Gastrulation: Making and Shaping Germ Layers

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Abstract

Gastrulation is a fundamental phase of animal embryogenesis during which germ layers are specified, rearranged, and shaped into a body plan with organ rudiments. Gastrulation involves four evolutionarily conserved morphogenetic movements, each of which results in a specific morphologic transformation. During emboly, mesodermal and endodermal cells become internalized beneath the ectoderm. Epibolic movements spread and thin germ layers. Convergence movements narrow germ layers dorsoventrally, while concurrent extension movements elongate them anteroposteriorly. Each gastrulation movement can be achieved by single or multiple motile cell behaviors, including cell shape changes, directed migration, planar and radial intercalations, and cell divisions. Recent studies delineate cyclical and ratchet-like behaviors of the actomyosin cytoskeleton as a common mechanism underlying various gastrulation cell behaviors. Gastrulation movements are guided by differential cell adhesion, chemotaxis, chemokinesis, and planar polarity. Coordination of gastrulation movements with embryonic polarity involves regulation by anteroposterior and dorsoventral patterning systems of planar polarity signaling, expression of chemokines, and cell adhesion molecules.

Contents

INTRODUCTION

Animals have bodies of diverse shapes with internal collections of organs of unique morphology and function. Such sophisticated body architecture is elaborated during embryonic development, whereby a fertilized egg undergoes a program of cell divisions, fate specification, and movements. One key process of embryogenesis is determination of the anteroposterior (AP), dorsoventral (DV), and left-right (LR) embryonic axes. Other aspects of embryogenesis are specification of the germ layers, endoderm, mesoderm, and ectoderm, as well as their subsequent patterning and diversification of cell fates along the embryonic axes. These processes occur very early during development when most embryos consist of a relatively small number of morphologically similar cells arranged in simple structures, such as cell balls or sheets, which can be flat or cup shaped. The term gastrulation, derived from the Greek word *gaster*, denoting stomach or gut, is a fundamental process of animal embryogenesis that employs cellular rearrangements and movements to reposition and shape the germ layers, thus creating the internal organization as well as the external form of developing animals.

Here we discuss both the differences in the cellular and molecular mechanisms of gastrulation as well as the many similarities that emerge as we learn more about this fascinating process in model organisms. First, we discuss the four evolutionarily conserved gastrulation movements, epiboly, internalization, convergence, and extension, each of which drives defined morphological tissue transformation. Second, we survey cellular mechanisms underlying these gastrulation movements, including cell migration, intercalation, epithelial mesenchymal transition, and cell shape changes. Next, we discuss the process of gastrulation as it occurs in several model organisms, highlighting how they employ epiboly, internalization, convergence, and extension movements as well as the specific cellular mechanisms deployed. Then we provide a short review of the basic cell properties, including cell adhesion, cortical tension,

and cytoskeletal systems, that mediate various gastrulation cell behaviors. The essence of various gastrulation cell movements is their polarized and directional nature that affords the transformation of an amorphous cellular mass or cell sheet into highly asymmetric and structured body rudiment. We review the significant progress achieved in recent years in delineating various molecular mechanisms that mediate and instruct asymmetric cellular behaviors during gastrulation and coordinate morphogenetic movements with embryonic polarity.

COMPONENT GASTRULATION MOVEMENTS: MORPHOGENETIC OUTCOMES AND UNDERLYING CELL BEHAVIORS

The process of gastrulation entails a set of evolutionarily conserved morphogenetic movements, emboly/internalization, epiboly, convergence, and extension, which are defined by their morphogenetic outcome (Keller et al. 1991). Emboly, or internalization, is the defining gastrulation movement, which transports the prospective mesodermal and endodermal cells beneath the future ectoderm (**Figure 1a–j**). Epibolic movements spread and thin germ layers (**Figure 1d,e,k,l,m**). Convergence movements narrow germ layers dorsolaterally/mediolaterally, whereas concurrent extension movements elongate them anteroposteriorly (**Figures 2** and **3**). Importantly, the same morphogenetic transformation of tissue, or each of these gastrulation movements, can be achieved by various motile cell behaviors or a combination of cell behaviors. Consequently, involvement of a specific gastrulation movement in a given animal species does not imply the underlying cellular mechanism, which must be experimentally determined.

Emboly

During emboly or internalization, mesodermal and endodermal progenitors move via a gateway known as the blastopore (**Figure 1**), a

structure central to the process of gastrulation, also known as blastoderm margin in fish and primitive streak in amniotes (Keller & Davidson 2004). Internalization is usually followed by migration of endodermal and mesodermal progenitors away from the blastopore as individual cells (Solnica-Krezel 2005). At the onset of gastrulation, prospective mesodermal and endoderm cells reside in epithelium (*Drosophila melanogaster*, *Caenorhabditis elegans*, chick, mouse) or within tightly packed and adherent mesenchymal tissue (frog, fish). Thus, emboly and migration of internalized mesodermal and endodermal cells must involve some form of epithelial to mesenchymal transition (EMT) (Wu et al. 2007). In this process, epithelial junctions are disassembled and cell adhesion molecules are downregulated, while intermediate filament network is formed and microtubule network is rearranged from acentrosomal to that radiating from a centrosome (Thiery et al. 2009).

The variations in the cellular mechanisms that drive internalization include the position of the blastopore in the gastrula and the timing of the EMT with respect to the internalization (preceding or following it) (**Figure 1**). Invagination is one type of emboly that occurs during gastrulation in *D. melanogaster*. Apical constriction of ventral midline epithelial cells creates a furrow where mesoderm folds inward (**Figure 1b,c**) (Kam et al. 1991, Leptin & Roth 1994). As the ventral furrow (blastopore) deepens, taking the nascent mesoderm deep inside the embryo, cells break away from the epithelium and start migrating on the internal layer of the future ectoderm. Involution is another example of internalization that precedes EMT. In the extensively studied example of involution during frog gastrulation, the prospective mesoderm and part of endoderm form a cohesive tissue above the prospective blastopore (Keller 1981). Involution is heralded by apical constriction of so-called bottle cells marking the nascent blastopore in the dorsal gastrula region, where the Spemann-Mangold organizer (SMO) resides (Hardin & Keller 1988). Through that opening, which will expand laterally in the course of gastrulation,

Internalization/Emboly

Epiboly

Worm
Caenorhabditis elegans

a
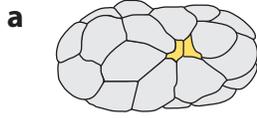
Fruit fly
Drosophila melanogaster
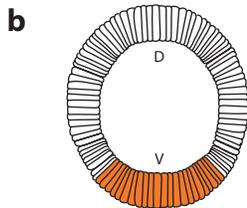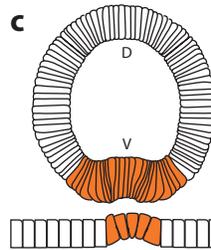
b

Invagination

c

D

V

Zebrafish
Danio rerio
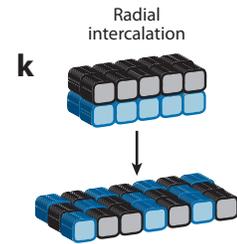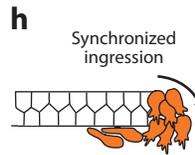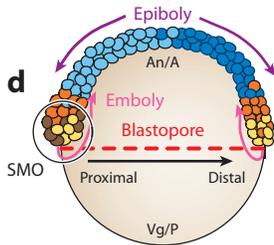
d

Epiboly

An/A

Emboly

Blastopore

SMO

Proximal   Distal

Vg/P

h

Synchronized
ingression

k

Radial
intercalation

Frog
Xenopus laevis
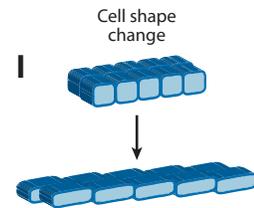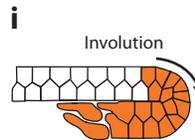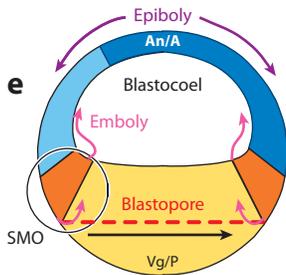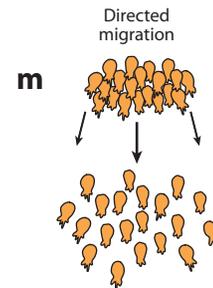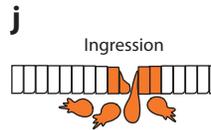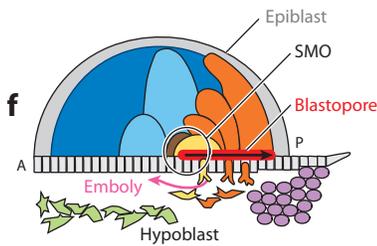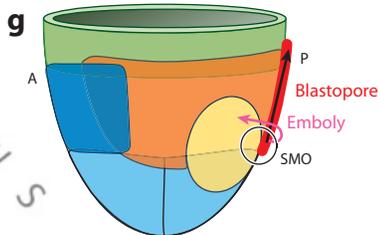
e

Epiboly

An/A

Blastocoel

Emboly

Blastopore

SMO

Vg/P

i

Involution

l

Cell shape
change

Chicken
Gallus gallus

f

Epiblast

SMO

Blastopore

A   P

Emboly

Hypoblast

j

Ingression

m

Directed
migration

Mouse
Mus musculus

g

A

P

Blastopore

Emboly

SMO

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the nascent mesoderm rolls as a coherent tissue (**Figure 1***i*). Only when inside the gastrula do the mesodermal cells break away from the involuted tissue mass to migrate on the internal side of the uninvoluted tissue (blastocoel roof) (Winklbauer & Nagel 1991). In the type of emboly known as ingression, best described during sea urchin (Fink & McClay 1985), or amniote gastrulation (Harrisson et al. 1991, Tam & Gad 2004, Tam et al. 1993), EMT precedes internalization. Thus, mesodermal and endodermal progenitors residing at the epithelial primitive streak (blastopore equivalent) undergo EMT to break away from the epithelium and move as individuals deep into the embryo, where they continue to migrate as individual cells (**Figure 1***j*). There are variations on these themes. For example, as described in more detail below, during zebrafish gastrulation, prospective mesoderm and endoderm cells of mesenchymal character move through the blastopore largely as individuals, but in a synchronized manner (Kane & Adams 2002), or as a more cohesive tissue as occurs during involution (**Figure 1***i*) (Keller et al. 2008).

Epiboly

Epiboly is a morphogenetic process that results in isotropic spreading of tissues, usually associated with its thinning (**Figure 1***d,e,k–m*) (Trinkaus & Lentz 1967). In the classic example of frog or fish epiboly, thinning and spreading of germ layers during gastrulation is achieved by radial intercalation of cells from deeper to more superficial layers (Keller 1980, Warga & Kimmel 1990). Because these intercalations are random (not polarized) with respect to embryonic axes, they result in isotropic expansion of tissues around the nascent embryo (**Figure 1***k*). Cell shape changes, such as flattening and narrowing of cells in a cell sheet, can drive or contribute to thinning and expansion of the cell sheet (**Figure 1***l*) (Keller & Hardin 1987). In zebrafish, directed migration of cells away from a tightly packed and thick cell mass at the embryo equator results in its thinning and spreading toward the vegetal pole (**Figure 1***m*) (Lin et al. 2009).

