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Conserved Patterns of Cell Movements during Vertebrate Gastrulation

Review

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Vertebrate embryogenesis entails an exquisitely coordinated combination of cell proliferation, fate specification and movement. After induction of the germ layers, the blastula is transformed by gastrulation movements into a multilayered embryo with head, trunk and tail rudiments. Gastrulation is heralded by formation of a blastopore, an opening in the blastula. The axial side of the blastopore is marked by the organizer, a signaling center that patterns the germ layers and regulates gastrulation movements. During internalization, endoderm and mesoderm cells move via the blastopore beneath the ectoderm. Epiboly movements expand and thin the nascent germ layers. Convergence movements narrow the germ layers from lateral to medial while extension movements elongate them from head to tail. Despite different morphology, parallels emerge with respect to the cellular and genetic mechanisms of gastrulation in different vertebrate groups. Patterns of gastrulation cell movements relative to the blastopore and the organizer are similar from fish to mammals, and conserved molecular pathways mediate gastrulation movements.

Introduction

Gastrulation is a fundamental process of animal embryogenesis that shapes the internal and external features of developing animals. Introduced by Haeckel, the term gastrulation is derived from the Greek word '*gaster*', meaning stomach or gut. It describes a set of morphogenetic processes that transform the rather unstructured early embryo into a gastrula with three germ layers — endoderm, mesoderm and ectoderm. Vertebrate embryos display a conserved body plan with an elongated rostrocaudal axis. Along the dorsoventral axis, the nervous system takes the most dorsal position, above the notochord flanked by bilateral somites, and the most ventral alimentary structures, including the gut (Figures 1 and 2).

Vertebrate gastrulation involves four evolutionarily conserved morphogenetic movements: internalization, epiboly, convergence and extension. Internalization brings cells of the prospective mesoderm and endoderm beneath the future ectoderm via the blastopore, an opening in the blastula, known as blastoderm margin in fish, and primitive streak in amniotes (Box 1). Epiboly movements spread and thin germ layers during gastrulation, while concurrent convergence and extension movements narrow them mediolaterally, and elongate the embryo from head to tail. Gastrulation is preceded and accompanied by inductive processes that specify and pattern the germ layers. These inductive processes are in large part controlled by the Spemann-Mangold organizer (SMO, hereafter referred to as the organizer), the key embryonic signaling center, which is located in the dorsal or axial aspect of the blastopore (Box 1) [1]. Work from the past two decades has revealed a great deal of conservation in the mechanisms of cell fate specification [2–4]. However, it is less clear whether this conservation also extends to the morphogenetic processes of gastrulation, in particular when the highly distinct architecture of gastrulae from different vertebrate groups is considered

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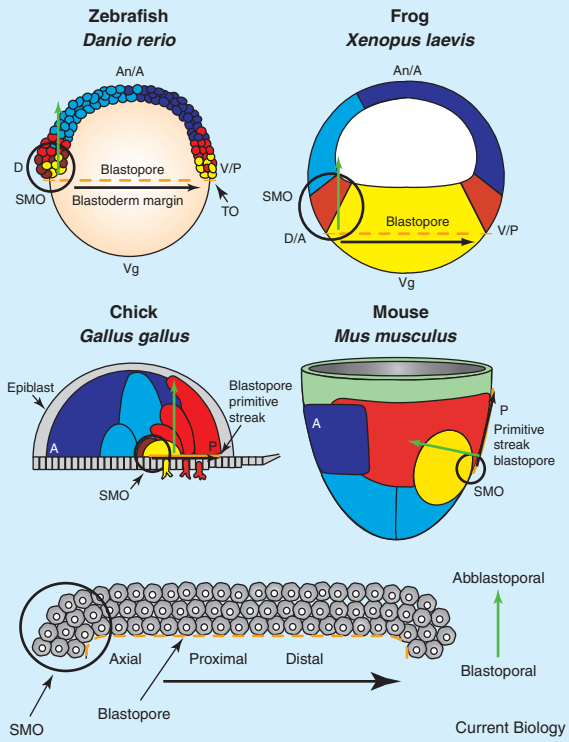
Diverse Pre-Gastrulation Histories of Vertebrate Embryos

The fertilized zygote contains all the instructions for its embryonic development in the zygotic genome and as maternally derived cytoplasmic substances. The relative contributions of zygotic and maternal regulation vary among vertebrates and this is reflected particularly in the speed and pattern of the early cleavages, and consequently in the morphology of the blastula. Fish and amphibian embryos develop externally and the fast rate of their early development ensures swift formation of independent larvae. These embryos rely on rich energy stores in the form of a yolk and on maternal determinants that mediate development until the midblastula stage, when the zygotic genome becomes transcriptionally active and takes control [5,6]. The yolk is generally concentrated vegetally and is either distributed between the blastomeres via complete cleavages, as in frog embryos, or deposited in a separate yolk cell, as seen in incompletely cleaving fish embryos (Figure 1A,B). Consequently, the fish blastula is a mound of blastomeres at the animal region on top of a large syncytial yolk cell (Figure 1E). By contrast, the frog blastula consists of smaller blastomeres at the animal hemisphere surrounding a blastocoel cavity and larger blastomeres in the vegetal region (Figure 1F).

The chick zygote, which is also endowed with large amounts of yolk, divides incompletely to partition the cytoplasmic island into centrally located small cells, and larger, yolky and open cells at the periphery (Figure 1C). Further divisions generate a superficial single-cell thick epithelium, known as the epiblast, which will give rise to the embryo proper, and which consists of a central *area pellucida* surrounded by the peripheral *area opaca*. Underlying the entire superficial layer is the primitive endodermal layer, known as the primary hypoblast, while the secondary hypoblast marks the prospective posterior of the blastoderm (Figure 1G) [7]. Although these tissues give rise only to extraembryonic structures, they are thought to play an active role in embryo patterning [8].

Box 1. Spatial coordinates in vertebrate gastrulae using blastopore and organizer as reference points.



According to the traditional view, the blastopore of fish and frog gastrulae is aligned with the dorsoventral axis, with the organizer (SMO) marking its dorsal end. Recently it was suggested that in the frog the blastopore marks the anteroposterior axis; the dorsoventral axis is perpendicular to the blastopore, thus corresponding to the animal-vegetal axis [32,33]. In the chick and the mouse, the blastopore is thought to be aligned with the anteroposterior embryonic axis and the position of the dorsoventral axis is not clear. Due to the distinct architecture of different vertebrate gastrulae and the dramatic cell movements during gastrulation it is not easy to assign the dorsoventral and anteroposterior gastrula axes, which has led some investigators to conclude that "all fate maps are wrong" [18]. To facilitate comparison, I suggest to use the blastopore and the organizer as conserved reference points for describing spatial coordinates in vertebrate gastrulae. In this view, the organizer marks the axial blastopore region (indicated by a circle), while the proximodistal axis (black arrow) indicates blastopore regions located further away from the organizer. In the most distal blastopore region of zebrafish gastrula, resides the tail organizer, a tissue which can invoke formation of partial tail upon transplantation [144]. The blastoporal–abblastoporal axis (green arrow), which is perpendicular to the blastopore, designates the distance from the blastopore. This simple terminology allows for a description of the position of a cell in any vertebrate gastrula, while abstracting from its future fate, and thus avoiding complications associated with the designation of dorsoventral and anteroposterior gastrula axes. However, this terminology can also be used to describe the positions of tissues on fate maps.
A, anterior; An, Animal; D, Dorsal; P, posterior; V, ventral; Vg, vegetal; SMO, Spemann-Mangold organizer; TO, tail organizer. Color code: red, mesoderm; yellow, endoderm; light blue, nonneural ectoderm; dark blue, neural ectoderm.

As mammalian embryos develop within the uterus, their development is rather slow and the amount of maternal energy supply in the completely cleaving blastomeres is minimal (Figure 1D). Likewise, the regulation of development is heavily shifted towards the zygotic genome, which becomes active already at the 2-cell stage [9]. The embryo communicates with the mother's uterus via extraembryonic structures, formation of which starts during the early cleavages. The mouse, human and chick blastulae are similar in that future embryonic tissues form a single-cell thick epithelium. This epithelium is flat in chicks and humans, but shaped as a cup in mice. In the murine embryo, extraembryonic tissues surround the cup on the outside (endoderm) and are gathered at its rim (ectoderm) (Figure 1H) [10].

