

# Wnt signaling in axon guidance

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**Recent studies have identified Wnt proteins as conserved axon guidance molecules in vertebrates and invertebrates. Wnt proteins are a large family of diffusible factors that play several important roles, both in embryonic development and in adult function. The signaling mechanisms of Wnt proteins are complex and, because Wnts are newly discovered as axon guidance cues, little is known about how Wnt signaling controls the direction of growth cone navigation – a process that is crucial in development of the nervous system. This review summarizes recent work on the role of Wnts in axon guidance and discusses the possible signaling mechanisms involved in growth cone guidance. Understanding how Wnts regulate axon wiring will not only help us to understand how the nervous system is connected but also provide possible tools for axon regeneration.**

Wnt family signaling proteins play several important roles in development and in function of the mature animal. It is well established that they are crucial in developmental processes such as axis patterning, cell fate specification and proliferation. In recent years, evidence suggesting that Wnt proteins also play important roles in axon development during nervous system formation has been accumulating. Wnt proteins were first found to be able to remodel axons and act as retrograde signals during synapse formation in the cerebellum [1,2]. More recent work showed that Wnt proteins are conserved axon guidance molecules [3,4]. Because several reviews already covered Wnts in axon remodeling and synaptogenesis [5,6], this review focuses on Wnts in axon guidance. Although much work has been done on Wnt signaling mechanisms [7–13], currently virtually nothing is known about the signal transduction pathways that lead to directed growth of axons; this promises to be an active area of investigation in the coming years. This review will briefly mention the current status of Wnt signaling studies and then explore the mechanisms that might be relevant to growth cone guidance.

## **Axon pathfinding: Wnts are bifunctional axon guidance molecules**

The first hint that Wnt proteins might be regulators of axon development came from the observation that DWnt3 (now named DWnt5) protein was found on axons of the ladder-like ventral nerve cord of the fly embryonic CNS [14]. Loss-of-function data were not available because of a lack of mutants at the time. Ubiquitous overexpression of

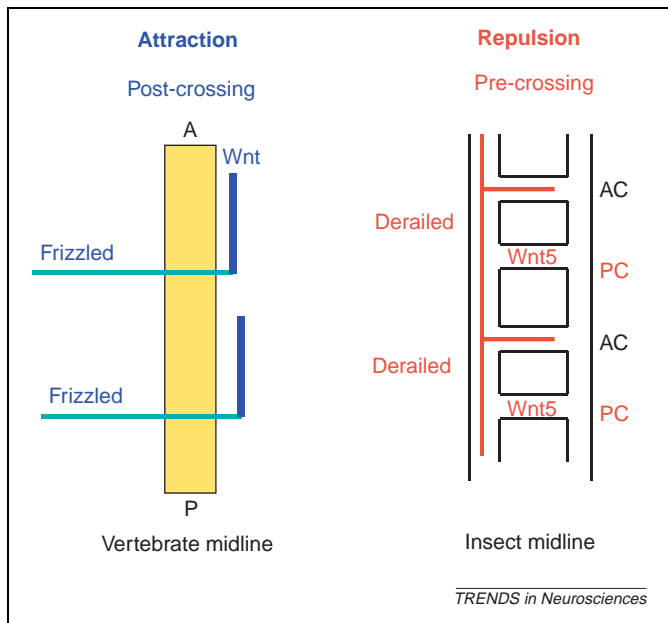
DWnt3 resulted in essentially wild-type cuticle morphology and *engrailed* gene expression domains during early segmentation (which is controlled by the *Wingless* gene), but at a later stage the overexpression caused severe disruption of CNS commissural axon tracts, such as reduced width of axon bundles crossing the ventral midline in the ventral nerve cord [14]. Although this study suggested that DWnt3 has a function distinct from the segment polarity gene *Wingless*, the role of DWnt3 in axons was not clear at the time. Several possible explanations were suggested to account for the defects caused by overexpression, including overproduction of the protein, inappropriate timing of expression, and changes in the fates of subsets of cells such as glia. In a separate line of research, an atypical receptor-tyrosine-kinase-type protein, Derailed, was shown to mediate a repulsive guidance mechanism at the midline controlling the pathway selection of commissural axons (i.e. the decision of which commissure to cross). An elegant study identified a genetic interaction of *Derailed* with DWnt5, and subsequent biochemical studies suggested that Derailed protein is a receptor for DWnt5 and mediates the repulsive function of DWnt5 in controlling pathway selection in the ventral nerve cord [3,15,16]. An independent study seeking guidance cues along the anterior–posterior (A–P) axis of the vertebrate CNS identified Wnt proteins as attractive cues for A–P guidance of spinal cord commissural axons after midline crossing [4]. Using a series of *in vitro* explant assays, this study provided direct evidence that Wnt proteins convey directional information for growth cone pathfinding. A Wnt receptor, Frizzled3, was implicated in mediating the attractive guidance function of Wnt in commissural axon pathfinding. Commissural axons ignore Wnt proteins until they reach the midline, and become attracted to Wnts immediately after midline crossing (Figure 1).