Convergence and Extension

Another evolutionarily conserved process that elongates the nascent germ layers from head to tail and narrows them from back to belly is convergence and extension (C&E) (**Figure 3**), which is also employed at other stages of embryogenesis such as during elongation of various tubular organs (Keller 2002, Zallen 2007). The best-studied type of C&E is so-called convergent extension (CE), described by the pioneering work of Keller et al. (1985) in *Xenopus*. During CE, simultaneous AP elongation and mediolateral (ML) narrowing of tissues is

C&E: convergence and extension

CE: convergent extension

Figure 1

Gastrulation movements and underlying cell behaviors in diverse animal models. (*a*) In *Caenorhabditis elegans*, the internalized endodermal cells (*yellow*) during gastrulation. (*b,c*) *Drosophila melanogaster*. A cross section is shown of the embryo at the onset of gastrulation, with the prospective mesoderm (*orange*) in the ventral region (*b*). Upon apical constriction, the prospective mesodermal cells acquire a bottle shape, resulting in the initiation of invagination and ventral furrow formation. Dorsal is up. (*d*) Zebrafish early gastrula fate map and the patterns of epiboly and emboly gastrulation movements. Cross section with animal/anterior up and dorsal is to the left. (*e*) Frog early gastrula fate map and the patterns of epiboly and emboly gastrulation movements. Cross section with animal/anterior up and dorsal is to the left. (*f*) Chick early gastrula fate map and the emboly gastrulation movements. A cross section of half of the embryo is shown. (*g*) Mouse early gastrula fate map and the patterns of emboly gastrulation movements. Lateral view with posterior to the right and anterior to the left. The tip of the embryonic cup corresponds to the distal side of the embryo. (*c,h–j*) Cellular basis of emboly: invagination in *Drosophila* (*c*), synchronized ingression in zebrafish (*h*), involution in *Xenopus* (*i*), ingression in amniotes (*j*). (*k–m*) Cellular basis of epiboly: radial intercalation in zebrafish and *Xenopus* (*k*), cell shape change (*l*), directed migration (*m*). Various elements are identified as follows: cytoplasm (*light gray*), mesoderm and its precursors (*orange*), prechordal mesendoderm (*brown*), definitive endoderm and its precursors (*yellow*), epidermis (*dark blue*), neuroectoderm (*lighter blue*), various extraembryonic tissues (green, brown, purple), blastopore (*red*). Abbreviations: A, anterior; An, animal; D, dorsal; P, posterior; SMO, Spemann-Mangold organizer; V, ventral; Vg, vegetal. Figure based on Solnica-Krezel (2005).
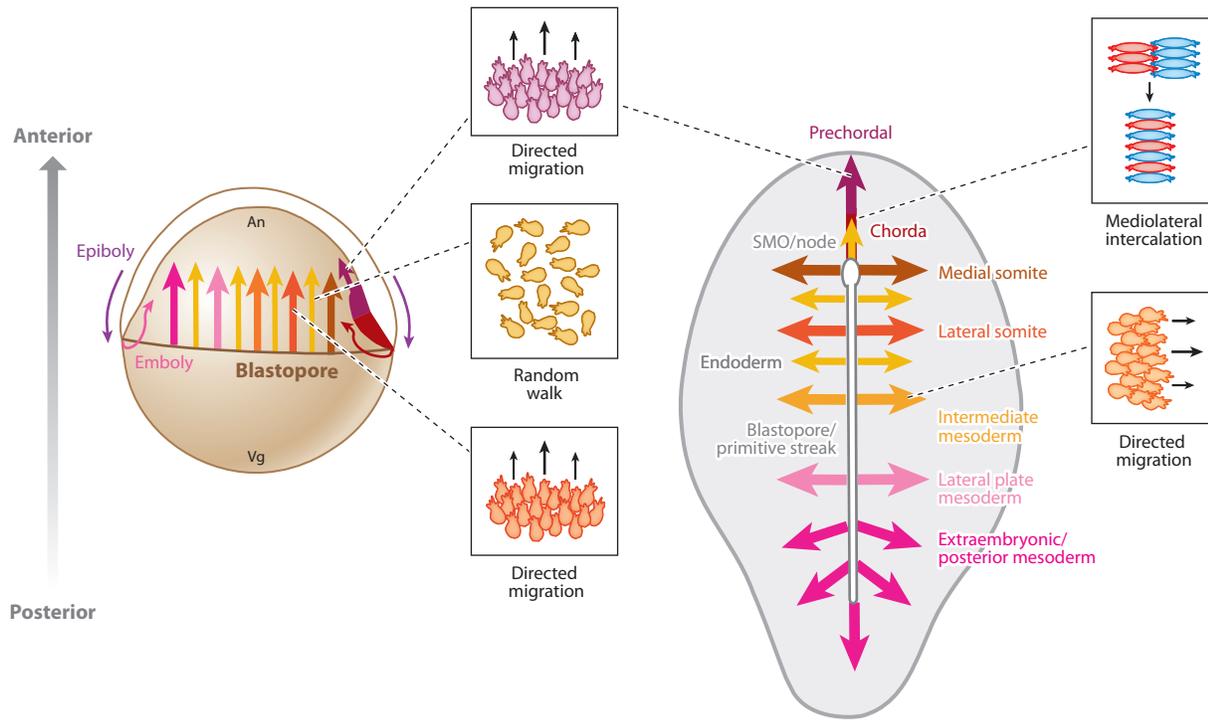
Figure 2

Movement patterns of internalized mesodermal and endodermal cells during early stages of vertebrate gastrulation in zebrafish and an idealized amniote embryo; also shown are the specific cell behaviors involved. Abbreviations: An, animal; SMO, Spemann-Mangold organizer; Vg, vegetal.

achieved by planar intercalation in either the medial or lateral direction of mediolaterally elongated cells that move between their anterior and posterior cell neighbors (**Figure 2**) (Shih & Keller 1992a). Similar AP tissue elongation associated with thinning can be achieved by polarized radial intercalation, whereby cells in multilayered tissue intercalate from one layer into another, preferentially separating their anterior and posterior neighbors, as observed during zebrafish gastrulation (**Figure 3**) (Yin et al. 2008). Polarized cell divisions can also contribute to tissue extension, where the cell division plane is polarized such that the daughters are aligned with the AP axis (Gong et al. 2004). Finally, cell migration affords another mechanism for C&E. For example, during zebrafish gastrulation, migration trajectories of cells in the lateral mesoderm point dorsally, such that this population converges toward the dorsal midline. However, trajectories of cells closer to the animal pole (anterior) are biased anteriorly, and those closer to the vegetal pole (posterior) are biased posteriorly. Therefore, the entire lateral mesoderm cell population converges to the embryonic midline and simultaneously extends (**Figure 3**) (Sepich et al. 2005). Interestingly, undirected cell migration (random walk) can also lead to tissue extension. This is illustrated by endodermal precursors that ingress beneath the ectoderm during zebrafish gastrulation via the circumferential blastoderm margin (blastopore) and migrate on the surface of the yolk cell in an undirected fashion, thus extending the nascent cell population in animal (anterior) (**Figure 2**) and later also in vegetal (posterior) direction (Pezeron et al. 2008). This type of tissue morphogenesis can be considered an extension without convergence, or alternatively as epiboly.

GASTRULATION MOVEMENTS IN MODEL ORGANISMS

Whereas the above-mentioned gastrulation movements are evolutionarily conserved, epiboly and C&E are employed in the same, but also distinct, aspects of gastrulation in various animal groups, in a manner dictated by the embryonic morphology. Below, we survey how the processes of emboly, epiboly, and C&E contribute to gastrulation and what cellular mechanisms they employ in select model organisms.