Induction and Patterning of the Germ Layers

Cleavage is followed by induction of the three germ layers: ectoderm, which will give rise to the epidermis and neural tissues; mesoderm, which will form muscles, cardiovascular, urogenital and skeletal elements of the body; and endoderm, which will generate the digestive tube and its accessory organs. After the mesoderm and endodermal precursors move through the blastopore and become internalized, the germ layers are patterned to produce specific tissues and organs along the dorsoventral and rostrocaudal embryonic axes.

The underlying molecular and genetic mechanisms involve a highly conserved cascade of transcription factors and signaling pathways resulting in a similar relative distribution of germ layers in different vertebrate embryos at the onset of gastrulation (Figure 1I–L; reviewed in [2,3]). Induction of mesoderm and endoderm precursors in all vertebrates entails signaling by Nodal ligands of the TGFβ superfamily, although the molecular pathways that activate expression of nodal genes in the first place might be distinct [11]. The crucial early symmetry-breaking events engage microtubule driven asymmetric transport of dorsal determinants in frog and zebrafish [12,13]. They culminate in the enrichment and nuclear localization of β-catenin, a transcriptional effector of canonical Wnt signaling, in the prospective dorsal region of the blastula known as the Nieuwkoop center [14]. In turn, β-catenin instigates a set of genetic pathways that establish the dorsal gastrula or organizer [15–17]. This specialized region of the dorsal gastrula was initially discovered by Hans Spemann and Hilde Mangold, who observed in newts that transplantation of the dorsal blastopore lip to a ventral region of another gastrula resulted in the formation of complete ectopic embryonic axes [1].

Current evidence indicates that the organizer activity encompasses all three germ layers of the dorsal gastrula and employs evolutionarily conserved genetic pathways that pattern germ layers and regulate gastrulation movements (Figure 1I–L) [2,3,18]. Many genes that are activated by β-catenin in the Nieuwkoop center continue to be expressed in the organizer, consistent with the notion that the Nieuwkoop center not only induces but also contributes to the organizer [19].

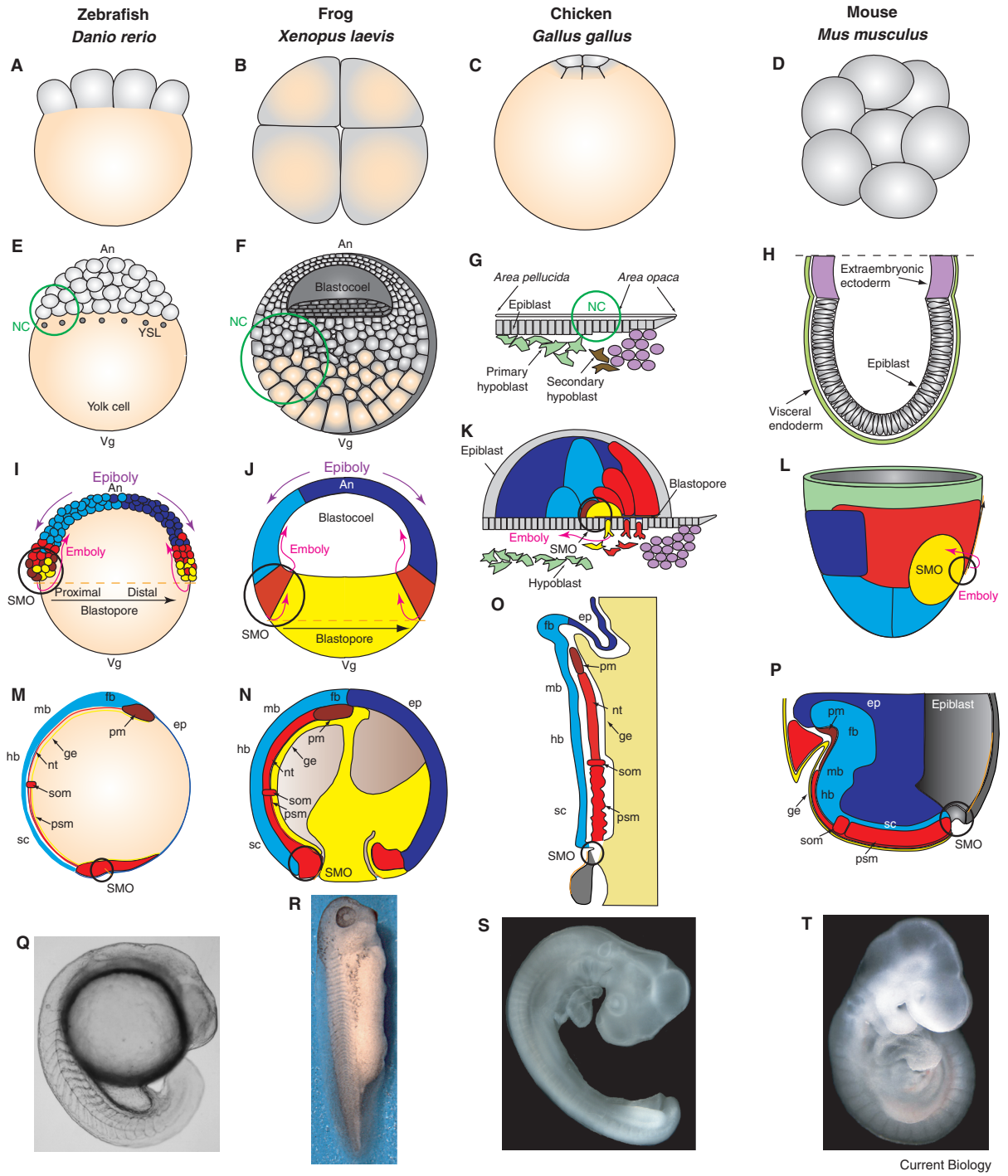


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Figure 1. Cleavage, blastula and gastrula stages of four vertebrate model organisms.

Developmental stages of zebrafish (A,E,I,M,Q), the frog *Xenopus laevis* (B,F,J,N,R), the chick (C,G,K,O,S) and the mouse (D,H,L,P,T). Cleavage, 8-cell stages (A–D). Note the incomplete cleavage in zebrafish (A) and chick (C) and the complete cleavage with differently sized blastomeres in *Xenopus* (B), and uniformly sized blastomeres in the mouse (D). Early blastula (E–H), late blastula–early gastrula (I–L), late gastrula (M–P) and pharyngula (Q–T). The position of the Nieuwkoop center (NC) and its equivalents is shown at cleavage stages and the position of the Spemann-Mangold organizer region (SMO) at early and late gastrula stages. Colors: light gray, cytoplasm; beige, yolk; dark gray, epiblast region of amniote embryos; red, mesoderm and its precursors; dark red, prechordal mesendoderm; yellow, definitive endoderm and its precursors; dark blue, epidermis; lighter blue, neuroectoderm; green, brown and violet, various extraembryonic tissues; orange, blastopore. Abbreviations: ep, epidermis; fb, forebrain; mb, midbrain; hb, hindbrain; sc, spinal cord; nt, notochord; pm, prechordal mesendoderm; som, somite; psm, presomitic mesoderm; ge, gut endoderm; an, animal; vg, vegetal.

