Cell. Mol. Life Sci. (2009) 66:2263–2273
DOI 10.1007/s00018-009-0018-2

Cellular and Molecular Life Sciences

REVIEW

Molecular basis of morphogenesis during vertebrate gastrulation

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Received: 16 January 2009 / Revised: 23 February 2009 / Accepted: 6 March 2009 / Published online: 4 April 2009

Abstract Gastrulation is a crucial step in early embryogenesis. During gastrulation, a set of morphogenetic processes takes place leading to the establishment of the basic body plan and formation of primary germ layers. A rich body of knowledge about these morphogenetic processes has been accumulated over decades. The understanding of the molecular mechanism that controls the complex cell movement and inductive processes during gastrulation remains a challenge. Substantial progress has been made recently to identify and characterize pathways and molecules implicated in the modulation of morphogenesis during vertebrate gastrulation. Here, we summarize recent findings in the analysis of signaling pathways implicated in gastrulation movements, with the aim to generalize the basic molecular principles of vertebrate morphogenesis.

Keywords Gastrulation · Morphogenesis · Convergent extension · Cytoskeleton · Cell adhesion · Protocadherin

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Introduction

"It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life." Lewis Wolpert (1986).

Vertebrate embryogenesis is a fundamental process in which various aspects of cellular activities including proliferation, division, cell fate determination, apoptosis, cell movement, and cell communication are delicately orchestrated. After induction of the germ layers, the blastula is transformed by gastrulation movements into a multilayered embryo with head, trunk, and tail rudiments. The Spemann organizer formed on the dorsal blastopore lip at the early gastrula stage is critical to the initiation of gastrulation. This important signaling center patterns the germ layers and regulates gastrulation movements. During the internalization process, cells of the mesendoderm move through the blastopore under the ectoderm. Epiboly movements expand and thin the prospective ectoderm. Convergence movements narrow the involuting mesoderm mediolaterally while extension movements elongate them from head to tail. Despite different morphologies, parallels emerge in different vertebrate species with respect to the cellular and molecular mechanisms of gastrulation. Patterns of gastrulation movements and the underlying regulatory pathways are conserved from fish to mammals [1].

Haeckel coined the term gastrulation, derived from the Greek word "*gaste*" (meaning stomach or gut), to describe a set of morphogenetic processes that transform the rather unstructured early embryo into a gastrula with several specific characteristics: (1) the three primary germ layers including ectoderm, endoderm, and mesoderm are formed; (2) the basic body plan is established, including the physical construction of the rudimentary primary body axes; and (3) the cells assume new positions, allowing them to

interact with cells that were initially not close to them. This is the basis for inductive interactions, which are hallmarks of neurulation and organogenesis.

To elucidate the mechanism that controls the complex cell movement and inductive processes during gastrulation has remained a major challenge for developmental biologists for over a century. The application of state-of-the-art forward and reverse genetic techniques helps us identify a growing number of signaling pathways and molecules and elucidate detailed molecular mechanisms underlying the morphogenetic events during gastrulation. Here we will highlight recent findings mainly in the *Xenopus* embryo with the aim to generalize the basic molecular principles of vertebrate gastrulation morphogenesis.

Morphogenetic movements in gastrulation

During gastrulation, cell movements reorganize the embryo from a simple spherical ball of cells, the blastula, into a multilayered organism. Many cells move to a new, more interior location, and the three primary germ layers are formed and organized in their proper locations during gastrulation. Ectoderm, the outer germ layer, gives rise to the epidermis and nervous system. Endoderm, the inner germ layer, forms the mucous membrane lining digestive and respiratory tract and digestive glands. Mesoderm, the middle germ layer, gives rise to skeletal, muscle, blood, bone, and connective tissues.

Although the details of gastrulation differ among different species, the cellular mechanisms involved in gastrulation are common to all animals. Embryos use a limited set of morphogenetic cell behaviors, but they employ them in different combinations, in different geometric and mechanical contexts, and with different timings [2]. As a result, during gastrulation a series of changes in cell motility, cell shape and cell adhesion occur and represent as four major modes of morphogenetic behaviors including bending of epithelial cell sheets, rearrangement of cells within sheets, dissociation of cells from sheets, and individual cell migration [3]. These four types of behaviors are often combined and integrated in diverse cell movements such as invagination, ingression, involution, epiboly, intercalation, convergent extension (CE) movements, and tissue separation. Since the focus of this review is on the molecular aspects of gastrulation, below we will only describe CE movements and tissue separation as two models of gastrulation morphogenesis since they have been studied relatively extensively and are understood better.