## **Wnt signaling: which pathway (or pathways) regulates axon guidance?**

Wnts are secreted, cysteine-rich proteins that are key players in several aspects of development, including axis formation, patterning, cell fate determination, proliferation, tissue polarity, morphogenesis, cell motility and synaptogenesis [7–11]. Wnt proteins bind to a large family of seven-transmembrane-domain proteins, the Frizzled proteins. Although recent studies converge on the conclusion that Wnt proteins are axon guidance molecules, very little is known about the Wnt signaling mechanisms in growth cone guidance. This is a very intriguing problem, not least because of the many roles played by Wnt family signaling proteins in developmental processes.

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**Figure 1.** Midline axon guidance by Wnts. At the vertebrate midline, commissural axons are attracted by Wnt proteins after midline crossing and are guided to grow anteriorly. The attractive function of Wnt proteins is likely to be mediated by Frizzled3. At the midline of the *Drosophila* ventral nerve cord, a subset of commissural axons that crosses through only anterior commissures (AC) is repelled by Wnt5 present in posterior commissures (PC) before entering the midline in each segment. The repulsive function of Wnt5 is mediated by Derailed.

The specificity of biological responses, therefore, lies in signaling mechanisms in the cells responding to Wnts. At present, it appears that Wnts can act as both repulsive and attractive cues in axon guidance. Repulsion of pre-crossing commissural axons in *Drosophila* is mediated by Derailed, a novel Wnt receptor, and the attractive response of post-crossing commissural axons in rodents involves Frizzled3.

#### Frizzled-dependent pathways in axon pathfinding

Wnt–Frizzled pathways have been studied intensely. Currently, there are three known major signaling pathways: the canonical Wnt– $\beta$ -catenin pathway, the planar cell polarity (PCP) pathway, and the Wnt– $\text{Ca}^{2+}$  pathway [7–13] (Figure 2). More recently, interactions among these pathways have begun to be explored.

