Gutsy moves in mice: cellular and molecular dynamics of endoderm morphogenesis

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Despite the importance of the gut and its accessory organs, our understanding of early endoderm development is still incomplete. Traditionally, endoderm has been difficult to study because of its small size and relative fragility. However, recent advances in live cell imaging technologies have dramatically expanded our understanding of this tissue, adding a new appreciation for the complex molecular and morphogenetic processes that mediate gut formation. Several spatially and molecularly distinct subpopulations have been shown to exist within the endoderm before the onset of gastrulation. Here, we review findings that have uncovered complex cell movements within the endodermal layer, before and during gastrulation, leading to the conclusion that cells from primitive endoderm contribute descendants directly to gut.

1. Introduction

The endoderm is one of three definitive germ cell layers generated at gastrulation. Cells descended from the endoderm will constitute the epithelial lining of the respiratory and digestive tracts, as well as the endocrine glands, and auditory and urinary systems. Although a large body of work exists concerning the specification and formation of the endoderm in different model systems, in the mouse, recent studies have called for a rethinking of the cellular dynamics that drive the initial phases of gut endoderm formation. It is now clear that, rather than respecting a strict embryonic–extraembryonic segregation of cell lineages, the gut endoderm is composed of cells derived from both definitive endoderm (DE) and so-called extraembryonic endoderm, and that these two cell populations are coordinately integrated into a single tissue, the gut endoderm, through a process of dispersion and intercalation.

2. A panoply of endoderm

At the time of implantation into the maternal uterus (at around embryonic day (E) 4.5), the late blastocyst stage mouse embryo comprises three tissue lineages: the pluripotent epiblast and two extraembryonic lineages, the primitive endoderm (PrE; figure 1 and table 1) and the trophectoderm. Shortly after implantation (E4.5–E5.0), the embryo elongates to form a radially symmetric egg cylinder, which concomitantly cavitates at its centre (reviewed in this issue [1]). At this point, the embryo comprises a bilaminar cup-shaped epithelium. The outer layer consists of the PrE-derived visceral endoderm (VE) that encapsulates the proximally positioned extraembryonic ectoderm (ExE), and the distally positioned, pluripotent epiblast that will go on to form the embryo proper as well as some extraembryonic mesoderm (figure 1).

As is the case in several vertebrates, in the mouse there are two recognized sources of endoderm tissue, one arising from the PrE and the other from the pluripotent epiblast [2,3]. Until recently, the canonical thinking dictated that the PrE gave rise only to extraembryonic lineages such as the parietal endoderm and VE of the early postimplantation conceptus, whereas endodermal cells that ingress through the streak made up the embryonic endoderm that comprises the gut.
At early postimplantation (E5.5–E6.5), the VE epithelium comprises several subpopulations, generally encompassing: the extraembryonic VE (exVE), a cuboidal epithelium overlying the ExE, and the embryonic VE (emVE), a squamous epithelium overlying the epiblast [4–9]. While exVE and emVE cells have different morphology and patterning abilities, it is important to note that these names (ex versus em) primarily relate to the position of the VE relative to the conceptus, but that all VE cells stem from the PrE. A further segmentation occurs within the emVE, its most distal cells forming the distal VE (DVE) at E5.5 [10]. Between E5.5 and E6.5, cells of the emVE undergo positional rearrangements and become further patterned, resulting in the establishment of the anterior VE (AVE) [11]. DVE and AVE express specific factors that are involved in patterning the underlying epiblast along its proximal–distal (P–D) and anterior–posterior (A–P) axes [2,12–14]. Additional marker-specific subpopulations of VE cells have been reported, although such regions have typically not been given names by their position [11,15,16].