Caenorhabditis elegans

In this nematode, gastrulation is initiated when the embryo contains 26 cells that flatten their innermost surfaces to separate 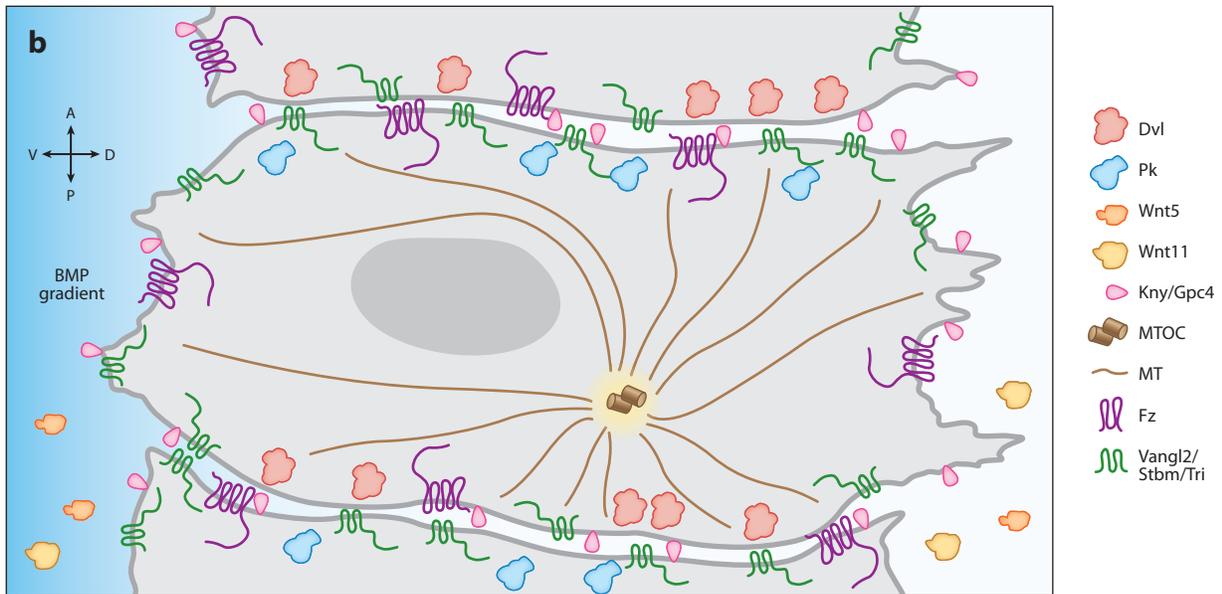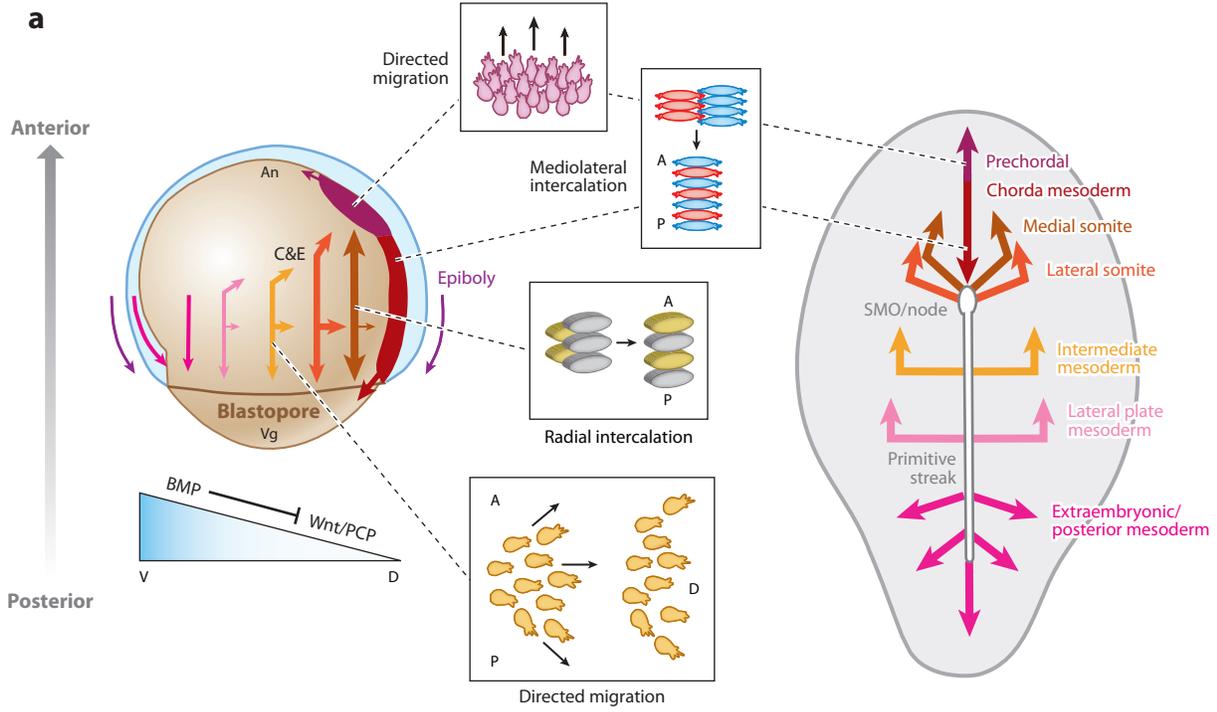from each other and thus create a small internal space, the blastocoel (Nance & Priess 2002, Nance et al. 2005). At this stage, the blastomeres are not connected via specialized cellular junctions and do not exhibit apical, basal, and lateral polarized membranes observed in typical epithelia. Prospective endodermal and mesodermal precursors, specified by a combination of maternal determinants and inductive cell interactions, are located at the ventral aspect of the embryo, whereas epidermal precursors occupy dorsal positions. Prospective endodermal cells ingress individually into the blastocoel (**Figure 1a**). This is followed by ingression of mesodermal precursors and then of germ cells. The ingressing blastomeres flatten their apical surfaces (Lee & Goldstein 2003, Nance & Priess 2002) and do not elaborate clear protrusions (Lee & Goldstein 2003), leaving open the question of the underlying cellular mechanism. Upon completion of internalization, epidermal precursors spread ventrally until they enclose the embryo in the process of epiboly, also known as epidermal or ventral enclosure (Simske & Hardin 2001). This process is initiated by bilaterally located cell pairs, termed leading cells, which elaborate filopodia and move ventrally until they make contact at the ventral midline and establish adherens junctions. The movement of the leading cells is followed by epiboly of their more posterior neighbors, until the ventral opening is sealed. The subsequent change of embryonic shape from an ellipsoid ball to a long tube is driven by contraction of the epidermal cells around the circumference of the body and, thus, a process of C&E that occurs via cell shape changes rather than cellular rearrangements (Williams-Masson et al. 1997).

Drososophila melanogaster

Gastrulation in *Drosophila* embryo starts after 3 h of development when the process of cellularization transformed a syncytium into a cellular embryo (Leptin 1995). Nearly 6,000 cells are arranged into a single-cell-thick epithelial egg-shaped ball with their apical surfaces facing outward (**Figure 1b**). The mesodermal precursors occupy most of the ventral aspect of the embryo, whereas prospective endodermal cells are gathered at the anterior-ventral and posterior most regions. The mesodermal territory is abutted by lateral territories of neuroblasts, whereas epidermal precursor fields lie dorsolaterally between the neuroblast territories and the single dorsal domain of extraembryonic amnioserosa. Internalization of the mesoderm is the first gastrulation movement and occurs via invagination of the mesodermal epithelium (**Figure 1c**). This process is heralded by smoothing of the ventral embryonic surface due to flattening of the apical surfaces of mesodermal cells (Leptin & Grunewald 1990, Turner & Mahowald 1977). Subsequently, a fraction of the most ventrally located mesodermal precursors undergo apical constriction, and the rest of the ventrally located cells follow, resulting in the indentation of the ventral epithelium, termed the ventral furrow, an equivalent of a blastopore (**Figure 1c**) (Leptin & Grunewald 1990). Following the apical constriction, the mesodermal cells continue their morphologic transformation from columnar into wedge shape, by translocating their nuclei basally and shortening their apicobasal dimensions. These morphological changes of individual cells within the epithelium deepen the ventral furrow and

a

Anterior

Posterior

Directed migration

Mediolateral intercalation

An

C&E

Epiboly

Blastopore

Vg

BMP

Wnt/PCP

V

D

A

P

Radial intercalation

A

P

D

Directed migration

Prechordal

Chorda mesoderm

Medial somite

Lateral somite

SMO/node

Intermediate mesoderm

Lateral plate mesoderm

Primitive streak

Extraembryonic/ posterior mesoderm

b

A

V   D

P

BMP gradient

Dvl

Pk

Wnt5

Wnt11

Kny/Gpc4

MTOC

MT

Fz

Vangl2/ Stbm/Tri

REVIEWS IN ADVANCE

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drive it inside the embryo, thus creating a mesodermal epithelial tube, which contacts the ventral aspect of the embryonic ectoderm (Sweeton et al. 1991). The nascent mesodermal tube flattens against the ectoderm and the cellular junctions are disassembled, freeing the mesodermal cells that spread on the ectodermal surface (McMahon et al. 2008, Stathopoulos & Levine 2004, Wilson & Leptin 2000). Some of the anterior endodermal precursors internalize at the anterior aspect of the ventral furrow, whereas others do so via separate invagination events. Neuroblasts internalize via ingression from the lateral epithelial surfaces.

The dorsolateral prospective epidermal ectoderm converges ventrally while dramatically increasing its AP length (Irvine & Wieschaus 1994). This process of C&E, termed germband extension (GBE), is described in more detail below and is driven via a suite of cell behaviors, including cell shape changes, cell divisions, and polarized rearrangements within the epithelial sheet (Blankenship et al. 2006, Butler et al. 2009).

1996, Solursh 1986). Following primary mesenchyme cell ingression, a group of cells forming the vegetal-plate epithelium, located in the center of the vegetal plate, change shape to drive the process of invagination of gut precursors into the blastocoel and form the archenteron (gut tube) (Gustafson & Kinnander 1956). The internalized gut tube quickly elongates, while narrowing its diameter via cell intercalations reminiscent of those underlying typical CE (Miller & McClay 1997). Meanwhile, the secondary mesenchyme cells located at the apical end of the nascent gut tube elaborate filopodia that stretch the length of the blastocoel to anchor the gut tube at the animal pole of the blastocoel, where the oral ectoderm is located and the mouth opening will form (Gustafson & Kinnander 1956, Hardin 1996). Hence, the sea urchin gastrulation employs several gastrulation movements, including invagination, involution, and CE. These movements are driven by a suite of cell behaviors, including EMT, cell shape changes, cell intercalation, and directed migration.

GBE: germ-band extension

Sea Urchin

Formation of the endoderm in sea urchin is considered to be the archetypal model of deuterostome gastrulation (Stern 2004a). In these small and translucent embryos, gastrulation starts with ingression of skeletogenic primary mesenchyme cells, which reside in the vegetal plate. These primary mesenchyme cells undergo EMT, ingress through the basal lamina into the blastocoel, where they migrate to eventually give rise to skeletal elements (Hardin

Zebrafish

When initiating gastrulation movements, the zebrafish embryo exhibits a simple architecture, with a mound of blastomeres, known as the blastoderm, residing atop the syncytial yolk cell (Kimmel et al. 1995). The blastoderm consists of a superficial enveloping layer and deep cells, which will give rise to all embryonic tissues. At this stage, the zygotic genome is transcriptionally active. In the prospective dorsal cells, β-catenin promotes expression of transcription

Figure 3

(*a*) Movement patterns of internalized mesodermal and endodermal cells during late stages of vertebrate gastrulation in zebrafish and an idealized amniote embryo; also shown are the specific cell behaviors involved. (*a,b*) Coordination of gastrulation movements with embryonic patterning in zebrafish gastrula. During polarized mediolateral and radial intercalations, mediolaterally elongated cells separate anterior and posterior neighbors, driving anteroposterior tissue extension. Components of Wnt/PCP (planar cell polarity) signaling become asymmetrically localized on the anterior or posterior membranes of mesenchymal cells engaged in intercalations (*b*). Ventral to dorsal gradient of bone morphogenetic protein (BMP) signaling inhibits expression of Wnt/PCP pathway components and cell adhesion, thus limiting convergence and extension (C&E) to the dorsolateral region. Abbreviations: A, anterior; An, animal; D, dorsal; Dvl, Dishevelled; Fz, Frizzled; Kny/Gpc4, Knypek/Glypican4; MT, microtubule; MTOC, microtubule organizing center; P, posterior; Pk, Prickle; V, ventral; Vangl2/Stbm/Tri, Vangogh-like2/Strabismus/Trilobite; Vg, vegetal.

factors and secreted signals that cooperate in the formation of the dorsal SMO (reviewed in Hibi et al. 2002, Langdon & Mullins 2011), and induction of the mesoderm and endoderm by Nodal signals is under way (Schier & Talbot 2005).

The first morphogenetic movement during zebrafish embryogenesis is epiboly, which begins when the flat yolk cell domes into the blastoderm and more deeply located blastomeres intercalate radially into more superficial layers (Warga & Kimmel 1990). Simultaneously, the blastoderm becomes thinner and expands toward the vegetal pole. When the blastoderm covers half of the yolk cell, the zebrafish blastula exhibits a distribution of germ-layer precursors (i.e., fate map) similar to those described for other vertebrate embryos (**Figure 1d**) (Kimmel et al. 1990). Prospective endodermal cells reside closest to the blastoderm margin, the zebrafish blastopore equivalent, and are intermingled with mesodermal precursors positioned farther away from the blastopore. The animal region of the blastoderm contains ectodermal precursors (Kimmel et al. 1990, Warga & Nusslein-Volhard 1999). During emboly, mesendodermal precursors move via the blastopore beneath the prospective ectoderm. In the dorsal blastoderm margin, the internalization involves ingression of individual blastomeres (Montero et al. 2005, Shih & Fraser 1995), whereas, in the lateroventral regions, mesendoderm precursors internalize in a synchronous manner reminiscent of involution, in the process of synchronized ingression (**Figure 1b**) (Kane & Adams 2002, Keller et al. 2008). Upon internalization, the mesodermal progenitors migrate away from the blastopore toward the animal pole via directed migration (**Figure 2**) (Sepich et al. 2005). Meanwhile, endodermal precursors also spread toward the animal pole via a random walk (**Figure 2**) (Pezeron et al. 2008). C&E movements are highly dynamic and vary in a spatiotemporal manner (Yin et al. 2009). In the ventral regions, mesodermal cells do not engage in C&E movements, but instead migrate toward the vegetal pole (Myers et al.

