

# Gastrulation dynamics: cells move into focus

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**During vertebrate gastrulation, a relatively limited number of blastodermal cells undergoes a stereotypical set of cellular movements that leads to formation of the three germ layers: ectoderm, mesoderm and endoderm. Gastrulation, therefore, provides a unique developmental system in which to study cell movements *in vivo* in a fairly simple cellular context. Recent advances have been made in elucidating the cellular and molecular mechanisms that underlie cell movements during zebrafish gastrulation. These findings can be compared with observations made in other model systems to identify potential general mechanisms of cell migration during development.**

The development of all multicellular organisms is driven by the dynamic rearrangement of different populations of cells. The macroscopic analysis of the morphogenetic processes in development, combined with forward and reverse genetic approaches, has led to the identification of many of the molecules involved in regulating tissue rearrangements during embryonic development. The cellular mechanisms by which these molecules control tissue morphogenesis during development are, however, only now beginning to be understood. Recent advances in the development of genetic tools, together with the arrival of new imaging techniques, have provided the necessary means with which to analyze morphogenesis *in vivo* on a cellular and subcellular level.

During morphogenesis, cells can move in different ways: some cells migrate freely over long distances to new locations in the embryo – a good example of this type of movement is the migration of germ cells at early embryonic stages (for review, see Ref. [1]); other cells embedded in tissues interact locally with neighboring cells to induce global changes in the arrangement and shape of the tissues – an example of this is the medio-lateral intercalation of mesodermal cells that underlies convergent extension movements in *Xenopus* gastrulation (for review, see Ref. [2]). Notably, although the molecules that control these various types of movement might differ depending on the precise cellular and developmental context in which they function, the basic cell biological processes that they are involved in, such as cell polarization and adhesion, remain the same (for review, see Ref. [3]).

For cells to move, they have to modulate their adhesive properties dynamically. The idea that differential

adhesiveness among the various cells and tissues controls cell movements was first proposed by Malcolm Steinberg more than 40 years ago. He suggested that the sorting of diverse embryonic cell types into separate tissues, which involves the movement of cells relative to each other, might be a result of their different adhesive properties [4]. Once segregated, this differential adhesiveness would also enable the various populations of cells to remain separated from each other, thereby restricting the movement of cells among those populations and stabilizing specific cellular arrangements. In support of this hypothesis, recent studies in zebrafish have provided evidence that the movement of muscle precursor cells in the developing myotome of zebrafish is driven by the differential expression of cadherin adhesion molecules [5].

In addition to regulating their adhesive properties, cells must be able to interpret their environment and to polarize in the direction of their movement to move to specific places in the embryo. Much work on cell movements *in vitro* has provided insight into the various intracellular signaling pathways that determine the cell polarization required for directed cell migration (for review, see Ref. [6]). Cell polarization involves the asymmetric localization of specific intracellular signaling mediators to the front and/or back of the cell followed by directional modifications of the cytoskeleton. In most cases, however, the relevance of these *in vitro* findings to the movement of cells *in vivo* has not been tested.

In this review, we summarize and discuss recent results on the role of cell adhesion and cell polarization in the regulation of cell movements during zebrafish gastrulation and relate these findings to observations made in other model systems of cell migration in development.

## Zebrafish gastrulation

Zebrafish embryos are ideal organisms in which to study the molecular and cellular mechanisms that underlie gastrulation movements. They are optically transparent and experimentally accessible throughout all stages of development. In addition, both forward and reverse genetic tools have been used to generate several mutants that show morphogenetic phenotypes in the early stages of embryonic development.

During zebrafish gastrulation, a unique combination of morphogenetic events and inductive processes forms – out of a seemingly unstructured cluster of cells sitting on top of a big yolk cell – an embryo that is subdivided into distinct germ layers and shows clear polarities along its

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anterio–posterior, dorso–ventral and left–right axes (Box 1). Gastrulation sets in when blastodermal cells at the animal pole begin to move over the yolk cell towards the vegetal pole in a movement that is commonly called ‘epiboly’. When epiboly has progressed about half way (50% epiboly), the different germ layers – ectoderm, mesoderm and endoderm – start to become morphologically distinct. These three cell layers will eventually give rise to every tissue and organ that is formed in the adult.

