[Frontiers in Bioscience 16, 2352-2366, June 1, 2011]

The role of Wnt in cell signaling and cell adhesion during early vertebrate development

Janssens Sylvie[1,2], Crabbe Ellen[1,2], Vleminckx Kris[1,2]

[1]Department for Molecular Biomedical Research, VIB, B-9052 Ghent, [2]Department of Biomedical Molecular Biology, Ghent University, B-9052 Ghent, Belgium

TABLE OF CONTENTS

1. ABSTRACT

During embryonic development, a group of dividing blastomeres is ultimately shaped into a structured, functional organism. To achieve this goal, individual cells and groups of cells need to move to new positions, organize themselves, and differentiate into specialized cell types. In these processes, intercellular contacts and contacts between cells and their environment play critical roles. The cells interact physically via cell adhesion molecule and communicate through signaling pathways. One of the pathways active during embryonic development is the Wnt pathway. Interestingly, Wnt and cell adhesion are often active in the same processes and crosstalk between them exists by reciprocal regulation and sharing of components. In this review, we will focus on how Wnt signaling cooperates with cell adhesion to ensure smooth processing of gastrulation, somitogenesis and neurulation.

2. INTRODUCTION

Attachment of cells is mediated by cell adhesion molecules on the cell surface. To achieve strong attachment, cell adhesion molecules can be complexed in focal adhesions, adherens junctions and desmosomes, where they cluster and form links with the cytoskeleton. The disruption and reestablishment of cell contacts are indispensable for processes that guide vertebrate morphogenesis, including cell sorting, cell movements, cell migration and separation or fusion of tissues. Several of these morphogenetic events are controlled by developmental signaling pathways. The importance of Wnt signaling in vertebrate development was discovered almost 20 years ago, when it was shown that injection of Wnt RNA in *Xenopus* embryos can induce formation of a complete secondary body axis (1). Since then, Wnt signaling has been implicated in a variety of cellular

processes, such as cell proliferation, differentiation, polarity and adhesion.

3. OVERVIEW OF THE WNT PATHWAY

Nowadays, a combination of genetic, cell biological, biochemical and developmental studies has led to the discovery of a huge network of proteins that interact in the Wnt signaling pathway. This network comprises several branches that lead to different endpoints and that can interact mutually or with other signaling pathways (2). The three 'main' branches are the canonical Wnt/beta-catenin pathway and two non-canonical branches: the PCP and the Wnt/calcium pathways (for a complete schematic overview of the non-canonical Wnt pathways see (3)).

In general, all three Wnt signaling pathways are activated by Wnt glycoproteins, which are ligands for the 7-transmembrane, G-protein-coupled receptors called Frizzled (Fz). Already 19 Wnt ligands and 10 Fz receptors have been identified in humans. Formerly, Wnts have been classified as canonical (Wnt1, Wnt3a, Wnt8) or non-canonical (Wnt5a, Wnt5b, Wnt11). However, this view of complete separation is no longer supported, and it is presumed that cell context dictates the downstream signaling pathway rather than the Wnt isoform itself (4).

In the canonical Wnt signaling pathway, Fz associates with LRP5/6 co-receptors to induce phosphorylation of the cytosolic tail of LRP5/6 and formation of the LRP5/6 signalosome (5). Dishevelled (DSH) and the beta-catenin destruction complex are recruited to the plasma membrane, resulting in inhibition of the activity of glycogen synthase kinase-3beta (GSK3beta) and accumulation of beta-catenin in the cytoplasm. Consequently, beta-catenin can translocate to the nucleus, where it binds to LEF1/TCF transcription factors and acts as a transcriptional activator for Wnt/beta-catenin target genes. In the absence of Wnts, beta-catenin interacts with APC in the destruction complex, where it is terminally phosphorylated by Casein Kinase 1 alpha (CK1alpha) and GSK3beta and subsequently targeted for proteosomal degradation by ubiquitination. This keeps the cytoplasmic level of beta-catenin low, so that in the nucleus LEF1/TCF factors interact with Groucho-related molecules, and Wnt target genes are repressed (6).

The Wnt/calcium pathway induces intracellular calcium release upon interaction of Wnt with Fz and signaling by the calcium-sensitive proteins calcium/calmodulin-dependent protein kinase II (CAMKII), Protein kinase C (PKC) and Calcineurin (CNA). Downstream events are gene activation via the transcription factor NFAT, regulation of cytoskeletal dynamics via the small GTPase Cdc42, and inhibition of canonical Wnt signaling via phosphorylation of TCF factors by nemo-like kinase (NLK) (3, 7).

The Wnt/JNK pathway was first delineated in *Drosophila melanogaster*, in which it is known as the PCP (planar cell polarity) pathway. The PCP pathway determines the polarity of cells in the plane of a tissue, as illustrated by the orientation of the epithelial cells of the pupal wing or by the organization of the ommatidia of the segmented fly eye. The PCP components are strongly conserved from flies to vertebrates and they are active in many processes, such as oriented cell division, convergent extension, and directional cell migration (8). In the PCP signaling pathway, Wnts signal through Fz/Dsh and downstream components such as Daam1, RhoA and Rac1 to regulate actin skeleton modifications or to activate the JNK pathway. Other components of PCP signaling are Flamingo (Fmi/Celsr) and the Dsh binding proteins Strabismus (Stbm/Vangl2), Diego (Dgo) or Prickle (Pk) (2, 9). Wnts can also signal through the Ror2 receptor to activate JNK signaling via the Rho GTPase Cdc42 (10-12).

4. INTERACTION BETWEEN WNT AND CELL ADHESION MOLECULES

There are four major families of cell adhesion molecules: the IgG superfamily cell adhesion molecules, the integrins, the cadherins and the selectins (13). Their potential link with Wnt signaling is the most obvious for cadherins because type I cadherins depend on beta-catenin for their anchoring to the actin cytoskeleton and thus they share a component with the canonical Wnt pathway. The coordination and distribution of the beta-catenin pool between cell adhesion and Wnt signaling is contingent on the phosphorylation status of beta-catenin or its binding partners (14). Besides beta-catenin and its dual function, other components of the cadherin adhesion complex, such as p120 catenin (15) and plakoglobin (16), interact with and regulate canonical Wnt signaling. In addition, Wnt signaling regulates cadherin-mediated cell adhesion via its target genes, such as *Twist*, *Snai1* (*Snail*) and *Snai2* (*Slug*). Both *Twist* and *Snai2* are under the direct control of beta-catenin/TCF signaling; *Snai1* is probably not, but Wnt signaling indirectly leads to increased levels of Snai1 by inhibiting GSK3beta activity. Twist, Snai1 and Snai2 regulate epithelial to mesenchymal transition because all three downregulate the expression of E-cadherin by binding to the E-box elements of its promoter. In addition, Twist can also promote the upregulation of N-cadherin (for overviews on canonical Wnt signaling and cadherins see (17, 18)).

Also other cell adhesion molecules are found to interact with the Wnt pathway. The PCP component, Flamingo, belongs to the seven-pass transmembrane cadherins (19) and provides a connection between cell adhesion and Wnt/PCP signaling. Integrins can be linked to Wnt signaling via integrin-linked kinase (ILK), which directly interacts with the intracellular domain of the beta1 and beta3 integrins and is activated by integrin clustering or upon interaction of cells with components of the extracellular matrix (ECM). Both *in vitro* (20) and *in vivo* (21) evidence support the hypothesis that ILK can stimulate beta-catenin nuclear translocation and its formation of a complex with LEF1. In addition, ILK can phosphorylate and inactivate GSK3beta, which eventually results in activation of canonical Wnt target genes. Within the IgG superfamily, the cell adhesion molecules L1 (22) and Nr-CAM (23) are proven Wnt/beta-catenin targets. No link

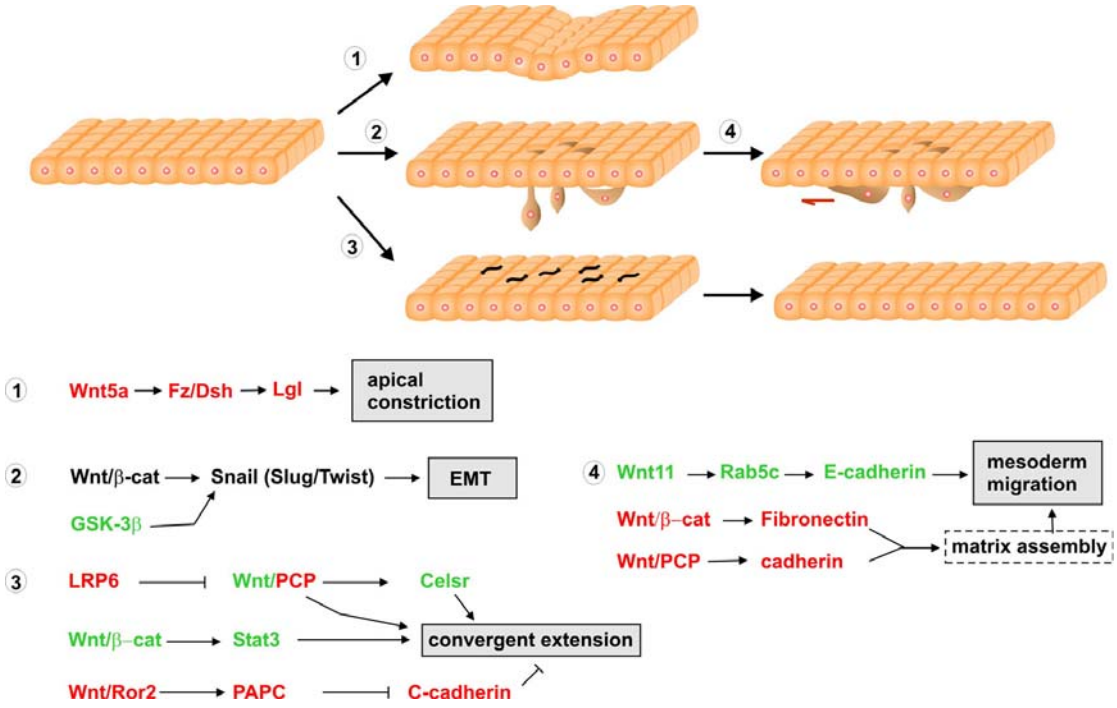

Figure 1. Morphogenetic movements during gastrulation processes rely on Wnt signaling and dynamic cadherin-mediated adhesion. Involution requires local indentation of the surface tissue that is realized by coordinated apical constriction of cells on the surface, leading to the formation of the bottle cells and the blastoporus in *Xenopus* (1). Gastrulation can also be associated with EMT processes, leading to invagination, delamination (2) and subsequent migration (4) of the mesodermal cells. Both in the fish and in *Xenopus*, convergent extension movements in the mesoderm, cause the elongation of the embryo (3). Adhesion and signaling processes discussed in the text are indicated (See text for details). The outcome of experiments in the mouse (blacktext), zebrafish (green) and *Xenopus* (red) are shown.