2002a). Cell populations located in the lateral blastopore region undergo convergence and extension movements of increasing speed (Jessen et al. 2002). The most intense C&E movements occur in the dorsal gastrula regions (Myers et al. 2002a,b; Sepich et al. 2000), where they are driven largely via planar intercalation (**Figure 2**) (Glickman et al. 2003). By contrast, in the paraxial regions, C&E movements involve a cooperation of planar ML intercalation and polarized radial intercalation during which cells intercalate between different layers to separate anterior and posterior cell neighbors (Yin et al. 2008). Therefore, zebrafish gastrulation entails all the conserved gastrulation movements, which are driven by a variety of cell behaviors, including cell migration, ingression, radial and planar intercalations, and cell shape changes.

Frog

Morphology and distribution of prospective germ layers in the frog blastula are similar to those described above for zebrafish; the prospective endoderm is the most vegetal and the mesodermal precursors form a broad band between the endodermal and animally located ectodermal precursors (**Figure 1e**) (Dale & Slack 1987, Lane & Sheets 2002). However, in the frog embryo, the yolk material is partitioned during cleavages into individual blastomeres; the vegetal blastomeres are the largest and decrease in size gradually along the vegetal to animal axis. Similar to the zebrafish, dorsal enrichment of β-catenin triggers a genetic cascade that establishes the SMO that will contribute to patterning of the germ layers and coordinate gastrulation movements (De Robertis et al. 2000, Heasman et al. 2000). Gastrulation entails internalization of mesoderm via the process of involution and epibolic expansion of germ layers toward the vegetal pole (**Figure 1e,i**) (Shih & Keller 1994). One key driving force of involution is vegetal rotation, an active distortion of the endodermal vegetal cell mass that causes turning around of the marginal zone toward the blastocoel

(Winklbauer & Schurfeld 1999). ML cell intercalations are the main morphogenetic behavior that simultaneously drives C&E, or CE (Keller 2002; Shih & Keller 1992a,b). In contrast to the mechanics of gastrulation in frog, however, this process in fish is driven largely by individual mesenchymal cells and, consequently, the main gastrulation movements in fish are independent. Indeed, zebrafish mutations blocking internalization do not interfere with the process of epiboly; mutants with dramatically impaired C&E also complete epiboly on time, and mutations impairing epiboly appear to impair C&E only mildly (Solnica-Krezel et al. 1996). By contrast, in the gastrulating frog embryos, mesenchymal cells are more tightly packed and connected, resulting in a much greater mechanical interdependence of gastrulation movements. For example, CE of the dorsal mesoderm is essential for normal involution as well as for normal completion of epibolic movements (Shih & Keller 1994).

Chick

Although the chick blastula contains relatively large amounts of yolk similar to those of frog or fish embryos, its architecture before the initiation of gastrulation movements is quite distinct (Schoenwolf & Sheard 1990, Stern 2004a). A flat island of epithelium, or epiblast, that will give rise to the embryo proper floats on a very large yolk cell. When the chick egg is laid, the single-cell-thick epiblast contains approximately 20,000 cells forming the central area pellucida surrounded by the area opaca. In the prospective posterior region of the epiblast, a group of small cells tightly adhering to the epiblast form Koller's sickle expressing the SMO genes. Below the epiblast, small cellular islands form by delamination of cells from the area pellucida epithelium (Schoenwolf 1991). These cell groups fuse to form the hypoblast proper. The blastopore, termed the primitive streak in the chick embryo, forms as a slit in the epiblast from the posterior region (**Figure 1***f*). It extends anteriorly during early gastrulation and subsequently shortens. Its formation has been known for a long time to be associated with large-scale cellular flows known as polonaise cell movements, so termed because the cells move in a manner reminiscent of a Polish dance (reviewed in Chuai & Weijer 2009). The lateral epiblastic cell populations converge symmetrically to the posterior midpoint of the area pellucida, where the flows from both directions merge and start to move anteriorly along the central midline to form the streak. These cyclical movements are associated with extension of the primitive streak along the midline. The cellular basis of these massive cell movements is a matter of an ongoing discussion (Chuai & Weijer 2009). According to one model, polonaise cell movements result from oriented cell divisions (Wei & Mikawa 2000). Alternatively, these are chemotactic cell movements directed by a combination of positive and negative cues (Chuai & Weijer 2008). According to a third model, these movements are driven by ML cell intercalation in the context of the epithelium (Lawson & Schoenwolf 2001, Voiculescu et al. 2007). As such, this type of CE is similar in terms of the underlying cellular mechanism to the process of GBE in *Drosophila*. Upon formation of the primitive streak, internalization movements occur as the streak extends anteriorly, with its anterior aspect known as the Hensen's node and corresponding to the SMO (**Figures 1** and **2**). Internalization occurs via ingression; individual endodermal and mesodermal progenitors undergo EMT and enter the space between the epiblast and the hypoblast. The internalized mesodermal cells initially move away from the streak (**Figure 2**). However, as the node regresses, leaving the embryonic midline in its wake, the trajectories of the migrating mesodermal cells turn, such that they start to move (converge) toward the midline (**Figure 3**) (Yang et al. 2002). Therefore, in contrast to other embryos, such as fish and mouse (see below), the avian embryo employs CE-like movements before forming the primitive streak. C&E movements at later gastrulation, driven largely by directed cell migration as well as intercalation in the axial mesoderm region, resemble those in

other vertebrates (reviewed in Solnica-Krezel 2005).

Mouse

The morphology of mammalian embryos differs in many respects from other embryos at the onset of gastrulation. In contrast to most other invertebrate and vertebrate embryos, mammalian embryos possess very limited amounts of maternal dowry and activate the zygotic genome as early as the two-cell stage (Guo et al. 2010, Schultz 2002). Moreover, mammalian embryos initiate gastrulation while having a very small number, just a few hundred, cells (Tam & Gad 2004). At this stage of development, mammalian embryos consist of the epiblast, a single cell layer pseudostratified epithelium, which is either flat (primates and marsupials) or cupshaped (rodents, including mouse). The epiblast will give rise to embryonic tissues as well as the visceral endoderm squamous epithelium that will develop into predominantly extraembryonic, and possibly some embryonic, tissues. In primates and rodents, the nascent gastrula is already implanted into the uterine wall, whereas in some other mammals, it is still freely moving within the oviduct (Eakin & Behringer 2004). Thus, in the mouse, the epiblastic cup's rim, considered to be the proximal aspect of the embryo, is in contact with the extraembryonic ectoderm tissues that give rise to the fetal portion of the placenta and facilitate the integration of the embryo into the uterine wall (**Figure 1***g*).

Gastrulation movements are initiated when a blastopore/primitive streak is formed in the prospective posterior proximal epiblast tissue (**Figure 1***g*). Current elegant time-lapse analyses of early mouse gastrulae revealed that the murine primitive streak forms in situ by initiating EMT and without any large-scale cell movements (Williams et al. 2012). This contrasts against avian gastrulation, in which the primitive streak forms in association with large-scale polonaise cell movements, as discussed above (Chuai & Weijer 2009). It will be important to determine whether any large-scale

movements precede primitive streak formation in the mouse. As in avian gastrula, the murine primitive streak is an equivalent of the blastopore, which serves as a gateway for the internalization of mesodermal and endodermal cells. Emboly in the mouse and other mammals occurs via ingression, whereby individual cells separate from the epiblast epithelium in the process of EMT (**Figure 1***j*) (Williams et al. 2012). Upon becoming individual motile cells, the prospective mesodermal cells invade the space between the epiblast and the visceral endoderm. The prospective extraembryonic mesoderm, as well as embryonic mesoderm cells, internalize via the posterior proximal aspect of the primitive streak. Concurrently, the primitive streak elongates distally along the posterior side of the gastrula until it reaches the distal tip of the embryonic epiblast. The nascent internalized mesoderm spreads away from the primitive streak (**Figures 1***g* and **2**).

Recent genetic and live-imaging studies in the mouse led to a revision of our view on gastrulation movements of the endoderm in the mouse. According to previous models, the nascent endodermal cells emerging largely from the distal aspect of the primitive streak establish the definitive endoderm layer that expands laterally to displace the visceral endoderm proximally toward the extraembryonic territory. However, Hadjantonakis and colleagues reported that the nascent endodermal cells intercalate between the cells of visceral endoderm epithelium, dispersing the visceral endoderm cells and expanding its surface (Kwon et al. 2008). Thus, endoderm gastrulation in the mouse entails an interesting combination of internalization movements via ingression of epiblast-derived endoderm precursors as well as epiboly of the visceral endoderm layer overlying the epiblast via radial intercalation of epiblast-derived definitive endodermal precursors into this layer. Cell divisions within the plane of the nascent epidermal epithelium lead to its further expansion. Because the visceral endoderm cells may persist during development, the endodermal derivatives are of both epiblast and visceral

endoderm origin, raising an interesting possibility that the segregation of the extraembryonic and embryonic tissues during mammalian gastrulation is not absolute (Kwon et al. 2008).

C&E movements during mouse gastrulation are driven via a number of cell behaviors, reminiscent of those observed in zebrafish and frog gastrulae. Recent studies revealed three distinct morphogenetic domains involved in the formation of the notochord (Yamanaka et al. 2007). Axial mesoderm precursors ingress via the most anterior aspect of the primitive streak, equivalent to the SMO (**Figure 2**). In the mouse gastrula at the late allantoic bud (E7.5–8) stage, this region acquires a characteristic horseshoe morphology, forming a structure known as the node. Interestingly, the most anterior axial mesoderm precursors become internalized and form a flat coherent sheet under the endoderm layer before the node structure becomes apparent. Subsequently, these cells converge to the midline to form the notochordal plate. However, the underlying cell behavior remains to be elucidated. In the second morphogenetic domain, prospective trunk notochord precursor cells internalize via the node. Later, when the node moves posteriorly, these cells become mediolaterally elongated and intercalate in a manner typical of the process of CE (**Figure 2**) (Yamanaka et al. 2007), which shapes the trunk axial mesoderm of frog and fish embryos (Glickman et al. 2003, Keller & Tibbetts 1989). Morphogenesis of the third and most caudal aspect of the notochord takes place at the early somite stages, when the node is no longer visible, and involves posterior migration of tail notochord precursors (Yamanaka et al. 2007).