The movements leading to formation of the different germ layers can be subdivided into two main morphogenetic events: first, internalization of the mesodermal and endodermal (mesendodermal) progenitors, which separates the ectodermal from the mesendodermal progenitors and organizes them into distinct layers of cells; and second, convergent extension movements of the mesendodermal and ectodermal germ layers, which leads to formation and elongation of the embryonic body

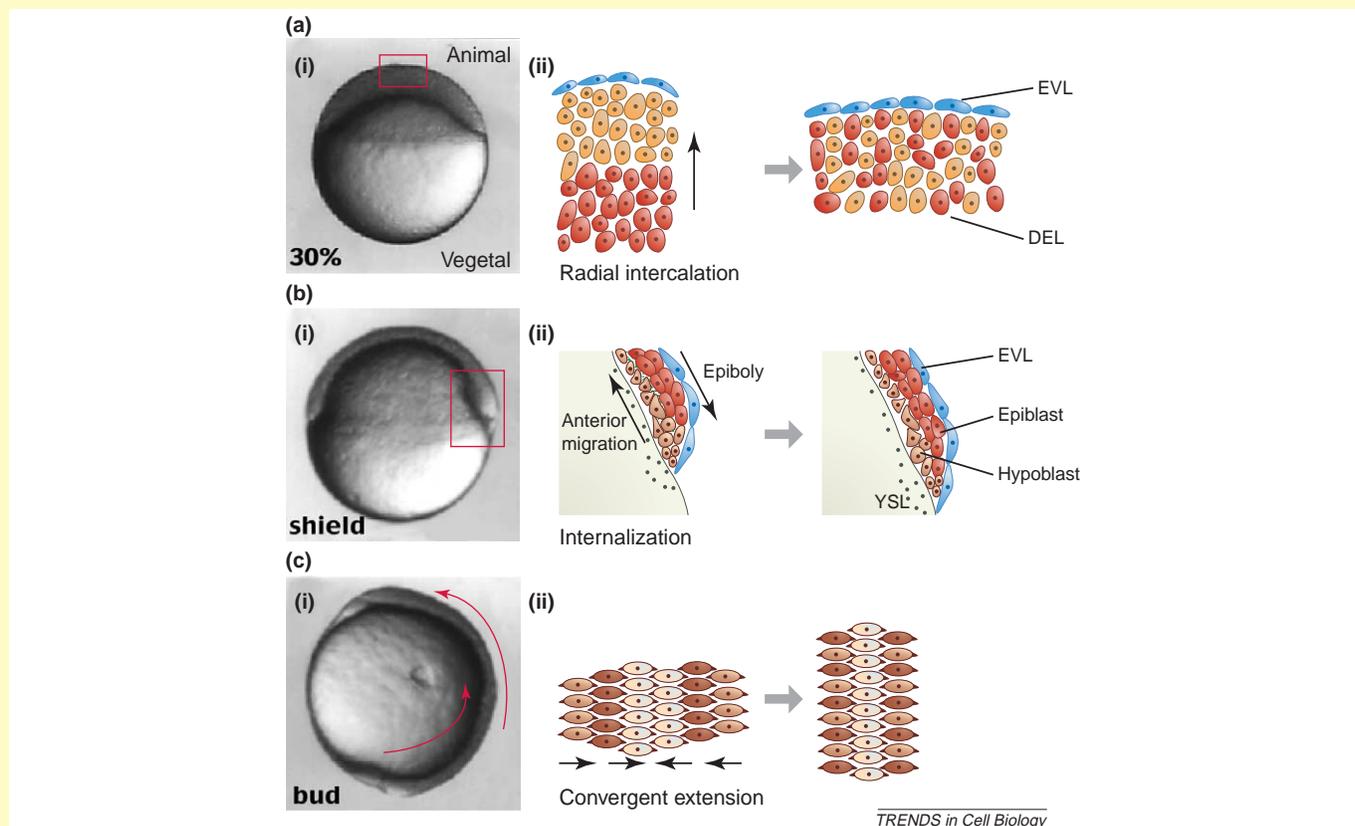
### Box 1. Gastrulation movements in zebrafish

Gastrulation in zebrafish starts when the embryo has reached the blastula stage. At this stage, the embryo consists of a mass of cells, the blastoderm, positioned on top of a big yolk cell. The blastoderm can be subdivided into an outer epithelium of enveloping layer (EVL) cells that covers the non-epithelial deep layer (DEL) cells from which the embryo proper will form. This is also the time when EVL and DEL cells begin to spread over the yolk cell in a movement commonly termed ‘epiboly’. In the DEL, epiboly is triggered by a radial intercalation of cells deep within the blastula that move upwards into more superficial layers, thereby thinning the DEL along its ‘inner–outer’ extent and extending its margin over the yolk cell (Figure 1a). The EVL layer, by contrast, does not undergo radial cell intercalations but instead connects at its margin to the yolk cell membrane and moves over the yolk cell towards the vegetal pole of the embryo.