with Wnt signaling has been described yet for selectins, which mediate adhesion between endothelial cells, leukocytes, and platelets and have an important role as inflammatory mediators (13).

5. INTERPLAY BETWEEN WNT AND CELL ADHESION DURING EARLY DEVELOPMENT

5.1. Gastrulation

The term gastrulation was first introduced by Haeckel and is derived from the Greek word ‘gaster’, which means belly or gut. In general, the gastrulation process can be defined as a transition from a simple group of rather unstructured cells to a more complex, organized and multilayered embryo containing three germ layers: the ectoderm, endoderm and mesoderm. Interestingly enough, even when the ultimate body plans are very much alike, the morphogenetic movements during gastrulation often differ greatly between different species (24). In general, however, four major modes of cell behavior can be distinguished during invertebrate and vertebrate gastrulation: (i) epithelial bending, (ii) dissociation of cells from epithelial structures, (iii) cell rearrangements within sheets, and (iv) cell migration (Figure 1) (25).

Dynamic modulation of cell adhesion is required for all four modes of gastrulation movements (26), and it is clear that many signaling pathways, including Wnt, contribute to this regulation. In the following subsections we will discuss the crosstalk between Wnt signaling and adhesion in each of the four aforementioned cell behavior modes. We stress, however, that classifying a gastrulation process under one movement does not exclude the involvement of other types of movement.

5.1.1. Epithelial bending

Bending of epithelial sheets can result in the translocation of a large group of cells from the surface into the interior of an epithelial sphere, ultimately creating a two-layered structure. This morphogenetic movement is observed, for example, during gastrulation in *Xenopus*, and it is initialized by apical constriction of the cells that makes them wedge-or bottle-shaped (Figure1). This induces local bending in the ectoderm, which is needed to form the blastopore and to allow mesoderm invagination. It was recently suggested that Wnt5a plays an important function in this process by regulating Lgl (lethal giant larvae) in the ectoderm via Frizzled and Dsh (27). Inhibition of Wnt5a was shown to prevent blastoporus formation, and this effect could be partially reversed by coinjection of Lgl RNA. In addition, overexpression of either Wnt5a or Lgl can induce ectopic apical constriction and elongation along the apical-basal direction, which are characteristics of bottle-cell formation (27). Although there are no reports specifically on the involvement of cell adhesion in epithelial bending during gastrulation, cadherins and cell adhesion do

contribute to the very similar process of neural plate bending, as will be discussed further on.

5.1.2. EMT and delamination of cells

Epithelial-mesenchymal transition (EMT) allows cells to dissociate from epithelial structures. This occurs either as delamination of single cells or as transition of part or all of an epithelium to a mesenchymal state (Figure 1). In amniotes, cells that ingress through the primitive streak undergo EMT (28). A crucial step in EMT is the switch from E- to N-cadherin. This switch is mediated by FGF (fibroblast growth factor) signaling and, as described above, Snai1, Snai2 and Twist perform important functions in EMT by regulating E-cadherin expression. In mice, *Snai1* is expressed in the primitive streak, but *Snai2* and *Twist* are not (29, 30). Also, *Snai1*-null mice exhibit defects in gastrulation because *Snai1* deficiency leads to retention of E-cadherin and causes cells to maintain their epithelial properties, resulting in defects in mesoderm formation (31). Note that the expression pattern of the chick homologue of *Snai2* is quite different from that of *Snai2* in mice: like mouse *Snai1*, it is expressed at the primitive streak and, moreover, it is implicated in the regulation of EMT during the formation of mesoderm (30, 32).

Several links between EMT-mediated gastrulation and Wnt signaling have been reported (Figure 1). First, Wnt signaling is required for the establishment of the primitive streak. In mice, Wnt reporters are active during streak formation, and *in vitro* experiments corroborated the requirement for Wnt signaling in EMT because *Wnt3a* controls *Snai1* upregulation and E-cadherin repression (33). Second, during zebrafish gastrulation, Snai1a degradation can be blocked by Gsk-3beta, but in a betacatenin independent way, resulting in impaired cell migration (34). These data indicate that Snai1 and Wnt signaling are interdependent in the important process of EMT during gastrulation.

5.1.3. Cell rearrangements – Convergent extension

Cell rearrangements within sheets take place to change the dimensions of epithelial or mesenchymal cell layers. Radial intercalations during *Xenopus* and zebrafish epiboly and convergent extension (CE) can be classified under this type of movement. Epibolic movements induce extension and often also thinning of epithelial layers, either by cell division or by radial intercalation, by which several layers integrate into one. In these situations, cell adhesion molecules, such as E-cadherin and Flamingo, are responsible for cell rearrangements, as was shown in zebrafish (35, 36).

CE movements take place not only during vertebrate gastrulation, but also during neurulation. CE causes the narrowing (convergence) and lengthening (extension) of a tissue (Figure 1). During these processes, cells change their relative positions within a cell sheet and intercalate between each other in a preferred direction. While *Xenopus* uses solely mediolateral intercalation during CE, fish are also able to use directed migration, thereby uncoupling convergence from extension. During gastrulation in fish, cells migrate from lateral parts towards the dorsal midline, a phenomenon called dorsal convergence, and it is only at the latest stages of gastrulation that these cells change neighbors by means of mediolateral intercalation. Mesoderm movements and CE require strong coordination of cohesion and dynamic regulation of cell-cell adhesion. Several cadherins have been proven to be involved in this process, including N-cadherin, the protocadherin PAPC, the atypical cadherin Flamingo, and the *Xenopus* XB/U-cadherin and C-cadherin.

XB/U-cadherin mediated adhesion is required for *Xenopus* gastrulation because a functional antibody to XB/U-cadherin dissociates mesoderm explants into individually migrating cells (37). On the other hand, the adhesive properties of C-cadherin have to be downregulated for normal tissue elongation, highlighting its crucial role in CE during gastrulation (38-40).

More recently, Chen and Gumbiner (2006) described a role for another cadherin, paraxial protocadherin (PAPC), in the regulation of C-cadherin activity during gastrulation. PAPC can control cell sorting and morphogenetic movements and thus plays an essential role in the CE movements of the paraxial mesoderm during early *Xenopus* development. In this process, PAPC itself does not function as a homophilic cell-cell adhesion molecule but influences mesoderm movements by downregulating C-cadherin adhesion activity (41). *Xenopus* Wnt5a morphants exhibit a phenotype comparable to PAPC loss-of-function in Keller open face explants (*i.e.* explants containing both ectoderm and mesoderm, reaching from the blastopore lip up to the animal pole, cultured flat under a cover slip to study CE). This observation led to the unraveling of a new cascade in which Wnt5a activates *Papc* transcription through the Ror2 receptor pathway. This is different from traditional Wnt/PCP, Wnt/calcium or canonical Wnt signaling. Pi3k, Cdc42 and JNK have been identified as downstream effectors of Wnt5a/Ror2 signaling. As no protein translation was required to induce *Papc* transcription, it can be assumed that Wnt5a/Ror2 directly regulates *Papc* transcription to induce its interference with adhesion during mesoderm movement and CE (12). Apart from its function in CE, PAPC also takes part in tissue separation. During *Xenopus* gastrulation, mesoderm and endoderm display tissue separation behavior to prevent mixing of the involuting mesendodermal cells with the overlying ectodermal cells. The border formed between the neurectoderm and the mesendoderm is called Brachet's cleft (42). Also Fz7 is involved in this process, and PAPC and Fz7 act synergistically in the regulation of tissue separation at Brachet's cleft. However, PAPC signals through the Wnt/PCP effectors Rho and JNK (43), while Fz7 signals through PKC-alpha in a Dsh independent way to control tissue separation (44). It is not yet clear how these pathways interact.

In amniotes, N-cadherin is indispensable for mesoderm development. It is also involved in mesoderm extension movements in zebrafish. Interestingly, N-cadherin mutants exhibit a phenotype that somewhat resembles the Wnt/PCP *vangl2/tri* mutant; this led to the

suggestion of genetic interaction between N-cadherin and Vangl2 (45). However, since injection of Vangl2 MO in N-cadherin mutants still provokes a more severe outcome and because N-cadherin levels are not changed in Vangl2/Tri mutants, this rather indicates that the Wnt/PCP pathway and N-cadherin function synergistically and in parallel to regulate mesoderm morphogenesis (45).

