

## Non-canonical Wnt signalling and regulation of gastrulation movements

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*Members of the Wnt family have been implicated in a variety of developmental processes including axis formation, patterning of the central nervous system and tissue morphogenesis. Recent studies have shown that a Wnt signalling pathway similar to that involved in the establishment of planar cell polarity in *Drosophila* regulates convergent extension movements during zebrafish and *Xenopus* gastrulation. This finding provides a good starting point to dissect the complex cell biology and genetic regulation of vertebrate gastrulation movements.*

**Key words:** Wnt / convergent extension / planar polarity / zebrafish / *Xenopus*

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### Introduction

The basic body plan of vertebrate embryos is established during gastrulation by a series of co-ordinated movements of cell groups that give rise to the three germ layers—ectoderm, mesoderm and endoderm—and overtly shape the embryonic axis. One such movement is convergent extension, a process that has been best characterised in amphibians and teleosts. During convergent extension, cells of the mesoderm and ectoderm accumulate on the dorsal side of the gastrula by means of highly directed and integrated movements. This results in both medio-lateral narrowing (convergence) and anterior-posterior elon-

gation (extension) of tissues to create the embryonic axis<sup>1,2</sup> (Figure 1(A)).

In recent years, members of the Wnt family of secreted glycoproteins have been implicated in the regulation of morphogenesis during gastrulation<sup>3</sup> and the signalling pathways through which these Wnt signalling molecules act are now beginning to be uncovered. It appears that the Wg/Wnt signalling pathway used for establishment of epithelial planar cell polarity (PCP) in *Drosophila*—the process by which epithelial cells within the eye, wing and thorax become polarised along the surface plane of these tissues<sup>4–6</sup>—also plays an important role in regulating convergent extension movements in both zebrafish and *Xenopus*.

The zebrafish has developed over the last two decades into an important model organism to study multiple aspects of vertebrate development. Zebrafish embryos combine several features that are ideal for a cellular and genetic analysis of early developmental processes. They develop *ex utero* into transparent embryos that are easily accessible for experimental manipulations such as cell and tissue transplantations. Moreover, large-scale genetic screens have generated a wide selection of mutant lines exhibiting defects in many different developmental processes. More recently, molecular and genetic tools have been developed which allow targeted knock-down of gene function by the application of morpholino antisense oligonucleotides<sup>7</sup> and the generation of transgenic lines.

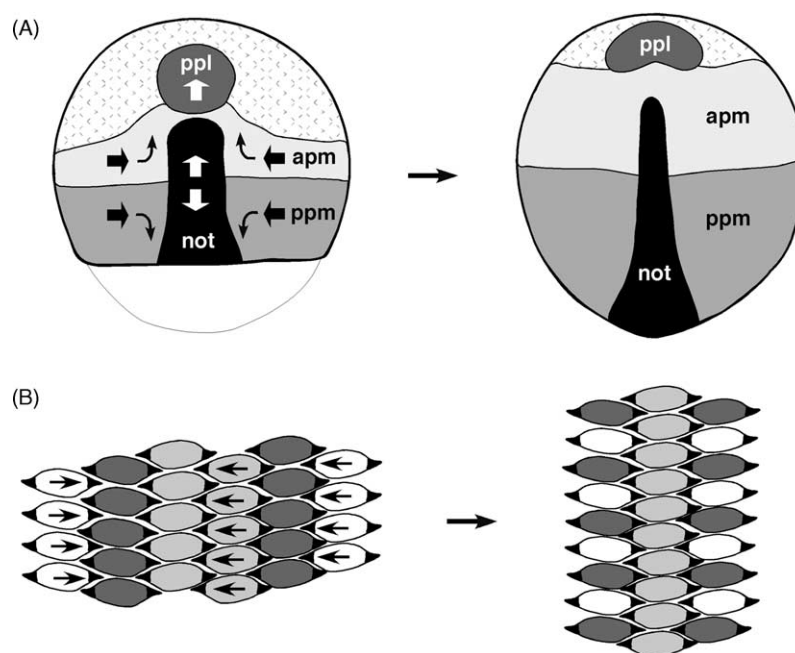
In this review we will focus on recent studies in zebrafish—and to a lesser extent in *Xenopus*—that suggest a role for non-canonical Wnt-signalling in regulating morphogenetic movements during vertebrate gastrulation.

### Cellular mechanisms underlying convergent extension in zebrafish and *Xenopus*

The cellular mechanisms underlying convergent extension have been extensively studied in the posterior mesoderm (notochord and somites) and

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**Figure 1.** Convergent extension in zebrafish. (A) Schematics of dorsal views of zebrafish embryos at 70% epiboly (left) and tailbud stages (right). Convergent extension of the mesoderm and ectoderm results from both medio-lateral narrowing (convergence, black arrows) and anterior-posterior elongation (extension, white arrows) of the embryonic axis. Mesodermal domains of expression of *wnt11* in the anterior paraxial mesoderm (apm), and *wnt5a* in the posterior paraxial mesoderm (ppm), are shown. Abbreviations: not (notochord), ppl: (prechordal plate). (B) Cellular basis of convergent extension of posterior tissues. Cells within the mesoderm and neural plate elongate along the medio-lateral axis while undergoing medio-lateral cell intercalation (left, arrows). This in turn leads to extension of embryonic tissues (right). Although the cell activities responsible for medio-lateral cell intercalation have not been analysed in detail in zebrafish, it is likely that they involve medio-laterally aligned protrusive activities similar to those described for *Xenopus*. See Reference 2.

