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Review

Wnt signaling: multiple functions in neural development

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Abstract. Wnt signaling has proven to be essential for neural development at various stages and across species. Wnts are involved in morphogenesis and patterning, and their proliferation-promoting role is a key function in stem cell maintenance and the expansion of progenitor pools. Moreover, Wnt signaling is involved in differentiation processes and lineage decision events during both central and peripheral nervous system development. Additionally, several reports point to a role of Wnt signaling in axon guidance and neurite outgrowth. This article reviews and consolidates the existing evidence for the functions of Wnt signaling in neural development.

Key words. Wnt signaling; β-catenin; Frizzled receptor; proliferation; apoptosis; stem cell maintenance; differentiation; lineage decision; axon guidance; neurite outgrowth.

Introduction

During the past few years, technical advances and improved methodology have helped to considerably increase our understanding of the mechanisms regulating neural development. However, our knowledge about neural development is still peppered with many unanswered questions. Various signaling molecules and signal transduction mechanisms, cell-cell interactions, as well as the extracellular matrix (ECM) have been implicated in neural development. Slowly, a complex scheme is emerging in which a plethora of factors and signaling cascades are orchestrated in a spatiotemporal manner. The signals involved include members of the transforming growth factor-β (TGF-β) superfamily [1] such as the bone morphogenic proteins (BMPs) [2–4] and the growth and differentiation factors (GDFs) [5, 6]. In addition, members of the Hedgehog family, fibroblast growth factor (FGF), and many other cues are crucial for neural development [7–11].

In this review article, we focus on the role of Wnt proteins in vertebrate neural development. After a short introduction to the Wnt family, we describe the canonical Wnt signaling pathway and discuss the effects of Wnt signaling on key developmental processes like proliferation, apoptosis, stem cell maintenance, lineage decision, differentiation, and axon guidance. Much of the data on Wnt signaling are related to embryonic development, such as the formation of Spemann's organizer and dorsalization of the vertebrate central nervous system (CNS), which involve Wnt signaling [12–15]. Moreover, a great deal of effort has been put into studies on postnatal requirement and function of Wnt signaling. Various requirements for Wnt signaling in different cortical cell populations were recently reported for postnatal mouse brains [16]. Equally important are the effects of Wnt signaling in the adult organism in cases of signal deregulation or alteration. In such situations, aberrant Wnt signaling can act as a pathomechanism in tumorigenesis [17–20]. Furthermore, Wnt signaling is thought to play an important role in the onset of Alzheimer's disease [21–23].

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The Wnt family

The Wnt family consists of a group of proteins encoded by 7 known genes in *Drosophila* and about 19 genes in vertebrates. The name Wnt is derived from the *Drosophila* gene wingless (wg), which plays a role in segment polarity, and the mouse gene int-1, which is required for midbrain and cerebellar formation as well as the generation of neural crest cells [24–27]. The size of Wnt proteins varies between 350 and 400 amino acids. They contain around 24 highly conserved cysteine residues most probably involved in disulfide bond formation, as is typical for extracellular proteins. The importance of Wnt proteins and their signaling pathways in development is reflected in the degree of conservation of protein structure across species [28–30]. Wnt proteins are mostly glycosylated prior to secretion. Their only lipid modification is palmitoylation, which is not strictly required for Wnt activity [31], but may be involved in tethering of the protein to the membrane thereby increasing its activity. Overexpression

of unpalmitoylated Wnt possibly overcomes the lack of membrane tethering [32]. Wnt proteins act in multiple disparate signaling pathways and bind to cell surface receptors to activate signaling cascades. In particular, three major pathways have been identified, all of which are thought to signal via Frizzled (Fz) receptors: (i) the Wnt/β-catenin pathway also referred to as the canonical Wnt signaling pathway, in which β-catenin – a *Drosophila* armadillo related protein – is crucially involved (fig. 1); (ii) the Frizzled/planar cell polarity (Fz/PCP) pathway, and (iii) the Ca^{2+} pathway [33, 34].