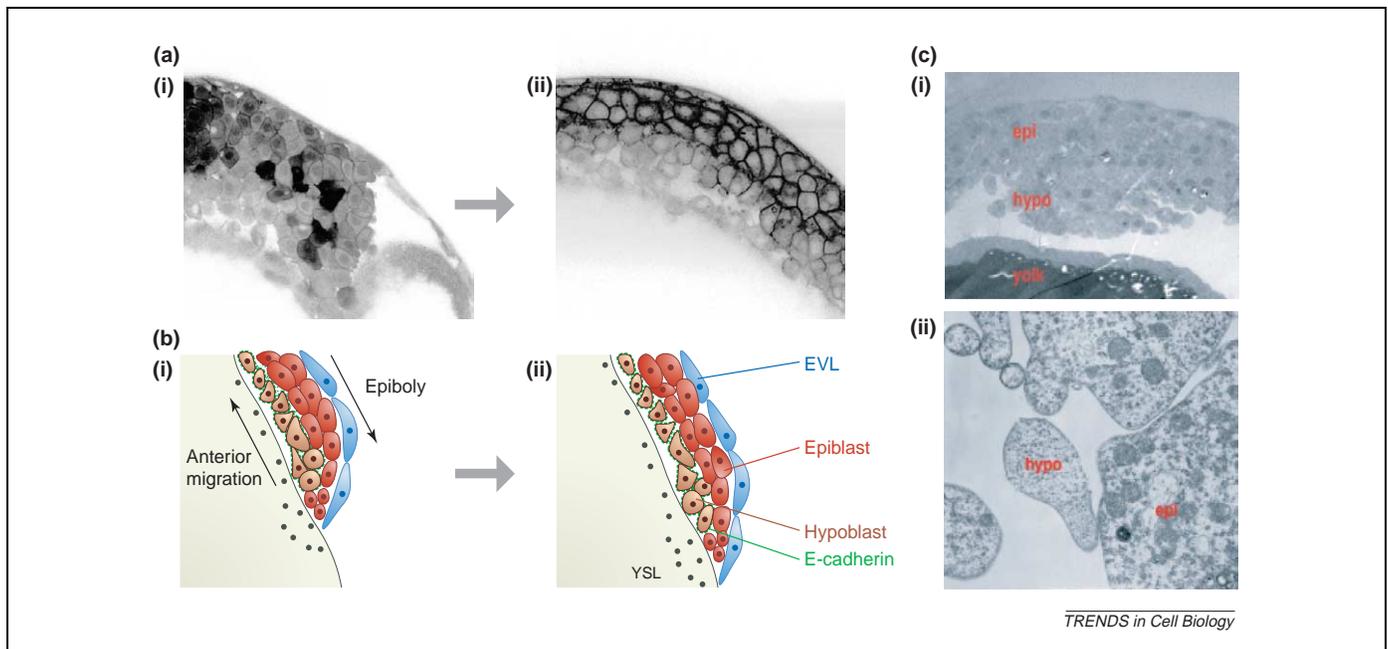
Mesendodermal cell internalization begins with the local accumulation of cells at the margin of the blastoderm, which form the embryonic germ ring. In the germ ring, mesendodermal progenitor cells will internalize to form the hypoblast layer, which gives rise to the mesodermal and endodermal germ layers. Positioned above the hypoblast is the layer of non-involuting ectodermal progenitor cells,

known as the epiblast, that will form the ectodermal germ layer. During internalization, mesendodermal progenitors first move towards the extreme margin of the germ ring and then change direction, moving down towards the yolk cell. Once they have reached the yolk cell, the mesendodermal progenitors change direction yet again, moving upwards towards the epiblast and eventually migrating along the epiblast away from the germ-ring margin towards the animal pole of the gastrula (Figure 1b).

At the same time as the germ ring forms and mesendodermal cell internalization starts, the ectodermal (epiblast) and mesendodermal (hypoblast) germ layers begin to converge towards the dorsal side of the gastrula and extend along the forming antero–posterior body axis. Convergence movements first become apparent in the germ ring by a local thickening at the dorsal side that gives rise to the embryonic organizer, which is called the ‘shield’ in zebrafish. Mesendodermal and ectodermal progenitors converging towards the dorsal side undergo medio–lateral cell intercalations, leading to a thinning of the forming body axis along its medio–lateral extent and a concomitant elongation along the antero–posterior axis (Figure 1c).



**Figure 1.** Gastrulation movements in zebrafish. (a) Radial cell intercalations of blastodermal cells drive epiboly movements at the onset of gastrulation (an embryo at 30% epiboly stage is shown). The cellular movements in the boxed area of (i) are illustrated schematically in (ii). (b) Internalization of mesendodermal progenitor cells at early stages of gastrulation (a shield-stage embryo is shown). The cellular movements in the boxed area of (i) are illustrated schematically in (ii). (c) Convergence and extension movements of mesendodermal cells during gastrulation (a bud-stage embryo is shown). Arrows in (a) (ii), (b) (ii) and (c) (i,ii) demarcate the directions of cell or tissue movements.