Convergent extension (CE) movements

CE movements are considered as the main driving force of *Xenopus* gastrulation [4]. At the onset of CE movements,

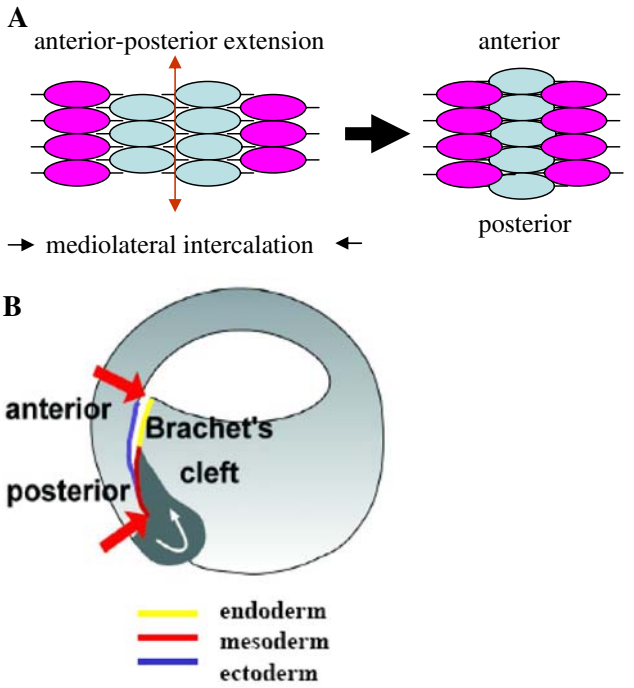

Fig. 1 Convergent extension movements and tissue separation in gastrulation. **a** Convergent extension involves polarization of cells with lamellipodia oriented mediolaterally ("-" at both ends of the cells represents these lamellipodia). As a result, cell intercalation leads to convergence along the mediolateral axis while extension occurs along the perpendicular anterior–posterior axis. **b** Tissue separation in *Xenopus* gastrula. Mesendodermal and ectodermal cells separate from each other and Brachet's cleft is formed between the ectoderm and the mesendoderm

the previously randomly oriented cell protrusions get polarized and stabilized along the mediolateral axis, forming the motile structures called lamellipodia [2]. These lamellipodia may serve as the driving force for cell intercalation, which results in the convergence of tissues along the mediolateral axis while extension occurs along the perpendicular anterior–posterior axis (Fig. 1a). CE movements are fundamental to the establishment of morphological and functional polarity of our body, with the head on one end and the tail on the other [5].

Tissue separation

The formation of tissue boundaries in the embryo is essential for the establishment of the body plan and the formation of organs. Tissues have to develop separation behaviors to prevent cells from mixing and to define borders between different groups of cells. In the amphibian blastula, a thin blastocoel roof (BCR) is formed as a wall enclosing the blastocoel cavity and most of the BCR will form ectoderm in later stages. During gastrulation, mesendodermal cells move as a coherent mass toward the animal pole and BCR serves

as the substrate for their translocation. Although BCR and internalized mesendoderm are in direct contact during the process, they do not fuse into a single cell mass but maintain a stable interface as a prerequisite for the movement of the two tissues past each other [6]. In other words, they display tissue separation behaviors. As a result of the separation, a visible cleft called Brachet's cleft is formed between the mesendoderm and the ectoderm (Fig. 1b).

Molecular basis of gastrulation movements

What is the molecular basis of the various gastrulation movements? Over the past 20 years a range of molecules and pathways have been identified that are implicated in the regulation of morphogenetic cell behaviors. Two key concepts emerge when summarizing these new findings: (1) Complex regulatory networks: Several classes of molecules contribute to the regulation of gastrulation movements in vertebrates. These include classic signaling pathways such as Wnt, Nodal, bone morphogenetic protein (BMP), and fibrobrast growth factor (FGF); transcriptional factors such as Brachyury and Snail; adhesion molecules including catenin, cadherin, and protocadherin; extracellular matrices such as fibronectin, cyr61, and syndecan; regulators of cytoskeleton including Rho, Rac, cdc42, and JNK; receptors such as ErbB epidermal growth factor receptor and G protein-coupled receptors; axon guidance molecules such as ephrin and slit; and even molecules involved in endocytosis including dynamin, Rab5, and μ2-adaptin. This fact emphasizes the complexity of gastrulation movements and indicates that diverse molecules have to be engaged to make sure that these movements go through perfectly. (2) Conservation of signaling modules: Despite the difference in morphology, different vertebrate species employ similar molecules and signaling pathways in gastrulation movements, showing the conserved aspect of vertebrate gastrulation movements. It is out of the scope of this review to comprehensively cover all the excellent work in this field. Instead we focus on molecular regulatory mechanisms underlying CE movements and tissue separation and intend to include the extensively studied signaling pathways such as Wnt, FGF, and BMP, and well established mediators of cell movement such as cytoskeleton, extracellular matrix, and cell adhesion systems. Moreover, we highlight new insights on the role of endocytosis in gastrulation and discuss how endocytosis is intertwined with signaling-pathway and cell-adhesion systems in gastrulation morphogenesis.

Wnt signaling

Wnt signaling is highly conserved in complex eukaryotes ranging from *Cnidaria* to humans. It is essential for development in that it regulates a variety of processes such as cell proliferation, cell differentiation, cell polarity, and cell migration. Wnt signaling is mediated by the canonical Wnt/β-catenin-dependent pathway and noncanonical Wnt pathways such as planar cell polarity (PCP) and Wnt/Ca^{2+}. Noncanonical Wnt pathways are β-catenin independent and play crucial roles in the regulation of gastrulation movements [7]. The first hint that Wnt signaling is involved in CE movements came from the observation that Xwnt5 overexpression blocks CE movements in *Xenopus* [8]. Furthermore, dominant negative (DN) Wnt11 blocks CE movements in *Xenopus*, and CE defects can be rescued by deleted form of Dsh that functions only in the PCP pathway, but cannot be rescued by components of canonical Wnt signaling such as β-catenin [9]. Similarly, *slb*/*Wnt11* mutant zebrafish displayed CE defects that are selectively rescued by Dsh constructs that activate PCP signaling, but not by activation of canonical Wnt signaling [10]. Later it was shown that vertebrate homologues of *Drosophila* PCP gene Stbm/Vang play an important role in CE movements during gastrulation in *Xenopus* and zebrafish [11]. Interestingly, PCP components Prickle and Dsh are localized asymmetrically in the dorsal mesoderm to bias cell intercalations during CE movements in zebrafish [12]. Recent mouse embryo studies revealed that Vangl2/Lp is also essential for CE movements in chordamesoderm during mammalian gastrulation [13]. All these data demonstrate that PCP is a highly conserved pathway that regulates CE movements in vertebrates.