The canonical Wnt signaling pathway

Extracellular Wnt molecules bind to Fz seven-pass transmembrane receptors and to low-density lipoprotein receptor-related proteins (LRP5 or LRP6) to form a ternary complex [35–38]. This receptor complex induces the phosphorylation of Dishevelled (Dsh). An alternative
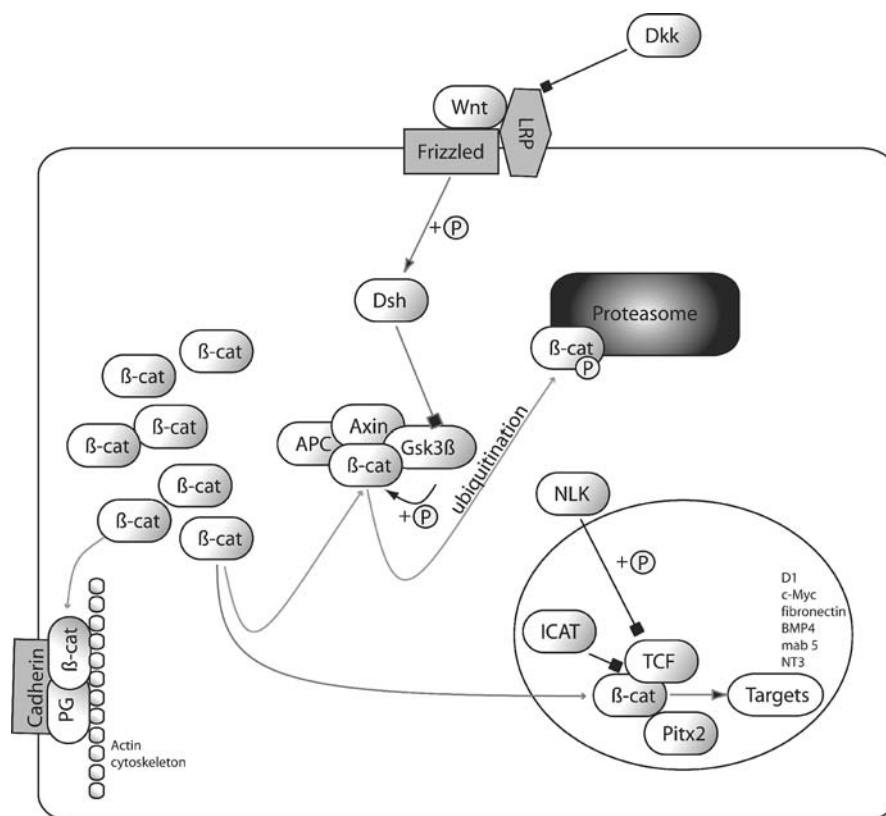

Figure 1. Model of canonical Wnt/β-catenin signaling pathway. Both adherens junctions and the canonical Wnt signaling pathway require β-catenin. By default, β-catenin is phosphorylated in the glycogen synthase kinase-3β (GSK3β), Axin, APC complex and thus directed into the ubiquitin/proteasome degradation pathway. Wnt forms together with Frizzled and LRP 5/6 a trimeric complex and activates intracellular dishevelled (Dsh) by phosphorylation. Dickkopf (DKK) inhibits the formation of the Wnt/receptor complex. Activated Dsh inhibits GSK3β and thus leads to stabilization and accumulation of β-catenin in the cytoplasm. Stabilized β-catenin is transported to the nucleus in a concentration-dependent manner. There it activates, together with transcription factors of the TCF/LEF family, the transcription of target genes. Nemo-like kinase (NLK) phosphorylates TCF and regulates its DNA-binding affinity. Pitx2 acts as a transcriptional repressor when bound to β-catenin.

activation of the canonical Wnt signaling pathway via Fz dimerization only has been proposed from experiments with *Xenopus laevis* [39]. Moreover, there is evidence that Wnt proteins are able to induce phosphorylation of Dsh via LRP5/6 by pathways other than the canonical, since phosphorylation of Dsh did not necessarily lead to β-catenin stabilization [40].