3. The displacement model of gut endoderm formation

About a quarter of a century ago, elegant fate-mapping experiments using single-cell iontophoretic injection of horseradish peroxidase resulted in the first conceptual reconstruction of the cellular dynamics involved in endoderm formation in mouse embryos [17,18]. In this set of studies, cells of the epiblast and VE were individually labelled, embryos incubated for various time periods and then the position of their cellular descendants noted. Derivatives of epiblast cells from the region in the vicinity of the anterior primitive streak (APS) were likely to end up on the embryo’s surface, often in the area of the DE, and at later stages appeared within the gut tube. By contrast, descendants of VE cells overlying the midline, which are referred to as axial VE cells, often appeared in extraembryonic regions of the conceptus. This gave rise to the notion that epiblast cells around the APS ingress (EMT) at the primitive streak (figure 2), and subsequently egress (MET) to emerge at the surface inserting into the VE at the distal tip of the embryo. As increasing numbers of cells undergo this process, the newly formed sheet of DE cells would form a contiguous epithelium with the VE, which would gradually and uniformly displace the latter to proximal regions, from where it would exclusively contribute to extraembryonic tissues. Additional, fate-mapping analyses using orthotopic grafting and other cell labelling techniques supported the notion that cells in the posterior epiblast near the APS end up on the embryo’s surface and give rise to gut endoderm cells [14,19] (figure 2). Few studies have looked directly at the fate of VE cells, but one in particular appeared to support the displacement model. VE
4. Proposing cell dispersal as a morphogenetic mechanism for gut endoderm

The displacement model remained in place for nearly two decades until recent advances in live cell imaging techniques allowed, for the first time, direct imaging of cell movements throughout the VE during gastrulation. These studies revealed surprising new details about how the gut endoderm is formed. In particular, generation of the Afp–GFP transgenic mouse strain permitted labelling of the entire VE and this allowed global imaging of the cellular dynamics occurring within the VE layer during gut endoderm morphogenesis [23]. In this strain, green fluorescent protein (GFP) is expressed under the enhancer/promoter sequence of alpha-fetoprotein (Afp). Afp is a VE-specific marker in early postimplantation embryos, and is also expressed at later stages of development in a series of tissues, including endoderm derivatives. At gastrula stages, all VE cells in Afp–GFP embryos are labelled with GFP. Additionally, owing to the perdurance of the GFP, immediate descendants of VE cells are labelled as well, even if the transgene is no longer being transcribed. This renders the Afp–GFP strain both a marker for pre-gastrula VE and a short-term cell lineage tracer for VE-derived cells.

Live imaging of Afp–GFP embryos resulted in a surprising finding [21]. In accordance with the displacement model, upon gastrulation, one might have expected an initially uniform sheet of GFP-positive cells covering the entire conceptus to be gradually displaced proximally by a new sheet of GFP-negative cells emerging from the distal tip of the embryo. This was not observed. Instead, GFP-negative areas, corresponding approximately to the size of single cells, began appearing within the region of the emVE. Over time, the GFP-negative areas grew in size, diluting the GFP-positive areas initially to approximately to the size of single cells, began appearing within the region of the emVE. Over time, the GFP-negative areas grew in size, diluting the GFP-positive areas initially to eventually fill the entire conceptus. This supported the notion that the VE is displaced at the distal tip of the conceptus at E5.5 were labelled with DiI and examined after 24 h. These labelled cells showed a marked directional movement towards the anterior of the forming embryo, ending up in the AVE. While these cells did not themselves cross over into the extraembryonic region, this finding strongly suggested that cells ‘ahead’ of those labelled must have migrated outside of the embryo proper [20]. Later, it was shown that VE cells abruptly stop their forward movement once they have reached the embryonic/extraembryonic border, and thereafter begin to migrate laterally [9]. Whether the organization of the VE epithelium overlying the ExE provides an impenetrable physical barrier, or a repulsive signal, to promote the cessation of anteriorward AVE migration remains an open question.

In addition to fate-mapping studies, several other observations appeared to support the notion that VE is displaced by the forming DE. Morphological analysis of cells on the embryo’s surface nicely fits in with the displacement model. Initially, heavily vacuolated cuboidal cells, typically associated with VE, surround the entire conceptus, but after gastrulation, cells with this shape are confined to extraembryonic regions while cells on the surface of the embryonic region are squamous, classically associated with the DE lineage [7]. Analysis of the localization of pan-VE markers also fit the displacement model. At first, VE markers such as Ttr, Afp, Sox7 or Hnf4a are present in a layer of cells surrounding the entire conceptus, but when gastrulation is complete, their domain of expression is restricted to proximal regions [4,21,22]. This supported the notion that the VE is displaced proximally as a uniform cellular sheet.

Table 1. Endodermal cell populations of the early mouse embryo.