neuroectoderm (spinal cord and hindbrain) of *Xenopus* embryos.<sup>1,2</sup> In these tissues, convergent extension is driven by tissue-autonomous activities that lead cells to become highly aligned along the major embryonic axes and which are integrated over time and space to generate forces that ultimately shape the entire gastrula. Within the mesoderm, cells predominantly develop a mode of bipolar medio-laterally oriented protrusive activity that results in medio-lateral cell intercalation<sup>8</sup> (Figure 1(B)). This in turn leads to extension of the mesoderm along the anterior-posterior and to some degree also thickening along the superficial to deep axes. Within the neural plate, the most prominent cell behaviour underlying convergent extension involves monopolar lateral to medial directed protrusive activity that depends on vertical signalling from the underlying mesoderm<sup>9</sup> and which also results in medio-lateral cell intercalation and elongation of the neural plate along the anterior-posterior axis.<sup>10</sup> Such aligned cell behavi-

ours likely require reorganisation of the cytoskeleton and modulation of cell adhesion but these issues have yet to be fully analysed.

Several observations suggest that the mechanisms of convergent extension in zebrafish are similar to those in *Xenopus*. The ability of tissues that undergo convergent extension movements to exhibit autonomous elongation when explanted<sup>11</sup> and to deform the yolk cell<sup>12</sup> indicates that this process is force generating in zebrafish as in frogs. Furthermore, the patterns of cell behaviours observed in the posterior mesoderm and neuroectoderm of zebrafish suggest that medio-lateral cell intercalation also contributes to convergent extension movements. Epiblast cells, for example, elongate along the medio-lateral axis while displaying autonomous protrusive activities.<sup>13</sup> This behaviour is contemporaneous with medio-lateral cell intercalation and anterior-posterior extension of the neural plate.<sup>13–15</sup> In the mesoderm, cells also become elongated along the medio-lateral axis<sup>16</sup>

while undergoing medio–lateral cell intercalation.<sup>14</sup> However, whether this pattern of cell elongation is a consequence of oriented protrusive activity is still unclear. Taken together, these observations suggest that convergent extension of the posterior mesoderm and neural plate makes use of a similar set of cell behaviours in both zebrafish and *Xenopus* although some variations are likely due to the dissimilar phylogenetic histories of these two species.

One aspect still unexplored in zebrafish is the extent to which mechanisms of convergent extension described for posterior tissues also operate for the anterior mesoderm (prechordal plate and anterior paraxial mesoderm) and neural plate (midbrain and forebrain). The observation that expression domains of different Wnt ligands implicated in convergent extension (e.g. *wnt11*<sup>17</sup> and *wnt5a*<sup>18</sup>) and other molecules (e.g. *papc*<sup>19</sup>) define anterior and posterior domains within the mesoderm of zebrafish gives a first indication that convergent extension in these two domains might differ (Figure 1(A)). Ongoing *in vivo* analyses of cell behaviour in zebrafish show that anterior axial and paraxial mesodermal cells seem not to undergo the classical medio–lateral cell intercalation behaviour described for posterior tissues, but instead exhibit a behaviour more typical of cells with directed migration on a substrate (MLC, MT, CPH, Steve Wilson, Richard Adams, unpublished observations) such as those described for the anterior migration of the prechordal plate in *Xenopus*.<sup>20</sup> Whether this difference in cell behaviour is reflected at the molecular level is still unclear and further analyses will be needed to corroborate this hypothesis.

## Zebrafish mutants exhibiting defective convergent extension movements

Several zebrafish mutants exhibit reduced convergent extension movements without major patterning defects of the gastrula (Table 1). The *silberblick* (*slb*) mutant shows a transiently shortened and broadened body axis at the end of gastrulation followed by a slight fusion of the eyes at later developmental stages.<sup>21,22</sup> In contrast, *pipetail* (*ppt*), *knypek* (*kny*) and *trilobite* (*tri*) mutant embryos exhibit a shortened body axis from late gastrulation stages onwards while the position of the eyes is only mildly affected.<sup>23–25</sup>

Cloning of the *slb*, *ppt* and *kny* loci has revealed that they all encode components associated with a Wnt signalling pathway. It was observed that *slb/wnt11* and *ppt/wnt5a* encode Wnt ligands<sup>17,18</sup> while *kny* encodes a member of the glypican family of heparan sulphate proteoglycans, proteins implicated in Wg/Wnt signal reception/transduction.<sup>16</sup> Positional cloning of the *tri* locus is well underway (Solnica-Krezel, personal communication) and it will be interesting to see whether it also encodes a component or modulator of the Wnt signalling cascade since *tri* genetically interacts with *kny* in regulating convergent extension.<sup>25</sup>

Mutations in other components of the Wnt signalling pathway have been identified in zebrafish but these primarily show defects in patterning rather than morphogenesis<sup>26–28</sup> (Table 1). It is therefore thought that these genes act in the canonical intracellular Wnt signalling cascade that may be relatively independent of the cascade regulating convergence extension.