Another set of time-lapse studies shed light on the C&E of presomitic mesoderm (PSM) precursors in the mouse gastrula (Yen et al. 2009). PSM cells ingress via the primitive streak proximally to the node upon undergoing EMT (**Figure 1**). These mesenchymal cells move, using multipolar, biased protrusive activity, first laterally, away from the streak; they later direct their trajectories anteriorly, thus contributing to tissue extension (**Figures 2** and **3a**). Subsequently, these cells elongate and align with the ML embryonic axis and, thus, perpendicular to the primitive streak and AP embryonic axis. These cells also bias their protrusive activity mediolaterally and intercalate mediolaterally within the tissue plane to contribute to the C&E of the nascent PSM (Yen et al. 2009).

In summary, recent, very informative time-lapse analyses of murine gastrulation reveal striking similarities among gastrulation movements in vertebrates, including internalization of mesodermal precursors via ingression, initial migration of the mesoderm away from the streak/blastopore, as well as C&E movements driven via a combination of mediolaterally polarized cell intercalations and directed cell migrations (**Figures 2** and **3**). Surprising differences in the formation of the primitive streak between mouse (in situ, without large-scale movements) and chick (large-scale polonaise C&E movements) also emerge and raise a question as to what degree one can extrapolate the cellular mechanisms of gastrulation from model systems to that of humans.

MECHANICS OF POLARIZATION OF CELL ARCHITECTURE AND ACTIVITY DURING GASTRULATION

Cell Shape and Motility Depend on Adhesion and Cytoskeleton

Above, we discuss a variety of cellular rearrangements, directed migrations, and shape changes that serve as morphogenetic tools during gastrulation of various animal species. Here we consider how a cell alters its shape, how it changes its position within an epithelial sheet, and how mesenchymal cells migrate as individuals or in a coherent group (**Figure 1c, h–m**). Shape changes, migration, and intercalation are driven largely by modulation of cell adhesion and the actomyosin and microtubule cytoskeletal systems. These components are asymmetrically delivered by polarized membrane transport and removed by endocytosis to polarize the cell (Nelson 2009). Cell-cell and cell-matrix adhesion are regulated by the

PSM: presomitic mesoderm

ECM: extracellular matrix

formation of adhesive complexes between a cell and its neighbor or between a cell and the extracellular matrix (ECM) from preexisting components and their insertion into, or removal from, the plasma membrane. Key mediators of cell-cell adhesion are classical cadherins, protocadherins, and tight-junction components (Halbleib & Nelson 2006, Nishimura & Takeichi 2009). Cadherin- and integrin-based adhesion responds to extracellular and intracellular conditions (receptor occupancy as well as extracellular and intracellular tension) that modulate composition of adhesion complexes and interaction with the actin cytoskeleton. Increasing tension generated by cortical actin can mature and stabilize adhesive contacts (Krens & Heisenberg 2011, Krieg et al. 2008). Small GTPases are central to modulation of the actin cytoskeleton but can also regulate microtubule association with the cell cortex (Etienne-Manneville & Hall 2002, Spiering & Hodgson 2011). In a simplified model of the regulation of actomyosin contractility, the small GTPase RhoA acts through its effector Rho kinase (Rok), which phosphorylates the myosin regulatory light chain and stimulates actomyosin contraction. Both Rho and myosin are targets of a number of factors that regulate their activity. Depending on whether the cell is in a mesenchymal or epithelial cell state, different factors control whether F-actin is organized into apical meshworks, circumferential bands at the level of adherens junctions, or linear and crosslinked filaments extending into the lamellipodia.

Recent studies of cell behaviors and cell migration in culture indicate that actomyosin contractility and polymerization occur in cyclical fashion (Gorfinkiel & Blanchard 2011). Often, shape changes occur gradually: Each cycle contributes a small change, and another mechanism preserves the new shape between cycles of activity (similar to a ratchet). Attachment of the actin cytoskeleton to adhesive contacts converts contractile force into motile force (variable linkage is invoked as a "clutch" to modulate motile force). How the force of actin contractility or polymerization is transmitted is determined by

the type of actin structure and adhesive contact (Mason & Martin 2011).

Microtubules are vital to the polarity of cell morphology and polarized motile behaviors and act by delivering cargo to restricted locales (Siegrist & Doe 2007). For example, polarized microtubule arrays are essential to protein transport and removal that underlies apical/basal polarity of the epithelia. In mesenchymal cells, the dynamic instability of microtubules is required for rapid modification of cell motility and adhesion. Microtubules engage in cycles of rapid growth and collapse (Kirschner & Mitchison 1986). The apparently random direction of growth enables microtubules to stochastically explore the cell and encounter factors on the plasma membrane that capture and protect microtubule ends from degradation, thus linking signals on the plasma membrane to the interior of the cell (Holy & Leibler 1994). Similarly, factors regulating adhesion and actomyosin contractility or remodeling can respond to those signals. Finally, microtubules can bind these factors and release them upon depolymerization (Kaverina & Straube 2011). Microtubule and actin cytoskeletal systems interact with the same cellular structures (e.g., adhesive complexes, cell cortex) and are critical for many cellular functions. Accordingly, they are coordinately regulated by factors such as small GTPases, APC, formins, and MACF7 (Kaverina & Straube 2011). In the following sections, we review recent progress in our understanding of how the activity of actomyosin and microtubule networks affects specific gastrulation cell behaviors.

Apical Constriction and Pulsed Actomyosin Contraction

Cells within an epithelium are typically columnar in shape and polarized so that adherens and tight junctions are near the apical surface, whereas integrin/ECM are found along the basolateral surfaces. Constriction of the apical cell surface, expansion of the basal surface, and elongation of the apical-basal cell height forms bottle-shaped cells within the epithelial

sheet and drives bending of the sheet, often into a tube that is internalized (Sawyer et al. 2010). Such shape changes accompany the gastrulation internalization movements of invagination (**Figure 1b,c**) (*Drosophila*, sea urchin*)*, involution (**Figure 1e,i**) (frog), or ingression (**Figure 1f,g,j**) (chick, mouse).

Mesodermal invagination in *Drosophila* occurs when cells at the ventral midline shrink their apical surfaces, first synchronously then stochastically (**Figure 4a,b**) (Oda & Tsukita 2001). Actin forms a mesh-like cytoskeleton at the apical surface and circumferential bands at the level of the adherens junctions (**Figure 4b,c**) (Martin et al. 2009). The apically secreted protein termed folded gastrulation (Fog) (Oda & Tsukita 2001) and a heterotrimeric G12/13 protein identified by the mutation *concertina* are required to initiate invagination (Costa et al. 1994). Myosin II and RhoGEF2 become apically localized downstream of *concertina* (Fox & Peifer 2007, Nikolaidou & Barrett 2004) and Fog (Dawes-Hoang et al. 2005). F-actin becomes apically localized under the influence of RhoGEF2 and Abelson tyrosine kinase (Fox & Peifer 2007, Kolsch et al. 2007). Adherens junctions are required for apical constriction and to maintain myosin and F-actin at the apical surface (Dawes-Hoang et al. 2005). Surprisingly, apical constriction seems to be driven by pulsed contraction of apical actin rather than constriction of the junctional actomyosin ring (**Figure 4d–f**) (Martin et al. 2009). During pauses in contraction, the apical surface remains shrunken, suggesting a ratchet mechanism that maintains the decreased size between pulsed contractions, possibly involving the junctional actomyosin ring. Interestingly, the later contractions are not synchronized between individual mesodermal cells; however, actomyosin appears to form a dynamic supracellular meshwork at the apical tissue surface (Martin et al. 2009). Pulsed contractions are also observed during dorsal closure, which is another morphogenetic movement in the *Drosophila* embryo (Blanchard et al. 2010, David et al. 2010).

In *Xenopus*, apical constriction of epithelial cells plays a role in the early phase of involution
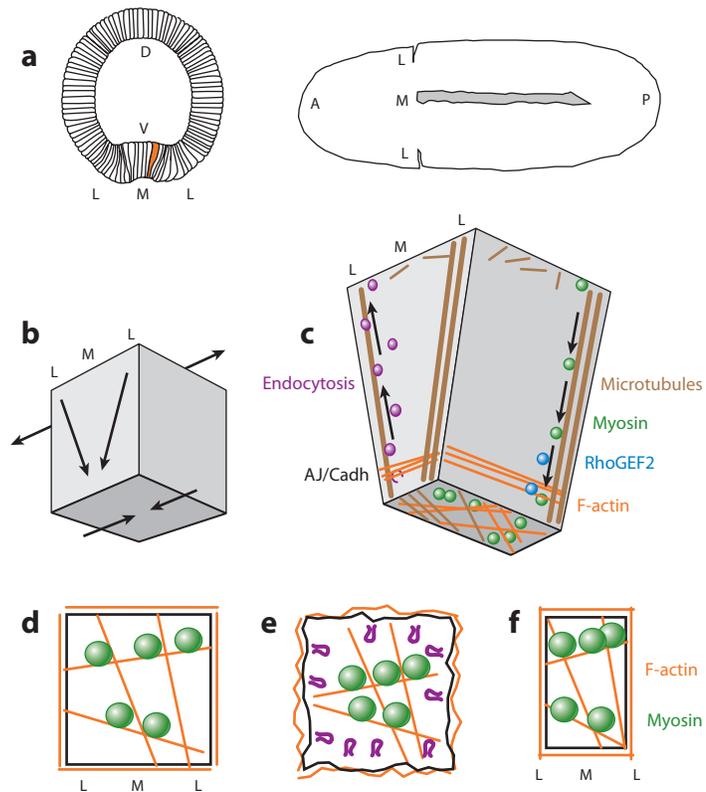

Figure 4

Apical constriction during mesoderm invagination. (*a*) Mesodermal cells at the ventral midline of a stage 7 *Drosophila* embryo undergoing apical constriction, shown in cross section and ventral view. One cell is highlighted in orange. (*b,c*) Shape changes are schematized for an idealized cell undergoing apical constriction. In general, cells constrict their apical surfaces, expand their basal surfaces, and elongate apical-basally. (*c*) Model of protein localization in apical constriction. F-actin is present in an apical meshwork and in cables at the level of the AJ. Apical-basal-oriented microtubules (*brown*) transport cargo myosin (*green*), RhoGEF2 (*blue*), actin (*orange*), and endocytic vesicles (*purple*). (*d*) Model of actomyosin contraction that drives apical constriction. A network of apical F-actin (*orange*) and myosin (*green*) contracts, reducing surface area; (*e*) when the actomyosin network relaxes, the diminished cell surface area is maintained, possibly by junctional actomyosin, and excess cell membrane is removed by endocytosis. (*f*) After repeated cycles, the cell surface is reduced. Abbreviations: A, anterior; AJ, adherens junction; Cadh, cadherin; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

during gastrulation. Bottle-shaped cells form in the dorsal superficial epithelium and promote the onset of involution and proper shaping of the archenteron (Keller 1981, Lee & Harland 2007). F-actin and myosin become enriched at the apical-cell surfaces while microtubules form apicobasally oriented arrays (**Figure 4b,c**). Both

are required for apical constriction (Lee & Harland 2007). Apical constriction can also drive the internalization of individual or small groups of cells. Ingression of mesoderm and endoderm during gastrulation in chick begins with an