<table>
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<tr>
<th>Endodermal Type</th>
<th>Description</th>
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<tr>
<td>primitive endoderm</td>
<td>PrE-derived cell population that lies adjacent to the mural trophectoderm. Fated to contribute to the endoderm layer of the parietal yolk sac as well as the deposition of Reichert’s membrane</td>
</tr>
<tr>
<td>parietal endoderm</td>
<td>PE</td>
</tr>
<tr>
<td>visceral endoderm</td>
<td>VE</td>
</tr>
<tr>
<td>distal visceral endoderm and anterior visceral endoderm</td>
<td>DVE and AVE two molecularly distinct subdomains of the VE, one located at the distal tip of conceptus (DVE), and the other demarcating the prospective anterior of the conceptus (AVE)</td>
</tr>
<tr>
<td>extraembryonic VE</td>
<td>eeVE subset of VE cells situated in the extraembryonic region of the postimplantation conceptus, overlying the extraembryonic ectoderm. Fated to give rise to the endoderm of the visceral yolk sac</td>
</tr>
<tr>
<td>embryonic VE</td>
<td>emVE subset of VE cells in the embryonic region of the early postimplantation conceptus, overlying the epiblast. Probably fated to give rise to some yolk sac endoderm as well as part of the gut tube</td>
</tr>
<tr>
<td>definitive endoderm</td>
<td>DE epiblast-derived cell population of cells that contributes to the gut endoderm</td>
</tr>
<tr>
<td>gut endoderm</td>
<td>Precuror of all endodermal tissues in the adult organism. It is an epithelium composed of emVE- and DE-derived cells</td>
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cells from an extraembryonic lineage persist within the gut of the fetus, and possibly even the adult. These observations were confirmed using other fluorescent protein reporter lines, as well as using genetic fate mapping with a pan-VE Ttr-Cre line and lacZ- and GFP-based Cre reporters [24].

These data suggest a new model that can account for both these new findings as well as previous fate-mapping and gene expression data. In what we refer to as the cell dispersion model, the epiblast-derived DE does not displace the emVE as a sheet. Instead, the DE initially migrates through primitive streak epithelial cell ingression (EMT) mesenchymal (mesoderm) cell

time

Figure 2. Relating the cellular dynamics of gut endoderm morphogenesis to epithelial–mesenchymal transitions. During gut endoderm formation, cells undergo both epithelial-to-mesenchymal (EMT) and mesenchymal-to-epithelial (MET) transitions in rapid succession. At the primitive streak, DE progenitors (purple) exit the epithelial pluripotent epiblast (blue) and enter the mesenchymal wings through the process of ingression. The ‘wings’ consist of DE precursors and mesoderm (orange). Once they have reached their final position, DE cells egress from the mesenchymal wings into the newly formed endodermal epithelium that is composed of DE cells and visceral endoderm cells (VE; blue).
beneath the emVE and gradually causes the emVE to disperse as increasing numbers of DE cells egress into it.

5. A stage-by-stage description of cellular dynamics that drive endoderm formation

(a) Pre-gastrulation and ingression through the primitive streak

Before gastrulation, the emVE encapsulates the epiblast, where the precursors of the DE lineage reside. The anterior part of the emVE epithelium, the AVE, expresses inhibitors of Nodal and Wnt3, restricting the activity of these genes to posterior regions [25,26]. In the posterior of the embryo, high levels of Nodal and Wnt3, in addition to bone morphogenetic protein 4 (BMP4), elicit the formation of the primitive streak, marking the onset of gastrulation [27]. Posterior epiblast cells become fated to the mesoderm and DE lineages, ingress through the primitive streak and undergo an epithelial-to-mesenchymal transition (EMT). Cells lose epithelial morphology and adopt a mesenchymal shape by breaking down apical–basal cell polarity, cell–cell junctions, as well as constituents of the basement membrane, freeing them to migrate away from the vicinity of the primitive streak. Fibroblast growth factor (FGF) signalling mutants [28–30] as well as embryos lacking Snail [31], Eomes [32] or Brachyury [33,34] have cells that ingress at the primitive streak but cannot correctly migrate away from it, as a result of incomplete EMT. These mutants invariably fail to establish both mesoderm and DE. It is therefore largely accepted that all epiblast cells that will give rise to DE lineage undergo EMT at the primitive streak.