Wnt signaling is regulated at the receptor level by various regulatory proteins, including LRP5/6-binding factors (Dickkopfs, DKKs) and secreted Fz-related proteins (SFRPs) [41–43]. DKKs inhibit the ternary complex formation between Wnt, Fz, and LRP5/6, and therefore inhibit canonical Wnt signaling [44]. Interestingly, DKK1 has been reported to be a direct transcriptional target of the canonical Wnt signaling pathway [45]. This might point to a feedback loop modulating canonical Wnt signaling. Moreover, Golan at al. [46] have shown that human Fz receptor 6 can act as a negative regulator for the canonical Wnt signaling pathway downstream of the β-catenin destruction complex (fig. 1). β-Catenin is not only a key component of the canonical Wnt signaling pathway, but also serves as a structural molecule that anchors the actin cytoskeleton to the intracellular domain of cadherins [47–49]. As such, β-catenin is also involved in cadherin-mediated cell-cell adhesion. Adherens junctions and canonical Wnt signaling possibly require distinct molecular forms of beta-catenin. While the cytosolic pool of β-catenin can bind to α-catenin and cadherins, Wnt signaling is thought to promote a specific conformation of β-catenin which can bind to certain transcription factors but not to cadherins [50].

Dsh inhibits the complex formation of Axin, adenoma poliposis coli protein (APC), glycogen synthase kinase-3β (GSK3β) and β-catenin. This complex is required for β-catenin phosphorylation, which directs the protein into the proteasome degradation pathway [51, 52]. If not degraded, β-catenin accumulates in the cytoplasm. Although β-catenin lacks a nuclear localization signal it can be translocated into the nucleus in a concentration-dependent manner. Translocation has been shown to be independent of the classical nuclear transport pathways that involve Ran or importin [53, 54]. However, β-catenin is too large for passive nuclear transport. A possible solution for the nuclear transport problem emanates from the fact that β-catenin interacts with the androgen receptor (AR), which might serve as a nuclear transporter. Indeed, AR agonists affected the nuclear transport kinetics of β-catenin and the AR in an analogous manner [55].

In the nucleus, β-catenin interacts with high-mobility group (HMG) box transcription factors, like the T cell factor (TCF, also known as lymphoid-enhancer factor LEF), and forms a transcriptional activator complex [56]. This activator complex targets genes such as cyclin D1, c-Myc, fibronectin, BMP4, mab-5, and NT-3 [27]. Complex formation between the TCF and β-catenin is inhibited

by a protein called inhibitor of β-catenin and TCF-4 (ICAT), which can bind to β-catenin armadillo repeats in a manner similar to that of the TCFs [57, 58]. In cells with elevated β-catenin, ICAT was proposed to sequester a subpopulation of β-catenin and thus to buffer increased β-catenin levels in the cytoplasm [59]. Phosphorylation of TCF/LEF by activated Nemo-like kinase (NLK) inhibits the DNA-binding affinity of the complex and thus indirectly regulates Wnt signaling in the nucleus [60]. Yet NLK has also been shown to act as a positive regulator of Wnt signaling in early zebrafish development [61]. Moreover, the factor Pitx2, which is involved in cell type-specific proliferation, has been shown to be converted from a transcriptional repressor into a transcriptional activator when bound to β-catenin [62]. Overall, the various levels of regulation of canonical Wnt signaling may indicate multiple possible interactions with other signaling cascades, required for differential signal integration.

Proliferation and apoptosis

Precise regulation of proliferation/apoptosis ratios is essential in neural development [63]. Unbalance results in severe malformations during embryonic development, and promotes cancer formation postembryonically. In vitro as well as in vivo studies have shown that Wnt signaling is required to expand and maintain neural precursor populations in the brain and the spinal cord [64–66]. Wnt-1 regulates precursor populations in the mid/hindbrain and is necessary for its development [67, 68]. Wnt-3a signaling seems to be involved in hippocampal development by regulating the size of the caudomedial cortex through progenitor pool regulation and/or stem cell maintenance [69]. Moreover, Wnt signaling regulates the size of the cerebral cortex in the mammalian system [70]. In the spinal cord, progenitors are specified by BMP signaling that determines domains of Wnt ligand, receptor, and antagonist expression, resulting in spinal cord patterning [71]. In these BMP-defined progenitor populations, Wnt signaling is thought to regulate cell cycle exit and thus progenitor expansion [70, 72]. Similar proliferative effects of Wnt signaling have been described for stem cells and progenitors in various tissues like the skin, intestine, and the hematopoietic system [31, 73, 74].