Although no genetic evidence in zebrafish exists yet to demonstrate the engagement of the Wnt/Ca^{2+} pathway in CE movements, it was shown that Cdc42 acts downstream of the Wnt/Ca^{2+} signaling pathway involving PKC activation to regulate CE movements in *Xenopus*, and importantly, Cdc42 does not seem to act downstream or upstream of Dsh [14], strongly indicating that this pathway is different from the PCP pathway that involves Dsh. Furthermore, G$\beta\gamma$ was shown to signal downstream of Wnt-11/xFz7 and upstream of PKC to regulate Cdc42 activity and play a role in CE movements in *Xenopus* [15]. These two studies provide strong evidence that Wnt/Ca^{2+} pathway regulates CE movements in *Xenopus*. Wnt/Ca^{2+} signaling is also implicated in the regulation of tissue separation [16]. Loss of Fz7 function led to a defective separation of mesodermal and ectodermal germ layers and this defect could be rescued by PKC but not by Dsh, Cdc42, β-catenin, or Tcf3. Furthermore, tissue separation behavior was blocked by heterotrimeric G protein inhibitor pertussin toxin and could be rescued by PKC but not by Fz7 [16]. These results clearly showed that Fz7 mediates tissue separation via the Wnt/Ca^{2+} branch distinct from the Wnt/PCP or Wnt/β-catenin branch.

FGF signaling

FGF signaling plays important roles in embryogenesis. In *Xenopus* embryos, FGF signaling impacts CE movements both directly and indirectly. In a direct manner, FGF signaling may crosstalk with the PCP pathway to regulate CE movements. FGF signaling activates PLCγ to produce IP3 and DAG. DAG leads to recruitment of PKCδ to the membrane, where it activates Dsh and regulates CE movements by PCP pathway [17]. Sproutys are induced by FGF signaling early in gastrulation and inhibit both calcium release and PKCδ translocation, therefore blocking FGF-mediated CE movements but permitting FGF-mediated cell specification. However, in late gastrulation and neurulation stages, the expression of Sproutys declines and expression of Spred (Sprouty-related proteins) increases dramatically, blocking cell specification while permitting mesodermal CE movements induced by FGF. In this way, FGF signaling fine-tunes both cell fate and cell motility in the same cell [18].

In an indirect manner, FGF signaling regulates the induction and maintenance of Xbra, which functions as a switch to promote CE movements and inhibit cell migration [19]. Furthermore, Xbra as a transcription factor directly induces Xwnt11 and prickle [20], both of which regulate CE movements by PCP pathway. *Xenopus* marginal coil (Xmc), another gene induced by FGF signaling, regulates CE movements with no impact on mesoderm induction or maintenance per se [21]. The FGF target gene neurotrophin receptor homolog (NRH) regulates the protrusive activity necessary for CE movements [22]. NRH activates GTPases including Rho, Rac, and Cdc42 as well as the cascade of MKK7-JNK independently of Dsh, suggesting that NRH signaling interacts with the PCP pathway downstream of Dsh [23].

BMP and nodal signaling

A ventral to dorsal gradient of BMP activity is established under control of the Spemann organizer, and this gradient coordinates cell fate determination with morphogenetic movements during early embryogenesis [24]. In *Xenopus*, high BMP activity blocks extension of dorsal mesodermal explants, whereas DN BMP receptor instigates ectopic CE movements of ventral mesoderm explants [25]. In zebrafish, the BMP activity gradient has been shown to play an instructive role in determining domains of distinct CE movements, possibly in parallel with, rather than downstream of cell-fate specification [26]. High ventral BMP activity levels inhibit CE movements and specify the NCEZ (no convergence, no extension zone); decreasing BMP activity levels in the lateral gastrula increase CE movements; and dorsally, low BMP activity promotes substantial extension with limited convergence. The three morphogenetic domains are reduced or expanded in mutants with excess or deficit of BMP activity, supporting the roles of BMP in regulation of CE movements [24]. Mechanistically, different BMP activity thresholds might regulate genes that mediate cell movement behavior and fate specification. For example, high BMP activity negatively regulates the expression of Wnt11 and Wnt5a, therefore limiting CE movements [24].