(b) Migration of definitive endoderm progenitors

The concept that DE emerges at the distal tip of the conceptus resulted from fate-mapping studies that showed that DE precursors reside near the APS [17–19]. The primitive streak is a dynamic structure: it starts out as a small region of cellular in regression in the most posterior part of the epiblast (at the epiblast–ExE border), which then gradually elongates distally and anteriorly until reaching the embryo’s distal tip.

In vivo lineage-tracing experiments using single-cell iontophoresis of horseradish peroxidase showed that in some cases a single labelled posterior epiblast cell could result in cells of both mesoderm and gut endoderm [17,18]. Investigations of Nodal mutants have provided several insights into this matter. In vivo, the levels of Nodal signalling regulate the selective allocation of both mesoderm and endoderm [41]. Mice lacking Nodal or its co-receptor Cripto fail to form a primitive streak and lack both mesoderm and gut endoderm [42–44]. Hypomorphic mutants with moderate levels of Nodal signalling can form some mesoderm and endoderm, whereas lower levels of Nodal signalling result in a loss of endoderm, but not mesoderm [45]. By contrast, elevated Nodal signalling caused by loss of the repressor DRAp1 resulted in an expanded primitive streak and production of excess mesoderm [46]. These findings suggest that the levels of Nodal signalling play an important role in regulating the selective allocation of mesoderm and endoderm from a common progenitor population. Further support for the existence of a bipotential mesendodermal population comes from studies on the Wnt/β-catenin pathway. Upon lineage-specific removal of β-catenin, mesoderm-like cells are present in the endoderm layer of mutant embryos suggesting a transfating of endoderm to mesoderm [47], and deletion of β-catenin specifically in Sox17-expressing cells prevents endoderm formation [48]. Nonetheless, based on marker expression, it has been suggested that DE and mesoderm cell lineages might already be predetermined before gastrulation [49], though it should be noted that markers indicate the state, not fate, of cells.
If a mesendodermal state indeed exists within the mouse embryo, it is worth considering how it might tie in with a dispersal model of endoderm morphogenesis. Bipotentiality could be restricted to stages immediately after ingression through the primitive streak, or extend on through migration of cells within the wings of mesoderm. While the levels of Nodal, seen by any individual cell as it passes through the primitive streak, are thought to affect its decision to become mesoderm or DE, we do not know if this is sufficient for the fate choice or if it merely primes cells to one or the other fate, and subsequent environmental cues seal their fate. DE progenitors express endoderm markers just prior to and during intercalation into the emVE [50]. It is conceivable that a signal from the emVE instructs adjacent cells to upregulate DE factors. Only cells in the outer layers of the wings of mesoderm making direct contact with the emVE show expression of DE markers [50]. Accordingly, ultrastructural examinations of cells in the wings of mesoderm have revealed that cells adjacent to the emVE are more closely opposed to the basement membrane and more tightly packed than their counterparts that lie closer to the epiblast [17,51,52], perhaps hinting at an intrinsic heterogeneity within the mesoderm, or a subpopulation primed to join the surface endoderm by virtue of its position.

Recently, miR-335 has been implicated in the fate choice between mesoderm and DE [53]. This intragenic microRNA is encoded in a mesoderm-specific transcript (Mest) and targets the 3’UTRs of Sox17 and Foxa2. Overexpression of miR-335 reduces Sox17 and Foxa2 levels and blocks endoderm differentiation in embryonic stem cells (ESCs) and ESC-derived embryos. Conversely, inhibition of miR-335 leads to increased Sox17 and Foxa2 protein accumulation and endoderm formation [53]. Yet, when, where and how cells are instructed to express miR-335 remain open questions.