**Table 1.** Mutations in genes encoding components of the canonical and non-canonical Wnt signals in zebrafish

Mutation	Gene product	Molecular role	Phenotype	References
Canonical pathway				
<i>wnt8</i>	Wnt8	Wnt ligand	Reduced posterior and ventral structures	28
<i>masterblind</i>	Axin1	Intracellular scaffolding protein	No eyes and telencephalon	27
<i>headless</i>	Tcf-3	Transcription co-factor	No forebrain	26
Non-canonical pathway				
<i>silberblick</i>	Wnt11	Wnt ligand	Weakly reduced CE (gastrula stage)	17
<i>pipetail</i>	Wnt5a	Wnt ligand	Weakly reduced CE (somitogenesis stage)	18
<i>knypek</i>	Glypican6	Wnt co-receptor?	Strongly reduced CE	16
<i>trilobite</i>	?	?	Strongly reduced CE	23, 24

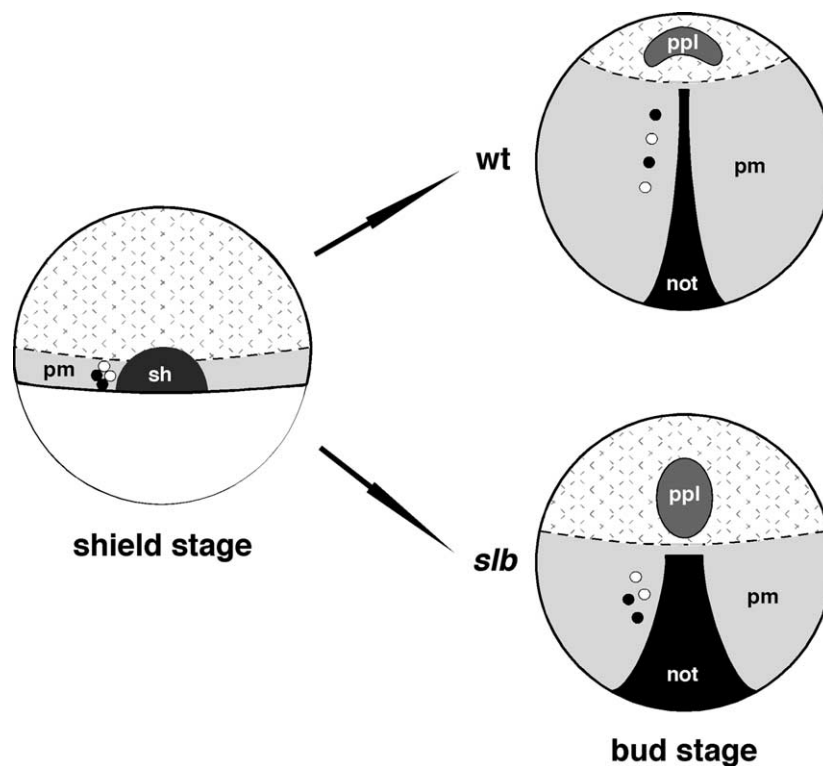
Notes: CE refers to convergent extension. Citations are selective and more details are given in the text.

### Identification of *silberblick* (*slb*) as a component of the Wnt/PCP pathway

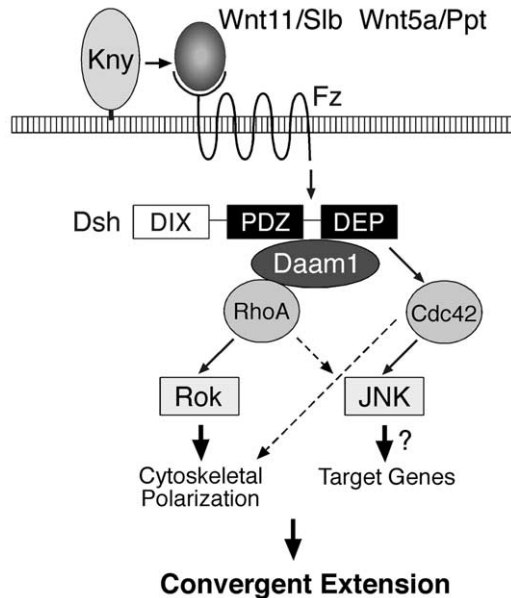
It was originally identified as a mutant affecting eye/forebrain development.<sup>21</sup> More detailed analysis, however, showed that the *slb* eye/forebrain phenotype is preceded by a reduced elongation of the body axis during gastrulation.<sup>22</sup> The *slb* locus encodes *wnt11*,<sup>17</sup> a gene initially expressed in the dorsal region of the germ ring at sphere/dome stage (4–5 hpf). Through the course of gastrulation *wnt11* expression expands to the lateral and ventral germ ring while becoming downregulated within the shield and its axial derivatives. In addition to the germ ring, *wnt11* is expressed in restricted domains within the anterior paraxial mesoderm and anterior lateral neuroectoderm by the end of gastrulation.

The phenotypic characterisation of *slb* mutant embryos has provided some initial insights into the potential genetic and cellular mechanisms of Slb function. Cell-labelling and tracking experiments have shown that the shortened body length of *slb* mutants is due to reduced cell intercalations along the medio–lateral axis during gastrulation<sup>17</sup> (Figure 2). Through cell- and tissue-transplantation experiments, it has been demonstrated that Slb acts cell-non-autonomously and that its function within paraxial tissues is sufficient to drive normal convergent extension movements of axial and paraxial tissues (Figure 3).

In *Drosophila*, establishment of epithelial PCP depends on a non-canonical Wg/Wnt-signalling cascade involving small GTPases (RhoA, Rac and Cdc42) and the Jun-N-terminal-kinase (JNK) cascade.<sup>4–6</sup> Various truncated forms of the Dsh protein, a downstream



**Figure 2.** Schematic diagram of the *slb* mutant phenotype. In *slb*<sup>−/−</sup> embryos, reduced medio–lateral cell intercalations of mesendodermal cells lead to a reduction in convergent extension of the embryonic axis. The schematised embryo on the left side represents a shield stage embryo (wild type or *slb*) in which a group of four cells (black and white) is labelled in the involuting paraxial mesendoderm (pm) next to the shield (sh). The schematised embryos on the right side represent a wildtype (top) and a *slb*<sup>−/−</sup> (bottom) embryo at tailbud stage. While the prechordal plate cells (ppl) have formed a polster at the anterior end of the axial mesendoderm and the notochord (not) has elongated along the anterior–posterior axis in the wild-type embryo, prechordal plate cells are displaced posteriorly and the notochord is short and broader in the *slb* mutant. Furthermore, the group of paraxial cells labelled at shield stage has undergone medio–lateral cell intercalations in the wild-type embryo whereas only reduced medio–lateral cell intercalations have occurred in the *slb* mutant.