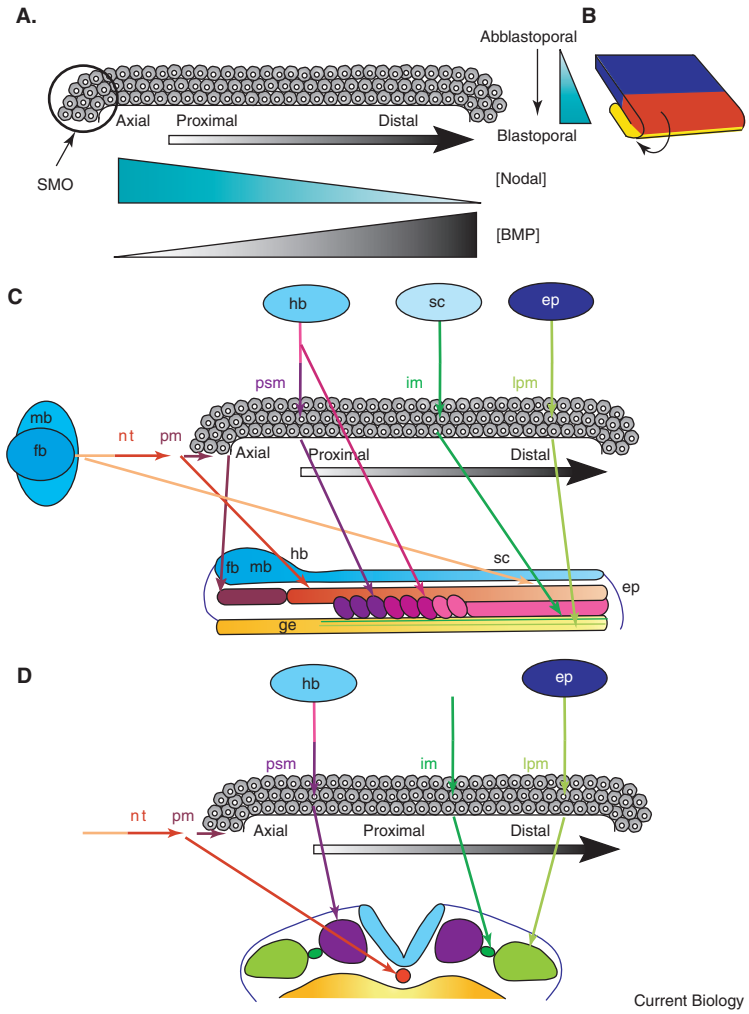

Figure 2. Anatomy of the vertebrate blastopore.
(A) Schematic of a blastopore (blastoderm margin in fish, primitive streak in amniotes). The Spemann-Mangold organizer (SMO), known as embryonic shield in fish, dorsal blastopore lip in frogs and node in amniotes, marks its axial region. The proximodistal axis of the blastopore designates regions of the blastopore relative to the organizer. A BMP activity gradient forms along the blastopore with its minimum at the axial (organizer) region and increasing towards the distal region. The Nodal gradient has an opposite polarity. The blastoporal–abblastoporal axis reflects the distance of a given cell layer from the blastopore edge. Nodal signaling might form a gradient along the blastoporal–abblastoporal axis, with the maximum at the blastopore edge. (B) Internalization movements through the blastopore illustrate that in most cases endodermal precursors become internalized before the mesodermal precursors. Color scheme as in Figure 1. (C) Relationship between the position and timing of internalization of mesodermal cells and their final rostrocaudal localization. A shaded arrow indicates the proximodistal axis of the blastopore. Colored arrows pointing towards the blastopore depict the movements of different types of mesoderm via this part of the blastopore. Oval shapes colored in blue depict positions of prospective ectodermal regions that neighbor different types of mesoderm. (D) Relationship between the position of internalization of mesodermal cells through the blastopore and their final localization along the dorsoventral embryonic axis, shown as a crossection through the trunk region. Color scheme: red, mesoderm; dark red, prechordal mesendoderm; lighter red, notochord (nt); violet and pink, somitic and presomitic mesoderm (psm); dark green, intermediate mesoderm (im); light green, lateral plate mesoderm (lpm); yellow, definitive endoderm and its precursors; blue, ectoderm; lighter blue, neuroectoderm. Abbreviations: fb, forebrain; mb, midbrain; hb, hindbrain; sc, spinal cord; nt, notochord; pm, prechordal mesendoderm; im, intermediate mesoderm; psm, presomitic mesoderm; ge, gut endoderm.

Transcriptional targets of β-catenin include *nodal* genes, or genes that promote expression of *nodal* genes, thus generating a dorsal to ventral gradient of Nodal signaling activity [20,21]. Probably, one of the most important roles of the organizer is to limit the ventralizing and posteriorizing activity of Bone morphogenetic proteins (BMPs) and canonical Wnt ligands during gastrulation [3]. Indeed, β-catenin directly promotes expression of secreted antagonists of BMPs and Wnts, as well as of transcription factors such as Bozozok in zebrafish or Siamois and Twin in *Xenopus* that repress expression of BMP and Wnt genes [22,23]. In zebrafish, β-catenin also promotes the expression of members of the Fibroblast Growth Factor (FGF) family as well as their negative regulators. Early FGF signaling on the dorsal side of the embryo contributes to embryonic patterning by restricting expression of BMP genes [24,25].

This genetic hierarchy within the organizer culminates in the establishment of gradients of BMP and probably also Wnt activity along the blastopore with their minima in the axial or organizer region of the blastopore. Intriguingly, the Nodal activity gradient has the opposite orientation with its maximum in the organizer region of the blastopore. Moreover, Nodal activity is highest in the cells at the blastopore and decreases in cells further away from the blastopore (Figure 2A). These gradients along the proximodistal and blastoporal–abblastoporal axes of the gastrula (Figure 2A) are thought to specify cell fates along the future dorsoventral and rostrocaudal axes [2,3,26]. In the zebrafish organizer, β-catenin was also shown to activate the Stat3 pathway, which mediates some gastrulation movements [27]. Hence, before the morphogenetic movements are initiated, a set of genetic pathways has been set in motion to induce and pattern germ layers and to mediate individual gastrulation movements.

Early Morphogenetic Movements

Gastrulation is preceded by morphogenetic movements that are either shared or unique to different

vertebrate embryos. For instance in murine embryos, extraembryonic anterior visceral endoderm (AVE), initially located at the base of the cup-shaped embryo, moves towards its rim near the junction with the extraembryonic ectoderm (Figure 1H). Upon reaching its destination, the AVE will find itself in close apposition to prospective anterior neural tissue and will play instructive roles in its patterning [10,28]. In chick embryos, so-called 'polonaise movements' bring future dorsal and anterior mesoderm from the lateral region of the epiblast towards its posterior edge where the blastopore ('primitive streak') will form and then towards its center as the primitive streak extends [8]. Similar movements are likely to occur in mammalian embryos as the prospective mesoderm initially distributed along the entire rim of the blastodermal cup becomes concentrated at the edge where the blastopore will start to form and extends towards the tip or center of the epiblast (Figure 1L) [29]. It is intriguing that many of these group-specific movements seem to work towards establishing a similar arrangement of germ layers and tissue progenitors at the onset of gastrulation movements (Figure 1I–L).

Conserved Arrangements of Tissue Progenitors at Early Gastrulation

At the late blastula stage, the position of prospective organs and tissues can be predicted by lineage tracing. Such experiments allow the construction of fate maps, which show the fate of each blastomere in terms of its position and the type of tissue it gives rise to [30]. Thus fate maps are two-dimensional representations of the future three-dimensional embryonic body. However, these fate maps are often difficult to compare between different vertebrates, largely due to differences in the naming traditions for embryonic axes in architecturally distinct vertebrate embryos (Box 1) [31]. Even in the case of frog and fish gastrulae where the animal–vegetal and dorsoventral axes are traditionally defined as perpendicular and parallel to the blastopore, respectively (Figure 1I–J), it is debatable whether the anteroposterior axis is parallel to the animal–vegetal axis or aligned with the blastopore [32, 33]. In mice the blastopore is thought to demarcate the anteroposterior embryonic axis, whereas the position of the dorsoventral axis is less clear [34].

I will argue that the relative position of tissues on fate maps, as well as the later patterns of gastrulation movements, can be readily compared between different vertebrate gastrulae when using the blastopore and the organizer as the main reference points (Box 1). These similarities are most striking when early fish and frog gastrulae are compared to amniote embryos in which the primitive streak (blastopore) is extending (Figure 1I–L), and in particular when it is fully extended and reaches the center of the epiblast (Figures 2 and 4). I will refer to tissues positioned along the blastopore as proximal and distal with respect to the organizer, which takes the axial blastopore position (Figure 2A). To describe positions within the blastoderm (the epiblast of amniote embryos) I will refer to the distance from the blastopore as defining the blastoporal–abblastoporal axis (Figure 2A).

In this somewhat simplified schematic of the early vertebrate gastrula, the positions of prospective germ layers and tissues residing at specific rostrocaudal and dorsoventral positions at the pharyngula stage are remarkably similar. Endoderm progenitors reside near the blastopore, mesodermal progenitors reside further away, whereas ectodermal precursors are positioned at the greatest distance from the blastopore (Figures 1I–L and 2B). Future midline tissues reside in the axial region of the blastopore, with prospective prechordal mesendoderm being found in the cell layers closest to the blastopore and chordamesoderm being further away (Figure 2C,D) [20]. Notably, the axial blastopore region gives rise to both anterior and posterior midline tissues in all vertebrates (Figure 2C) [1,35,36]. Presomitic mesoderm occupies a proximal position, whereas intermediate and lateral plate mesoderm reside at progressively more distal blastopore regions. Prospective posterior mesendodermal tissues with the notable exception of posterior axial mesoderm are located at the most distal positions of the blastopore (Figure 2C,D). The similarity between vertebrate fate maps at this stage of gastrulation is also reflected in the similar expression patterns of genes that determine cell fates. For example, in all vertebrates the prechordal mesendoderm marker *goosecoid* (*gsc*) and the chordamesoderm marker *not* are expressed in the organizer region [37,38], while *Brachyury* homologs are expressed throughout the blastopore [39–41].