**Figure 1.** Internalization and anterior migration of mesendodermal progenitor cells at the onset of zebrafish gastrulation. **(a)** Confocal images showing a cross-section of the forming germ ring at the region of the shield (embryonic organizer) at 60% (i) and 75% (ii) epiboly. Images were taken from time-lapse videos of the cellular movements in the germ ring (shield) between 60% and 75% epiboly (see also Supplementary Video 1). The cells were labeled with a mixture of membrane-bound green fluorescent protein and fluorescein-dextran. **(b)** Illustration of the different cell types and movements involved in mesendodermal cell internalization and migration at 60% (i) and 75% (ii) epiboly. The diagrams in [b(i),(ii)] correspond to the images in [a(i),(ii)], respectively. **(c)** Electron microscopy pictures of the border region between the epiblast and the hypoblast in the forming shield at the onset of gastrulation (60% epiboly; frontal view). Shown are an overview (i) and a close-up (ii) of cells at the border at which the epiblast and the hypoblast contact each other directly. Abbreviations: epi, epiblast; EVL, enveloping layer cells; hypo, hypoblast.

axis. Gastrulation is finished when the epibolizing blastoderm has completely engulfed the yolk cell, the blastopore has closed, and the tailbud has formed.

### Cell adhesion

Once the mesendodermal progenitors (hypoblast cells) have internalized, they move between the overlying ectodermal progenitors (epiblast cells) and the underlying yolk cell towards the animal pole of the gastrula (Figure 1 and Supplementary Video 1). Epiblast cells themselves undergo epiboly movements towards the vegetal pole of the gastrula. Cells in the epiblast and hypoblast are therefore moving on top of each other, but in opposite directions. This movement implies that the adhesive contact between these two layers of cells must be regulated in a highly dynamic manner such that the hypoblast not only can compensate for the oppositely directed movement of its adjacent cell layer, the epiblast, but also can create some net movement towards the animal pole of the gastrula.

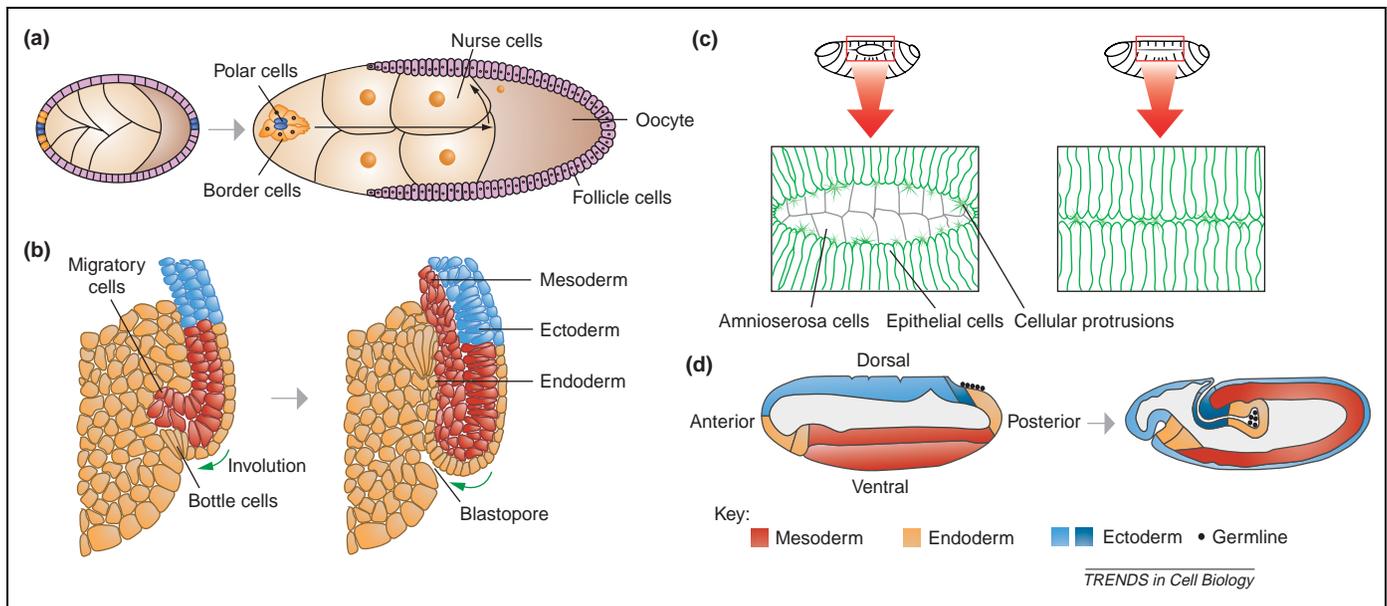
Indeed, recent observations (J-A. Montero and C-P. Heisenberg, unpublished) indicate that a direct and dynamic protrusive contact exists between epiblast and hypoblast cells at the interface between these two cell layers and that no obvious extracellular matrix (ECM) separates them from each other (Figure 1 and Supplementary Video 1). Below, we discuss different cellular and molecular mechanisms by which the epiblast and hypoblast might interact to mediate their movements in opposite directions.

