarily play a signaling role, for instance to stabilize apical-basal polarity or locally coordinate cell behaviors, alternatively it could be required for cells to obtain traction for active movement. Recent observations in the chick suggesting movement of the ECM underlying nascent mesoderm are tantalizing, but need to be substantiated and shown to occur in the mouse. If the ECM does move, it will be important to determine whether it does so passively, where cells take the ECM with them or the ECM scaffold is being deformed perhaps as a result of the overall growth of the embryo. Indeed, cells could modulate ECM composition by localized remodeling involving the making and breaking of interactions between various matrix components. Alternatively, ECM might move actively itself, thus carrying cells along with it. Future genetic studies of ECM mutants coupled with live imaging of ECM assembly and movement, should provide some answers to these questions.

Lineage allocation

After its induction the primitive streak elongates distally until it reaches the tip of the embryo (E7.5, Figure 1). During this time the allocation of the distinct lineages takes place in an anterior-to-posterior sequence determined by both the time and position of cell ingression [33]. Extraembryonic mesoderm, which gives rise to the mesoderm of the yolk sac, chorion, and the blood islands, is the first lineage that emerges from the streak at its most posterior end. BMP4, a Tgf- β signaling molecule expressed in the ExE, is involved in patterning this lineage [34]. Later ingressing cells from middle and anterior streak regions give rise to the cardiac, lateral plate and paraxial mesoderm [35,36]. Establishment of a resident stem cell population contributing to the production of paraxial mesoderm progenitor cells has been proposed, but not yet confirmed [37,38]. The last cell population to emerge, from the anterior primitive streak, gives rise to axial mesendoderm (prechordal plate, notochord, and node) as well as definitive endoderm (DE).

Live imaging studies combined with the analysis of mutants have revealed three distinct morphogenetic processes occurring along the A-P length of the axial mesendoderm. Convergence of dispersed notochord progenitors cells forms the most anterior segment, known as the anterior head process, while medial-lateral cell intercalation gives rise to trunk notochord, and active cell migration of node-derived cells forms the posteriorly-positioned tail notochord [39].

Intercalation and endoderm morphogenesis

Endoderm morphogenesis poses a unique topological hurdle to the embryo: cells exit an epithelium (the EPI) which possesses a reversed polarity to the one they will end up in (the endoderm). Prospective DE cells insert into the surface epithelial layer of the embryo and in doing so undergo a mesenchymal-epithelial transition (MET) soon after EMT at the streak. The prevailing view of endoderm morphogenesis has posited that as increasing numbers of DE cells insert focally into the pre-existing VE epithelium at the distal tip of the embryo's

surface. From there they displace preexisting emVE cells proximal-ward towards extraembryonic territories, thereby completing the segregation of embryonic from extraembryonic tissues (displacement model). Fate mapping studies and the analysis of molecular markers have supported this model, which is predicted to involve the unidirectional traction of emVE epithelium by the expansion of distally positioned DE epithelium [40–44]. Elegant imaging studies combined with the use of embryo chimeras suggest that DE cells may already be specified within the streak, and that they polarize as they insert into the VE epithelium comprising the embryo's surface [45].

A recent study from our laboratory using genetic labeling and live imaging, refined this model by demonstrating that emVE cells are rapidly dispersed and not displaced *en masse* during endoderm morphogenesis (E7.5, Figure 1). This (dispersal) model posits the widespread multifocal intercalation of DE cells into the emVE epithelium situated on the surface of the embryo. This results in scattering, and dilution, of emVE cells. If our model is correct, egressing DE cells likely reside in the wings of mesoderm or take the same trajectory as they emerge from the streak. For egression to occur, cell-cell junctions must be remodeled and ECM must be degraded or contorted. After insertion into the emVE, egressing DE cells are predicted to undergo MET and upregulate junctional proteins [2]. Additional studies are required to determine the forces and mechanisms driving cell egression. However, rapid growth of the EPI and its derivatives, which exhibit increased levels of cell proliferation compared to the VE may exert the necessary force to facilitate egression. Multifocal egression represents a mechanism that can rapidly increase the surface area of the embryo to accommodate for its rapid growth.