The fate map of the neuroectoderm is complex, yet the forebrain takes the axial position, midbrain precursors are found in proximal positions, and hindbrain and spinal cord precursors reside in progressively distal locations with respect to the blastopore (Figures 1I–L and 2). Again, the exception is the prospective midline neural tissue, which — whether destined for tail floorplate or head floorplate and hypothalamus — is derived from the vicinity of the organizer (Figure 2C). In this view both future dorsoventral and rostrocaudal axes of the embryo are aligned with the proximodistal axis of the blastopore. In addition further rostrocaudal diversification of tissues is seen along the blastoporal–abblastoporal axis (Figure 2A,C).

Formation of the Germ Layers via Stereotyped Gastrulation Movements

Despite the morphologies of early vertebrate embryos being quite diverse (Figure 1), there are four predominant gastrulation movements shared by all vertebrates: epiboly, mesendoderm internalization or emboly, convergence and extension. These movements can be defined most generally in terms of the morphogenetic changes they produce (Figure 3A). Epiboly leads to an expansion of tissue, often accompanied by its thinning. Emboly or internalization entails movements of mesodermal and endodermal precursors from the blastula surface beneath the ectodermal layer. Convergence movements narrow embryonic tissues mediolaterally, whereas extension movements elongate them from head to tail. These different morphogenetic movements can be achieved by a variety of cellular activities (Figure 3B–H).

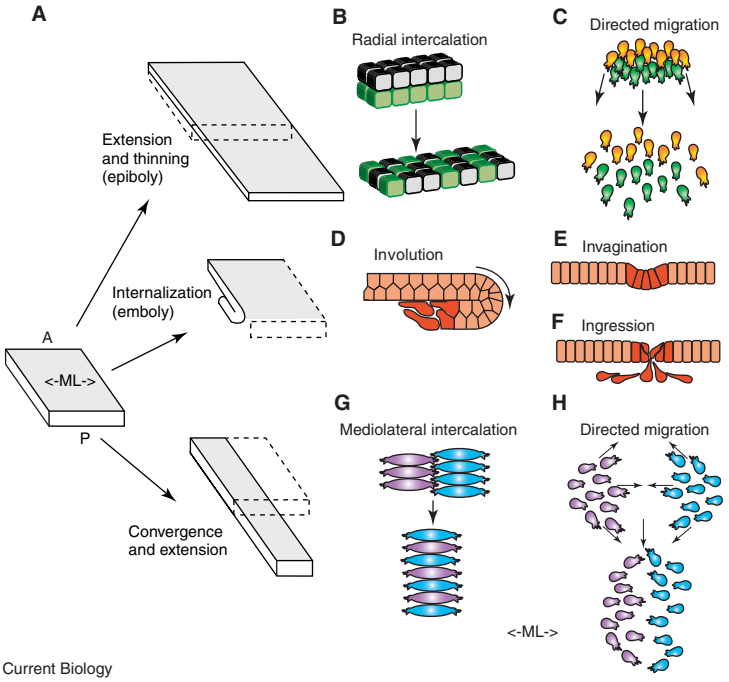

Figure 3. Gastrulation movements and cell behaviors.

(A) Gastrulation movements can be classified based on the morphogenetic changes they produce. Epiboly leads to expansion of tissue, often accompanied by thinning. Emboly or internalization entails movement of mesodermal and endodermal precursors from the blastula surface beneath the prospective ectodermal layer. Convergence narrows tissues mediolaterally, whereas extension elongates them from head to tail. (B–H) Different morphogenetic endpoints of the gastrulation movements can be achieved by a variety of cellular activities. (B) Radial cell intercalation entails intercalation movements of cells between superficial and deeper layers, resulting in thinning and surface expansion of tissue. (C) Directed migration of tightly packed cells leads to their spreading and thus surface expansion of tissue. (D–F) Cell behaviors driving internalization. (D) Involution entails rolling of a cell sheet over an edge and frequently over itself. Upon involution, cells at the leading edge can undergo epithelial mesenchymal transition (EMT) and move on the overlying sheet. (E) Invagination, or formation of a groove in a sheet of tissue occurs via cell shape changes such as apical constriction. (F) Invagination is often followed by ingression, whereby cells in the groove undergo EMT and move freely beneath the surface layer. (G–H) Cell behaviors that drive convergence and extension. (G) Intercalation of mediolaterally elongated cells between their medial and lateral neighbors results in simultaneous convergence and extension. (H) Directed migration of cells in two populations towards the dorsal midline can also lead to convergence and extension of tissue.

Spreading out — Epiboly Movements

Epibolic expansion starts before the germ layers arise. In blastulae organized as a single-layered epithelium, like those of mammalian embryos, epiboly entails expansion of the surface area with thickness remaining constant. In multilayered blastulae, epibolic expansion is usually accompanied by a thinning of the tissue. Many different cell behaviors can contribute to epiboly. In embryos that experience significant cell division and cell growth, such as amniotes, cell division within the plane of the epithelium followed by an increase of cell volume drives the expansion of embryonic tissues. The key cell behavior during epiboly in the frog *Xenopus laevis* [42] and in teleost fish [35,43] is radial cell intercalation, whereby cells from deeper layers interdigitate between more superficial cells or *vice versa*. This results in fewer layers of cells occupying a bigger area and thus in a thinning and spreading of tissues (Figure 3B). Cell shape changes, namely flattening of columnar cells, can also produce an increase in tissue area [42]. Finally, directed cell migration, which spreads densely packed cells can also lead to epibolic expansion of tissue as observed during epiboly of the mesoderm in the zebrafish (Figure 3C) [44].

While our understanding of molecular determinants of these diverse epibolic cell behaviors is still very limited (Table 1), the first insights have been gained into radial intercalation. In *Xenopus*, the extracellular matrix protein Fibronectin and its signaling via the α5β1 Integrin receptor is required for the radial intercalation of prospective ectoderm and mesoderm cells [45]. Fibronectin might provide a permissive signal for elongation and alignment of cells along the axis of radial intercalation [45]. Regulation of intercellular adhesion via members of the cadherin family of cell adhesion molecules has been also implicated in epiboly. E-cadherin is maternally expressed in fish, chick and mammals [46], whereas in *Xenopus* its expression is initiated at early gastrulation [47]. E-cadherin is essential for normal epibolic movements in zebrafish [48,49]. In E-cadherin deficient zebrafish mutants cells from deeper layers fail to undergo the shape changes characteristic of the superficial layer and eventually sink back [48].

Gaining Guts — Emboly

Internalization of mesodermal and endodermal precursors is the defining event of gastrulation. Mesodermal and endodermal precursors move inside via a gateway that is known as the blastopore lip in the frog, the blastoderm margin in fish and the primitive streak in amniotes. In terms of cellular behavior, internalization can occur via invagination, which involves bending of cell sheets, often due to coordinated constriction of cells on one side of the sheet (Figure 3E) [8]. In the frog, emboly starts with the invagination of so-called bottle cells, which shape the axial blastopore lip. Invagination is followed by involution, whereby prospective mesodermal and endodermal precursors move as one cohesive sheet around the blastopore lip (Figure 3D) [50–52]. By contrast, during ingression movements in chick and mouse gastrulae individual cells translocate from superficial to deeper positions at or near the blastopore

lip [53]. In general, internalization is thought to involve an epithelial to mesenchymal transition (EMT), with cells breaking free of the epithelial sheet and moving individually through the blastopore (Figure 3F). In fish embryos, internalization might represent an intermediate mechanism between ingression and involution (also named 'synchronized ingression'), whereby prospective mesendodermal cells approach the blastopore as a coherent sheet, but at the blastopore the coherence is lost and cells move in a coordinated manner but as individuals [54,55].