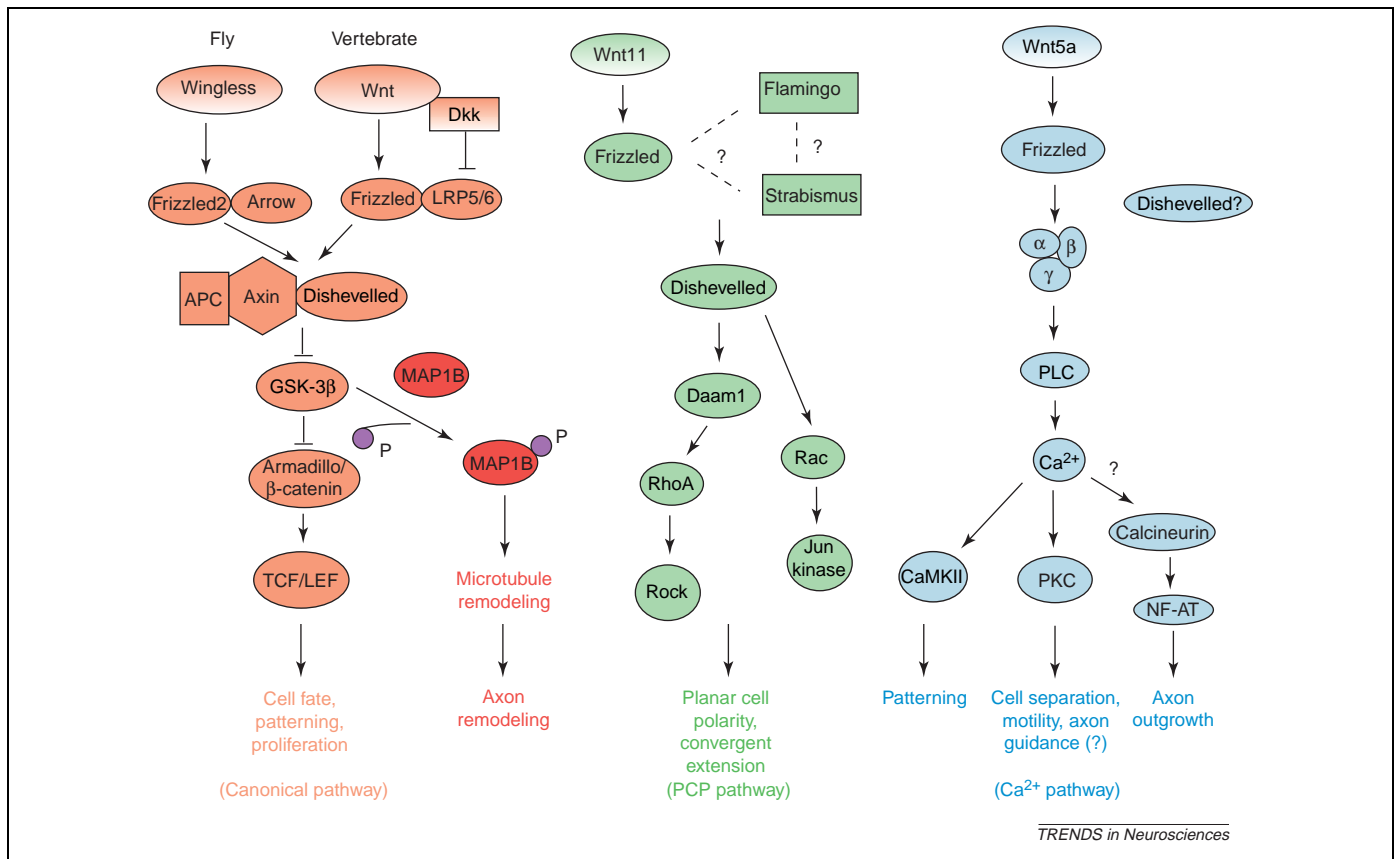
#### Can the canonical pathway regulate axon pathfinding?

The canonical Wnt– $\beta$ -catenin pathway, exemplified by the *Drosophila* *Wingless* pathway that is required for the formation of parasegmental boundaries, involves DFrizzled2 as the cell surface receptor and Dishevelled and Armadillo as downstream intracellular signaling components (Figure 2). Armadillo and its vertebrate homologue  $\beta$ -catenin are normally actively degraded in the cytoplasm. Upon activation of the canonical Wnt signaling pathway, Armadillo or  $\beta$ -catenin is stabilized and translocated into the nucleus and activates the high-mobility group (HMG)-box transcription factor T-cell factor (TCF; also known as lymphoid-enhancer factor or LEF), which activates the downstream Wnt response genes. Low-density lipoprotein (LDL)-receptor-related protein (LRP) family members LRP5 and LRP6 are the Wnt coreceptors specific to the canonical Wnt– $\beta$ -catenin pathway. The Wnt–Frizzled–LRP complex is required for

stabilization and nuclear translocation of  $\beta$ -catenin, and in vertebrates, LRP5- and LRP6-binding proteins (Dickkopfs) block the formation of such a complex and, thus, specifically inhibit the canonical pathway [8].

Wnt signaling through glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) has been implicated in axon remodeling before synapse formation, presumably through microtubule remodeling by regulating phosphorylation of microtubule-associated protein (MAP)1B [17,18]. This was proposed to be a divergent canonical pathway (Figure 2). For growth cone pathfinding, it is currently unclear whether the canonical pathway is involved. No direct connections to the actin cytoskeleton have been found in the canonical pathway. LRP6, a coreceptor that is required for the canonical pathway, was found not to be required for A–P axon guidance of spinal cord commissural axons, although patterning defects were found in the spinal cord and other parts of the embryo in LRP6-knockout mice [4]. This would suggest that the canonical pathway is not involved in axon guidance. Further studies will be needed to determine whether LRP5 is compensating for the loss of LRP6 function in these mutant mice. The canonical pathway is worth further attention in axon guidance because stabilization of  $\beta$ -catenin not only leads to transcriptional responses but also could modulate cadherin-mediated adhesion, potentially contributing to axon pathfinding.