Flamingo (Fmi) was first identified in *Drosophila* as a seven-pass transmembrane cadherin regulating PCP under the control of Fz. Fmi likely mediates homophilic cell adhesion because it can induce aggregation of *Drosophila* S2 cells (46). During zebrafish gastrulation, the Celsr proteins (the vertebrate homologues of Fmi) are active in both CE and epiboly. While CE movements are subject to PCP signaling, it appears that mainly the extracellular domains of Celsr proteins contribute to epibolic movements by modulating cell adhesion and cohesion independently of other core PCP elements. In contrast, for the regulation of CE movements, Celsrs interact with other Wnt/PCP elements via their intracellular SE/D domains. Here, this SE/D domain is essential for Fz7-mediated localization of Dsh to the cell membrane (36). Finally, Wnt11 can induce Fz7 and Dsh accumulation at distinct sites on the plasma membrane, which results in increased persistence of cell contacts in a Celsr-dependent way (47). This means that Celsrs have multiple roles in regulating cell adhesion during gastrulation. On the one hand, they regulate cell cohesion during epiboly in a Wnt/PCP-independent manner. On the other hand, together with other Wnt/PCP proteins, they regulate persistence of cell contact in gastrulating cells.

In *Xenopus*, normal CE also requires strict regulation of other Wnt/PCP components, such as Vangl2 (48), Prickle (49), Dsh (50) and PKC-delta (51), and, surprisingly, also LRP6 (52). LRP6 is a good example of crosstalk between Wnt/PCP signaling and the canonical Wnt branch. LRP6 morphants exhibit, aside from the classical defects caused by derangement of Wnt/beta-catenin signaling (anteriorization and expansion of head and cement gland), also elongation defects, which is consistent with CE abrogation; those defects could not be rescued by co-injection of beta-catenin DNA. Furthermore, activated LRP6 seems to be a negative regulator of the Wnt/PCP pathway, which makes it plausible that canonical Wnt inhibitors that bind LRP6 (Dkk, SOST) could potentiate the Wnt/PCP pathway by relieving the inhibition through LRP6 (52). Moreover, although canonical Wnt signaling is mainly linked to proliferation and cell fate decisions during gastrulation, it can also be associated with cell movements. It even seems to be essential for proper CE during gastrulation, possibly through the induction of the direct target gene *Xnr3* (53). Further, crosstalk between the Wnt/beta-catenin and JAK/STAT pathways determines gastrulation movements because Stat3 activation is induced by maternal canonical Wnt signaling, which is necessary for cell migration and CE (54).

5.1.4. Cell migration

Cell migration involves their movement across a substratum, which can be the ECM or other cells (Figure 1). When migrating, cells form focal adhesions with ECM components or intercellular contacts with other cells. Both types of contact need to be dynamically regulated. Cells can migrate individually, but in many cases they move as groups.

As already mentioned, shaping of the mesodermal layer in fish after internalization of the mesendodermal progenitors is determined by a balance between CE movements and cell migration. Again, E-cadherin is involved in this process. At the onset of gastrulation, the mesendodermal progenitors internalize at the embryonic organizer or "shield" (the equivalent of the blastopore lip in *Xenopus*) by single cell delamination. Once internalized, E-cadherin is upregulated and the mesoderm moves along the overlying ectoderm towards the animal pole. Knock-down of E-cadherin does not cause failure of internalization, but it reduces cell elongation and further migration along the epiblast of the most anterior axial mesendodermal (prechordal plate) progenitors (55).

In zebrafish *wnt11/slb* mutants, which carry a loss-of-function mutation in the Wnt/PCP gene, migration of the anterior mesoderm is disturbed, and E-cadherin turned out to be a target of Wnt11 in this process. It appears that Wnt11 is essential for the proper E-cadherin-mediated cohesion between mesendodermal cells, which is required for their movement. Further insight into the mechanisms involved revealed that Wnt11 functionally interacts with E-cadherin by promoting Rab5c-mediated endocytosis and recycling of E-cadherin at the plasma membrane, and so it controls the rapid de-adhesion and re-adhesion of cells that is indispensable for proper migration (56). Moreover, *wnt11/slb* mutants show reduced adhesion to fibronectin, raising the possibility that Wnt/PCP also modulates other adhesion molecules, such as integrins (57).

The ECM is a three-dimensional fibrillar network composed mainly of collagens and fibronectin. Fibronectin is an essential component for embryogenesis, because mice lacking fibronectin cannot complete gastrulation and have severe defects in mesoderm formation and migration (58). In *Xenopus*, migrating mesoderm cells spread towards the animal pole of the embryo using the fibronectin matrix on the blastocoel roof as a substrate (59). Perturbation of the assembly of the fibronectin matrix on the roof causes problems in radial intercalation during epiboly and hinders blastopore closure (60, 61). According to Dzamba *et al.*, matrix assembly on the blastocoel roof is controlled by Wnt/PCP signaling and both cell-cell adhesion and tissue tension contribute to this process. An increase in cadherin activation, induced by Wnt11 and Rac, is believed to generate enough cytoskeletal tension to activate integrins, which in turn aid in fibril assembly (62). Interestingly, fibronectin also seems to be upregulated by Wnt3a (*i.e.* by canonical Wnt signaling) in an *in vitro* murine ES model of gastrulation (33), and it was proven to be a direct canonical Wnt target gene in *Xenopus* (63).

5.2. Somitogenesis

Somitogenesis, which has been studied mostly in chick and mouse embryos, includes processes such as

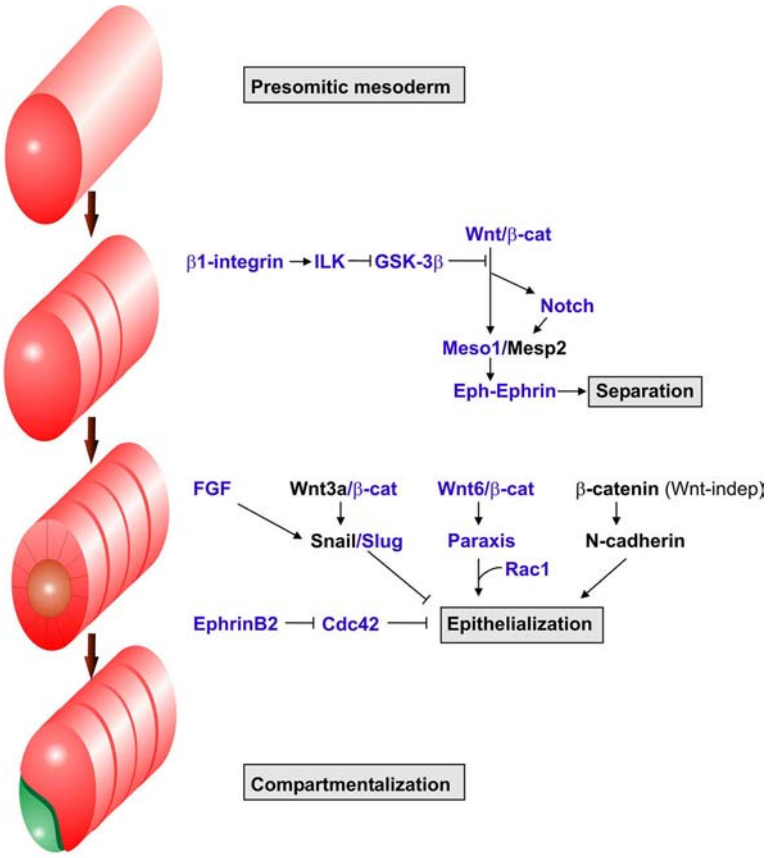

Figure 2. The interplay between Wnt signaling and cell adhesion during somitogenesis. The different steps involved in somite formation (separation, epithelialization and compartmentalization) are controlled by cell adhesion and repulsion events. These involve the molecular activity of cadherins and Eph/Ephrins, which are regulated by Wnt and other signaling pathways. See text for details. Most interactions have been identified in the chick (blue text) and the mouse (black text).

periodicity, epithelialization, separation, specification and differentiation (64). The gradual formation of bilateral somites from the paraxial mesoderm (PM) in the anterior-posterior direction is regulated by a so-called periodic clock manifested in signals (mediated by Notch, Wnt and FGF) that determine the temporal and spatial formation of new somites. The role of Wnt in this process has been well documented but it will not be discussed here, as it falls outside the scope of this review.

Specification takes place during and shortly after gastrulation and determines the fate of the tissues along the anteroposterior axis. The presomitic tissue undergoes epithelialization, *i.e.* conversion of mesenchymal tissue into a block surrounded by epithelium. Meanwhile, separation of the sequential somites takes place in an anterior-to-posterior wave. By a process known as compartmentalization, the newly formed epithelialized somites become patterned to form the sclerotome ventrally and the dermomyotome dorsally. The dermomyotome initially remains epithelial and afterwards becomes subdivided into dermatome and myotome. Next, the different somite compartments differentiate into specific derivatives, such as vertebrae, muscles, cartilage, dermis, and blood vessels (64).

As cell adhesion is intimately involved in separation, epithelialization and compartmentalization, we will focus on these processes in the context of Wnt signaling and interference with cell adhesion (Figure 2).