There are parallels but also differences between vertebrates with regard to the order in which germ layers undergo internalization. In the frog gastrula, formation of the dorsal (axial) blastopore lip heralds the onset of internalization. Subsequently, the blastopore lip expands in the lateroventral (proximodistal) direction [50]. Likewise, in the zebrafish gastrula, internalization movements are initiated in the axial blastoderm margin, where the organizer will form, and then rapidly spread around the entire blastoderm margin [35,56]. Hence, in frog and fish the first tissues to be internalized are dorsoanterior mesoderm and endoderm. This contrasts the situation in the chick and the mouse, where the primitive streak first arises at the future caudal end of the embryo, and then elongates towards the future rostral end assuming a linear shape [57]. The expansion of the streak is accompanied by ingression of mesendodermal cells fated to become extraembryonic and posterior tissues. Only when the primitive streak achieves its maximal length, its rostral aspect forms Hensen's node, which becomes a conduit for the ingression of dorsoanterior mesendoderm [29,58]. Therefore, early internalization in fish and frog engages dorsoanterior tissues, whereas in amniotes extraembryonic and posterior tissues undergo internalization first.

There is a remarkable degree of conservation of the temporal order of germ layer internalization and of the spatial order in which different tissues become internalized with respect to the organizer. I will continue to use here the term proximal to describe regions of the blastopore close to the organizer, while distal refers to blastopore regions farther away (Box1; Figure 2A). Different vertebrate gastrulae share a number of similarities in endoderm internalization and its subsequent morphogenetic behaviors. In general, endoderm becomes internalized before mesoderm (Figure 2B). This is probably most apparent during fish gastrulation, where the endoderm precursors are localized closest to the blastoderm margin (blastopore), and most of them become internalized by early gastrulation [59]. Strikingly, in fish, chick and mouse the majority of endoderm is internalized via the blastopore region proximal to the organizer [60,61]. Upon internalization, endodermal cells in these vertebrates move initially as individuals. In fish, endodermal cells assume a morphology distinct from that of the mesoderm and move on the surface of the yolk cell to eventually form an endodermal layer along the rostrocaudal embryonic axis (Figures 1M and 2B) [59]. In chick, and probably also in the mouse, the precursors of definitive endoderm invade between the cells of the hypoblast (primitive endoderm), and gradually establish a congruent layer of definitive endoderm, which will displace the primitive endoderm towards extraembryonic tissues.

With respect to mesoderm formation, there is a trend in all vertebrates that the rostrocaudal organization of mesodermal tissues reflects the temporal order of their internalization via a specific proximodistal position of the blastopore (Figure 2C). Fate mapping of cells moving at specific proximodistal blastopore positions revealed that emboly of cells giving rise to more anterior structures precedes the movement of cells that generate more posterior structures (Figure 2). Another common aspect of emboly is that the mediolateral organization of mesodermal and perhaps endodermal tissues is linked to the spatial order in which they move through the blastopore with respect to the organizer region. The axial mesoderm moves through the axial aspect of the blastopore (Figure 2C,D), i.e. the dorsal blastopore lip in frogs, the shield in fish, and the node in amniotes, with the emboly of more rostral, prechordal mesoderm preceding the internalization of chordamesoderm. Presomitic mesoderm moves through the blastopore region proximal to the organizer, while intermediate mesoderm and lateral plate mesoderm are internalized via more distal blastopore regions (Figures 2 and 4; reviewed in [61,62]). In zebrafish the most posterior (tail) mesendoderm becomes internalized via the distalmost part of the blastopore [63,64]. Studies in amniotes show that the distalmost blastopore is a gateway predominantly for extraembryonic mesoderm [65,66].

The distinct internalization behaviors, involution of cell sheets and ingression of individual cells, appear to be mediated by similar gene cascades (Table 1). Mesendoderm specification and internalization is absolutely dependent on Nodal signals [11]. In mouse and fish embryos with mutations that impair Nodal signaling, prospective mesoderm and endoderm fail to express the proper markers and do not become internalized [67–72]. Instead, they assume neuroectodermal or tail fates [73,74]. Reception of Nodal signaling is sufficient for the execution of the internalization program by individual cells in the blastopore region and also in ectopic positions [74,75]. In fact, Nodal signaling must be restricted before the onset of gastrulation to prevent excess internalization and formation of ectopic blastopores. This is achieved in chick and mouse embryos by the Nodal antagonists Cerberus and/or Lefty, which emanate from extraembryonic tissues [76,77]. During gastrulation in fish, frog and amniotes, secreted Lefty proteins act as feedback antagonists of Nodal signaling in the mesoderm and are essential to prevent excess mesoderm specification and internalization [78–81]. The molecular effectors of Nodal signaling that regulate internalization behaviors remain to be elucidated.

Mutational analyses in the mouse and overexpression of dominant negative receptors in *Xenopus* revealed that FGF signaling is also essential for mesendoderm specification and internalization [82,83]. Mouse embryos homozygous for null alleles of genes encoding FGF receptor 1 (Fgfr1) and the Fgf8 ligand show similar gastrulation defects. In such embryos,

Table 1. Molecular regulators of gastrulation movements in vertebrates.

Gene product	Gastrulation movement	Cellular behavior and/or fate	Species	References
BMPs	Convergence and extension	Cell fate, negative regulator of mediolateral elongation (intercalation, migration)	zebrafish, *Xenopus*	[64]
Nodal	Emboly	Mesendoderm fate, involution, ingression	zebrafish, *Xenopus,* mouse	[67,74]
	Convergence and extension	Unknown	zebrafish	[73,143]
FGFs	Mesendoderm migration away from the blastopore	EMT, directed cell migration	chick, mouse	[83,84,86,117]
	Convergence and extension	Directed cell migration	*Xenopus*, zebrafish	[82,117,145,146]
Brachyury	Convergence and extension, mesendoderm migration away from the blastopore, mesodermal cell fate	Cell intercalation, directed migration	*Xenopus*, zebrafish, mouse	[113,120,147,148]
Spadetail (Tbx 16)	Convergence and extension,	Directed cell migration, mesodermal cell fate	zebrafish	[149,150]
Slit	Convergence and extension	Unknown	zebrafish	[151]
Neogenin	Convergence and extension	Unknown	zebrafish	[152]
E-cadherin	Epiboly	Radial intercalation	zebrafish, mouse, *Xenopus*	[48,49]
	Convergence and extension	Unknown	zebrafish	[153]
Fibronectin	Epiboly	Cell polarity, radial intercalation	*Xenopus*	[45]
	Mesendoderm migration	Directed migration	*Xenopus*	[98]
Snail	Convergence and extension, mesendoderm migration	EMT, directed migration	mouse	[87,88]
Liv1	Convergence and extension, mesendoderm migration	EMT(?), directed migration	zebrafish	[95]
Stat3	Convergence and extension	Mediolateral elongation	zebrafish	[27]
Wnt11/ Silberblick	Convergence and extension	Mediolateral elongation (intercalation, migration)	zebrafish, *Xenopus*	[90,120,121]
Wnt5/ Pipetail	Convergence and extension	Mediolateral elongation (intercalation, migration)	zebrafish	[125,154,155]
Knypek (Gpc 4)	Convergence and extension	Mediolateral elongation (intercalation, migration)	zebrafish, *Xenopus*	[119,156,157]
Stbm (Vangl2)	Convergence and extension	Mediolateral elongation (intercalation, migration)	zebrafish, *Xenopus*	[114,127,156]
PDGF	Convergence and extension, mesendoderm migration	Directed migration	zebrafish, *Xenopus*	[100,101]
PI3K	Convergence and extension, mesendoderm migration	Directed migration	zebrafish	[101]
PAPC	Convergence and extension	Mediolateral cell polarization	*Xenopus,* zebrafish	[158,159]
Quatro	Convergence and extension, mesendoderm migration	Directed migration	zebrafish	[160]
Cap1	Convergence and extension, mesendoderm migration	Directed migration	zebrafish	[160]

mesoderm and endoderm derived tissues are severely reduced, presumably because progenitor cells fail to migrate and accumulate at the blastopore (primitive streak) and retain epithelial character instead [83–85]. Elegant studies in mice revealed that FGF signaling promotes EMT and consequently movement of mesodermal progenitors through the blastopore by positively regulating expression of the transcriptional repressor Snail (Sna), which in turn downregulates expression of E-cadherin [86]. In *Sna*-deficient mouse embryos, mesoderm forms but fails to completely downregulate E-cadherin expression and retains epithelial characteristics [87]. The milder gastrulation defects observed in *Sna* mutants compared to FGF-deficient embryos

suggest that additional factors execute the FGF-mediated morphogenetic program. Strikingly, Snail function is also necessary for mesoderm internalization during gastrulation in *Drosophila melanogaster*, underscoring the evolutionary conservation of genetic hierarchies that regulate internalization (reviewed in [88,89]).