### Cadherins and cell–cell adhesion

At the onset of gastrulation, hypoblast cells must lose affinity for the epiblast, from which they originate, to

internalize. At the same time, they must acquire and/or retain some affinity for epiblast cells because, once internalized, they migrate along this cell layer in the direction of the animal pole of the gastrula (Figure 1 and Supplementary Video 1). This movement implies that during internalization hypoblast cells change their adhesive properties relative to epiblast cells. Notably, the expression of E-cadherin, a well-known regulator of cell–cell adhesion that is expressed in both the epiblast and the hypoblast at the onset of gastrulation, increases in hypoblast cells once they have internalized [7], suggesting that upregulation of E-cadherin might be one mechanism by which hypoblast cells can segregate from epiblast cells but still adhere to them.

A similar upregulation of E-cadherin has been observed during *Drosophila* oogenesis, when border cells in the egg chamber migrate towards the oocyte [8] (Figure 2a). In the *Drosophila* ovary, border cells originate in the anterior extent of a somatic epithelium of cells known as ‘follicle cells’, which surround the egg chamber comprising nurse cells and the oocyte. Shortly before detaching from the follicle epithelium, the border cells strongly upregulate their expression of *Drosophila* E-cadherin (DE-cadherin). This upregulation of DE-cadherin is required for the efficient migration of border cells towards the oocyte located in the posterior of the egg chamber, because inactivation of DE-cadherin in either the border cells or the surrounding germline cells disrupts migration [9]. Furthermore, it has been speculated that DE-cadherin functions in this process by mediating homophilic cell–cell adhesion between the border cells and the surrounding germline



**Figure 2.** Main morphogenetic movements and cell types. **(a)** During border cell migration in *Drosophila*, somatic follicle cells at the anterior end of the egg chamber delaminate, become motile and migrate as a cluster of cells along the surrounding nurse cells, first posteriorly towards the oocyte and then along the oocyte in direction to the dorsal side of the egg chamber. The border cells carry with them a pair of polar cells that keep the border cells motile. Once the border cells have reached their final destination at the dorsal side of the oocyte, they form the micropyle – a structure needed for successful fertilization of the oocyte. Arrows indicate the directions of border cell migration during oogenesis. **(b)** During involution in *Xenopus*, mesodermal and endodermal progenitors involute as a continuous sheet of cells at the blastopore and migrate along the overlying sheet of non-involuting ectodermal progenitors (blastocoel roof) towards the animal pole of the gastrula. **(c)** During dorsal closure in *Drosophila*, an opening in the dorsal epithelium closes through the synchronous movements of lateral epithelial cells towards the dorsal midline. The two lateral epithelial sheets eventually meet and fuse at the dorsal midline. Epithelial cells at the margin form multiple processes directed towards the dorsal midline. **(d)** During *Drosophila* gastrulation, the germ band extends along the anterior–posterior axis and narrows along the dorso–ventral axis. This movement is triggered by a medio–lateral conversion of epithelial cells along the dorso–ventral axis.

cells, thereby providing the necessary traction for the border cells to migrate.

Analogous to the situation in *Drosophila*, hypoblast cells at the onset of zebrafish gastrulation might need high expression of E-cadherin to adhere and to migrate on the overlying epiblast cells. In support of this notion are recent observations in zebrafish showing that interfering with E-cadherin function at the onset of gastrulation leads to defects in the extension of the forming hypoblast cell layer from the blastoderm margin towards the animal pole of the gastrula [7].

An equally important role of cadherin-mediated cell–cell adhesion in mesodermal cell migration has been observed in the early stages of *Xenopus* gastrulation. In *Xenopus*, mesodermal cells, once they have involuted, migrate away from the blastopore towards the animal pole of the gastrula by using the blastocoel roof, which consists of non-involuting ectodermal progenitor cells, as a substrate on which to move [10] (Figure 2b). When the function of different *Xenopus* cadherin molecules such as XB/U- and/or EP/C-cadherin is blocked, the separation behavior between the involuted mesodermal progenitors and the blastocoel roof is impaired, indicating that cadherin-mediated cell–cell adhesion is required for germ-layer separation at the onset of *Xenopus* gastrulation [11].

Notably, this separation behavior seems to be controlled by the Wnt receptor Frizzled-7 (Fz7), which signals through a pathway that differs from the ‘canonical’,  $\beta$ -catenin-dependent Wnt signaling pathway [12]. Furthermore, it has been postulated that heterotrimeric G proteins and protein kinase C function as downstream mediators of Fz-7 in this process, although genetic

evidence for the involvement of these molecules is lacking. Taken together, these data suggest that Wnt signaling regulates the differential adhesiveness between the mesodermal and ectodermal germ layers, possibly through the upregulation and/or activation of cadherin adhesion molecules.

#### Growth factors and cell–matrix adhesion

Not only cell–cell adhesion but also cell–matrix adhesion has been shown to be important in regulating mesodermal cell movement at the onset of *Xenopus* gastrulation. In *Xenopus*, the ECM protein fibronectin accumulates at the interface between migrating mesodermal progenitors and the blastocoel roof, and this accumulation is required for the spreading and protrusive activity of mesodermal progenitors [13].

