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Back and Forth between Cell Fate Specification and Movement during Vertebrate Gastrulation

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Summary of recent advances

Animal body plan arises during gastrulation and organogenesis by the coordination of inductive events and cell movements. Several signaling pathways, such as BMP, FGF, Hedgehog, Nodal and Wnt, have well recognized instructive roles in cell fate specification during vertebrate embryogenesis. Growing evidence indicates that BMP, Nodal and FGF signaling also regulate cell movements, and that they do so through mechanisms distinct from those that specify cell fates. Moreover, pathways controlling cell movements can also indirectly influence cell fate specification by regulating dimensions and relative positions of interacting tissues. The current challenge is to delineate the molecular mechanisms via which the major signaling pathways regulate cell fate specification and movements, and how these two processes are coordinated to ensure normal development.

Introduction

One striking feature of animal development is the intricate relationship between cell fate specification and movement. Many developmental processes start with a uniform pool of multipotent progenitors, which subsequently acquire diverse cell fates and engage in cell movements to generate structures of unique morphology and function. This is obvious during gastrulation, the process by which the body plan is established [1,2]. During vertebrate gastrulation a ball or a sheet of early blastomeres receives anteroposterior and dorsoventral positional information instructing the formation of three germ layers: the most internal endoderm and superficial ectoderm, separated by mesoderm. Concurrent epiboly movements spread and thin these germ layers, whereas convergence and extension (C&E) movements narrow them mediolaterally and elongate them from head to tail [3,4].

Thus the patterning, cell fate specification and morphogenetic processes of gastrulation take place concurrently, which raises the question of how they are regulated to achieve normal development. The issue is further complicated by recent discoveries that many signaling pathways instructing cell fate decisions also influence cell movements [5–9]. One possibility is that cell fate specification and movement are controlled by a linear pathway where a signal establishing positional information in the embryo, also specifies cell fates, and part of that instruction subsequently determines specific movement behaviors. In this model, cell movement behavior determinants are downstream of events specifying positional information and cell fate specification; e.g. a program that specifies heart progenitors would contain not

only the information about their future fate and function, but also a unique program of gastrulation cell movements. Another model proposes that a pathway that specifies positional information, triggers parallel programs in cells at different embryonic positions, one program specifying cell fates and another regulating cell movement behaviors. This, however, does not necessarily imply cell movement and fate specification are independent processes as the cell movement program can influence cell fate specification by placing cells in the appropriate embryonic positions to receive the next set of inductive signals. Here, we will review recent findings on the roles the major pathways implicated in gastrulation play in cell fate specification and movement.

Wnt

Wnt signaling controls a wide array of embryonic and adult processes ranging from gastrulation to aging [10]. Generally, Wnt signaling can be subdivided into canonical and non-canonical pathways. Canonical Wnt signaling is characterized by its regulation of gene transcription through β -catenin and TCF. The earliest role of the canonical/ β -catenin pathway in frog and fish embryos is the breaking of embryonic symmetry to establish the dorsal blastula organizer, or the Nieuwkoop center. Accumulation of β -catenin in nuclei of the dorsal blastula activates expression of a suite of genes to establish the Spemann-Mangold gastrula organizer, which controls both cell fates and C&E movements during gastrulation [11]. Elegant studies in zebrafish demonstrated that parallel pathways activated by β -catenin regulate cell fate specification as well as cell movements. β -catenin dependent transcription of secreted antagonists of BMP signaling or repressors of genes encoding BMP ligands have been implicated in the establishment of a ventral to dorsal gradient of BMP signaling [12], which influences specification of cell fates and movements during later gastrulation [6•,13](see below). β -catenin also cooperates with and enhances Nodal signaling to promote dorsal fates [14]. However, independently of regulation of BMP and Nodal signaling, β -catenin also indirectly promotes phosphorylation of Stat3, required cell autonomously for axial mesoderm C&E and cell non-autonomously for dorsal convergence of lateral cells in the zebrafish gastrula [15]. During gastrulation canonical Wnt signaling reverses its action and inhibits the gastrula organizer maintenance and function, and promotes posterior fates in the nascent neuroectoderm [16].