Figure 5

Intercalation during germ-band extension. (*a,b*) Cells exchange neighbors, causing the ventral epidermis of a *Drosophila* embryo to narrow mediolaterally and extend anterior-posteriorly. (*a*) Rosette formation in intercalation, drawn from Blankenship et al. (2006). (*b*) Junctional remodeling in intercalation, drawn from Bertet et al. (2004). (*c*) Adhesive and polarity molecules (*blue*) accumulate on anteroposterior (AP)-oriented membranes, while cytoskeletal molecules (*orange*) accumulate on mediolateral (ML)-oriented membranes. (*d*) Model of actomyosin contraction that drives intercalation. Apical actin/myosin web contracts. (*e*) Contracted actin flows to the ML cell membrane. (*f*) Cell membranes shorten, and junctional actin shortens forming rosette or type II junctions. Abbreviations: αcat, α-catenin; βcat, β-catenin; A, anterior; aPKC, atypical protein kinase C; D, dorsal; Ecad, E-cadherin; L, lateral; M, medial; P, posterior; Rok, Rho kinase; V, ventral.

apical constriction that bends the center of the primitive streak. The epithelium of the primitive streak is abutted by a delicate basement membrane at its basal surface as well as robust tight and adherens junctions near its apical surface. Microtubule instability and inhibition of RhoA are required to break down the basement membrane (**Figure 4b,c**). Cells in the primitive streak assume an extreme bottle shape and are released when tight junctions at the apical surface dissolve, thus undertaking an EMT (Nakaya & Sheng 2008, 2009).
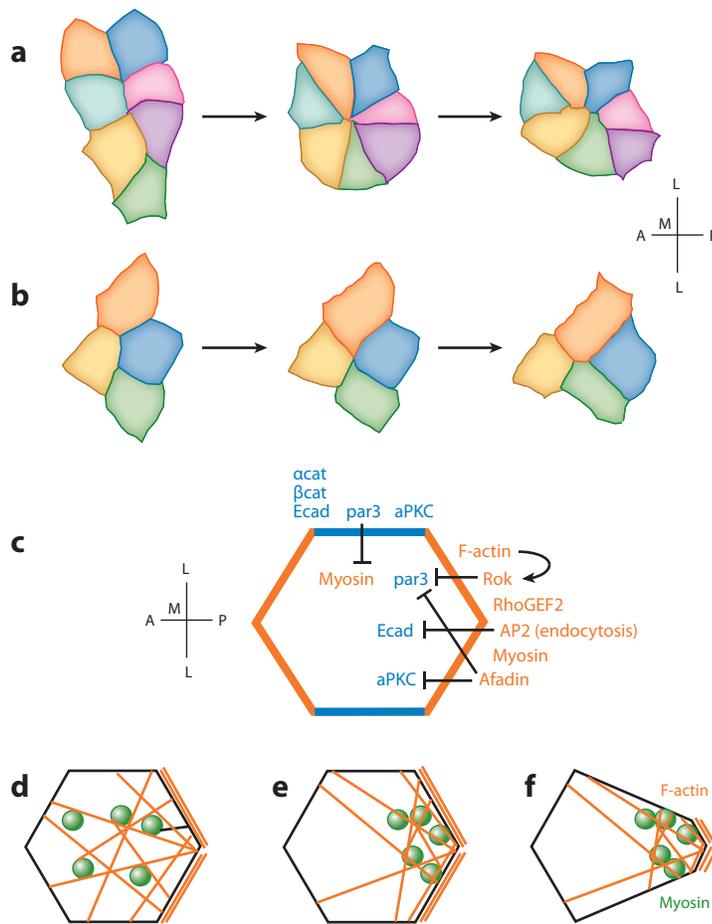
Cell Intercalation

Cell rearrangements, such as planar and radial intercalations, can drive gastrulation movements of epiboly and C&E. During the process of GBE that follows invagination of the ventral mesoderm in *Drosophila* embryos, a combination of cell behaviors, including asymmetric cell shape changes and rearrangements, cooperate to narrow the ventrolateral epidermis mediolaterally (dorsoventrally) while extending it anteroposteriorly (Zallen 2007). Interestingly, these GBE morphogenetic cell behaviors occur in the context of the epithelium, similar to the invagination described above, driven by apical constriction. Mesodermal invagination leaves adjacent epithelial cells stretched mediolaterally. Between invagination and GBE, cells relax their ML elongated shape (Butler et al. 2009) then actively stretch (Sawyer et al. 2010) to elongate in an AP direction. Similar to what is observed in mesodermal invagination, actin forms an apical network. However, in contrast to mesodermal invagination, actin also forms multicellular cables at cell junctions during GBE. Asymmetric constriction of the apical actin occurs before the ML cell junctions shortening, which precedes contraction of junctional actin cables (**Figure 5d–f**) (Bertet et al. 2004, Blankenship et al. 2006, Fernandez-Gonzalez & Zallen 2011, Rauzi et al. 2010, Sawyer et al. 2011). Constriction over 4–11 adjacent cells along the ML axis creates multicellular clusters, called rosettes, and groups of four cells that engage in type 2 transitions (**Figure 5a,b**) (Bertet

et al. 2004, Blankenship et al. 2006). Multicellular actin cables are proposed to pull cells into straight rows during GBE and at compartment boundaries (Blankenship et al. 2006, Monier et al. 2010). Subsequent loss of myosin and lengthening of junctional membranes along the AP axis resolve the cell clusters to yield AP extension (Bertet et al. 2004, Blankenship et al. 2006, Zallen & Wieschaus 2004). Interestingly, the polarized distribution of cytoskeletal molecules and E-cadherin endocytosis (along the ML axis) with adhesion and polarity molecules (along the AP axis) are required for cell intercalation and elongation (**Figure 5c**) (Levayer et al. 2011). Further, the apical actin web is dependent on Afadin for linkage to boundaries oriented along the ML axis (Sawyer et al. 2011). This molecular asymmetry may transmit force asymmetrically from the apical actin web to multicellular cables, thus causing intercalation behavior (Sawyer et al. 2011). Finally, tension along cell boundaries recruits myosin to the boundaries; this increases tension that can then spread to adjacent cells, thereby enhancing and coordinating tissue elongation over several cells (Fernandez-Gonzalez et al. 2009). During vertebrate gastrulation, polarized planar and radial intercalations are some of the main cellular mechanisms underlying CE movements that simultaneously narrow and elongate the embryonic tissues (**Figure 3a**). In contrast to the GBE, these cell intercalations take place in the context of a closely packed mesenchyme lacking the typical epithelial architecture marked by tight junctions. Dorsal mesodermal cells in *Xenopus* and zebrafish gastrulae lengthen and align mediolaterally while elaborating actin-rich protrusions at the medial and lateral edges (**Figure 3b**) (Keller et al. 1989, Myers et al. 2002a, Shih & Keller 1992a, Wallingford et al. 2000).

How are these changes in cell shape and behavior achieved? Actomyosin dynamics in the cells engaged in the polarized intercalation behaviors is similar to that observed in cell intercalations in *Drosophila* epithelia. Actin is organized in cables and medial webs that align with the long axis of the cell and that cyclically shorten and lengthen (Kim & Davidson 2011, Skoglund et al. 2008). Myosin IIB is required for effective cell motility and protrusion retraction, but not for extension of protrusions (Skoglund et al. 2008). These punctuated actin contractions are thought to be regulated by both myosin contractility and F-actin polymerization, and during CE, they depend on Wnt/planar cell polarity (PCP)-pathway activity (Kim & Davidson 2011, Skoglund et al. 2008). Cytoskeletal changes are regulated by small GTPases, Rac and Rho, and Rho's downstream effector, Rho kinase, which is activated by Wnt/PCP signaling (see below) (Habas et al. 2003, Kim & Han 2005, Marlow et al. 2002) and is cell autonomously required for cell elongation (Marlow et al. 2002). Myosin phosphatase downstream of Wnt/PCP signaling limits protrusive activity during gastrulation (Weiser et al. 2009). Gravin (a protein kinase A interactor) is essential for the initiation of the intercalation behavior (Weiser et al. 2007). In addition to its role in cell motility, actomyosin contractility stiffens the axis through cortical tension (Kwan & Kirschner 2005; Zhou et al. 2009, 2010). Here, cortical actin polymerization is stimulated by the release of Rho-GEF-H1 from depolymerized microtubules. Local release of Rho-GEF-H1 was proposed to control motility (Kwan & Kirschner 2005). This function was observed in cultured HeLa cells where local microtubule depolymerization releases Rho-GEF-H1 to activate RhoA at the cell's leading edge (Nalbant et al. 2009). It will be important to understand how both the internal (cyclic actomyosin contraction, protrusion formation) and the external (supracellular actin cables and tension, ECM-mediated movement and tension) forces as well as the signals (Wnt/PCP signaling, among others) are integrated to move cells.

Wnt/PCP:
Wnt/planar cell polarity

Directed Migration

Recent work in cell culture offers a detailed mechanistic model of migration over 2D substrata (Gardel et al. 2010). In this model, the leading lamellipodium expands in cycles

FGF: fibroblast
growth factor

as branched and linear actin are polymerized. Behind the lamellipodium, in the lamella, actin filaments are compressed by myosin II and swept rearward. There, adhesive contacts are strengthened by myosin-dependent tension. The extent of coupling of actin to adhesive complexes determines the force providing forward movement (Mason & Martin 2011). Cells in 3D culture are less spread, but similar to cells in vivo, they have several modes of migration available to them (Friedl & Wolf 2009, Mogilner & Keren 2009). Examples of directed migration during gastrulation include migration of internalized nonaxial mesoderm away from the blastopore in fish and chick gastrulae (**Figures 2** and **3**) (Schoenwolf et al. 1992, Warga & Kimmel 1990), anterior migration of prechordal mesoderm in fish and frog (**Figure 2b**) (Heisenberg et al. 2000, Keller et al. 2003), dorsal convergence of the lateral mesoderm in fish (Jessen et al. 2002, Sepich et al. 2005, Trinkaus et al. 1992), and extension of the mesodermal mantle in *Xenopus* (Davidson et al. 2002). Migration of lateral mesoderm in zebrafish involves cycles of preferential dorsally oriented protrusion and attachment, followed by cell body movement (von der Hardt et al. 2007). An interesting example of cell migration during gastrulation is the random walk of endodermal cells in zebrafish gastrulae (**Figure 2**) (Pezeron et al. 2008). It will be important to understand to what extent cyclic contraction of the actomyosin network and actin polymerization as a driving force of protrusion formation apply to gastrulation. Also important is identification of the molecular component that serves as a "clutch" in these various cell migrations during gastrulation.