The nodal class of TGFβ plays a critical role in early vertebrate development, essential for the establishment of mesodermal and endodermal lineages and cell movements involved in gastrulation. Xnr3 is a special nodal-related protein in *Xenopus* in that it is structurally different from other Xnrs, and, uniquely among them, induces protrusions when ectopically expressed. This led to the investigation of its role in CE movements during embryogenesis. Loss of Xnr3 function led to CE defects in embryos and explants. Moreover, Xnr3 requires the FGF receptor FGFR1 to activate Xbra expression and induce CE movements [27]. This finding demonstrates the crosstalk of Nodal and FGF signaling in morphogenesis. Another recent study found that nodal-induced notochord-somite tissue boundary is essential for the cell polarity and cell alignment that drive CE movements in *Xenopus* [28].

Endocytosis

Signaling and endocytosis are inseparable companions. On one hand, endocytosis downregulates signaling initiated at the membrane. On the other hand, recently endocytosis has been shown to play an active role in signal transduction, especially in terms of regulating the activity and distribution of developmental signals. For example, endocytosis of delta, the notch ligand, is required for signal activation. In Hedgehog signaling, endocytic trafficking segregates an inhibitory receptor (Patched) from the positive effector (Smoothened). Endocytosis also powers the transport of morphogens along epithelia [29]. Unfortunately, how endocytosis is engaged in morphogenetic movements is seldom addressed, although a variety of signals have impact on gastrulation morphogenesis as described above. Nevertheless, it is encouraging that recent reports demonstrated that endocytosis of Fz and Dsh play an active role in PCP signaling to regulate CE movements [30, 31]. The interaction of Dsh with μ2-adaptin, a subunit of clathrin adaptor AP-2, is required to engage activated Fz4 with the endocytic machinery for its internalization. Interestingly, AP-2-mediated Fz4 endocytosis plays a specific role in PCP signaling with no effect on the canonical Wnt/β-catenin pathway [30]. Via the interactions with both Wnt11 and β-arrestin2,

Ryk cooperates with Fz7 to mediate Wnt11-induced endocytosis of Dsh and actively transduces the noncanonical Wnt signal in CE movements in *Xenopus* [31]. Thus, gastrulation movements are regulated by endocytosis of Fz and Dsh via the engagement of endocytic molecules such as AP-2 and arrestin. Along this line, it was observed earlier that DN dynamin, a GTPase essential for clathrin-mediated endocytosis, significantly blocked the elongation of animal cap explants induced by activin, accompanied by inhibition of C-cadherin endocytosis. It is proposed that dynamin-dependent endocytosis of C-cadherin is crucial in remodeling adhesive contacts during CE movements [32]. But taking into account the new results, we can assume that the effect of dynamin on CE movements is mediated not only by C-cadherin endocytosis, but also by endocytosis of other molecules such as Fz and Dsh. In another interesting study, Wnt11 was shown to regulate the cohesion and migration of mesendodermal progenitor cells during zebrafish gastrulation by modulating endocytosis of E-cadherin through Rab5c, another GTPase engaged in early endocytosis [33]. It is expected that a more detailed mechanism by which endocytosis controls gastrulation morphogenesis will be revealed in the near future.

Cytoskeleton

Cytoskeleton forms a complex and dynamic network that plays a critical role in the establishment and maintenance of cell and embryonic polarity, cell shape, cell adhesion, and cell motility. The Rho family of GTPases including Rho, Rac, and Cdc42 are good candidates to be mediators of morphogenetic events in gastrulation due to their ability to regulate the cytoskeleton remodeling underlying cell motility and shape changes. Rho mediates formation of stress fibers, contractile microfilaments bundles spanning the cell, and focal adhesions, the attachments of stress fibers to the substrate. Rac mediates formation of lamellipodia, flattened protrusions important in cell motility. Cdc42 mediates cell polarity and formation of filopodia, thin protrusions that mediate cell motility and contact interactions [34]. In *Xenopus* embryos, Rho GTPases are expressed in tissues undergoing extensive morphogenesis and are activated downstream of noncanonical Wnt signaling during gastrulation. The picture of how Wnts mediate cytoskeleton remodeling to control gastrulation movements emerges with the identification of an expanding number of effectors that modulate the cytoskeleton (Fig. 2).

WGEF, a guanine nucleotide exchange factor (GEF), was identified to form a complex with Dsh and Daam1 and activate RhoA specifically. WGEF can rescue CE movements impaired by DN Wnt11, providing the missing link between Dsh/Daam1 and Rho activation in PCP signaling [35]. xNET1, another RhoA-specific GEF, was found to impair CE movements when overexpressed. Although xNET1 co-immunoprecipitated with Dsh, its localization in animal caps was not changed upon the activation of PCP signaling [36]. Thus, upstream events other than Wnts may regulate the activity of xNET1 to activate RhoA. Eps8 acts as a scaffold to promote the formation of complexes including Abi1 that are essential for Rac activation and Rac-dependent actin remodeling and membrane ruffling [37]. Interestingly, Eps8 was shown to recruit Dsh to actin filaments and cell membrane in *Xenopus* and impair CE