While most results indicate a proliferative function of Wnt signaling, there are also reports which claim that Wnt signaling inhibits proliferation in certain cell types, such as human endothelial vein cells (HUVECs) [75]. This effect is thought to result from non-canonical Wnt/Ca^{2+} pathway signaling that inhibits the proliferative effects of canonical Wnt signaling [76–78].

Wnt signaling not only affects proliferation but has also been implicated in apoptosis. In cancer research, for example, Wnt signaling has been related to drug resis-

tance in cancer therapy where vinblastine and vincristine are used as apoptosis-inducing drugs. The effect of these drugs has been overruled by Wnt signaling and cell survival maintained, while inhibition of Wnt signaling by expression of dominant-negative TCF4 rendered the cells responsive to the drugs [79]. Another study on 3T3-L1 cells revealed a TCF4-independent mechanism by which Wnt signaling inhibits apoptosis. While overexpression of dominant-negative TCF4 triggered the expression of apoptotic genes, Wnt signaling upregulated the expression of insulin-like growth factors (IGF I/II) which mediate antiapoptotic effects [80]. Moreover, conditional ablation of β-catenin in the dorsal spinal cord led to increased apoptosis, although whether this was due to impaired canonical Wnt signaling or to disturbed cell-cell interactions was difficult to assess [F. Ille, R. Kemler and L. Sommer, unpublished data]. Canonical Wnt signaling has been proposed to suppress apoptosis by inhibiting c-Myc-induced release of cytochrome c and caspase activation [81, 82]. In addition to the antiapoptotic effects of canonical Wnt signaling, apoptotic inhibition has been attributed to β-catenin-independent Wnt signaling via Janus kinase (Jnk) [83]. Despite the evidence for antiapoptotic effects of Wnt signaling, Wnt may also induce

apoptosis. In particular, Hasegawa and colleagues have shown that stabilizing β-catenin by conditional APC ablation leads to massive induction of apoptosis in neural crest cells in the mouse model [84].

Stem cell maintenance, differentiation, and lineage decision

Stem cell maintenance and self-renewal are cellular processes closely associated with proliferation. Given the role of Wnt signaling in cell cycle regulation, Wnt signaling has not surprisingly also been implicated in the control of stem cell development [85]. Activation of Wnt/β-catenin signaling in human and mouse embryonic stem cells (ESCs) by administration of pharmacological GSK3β inhibitor maintains their self-renewal capacity as well as their pluripotency [86]. Exposure of hematopoietic stem cells to Wnt molecules and sustained expression of β-catenin in long-term cultures maintains self-renewal as well as the capacity of these cells to reconstitute the hematopoietic lineages in vivo [87]. Nonetheless, ablation of β-catenin seems to impair neither hematopoiesis nor lymphopoiesis under physiological conditions [88]. For
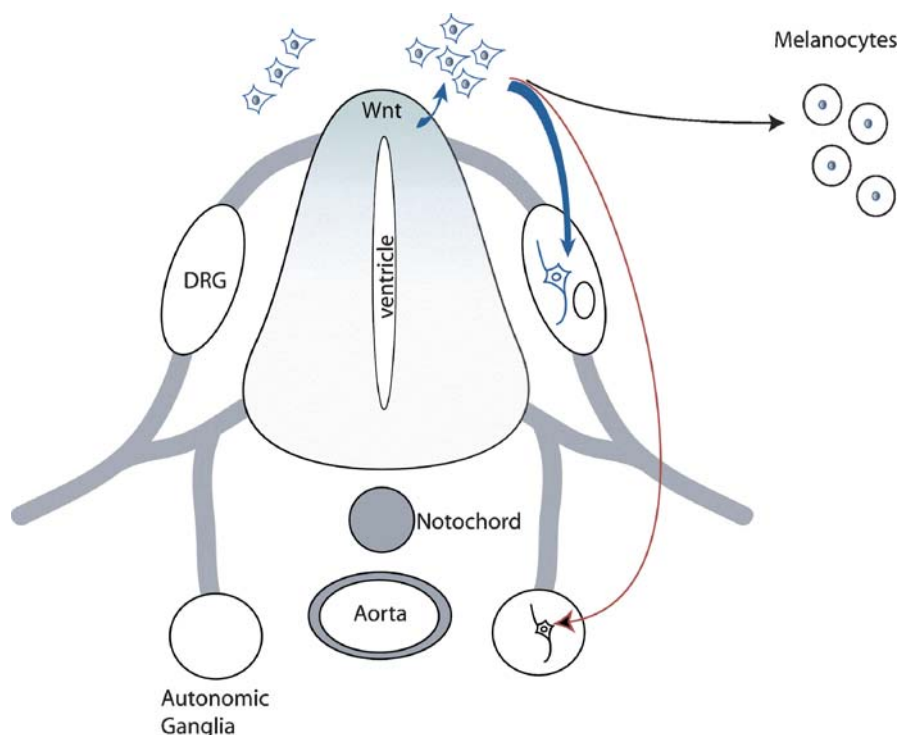