**Figure 3.** A model for the non-canonical Wnt/PCP pathway regulating convergent extension during zebrafish/*Xenopus* gastrulation. The secreted ligands, Wnt11/Slb and Wnt5a/Ppt, bind to the receptor Frizzled-7, an interaction possibly facilitated by the GPI-anchored proteoglycan Kny. This leads to translocation of Dsh to the membrane. The PDZ and DEP domains of Dsh are responsible for the specific activation of the PCP/convergent extension pathway, but not of the canonical pathway, through mediation of the activity of members of the Rho family of small GTPases. RhoA, which is linked by Daam1 with Dsh, activates an effector Rok that in turn directly regulates the actin cytoskeleton. Alternatively or additionally, Dsh-mediated activation of Cdc42 signals to JNK that in turn regulates transcription of target genes. These two branches potentially collaborate to mediate convergent extension during zebrafish/*Xenopus* gastrulation. (see also text). The Wnt/ $\text{Ca}^{2+}$  pathway is not shown.

mediator of Wnt signalling, have been used to specifically activate/block the PCP signal transduction pathway.<sup>29,30</sup> In *slb* mutants, ubiquitous over-expression of full-length Dsh dorsalises embryos (through activation of the canonical signalling cascade), while injection of an N-terminal truncated form of Dsh, which specifically transduces the PCP pathway in *Drosophila*, is able to fully rescue the *slb* mutant phenotype.<sup>17</sup> Conversely, in wild type embryos, ubiquitous over-expression of a form of Dsh that specifically inhibits PCP signalling in *Drosophila* causes a phenotype reminiscent of the *slb* mutant. Together with studies in *Xenopus* demonstrating that Dsh directly controls

cell polarity in cells undergoing convergent extension via the non-canonical pathway,<sup>31,32</sup> these studies suggest that Slb/Wnt11 regulates convergent extension movements in zebrafish through activation of a pathway similar to the PCP signalling cascade in *Drosophila*.

Further experiments will have to address precisely where and when Slb/Wnt11 function is required in the gastrulating embryo and determine the function of Slb/Wnt11 on a cellular level. As Slb/Wnt11 is likely to signal through a pathway similar to the *Drosophila* PCP pathway, it will be interesting to determine whether Slb/Wnt11 can directly influence cell morphology/polarity and if so, how this might influence cell movement and tissue morphogenesis during gastrulation.

### Other zebrafish loci/genes genetically interacting with the Wnt/PCP pathway

Several other loci/genes are known to genetically interact with *slb/wnt11* in the regulation of convergent extension movements. The *ppt/wnt5a* mutant embryos display mild defects in convergent extension movements of the posterior body axis.<sup>18</sup> However, embryos mutant for both *slb/wnt11* and *ppt/wnt5a* show a strong reduction in convergent extension suggesting that Ppt/Wnt5a and Slb/Wnt11 have partially overlapping functions (CPH, MT, unpublished observations). This notion is also supported by previous studies in *Xenopus* showing that Wnt5, like Wnt11, can signal through a non-canonical Wnt-pathway to influence convergent extension movements during gastrulation.<sup>33–35</sup> Given the observed genetic interaction, it is surprising that zebrafish *wnt11* and *wnt5a* are expressed in largely non-overlapping domains (anterior *versus* posterior; also see Figure 1(A)), suggesting that they possess some far-reaching cell-non-autonomous activity during gastrulation.

In *kny* mutant embryos, convergent extension movements of the posterior body axis are strongly reduced.<sup>24</sup> The *kny* locus encodes a member of the glypican family of heparan sulphate proteoglycans<sup>16</sup> and embryos mutant for both *slb* and *kny* display an additive phenotype compared to either single mutant. This indicates that Slb/Wnt11 and Kny act in the same or parallel pathways. Furthermore, the ability of Slb/Wnt11 to rescue the *slb* phenotype can be enhanced by co-injection of *kny* suggesting that Kny can interact with the Slb/Wnt11 signal transduction cascade.<sup>16</sup> Similar to the *Drosophila* Dally (dly) and



Dally-like (dll), Kny could be involved in Wg/Wnt signal reception and/or extracellular distribution of Wnt proteins.<sup>36–38</sup> It is therefore conceivable that Kny enhances Slb/Wnt11 signalling by directly localising the ligand to the cell surface, thereby facilitating receptor–ligand interaction. Considering the fact that the *kny* mutation does not affect canonical Wnt signalling during zebrafish gastrulation, it is possible that Kny determines the preference of a cell to activate the Wnt/PCP pathway in response to Wnt11/Slb. Kny might thereby act in a similar way to LDL-receptor related proteins (LRPs) which facilitate Wnt signalling via the canonical pathway.<sup>39–42</sup>