*Is the PCP pathway involved in axon guidance?* In *Drosophila*, the PCP pathway controls the tissue polarity of epithelial structures such as the orientation of cuticle hairs and sensory bristles [9–11] (Figure 2). Frizzled mutations lead to distortion of the tissue polarity in *Drosophila*. Wnts have been implicated as the ligands in this process, although the actual *Drosophila* Wnt protein involved has not been determined. Several PCP genes have been identified – such as *Flamingo* (also known as *Starry night*) and *Strabismus* (also known as *Van Gogh*), which encode membrane bound proteins essential to the establishment of tissue polarity – although the exact mechanisms of their action have not been elucidated [19,20]. In vertebrates, convergent extension is a morphogenetic process during gastrulation whereby the mesoderm and ectoderm tissues undergo mediolateral narrowing and A–P elongation. This process is mediated by highly directed cell movement and intercalation, appears to involve several key genes in the PCP pathway, and requires *Wnt11* and *Wnt5a* [10,11,21–23]. Therefore, the same signaling pathway (the PCP pathway) is used for regulating tissue polarity in *Drosophila* and cell intercalation during convergent extension in vertebrates. Frizzled7 is required in the PCP pathway, which depends on Dishevelled but does not require LRP5, LRP6 or  $\beta$ -catenin. It requires a Formin-homology protein Daam1, which is not required in the canonical pathway [24]. The PCP pathway leads to changes in the actin cytoskeleton and provides directionality for morphogenesis and cell movement. Other vertebrate developmental processes, such as cochlear hair cell orientation, depend on PCP signaling. Mice with mutations in genes of the PCP pathway display defects in stereociliary



**Figure 2.** Three major Frizzled-dependent Wnt pathways. In the canonical Wnt- $\beta$ -catenin pathway, Wnt proteins (Wingless in *Drosophila*) activate Frizzled proteins that are associated with coreceptors [Arrow in *Drosophila* and low-density lipoprotein (LDL)-receptor-related proteins (LRPs) in vertebrates; formation of the LRP-Frizzled complex can be inhibited by Dickkopf proteins (Dkk)]. Both the canonical Wnt- $\beta$ -catenin pathway and planar cell polarity (PCP) pathway clearly involve Dishevelled; in the canonical Wnt- $\beta$ -catenin pathway, Dishevelled is associated with adenomatous polyposis coli (APC) and Axin. It was recently suggested that Dishevelled is also required in the Wnt- $\text{Ca}^{2+}$  pathway. The canonical pathway involves stabilization and nuclear localization of Armadillo ( $\beta$ -catenin) and transcriptional activation of Wnt-response genes by T-cell factor/lymphoid-enhancer factor (TCF/LEF). A divergent pathway downstream of glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) was suggested to mediate axon microtubule remodeling via phosphorylation of microtubule-associated protein 1B (MAP1B) prior to synapse formation. The PCP pathway involves Daam1, which connects Dishevelled to regulators of the actin cytoskeleton, such as the small GTPases RhoA and Rock. Another branch of PCP signaling involves Rac and Jun kinase. In the PCP pathway, transmembrane proteins such as Flamingo and Strabismus are required, but it is not clear whether there are direct protein-protein interactions (question marks). These proteins might also function in pathways other than the PCP pathway. The Wnt- $\text{Ca}^{2+}$  pathway involves G proteins and phospholipase C (PLC), and can lead to activation of  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), calcineurin and nuclear factor of activated T cells (NF-AT). Interactions among the three pathways are discussed in the main text. ' $\alpha$ ', ' $\beta$ ' and ' $\gamma$ ' represent G-protein subunits and 'P' indicates phosphorylation.

bundle formation [25]; Wnt7a has been implicated in this process [26].

It is attractive to consider a possible role for the PCP pathway, or a variation of it, in axon guidance. Cellular polarity created by the function of this pathway is analogous to the polarized migration of growth cones. PCP proteins are essential to directed cell movement in convergent extension during vertebrate gastrulation, a process analogous to directed migration of growth cones. Whether PCP proteins are present and control the direction of growth cone migration has not been evaluated. It should be noted that one of the core PCP components has been reported to have a role in axon target selection, a process that occurs after axons reach the vicinity of their targets. In visual system development in *Drosophila*, photoreceptor axon target selection requires Flamingo. It is interesting that this process does not require other core PCP proteins, such as Frizzled, Dishevelled, Strabismus or Prickle-spiny legs [27,28]. It has not been determined whether any Wnt proteins are involved in this interaction. Therefore, it is possible that Flamingo might function in an entirely different pathway from the PCP pathway in

axon target selection in the fly visual system. Flamingo was shown to mediate repulsion of dendrites from homologous neurons in dendritic morphogenesis in *Drosophila* peripheral nervous system and also appears to function in a Frizzled-independent pathway [29]. Taken together, it is possible that these Flamingo-dependent processes of target selection and dendrite development are likely not to be part of the PCP pathway, or to require only a subset of the PCP machinery. It remains to be tested, however, whether Flamingo is involved in axon pathfinding and, if so, whether Flamingo regulates axon pathfinding via the PCP pathway.