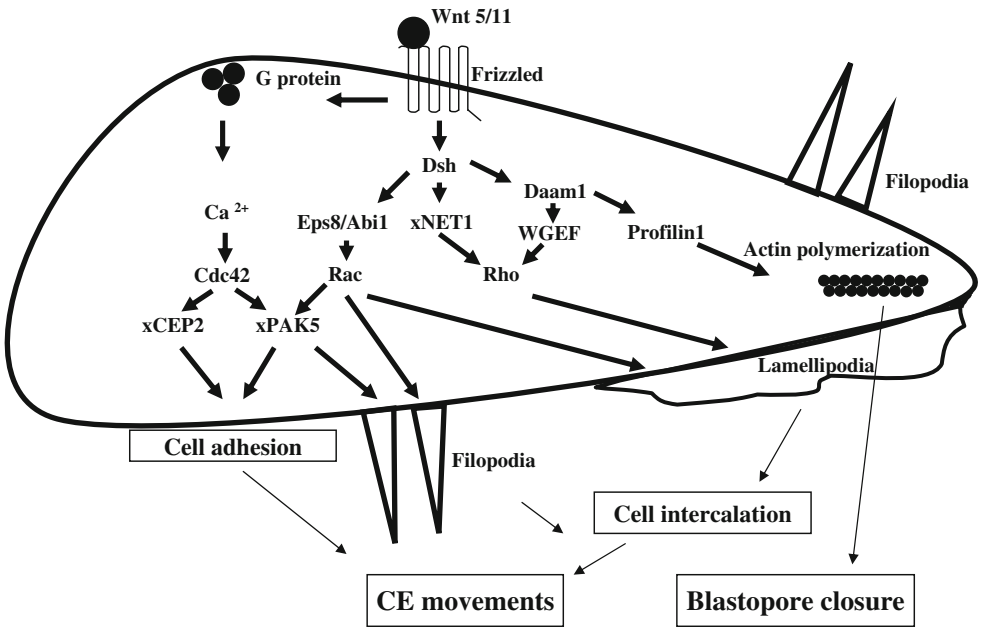

Fig. 2 Cytoskeleton remodeling mediated by Wnt and their effectors during gastrulation movements in *Xenopus*. See text for details

movements [38]. Dsh can activate both RhoA and Rac while Daam1 is required for Dsh-mediated RhoA but not Rac activation [39]. Therefore, Eps8 may provide a crucial link between Dsh, Rac, and the actin cytoskeleton during gastrulation. That Rho and Rac are activated via a different branch downstream of Dsh is in accordance with the distinct and overlapping roles that Rho and Rac play in cytoskeleton remodeling necessary for CE movements. Rac is important for filopodia formation along the elongate sides of intercalating mesoderm cells, while Rho regulates their bipolar morphology. Both Rac and Rho contribute to the mediolateral extension of tractive lamellipodia [40].

Cdc42 is not activated by PCP pathway [41], but is activated by Wnt/Ca^{2+} pathway and functions in gastrulation by regulating Ca^{2+}-mediated cell adhesion [14, 16]. *Xenopus* Cdc42 effector protein 2 (XCEP2) and *Xenopus* p21-activated kinase 5 (X-PAK5) were identified to act downstream of Cdc42 to modulate Ca^{2+}-mediated cell–cell adhesion, therefore balancing the need for tissue integrity and plasticity during the dynamic cellular rearrangements of gastrulation [42, 43]. Recently, Profilin1 was found as an interacting partner of Daam1, and it is localized with Daam1 to actin stress fibers in response to Wnt signal. Inhibition or depletion of Profilin1 inhibited stress fiber formation and specifically inhibited blastopore closure but not CE movements, tissue separation, or neural fold closure in *Xenopus* [44]. Taken together, it seems that different effectors downstream of Wnt mediate different aspects of cytoskeleton reorganization both independently and cooperatively and contribute to the coordination of gastrulation movements (Fig. 2).

Extracelluar matrix

Extracelluar matrix (ECM) consists of collagen, proteoglycans, and a variety of glycoproteins secreted by cells. Cell adhesion, cell migration, and the formation of epithelial sheets and tubes all depend on the ability of cells to form attachments to ECM. In some cases, as in the formation of epithelia, these attachments have to be extremely strong. In other instances, as in the migration of cells, attachments have to be made, broken, and made again. In some cases, ECM serves as a permissive substrate to which cells can adhere, or upon which they can migrate. In other instances, ECM provides the direction for cell movements or the signals for a development event. Therefore, it is not surprising that ECM plays an important role in morphogenesis. Here we just highlight the mechanisms by which ECM modulates CE movements in gastrulation (Fig. 3).

In *Xenopus* embryos, Integrin–ECM interaction modulates cadherin-mediated cell adhesion and is required for mediolateral cell intercalation behaviors that drive CE movements [45]. It was demonstrated recently that ECM component fibronectin may play an instructive role in the coordination of protrusive activity underlying CE movements [46]. Cyr61, a CCN-family, secreted, heparin-binding ECM-associated protein, is an important regulator of CE movements in *Xenopus* by assembling ECM to regulate cell–cell and cell–matrix adhesion and modulating Wnt signaling [47].