Notably, fibronectin has been also shown to promote the function of C-cadherin in mediating homophilic cell–cell adhesion and cell invasiveness of gastrulating mesodermal cells *in vitro* and *in vivo* in *Xenopus* [14,15]. Similarly, *fibronectin-1* in zebrafish is required for E-cadherin localization, epithelial organization and the migration of myocardial precursors during heart formation [16]. It is therefore conceivable that during zebrafish gastrulation fibronectin controls the movement of involuting mesodermal cells by upregulating and/or activating E-cadherin in these cells, thereby enabling them to adhere and to migrate efficiently on the overlying epiblast surface.

The ECM can also influence cell movements by binding and thereby localizing signaling molecules such as platelet-derived growth factors (PDGFs). These growth factors

possess binding sites for heparan-sulfate proteoglycans found in the ECM [17,18] and can function as chemoattractants that direct the migration of various cell types during development [19]. Probably the best example of this function of PDGFs is seen during border cell migration in *Drosophila*, in which Pvf-1, a *Drosophila* homolog of PDGF and vascular endothelial growth factor, shows a graded distribution along the antero–posterior axis of the egg chamber, with highest expression in the oocyte to which the border cells migrate [20,21]. When *pvf-1* and/or its receptor *pvr* are over- or underexpressed in the egg chamber, border cells show defects in process formation and directed cell migration, indicating that a gradient of Pvf-1 directs the polarization and migration of these cells [20,21].

A similar function of PDGFs has been observed during *Xenopus* and zebrafish gastrulation. In the early *Xenopus* gastrula, the blastocoel roof expresses high levels of PDGF $\alpha$ , whereas the underlying mesoderm expresses the corresponding receptor PDGFR $\alpha$  [22]. Furthermore, it has been shown that PDGF $\alpha$  is required for the oriented migration and survival of mesodermal progenitors once they have involuted, indicating that PDGF $\alpha$  secreted from the blastocoel roof promotes the migration and survival of the underlying mesodermal progenitors [23,24].

In zebrafish, PDGF $\alpha$  and its receptor PDGFR $\alpha$  are expressed ubiquitously throughout the gastrula [25,26], and interfering with PDGF activity leads to defects in process formation in involuted mesendodermal progenitors during their initial migration [27]. So far, however, a graded distribution of PDGFs during the early stages of gastrulation has not been detected either in *Xenopus* or in zebrafish, raising the issue of whether or not PDGFs function endogenously as chemoattractants that direct the migration of mesodermal cells.

### Cell polarization

To migrate in a specific direction, cells not only need sufficient adhesion but also must polarize along the direction of their migration. Notably, it is often the same intracellular signaling mediators that control both cell polarization and adhesion in migrating cells (e.g. the small GTPases Rho, Rac and Cdc42), indicating that these processes are highly interconnected (Table 1 contains an overview of the function of small GTPases in cell adhesion and polarization *in vivo*).

#### Polarized cellular protrusions

During *Xenopus* and zebrafish gastrulation, phosphatidylinositol 3-kinases (PI3Ks) are thought to function downstream of PDGFs and to be required for cell polarization, filamentous actin (F-actin) localization and process

formation in involuted mesendodermal cells [24,27,28]. Notably, mesendodermal cells that lack PI3K activity retain their ability to migrate, albeit slightly slower, in a coordinated and directed manner towards the animal pole [27]; this suggests that the formation of polarized protrusions is not essential for the directed migration of mesendodermal progenitors at the onset of gastrulation, but instead might be required for the fine-tuning of this process.

Similar observations about the role of polarized cellular protrusions in the movement of cells have been also made in a process called ‘dorsal closure’ in *Drosophila* [29] (Figure 2c). During dorsal closure, an opening in the epithelium at the dorsal side of the embryo is closed at the end of embryogenesis by the synchronous advancement of epithelial cells at the margin of this opening. The margins of the epithelium move dorsally from both sides, thereby replacing an exposed layer of cells in the middle, the ‘amnioserosa’, and eventually meeting and fusing at the midline. Cells advancing at the margin of this epithelium accumulate large amounts of actin and myosin at their leading edges and form several F-actin-based cellular protrusions with which they contact the amnioserosa [30,31]. Similar to the situation in zebrafish mesendodermal cells, however, the formation of cellular processes in marginal cells is not required for the movement of these cells but for fusion of the epithelia margins at the midline [30,32].

#### The Wnt/Wg signaling pathway

Not only the specific requirements for actin accumulation and cellular protrusion formation but also some of the molecules that control these processes seem to be conserved between zebrafish gastrulation and dorsal closure in *Drosophila*. Wnt signaling has an important role in cell polarization, F-actin accumulation and directed cell and tissue movements in various developmental processes both in vertebrates and in invertebrates [33]. In *Drosophila* embryos mutant for *wingless* (*wg*) or its intracellular signaling mediator *dishevelled* (*dsh*), epithelial cells during dorsal closure fail to polarize along their dorso–ventral axis and at the same time show reduced dorsal movements [32], indicating that Wg-mediated epithelial cell polarization controls the directed movement of these cells during *Drosophila* dorsal closure.