Mouse ESCs that are treated with Activin A in many respects provide an in vitro model of primitive streak formation and gastrulation-like EMT. Molecular studies in this model suggest that EMT at gastrulation involves a gene-regulatory feedback loop consisting of Brachyury, Foxa2 and Sox17 [54] such that upon Activin A treatment, cells changed their molecular identity, heterogeneously expressing previously silenced Brachyury, Foxa2 and Sox17. Interestingly, Brachyury depletion decreased Sox17 and Foxa2 expression at both transcript and protein levels, suggesting that expression of Brachyury might be a prerequisite for a cell to enter a mesendodermal state. Supporting this mechanism, chromatin immunoprecipitation sequencing experiments revealed direct binding of Brachyury to regulatory sequences of Sox17 and Foxa2. By contrast, overexpression of Sox17 in embryonic bodies resulted in a significant downregulation of Brachyury, pointing to a negative feedback loop. How the post-EMT heterogeneity of cells is achieved remains an open question. Further studies will be needed to address the question of how cells in the wings of mesoderm sort into mesodermal and DE populations, and specifically whether a predetermination to one lineage is established at the primitive streak, or if migrating cells become programmed by a positional cue, possibly emanating from the emVE.

### 6. Egression of definitive endoderm cells into the overlying embryonic visceral endoderm

We have already described how cells fated to contribute to the mesoderm and endoderm enter the mesodermal wings by undergoing EMT at the primitive streak. These cells migrate anteriorly until, shortly thereafter, certain cells in their outer edges begin to express DE markers and intercalate into the emVE. As they leave a mesenchymal population and join an epithelial layer, they undergo a mesenchymal-to-epithelial transition (MET). This rapid EMT–MET cycle raises the question of whether DE cells undergo a complete transition between epithelial and mesenchymal states, or rather whether they might loosen their epithelial characteristics temporarily to migrate, then re-epithelialize during egression. An incomplete or partial EMT and MET have been described in other contexts [55,56], and further work will be needed to elucidate these details in mouse gut endoderm morphogenesis.

It is not known how Foxa2 and Sox17, the evolutionarily conserved DE transcription factors expressed in egressing cells [50], control cell egression. Mutants in either one of these factors exhibit severe reductions of the DE lineage in the gut endoderm [57–59]. Evidence is accruing to suggest that Foxa2, as well as Sox17, regulate factors necessary for epithelialization, such as cell polarity, cell–cell junctions and basement membrane components. An embryo chimera study focusing on the cell dynamics in the axial midline has reported that Foxa2 mutant cells are capable of inserting into the VE layer, but fails to acquire epithelial polarity or establish new adherens or tight junctions with neighbouring emVE cells, and consequently regress back into deeper (mesoderm) layers [49]. Accordingly, proteins involved in cell adhesion during epithelialization have been proposed as direct targets of Foxa2, including Claudin4, Flr2, Flr3, Pcdh19 and Iga3 [60].

While experimental evidence has yet to connect Sox17 to cell polarity or cell–cell junctions, Sox17 has been shown to bind promoter regions and directly regulates the expression of genes encoding basement membrane factors in some endodermal cell types [61–63]. Furthermore, a study on mouse blastocyst stage embryos revealed a requirement for Sox17 in basement membrane formation in the PrE [64].

Another recent study has highlighted the importance of DE epithelialization as a requisite intermediate step in the differentiation of DE progenitors. The RhGTPase Rhou controls F-actin distribution and cytoskeletal organization in epithelial cells. Knockdown of Rhou impairs endoderm differentiation in tissue culture as well as in the embryo [65]. It will be interesting to determine if and how Rhou and other factors regulating cell morphology could function downstream of Sox17 and/or Foxa2.

Loss of Foxa2 leads to absence of anterior DE (which would make the foregut) and axial mesoderm [59,66], but formation of mid- and hindgut is largely unaffected [35]. Sox17 mutants appear to display the converse phenotype. Foregut endoderm forms, whereas the regions of the prospective mid- and hindgut are devoid of DE cells [58]. Furthermore, the Afp–GFP transgenic reporter reveals the emVE only shows limited dispersal in anterior regions and no dispersal in mid- and posterior regions [50], suggesting that DE cells fail to egress in the mid- and posterior regions. Nonetheless, we have also noted that Sox17 mutants properly specify axial midline structures [50]. Interestingly, Sox17 is localized in the majority of gut endoderm cells, but is absent from axial midline structures as well as the gut endoderm area around the anterior intestinal portal [50]. Chimera studies have shown that cells lacking Sox17 cannot contribute to mid- or hindgut, but are capable of contributing to foregut [58]. Conversely,
cells lacking Foxa2 can colonize the hindgut, but not the fore- and midgut [67]. Taking these data into account, one might envision a mechanism whereby MET of DE cells is controlled by Sox17 in the mid- and posterior areas of the emVE (which will give rise to mid- and hindgut), and by Foxa2 in the anterior (which will give rise to foregut). Additionally, Foxa2 regulates the epithelialization of cells in the axial midline [49].