Heparan sulphate proteoglycans (HSPG) are abundant molecules in ECM, consisting of a protein core to which

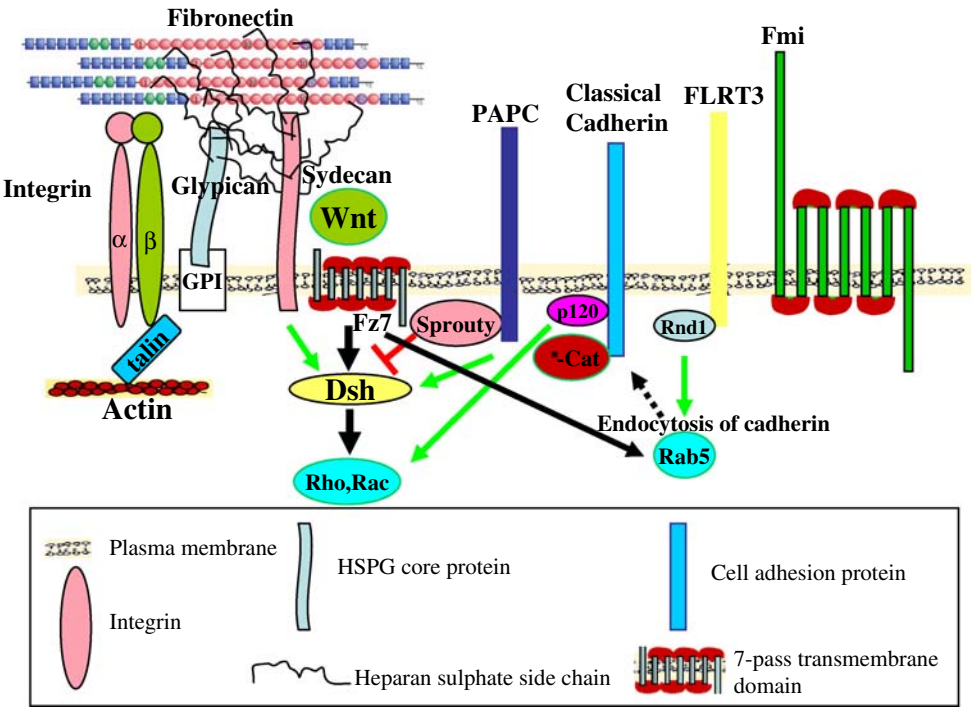

Fig. 3 Extracellular matrix and cell adhesion molecules initiate downstream signaling that modulates gastrulation morphogenesis. *Black arrows* represent the main Wnt signaling cascade that drives morphogenesis, *green arrows* represent positive modulation of this cascade, while *red arrow* represents negative modulation of this cascade. *Black dashed arrow* represents modulation of cadherin endocytosis by Rab5

heparan sulphate glycosaminoglycan (GAG) chains are attached. Glypicans and syndecans represent the two main cell-surface HSPGs. Glypicans are linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor whereas syndecans are type I transmembrane proteins with up to five GAG attachment sites. In mammals, six glypican and four syndecan genes have been identified, and they are of interest in the context of morphogen gradient formation [48]. Glypican Knypek potentiates Wnt11/PCP signaling to regulate CE movements in zebrafish [49]. *Xenopus* glypican 4 (Xgly4, *Xenopus* ortholog of *kny*) physically binds Wnt ligands and Fz7 and likely functions as a co-receptor to modulate CE movements [50]. *Xenopus* syndecan-4 (xSyn4) was also shown to regulate CE movements by interacting with Fz7 and Dsh to recruit Dsh to the plasma membrane, resulting in the activation of the PCP pathway. Importantly the recruitment of Dsh by xSyn4 is regulated by fibronectin [51]. Therefore, a model was proposed in which xSyn4 and fibronectin cooperate with xFz7 and Wnt in the specific activation of PCP pathway. In this aspect, xSyn4 functions as a co-receptor for PCP signaling, similar to Xgly4 as described above (Fig. 3).

Cell adhesion molecules

The formation, maintenance, and turnover of adhesion between cells are crucially involved in all morphogenetic events. A plethora of cell adhesion molecules control these adhesive contacts between cells. Among them, cadherins are major players for dynamic regulation of adhesive contacts associated with diverse morphogenetic processes. Perhaps the large size and the structural and functional diversity of the cadherin family members have evolved to allow different cell interactions necessary for tissue morphogenesis in complex organisms [52]. Cadherins are a superfamily of membrane proteins characterized by the presence of extracellular cadherin (EC) repeats in the extracellular domain. Different cadherins are classified into several subfamilies by the gross organization of their EC motifs and sequence similarities in their extracellular and cytoplasmic domains. These subfamilies of cadherins and their functions in gastrulation morphogenesis are described below (see also Fig. 3).

Classic cadherins

Classic cadherins were identified first among the cadherin superfamily (so named as "classic") and are defined by their characteristic cytoplasmic sequence for binding to catenins. More than 20 different classic cadherin subtypes found so far play key roles in a variety of morphogenetic processes by mediating cell sorting, coordinated cell movements, and planar cell division [52]. During gastrulation, modulation of adhesion plays a major role in cell rearrangements within cell sheets including CE movements and in epithelial mesenchymal transitions. Modulation of the adhesive function of C-cadherin is engaged in CE movements in *Xenopus* [45]. Cadherins are also involved in the tissue separation of germ layers in *Xenopus* [6].