*The Wnt- $\text{Ca}^{2+}$  pathway is an appealing candidate.* The Wnt- $\text{Ca}^{2+}$  pathway is involved in cell separation during convergent extension and cell migration (Figure 2). Cell separation, an integral part of the convergent extension, refers to cell movement from the adjacent cell layer, distinct from the process of the highly directed medio-lateral cell intercalation, which is mediated by the PCP pathway [10,11]. *Xenopus* Wnt5a can stimulate intracellular  $\text{Ca}^{2+}$  release via Frizzled7, leading to activation of protein kinase C (PKC) in a G-protein-dependent



manner. During ovarian organogenesis in *Drosophila*, Wnt4 facilitates cell movement through activation of PKC and focal adhesion kinase [30]. Wnt5a has also been reported to increase PKC activity, cell motility and invasiveness of metastatic melanoma. This process appears to be mediated by Frizzled5 and is independent of  $\beta$ -catenin [31]. Therefore, the Wnt–Ca<sup>2+</sup>–PKC pathway represents a third Wnt signaling pathway that regulates processes such as cell separation and cell motility. More recently, Dishevelled was found to be required for the Wnt–Ca<sup>2+</sup> pathway as well [32], suggesting that the PCP and the Ca<sup>2+</sup> pathways might be intimately connected. Interestingly, Dishevelled is a target of PKC $\delta$  in the Jun N-terminal kinase (JNK) pathway [33]. Although PKC $\delta$  is not Ca<sup>2+</sup>-dependent, it is possible that Dishevelled can also be regulated directly or indirectly by other PKC isoforms, including Ca<sup>2+</sup>-dependent PKCs. Interactions among the Wnt intracellular pathways could be an integral part of the regulatory networks. For example, activation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) leads to inhibition of the  $\beta$ -catenin pathway [34].

The Wnt–Ca<sup>2+</sup> pathway is another good candidate for being involved in axon guidance. The PKC pathway is required in G-protein-coupled-receptor-mediated axon repulsion by stromal-cell-derived factor (SDF-1), a chemokine that attracts migrating leukocytes and cerebellar granule cells [35]. The repulsion can be converted into attraction when the cGMP level is increased. The cGMP-induced attraction to SDF-1 is also PKC-dependent. Although direct demonstration that Wnt proteins lead to activation of PKC in growth cone guidance has been lacking, it is an intriguing possibility and worth further attention. It should be noted that Ca<sup>2+</sup> is involved in Netrin-1-induced axon outgrowth [36] and also activates the calcineurin–nuclear factor of activated T cells (NF-AT) pathway and stimulates axon outgrowth [37]. Therefore, the Wnt–Ca<sup>2+</sup> pathway could trigger an attractive guidance pathway and might in fact be involved in the extension of long axons such as commissural axons after midline crossing [4].

#### *Derailed and axon repulsion by Wnts*

In the study of *Drosophila* midline axon pathfinding, Derailed was identified as a repulsive receptor in response to Wnt5. Derailed is a receptor-tyrosine-kinase-like protein, the signaling mechanism of which is unknown. Although its kinase domain is not active, it is required for the function [38]. It was proposed that there is crosstalk between the vertebrate homologue of Derailed, Ryk, and the EphB signaling pathway [39,40]. EphB is a major axon guidance receptor that is well established as a mediator of repulsion [41]. More recent studies also show that EphB can mediate attraction [42,43]. Is the Ephrin–Eph pathway involved in *Drosophila* midline axon guidance? Indeed, a *Drosophila* Eph-like receptor, Dek/Deph, mediates the repulsive function of Dephrin, a *Drosophila* ephrin orthologue [44,45]. It is attractive to hypothesize that Derailed/Ryk could interact with the Eph pathway in mediating axon repulsion by Wnts. An obvious test would be whether DWnt5 and Dek/Deph, or Derailed and

Dek/Deph, interact genetically. Alternatively, Derailed/Ryk could activate a separate repulsive signaling pathway. Identification of downstream signaling components of Derailed/Ryk will be informative for an understanding of the Ryk-dependent pathway(s).