5.2.1. Separation of somites

Somite separation is the process by which a morphological boundary is sculpted between the forming somite and the presomitic mesoderm. At this intersomitic boundary, cells of the anterior and posterior border need to change their shape and their cell-cell interactions. In vertebrates, tyrosine Eph receptors and their Ephrin ligands are responsible for the separation of the somites (65). In chick, one of the molecules acting upstream of the Eph4-EphrinB2 interaction to mediate somite gap formation is the transcription factor Meso-1 (66). Mesp2 is the mouse homolog of Meso-1, and its knockout results in development of embryos lacking segmented somites (67). Fibronectin and members of the integrin family, such as alpha5-integrin (shown in mice and zebrafish) and beta1-integrin (shown in chick), also affect segmentation (68-71). Interestingly, beta1-integrin seems to regulate boundary formation in a cell-autonomous manner and acts upstream of canonical Wnt and Notch signaling. In this model, beta1-integrin can activate canonical Wnt signaling, presumably

via the cytoplasmic domain of beta1-integrin, intracellular signaling by ILK, and inhibition of GSK3beta (Figure 2). Next, Notch signaling and Mesp2 expression are induced downstream of Wnt signaling (68). The remaining question is whether ILK acts directly to enhance phosphorylation of GSK3beta, or if an indirect mechanism mediates Wnt activation by beta1-integrin. Additionally, it is unknown whether beta1integrin-mediated Wnt/beta-catenin signaling could furthermore act on the Eph-Ephrin interaction, possibly via Mesp2. Certainly, Wnt does not regulate Mesp2 directly (72). Still, Wnt and Notch signaling seem to cooperatively activate Mesp2 (68).

5.2.2. Somite epithelialization

As mentioned before, epiblast cells in amniotes downregulate E-cadherin and upregulate N-cadherin when they enter the primitive streak to form the endomesoderm. Hence N-cadherin is the main adhesion molecule present in the mesoderm during somitogenesis. When a new somite pinches off from the presomitic plate, the cells at the newly generated anterior border soon undergo epithelialization. Hence, the somites consist of a somitocoel of mesenchymal cells surrounded by epithelial tissue (Figure 2). During this process, N-cadherin concentrates at the apical surfaces of the epithelial cells, whereas in the core mesenchymal cells N-cadherin remains uniformly distributed (73-75). N-cadherin-null mice still undergo somitogenesis, but their somites are smaller, irregular and fairly disorganized (73). The rostral and caudal subdivision of the somites in these mice is segregated, and additional mutation of cadherin-11 causes further fragmentation of each somitic cell mass (74). This proves that N-cadherin mediates cell adhesion and is crucial for keeping cells together during somitogenesis.

While the role of Wnt/beta-catenin signaling in the somitogenesis-associated periodic clock has been studied thoroughly, its potential contribution to somite epithelialization is less clear. Studies on chick embryos (76, 77) revealed a cascade in which Wnt6 signals from the overlaying ectoderm and binds to the Fz7 receptor expressed in the segmental plate cells. This results in beta-catenin-dependent signaling that activates the bHLH protein Paraxis (Tcf15), a transcription factor required for somite epithelialization (78). Moreover, overexpression of the Wnt inhibiting protein Sfrp2, results in loss of epithelialization (76). In addition, by using an inducible *T-Cre;Ctnnb1*$^{fILOF/\Delta}$ mutant mouse, Duntry *et al.* showed that beta-catenin loss-of-function disturbs somite epithelialization. However, the authors claimed that the influence of beta-catenin on somite epithelialization is independent from Paraxis and they supported the notion that beta-catenin participates in somite epithelialization by acting both as a cell adhesion molecule and as a component of Wnt signaling (72). It has also been suggested that the Wnt pathway is important in the formation of the dermomytome and in maintaining, rather than inducing, the epithelial state of somites (77) (see below). Actually, experiments in embryos of chick and mouse revealed that Wnt signaling in the presomitic mesoderm could also counteract epithelialization via the Snai proteins, which control EMT. Just as in gastrulation, Snai1 in mice and Snai2 in chick seem to regulate EMT during somitogenesis. *Snai* genes have been demonstrated to be cycling genes, as they show a periodic expression pattern in the PM during somite segmentation. *Snai1* expression, which is induced by FGF, seems also to be dependent on Wnt3a signaling. The importance of Snai1/Snai2 in counteracting epithelialization is clearly shown in chick embryos, in which overexpression of Snai2 is associated with loss of epithelial structures in the somites. Moreover, Paraxis is downregulated in a cell autonomous way in these regions, and so the cells are blocked in a mesenchymal state (79).

Separation of the sequential somites seems to be mainly regulated by Ephrin-mediated repulsion (see 1.2). Recent findings couple the MET processes that occur during the formation of the somites with the Eph4-EphrinB2 mediated tissue separation and repulsion of the newly forming somites. It seems that EphrinB2 can signal intracellularly (via reverse signaling) to repress Cdc42 expression, which is a prerequisite for somite separation and the MET of the tissue surrounding the somitocoel (66, 80) (recently reviewed in (81)).

In summary, although Wnt signaling during somitogenesis is associated mainly with its function within the periodic clock, it is also involved in regulating the epithelial state of the newly formed somites, but the mechanisms are not completely understood. N-cadherin and Ephrin signaling also appear to be essential for epithelialization.

5.2.3. Compartmentalization

After separation and epithelialization, the ventral region of each somite de-epithelializes again, and together with the somitocoel, it gives rise to the sclerotome. The dorsal half of the somite initially remains epithelial and becomes the dermomyotome, which gives rise to the myotome and the dermatome. During further differentiation and formation of the myoblasts and dermoblasts, the dermomytome epithelium eventually undergoes EMT (64). The N-cadherin expression levels seem to correlate well with the epithelial-versus-migratory status of the somitic cells and derived precursor cells. During sclerotome formation, EMT is associated with N-cadherin downregulation and leads to a decrease in cell adhesion and an increase in cell motility (64, 75). In the myotome, migrating pioneer myoblasts lose their N-cadherin expression, but the protein is re-expressed during differentiation, and it appears that N-cadherin mediated cell adhesion is required for proper myogenic specification (82).

During compartmentalization, Wnt/beta-catenin signaling determines the epithelial status. In the chick, Wnt6 signals from the overlaying ectoderm and other Wnts, including Wnt1, Wnt3a and Wnt4, signal from surrounding tissues to determine the epithelial status and to control compartmentalization of the somites (77, 83, 84). In addition, Wnt11 is expressed in the dorsomedial lip of the dermomyotome itself, where it acts as a mesoderm-intrinsic epithelializing factor to maintain the epithelial status of these cells (84). Thus, Wnts act as epithelializing factors, and their actions are mediated, at least in part, by

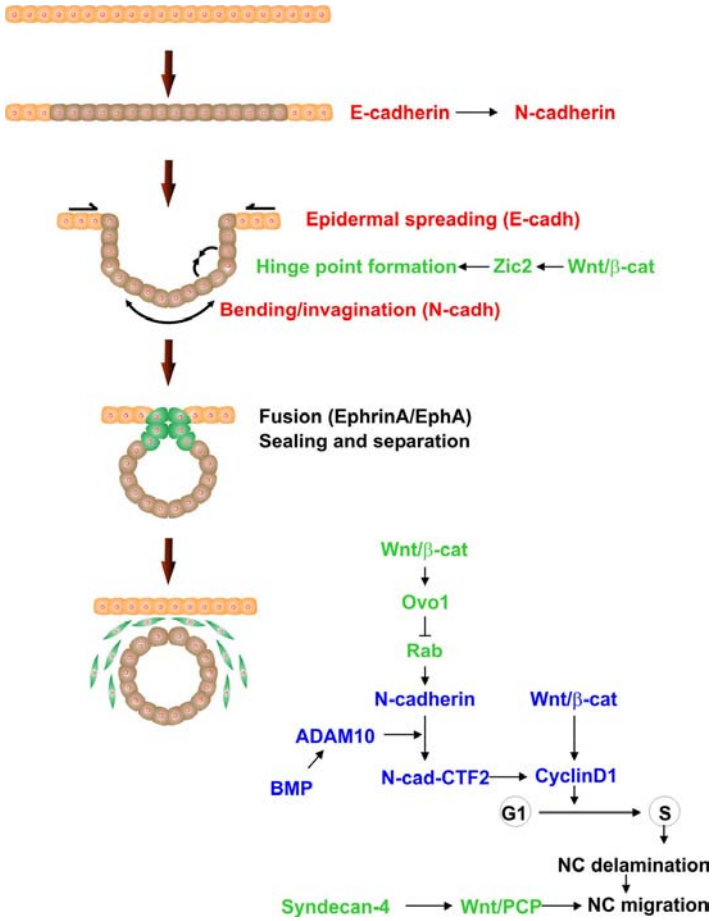

Figure 3. Adhesion and Wnt signaling events associated with morphogenetic processes during neurulation. Induction of the neural plate is associated with a cadherin switch. During the invagination of the neural plate, both spreading activity in the epidermis and bending behaviour in the neural plate proper are driven by cadherin activity. Fusion of the neural folds is mediated by Eph/Ephrin interactions and a complex interplay between different Wnt signaling pathways and cadherin-mediated adhesion and signaling control the delamination and migration of neural crest cells. The molecular interactions have been identified via experiments in the mouse (black text), the chick (blue), zebrafish (green) and *Xenopus* (red). See text for details.

downstream activation of Paraxis. The details of Paraxis functions are not completely understood, but some *in vivo* experiments show that during somitogenesis, Paraxis interacts with Rac1 and Cdc42, two members of the Rho family GTPases known to maintain/stabilize epithelial structures by regulating cadherin-mediated cell-cell adhesion (80).