The function of classical cadherins is modulated by a group of cytoplasmic proteins called catenins that interact with the cadherin intracellular domain. A major role of catenins is to anchor the cadherin complex to the actin cytoskeleton. β-catenin and plakoglobin (γ-catenin) bind the C-terminal region of cadherin, acting as bridges connecting E-cadherin to α-catenin, which in turn associates with actin filaments directly or indirectly. β-catenin also has signaling roles in the Wnt/β-catenin pathway. In contrast, p120 catenin subfamily members including p120 and armadillo repeat gene deleted in velo-cardio-facial syndrome (ARVCF) bind the juxtamembrane region of cadherin. p120 regulates cell adhesion and motility positively or negatively depending on its effects on cadherin stability and clustering or on small GTPases and cytoskeleton. p120 and ARVCF inhibit RhoA activity as a guanine nucleotide dissociation inhibitor (GDI) but activate Rac1 and Cdc42 through interaction with Rho GEF VAV2 [53]. p120 and ARVCF are essential for CE movements in *Xenopus*, perhaps resulting from the modulation of cadherin and Rho GTPase activity [52]. Moreover, p120 can bind transcriptional repressor Kaiso and relieve repression of Kaiso target genes such as Wnt11, contributing to gastrulation movements indirectly by upregulation of PCP pathway [54]. Thus, p120 acts as a double-edged sword to regulate morphogenesis by modulating not only adhesion but also gene expression, in a way similar to β-catenin.

Protocadherins

Protocadherins make up the largest subfamily of cadherins, expressed mostly in but not limited to the nervous system. Compared with classic cadherins, protocadherins have six to seven EC repeats in the extracellular domain and no catenin-binding sites in the intracellular domain. Much less is known about the function of protocadherins, but increasing evidence demonstrates their roles in tissue morphogenesis and neural development [55]. Here we focus on the roles of *Xenopus* protocadherins in gastrulation morphogenesis since the developmental roles of protocadherins are mainly investigated using *Xenoups* as a model [56]. Nevertheless, how their orthologs function in other species will be discussed where data are available.

Axial protocadherin

Axial protocadherin (AXPC) is expressed exclusively in the axial mesoderm notochord. At the tailbud stage, it is also expressed in the pronephros, somites, heart, optic vesicle, otic vesicle, and distinct parts of the brain. AXPC is necessary and sufficient for prenotochord cell sorting in the gastrulating embryo. Importantly, cell sorting requires extracellular domain, implying that EC-mediated cell adhesion is involved in cell sorting [57]. Protocadherin-1 (Pcdh1) is the mammal ortholog of AXPC. In transfected cells, Pcdh1 localizes to the cell–cell junction and mediates weak homophilic cell–cell adhesion. Except for mediating cell–cell adhesion, no other function of AXPC or Pcdh1 has been reported yet.

Paraxial protocadherin

Paraxial protocadherin (PAPC) incites more research interest, perhaps due to its initial specific expression in the Spemann organizer. It is expressed in the dorsal marginal zone at gastrula and in paraxial mesoderm at a later stage. Interestingly, PAPC is expressed in a complementary pattern to AXPC, and this pattern is important for boundary formation and sorting of cells into the paraxial (PAPC) and axial (AXPC) mesoderm that form the somites and notochord, respectively [58, 59]. Surprisingly, PAPC expression is regulated strongly by both the maternal β-catenin and nodal-related signaling in gastrulation [60], highlighting the important role that PAPC may play in gastrulation movements. Indeed PAPC has been revealed to have multiple functions in a variety of developmental systems. During *Xenopus* gastrulation, the extracellular domain of PAPC may mediate cell sorting but in an indirect manner by modulating C-cadherin adhesion through an unknown mechanism [61], while the intracellular domain is implicated in the regulation of CE movements and tissue separation [58, 62, 63]. Importantly, the regulation of CE movements and tissue separation by PAPC depends on the signaling function of PAPC to modulate the activity of Rho GTPase and JNK and may involve the interaction of PAPC and Fz7 ectodomains [62, 63].

A recent study revealed the first direct link between PAPC and PCP pathway [64]. In this study it was established that Sprouty is a negative regulator of the PCP pathway by inhibiting Dsh membrane recruitment and RhoA activation, explaining the previously described inhibition of CE movements by Sprouty [18]. The cytoplasmic domain of PAPC sequesters and inhibits Sprouty, relieving the inhibitory effect of Sprouty on PCP pathway [64]. Thus, this new finding provided mechanistic insight into how PAPC promotes CE movements and tissue separation. Interestingly, another study demonstrated that a novel FGF target, ANR5, mediates downstream signaling of PAPC to regulate CE movements and tissue separation in *Xenopus* [65]. PAPC is also required for CE movements during gastrulation in zebrafish; in this context, PAPC is a downstream target of spadetail, a transcription factor required for mesodermal morphogenetic movements [59].

It is not currently well established whether the function of PAPC in gastrulation is conserved in mammals. The putative mammalian PAPC ortholog, Pcdh8, while expressed in the primitive streak, paraxial mesoderm, somites, and CNS, does not have a significant loss-of-function phenotype as the authors observed [66]. But another study suggested that PAPC regulates gastrulation movements in mice. Lim1 is a transcriptional factor that promotes PAPC expression in both mice and frogs. *Xenopus* embryos depleted of Lim1 lack anterior head structures and fail to form a proper axis as a result of gastrulation-movements defects. Similar disruption of cell movements in the mesoderm is also observed in Lim1 knockout mice. PAPC expression is lost in the nascent mesoderm of Lim1 knockout mouse embryos and in the organizer of Lim1-depleted *Xenopus* embryos. Importantly the defects caused by loss of Lim1 can be rescued to a considerable extent by supplying PAPC exogenously [67]. Therefore it is likely that Lim1 and its downstream target, PAPC, function in gastrulation movements in both *Xenopus* and mammals. In conclusion, PAPC represents a link between regulatory genes in the Spemann organizer and the execution of cell movements during morphogenesis.