As is the case with almost all endoderm markers, both Sox17 and Foxa2 are expressed in the extraembryonic and embryonic endoderm lineages, at different stages of development and even adulthood [27]. They are both first expressed in the PrE [54], the first epithelium formed in the embryo, and absence of Sox17 leads to a disorganized PrE epithelium in mouse blastocysts [64]. It is therefore tempting to speculate that Sox17 and Foxa2 are key regulators of endodermal tissue epithelialization throughout development and in adult homeostasis.

emVE cells downregulate cell–cell junctions to accommodate egression of DE cells [21]. This response by the emVE reveals a tight-knit coordination with egressing DE cells, especially taking into account the multifocal egression of DE progenitors into different areas of the emVE. The preferential egression into emVE cells with relaxed cell–cell junctions rather than already egressed DE cells might account for the full dilution of the emVE to single cells by the end of gut endoderm morphogenesis. If a bias to egress into neighbouring emVE cells did not exist, one could expect to find small cohorts of emVE-derived cells in the gut endoderm. The communication signals between cell donor tissue (DE) and the epithelial acceptor tissue (the emVE) remain to be elucidated, and might have implications in other contexts of epithelial reorganization and metastasis.

7. Gut endoderm patterning and gut tube formation

In lateral regions of the gut endoderm, emVE-derived cohorts of cells become diluted until they are single cells surrounded by cells of the DE lineage. By contrast, in the axial midline region, emVE-derived cells remain organized around the node and notochord (figure 3), suggesting a distinct morphogenetic behaviour is likely to have occurred and that the egression mechanism described above may not apply to midline regions [21]. One could envision axial mesoderm precursors inserting into the distal tip of the embryo, pushing emVE cells aside, which consequently remain organized around the emergent midline and node. As the node expands anteriorly forming the notochord, those axial mesoderm cells could displace a portion of emVE cells anteriorly up to, but not beyond, the embryonic–extraembryonic junction. This would be in concordance with the lineage-tracing and live cell imaging experiments, where pre-gastrulation axial emVE or AVE cells were labelled and the majority of their descendants ended up in anterior regions of the conceptus, but respected the embryonic–extraembryonic border by remaining in the embryonic region [9,17,18,20,68]. Another portion of axial emVE cells could be pushed aside, thereby explaining the manner in which emVE cells lie on either side of the notochord in Afp–GFP embryos [21].

By the late bud stage (approx. E7.5), no more DE cells egress onto the surface, and the resulting gut endoderm is a monolayered epithelium. Different regions of the gut endoderm already have distinct molecular characteristics, and have thus started transitioning from a naive epithelium to a patterned tissue. Over time, broad regions of gene expression become refined and result in three distinct domains: foregut, midgut and hindgut. Gut endoderm patterning is, at least in part, directed by environmental cues, for example from the neighbouring mesoderm [69–72]. Evidence from two in vitro studies of mouse ESC differentiation into endoderm suggests that the gut endoderm is partly patterned by the underlying basement membrane [73,74]. In particular, FGF-induced PI3K/Akt1 signalling was shown to modulate regional extracellular matrix deposition along the A–P axis, which in turn dictates positional cell-type specification [73]. These findings are substantiated by the observed transfecting of mesoderm to endoderm in zebrafish embryos upon deletion of fibronectin [74].
With the onset of gut tube formation, first a foregut pocket, constituting a local involution of the gut endoderm, and then a hindgut pocket emerge. Over time, these two pockets extend and finally meet, forming the gut tube. The gut tube exhibits extensive contribution of emVE-derived cells up until (at approx. E8.75) the 15-somite stage [21] (figure 4).

8. A universal cell dispersal mechanism driving endoderm morphogenesis?

The core components of the gene-regulatory network involved in endoderm specification are largely conserved across species [40]. The main transcription factors are orthologues of Foxa2, Sox17, T-box family members, Mix-like proteins and Gata factors (figure 5). These factors are regulated by Nodal, Wnt and FGF signalling inputs [75]. But are the cellular dynamics of endoderm morphogenesis conserved, and to what extent might the dispersal model proposed in the mouse be applicable to other vertebrates?