Figure 2. Wnt signaling in neural crest stem cell development. A gradient of Wnt molecules is established in the dorsal neural tube, which is involved in neural tube patterning and the generation of the neural crest cell population. Some of the early neural crest stem cells (eNCSC; blue-colored cells) are thought to become committed to the sensory lineage (blue arrow) in response to canonical Wnt/β-catenin signaling in the dorsal neural tube. Delaminated eNCSCs migrate toward their homing positions. At these positions, NCSCs respond to local signals and are involved in the generation of structures such as the dorsal root ganglia (DRG), the autonomic ganglia (red arrow), and structures of the skin (black arrow), and others.

these processes, the data suggest either an alternative rescue pathway or the involvement of a factor upstream of β-catenin in the Wnt signaling cascade.

The effects of Wnt signaling on stem cells seem to be diverse. While it inhibits neural differentiation and maintains pluripotency in ESCs [86, 89], it can also promote differentiation in other stem and progenitor cells [85]. Marretto et al. [90] identified a TCF-dependent reporter gene in differentiating cortical neurons during development, suggesting a potential role of Wnt/β-catenin signaling in the differentiation process. In agreement with this, Wnt-7a signaling induces differentiation in neural precursor cells (NPCs) of the neocortex [91]. This process reduces the NPC pool at late developmental stages (E13.5) whereas NPCs at earlier developmental stages (E10.5) do not seem to differentiate in the presence of Wnt. Recently, Wnt-3a has been reported to promote differentiation into the neural and astrocyte lineage by inhibiting neural stem cell maintenance [92]. Moreover Wnt/β-catenin signaling is required for neural differentiation in ESCs [93].

In neural crest stem cells (NCSCs), Wnt signaling has been linked to cell fate decision (fig. 2). Neural crest cells generate various cell types of the peripheral nervous system (PNS) as well as craniofacial, skin, and heart structures [94]. These cells are derived from the border of the neural plate adjacent to the ectoderm, and are strictly dependent on Wnt signaling [95, 96]. Wnt/β-catenin signaling regulates cell fate decisions in early neural crest stem cells (eNCSCs) by driving these cells into the sensory lineage, rather than affecting the population size. Hari at al. [97] have shown by cell type-specific gene ablation that loss of β-catenin-function in NCSCs prevents sensory