Migration of Internalized Mesendoderm

While the specific direction in which mesendoderm migrates upon internalization depends on the overall shape of the embryo (Figure 1), mesendodermal cells generally move away from the blastopore (Figures 2 and 4). The evolutionarily most conserved migration behavior is observed for mesendoderm internalized at the axial region of the blastopore (Box 1); these prospective axial mesendoderm cells move rostrally (and also animally in frogs and fish), away from the blastopore and towards the future forebrain (Figure 2C). By doing so, these cells contribute to axis extension. In all vertebrates, mesendodermal cells emerging from proximal and proximodistal blastopore regions also move away from the blastopore. However, in frog and fish gastrulae they move from the rim of a 'cup' towards its base at the animal pole, and thus anteriorly, and parallel to the axial mesoderm (Figures 1 and 4A). In mouse gastrulae mesendodermal cell populations move from the bottom of a cup on its outside wall towards its rim; thus, their pathways diverge initially from those of the axial mesoderm. In the flat chicken gastrulae, paraxial and lateral mesendoderm initially move perpendicular to the axial mesendoderm (Figure 4B).

The morphology and behavior of migrating mesodermal cells in different vertebrate gastrulae is similar. In amniotes and fish, mesodermal cells migrate as a loose association of mesenchymal monopolar cells, which form numerous filopodia and lamellipodia at the leading edge facing the direction of movement [90,91]. In *Xenopus,* prechordal mesendoderm cells move as a partially coherent mass, in which individual cells overlap such that protrusions of a given cell underlie the posterior part of the cells anterior to it [92,93]. This geometrical arrangement is likely to be important for normal movement, as the migration rates of individual cells in explants are not sufficient to explain normal movement of the prechordal mesoderm tissue *in vivo* [94]. The mesenchymal nature of migrating mesodermal cells is a consequence of EMT, which either precedes internalization, as in amniote embryos, or follows internalization, as in frogs and possibly fish. Studies in zebrafish identified a separate EMT pathway in the dorsal gastrula organizer, in parallel to downregulation of E-cadherin by Snail [27,95]. Phosphorylation and consequent activation of the Stat3 in the organizer region is required for the normal anterior migration of the organizer cells [27]. The main target of Stat3 appears to be Liv1, a breast cancer-associated zinc transporter protein, which promotes nuclear localization of Snail and thus its ability to repress epithelial character [95]. Similar to mouse gastrulation, a full EMT (and Snail1 function) appears not to be essential for internalization, but for the effective migration of internalized cells away from the blastopore [87,95].

After internalization, mesenchymal mesendodermal cells find themselves between the superficial tissue, largely prospective ectoderm, and extraembryonic tissues in amniotes and fish. In frogs and fish, the mesendoderm faces non-involuted tissues that form the blastocoel roof on the inner wall of the blastodermal cup, while having a nascent archenteron space beneath them (Figure 1I,J). Mesendodermal cells form rich protrusive contacts with overlying and underlying tissues [90,93,96]. The relationship of migrating mesodermal cells with the overlying noninvoluted ectoderm is especially complex and instructive. Elegant work in *Xenopus* demonstrated that the mesodermal cells must be able to move on the overlying surface without adhering to or 'sinking' into it. This separation behavior is mediated by transcription factors Mix1 and Gsc, as well as by non-canonical Wnt signaling via Protein Kinase C [97]. Importantly, the ectodermal roof produces a number of factors that guide mesoderm migration. The basal surface of the ectodermal roof is covered by a sparse network of extracellular matrix (ECM), the density of which decreases during gastrulation. In frog and chick, interference with the main ECM component Fibronectin or its Integrin receptors expressed by mesodermal cells, does not prevent their adhesion to the ECM. However, their protrusive activity is severely reduced, and they fail to migrate away from the blastopore [98]. Whether Fibronectin has a similarly important role in mesoderm migration in all vertebrates is questioned by the finding that in murine embryos with inactivated Fibronectin genes the mesoderm migrates normally [99]. The ECM network *per se* is not likely to guide mesoderm migration, rather it harbors instructive cues and permissive factors secreted by the ectoderm and mesendoderm. One such molecule, Platelet Derived Growth Factor (PDGF), which signals via a receptor tyrosine kinase, has been implicated in mesoderm spreading on Fibronectin in tissue culture, and in survival and migration of mesodermal cells in *Xenopus* [100]. In mouse embryos, inactivation of the PDGF receptor results in mesendodermal cells migrating abnormally. Recent studies show that during zebrafish gastrulation PDGF functions upstream of PI3 Kinase to promote formation of cellular protrusions, to polarize migrating prechordal mesoderm cells and localize protein kinase B (PKB) to the leading edge of the moving cells [101]. In fish embryos, the ectodermal roof also produces Wnt11, which is required for normal orientation of mesodermal cell protrusions in the direction of migration, as well as for effective migration away from the blastopore [90]. It will be important to determine whether non-canonical Wnt11 signaling also contributes to the separation of mesoderm from ectoderm in fish, as it does in the frog gastrula [97].

Becoming Lanky — Convergence and Extension Movements

Convergence and extension movements narrow (convergence) the mediolateral aspect and elongate (extension) the rostrocaudal aspect of the vertebrate body (Figure 3A). Convergence and extension pushes the neural and mesendodermal head components

away from trunk and tail chordamesoderm precursors, which are positioned close together in the blastula fate maps (Figure 2C) [102]. In fish and frog, these movements are initiated after the formation of the three germ layers and when epibolic movements are well underway. By contrast, in amniote embryos anterior expansion of the primitive streak (blastopore) is considered an integral part of convergence and extension movements [91].

Depending on the underlying cellular mechanisms, convergence and extension can be independent, or they can be linked in the process known as convergent extension [103,104]. Pioneering work by Keller [105,106] and colleagues in *Xenopus* has shown that, as gastrulation proceeds, mesodermal cells lose their irregular shape and become elongated along the mediolateral embryonic axis. These elongated cells intercalate between their medial and lateral neighbors, thereby simultaneously narrowing (mediolaterally) and elongating (rostrocaudally) the body axis (Figure 3G) [105,106]. In the mesoderm, convergent extension is driven by a bipolar protrusive activity: medially and laterally projected lamelliform protrusions attach to neighboring cells and facilitate intercalation by wedging between cells as well as maintain supportive connections that become increasingly necessary as the tissue stiffens. Initially neural ectodermal cells also exhibit bipolar protrusive activity, but after induction by an unknown midline derived signal they show medially directed monopolar protrusions [107,108]. Studies in chick and zebrafish indicate that additional cell behaviors, such as polarized cell division and apical-basal elongation of cells, also contribute to neural convergence and extension [109,110].

John Trinkaus, through his original time-lapse analyses of gastrulation in the fish *Fundulus heteroclitus*, demonstrated that several cell behaviors can contribute to convergence and extension [111,112]; this has now also been shown in zebrafish [90,113,114]. In the axial gastrula, mediolateral cell intercalation seems to drive convergence and extension of axial mesoderm and the adjacent presomitic mesoderm, as in *Xenopus* [113]. In proximodistal regions, mesodermal and ectodermal cell populations move dorsally at an increasing speed [112,115]. Time-lapse analyses revealed that cells in the more distal domain are only slightly mediolaterally elongated and migrate as individuals with a slow net dorsal speed along indirect paths. When these cells move closer to the midline they become mediolaterally highly elongated and migrate dorsally at an increased net speed along more direct trajectories [114]. As some of these dorsally migrating cells bias their trajectories towards the rostral and some towards the caudal region, the entire population contributes to tissue convergence by moving dorsally, but also to extension, as it becomes elongated rostrocaudally (Figure 3H) (Sepich and LSK, unpublished observations). Hence, in *Fundulus* and zebrafish convergence and extension movements involve directed migration and intercalation of mediolaterally elongated cells (Figure 3G,H) [104].

In recent years, many inroads have been made into the molecular mechanisms of polarized cell behaviors associated with convergence and extension movements. The directed migration of mesodermal cells towards the midline in fish and chick is reminiscent of chemotactic cell movements [111,112,114,116,117]. In chick, FGF4 was proposed to act as a the midline chemoattractant [117]. Whereas the chemoattractants have not been identified in zebrafish, a putative secreted convergence factor has been proposed to be produced in the organizer downstream of Stat3 [27]. Inhibition of Stat3 impairs anterior movement of axial mesoderm and dorsal convergence and extension movements of lateral cells, without significant effect on cell fates in the gastrula. Although Stat3 is expressed ubiquitously at blastula stages, tyrosyl-phosphorylation and presumed activation of Stat3 protein is observed exclusively in the dorsal aspect of the embryo and is dependent on the β-catenin pathway. Elegant transplantation experiments demonstrated that Stat3 is required in axial tissues for convergence and extension movements of distal cells, thus implying that Stat3 promotes production of a secreted convergence factor [27].