In addition to the canonical Wnt signaling pathway, multiple, partially overlapping, non-canonical Wnt pathways have been identified. It has been well established that non-canonical Wnt signaling has critical roles in the regulation of gastrulation movements, mainly C&E [17]. In zebrafish, components of the non-canonical Wnt/planar cell polarity (Wnt/PCP) pathway are required for C&E movements and anterior migration of internalized prechordal mesoderm progenitors [18]. In *Xenopus laevis* embryos, a distinct non-canonical Wnt signaling pathway including the Wnt receptor Frizzled and the intracellular signaling mediator PKC, ensures that internalized prechordal mesoderm separates from the overlying non-involuting tissues allowing normal anterior migration [19]. At later gastrulation, Wnt/PCP signaling mediates the mediolateral elongation of mesodermal and ectodermal cells and is required for effective dorsal migration, and polarized mediolateral and radial intercalations that drive C&E in different regions of the fish gastrula [20]. Recent cell tracing studies in the mouse embryos demonstrate a cell-autonomous requirement for *vangl2* (*loop-tail*) in C&E of both axial mesoderm and neuroectoderm. As in other vertebrates, the murine *Vangl2*/Lp affects cell movement rather than cell fate specification and is likely to function in cell intercalation [•21]. It is likely that there are additional roles for the Wnt/PCP during vertebrate development yet to be discovered. For example, new studies in avian embryos implicate this pathway in morphogenetic movements that generate the primitive streak, the amniote blastopore equivalent [••22].

Interestingly, Wnt/PCP signaling not only controls cell movements but secondarily can also interfere with cell fate specification. For example, in zebrafish embryos with compromised Wnt/PCP signaling the interface between axial and adaxial tissues is reduced, and the distance of cells that require a specific signal to its source is increased, leading to diminished or aberrant induction of adaxial muscle precursor cells in the mesoderm and optic stalk tissue in the neuroectoderm [23–26]. This suggests Wnt signaling can interfere with embryonic patterning both directly via its canonical signal transduction pathway and indirectly by controlling the relative position of signaling and receiving cells/tissues during induction processes.

Nodal

Nodal/TGF β signaling plays key roles in axes formation, mesendoderm induction, neural patterning and left-right asymmetry [27]. In addition, it has been implicated in tissue morphogenesis [7–9], although only little is known about the mechanisms by which it functions in this process. Recent studies in gastrulating *Xenopus* embryos have provided evidence that a gradient of TGF β signaling along the anteroposterior axis not only induces different mesendoderm cell fates, but is also required for mediolateral mesoderm progenitor polarization and intercalation driving C&E movements [28]. A similar function of Nodals in cell specification and polarization has been observed in *Xenopus* embryos where Nodal-signaling mediated formation of tissue boundaries, influences mediolateral polarization and intercalation of mesoderm progenitors [29]. Cell fate specification along the anteroposterior axis has also been linked to cell behavior in chick embryos, where Hox-gene mediated anteroposterior patterning is able to induce differential cell behavior of paraxial mesoderm progenitors along this axis during gastrulation [••30]. How different levels of Nodal signaling determine specific cell behaviors is not yet understood. Interestingly, recent studies have demonstrated that Nodal signaling is able to modulate cell adhesion through adhesion molecule endocytosis/recycling [31,32] and acto-myosin dependent cell cortex tension [••33]. Considering that both differential adhesion and tension have been proposed to constitute key parameters driving cell sorting and tissue envelopment during zebrafish gastrulation [31], it is conceivable that Nodals modulate cell behavior by determining their specific adhesive and tensile properties.

BMP

The bone morphogenetic proteins (BMPs) function in a wide range of different cellular processes including cell proliferation, differentiation, motility, adhesion and death [34]. At the onset of gastrulation, a ventral to dorsal gradient of BMP signaling is established, playing a key role in mesoderm induction and dorsoventral patterning of all germ layers [12]. New studies revealed the BMP signaling gradient confers dorsoventral pattern in a temporally progressive fashion along the anteroposterior axis, likely due to a temporal cue that regulates a cell's competence to respond to BMP signaling [•13]. Interestingly, this gradient of BMP activity appears to also be responsible for determining differential cell movements along the dorsoventral axis [6]. Lower BMP activity at the lateral and dorsal side of the gastrula promotes C&E movements by allowing mediolateral cell elongation underlying fast dorsal migration and dorsally biased intercalation. Conversely, high levels of BMP activity at the ventral side promote epibolic migration of cells into the tailbud. BMPs have been proposed to function in this process both indirectly by specifying different cell fates and more directly by regulating the expression of components of the Wnt/PCP pathway essential for polarized cell behaviors underlying C&E [6] and by modulating Cadherin-mediated cell adhesion [•35]. In zebrafish gastrulae, a ventral-to-dorsal BMP gradient establishes a reverse gradient of cell-cell adhesiveness directing lamellipodia-driven dorsal convergence of mesoderm progenitors. Importantly, BMPs are thought to modulate progenitor cell adhesion without changing cell fates, suggesting that the BMP pathways involved in induction of cell fate and morphogenesis are separable [•35].