ganglia and melanocyte formation in vivo [97]. Intriguingly, β-catenin-deficient NCSCs emigrate and proliferate normally in culture, but they fail to acquire a sensory neuronal fate [97]. Conversely, a β-catenin gain-of-function study shows that, in vivo, continuous Wnt/β-catenin signaling in NCSCs leads to sensory neurogenesis at the expense of all other neural crest derivatives [98]. However, stabilized β-catenin does not affect NCSC migration and proliferation. Consistent with the in vivo data, practically all mutant cells lose NCSC features and adopt a sensory fate in cell culture. Although all eNCSCs are Wnt responsive, only a subpopulation of the cells generate sensory cells in vivo, indicating the presence of factors counteracting Wnt activity [85]. The development of the hippocampus, and in particular the generation of dentate gyrus granule cells, also appears to be regulated by LEF1/TCF transcription factors [99]. Similar effects have been reported for skin stem cells, in which β-catenin signaling determines differentiation into follicular and epidermal lineages [100, 101]. Taken together, these results underline the role of Wnt signaling in lineage decision, but the molecular mechanisms remain to be resolved.

Axon guidance and neurite outgrowth

One essential aspect in CNS development is 'wiring,' a process which involves patterning, migration, axon guidance, and synapse formation. As mentioned before, Wnt molecules have been implicated in morphogenesis, where they establish tissue patterning together with other
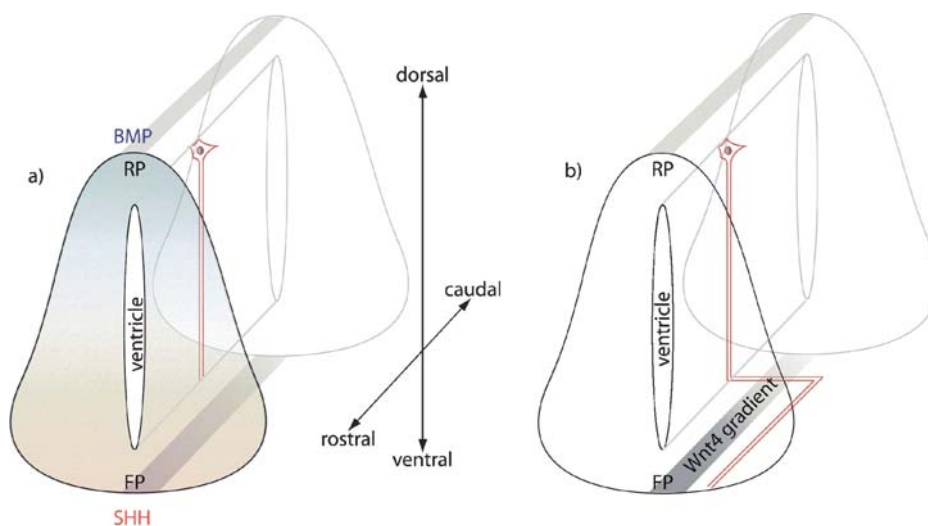

Figure 3. Guidance of commissural axons in the developing spinal cord. (*a*) Commissural axons of certain neurons are repelled by the dorsal bone morphogenetic protein (BMP) gradient (blue) that originates from the roofplate (RP). At the same time, the axons are attracted by the ventral sonic hedgehog (SHH) gradient in the floorplate (FP). Therefore, commissural axons extend ventrally toward the floorplate. (*b*) Once the axons reach the floorplate, they cross it and turn rostrally. After turning toward the head, the axons extend along a Wnt-4 gradient until they reach their target area and form synapses.

proteins like sonic hedgehog (SHH) and BMPs. In the early neural tube, for example, BMP4 expressed in the roofplate and SHH expressed in the floorplate generate two dorsoventral countergradients which influence proneural gene expression. Wnt-1 and Wnt-3a are expressed in the dorsal neural tube at later stages, and are thought to determine proneural domains of the dorsal neural tube by mechanisms which still remain to be elucidated [102, 103]. Wnt-4, however, is expressed in the ventral neural tube and has been related to axon guidance in commissural axons [104]. Commissural axon migration is repelled from the roofplate by BMP signaling, and at the same time, these axons are attracted toward the floorplate by SHH. Once these axons reach the floorplate, they cross it, turn rostrally, and extend toward the brain (fig. 3). Lyuksyutova et al. [104] have found Wnt-4 to be expressed in a rostrocaudal gradient in the floorplate, which attracts postcrossing commissural axons. Likewise, commissural axons which lack the Wnt receptor Fz3 exhibit rostrocaudal guidance defects in postcrossing commissural axons [104]. Since these results have not been tested in Wnt-4 knockout animals, the direct involvement of Wnt-4 in axon guidance remains to be proven. Nevertheless, evidence from invertebrate systems favors the hypothesis that Wnt molecules can act as guidance molecules. In *Drosophila*, Wnt-5 seems to fulfill a guidance function. By signaling through the Derailed receptor expressed in the growth cones, Wnt-5 acts as a chemorepellent [105–107].