The mediolateral cell polarization underlying convergence and extension is dependent on a non-canonical Wnt signaling pathway, similar to the planar cell polarity (PCP) pathway in *Drosophila* [114,118–122]. In this pathway, which mediates cell polarization along the proximal-distal axis within the plane of an epithelium, the transmembrane protein Frizzled (Fz) signals through downstream factors including Dishevelled (Dsh), small GTPases of the Rho family, Rho kinase and JNK [123,124]. In contrast to fruit flies, however, this pathway employs Wnt ligands in vertebrates. Zebrafish mutants for *wnt11* and *wnt5* result in shorter and broader embryos [121,125]. Fruit flies and vertebrates share components of this pathway, such as Dsh, the transmembrane protein Strabismus/Trilobite, Rho, Rho kinase 2, and Prickle [114,118,126–131]. Recent studies revealed new vertebrate specific components, including the glypican Knypek, which promotes Wnt11 signaling [119], and the intracellular protein Daam1, which links Dsh and Rho [132]. As the small GTPase Rac has been implicated as a downstream component of Wnt/PCP signaling [133], regulation of small GTPases, such as Rho, Rac and Cdc42, is likely to play a general role in mediating cell behaviors driving vertebrate gastrulation [134,135].

Expression of dominant negative Dsh in explants of frog gastrulae revealed a critical requirement of this pathway for mediolateral cell intercalation [118]. The failure of cells in zebrafish *knypek* and *trilobite* mutants to elongate and align mediolaterally links impaired mediolateral cell polarization with defective convergence and extension movements *in vivo* [119], and with the ability of cells to move dorsally along straight paths [114]. Non-canonical Wnt/PCP not only regulates the elongation and orientation of the cell but also polarization and stabilization of cellular protrusions [90,118]. Moreover, decreased cell adhesion is associated with the axis extension defect in embryos in which Wnt4 is overexpressed [136].

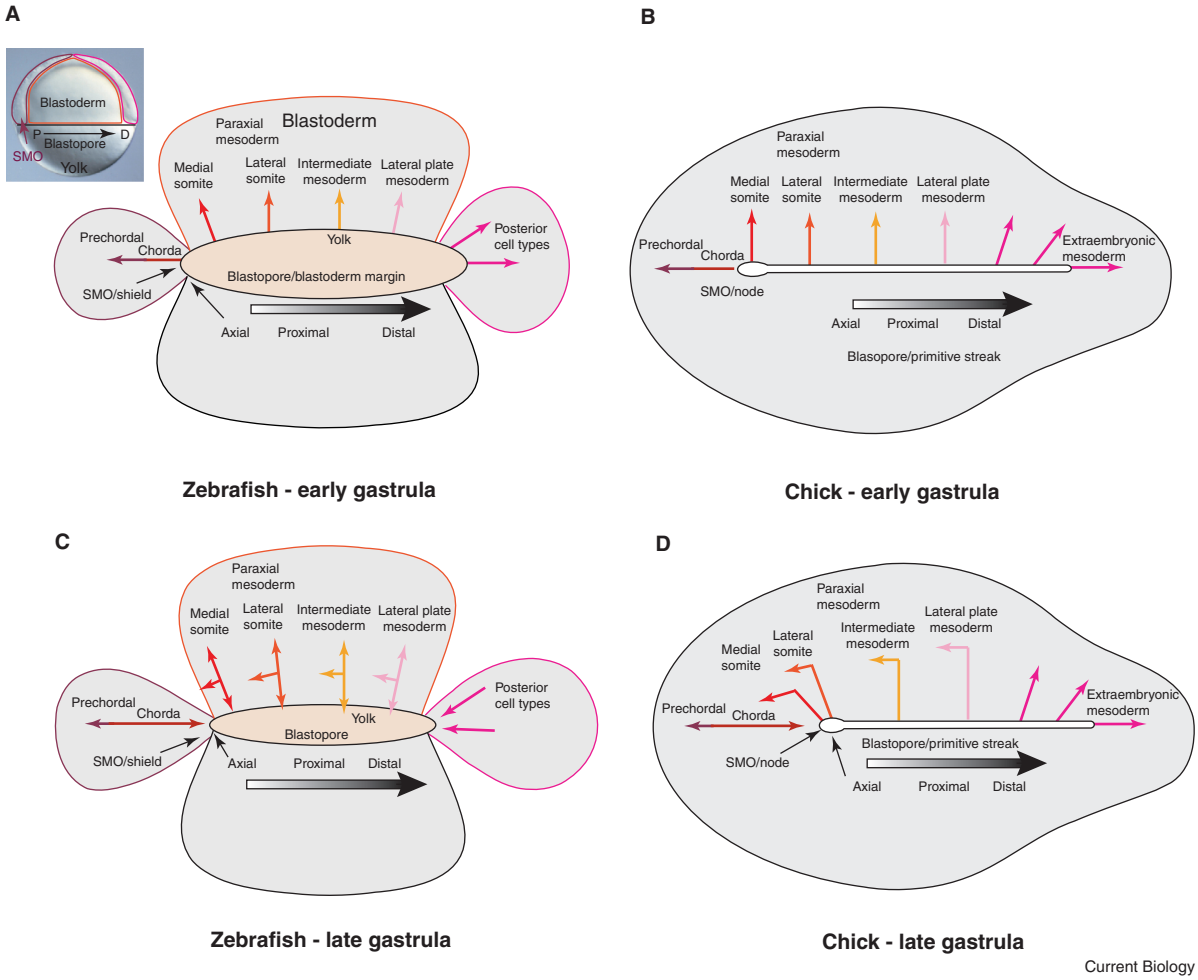

Figure 4. Conserved patterns of cell movements during vertebrate gastrulation.

Cell movements during early (A,B) and late (C,D) gastrulation in zebrafish (A,C) and chick (B,D). (A) Inset shows early zebrafish gastrula, in lateral view with animal to the top, vegetal to the bottom and dorsal to the left. The blastopore is located at the blastoderm margin with the organizer taking its axial position. The arrow shows proximaldistal axis of the blastopore. The schematic shows a flattened zebrafish embryo with the yolk cell in the center, surrounded by the blastopore. The blastoderm is divided into three regions, axial, proximodistal and distalmost, as illustrated by color lines on the inset. (B) Schematic of a chick gastrula at stage 4+, when the primitive streak is most elongated. Several similarities are apparent between (A) and (B): Types of mesoderm become internalized along similar regions of the blastopore: prechordal and chorda mesoderm through the axial blastopore, precursors of somites via proximal regions, and intermediate as well as lateral plate mesoderm through distal regions. In the chick, the most distal aspect of the blastopore provides a conduit for internalization of extraembryonic tissues, whereas in zebrafish precursors of the most posterior (tail) somites, intermediate and lateral plate mesoderm move via the distal blastopore. Upon internalization all types of mesoderm move away from the blastopore. (C,D) At later stages of gastrulation, the blastopore becomes smaller in fish, due to epiboly, and in chick, due to regression of the primitive streak. This is associated with extension of axial mesoderm (dark red). Streams of mesodermal cells that were internalized via proximal and proximodistal regions of the blastopore move dorsally towards the extending midline. Whereas in the distal region of the zebrafish blastopore, prospective posterior mesoderm cells move back towards the blastopore to form the tail bud (C), there is a continued movement of extraembryonic mesoderm away from the blastopore in the chick (D). (B) and (D) are based on figures presented in [23] and [62].

Non-canonical Wnt signaling is required for cell behaviors that drive convergence as well as extension movements, and defects in extension are not simply secondary to defective convergence [115]. Providing further support for the notion that convergence and extension can be separated, zebrafish *no tail* mutants, in which the *Brachyury* homolog is mutated, have a pronounced defect in convergence, but exhibit normal extension of axial mesoderm during early gastrulation [113]. Current work revealed that Hyaluronan synthesizing enzyme 2 (Has2) acts upstream of Rac1 in zebrafish mesoderm cells to promote the formation of lamellipodia. Intriguingly, Has2- and Hyaluronan-deficient gastrulae show a dramatic defect in dorsal convergence but not in extension movements of lateral mesoderm [137]. Given that non-canonical Wnt signaling also impacts Rac1 activity [133], it will be interesting to see how these pathways interact to regulate convergence and extension movements.