Studies in *Xenopus* have identified Frizzled 7 (Fz7) as a potential receptor for Wnt11. Fz7 can bind directly to Wnt11<sup>43</sup> and both constitutively active and dominant negative forms of Fz7 interfere with normal convergent extension movements.<sup>43–45</sup> Similarly, inactivation of Fz7 by morpholino antisense oligonucleotides leads to reduced convergent extension movements<sup>46</sup> and a failure of tissue separation during involution of prospective mesendodermal cells.<sup>47</sup> In zebrafish and *Xenopus* during gastrulation,<sup>43–45, 48</sup> *fz7* is also expressed in largely overlapping domains with *wnt11*, which provides further evidence that Fz7 might indeed act as a Wnt11 receptor. Similar to Fz7, Fz2 may act as a receptor for Wnt5a since injection of *fz2* morpholinos into zebrafish embryos results in a phenotype reminiscent of *ppt/wnt5a*.<sup>49</sup>

### Intracellular mediators of Slb/Wnt11

What are the potential components of the signalling cascade downstream of Slb/Wnt11/Fz? Strong candidates are members of the family of small GTPases such as RhoA, Rac and Cdc42 that are considered to be direct regulators of cytoskeletal architecture.<sup>50</sup> In the establishment of PCP within the *Drosophila* eye, *rhoA* genetically interacts with *fz1* and *dsh* and acts downstream of these genes.<sup>51</sup> Similarly in *Xenopus*, Wnt11/Fz7 signalling activates *rhoA* during gastrulation<sup>52</sup> and Cdc42 may mediate the function of Wnt11/Fz7 in cells undergoing convergent extension.<sup>43</sup>

Most recently, Daam1 has been identified as a key factor linking Dsh to the small GTPase RhoA.<sup>52</sup> Daam1 can bind to Dsh and also directly to RhoA thereby mediating Wnt-induced Dsh/RhoA complex formation. Daam1 function is required for RhoA activation and gastrulation movements in *Xenopus* embryos, while defective morphogenetic movements caused

by loss of Wnt11, Fz7 or Dsh activity are rescued by an active form of Daam1. These observations provide strong evidence that Daam1 functions downstream of Wnt11/Fz7.

In *Drosophila*, Rho-associated kinase (*Drok*), a RhoA effector links Fz-mediated PCP signalling directly to the rearrangement of the actin cytoskeleton.<sup>53</sup> Similarly in zebrafish, inhibition of Rok2 function by use of a dominant-negative version of Rok2 causes reduced convergent extension movements, while over-expression of *rok2* can rescue the *slb/wnt11* mutant phenotype (Lila Solnica-Krezel, personal communication). This suggests that Rok2 acts downstream of Wnt11 to influence the actin cytoskeleton during gastrulation.

One of the remaining questions is whether the Wnt/PCP pathway transcriptionally regulates target genes that influence cytoskeletal rearrangements. In the *Drosophila* eye, the small GTPases Rac and RhoA act downstream of Fz/Dsh and signal via MAP kinase effectors such as Misshapen (Msn) through to the JNK cascade. In turn this cascade activates transcriptional targets, such as *delta*, involved in the establishment of PCP.<sup>5, 54</sup> There is mounting evidence that transcriptional targets are also required for Wnt/PCP signalling during vertebrate gastrulation. First, Dsh can activate JNK in cultured cells and this activation is at least in part mediated by Cdc42.<sup>29, 55, 56</sup> Second, Wnt5a protein is also capable of activating JNK in cultured cells.<sup>57</sup> Third, modulators of Dsh function including Naked cuticle (Nkd), casein kinase I (CKI) and Strabismus/Van Gogh (Stbm/Vang) are able to affect convergent extension cell behaviours as well as to activate JNK.<sup>58–60</sup> However, as yet there is no direct evidence that the Wnt/PCP pathway requires transcriptional targets to regulate convergent extension movements in vertebrates. Although XFz8 has the capacity to activate JNK<sup>61</sup> and a dominant-negative XFz8 can modulate convergent extension in *Xenopus* embryos,<sup>32, 62, 63</sup> this Fz-mediated activation of JNK occurs in a Dsh-independent manner and is closely associated with inducing apoptosis rather than regulating convergent extension.<sup>61</sup> This argues that the activation of JNK might be uncoupled from the output of the Wnt/PCP pathway that regulates cell polarity. The only currently known potential transcriptional target for the Slb/Wnt11 pathway in regulating convergent extension is *wnt11* itself since *wnt11* expression is down-regulated in *slb* embryos.<sup>17</sup> The identification of further transcriptional targets is needed to clarify the role of transcription in Wnt/PCP signalling during vertebrate gastrulation.

### Involvement of other PCP-specific genes in the regulation of convergent extension

A fundamental and unresolved question regarding both the establishment of PCP in flies and the generation of convergent extension movements in vertebrates is how cells interact with one another in order to co-ordinate the response to a polarizing signal within a large population of cells. Genes shown to function cell-non-autonomously in the establishment of PCP in *Drosophila* are *fz*, *flamingo* (*fmi*) and *stbm/vang* (reviewed in Reference 4). A cadherin-related molecule is encoded by *fmi* that plays a pivotal role in establishing PCP in part by mediating cell adhesion with surrounding cells.<sup>64</sup> In *Drosophila*, membrane localisation of Dsh is thought to be one of the key processes in the establishment of PCP,<sup>30</sup> presumably leading to the formation of a functional signalling complex, including Fmi, Fz and Diego.<sup>65–68</sup>

The *stbm/vang* gene encodes a novel four-pass membrane protein with a putative C-terminal PDZ-binding motif and cell-non-autonomously affects polarity of adjacent cells in the *Drosophila* wing disc.<sup>69,70</sup> A recent analysis has revealed that a vertebrate homologue of *stbm/vang* regulates convergent extension in *Xenopus* and zebrafish.<sup>60</sup> The mode of action of Stbm/Vang in the regulation of convergent extension is still unclear, but might in part be explained by the ability of Stbm/Vang to bind and recruit Dsh to the membrane thereby facilitating the activation of JNK.