MOLECULAR CUES GUIDING POLARIZED GASTRULATION CELL BEHAVIORS

The hallmark of gastrulation movements is their polarization. Most cell intercalations, cell shape changes, and cell migrations are anisotropic, resulting in polarized tissue transformations such as internalization, conver-

gence, and/or extension. Key questions regard the molecular nature of the cues that polarize gastrulation movements and how these directional cues direct the actomyosin and microtubule networks that drive cell shape changes and movements. In the following section, we focus on the recently delineated mechanisms that guide gastrulation movements, including the role of cell-cell or cell-matrix adhesion, Wnt/PCP-dependent planar and radial intercalations, and the role of the fibroblast growth factor (FGF) family members in chemotaxis and chemokinesis during avian gastrulation.

Cell-Cell Adhesion

Intercellular adhesion has roles in germ-layer separation in frogs and fish, radial intercalation, EMT, and dorsal migration of mesoderm during zebrafish gastrulation. Our focus here is how differential adhesion can instruct directional gastrulation movements. The pioneering work of Townes & Holtfreter (1955) established that embryonic cells, if separated from each other, could both reaggregate and subsequently sort into previously specified germ layers. Steinberg (2007) proposed that these abilities reflected quantitative differences in surface adhesion, a concept known as the differential adhesion hypothesis. A complementary idea is the differential surface contraction hypothesis, in which a cell's stiffness or ability to contract its cortex influences cell sorting (Krens & Heisenberg 2011). Differences in the relative adhesiveness and stiffness of the germ layers in zebrafish gastrula cells allow these hypotheses to be compared. Ectodermal progenitors in zebrafish display lower surface adhesion than do endodermal cells, which, in turn, display lower adhesion than do mesodermal progenitors. However, the germ layers are ordered differently with respect to surface contractility or stiffness: Ectoderm progenitors are stiffer than mesodermal ones, which are stiffer than endoderm cells (Krieg et al. 2008). Consistent with the differential surface contraction hypothesis, when intermixed, ectodermal cells sort to the interior of the

mesoderm or the endoderm. However, when differences in stiffness are abolished by inhibiting actinomyosin contractility, ectoderm cells sort to the outside of the mesoderm, as predicted by the differential adhesion hypothesis (Krieg et al. 2008). These results reflect our current understanding that both adhesion and stiffness contribute to cell-sorting behavior.

In zebrafish, reduction of E-cadherin adhesion by hypomorphic mutations or by injection of antisense morpholino oligonucleotides does not block germ-layer formation, but it does decrease successful radial cell intercalation, attachment to the superficial enveloping layer, and, consequently, the process of epiboly (Babb & Marrs 2004, Kane et al. 2005, Shimizu et al. 2005, Winklbauer 2009). During epiboly, deeper blastomeres intercalate between more superficial cells to reach a position against the enveloping layer (**Figure 1***k*). In embryos with reduced levels of E-cadherin, cells still intercalate superficially, but they frequently return to the deeper layer, impairing both thinning and spreading of the blastoderm (Kane et al. 2005, Montero et al. 2005). On the basis of transcript levels, Kane et al. (2005) suggested that higher levels of E-cadherin in more superficial ectoderm layers determined directionality of intercalation. Antibody labeling shows equivalent E-cadherin levels in deeper and more superficial layers, leaving open whether a differential level of E-cadherin is instructive for radial intercalation (Montero et al. 2005). Electron microscopy studies in E-cadherin-depleted embryos reveal striking gaps between the enveloping layer and superficial ectoderm, supporting the idea that reduced adhesion between the enveloping layer and superficial ectoderm contributes to the radial intercalation defect (Shimizu et al. 2005). Further, reduced intercalation and rounded cell shape were found within the anterior dorsal mesoderm (Montero et al. 2005). E-cadherin depletion also slows migration of axial and lateral mesoderm on the ectoderm, and consequently impairs C&E (Montero et al. 2005). Several studies underscore the significance of the precise and dynamic regulation of E-cadherin expression and activity for normal gas-

trulation movements, as found for movements of other cell types, such as primordial germ cells (Blaser et al. 2005). Increased expression of E-cadherin, due to reduced prostaglandin levels, impairs epiboly in zebrafish embryos (Speirs et al. 2010). Moreover, gain and loss of function of Gα12/13, a heterotrimeric G protein that binds to E-cadherin and inhibits its activity without altered membrane distribution, also impairs epiboly (Lin et al. 2009).

Cell adhesion was also proposed to have an instructive role in guiding dorsal convergence movements during zebrafish gastrulation (von der Hardt et al. 2007). Here, gradients of cadherin-dependent cell adhesion, increasing from ventral to dorsal, are established by the reverse bone morphogenetic protein (BMP) activity gradient that also instructs cell fates during vertebrate gastrulation (De Robertis & Kuroda 2004, Langdon & Mullins 2011). When a local BMP gradient was generated ectopically by implanting BMP-loaded beads at early gastrulation, cells migrated away from high BMP levels. In zones of high BMP activity, cells touched each other transiently and did not migrate, whereas, in zones of low BMP, cells retained contact and moved toward each other. In support of the notion that these movements are dependent on cadherin, which requires extracellular Ca^{2+} to form adhesive contacts, cells migrated away from beads loaded with Ca^{2+} chelators. Presumably by reducing local Ca^{2+}, cadherin function was inhibited locally, establishing a gradient of high cadherin activity away from the bead. In other studies, reduction of E-cadherin expression left cells with unstable cell-cell contacts and significant defects in effective directed migration (Arboleda-Estudillo et al. 2010). It is not clear which calcium-dependent adhesion molecules are negatively regulated by BMP during zebrafish gastrulation. BMP and N-cadherin compound heterozygotes exhibit worse convergence than either single mutant, without additional changes in cell fate, suggesting N-cadherin plays a role in migration (von der Hardt et al. 2007). Accordingly, N-cadherin mutants exhibit mesoderm migration defects (Warga & Kane 2007). However,

BMP: bone morphogenetic protein

FN: fibronectin

studies using atomic force microscopy have so far demonstrated only E-cadherin and fibronectin (FN) adhesion in mesodermal precursors (Krieg et al. 2008, Puech et al. 2005). In other vertebrates (chicken), N-cadherin may serve as an essential adhesive molecule in gastrulation, as it is required for mesodermal cells to respond to several directional signals (Yang et al. 2008).

Cell-Matrix Adhesion

The ECM is the assortment of secreted glycoproteins that surround cells and tissues. ECM can provide a scaffold for migration or transmission of force, and it can bind and influence dispersal of directional cues. Movement of meshworks of ECM beneath cells likely provides a motile substratum that displaces cells in early chick primitive-streak formation and later in extension of the axis (Benazeraf et al. 2010, Zamir et al. 2008). FN is found assembled on surfaces used by mesoderm migration during gastrulation (on the blastocoel roof in amphibians and at the basal surface of the ectoderm in chicks). In amphibians, adhesion to FN supports mesoderm spreading on the blastocoel roof and its anteriorward migration (Boucaut et al. 1996; Davidson et al. 2004, 2006; Winklbauer 2009). Disruptions of FN expression cause defects in heart, notochord, and somite patterning in mice and zebrafish (Schwarzbauer & DeSimone 2011). Interestingly, assembly of FN into fibrils is responsive to cell adhesion and tension (Dzamba et al. 2009, Winklbauer 1998).

Studies in zebrafish reveal new mechanisms through which ECM can regulate polarized tissue morphogenesis by mediating a random walk of endodermal precursors (Nair & Schilling 2008). After internalization, endodermal cells, unlike mesodermal cells, do not undergo directed migration away from the blastopore/margin, but rather they engage in a randomly oriented and nonpersistent migration (**Figure 2**). This random migration disperses endodermal cells in the space between the yolk cell and the nascent mesoderm,

resulting in animal/anterior expansion of the endoderm (Pezeron et al. 2008). The molecular mechanism guiding the endoderm involves cell-matrix adhesion mediated by integrin and FN and a chemokine/G protein–coupled receptor pair. FN and integrin are first expressed at early gastrulation in small patches on the surfaces of the germ layers and the yolk cell, and they become continuous layers at later gastrulation (Latimer & Jessen 2010). RGD peptides block integrin/FN adhesion and disrupt the migration of endodermal cells in zebrafish gastrulae, causing the endoderm to migrate too far anteriorly (Nair & Schilling 2008). Interestingly, depletion of the chemokines Cxcl12a and Cxc112b (Sdf1a and Sdf1b) expressed on mesodermal cells, or their receptor Cxcr4a expressed on endoderm cells, yields a similar endodermal migration defect (Mizoguchi et al. 2008, Nair & Schilling 2008). One possibility is that Cxcl12-secreting mesodermal cells attract the endoderm, which limits their migration, a suggestion supported by the ability of cells overexpressing Cxcl12 to cluster endodermal cells (Mizoguchi et al. 2008). An alternative view is that chemokine signaling regulates integrin-FN adhesion between the endoderm and mesoderm. This idea is supported by the finding that Cxcr4a-depleted endoderm is less adhesive to FN-coated surfaces and this defect is suppressed by integrin overexpression (Nair & Schilling 2008). Both perturbations (depletion of FN or chemokine signaling) result in excessive anterior migration of the endoderm and a vacant region near the margin/blastopore. Whether by modulating chemoattraction or by adhesion to the FN/mesoderm, chemokine signaling limits the anterior spread of the endoderm via its random walk (Nair & Schilling 2008).

Planar Polarity

Planar polarity is revealed by coordinated cellular orientation over a tissue. For example, hairs coordinate growth direction over the plane of the skin in mammals and bristles over the *Drosophila* wing to point distally. Such planar

polarization can also bias and coordinate gastrulation cell behaviors. One of the evolutionarily conserved molecular mechanisms underlying planar polarity, Wnt/PCP, was first described in *Drosophila* (Gubb & Garcia-Bellido 1982). Complex interactions of the components of the PCP-signaling network between the cells, as well as intracellularly via feedback loops, result in asymmetric distribution of PCP components on cell membranes (Strutt & Strutt 2009). The core molecular PCP components in *Drosophila* include the Frizzled receptor, which recruits the cytosolic effector, Dishevelled (Dvl), to the distal side of the cell. On the proximal side, antagonistic components accumulate, i.e., the four-pass transmembrane protein Strabismus/VanGogh, which interacts with another cytoplasmic component, Prickle (Pk) (Goodrich & Strutt 2011). The Flamingo adhesion GPCR is necessary for both complexes but is not asymmetrically localized (Usui et al. 1999). In vertebrates, this so-called Wnt/PCP-signaling network features additional components, including Wnt ligands, and several membrane components (Ror2, Glypican) (Gray et al. 2011). In addition, Wnt/PCP signaling is needed during *Xenopus* and zebrafish gastrulation for efficient C&E movements of mesenchymal cells (Heisenberg et al. 2000, Jessen et al. 2002, Sokol 1996, Tada & Smith 2000, Topczewski et al. 2001, Wallingford & Harland 2001, Wallingford et al. 2000).