In vertebrates, ablation of Ryk, the mammalian homologue of *Drosophila* Derailed, leads to axon guidance defects in vivo [108]. Unlike Derailed, Ryk acts as a Fz coreceptor and forms a ternary complex with Fz and Wnt-1. It also mediates TCF activation induced by Wnt-1 and is able to bind to Wnt-3a. Moreover, Ryk is required for neurite outgrowth in dorsal root ganglion neurons, induced by Wnt-3a [108]. Similarly, Wnt-7a has been described to remodel axon spreading and branching in developing cerebellar granule cells [109]. Axonal remodeling involves MAP-1B, a microtubule-associated protein has been identified as substrate for GSK3β and implicated in axonal outgrowth [110]. Upon GSK3β inhibition, the amount of phosphorylated MAP-1B decreases in the cells, a process that naturally occurs prior to axonal remodeling and leads to changes in microtubule dynamics. Recently, Ciani et al. [111] have shown that the Wnt/β-catenin signaling component Dsh also induces axonal remodeling and stabilizes microtubules in developing neurons. Intriguingly, this process seems to involve neither β-catenin nor TCF factors, suggesting an alternative regulatory pathway for canonical Wnt signaling, downstream of GSK3β. Similar evidence comes from experiments in neuroblastoma [112]. Here, GSK3β and Axin have been reported to promote neurite outgrowth in a β-catenin-independent manner.

Conclusion

The roles of Wnt signaling in neural development are manifold, and seem at times contradictory. While one of its most important functions is promotion of proliferation, in some contexts, Wnt signaling is able to inhibit it. Similarly, apoptosis is abolished by Wnt signaling in certain cases whereas in others it is induced. As much as Wnt signaling is responsible for stem cell maintenance, it is to a similar extent essential for differentiation and lineage decisions, axon guidance, and neurite outgrowth. With every newly described aspect of Wnt signaling, more questions arise. Some answers to these questions may lie in understanding the intrinsic status of a cell that reflects its 'history' and that is modified depending on the position of the cell in the organism at a given timepoint. In other words, the effects of Wnt signaling are tissue and cell type specific and represent the results of combinatorial signal integration in an environment of dynamic signaling networks.

1   Derynck R. and Zhang Y. E. (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature **425:** 577–584

2   Takahashi, Y., Tonegawa, A., Matsumoto, K., Ueno, N., Kuroiwa, A., Noda, M. et al. (1996) BMP-4 mediates interacting signals between the neural tube and skin along the dorsal midline. Genes Cells **1:** 775–783

3   Mehler M. F., Mabie P. C., Zhang D. and Kessler J. A. (1997) Bone morphogenetic proteins in the nervous system. Trends Neurosci. **20:** 309–317

4   Timmer J. R., Wang C. and Niswander L. (2002) BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. Development **129:** 2459–2472

5   Lee S. J. (1990) Identification of a novel member (GDF-1) of the transforming growth factor-beta superfamily. Mol. Endocrinol. **4:** 1034–1040

6   Rankin C. T., Bunton T., Lawler A. M. and Lee S. J. (2000) Regulation of left-right patterning in mice by growth/differentiation factor-1. Nat. Genet. **24:** 262–265

7   Guerrero I. and Ruiz i Altaba A. (2003) Development. Longing for ligand: hedgehog, patched, and cell death. Science **301:** 774–776

8   Thibert C., Teillet M. A., Lapointe F., Mazelin L., Le Douarin, N. M. and Mehlen, P. (2003) Inhibition of neuroepithelial patched-induced apoptosis by sonic hedgehog. Science **301:** 843–846