Further studies on the function of vertebrate homologues of other *Drosophila* PCP-specific genes will be needed to better understand the mechanisms by which these genes regulate convergent extension in vertebrate gastrulation.

### Involvement of Wnt/Ca<sup>2+</sup> pathway in regulating convergent extension

Several lines of evidence suggest that a further branch of the Wnt pathway that modulates intracellular Ca<sup>2+</sup> levels could be involved in regulating convergent extension movements. Xwnt5a is capable of stimulating intracellular Ca<sup>2+</sup> release in combination with Fz2 in a G-protein coupled manner<sup>71</sup> and can thereby activate Ca<sup>2+</sup>-sensitive enzymes including Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CamKII) and protein kinase C (PKC).<sup>72–74</sup> Interestingly, interfering with *fz7* function leads to defective separation of mesodermal and ectodermal germ layers in *Xenopus* gastrula embryos, and this function ap-

pears to be mediated by PKC in a G-protein-dependent manner.<sup>47</sup> This indicates that Fz7 may regulate the adhesive properties of tissues during gastrulation through the activation of Ca<sup>2+</sup> signalling.

There is, as yet, no direct genetic evidence that the Wnt/Ca<sup>2+</sup> pathway is directly involved in regulating convergent extension in zebrafish gastrula embryos. Validation of a role for Ca<sup>2+</sup> signalling will require the identification of mutant loci encoding intracellular mediators of the Wnt/Ca<sup>2+</sup> pathway that exhibit defective convergent extension movements. It will also be of interest to test whether intracellular Ca<sup>2+</sup> signalling is altered in *slb/wnt11* or *ppt/wnt5a* mutant embryos.

In addition to intracellular Ca<sup>2+</sup> signals, recent reports suggest that, at least in *Xenopus*, intercellular Ca<sup>2+</sup> signals could play an important role in the co-ordination of individual cell behaviour within tissues undergoing convergent extension movements.<sup>75,76</sup> Calcium waves are generated within the dorsal tissues of gastrula embryos at some frequency and propagated to surrounding cells over long distances. This dynamic event is closely associated with the generation of convergent extension within the dorsal tissue. It is likely that Wnt signals are involved in the regulation of some aspects of these Ca<sup>2+</sup> waves as their frequency is reduced through over-expression of a dominant-negative form of *xfz8* which strongly inhibits both canonical and non-canonical Wnt signalling.<sup>75</sup> Further experiments are required to elucidate the mechanisms that underlie intercellular Ca<sup>2+</sup> signals in relation to non-canonical Wnt/PCP signals. These may lead us to a better understanding of how the Wnt/PCP signals co-ordinate convergent extension movements in large populations of cells.

### Perspective

Genetic evidence in zebrafish, together with functional analyses in *Xenopus*, strongly support the notion that there are similarities between the Wnt/Wg signalling pathways controlling gastrulation movements in zebrafish/*Xenopus* and those controlling PCP in *Drosophila*. However, despite the similarities between these signalling pathways, there are also significant differences both molecular and also with respect to cellular contexts and outcomes. For instance, in *Drosophila* PCP signalling no ligand for the Fz receptor has yet been identified while in zebrafish and *Xenopus* it is clear that both Wnt11 and Wnt5a constitute central components of the pathway regulating gastrulation movements. Furthermore, while PCP is

exclusively established within an epithelial sheet of cells, Wnt-signalling in vertebrate gastrulation affects dynamic cellular assemblies that exhibit both epithelial as well as mesenchymal features. It is one of the main challenges for the future to identify not only the shared features of these signalling cascades but also the evolutionary diverse adaptations that these pathways have undergone. Continued isolation of genes from screens for convergent extension phenotypes, coupled with cell behaviour analyses using genetic and molecular approaches, will undoubtedly lead to a better understanding of how vertebrate gastrulation movements are regulated by mediators of Wnt/PCP signalling.