5.3. Neurulation

After the formation of the three germ layers, the neural tube (NT) and neural crest (NC) are formed in the early embryo by a process known as neurulation. NT formation is driven by two different mechanisms: primary neurulation and secondary neurulation. In primary neurulation, a combination of intrinsic and external mechanisms orchestrate the invagination and rolling up of the neural plate (originating from the ectoderm) into a hollow tube, and its separation from the surface ectoderm (Figure 3). Secondary neurulation is the condensation of mesenchyme-like cells into a solid strand, which is subsequently hollowed out. Generally, in amniotes and amphibians, primary and secondary neurulation lead to the formation of an anterior and a posterior tube, respectively. These tubes then join to form the entire neural tube. In this section, we will focus on primary neurulation because it has been investigated more thoroughly.

Just as in gastrulation, cell movements –as well as related processes such as cell sorting and cell adhesion– have a major function in neurulation. Furthermore, during neural development, Wnt signaling plays an important role in the proliferation and differentiation of neural progenitors and in processes such as axon guidance and synapse formation (85). In the following subsections, we will sketch the interaction of Wnt signaling with cell adhesion during neural tube formation and neural crest initiation and migration.

5.3.1. Neural tube formation: shaping, bending and fusion

Before the neural plate (NP) folds, it lengthens and narrows by CE movements. The NP then folds by

epithelial bending (see 5.1.1) at one medial and two dorsolateral hinge points. For the neural tube to invaginate, cells at the hinge points have to become wedge-shaped. Finally, the neural folds move towards each other, make contact, and close the NT. In addition, extrinsic forces, from the flanking non-neural ectoderm and the underlying endoderm, co-operate to achieve neural tube formation (86).

Cell adhesion molecules play a decisive role in neurulation movements. First of all, cells of the neural plate originally express E-cadherin, but as the neural tube forms, they start to express N-cadherin and N-CAM instead (Figure 3). Experiments on *Xenopus* indicate that this switch in cell adhesion molecules is indispensable for the separation of the neural tube from the overlying ectoderm, as ectopic expression of N-cadherin in the ectoderm prevents separation and normal development of the neural tube (87, 88). N-cadherin, which is expressed at the apical adherens junctions of the neural ectoderm, has a crucial function. *N-cadherin* null mice and zebrafish, as well as *Xenopus* embryos overexpressing N-cadherin and chick embryos treated with N-cadherin blocking antibodies, all show defects and malformations of the neural tube (73, 87, 89, 90). Controversially, neural tube closure still occurs in N-cadherin knockout mice. Nevertheless, this closure is not complete at the cranial neuropore (91). An accepted explanation for the closure in *N-cadherin* knockout mice is that other cell adhesion molecules substitute for N--cadherin. Furthermore, beta-catenin is crucial in the adherens complexes of the neural ectoderm, because neuroepithelial cells deficient in beta-catenin delaminate from the neural tube and undergo apoptosis (92). In contrast to mice deficient in N-cadherin, N-CAM deficient mice appear healthy and do not show any defects in neural tube closure (93). Finally, fusion of the tips of the neural folds at the midline seems to be made possible by the adhesion mediated by EphrinA-EphA (94).

NT formation is also determined by expansion of the flanking epidermal ectoderm, where E-cadherin is the main cadherin. Nandandasa *et al.* showed by injecting morpholinos into *Xenopus* that depletion of E-cadherin leads to delayed NT closure due to reduced spreading movements of the non-neural ectoderm, whereas depletion of N-cadherin results in failure of the neural plate to undergo normal invagination movements (Figure 3). Furthermore, they suggested that E- and N-cadherin control these characteristic movements by the assembly of F-actin in a cadherin and tissue specific way, wherein E-and N-cadherin can not substitute for each other (95).

As neurulation depends on CE, it is not surprising that many Wnt/PCP components responsible for CE during gastrulation (see 5.1.3) also contribute to neural tube formation. *Xenopus* morphants and mice with mutations in PCP genes (such as *Dsh*, *Celsr1*, *Pk* and *Vangl2*) also have defects in closure of the neural tube (reviewed in (96)), as well as gastrulation defects. The way Wnt/PCP regulates neural CE may involve N-cadherin mediated cell adhesion. Mice overexpressing Wnt7a in the neural tube have a defect in closure of the neural tube, and this phenotype goes along with elevated Vangl2 expression and seems to be caused by reduced levels and impaired distribution of actin microfilaments, beta-catenin and N-cadherin at the adherence junctions (97). Further research revealed that Vangl2 affects the cytoskeleton and cell adhesion by directly interacting with the small GTPase Rac1, downstream in the Wnt/PCP pathway. It seems that precisely regulated levels of Vangl2 are crucial for local recruitment of Rac1 to the cell membrane, which is necessary for appropriate regulation of adherens junctions and neural tube development (98). In the same study, Lindqvist *et al.* also showed that Vangl2 can complex with RhoA, but they could not show that Vangl2 controls RhoA distribution in the cell. Nevertheless, Wnt11/Fz7-mediated PCP signaling seems to be required for the apical accumulation of Rho GTPases in neural plate cells and for normal neural tube formation (99).

Research on zebrafish embryos indicates that Wnt is involved in hinge-point formation by regulating cytoskeleton organization via the targeting of z*ic* genes (100). In this model, *zic* genes, which encode a family of zinc-finger transcription factors, do not act in their better-known proliferative role, but by regulating apical actomyosin contraction and establishing and/or maintaining apical junctions. Note that neural tube formation in zebrafish occurs only by secondary neurulation. Nevertheless, the authors claim that the model they propose is strongly conserved and that it can be extrapolated to the molecular mechanisms regulating DHLP formation in mammals and birds (100).

5.3.2. Neural crest induction and migration

Cells of the neural crest (NC) originate from the dorsal folds. They undergo EMT, detach from the neuroepithelium, and migrate to their homing sites, where they differentiate. During this process, transient cell-cell contacts and changes in expression of cadherins are of key importance. In mouse, chick and *Xenopus* models, N-cadherin, cadherin-6/6b, cadherin-7 and/or cadherin-11 are implicated in NC migration, and anomalous expression of these molecules can result in aberrant NC migration (101). In the chicken embryo, EMT is necessary for NC migration and is accompanied by downregulation of N-cadherin, and so overexpression of N-cadherin prevents delamination of premigratory NC cells (102). After delamination, N-cadherin is probably re-expressed in the migrating NC cells, as was shown *in vitro* (103, 104). Still, this re-expression of Ncadherin remains disputed due to the lack of *in vivo* evidence (for review see (101)).

Besides its function in specifying NC (105), canonical Wnt signaling contributes to NC delamination (Figure 3). The group of Kalcheim proposed a model integrating Wnt and BMP signaling with cell cycle regulation and cell adhesion. First, delamination of NC depends on successful G1/S cell cycle transition, which is controlled by Wnt signaling via transcription of *cyclinD1* (106). Second, NC migration requires BMP-dependent N-cadherin cleavage, which not only causes disruption of N-cadherin mediated adhesion, but also triggers intracellular signaling via the remaining C-terminal part of N-cadherin.

Upon BMP4 signaling, ADAM10 (A Disintegrin And Metalloprotease), cleaves N-cadherin. The generated C-terminal part (CTF1) is further processed to a soluble intracellular CTF2 that interacts with beta-catenin, translocates to the nucleus, activates beta-catenin transcriptional activity and consequently enhances *CyclinD1* expression. The latter subsequently induces the G1 to S transition. How the CTF2-beta-catenin interaction leads to elevated transcriptional activity remains speculative (107). Interestingly, ADAM10 is a Wnt target in colon cancer cells, in which it cleaves the adhesion molecule L1-CAM (108). Noteworthy, L1-CAM is also required for normal migration of NC cells to the developing gut (109).

During NC migration, intracellular N-cadherin trafficking seems to be controlled by canonical Wnt signaling. In zebrafish, one possible downstream mechanism acts via the Wnt target gene *ovo1*. Proper NC cell migration requires localization of N-cadherin to the tips of the filopodia, and *ovo1* expression seems to be required for this localization of N-cadherin to the cell membrane. Hereby, Ovo1 mediates, likely under the control of canonical Wnt signaling, repression of *Rab* genes which subsequently leads to accumulation of N-cadherin at the cell membrane (110).

The directionality of NC cell migration seems to be coordinated by non canonical Wnt signaling. It has been postulated that NC cell migration is directional independently of any external chemoattractant and that it is propelled by intracellular interaction between Syndecan-4 and Wnt/PCP signaling (111). After delamination, the NC cells come into contact with fibronectin, and interaction of this extracellular matrix component with the transmembrane proteoglycan Syndican-4, results in activation of Wnt/PCP signaling. Then, cooperation between Syndecan-4 and Wnt/PCP leads to polarized Rac activity in the NC cells. Rac expression is low at the back of the cell and enriched at the leading edge, which enables formation of lamellipodia and directional migration of the cell (further elaborated in (111)).

In conclusion, both the canonical and non-canonical Wnt pathways contribute to cell adhesion and cadherin expression during NC induction and migration. Remarkable is the cooperation and crosstalk with multiple other signaling pathways to influence a broad range of cellular events, such as intracellular trafficking, signal transduction, cell cycle transition and cytoskeleton rearrangements.