#### Summary

Wnts are multifunctional axon guidance molecules that can both attract and repel axons. The attractive response is likely to be mediated by a Frizzled-dependent pathway and to be independent of Derailed/Ryk, because spinal cord commissural axons do not express Ryk [4]. The repulsion is mediated by Derailed and does not seem to involve Frizzled [3]. As more studies on Wnts in axon guidance are carried out, we will have the opportunity to test whether this pattern of Frizzled mediating attraction and Ryk mediating repulsion is true for all neurons that respond to Wnts. More studies will also be needed for further information on the downstream signaling mechanisms that mediate Wnt responsiveness and for understanding how dynamic responsiveness to Wnts is regulated. Currently known Wnt pathways, such as the PCP pathway and the Ca<sup>2+</sup> pathway, are very appealing candidates. These pathways might not necessarily exclude one other in axon guidance. Instead, they could also function in a cooperative way, or they could control different aspects of the cellular processes of growth cone guidance. More and more recent studies suggest that these two pathways might not be separable. It is also possible that new Frizzled-dependent pathways could lead to axon attraction by Wnts. Different Wnt proteins appear to have different functions in development. It will be important to address how many of these Wnt proteins can regulate axon guidance, which Wnt proteins signal through which downstream signaling pathways, and which Wnt proteins interact with which Frizzled proteins in nervous system wiring.

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#### References

- Lucas, F.R. and Salinas, P.C. (1997) WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. *Dev. Biol.* 192, 31–44
- Hall, A.C. *et al.* (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* 100, 525–535
- Yoshikawa, S. *et al.* (2003) Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. *Nature* 422, 583–588
- Lyuksytova, A.I. *et al.* (2003) Anterior–posterior guidance of commissural axons by Wnt–frizzled signaling. *Science* 302, 1984–1988
- Burden, S.J. (2000) Wnts as retrograde signals for axon and growth cone differentiation. *Cell* 100, 495–497
- Salinas, P.C. (2003) Synaptogenesis: Wnt and TGF- $\beta$  take centre stage. *Curr. Biol.* 13, R60–R62
- Wodarz, A. and Nusse, R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* 14, 59–88
- Zorn, A.M. (2001) Wnt signalling: antagonistic Dickkopfs. *Curr. Biol.* 11, R592–R595
- Adler, P.N. and Lee, H. (2001) Frizzled signaling and cell–cell interactions in planar polarity. *Curr. Opin. Cell Biol.* 13, 635–640
- Tada, M. *et al.* (2002) Non-canonical Wnt signalling and regulation of gastrulation movements. *Semin. Cell Dev. Biol.* 13, 251–260