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## References

- Keller R, Shih J, Domingo C (1992) The patterning and functioning of protrusive activity during convergence and extension of the *Xenopus* organiser. *Dev Suppl* 81–91
- Keller R, Davidson L, Edlund A, Elul T, Ezin M, Shook D, Skoglund P (2000) Mechanisms of convergence and extension by cell intercalation. *Philos Trans R Soc Lond B Biol Sci* 355:897–922
- Moon RT, Brown JD, Torres M (1997) WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet* 13:157–162
- Adler PN, Lee H (2001) Frizzled signaling and cell–cell interactions in planar polarity. *Curr Opin Cell Biol* 13:635–640
- Mlodzik M (1999) Planar polarity in the *Drosophila* eye: a multifaceted view of signaling specificity and cross-talk. *EMBO J* 18:6873–6879
- Shulman JM, Perrimon N, Axelrod JD (1998) Frizzled signaling and the developmental control of cell polarity. *Trends Genet* 14:452–458
- Nasevicius A, Ekker SC (2000) Effective targeted gene ‘knock-down’ in zebrafish. *Nat Genet* 26:216–220
- Shih J, Keller R (1992) Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* 116:901–914
- Elul T, Keller R (2000) Monopolar protrusive activity: a new morphogenic cell behavior in the neural plate dependent on vertical interactions with the mesoderm in *Xenopus*. *Dev Biol* 224:3–19
- Keller R, Shih J, Sater A (1992) The cellular basis of the convergence and extension of the *Xenopus* neural plate. *Dev Dyn* 193:199–217
- Laale H (1982) Fish embryo culture: observations on axial cord differentiation in presomitic isolates of the zebrafish *Brachydanio rerio*. *Can J Zool* 60:1710–1721
- Baumann M, Sander K (1984) Bipartite axiation follows incomplete epiboly in zebrafish embryos treated with chemical teratogens. *J Exp Zool* 230:363–376
- Concha ML, Adams RJ (1998) Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. *Development* 125:983–994
- Warga RM, Kimmel CB (1990) Cell movements during epiboly and gastrulation in zebrafish. *Development* 108:581–591
- Kimmel CB, Warga RM, Kane DA (1994) Cell cycles and clonal strings during formation of the zebrafish central nervous system. *Development* 120:265–276
- Topczewski J, Sepich DS, Myers DC, Walker C, Amores A, Lele Z, Hammerschmidt M *et al.* (2001) The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev Cell* 1:251–264
- Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, Geisler R, Stemple DL *et al.* (2000) Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405:76–81
- Rauch GJ, Hammerschmidt M, Blader P, Schauerte HE, Strahle U, Ingham PW, McMahon AP *et al.* (1997) Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb Symp Quant Biol* 62:227–234
- Yamamoto A, Amacher SL, Kim SH, Geissert D, Kimmel CB, De Robertis EM (1998) Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. *Development* 125:3389–3397
- Winklbauer R, Nagel M (1991) Directional mesoderm cell migration in the *Xenopus* gastrula. *Dev Biol* 148:573–589
- Heisenberg CP, Brand M, Jiang YJ, Warga RM, Beuchle D, van Eeden FJ, Furutani-Seiki M *et al.* (1996) Genes involved in forebrain development in the zebrafish, *Danio rerio*. *Development* 123:191–203
- Heisenberg CP, Nusslein-Volhard C (1997) The function of *silberblick* in the positioning of the eye anlage in the zebrafish embryo. *Dev Biol* 184:85–94
- Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, Brand M, van Eeden FJ, Furutani-Seiki M *et al.* (1996) Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, *Danio rerio*. *Development* 123:143–151
- Solnica-Krezel L, Stemple DL, Mountcastle-Shah E, Rangini Z, Neuhauss SC, Malicki J, Schier AF *et al.* (1996) Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. *Development* 123:67–80
- Marlow F, Zwartkruis F, Malicki J, Neuhauss SC, Abbas L, Weaver M, Driever W *et al.* (1998) Functional interactions of genes mediating convergent extension, *knypek* and *trilobite*, during the partitioning of the eye primordium in zebrafish. *Dev Biol* 203:382–399
- Kim CH, Oda T, Itoh M, Jiang D, Artinger KB, Chandrasekharappa SC, Driever W *et al.* (2000) Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* 407:913–916
- Heisenberg CP, Houart C, Take-Uchi M, Rauch GJ, Young N, Coutinho P, Masai I *et al.* (2001) A mutation in the Gsk3-binding domain of zebrafish Masterblind/Axin1 leads to a fate transformation of telencephalon and eyes to diencephalon. *Genes Dev* 15:1427–1434