Many other molecules and pathways have been implicated in convergence and extension movements based on phenotypes of gain and loss-of-function

experiments (Table 1). As cell movements have not been analyzed in these studies, the critical questions regarding the involvement of these molecules in convergence and extension remain open.

Conserved Patterns of Cellular Flow in Vertebrate Gastrulae

Recent studies in which movements of large mesodermal cell populations have been traced over the course of gastrulation uncovered striking similarities in patterns of cell movements with respect to the main landmarks of vertebrate gastrulae, the blastopore, the organizer and the nascent embryonic midline (Figure 4) [64,117].

The migration patterns of cells emerging at discrete positions from the fully extended streak of chick gastrulae have been revealed by time-lapse analyses of GFP-labeled cells. Cells labeled in the node move beneath the neuroectoderm towards the future forebrain and form rostrocaudally elongated arrays marking the axial mesoderm. The cells initially move away from the streak (Figure 4B). However, as the node (the organizer) regresses leaving the embryonic midline in its wake and passes the position in the streak where the labeled cells originate, the trajectories of the labeled cells turn, such that they start to move towards the midline (Figure 4D). A similar pattern of movement was observed for cells proximal to the node that contribute to the somites, as well as for cells originating from more distal streak positions that give rise to lateral plate mesoderm. Cells emerging from the very distal positions (posterior) in the streak move outward toward the boundary of *area pellucida* and *area opaca* (Figure 4B,D) [117].

In zebrafish, cell populations labeled in the organizer region (embryonic shield) of the blastoderm margin move towards the future forebrain and subsequently also posteriorly. Thus, at the end of the gastrula period they form an elongated array spanning from forebrain to tailbud (Figure 4A). Cells originating from the proximal or distal blastopore regions, in striking similarity to behavior of cells in the chick gastrula, initially move away from the blastopore without any bias towards the midline (Figure 4A). However, close to the midgastrula stage when the organizer has moved posteriorly, these cells turn towards the embryonic midline, thus initiating dorsal convergence (Figure 4C) [115] (Sepich & LSK, unpublished observations). It is noteworthy that similarly to node regression in the chick, epibolic expansion of blastoderm around the yolk cell in zebrafish gastrulae, decreases blastopore size and moves the axial blastopore region away from rostral tissues (Figures 4C,D). In another remarkable similarity with chick gastrulae, cell populations residing in the distal blastopore (ventral blastoderm margin in fish and posterior primitive streak in chick) do not engage in convergence towards the axial midline, but rather move towards the prospective tailbud or extraembryonic region [64,117]. In the chick, the movement of mesendodermal cells away from the blastopore has been proposed to be mediated by FGF8 acting as a chemorepellent, whereas their later movement towards the midline to be promoted by FGF4 [117]. It will be important to test the universality of these signals in other vertebrates.

The Complex Relationship between Cell Fates and Movements during Gastrulation

Gastrulation simultaneously entails cell fate specification and movements. How are the two related? While the answer to this question is far from clear, two relevant examples are given below: As discussed above, Nodal signaling is sufficient and necessary for mesendoderm induction and internalization. Indeed, excess Nodal signaling leads to increased internalization in fish, chick and mouse [76,77,81]. Is the internalization cell behavior simply a consequence of specification of mesendodermal cell fate? Or rather, does Nodal signaling activate a parallel gene cascade that mediates internalization and that can be uncoupled from mesendodermal cell identity? After all, both mesodermal and endodermal progenitors undergo internalization, suggesting a common morphogenetic program. EMT is a hallmark of internalization and migration and might require intersection of several signaling events. For example, in mouse, Snail activation and E-cadherin activation require FGF signaling, and FGF signaling also contributes to mesoderm patterning. However, in the organizer region of the zebrafish blastopore, nuclear localization of Snail is controlled by the Stat3–Liv1 pathway, which acts in parallel to Nodal signaling and is not required for mesodermal cell fate [95]. Studies in chick have revealed that the extent of mesoderm internalization during gastrulation might be limited by expression of the transcription factor Churchill and downregulation of Brachyury expression in the prospective ectoderm [138]. Thus, internalization and migration of mesoderm, in part mediated by Snail and Brachyury, might involve integration of several genetic pathways, linked to but also separate from the specification of mesodermal cell identity.

A similar relationship exists between BMP signaling and convergence and extension movements. As discussed above, BMP activity forms a distal to proximal gradient and is thought to promote ventroposterior and to inhibit dorsoanterior cell fates in a concentration dependent manner [18,26]. The BMP activity gradient has been also proposed to establish domains of distinct convergence and extension movements in the zebrafish gastrula [64]. In this model, cells that become internalized via the distalmost part of the blastopore, at high levels of BMP activity, do not engage in convergence movements (Figures 2A and 4A,C). Mesodermal cell populations that become internalized through the distal-proximal region of the blastopore and thus at moderate BMP levels, translocate towards the midline and extend at increasing speeds as they move down the BMP activity gradient. At the level of the axial blastopore and thus at low BMP activity, slow convergence and fast extension occur, driven by mediolateral intercalation (Figures 2A and 4A,C). In *Xenopus*, high levels of BMP activity can also inhibit convergence and extension of tissue explants [139]. Again, the question arises whether the specific convergence and extension behaviors of a

cell at a given level of BMP activity are simply a consequence of its BMP-specified cell fate? Or alternatively, do different thresholds of BMP activity set off parallel pathways that control cell fates and cell movements? In support of the latter, inhibition of convergence and extension of paraxial mesoderm cells in ventralized zebrafish gastrulae is associated with reduced expression of *wnt5*, which regulates convergence and extension movements, but persistent expression of the cell fate regulator MyoD [64].

The concept of positional information specifying cell fate and movement in parallel is attractive. BMP signaling activity forms a gradient with a high point at the distal blastopore, decreasing in its proximal regions and with a minimum in the axial blastopore, where the organizer produces BMP antagonists (Figure 2A). Conservation of this gradient along the blastopores of different vertebrate gastrulae could account for the similarities in the patterns of cell movements (Figures 2 and 4). In the frog embryo, elongation of axial mesoderm by the process of convergence and extension is thought to be coordinated by anteroposterior tissue polarity [140]. Whereas the nature of this hypothetical anteroposterior polarity signal that determines the direction of tissue elongation remains to be determined, Nodal has been proposed to act as a morphogen for mesodermal tissues and to affect the global anteroposterior embryo pattern in a direct or indirect manner [141–143]. Therefore, it is intriguing that Nodal activity is thought to form a gradient decreasing along the proximal–distal, and blastoporal–abblastoporal axes (Figure 2A), and that Nodal signaling is essential for extension movements [73,143]. Interpretation of positional information could account for the coordinated morphogenetic behaviors of cells in different germ layers. It will be important to determine whether Nodal and BMPs indeed provide such positional information in vertebrate gastrulae and delineate pathways that link positional information to specific cell behaviors during gastrulation.

Conclusions

This is an exciting time for gastrulation research: signaling pathways and molecules can be linked to individual gastrulation movements, to discrete gastrulation cell behaviors or even to specific cell properties underlying these behaviors. However, a complete picture of gastrulation is still lacking, and it is likely that many novel cellular and molecular mechanisms will be discovered. How similar the molecular mechanisms of gastrulation are in different vertebrates remains to be determined. It will be important to test whether mechanisms uncovered in one species, such as the Stat3–Liv1–Snail pathway in zebrafish also operate in other vertebrates [27,95]. It will also be essential to elucidate further the pathways specifying cell fates and how they relate to cell movements.

Despite the focus resting on molecules, a deeper understanding at the cellular level is also required. Indeed, what John Trinkaus wrote two decades ago in his book 'Cells into Organs: Forces that Shape the Embryo' [51] remains highly relevant to the gastrulation field today: "It is well to be reminded that *molecular* does not necessarily mean *analytical* nor, by contrast, does *cellular* or *histological* necessarily imply *descriptive*. Sound descriptive and analytical studies are obviously required at both (and all) levels of organization. Snobbish references to certain morphological studies as *merely descriptive* only serve to reveal naiveté of the authors and, if insisted upon, could impede advance of the field".

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I wish to dedicate this review to the memory of Dr. J.P. Trinkaus, whose pioneering studies on gastrulation cell behaviors in *Fundulus heteroclitus* and enthusiasm for gastrulation, continue to inspire our work.

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