FGF

Signaling of Fibroblast growth factors (FGFs) via their tyrosine kinase receptors regulates both specification and movement of mesendodermal precursors. During mouse gastrulation, FGF signaling in the primitive streak activates expression of the Snail transcriptional repressor to downregulate E-cadherin and to promote the epithelial to mesenchymal transition. This is necessary for mesodermal progenitors to undergo ingression and subsequent migration away from the primitive streak [5,36]. Studies in the chick suggested yet another role for FGF in guidance of internalized mesodermal cells. During early gastrulation, FGF8 expressed in the primitive streak is proposed to act as a chemorepellant directing migration of mesodermal cells away from the blastopore. At later gastrulation, FGF4 expressed in the extending axial mesoderm serves as a chemoattractant for the dorsal convergence of the lateral mesoderm [37]. Whereas evidence for such a chemotactic role of FGF signaling is lacking in other vertebrate models, recent genetic experiments in the mouse argue that a higher level of FGF8 signaling is required for normal mesoderm migration than for mesoderm specification [•38]. Interestingly, inactivation of maternal and zygotic function of FGF receptor 1 in medaka fish impairs anterior migration of prechordal mesoderm and extension movements of chordamesoderm, but not convergence of the lateral mesoderm or initial mesoderm induction [39]. Given that three additional FGF receptor genes are expressed in medaka gastrulae, it is conceivable that higher FGF signaling levels are required for the migration than for the specification of mesoderm. Additional support for distinct roles of FGF in mesoderm movement and specification comes from *Xenopus*, where Sprouty2 was shown to inhibit FGF-mediated gastrulation movements without affecting mesoderm induction and patterning [40]. Interestingly, recent studies demonstrated that Sprouty can be sequestered and inhibited by Paraxial Protocadherin, which is associated with increased C&E movements and Wnt/PCP signaling [•41].

G Protein Coupled Receptors

Directed cell migration plays an important role in vertebrate gastrulation. Internalized mesendodermal progenitors in most vertebrates undergo directed migration away from the blastopore. Later during gastrulation, mesodermal cells in zebrafish and in amniote embryos, change their trajectories and undergo directed migration towards the dorsal midline. The behavior and trajectories of these lateral mesodermal cells in zebrafish embryos suggest a chemotactic movement [42]. Interesting new evidence from zebrafish indicates that G protein-coupled receptor (GPCR) signaling, which is a hallmark of chemotaxis in other systems, plays an important role during vertebrate gastrulation. Forward and reverse genetic studies have implicated *Agtr1b*, a GPCR associated with heart physiology in humans, in heart progenitor movement. During gastrulation, *agtr1b* is expressed in the lateral plate mesoderm, whereas expression of its ligand, *Apelin*, is confined to the midline. Reduced or excess *Agtr1b* or *Apelin* function impaired C&E movements particularly of the cardiac precursors, and led to heart deficiencies, with little effect on other organs [•43,•44]. Further evidence for the involvement of GPCRs in gastrulation comes from studies of *miles apart (mil)*, encoding Edg5, a sphingosine-1-phosphate (S1P) receptor previously implicated in fusion of bilateral heart primordia in zebrafish and in cytoskeletal rearrangements, cell motility and adhesion in several cell types [45]. During gastrulation, *Mil* regulates motility, cohesion and polarization of migrating prechordal mesoderm cells (Tada, M, CPH, unpublished observations). These studies suggest an interesting model of vertebrate gastrulation where the pathways discussed above regulate gastrulation movements of all or individual germ layers to sculpt the body plan, whereas GPCR signaling regulates movements of discrete cell populations that establish organ rudiments. Consistent with this notion, numerous chemokine GPCRs are expressed during gastrulation in zebrafish (Wu, S., Lin, F. and LSK, unpublished observations).