The formulation of a model for gut endoderm morphogenesis in the chick has had a remarkably similar history to the mouse. During gastrulation, the chick embryo needs to reorganize its extraembryonic endodermal tissues (called hypoblast and endoblast) around newly generated DE. A series of fate-mapping experiments initially suggested an equivalent to the murine displacement model, namely that DE precursors ingressed at the primitive streak and inserted into the extraembryonic endoderm tissues, gradually displacing them to the periphery of the embryo where they would contribute primarily to extraembryonic tissues [76–80]. These fate-mapping experiments were performed with techniques such as carbon particle and vital dye staining that proved sufficient to look at mass movements, but did not provide sufficient resolution to address the movements of all cells and their individual dynamics.

A lineage-tracing experiment using [H³] thymidine revealed that the hypoblast does not move away from the primitive streak as a uniform epithelium [81]. Instead, during gastrulation, the gut endoderm is a mosaic layer
made of hypoblast and DE cells. After gastrulation, hypoblast cells had sorted to the germinall crescent (the extraembryonic region), but for a time, extraembryonic and embryonic endoderm lineages intermingled just as in the mouse. This was recently supported by a cell-labelling study which additionally showed that DE progenitors migrate in the mesoderm layer and then insert as single cells into different regions of the endoderm a few cell diameters away from the primitive streak [82]. While the precise fate of the endoblast has not yet been directly examined, chick–quail grafting experiments have suggested that the endoblast might not entirely sort to extraembryonic regions during DE formation, as is the case for the hypoblast [83]. Further analysis using the endoderm-specific marker ApoA1 suggests that endoblast cells remain interspersed between primitive streak-derived DE cells to form the gut endoderm and eventually contribute to the developing liver [84]. Together, these observations beg the question of whether mixing of cells of two different origins may represent a general mode of endoderm formation across vertebrates.

In Xenopus, it is well established that three spatially and morphologically distinct populations of cells in the gastrula stage embryo contribute cells to the gut [85,86]. In addition, co-migration of mesoderm and DE progenitors was observed in a study in rabbit embryos in which the authors analysed the distribution of Sox17 expression during gastrulation [87]. In zebrafish, on the other hand, it was shown that endoderm progenitors are restricted to the epiblast [37,88]. Together, these findings suggest that some elements of the dispersal model can be found in different vertebrates, arguing for conservation of the mechanism across species (table 2). Further studies will be necessary to determine the cell dynamics of the murine dispersal model across vertebrates and, notably, whether endoderm tissues in the adult human contain an extraembryonic component. In worms and flies, different germ layers contribute to the gut tube [91], making a comparison difficult with vertebrates.

9. Concluding remarks

The advent of high-resolution live imaging techniques, which enable the visualization of entire populations of cells in real-time, is adding new insights to our understanding of the cellular dynamics that underlie critical morphogenetic events in vertebrate development. Nowhere is this more striking than the recent finding that so-called extraembryonic VE cells contribute to the gut endoderm. This finding opens up exciting possibilities with several important questions that remain to be answered. Do VE cells persist in the gut of the adult, or are they lost as embryonic development proceeds? If they do indeed persist, to which organs or tissues do VE descendants contribute? Do they behave any differently than their DE neighbours? What is their potential role in the adult? The next generation of live cell imaging tools that will allow for longer and more temporally regulated expression of lineage labels will allow us to investigate the fate of emVE-derived cells persisting within the gut endoderm.

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References


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<th>Table 2. Endoderm dynamics and fate across vertebrates.</th>
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<td>comigration of mesoderm and DE progenitors</td>
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37. Hashimoto K, Fujimoto H, Nakatsuji N. 1987 An ECM substratum allows mouse mesodermal cells isolated from the primitive streak to exhibit motility similar to that inside the embryo and reveals a deficiency in the T1/7 mutant cells. Development 100, 587 – 598.


75. Loebel DA et al. 2011 Rhein maintains the epithelial architecture and facilitates differentiation of the foregut endoderm. Development 138, 4511–4522. (doi:10.1242/dev.063867)