6. SUMMARY AND PERSPECTIVES

Wnt signaling and cell adhesion function interdependently in the processes of gastrulation, somitogenesis, neurulation, and other morphogenetic processes. Organogenesis and tissue formation in general require an orchestrated interplay between cellular differentiation and morphogenetic movements and organization. Developmental signaling pathways can affect morphogenetic processes: indirectly via transcriptional activation of specific target genes, and by direct effects on the cytoskeleton and intracellular trafficking. The cell adhesion molecules (and cytoskeletal regulators) can be considered the morphogenetic effectors of the developmental signaling pathways. Cooperation between signaling pathways further fine tunes the processes that depend on cell adhesion. Conversely, it has become clear that cell adhesion molecules can directly or indirectly interact with signaling components, which ensures a reciprocal interaction. Better knowledge of the interplay between cell signaling and cell adhesion will be essential for successful applications in tissue and organ reconstitution and regenerative medicine in general.

7. ACKNOWLEDGEMENTS

S. Janssens is supported by a Ph.D. grant of the Research Foundation -Flanders (FWO). We thank Amin Bredan for editing the manuscript and Wim Drijvers for graphical work. This work is supported by grants from Research Foundation -Flanders (FWO), the Belgian Federation against Cancer and the "Geconcerteerde Onderzoeksacties of Ghent University"

8. REFERENCES

1. S. Sokol, J. L. Christian, R. T. Moon and D. A. Melton: Injected Wnt RNA induces a complete body axis in Xenopus embryos. *Cell*, 67(4), 741-52 (1991)

2. H. A. Kestler and M. Kuhl: From individual Wnt pathways towards a Wnt signalling network. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 363(1495), 1333-47 (2008)

3. M. V. Semenov, R. Habas, B. T. Macdonald and X. He: SnapShot: Noncanonical Wnt Signaling Pathways. *Cell*, 131(7), 1378 (2007)

4. J. Chien, W. H. Conrad and R. T. Moon: A Wnt survival guide: from flies to human disease. *The Journal of investigative dermatology*, 129(7), 1614-27 (2009)

5. J. Bilic, Y. L. Huang, G. Davidson, T. Zimmermann, C. M. Cruciat, M. Bienz and C. Niehrs: Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science*, 316(5831), 1619-22 (2007)

6. K. M. Cadigan and M. Peifer: Wnt signaling from development to disease: insights from model systems. *Cold Spring Harbor perspectives in biology*, 1(2), a002881 (2009)

7. D. Kohn and R. T. Moon: Wnt and calcium signaling: beta-catenin-independent pathways. *Cell calcium*, 38(3-4), 439-46 (2005)

8. E. K. Vladar, D. Antic and J. D. Axelrod: Planar cell polarity signaling: the developing cell's compass. *Cold Spring Harbor perspectives in biology*, 1(3), a002964 (2009)

9. M. T. Veeman, J. D. Axelrod and R. T. Moon: A second canon. Functions and mechanisms of beta-catenin-

independent Wnt signaling. *Developmental cell*, 5(3), 367-77 (2003)

10H. Hikasa, M. Shibata, I. Hiratani and M. Taira: The Xenopus receptor tyrosine kinase Xror2 modulates morphogenetic movements of the axial mesoderm and neuroectoderm via Wnt signaling. *Development*, 129(22), 5227-39 (2002)

11. Oishi, H. Suzuki, N. Onishi, R. Takada, S. Kani, B. Ohkawara, I. Koshida, K. Suzuki, G. Yamada, G. C. Schwabe, S.Mundlos, H. Shibuya, S. Takada and Y. Minami: The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNKsignalling pathway. *Genes to cells: devoted to molecular & cellular mechanisms*, 8(7), 645-54 (2003)

12. Schambony and D. Wedlich: Wnt-5A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. *Developmental Cell*, 12(5), 779-92 (2007)

13. Joseph-Silverstein and R. L. Silverstein: Cell adhesion molecules: an overview. *Cancer investigation*, 16(3), 176-82 (1998)

14. R. L. Daugherty and C. J. Gottardi: Phospho-regulation of Beta-catenin adhesion and signaling functions. *Physiology*, 22,303-9 (2007)

15. I. Park, S. W. Kim, J. P. Lyons, H. Ji, T. T. Nguyen, K. Cho, M. C. Barton, T. Deroo, K. Vleminckx, R. T. Moon and P. D.McCrea: Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt gene targets. *Developmental cell*, 8(6), 843-54 (2005)

16. J. Zhurinsky, M. Shtutman and A. Ben-Ze'ev: Plakoglobin and beta-catenin: protein interactions, regulation and biological roles. *Journal of cell science*, 113 (Pt 18), 3127-39 (2000)

17. J. Heuberger and W. Birchmeier: Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. *Cold Spring Harbor perspectives in biology*, 2(2), a002915 (2010)

18. W. J. Nelson and R. Nusse: Convergence of Wnt, beta-catenin, and cadherin pathways. *Science*, 303(5663), 1483-7 (2004)

19. T. Yagi and M. Takeichi: Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes & development*, 14(10), 1169-80 (2000)

20. Novak, S. C. Hsu, C. Leung-Hagesteijn, G. Radeva, J. Papkoff, R. Montesano, C. Roskelley, R. Grosschedl and S. Dedhar: Cell adhesion and the integrin-linked kinase regulate the LEF-1 and beta-catenin signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America*, 95(8), 4374-9 (1998)

21. Tan, P. Costello, J. Sanghera, D. Dominguez, J. Baulida, A. G. de Herreros and S. Dedhar: Inhibition of integrin linked kinase (ILK) suppresses beta-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in APC-/- human colon carcinoma cells. *Oncogene*, 20(1), 133-40 (2001)

22. N. Gavert, M. Conacci-Sorrell, D. Gast, A. Schneider, P. Altevogt, T. Brabletz and A. Ben-Ze'ev: L1, a novel target of betacatenin signaling, transforms cells and is expressed at the invasive front of colon cancers. *The Journal of cell biology*, 168(4),633-42 (2005)

23. E. Conacci-Sorrell, T. Ben-Yedidia, M. Shtutman, E. Feinstein, P. Einat and A. Ben-Ze'ev: Nr-CAM is a target gene of the beta-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis. *Genes & development*, 16(16), 2058-72 (2002)

24. Solnica-Krezel: Conserved patterns of cell movements during vertebrate gastrulation. *Current biology* : CB, 15(6), R21328 (2005)

25. Leptin: Gastrulation movements: the logic and the nuts and bolts. *Developmental Cell*, 8(3), 305-20 (2005)

26. M. Hammerschmidt and D. Wedlich: Regulated adhesion as a driving force of gastrulation movements. *Development*, 135(22), 3625-41 (2008)

27. S. C. Choi and S. Y. Sokol: The involvement of lethal giant larvae and Wnt signaling in bottle cell formation in Xenopus embryos. *Dev Biol*, 336(1), 68-75 (2009)

28. K. Hatta and M. Takeichi: Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature*, 320(6061), 447-9 (1986)

29. E. M. Fuchtbauer: Expression of M-twist during postimplantation development of the mouse. *Developmental dynamics: an official publication of the American Association of Anatomists*, 204(3), 316-22 (1995)

30. R. Jiang, Y. Lan, C. R. Norton, J. P. Sundberg and T. Gridley: The Slug gene is not essential for mesoderm or neural crest development in mice. *Dev Biol*, 198(2), 277-85 (1998)

31. E. A. Carver, R. Jiang, Y. Lan, K. F. Oram and T. Gridley: The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition. *Molecular and Cellular Biology*, 21(23), 8184-8 (2001)

32. M. A. Nieto, M. G. Sargent, D. G. Wilkinson and J. Cooke: Control of cell behavior during vertebrate development by Slug, a zinc finger gene. *Science*, 264(5160), 835-9 (1994)

33. D. ten Berge, W. Koole, C. Fuerer, M. Fish, E. Eroglu and R. Nusse: Wnt signaling mediates self-organization and axis formation in embryoid bodies. *Cell stem cell*, 3(5), 508-18 (2008)

34. C. K. Speirs, K. K. Jernigan, S. H. Kim, Y. I. Cha, F. Lin, D. S. Sepich, R. N. DuBois, E. Lee and L. Solnica-Krezel:Prostaglandin Gbetagamma signaling stimulates

gastrulation movements by limiting cell adhesion through Snai1a stabilization. *Development*, 137(8), 1327-37 (2010)

35. D. A. Kane, K. N. McFarland and R. M. Warga: Mutations in half baked/E-cadherin block cell behaviors that are necessary for teleost epiboly. *Development*, 132(5), 1105-16 (2005)

36. F. Carreira-Barbosa, M. Kajita, V. Morel, H. Wada, H. Okamoto, A. Martinez Arias, Y. Fujita, S. W. Wilson and M. Tada:Flamingo regulates epiboly and convergence/extension movements through cell cohesive and signalling functions during zebrafish gastrulation. *Development*, 136(3), 383-92 (2009)

37. R. Winklbauer, A. Selchow, M. Nagel and B. Angres: Cell interaction and its role in mesoderm cell migration during Xenopus gastrulation. *Developmental Dynamics*, 195(4), 290-302 (1992)

38. W. M. Brieher and B. M. Gumbiner: Regulation of C-cadherin function during activin induced morphogenesis of Xenopus animal caps. *J Cell Biol*, 126(2), 519-27 (1994)

39. C. H. Lee and B. M. Gumbiner: Disruption of gastrulation movements in Xenopus by a dominant-negative mutant for C-cadherin. *Dev Biol*, 171(2), 363-73 (1995)

40. Y. Zhong, W. M. Brieher and B. M. Gumbiner: Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J Cell Biol*, 144(2), 351-9 (1999)

41. X. Chen and B. M. Gumbiner: Paraxial protocadherin mediates cell sorting and tissue morphogenesis by regulating Ccadherin adhesion activity. *J Cell Biol*, 174(2), 301-13 (2006)