This finding is reminiscent of the situation in zebrafish and *Xenopus*, in which Wnt signaling is required for process orientation and directed cell migration in mesendodermal progenitors at both early and late stages of gastrulation. In both organisms, interfering with the function of several components of the Wnt signaling pathway, including the Wnt signal Wnt11 and the

**Table 1. Cellular functions of small GTPases in morphogenetic processes during *Drosophila*, *Xenopus* and zebrafish development**

Morphogenetic processes	Cell polarization	Cell adhesion	Process formation	Cytoskeletal organization	Cell or tissue movements	Refs
<i>Drosophila</i> dorsal closure	Cdc42, RhoA, Rac	Cdc42, RhoA	Cdc42, Rac	RhoA, Rac, Cdc42	Rac, Cdc42	[30,58–63]
<i>Drosophila</i> border cell migration		Rac	Rac	Rac		[64–66]
<i>Drosophila</i> gastrulation				RhoA		[67]
<i>Xenopus</i> gastrulation	RhoA, Rac	Rac, RhoA, Cdc42	RhoA, Rac	RhoA, Rac	Cdc42, RhoA, Rac	[68–73]
Zebrafish gastrulation	Rac		Rac		Rac	[74]

intracellular signaling mediator Dsh, leads to defects in the orientation and stabilization of cellular protrusions in mesodermal cells during gastrulation [34–39]. Although the target mechanisms that Wnt signaling uses to control cellular protrusion orientation and stabilization remain largely unclear, several observations point to actin as an important downstream mediator in this process [39].

The downstream signaling pathway by which Wnt signals transduce their morphogenetic function during zebrafish gastrulation and *Drosophila* dorsal closure are similar but not identical. In zebrafish gastrulation, Wnt11 has been proposed to signal through a non-canonical,  $\beta$ -catenin-independent Wnt signaling pathway that shares some similarities with the Fz and planar cell polarity (Fz/PCP) pathway that was originally identified to polarize cells in the plane of an epithelium in *Drosophila* (for review, see Refs [40,41]). By contrast, although the planar cell polarity of epithelial cells in *Drosophila* dorsal closure is dependent on Wg signaling [32], Wg functions in this process via  $\beta$ -catenin, suggesting that canonical Wnt/Wg signaling is involved [42]. However, because  $\beta$ -catenin not only regulates target gene expression in the canonical Wnt signaling pathway but also controls cell adhesion by linking cadherins to the actin cytoskeleton (for review, see Ref. [43]), additional evidence for the involvement of canonical Wnt signaling in dorsal closure is needed to support this claim.

Rho kinase 2 (Rok2) can function as a downstream effector of Wnt11 signaling during zebrafish gastrulation [39]. Similarly, *Drosophila* Rho kinase (Drok) has been shown to function downstream of Fz and Dsh to mediate a branch of the Fz/PCP involved in actin bundle formation in developing wing cells [44]. Once activated in these cells, Drok modulates the actin cytoskeleton by phosphorylating the non-muscle myosin regulatory light chain and hence the activity of myosin II. Notably, myosin II has been recently shown [45,46] to be a crucial factor in the regulation of germ-band extension during *Drosophila* gastrulation [47] (Figure 2d). This observation points to the intriguing possibility that activation of myosin II serves as a common mechanism by which Wnt/Fz signaling regulates gastrulation movements in both zebrafish and *Drosophila*.

During *Drosophila* germ-band extension, myosin II shows a polarized localization in the plane of the epithelium, accumulating at the interface between cells along the antero–posterior axis of the embryo. At this interface, myosin II specifically induces E-cadherin-based junctional remodeling, which causes a local shrinking of the interface that eventually triggers the medio–lateral convergence of cells along the dorso–ventral axis [45]. Assuming that Wg might function upstream of myosin II in this process, this finding suggests that remodeling of cadherin-based junctional complexes could function as a downstream effector mechanism that mediates the morphogenetic function of Wg signaling during *Drosophila* gastrulation.

This hypothesis is attractive because it would provide a mechanistic explanation of how Wg/Wnt signaling controls tissue morphogenesis not only in *Drosophila* germ-band extension but also in other related processes such

as convergent extension movements during vertebrate gastrulation. Notably, junctional remodeling is not a process that is restricted to epithelial cells; for example, the capacity of mesenchymal cells to migrate depends heavily on their ability to assemble and disassemble junctional complexes such as integrin-based focal adhesions [48].

#### The JAK/STAT signaling pathway

The Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway has been suggested to be important in mesendodermal cell polarization, directed cell migration and germ-layer separation at the onset of zebrafish gastrulation. In zebrafish, several JAK and STAT homologs have been identified and are expressed during gastrulation [49–52]. Interfering with the function of JAK1 and STAT3 during zebrafish gastrulation leads to defects in both radial cell intercalations of epiblast cells and anterior migration of hypoblast cells, indicating that JAK/STAT signaling controls cellular movements during gastrulation [49,52].