However, some GPCRs are likely to have more global effect on cell movement. For example prostaglandin E2 signaling, at least in part via the EP4 receptor, is essential for epiboly, C&E and probably also internalization in zebrafish [46]. Moreover, studies in *Xenopus* have established a general role for GPCR signaling (receptors for lysophosphatidic acid and an orphan GPCR, Xflop) in the regulation of cortical actin assembly underlying the shape and rigidity of the early embryo, by controlling cell-surface cadherin expression [•47].

Outlook and open questions

Progenitors of the different germ layers not only show distinct gene expression profiles, but also exhibit pronounced differences in their morphogenetic behaviors. Ectoderm progenitors exhibit a tightly packed pseudo-epithelial organization and primarily undergo cell-cell intercalations, whereas mesoderm and endoderm progenitors migrate as more loosely associated mesenchymal cells. Recently, endoderm and mesoderm progenitors in zebrafish have also been suggested to possess different movement behaviors with mesoderm showing directed cell migration [43] and endoderm exhibiting a “random walk” behavior [••48]. The induction of different germ layer progenitor cell fates critically depends on Nodal signaling with the highest levels specifying endoderm, while lower levels or no Nodal signaling leading to mesoderm and ectoderm specification, respectively [27]. This points at the possibility that Nodal signaling directly controls the specific movement behaviors of germ layer progenitors in addition to its cell fate induction activity. The observations that Nodal signaling regulates the internalization of mesoderm progenitors by modulating Cadherin endocytosis and/or recycling [32] and influences germ layer formation and organization by modulating actomyosin dependent progenitor cell cortex tension [••33], clearly supports this assumption. However, progenitor cell behavior might also be influenced by spatial organization of the forming germ layers. Mesoderm progenitors, for example are positioned between forming endoderm and ectoderm germ layers and use the overlying ectoderm germ layer as a substrate for migration [49]. In contrast, endoderm progenitors in zebrafish move in close proximity to the yolk cell [48,50]. Future studies analyzing the contribution of intrinsic and extrinsic factors in germ layer progenitor cell movement will be needed to understand the basis for differential progenitor cell behavior.

As anticipated, the emerging mechanisms underlying the coordination of cell fate specification and cell movements during vertebrate gastrulation are complex. Clearly the major regulators of gastrulation, the Nodal, BMP, Wnt and FGF signaling pathways, can regulate both cell fates and movements. As far as Wnt signaling is concerned, the situation may be relatively simple with the canonical pathway triggering two parallel mechanisms that specify cell fates and regulate cell movements (Stat3 phosphorylation) [15], and the Wnt/PCP pathway directly controlling morphogenetic cell movements and influencing cell fate only indirectly [23–26]. In contrast, BMP, Nodal and FGF signaling appear to regulate both cell fates and movements. What determines the balance between patterning versus morphogenetic function of these signaling pathways? As discussed above, higher levels of FGF signaling might be needed for mesodermal cell migration than for fate specification [•38]. In the zebrafish paraxial mesoderm, different thresholds of BMP signaling induce expression of the cell fate determinant *myoD* and of *wnt5*, a regulator of cell movement [6]. Indeed, the idea of regulatory interactions between signaling pathways that control both cell fate and movements and those that primarily regulate cell movements provides one attractive mechanism for the separation of the two functions. Thus, regulation of the expression of Wnt/PCP components by BMP could coordinate C&E movements with dorso-ventral patterning. Moreover, Wnt/PCP signaling must also be linked to anteroposterior patterning, as some PCP components in mediolaterally polarized cells in fish gastrulae are enriched at the anterior cell edges (Prickle) [51], whereas others (Dsh and Daam1) are enriched at the posterior cell edges [20,31].

Further confirmation and elaboration of the distinct mechanisms used by BMP or FGF to regulate cell fate specification and movement will require identification of their downstream targets in these processes. If, indeed, these major signals trigger parallel pathways to regulate both cell fate and movement behaviors, how are the two coordinated? Do checkpoints, similar to those ensuring coordinate progress of cell cycle events, operate during gastrulation to orchestrate specification of cell fates and movements?

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Figure 1. Relative timing of cell fate specification and morphogenetic events during early zebrafish morphogenesis. The roles of the major signaling pathway in cell fate specification and morphogenetic events are indicated.