- 11 Keller, R. (2002) Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* 298, 1950–1954
- 12 Huelsken, J. and Behrens, J. (2002) The Wnt signalling pathway. *J. Cell Sci.* 115, 3977–3978
- 13 Kuhl, M. (2004) The WNT/calcium pathway: biochemical mediators, tools and future requirements. *Front. Biosci.* 9, 967–974
- 14 Fradkin, L.G. *et al.* (1995) The *Drosophila* Wnt protein DWnt-3 is a secreted glycoprotein localized on the axon tracts of the embryonic CNS. *Dev. Biol.* 168, 202–213
- 15 Callahan, C.A. *et al.* (1995) Control of neuronal pathway selection by a *Drosophila* receptor protein-tyrosine kinase family member. *Nature* 376, 171–174
- 16 Bonkowski, J.L. *et al.* (1999) Axon routing across the midline controlled by the *Drosophila* Derailed receptor. *Nature* 402, 540–544
- 17 Lucas, F.R. *et al.* (1998) Inhibition of GSK-3 $\beta$  leading to the loss of phosphorylated MAP-1B is an early event in axonal remodelling induced by WNT-7a or lithium. *J. Cell Sci.* 111, 1351–1361
- 18 Ciani, L. *et al.* (2004) A divergent canonical WNT-signaling pathway regulates microtubule dynamics: Dishevelled signals locally to stabilize microtubules. *J. Cell Biol.* 164, 243–253
- 19 Usui, T. *et al.* (1999) Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* 98, 585–595
- 20 Taylor, J. *et al.* (1998) Van Gogh: a new *Drosophila* tissue polarity gene. *Genetics* 150, 199–210
- 21 Rauch, G.J. *et al.* (1997) Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb. Symp. Quant. Biol.* 62, 227–234
- 22 Heisenberg, C.P. *et al.* (2000) Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405, 76–81
- 23 Smith, J.C. *et al.* (2000) Xwnt11 and the regulation of gastrulation in *Xenopus*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 355, 923–930
- 24 Habas, R. *et al.* (2001) Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. *Cell* 107, 843–854
- 25 Montcouquiol, M. *et al.* (2003) Identification of Vangl2 and Scrb1 as planar polarity genes in mammals. *Nature* 423, 173–177
- 26 Dabdoub, A. *et al.* (2003) Wnt signaling mediates reorientation of outer hair cell stereociliary bundles in the mammalian cochlea. *Development* 130, 2375–2384
- 27 Senti, K.A. *et al.* (2003) Flamingo regulates r8 axon–axon and axon–target interactions in the *Drosophila* visual system. *Curr. Biol.* 13, 828–832
- 28 Lee, R.C. *et al.* (2003) The protocadherin Flamingo is required for axon target selection in the *Drosophila* visual system. *Nat. Neurosci.* 6, 557–563
- 29 Gao, F.B. *et al.* (2000) Control of dendritic field formation in *Drosophila*: the roles of flamingo and competition between homologous neurons. *Neuron* 28, 91–101
- 30 Cohen, E.D. *et al.* (2002) DWnt4 regulates cell movement and focal adhesion kinase during *Drosophila* ovarian morphogenesis. *Dev. Cell* 2, 437–448
- 31 Weeraratna, A.T. *et al.* (2002) Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 1, 279–288
- 32 Sheldahl, L.C. *et al.* (2003) Dishevelled activates Ca<sup>2+</sup> flux, PKC, and CaMKII in vertebrate embryos. *J. Cell Biol.* 161, 769–777
- 33 Kinoshita, N. *et al.* (2003) PKC delta is essential for Dishevelled function in a noncanonical Wnt pathway that regulates *Xenopus* convergent extension movements. *Genes Dev.* 17, 1663–1676
- 34 Kuhl, M. *et al.* (2001) Antagonistic regulation of convergent extension movements in *Xenopus* by Wnt/ $\beta$ -catenin and Wnt/Ca<sup>2+</sup> signaling. *Mech. Dev.* 106, 61–76
- 35 Xiang, Y. *et al.* (2002) Nerve growth cone guidance mediated by G protein-coupled receptors. *Nat. Neurosci.* 5, 843–848
- 36 Hong, K. *et al.* (2000) Calcium signalling in the guidance of nerve growth by netrin-1. *Nature* 403, 93–98
- 37 Graef, I.A. *et al.* (2003) Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. *Cell* 113, 657–670
- 38 Yoshikawa, S. *et al.* (2001) The derailed guidance receptor does not require kinase activity *in vivo*. *J. Neurosci.* 21, RC119
- 39 Halford, M.M. *et al.* (2000) Ryk-deficient mice exhibit craniofacial defects associated with perturbed Eph receptor crosstalk. *Nat. Genet.* 25, 414–418
- 40 Trivier, E. and Ganesan, T.S. (2002) RYK, a catalytically inactive receptor tyrosine kinase, associates with EphB2 and EphB3 but does not interact with AF-6. *J. Biol. Chem.* 277, 23037–23043
- 41 Wilkinson, D.G. (2000) Eph receptors and ephrins: regulators of guidance and assembly. *Int. Rev. Cytol.* 196, 177–244
- 42 Mann, F. *et al.* (2002) Topographic mapping in dorsoventral axis of the *Xenopus* retinotectal system depends on signaling through ephrin-B ligands. *Neuron* 35, 461–473
- 43 Hindges, R. *et al.* (2002) EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping. *Neuron* 35, 475–487
- 44 Scully, A.L. *et al.* (1999) Isolation and characterization of Dek, a *Drosophila* eph receptor protein tyrosine kinase. *Mol. Cell. Neurosci.* 13, 337–347
- 45 Bossing, T. and Brand, A.H. (2002) Dephrin, a transmembrane ephrin with a unique structure, prevents interneuronal axons from exiting the *Drosophila* embryonic CNS. *Development* 129, 4205–4218

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