42. S. Wacker, K. Grimm, T. Joos and R. Winklbauer: Development and control of tissue separation at gastrulation in Xenopus. *Dev Biol*, 224(2), 428-39 (2000)

43. Medina, R. K. Swain, K. M. Kuerner and H. Steinbeisser: Xenopus paraxial protocadherin has signaling functions and is involved in tissue separation. *EMBO J*, 23(16), 3249-58 (2004)

44. R. Winklbauer, A. Medina, R. K. Swain and H. Steinbeisser: Frizzled-7 signalling controls tissue separation during Xenopus gastrulation. *Nature*, 413(6858), 856-60 (2001)

45. M. J. Harrington, E. Hong, O. Fasanmi and R. Brewster: Cadherin-mediated adhesion regulates posterior body formation. *BMC Dev Biol*, 7, 130 (2007)

46. T. Usui, Y. Shima, Y. Shimada, S. Hirano, R. W. Burgess, T. L. Schwarz, M. Takeichi and T. Uemura: Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell*, 98(5), 585-95 (1999)

47. S. Witzel, V. Zimyanin, F. Carreira-Barbosa, M. Tada and C. P. Heisenberg: Wnt11 controls cell contact persistence by local accumulation of Frizzled 7 at the plasma membrane. *J Cell Biol*, 175(5), 791-802 (2006)

48. T. Goto and R. Keller: The planar cell polarity gene strabismus regulates convergence and extension and neural fold closure in Xenopus. *Dev Biol*, 247(1), 165-81 (2002)

49. M. Takeuchi, J. Nakabayashi, T. Sakaguchi, T. S. Yamamoto, H. Takahashi, H. Takeda and N. Ueno: The prickle-related gene in vertebrates is essential for gastrulation cell movements. *Current biology : CB*, 13(8), 674-9 (2003)

50. J. B. Wallingford and R. M. Harland: Xenopus Dishevelled signaling regulates both neural and mesodermal convergent extension: parallel forces elongating the body axis. *Development*, 128(13), 2581-92 (2001)

51. Kinoshita, H. Iioka, A. Miyakoshi and N. Ueno: PKC delta is essential for Dishevelled function in a noncanonical Wnt pathway that regulates Xenopus convergent extension movements. *Genes Dev*, 17(13), 1663-76 (2003)

52. E. Tahinci, C. A. Thorne, J. L. Franklin, A. Salic, K. M. Christian, L. A. Lee, R. J. Coffey and E. Lee: Lrp6 is required forconvergent extension during Xenopus gastrulation. *Development*, 134(22), 4095-106 (2007)

53. M. Kuhl, K. Geis, L. C. Sheldahl, T. Pukrop, R. T. Moon and D. Wedlich: Antagonistic regulation of convergent extension movements in Xenopus by Wnt/beta-catenin and Wnt/Ca2+ signaling. *Mech Dev*, 106(1-2), 61-76 (2001)

54. S. Yamashita, C. Miyagi, A. Carmany-Rampey, T. Shimizu, R. Fujii, A. F. Schier and T. Hirano: Stat3 Controls Cell Movements during Zebrafish Gastrulation. *Dev Cell*, 2(3), 363-75 (2002)

55. J. A. Montero, L. Carvalho, M. Wilsch-Brauninger, B. Kilian, C. Mustafa and C. P. Heisenberg: Shield formation at the onsetof zebrafish gastrulation. *Development*, 132(6), 1187-98 (2005)

56. F. Ulrich, M. Krieg, E. M. Schotz, V. Link, I. Castanon, V. Schnabel, A. Taubenberger, D. Mueller, P. H. Puech and C. P. Heisenberg: Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. *Dev Cell*, 9(4), 555-64 (2005)

57. H. Puech, A. Taubenberger, F. Ulrich, M. Krieg, D. J. Muller and C. P. Heisenberg: Measuring cell adhesion forces ofprimary gastrulating cells from zebrafish using atomic force microscopy. *J Cell Sci*, 118(Pt 18), 4199-206 (2005)

58. E. L. George, E. N. Georges-Labouesse, R. S. Patel-King, H. Rayburn and R. O. Hynes: Defects in mesoderm,

neural tube and vascular development in mouse embryos lacking fibronectin. *Development*, 119(4), 1079-91 (1993)

59. Winklbauer and R. E. Keller: Fibronectin, mesoderm migration, and gastrulation in Xenopus. *Dev Biol*,177(2), 413-26 (1996)

60. T. Darribere and J. E. Schwarzbauer: Fibronectin matrix composition and organization can regulate cell migration during amphibian development. *Mechanisms of development*, 92(2), 239-50 (2000)

61. T. Rozario, B. Dzamba, G. F. Weber, L. A. Davidson and D. W. DeSimone: The physical state of fibronectin matrix differentially regulates morphogenetic movements in vivo. *Dev Biol*, 327(2), 386-98 (2009)

62. B. J. Dzamba, K. R. Jakab, M. Marsden, M. A. Schwartz and D. W. DeSimone: Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. *Developmental cell*, 16(3), 421-32 (2009)

63. D. Gradl, M. Kuhl and D. Wedlich: The Wnt/Wg signal transducer beta-catenin controls fibronectin expression. *Molecular and cellular biology*, 19(8), 5576-87 (1999)

64. B. Christ, R. Huang and M. Scaal: Amniote somite derivatives. *Developmental dynamics: an official publication of the American Association of Anatomists*, 236(9), 2382-96 (2007)

65. N. Holder and R. Klein: Eph receptors and ephrins: effectors of morphogenesis. *Development*, 126(10), 2033-44 (1999)

66. T. Watanabe, Y. Sato, D. Saito, R. Tadokoro and Y. Takahashi: EphrinB2 coordinates the formation of a morphologicalboundary and cell epithelialization during somite segmentation. *Proceedings of the National Academy of Sciences of the United States of America*, 106(18), 7467-72 (2009)

67. Y. Saga, N. Hata, H. Koseki and M. M. Taketo: Mesp2: a novel mouse gene expressed in the presegmented mesoderm and essential for segmentation initiation. *Genes & development*, 11(14), 1827-39 (1997)

68. C. Rallis, S. M. Pinchin and D. Ish-Horowicz: Cell-autonomous integrin control of Wnt and Notch signalling during somitogenesis. *Development*, 137(21), 3591-601 (2010)

69. D. Julich, R. Geisler and S. A. Holley: Integrinalpha5 and delta/notch signaling have complementary spatiotemporal requirements during zebrafish somitogenesis. *Developmental cell*, 8(4), 575-86 (2005)

70. J. T. Yang, B. L. Bader, J. A. Kreidberg, M. Ullman-Cullere, J. E. Trevithick and R. O. Hynes: Overlapping and independentfunctions of fibronectin receptor integrins in early mesodermal development. *Dev Biol*, 215(2), 264-77 (1999)

71. Koshida, Y. Kishimoto, H. Ustumi, T. Shimizu, M. Furutani-Seiki, H. Kondoh and S. Takada: Integrinalpha5-dependent fibronectin accumulation for maintenance of somite boundaries in zebrafish embryos. *Developmental cell*, 8(4), 587-98 (2005)

72. W. C. Dunty, Jr., K. K. Biris, R. B. Chalamalasetty, M. M. Taketo, M. Lewandoski and T. P. Yamaguchi: Wnt3a/beta-catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation. *Development*, 135(1), 8594 (2008)

73. G. L. Radice, H. Rayburn, H. Matsunami, K. A. Knudsen, M. Takeichi and R. O. Hynes: Developmental defects in mouse embryos lacking N-cadherin. *Dev Biol*, 181(1), 64-78 (1997)

74. K. Horikawa, G. Radice, M. Takeichi and O. Chisaka: Adhesive subdivisions intrinsic to the epithelial somites. *Dev Biol*, 215(2), 182-9 (1999)

75. J. L. Duband, S. Dufour, K. Hatta, M. Takeichi, G. M. Edelman and J. P. Thiery: Adhesion molecules during somitogenesisin the avian embryo. *The Journal of cell biology*, 104(5), 1361-74 (1987)

76. C. Schmidt, M. Stoeckelhuber, I. McKinnell, R. Putz, B. Christ and K. Patel: Wnt 6 regulates the epithelialisation process oft he segmental plate mesoderm leading to somite formation. *Dev Biol*, 271(1), 198-209 (2004)

77. C. Linker, C. Lesbros, J. Gros, L. W. Burrus, A. Rawls and C. Marcelle: beta-Catenin-dependent Wnt signalling controls the epithelial organisation of somites through the activation of paraxis. *Development*, 132(17), 3895-905 (2005)

78. Burgess, A. Rawls, D. Brown, A. Bradley and E. N. Olson: Requirement of the paraxis gene for somite formation and musculoskeletal patterning. *Nature*, 384(6609), 570-3 (1996)

79. J. K. Dale, P. Malapert, J. Chal, G. Vilhais-Neto, M. Maroto, T. Johnson, S. Jayasinghe, P. Trainor, B. Herrmann and O.Pourquie: Oscillations of the snail genes in the presomitic mesoderm coordinate segmental patterning and morphogenesis invertebrate somitogenesis. *Developmental cell*, 10(3), 355-66 (2006)

80. Y. Nakaya, S. Kuroda, Y. T. Katagiri, K. Kaibuchi and Y. Takahashi: Mesenchymal-epithelial transition during somatic segmentation is regulated by differential roles of Cdc42 and Rac1. *Developmental cell*, 7(3), 425-38 (2004)

81. Watanabe and Y. Takahashi: Tissue morphogenesis coupled with cell shape changes. *Current opinion in genetics &development*, 20(4), 443-7 (2010)