Recently, STAT3 has been shown to upregulate the  $Zn^{2+}$  transporter LIV1; in addition, it has been proposed that LIV1 promotes hypoblast (mesendodermal) cell migration downstream of STAT3 by inducing an ‘epithelial to mesenchymal’ transition in mesendodermal progenitors when they internalize [53]. As yet, however, there is no compelling evidence to show that mesendodermal progenitors in zebrafish are organized into an epithelium before they internalize, nor has the type of mesenchymal character that LIV1 transfers to these cells once they have internalized been characterized. This indicates that STAT3 and its downstream effector LIV1 are needed for some aspects of mesendodermal progenitor cell motility, although the precise way in which these molecules function during zebrafish gastrulation remains to be elucidated.

Similar to the situation in zebrafish, JAK/STAT signaling is important in the migration of border cells during *Drosophila* oogenesis [54]. Embedded in the border cells are the ‘polar cells’ – a pair of cells that cannot migrate by themselves [55] but are carried by the border cells towards the oocyte. The polar cells express the secreted cytokine Unpaired, which is bound by surrounding border cells expressing the Unpaired receptor Domeless [56,57]. Binding of Unpaired to Domeless in border cells activates JAK/STAT signaling, which in turn induces a general motile state in border cells and maintains them in a clustered state during their migration [54,56,57]. Thus, the polar cells, by expressing the ligand that activates JAK/STAT signaling in border cells, represent a source of signal that keeps the border cells motile. In turn, the border cells carry with them the source of the signal – the polar cells – that maintains their motility during the course of their migration.

It is tempting to speculate that the upstream signals that activate JAK/STAT signaling in mesendodermal progenitors in zebrafish are also secreted by cells in their near vicinity. One possibility is that the epiblast cells, on which the mesendodermal progenitors migrate, could be a source of these activating signals, thereby

maintaining the motile state of mesendodermal cells on their way to the animal pole of the gastrula. Alternatively, mesendodermal progenitors themselves might express both ligand and receptor to maintain their motility through autocrine and/or paracrine signaling. Future experiments are needed to identify the molecular nature of the signals and receptors that function upstream of JAK and STAT and to determine their temporal and local distribution in the epiblast and hypoblast during the course of gastrulation.

### Concluding remarks

During zebrafish gastrulation, the hypoblast (mesendodermal germ layer) forms between the overlying epiblast (ectodermal germ layer) and the underlying yolk cell and moves along both cell layers towards the animal pole of the gastrula. At the same time as the hypoblast moves towards the animal pole, the overlying epiblast undergoes epiboly movements in the opposite direction towards the vegetal pole. These observations raise issues of how the two germ layers interact with each other and how this interaction influences cell polarization and directed cell migration. By comparing this process with other more extensively characterized model systems of cell migration in development, a series of potential cellular and molecular mechanisms that regulate interactions between the ectodermal and mesendodermal germ layers can be proposed.

On the one hand, these mechanisms include the differential distribution of adhesion molecules such as cadherins between the two germ layers, which might help to keep the germ layers separated from each other, but also might enable them to use each other as substrates for their movements in opposite directions. On the other hand, signaling molecules such as PDGFs, Wnts or chemokines that activate JAK/STAT signaling might function as guidance cues or 'chemoattractants' that polarize cells from both germ layers and/or direct the migration of these cells to specific places in the developing embryo.

Future experiments will have to address, first, the distribution of these different adhesion and signaling molecules at a cellular and subcellular level in the zebrafish embryo; second, the endogenous requirements of these molecules in terms of cellular movements and morphology during gastrulation; and last, the ways in which these molecules function in these processes. How quickly these issues can be resolved will depend crucially on the continuous development of new techniques in zebrafish that will facilitate the efficient generation of antibodies directed against various adhesion and signaling proteins, the conditional knockdown of genes of interest, and further advancements in image acquisition and analysis.

In addition, the development of assay systems by which cells and tissues can be analyzed in isolation would be extremely helpful for gaining an initial insight into the potential morphogenetic functions of molecules of interest, away from the huge complexity of cellular interactions in the embryo. Finally, quantitative analysis of the different morphogenetic processes during gastrulation could be used to develop mathematical models that can mimic specific aspects of these processes and, thereby, serve as a

powerful tool with which to elucidate the basic principles that underlie cellular movements during gastrulation in zebrafish. Although many of these techniques and/or approaches do not exist or need to be improved in zebrafish, the rapid progress in this field in recent years has already put zebrafish at the forefront of model organisms in which to study the molecular and cellular mechanisms that underlie gastrulation movements in vertebrates.

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### Supplementary data

Supplementary data associated with this article can be found at [doi:10.1016/j.tcb.2004.09.008](https://doi.org/10.1016/j.tcb.2004.09.008)

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