When Wnt/PCP signaling is compromised by loss or gain of function of Wnt/PCP components, the polarized ML and radial intercalation behaviors that drive C&E movements are perturbed, such that the normal bias of intercalating cells to separate preferentially anterior and posterior neighbors is reduced or lost (Davidson et al. 2002, Yin et al. 2008). Among the morphology defects, cells are less elongated and less mediolaterally aligned (Jessen et al. 2002, Topczewski et al. 2001, Ulrich et al. 2003). Protrusions are misaligned and less stable (Goto et al. 2005, Ulrich et al. 2003, Wallingford et al. 2000). Within the cell, Wnt/PCP signaling is needed for asymmetric position of centrosomes during C&E (Borovina et al. 2010, Sepich et al.

2011) as well as polarized accumulation of Pk and Dvl (**Figure 3b**) (Ciruna et al. 2006, Yin et al. 2008).

How does Wnt/PCP signaling polarize cell behavior? Wnt/PCP signaling alters E-cadherin adhesion, and likely distribution, through endocytosis (Ulrich & Heisenberg 2008). Moreover, it controls lamelli formation and myosin contractility, essential aspects of cell motility, through Rac and RhoA (Habas et al. 2001, 2003) as well as actomyosin contractility through Rho kinase (Marlow et al. 2002) and myosin phosphatase (Weiser et al. 2009). Cell elongation is effected by Rho kinase (Marlow et al. 2002), the PCP effector Fritz, and the cytoskeletal molecule Septin (Kim et al. 2010). The biased position of the microtubule-organizing center could afford asymmetric microtubule-based intracellular transport of Wnt/PCP components, as demonstrated in *Drosophila* (Shimada et al. 2006). Such asymmetric transport could account for the asymmetric localization of Pk and Dvl. It may also explain the localization of the cell adhesion molecules as shown in *Xenopus* or of other molecules such as the Eph receptors that could influence cell movements (Kida et al. 2007).

Chemotaxis

Chemotaxis is movement of cells in a direction relative to a chemical gradient in the environment without change in the instantaneous speed of the cell. FGFs have several roles in gastrulation, including specification of cell fate and differentiation and regulation of E-cadherin levels (Ciruna & Rossant 2001). Here we discuss how FGFs organize mesendodermal movements in the chick gastrula. As described above, after ingression through the primitive streak, mesendodermal cells migrate in a perpendicular direction away from the streak (**Figure 2**). These lateral-directed movements appear to be driven by repulsion to FGF8 expressed in the primitive streak. Cells that leave the anterior primitive streak migrate laterally then turn anteriorly and migrate toward the

Dvl: Dishevelled

Pk: Prickle

notochord, which forms anterior to the streak and expresses FGF4 (**Figure 3**). Chemorepulsion and chemoattraction to these two different FGFs were shown by implanting FGF-loaded beads and observing that mesendodermal cells move away from FGF8 and toward FGF4 (Yang et al. 2002). Other molecules, such as Wnt3a, are expressed in the primitive streak and may exert chemotactic effects on specific cell migrations similar to the action of FGF8. In a chemotaxis assay, Wnt3a repelled cardiac progenitors independently of FGF signaling and without disturbing the migration of other mesodermal cells (Yue et al. 2008). Other embryonic regions may also supply directional cues to guide cell migration. The region caudal to the primitive streak can also attract mesendodermal cells, suggesting that a natural chemoattractant, possibly VEGF, resides in that area (Yang et al. 2002). Hence, local gradients of chemotactic molecules may instruct migration of subpopulations of embryonic cells.

Chemokinesis

At first glance, chemokinesis, increased random motility in response to a chemical cue, seems an unlikely mechanism for directional movement. In the following example, a gradient of chemokinesis paired with what is essentially a boundary, an opposing cell-density gradient, is proposed as the mechanism that yields directional elongation during late chick gastrulation. During trunk and tail formation, the posterior axis of the chick embryo elongates caudally in an FGF-dependent manner. Laser ablation through both the ectoderm and mesoderm in the posterior axis reveals that the region containing posterior PSM is most important for robust axial elongation, whereas ablations lateral to this region, or of the posterior axial tissue, are much less detrimental. Cells in the posterior PSM are displaced posteriorly, with greatest displacement of the most posterior PSM, suggesting a linked and additive component to axis elongation (Benazeraf et al. 2010). The posterior motion could be separated into random active cell motility and passive posterior dis-

placement that exactly matches the displacement of the underlying ECM (i.e., the ECM moves posteriorly). Analyzed in this way, active cell motility in the PSM was revealed to be randomly, rather than posteriorly, oriented. Cell motion was graded from low anterior to high posterior motility and was dependent on posteriorly increasing FGF levels. In a computational model, a gradient of random cell motility, if paired with an impervious boundary, could yield movement away from the boundary (Benazeraf et al. 2010). Here, the PSM is confined by high cell density, medially by the neural tube and laterally by the lateral plate mesoderm. The third boundary is formed by cell density within the PSM. Anterior PSM regions have high cell density, which decreases posteriorly. Consistent with this model, overexpression of FGF8 increases cell motility everywhere and flattens the density gradient. In this chemokinesis model, boundaries limit the movement direction of the motile PSM, forcing it posteriorly. A similar model for adhesion-mediated cell sorting, using a boundary composed of increasing cell density, has been proposed; simulations show similar motion away from the boundary (Kafer et al. 2006). Hence, the combination of impermeable boundaries and opposing gradients of cell density and cell speed can direct tissue elongation.

COORDINATION OF GASTRULATION MOVEMENTS WITH BODY AXES

The animal body plan established during gastrulation displays AP and DV asymmetries, indicating that cues guiding gastrulation movements must be precisely coordinated with the nascent embryonic polarity. We describe several examples of gastrulation cell movements that are instructed by chemoattractive, chemokinetic, or adhesive gradients. How are the cues that instruct gastrulation cell behaviors coordinated with the embryonic axes? Although the full story remains to be revealed, we have started to understand some aspects of such global and local coordination.

CE in *Xenopus* mesoderm explants proceeds only if the explants contain mesoderm cells of significantly different AP identity; cultured mesoderm composed of two explants of similar AP level do not initiate CE (Ninomiya et al. 2004). Similarly, in *Drosophila* GBE, embryos lacking AP-patterning information, although able to form rosettes, are unable to organize asymmetrical F-actin structures and orient cell rearrangements (Blankenship et al. 2006). These observations imply that CE movements are regulated or coordinated with AP embryonic patterning. Signaling systems, such as Wnt/PCP, could afford a mechanism for coordination of AP embryonic and cellular polarity. Thus, they may coordinate embryonic patterning with morphogenesis during gastrulation (Gray et al. 2011, Yin et al. 2008). Current evidence indicates that during C&E, cells bias radial and ML intercalation relative to the AP (and ML) axis (**Figures 2** and **3**) (Davidson et al. 2002, Yin et al. 2008). Cell morphology (ML cell elongation, location of protrusions, centrosomes, and cilia) appears to be coordinated with AP polarity (**Figure 3b**) (Borovina et al. 2010, Sepich et al. 2011). Finally, components of Wnt/PCP signaling become asymmetrically distributed in zebrafish gastrulae: Pk accumulates at the anterior cell membranes (Ciruna et al. 2002, Yin et al. 2008), whereas Dvl is enriched at the posterior cell membranes (**Figure 3b**) (Yin et al. 2008). Key questions remain: How does the AP-polarity information regulate the Wnt/PCP pathway? How does the asymmetric distribution of Wnt/PCP components mediate polarization of motile cell behaviors?

Hox genes are required for acquisition of AP polarity in *Drosophila* and vertebrates (Mallo et al. 2010). On the basis of chick studies, it has been proposed that Hox genes regulate the timing of mesoderm internalization (Iimura & Pourquie 2006). Although, work in *Xenopus* suggests that timed interactions of Hox genes with the SMO impart AP identity on the mesoderm (Durston et al. 2009). How this positional information is read, interpreted, and translated into cellular changes remain open issues.

Current data suggest that homeodomain Cdx transcription factors could contribute to the coordination of AP patterning with Wnt/PCP signaling and gastrulation. In the mouse, expression of the *Ptk7* gene, which encodes a protein phosphatase involved in PCP, is markedly reduced in Cdx1-Cdx2 double mutants, which exhibit truncated embryonic axis (Savory et al. 2011).

We have also gained some insight into the mechanisms via which the embryonic pattern along the DV embryonic axis is coordinated with C&E movements. Vertebrate embryos establish a high ventral to low dorsal gradient of BMP activity that patterns cell fates during gastrulation (De Robertis & Kuroda 2004, Langdon & Mullins 2011). In zebrafish, C&E cell movements are also patterned along the DV gastrula axis. Experimental evidence indicates that the BMP activity gradient coordinates both cell movements and fate specification. Accordingly, C&E movements are inhibited in the ventral gastrula region at the highest BMP activity levels. In the lateral regions with decreased BMP activity levels, C&E movements of increased speed are driven largely by dorsally directed cell migration. Near the dorsal midline, where BMP levels are lowest, polarized planar and radial cell intercalation produce strong extension and modest convergence. Because BMP activity thresholds that regulate C&E movements are different from those regulating cell fates, BMP may regulate cell movements in parallel to its instructive role in cell-fate decisions (Myers et al. 2002a,b). The BMP gradient may also regulate C&E movements by inhibiting expression of Wnt/PCP pathway components to dorsolateral gastrula regions, thus limiting the ML cell polarization that is required for polarized directed migration and cell intercalations (Myers et al. 2002a).

As the tissues and organ rudiments form during gastrulation, they can provide cues instructing continued gastrulation movements. For example, during avian gastrulation, FGF4 expressed in the primitive streak is thought to serve as a chemorepellant to guide movement

of mesodermal cells away from the streak (**Figure 2**) (Yang et al. 2002). Later during gastrulation, FGF8 emanating from the regressing primitive streak was proposed to serve as a chemoattractant to guide convergence movements (**Figure 3**). In the frog gastrulae, notochord forms a lateral boundary that seems to be essential for CE of the paraxial mesoderm. Protrusions that touch the boundary become quiescent, leaving the cell with a medially oriented protrusion. Eventually, all cells are monopolar and intercalate medially (Keller et al. 2000). The notochord/somite boundary provides a special cue orienting microtubule growth (Shindo et al. 2008).

OUTLOOK

Recent decades have witnessed remarkable progress in our understanding of gastrulation in invertebrate and vertebrate animals. Advances in molecular genetic, genomic, and imaging methods afford studying gastrulation movements at the levels of whole embryo and individual cells as well as at cytoskeletal dynamics in vivo. Further progress and integration of information across the levels of biological complexity will lead, in the coming years, to a comprehensive understanding of gastrulation movements, from the mechanics of motility of individual cells to collective cell migrations and how they are coordinated with embryonic polarity and ongoing cell-fate specification. Studies of gastrulation inform our understanding of birth defects, such as spina bifida or LR asymmetry abnormalities. Moreover, striking parallels exist between the molecular mechanisms that regulate tumor growth and metastasis and those that govern gastrulation, especially the processes of EMT, collective cell migration, chemotaxis, and chemokinesis, thus further motivating continued interest in this fascinating and fundamental process of animal embryogenesis.

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