These two proposed models for endoderm morphogenesis need not be irreconcilable. An organized arrangement of emVE descendants persists around, but is excluded from, the axial midline [2]. Therefore, VE cell dispersal and displacement may both occur, and the mechanisms directing DE and axial mesendoderm (midline) formation may be distinct.

Several transcription factors and signaling pathways have been shown to be required for DE formation including members of the forkhead [45–48], T-box [28], HMG box [49], Mix/Bix homeobox [50] and GATA families, as well as Tgfb signaling components (reviewed in [51] and [52]). These mutants provide a molecular inroad into the problem, and visualizing mutant cell behaviors will refine our understanding.

In addition to contributing to extraembryonic tissues (the endoderm of the yolk sac - Figure 2), VE derivatives have been identified in the gut tube at midgestation [2]. Though intriguing to speculate, it still needs to be determined whether VE-derived cells persist in the fetus and adult, and if they do, whether they are distinguishable from their EPI-derived neighbors.

Looking to the future

The transformation of the egg cylinder into a recognizable body plan (compare E5.5 to E8.5, Figure 1) relies on the rapid growth of the embryo, a process which is coordinated with the exquisite regulation of at least three key morphogenetic events: (1) cell migration in the VE and P-D axis rotation, (2) ingression, EMT and cell migration at the primitive streak, and (3) widespread intercalation and endoderm morphogenesis. A genetic framework for the early development of the mouse may be in place, but the cellular details are still elusive, and we are only beginning to glean the cellular dynamics directing key morphogenetic events. Beyond the availability of reporters providing improved resolution, we can anticipate the formulation of methods to quantify not just cellular behaviors but also the physical forces existing between cells, as well as between cells and their substrata. As a starting point, we can expect to define the position and morphologies of cells in 4D space. With this positional

scaffold, cell rearrangements can be cataloged, cell migrations tracked and trajectories determined, and the mechanical coupling between cell populations calculated. Quantifying these parameters will allow us to determine if they can be used as predictive tools. Ultimately, by combining genetics and embryology with imaging tools, we can look forward to elucidating the fundamental, quantitative rules of mouse development, from a cellular to organismal scale.

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Figure 1. Schematic representation of still images depicting different tissue layers in the early mouse embryo: from the late blastocyst (E4.5) to early somite stage (E8.5)

Sagittal views are shown in the top row. Beneath them are depicted wholemount views of the egg cylinder at E5.5 and E5.75, and cut-away views (to illustrate the 3D apposition of tissues) at E6.5 and E7.5. The lower series of panels depicts high magnification views of three key morphogenetic events that serve to shape the early postimplantation mouse embryo. Abbreviations: A-P, anterior-posterior; al, allantois; amn, amnion; AVE, anterior visceral endoderm; DE, definitive endoderm; DVE, distal visceral endoderm; E, embryonic day; epc, ectoplacental cone; ExE, extraembryonic ectoderm; EPI, epiblast; emVE, embryonic visceral endoderm; exVE, extraembryonic visceral endoderm; h, heart; hf, headfolds; M, mesoderm; n, node; P-D, proximal to distal; PE, parietal endoderm; PrE, primitive endoderm; PS, primitive streak; som, somite; TE, trophectoderm; VE, visceral endoderm.

Figure 2. Cell lineage relationships in the early mouse embryo

Adapted from Lu et al., [53]. The grey line and question mark refer to the uncertainty concerning the fate of the visceral endoderm overlying the embryo, which is dispersed through widespread egression of epiblast-derived definitive endoderm. Abbreviations: emVE, distally positioned embryonic visceral endoderm; exVE, proximally positioned extraembryonic visceral endoderm from [3].