28. Lekven AC, Thorpe CJ, Waxman JS, Moon RT (2001) Zebrafish *wnt8* encodes two Wnt8 proteins on a bicistronic transcript and is required for mesoderm and neurectoderm patterning. *Dev Cell* 1:103–114
29. Boutros M, Paricio N, Strutt DI, Mlodzik M (1998) Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell* 94:109–118
30. Axelrod JD, Miller JR, Shulman JM, Moon RT, Perrimon N (1998) Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. *Genes Dev* 12:2610–2622
31. Tada M, Smith JC (2000) *Xwnt11* is a target of *Xenopus* Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* 127:2227–2238
32. Wallingford JB, Rowning BA, Vogeli KM, Rothbacher U, Fraser SE, Harland RM (2000) Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* 405:81–85
33. Wallingford JB, Vogeli KM, Harland RM (2001) Regulation of convergent extension in *Xenopus* by Wnt5a and Frizzled-8 is independent of the canonical Wnt pathway. *Int J Dev Biol* 45:225–227
34. Du SJ, Purcell SM, Christian JL, McGrew LL, Moon RT (1995) Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in *Xenopus* embryos. *Mol Cell Biol* 15:2625–2634
35. Moon RT, Campbell RM, Christian JL, McGrew LL, Shih J, Fraser S (1993) Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. *Development* 119:97–111
36. Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, Fox B, Humphrey M *et al.* (1999) The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. *Nature* 400:276–280
37. Lin X, Perrimon N (1999) Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling. *Nature* 400:281–284
38. Baeg GH, Lin X, Khare N, Baumgartner S, Perrimon N (2001) Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* 128:87–94
39. Mao B, Wu W, Li Y, Hoppe D, Stanek P, Glinka A, Niehrs C (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 411:321–325
40. Mao J, Wang J, Liu B, Pan W, Farr III GH, Flynn C, Yuan H *et al.* (2001) Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. *Mol Cell* 7:801–809
41. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA (2001) Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nat Cell Biol* 3:683–686
42. Semenov MV, Tamai K, Brott BK, Kuhl M, Sokol S, He X (2001) Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr Biol* 11:951–961
43. Djiane A, Riou J, Umbhauer M, Boucaut J, Shi D (2000) Role of Frizzled 7 in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*. *Development* 127:3091–3100
44. Sumanas S, Strege P, Heasman J, Ekker SC (2000) The putative wnt receptor *Xenopus* frizzled-7 functions upstream of beta-catenin in vertebrate dorsoventral mesoderm patterning. *Development* 127:1981–1990
45. Medina A, Reintsch W, Steinbeisser H (2000) *Xenopus* frizzled 7 can act in canonical and non-canonical Wnt signaling pathways: implications on early patterning and morphogenesis. *Mech Dev* 92:227–237
46. Sumanas S, Ekker SC (2001) *Xenopus* frizzled-7 morphant displays defects in dorsoventral patterning and convergent extension movements during gastrulation. *Genesis* 30:119–122
47. Winklbauer R, Medina A, Swain RK, Steinbeisser H (2001) Frizzled-7 signalling controls tissue separation during *Xenopus* gastrulation. *Nature* 413:856–860
48. El-Messaoudi S, Renucci A (2001) Expression pattern of the Frizzled 7 gene during zebrafish embryonic development. *Mech Dev* 102:231–234
49. Sumanas S, Kim HJ, Hermanson S, Ekker SC (2001) Zebrafish frizzled-2 morphant displays defects in body axis elongation. *Genesis* 30:114–118
50. Hall A (1998) Rho GTPase and the actin cytoskeleton. *Science* 297:509–514
51. Strutt DI, Weber U, Mlodzik M (1997) The role of RhoA in tissue polarity and Frizzled signalling. *Nature* 387:292–295
52. Habas R, Kato Y, He X (2001) Wnt/Frizzled activation of rho regulates vertebrate gastrulation and requires a novel formin homology protein daam1. *Cell* 107:843–854
53. Winter CG, Wang B, Ballew A, Royou A, Karess R, Axelrod JD, Luo L (2001) *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* 105:81–91
54. Fanto M, Weber U, Strutt DI, Mlodzik M (2000) Nuclear signaling by Rac and Rho GTPases is required in the establishment of epithelial planar polarity in the *Drosophila* eye. *Curr Biol* 10:979–988
55. Moriguchi T, Kawachi K, Kamakura S, Masuyama N, Yamanaka H, Matsumoto K, Kikuchi A *et al.* (1999) Distinct domains of mouse dishevelled are responsible for the c-Jun N-terminal kinase/stress-activated protein kinase activation and the axis formation in vertebrates. *J Biol Chem* 274:30957–30962
56. Li L, Yuan H, Xie W, Mao J, Caruso AM, McMahon A, Sussman DJ *et al.* (1999) Dishevelled proteins lead to two signaling pathways. Regulation of LEF-1 and c-Jun N-terminal kinase in mammalian cells. *J Biol Chem* 274:129–134
57. Yamanaka H, Moriguchi T, Masuyama N, Kusakabe M, Hanafusa H, Takada R, Takada S *et al.* (2002) JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates. *EMBO Rep* 3:69–75
58. Yan D, Wallingford JB, Sun TQ, Nelson AM, Sakanaka C, Reinhard C, Harland RM *et al.* (2001) Cell autonomous regulation of multiple Dishevelled-dependent pathways by mammalian Nkd. *Proc Natl Acad Sci USA* 98:3802–3807
59. McKay RM, Peters JM, Graff JM (2001) The casein kinase I family: roles in morphogenesis. *Dev Biol* 235:378–387
60. Park M, Moon RT (2002) The planar cell-polarity gene *stbm* regulates cell behaviour and cell fate in vertebrate embryos. *Nat Cell Biol* 4:20–25
61. Lisovsky M, Itoh K, Sokol SY (2002) Frizzled receptors activate a novel JNK-dependent pathway that may lead to apoptosis. *Curr Biol* 12:53–58
62. Deardorff MA, Tan C, Conrad LJ, Klein PS (1998) Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* 125:2687–2700
63. Itoh K, Jacob J, Sokol SY (1998) A role for *Xenopus* Frizzled 8 in dorsal development. *Mech Dev* 74:145–157
64. Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M *et al.* (1999) Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* 98:585–595

65. Strutt DI (2001) Asymmetric localization of frizzled and the establishment of cell polarity in the *Drosophila* wing. *Mol Cell* 7:367–375
66. Axelrod JD (2001) Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. *Genes Dev* 15:1182–1187
67. Shimada Y, Usui T, Yanagawa S, Takeichi M, Uemura T (2001) Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. *Curr Biol* 11:859–863
68. Feiguin F, Hannus M, Mlodzik M, Eaton S (2001) The ankyrin repeat protein Diego mediates Frizzled-dependent planar polarization. *Dev Cell* 1:93–101
69. Taylor J, Abramova N, Charlton J, Adler PN (1998) *Van Gogh*: a new *Drosophila* tissue polarity gene. *Genetics* 150:199–210
70. Wolff T, Rubin GM (1998) *strabismus*, a novel gene that regulates tissue polarity and cell fate decisions in *Drosophila*. *Development* 125:1149–1159
71. Slusarski DC, Yang-Snyder J, Busa WB, Moon RT (1997) Modulation of embryonic intracellular  $\text{Ca}^{2+}$  signaling by Wnt-5A. *Dev Biol* 182:114–120
72. Kuhl M, Sheldahl LC, Malbon CC, Moon RT (2000)  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in *Xenopus*. *J Biol Chem* 275:12701–12711
73. Sheldahl LC, Park M, Malbon CC, Moon RT (1999) Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr Biol* 9:695–698
74. Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT (2000) The Wnt/ $\text{Ca}^{2+}$  pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 16:279–283
75. Wallingford JB, Ewald AJ, Harland RM, Fraser SE (2001) Calcium signaling during convergent extension in *Xenopus*. *Curr Biol* 11:652–661
76. Tada M, Concha ML (2001) Vertebrate gastrulation: calcium waves orchestrate cell movements. *Curr Biol* 11:R470–R472