Protocadherin in neural crest and somites

Recently a novel protocadherin was identified in *Xenopus* that is initially expressed in the mesoderm during gastrulation, followed by expression in the cranial neural crest (CNC) and somites. Therefore, it is named Protocadherin in neural crest and somites (PCNS). PCNS shares 65% amino acid identity with *Xenopus* PAPC and 42–49% amino acid identity with Pcdh 8 in human, mouse, and zebrafish genomes. Overexpression of PCNS resulted in gastrulation failure but conferred little if any specific adhesion on ectodermal cells, while loss of PCNS function resulted in failure of CNC migration, leading to severe defects in the craniofacial skeleton. Somites and axial muscles also failed to undergo normal morphogenesis in these embryos. Thus, PCNS is essential for CNC migration and somite morphogenesis in *Xenopus* [68].

Atypical cadherins

Atypical cadherins are large cadherins that have great number of EC repeats in the extracellular domains. For example, Dachsous and Fat have 27 and 34 ECs,

respectively, while Flamingo is the only member of cadherins family that has seven-pass rather than single transmembrane domain (Fig. 3). These three atypical cadherins have been shown to interact with each other and regulate asymmetrical localization of Fz, therefore coordinating the polarity required for a variety of morphogenesis functions in *Drosophila* [69]. Characterization of these atypical cadherins orthologs in vertebrates is ongoing, and limited study has already shown that these atypical cadherins function in vertebrates in a similar manner. CE movements and neural tube closure are the major morphogenetic processes regulated by PCP signaling in vertebrates. Flamingo mediates CE movements during gastrulation in zebrafish [70]. Further study recently showed that in zebrafish embryos, Wnt11-induced Fz7 accumulation partially depends on Flamingo to increase cell contact persistence, but independent of Wnt11 downstream signaling via RhoA and Rok. Thus, Flamingo can interact with Fz7 and Wnt11 to modulate local cell contact persistence to coordinate cell movements during gastrulation [71]. This study for the first time showed that Flamingo performs a conserved role in PCP signaling by recruiting other PCP components to local sites on the plasma membrane to regulate cell adhesion both in the fly and in vertebrates. Interestingly, mice with Flamingo mutations exhibited failure of neural tube closure [72], and Flamingo is upregulated in the chick neural epithelium at the initiation of neural tube closure [73]. How these atypical cadherins are engaged in gastrulation movements in *Xenopus* awaits further study. But taking into account available data, it seems that atypical cadherins participate in a complex and highly conserved signaling cascade to maintain polarity in a range of tissues and coordinate morphogenetic movements during development.

Fibronectin leucine-rich repeat transmembrane 3 (FLRT3)

Fibronectin leucine-rich repeat transmembrane 3 (FLRT3) is a member of the fibronectin leucine rich transmembrane protein (FLRT) family. The protein structure of FLRT resembles small leucine-rich proteoglycans found in the extracellular matrix. FLRT3 is a type I transmembrane protein containing 10 leucine-rich repeats flanked by N-terminal and C-terminal cysteine-rich regions, a fibronectin-like domain, and an intracellular tail. Mediated by extracellular leucine-rich repeats, FLRT3 promotes cell sorting in a calcium-dependent manner [74]. A recent study elucidated a new mechanism by which FLRT3 modulates cell adhesion in CE movements in *Xenopus* gastrulation. The cytoplasmic domain of FLRT3 binds Rnd1, a small GTPase that inhibits cell adhesion. The binding promotes a dynamin-dependent endocytosis of C-cadherin. Interestingly, both FLRT3 and Rnd1 are induced by nodal signaling [75]. This scenario impressively demonstrates how signaling and cell adhesion are intertwined and fine-tuned to precisely control morphogenesis movements (Fig. 3).

Conclusions

Novel approaches in molecular biology, such as microarray and RNAi screen, as well as quantitative analysis of embryonic cell polarity and motility in vivo have substantially expanded our understanding of morphogenesis during vertebrate gastrulation. The morphogenetic processes that appear highly divergent in different species are in fact controlled by fundamentally similar molecular mechanisms. It was the aim of this review to highlight some of these recent findings and indicate possible directions of future investigations. One conclusion that we can draw is that signaling pathways that control gastrulation morphogenesis are evolutionarily conserved. Second there is substantial cross-talk between signaling pathways to orchestrate the complex spatial–temporal decision regarding cell movements as well as cell fate specification during gastrulation. Third, we should open our view when searching for novel modulators of gastrulation morphogenesis since previously unappreciated molecules have emerged as critical players in morphogenesis. With these thoughts in mind and with the emergence of new techniques and methods, we are in a position to decipher the molecular basis of gastrulation morphogenesis and contribute to the understanding of a critical process in our life history.

Acknowledgments We apologize to colleagues whose work has not been cited due to space limitations and the broad scope of this review. This work was supported by a research grant of the Deutsche Forschungsgemeinschaft (STE-613/4-1).

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