82. Y. Cinnamon, R. Ben-Yair and C. Kalcheim: Differential effects of N-cadherin-mediated adhesion on the

development ofmyotomal waves. *Development*, 133(6), 1101-12 (2006)

83. J. Wagner, C. Schmidt, W. Nikowits, Jr. and B. Christ: Compartmentalization of the somite and myogenesis in chick embryos are influenced by wnt expression. *Dev Biol*, 228(1), 86-94 (2000)

84. P. Geetha-Loganathan, S. Nimmagadda, R. Huang, B. Christ and M. Scaal: Regulation of ectodermal Wnt6 expression by the neural tube is transduced by dermomyotomal Wnt11: a mechanism of dermomyotomal lip sustainment. *Development*, 133(15), 2897-904 (2006)

85. L. Ciani and P. C. Salinas: WNTs in the vertebrate nervous system: from patterning to neuronal connectivity. *Nature reviews. Neuroscience*, 6(5), 351-62 (2005)

86. G. C. Schoenwolf and J. L. Smith: Mechanisms of neurulation: traditional viewpoint and recent advances. *Development*,109(2), 243-70 (1990)

87. R. J. Detrick, D. Dickey and C. R. Kintner: The effects of N-cadherin misexpression on morphogenesis in Xenopus embryos.Neuron, 4(4), 493-506 (1990)

88. Fujimori, S. Miyatani and M. Takeichi: Ectopic expression of N-cadherin perturbs histogenesis in Xenopus embryos. *Development*, 110(1), 97-104 (1990)

89. Z. Lele, A. Folchert, M. Concha, G. J. Rauch, R. Geisler, F. Rosa, S. W. Wilson, M. Hammerschmidt and L. Bally-Cuif: parachute/n-cadherin is required for morphogenesis and maintained integrity of the zebrafish neural tube. *Development*, 129(14) ,3281-94 (2002)

90. S. I. Ganzler-Odenthal and C. Redies: Blocking N-cadherin function disrupts the epithelial structure of differentiating neural tissue in the embryonic chicken brain. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 18(14),5415-25 (1998)

91. Y. Luo, M. Ferreira-Cornwell, H. Baldwin, I. Kostetskii, J. Lenox, M. Lieberman and G. Radice: Rescuing the N-cadherin knockout by cardiac-specific expression of N-or E-cadherin. *Development*, 128(4), 459-69 (2001)

92. Hierholzer and R. Kemler: Beta-catenin-mediated signaling and cell adhesion in postgastrulation mouse embryos. *Developmental dynamics: an official publication of the American Association of Anatomists*, 239(1), 191-9 (2010)

93. H. Cremer, R. Lange, A. Christoph, M. Plomann, G. Vopper, J. Roes, R. Brown, S. Baldwin, P. Kraemer, S. Scheff and et al.: Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature*, 367(6462), 455-9 (1994)

94. N. M. Abdul-Aziz, M. Turmaine, N. D. Greene and A. J. Copp: EphrinA-EphA receptor interactions in mouse spinal neurulation: implications for neural fold fusion. *The International journal of developmental biology*, 53(4), 559-68 (2009)

95. S. Nandadasa, Q. Tao, N. R. Menon, J. Heasman and C. Wylie: N-and E-cadherins in Xenopus are specifically required in the neural and non-neural ectoderm, respectively, for F-actin assembly and morphogenetic movements. *Development*, 136(8),1327-38 (2009)

96. N. Ueno and N. D. Greene: Planar cell polarity genes and neural tube closure. *Birth defects research*. Part C, Embryo today: reviews, 69(4), 318-24 (2003)

97. M. Shariatmadari, J. Peyronnet, P. Papachristou, Z. Horn, K. M. Sousa, E. Arenas and T. Ringstedt: Increased Wnt levels in the neural tube impair the function of adherens junctions during neurulation. *Molecular and cellular neurosciences*, 30(3), 437-51(2005)

98. M. Lindqvist, Z. Horn, V. Bryja, G. Schulte, P. Papachristou, R. Ajima, C. Dyberg, E. Arenas, T. P. Yamaguchi, H. Lagercrantz and T. Ringstedt: Vang-like protein 2 and Rac1 interact to regulate adherens junctions. *Journal of cell science*, 123(Pt 3), 472-83 (2010)

99. N. Kinoshita, N. Sasai, K. Misaki and S. Yonemura: Apical accumulation of Rho in the neural plate is important for neural plate cell shape change and neural tube formation. *Molecular biology of the cell*, 19(5), 2289-99 (2008)

100. M. K. Nyholm, S. Abdelilah-Seyfried and Y. Grinblat: A novel genetic mechanism regulates dorsolateral hinge-point formation during zebrafish cranial neurulation. *Journal of cell science*, 122(Pt 12), 2137-48 (2009)

101. L. A. Taneyhill: To adhere or not to adhere: the role of Cadherins in neural crest development. *Cell adhesion & migration*, 2(4), 223-30 (2008)

102. S. Nakagawa and M. Takeichi: Neural crest emigration from the neural tube depends on regulated cadherin expression. *Development*, 125(15), 2963-71 (1998)

103. F. Monier-Gavelle and J. L. Duband: Control of N-cadherin-mediated intercellular adhesion in migrating neural crest cells in vitro. *Journal of cell science*, 108 (Pt 12), 3839-53 (1995)

104. X. Xu, W. E. Li, G. Y. Huang, R. Meyer, T. Chen, Y. Luo, M. P. Thomas, G. L. Radice and C. W. Lo: Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. *The Journal of cell biology*, 154(1), 217-30 (2001)

105. B. Steventon, C. Carmona-Fontaine and R. Mayor: Genetic network during neural crest induction: from cell

specification to cell survival. *Seminars in cell & developmental biology*, 16(6), 647-54 (2005)

106. T. Burstyn-Cohen, J. Stanleigh, D. Sela-Donenfeld and C. Kalcheim: Canonical Wnt activity regulates trunk neural crest delamination linking BMP/noggin signaling with G1/S transition. *Development*, 131(21), 5327-39 (2004) .

107. Shoval, A. Ludwig and C. Kalcheim: Antagonistic roles of full-length N-cadherin and its soluble BMP cleavage product in neural crest delamination. *Development*, 134(3), 491-501 (2007)

108. N. Gavert, M. Sheffer, S. Raveh, S. Spaderna, M. Shtutman, T. Brabletz, F. Barany, P. Paty, D. Notterman, E. Domany and Ben-Ze'ev: Expression of L1-CAM and ADAM10 in human colon cancer cells induces metastasis. *Cancer research*, 67(16),7703-12 (2007)

109. R. B. Anderson, K. N. Turner, A. G. Nikonenko, J. Hemperly, M. Schachner and H. M. Young: The cell adhesion molecule l1 is required for chain migration of neural crest cells in the developing mouse gut. *Gastroenterology*, 130(4), 1221-32 (2006)

110. S. Piloto and T. F. Schilling: Ovo1 links Wnt signaling with N-cadherin localization during neural crest migration. *Development*, 137(12), 1981-90 (2010)

111. C. Carmona-Fontaine, H. Matthews and R. Mayor: Directional cell migration in vivo: Wnt at the crest. *Cell adhesion & migration*, 2(4), 240-2 (2008)

Abbreviations: PCP: planar cell polarity Fz: frizzled LRP5/6: low-density lipoprotein receptor-related protein 5/6Dsh: disheveled GSK3beta: glycogen synthase kinase 3 beta LEF1: lymphoid enhancer-binding factor 1 TCF: transcription factor APC: adenomatous polyposis coli protein CK1alpha: casein kinase 1 alpha CAMKII: calcium/calmodulin-dependent protein kinase type IIP KC: protein kinase CNA: calcineurin NFAT: nuclear factor of activated T-cells Cdc42: cell division control protein 42 NLK: nemo-like kinase JNK: c-Jun N-terminal kinase Daam1: disheveled-associated activator of morphogenesis 1 RhoA: ras homolog gene family, member A Rac1: ras-related C3 botulinum toxin substrate 1 Fmi: flamingo Celsr: cadherin EGF LAG seven-pass G-type receptor Stbm: strabismus Vangl2: van Gogh-like protein 2 Dgo: diego PK: prickle Ror2: receptor tyrosine kinase-like orphan receptor 2 IgG: immunoglobulin G ILK: integrin linked kinase ECM: extracellular matrix lgl: lethal giant larvae EMT: epithelial-mesenchymal transition FGF: fibroblast growth factor CE: convergent extrension PAPC: paraxial protocadherin PI3K : Phosphatidylinositol 3-kinase MO: morpholino Sost: sclerostin Xnr3: Xenopus nodal related 3 JAK/STAT: janus kinase/signal transducers and activators of transcription Slb: silberblick PM: paraxial mesoderm Mesp2: Mesoderm posterior protein 2 bHLH: basic helix-loop-helix Sfrp2: secreted frizzled-related protein 2 NT: neural tube NC: neural crest NP : neural plate N-CAM : neural cell adhesion molecule Zic: zinc finger protein DHLP: dorsolateral hinge point BMP: bone morphogenetic protein ADAM: a disintegrin and metalloprotease CTF1/2: c-terminal fraction ½ L1-CAM: neural cell adhesion molecule L1

Key Words: Wnt, Cell Adhesion, Gastrulation, Somitogenesis, Neurulation, Review

Send correspondence to: Kris Vleminckx, Department of Molecular Biomedical Research, VIB, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium, Tel 32-9-33-13720, Fax 32-9-33 13609, E-mail: kris.vleminckx@UGent.be

http://www.bioscience.org/current/vol16.htm