9   Gritli-Linde A., Lewis P., McMahon A. P. and Linde A. (2001) The whereabouts of a morphogen: direct evidence for short- and graded long-range activity of hedgehog signaling peptides. Dev. Biol. **236:** 364–386

10 Kuschel S., Ruther U. and Theil T. (2003) A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon. Dev. Biol. **260:** 484–495

11 Lobjois V., Benazeraf B., Bertrand N., Medevielle F. and Pituello F. (2004) Specific regulation of cyclins D1 and D2 by FGF and Shh signaling coordinates cell cycle progression, patterning, and differentiation during early steps of spinal cord development. Dev. Biol. **273:** 195–209

12 Sokol S., Christian J. L., Moon R. T. and Melton D. A. (1991) Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. Cell **67:** 741–752

13 Crease D. J., Dyson S. and Gurdon J. B. (1998) Cooperation between the activin and Wnt pathways in the spatial control of organizer gene expression. Proc. Natl. Acad. Sci. USA **95:** 4398–4403

14 Niehrs C. (1999) Head in the WNT: the molecular nature of Spemann's head organizer. Trends Genet. **15:** 314–319

15 Xanthos J. B., Kofron M., Tao Q., Schaible K., Wylie C. and Heasman J. (2002) The roles of three signaling pathways in the formation and function of the Spemann Organizer. Development **129:** 4027–4043

16 Shimogori T., VanSant J., Paik E. and Grove E. A. (2004) Members of the Wnt, Fz, and Frp gene families expressed in postnatal mouse cerebral cortex. J. Comp. Neurol. **473:** 496–510

17 Brown A. M. (2001) Wnt signaling in breast cancer: have we come full circle? Breast Cancer Res. **3:** 351–355

18 Giles R. H., Es J. H. van and Clevers H. (2003) Caught up in a Wnt storm: Wnt signaling in cancer. Biochim. Biophys. Acta **1653:** 1–24

19 Uren A., Wolf V., Sun Y. F., Azari A., Rubin J. S. and Toretsky J. A. (2004) Wnt/Frizzled signaling in Ewing sarcoma. Pediatr. Blood Cancer **43:** 243–249

20 Worm J., Christensen C., Gronbaek K., Tulchinsky E. and Guldberg P. (2004) Genetic and epigenetic alterations of the APC gene in malignant melanoma. Oncogene **23:** 5215–5226

21 De Ferrari G. V. and Inestrosa N. C. (2000) Wnt signaling function in Alzheimer's disease. Brain Res. Brain Res. Rev. **33:** 1–12

22 Inestrosa N., De Ferrari G. V., Garrido J. L., Alvarez A., Olivares G. H., Barria M. I. et al. (2002) Wnt signaling involvement in beta-amyloid-dependent neurodegeneration. Neurochem. Int. **41:** 341–344

23 Caricasole A., Copani A., Caruso A., Caraci F., Iacovelli L., Sortino M. A. et al. (2003) The Wnt pathway, cell-cycle activation and beta-amyloid: novel therapeutic strategies in Alzheimer's disease? Trends Pharmacol. Sci. **24:** 233–238

24 McMahon A. P. and Bradley A. (1990) The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell **62:** 1073–1085

25 McMahon A. P., Joyner A. L., Bradley A. and McMahon J. A. (1992) The midbrain-hindbrain phenotype of Wnt-1–/Wnt-1– mice results from stepwise deletion of engrailed-expressing cells by 9.5 days postcoitum. Cell **69:** 581–595

26 Ikeya M., Lee S. M., Johnson J. E., McMahon A. P. and Takada S. (1997) Wnt signalling required for expansion of neural crest and CNS progenitors. Nature **389:** 966–970

27 Nusse R. (2004) http://www.stanford.edu/~rnusse/wntwindow.html

28 Nusse R. and Varmus H. E. (1992) Wnt genes. Cell **69:** 1073–1087

29 Cadigan K. M. and Nusse R. (1997) Wnt signaling: a common theme in animal development. Genes Dev. **11:** 3286–3305

30 Miller J. R. (2001) The Wnts. Genome Biol. **3:** REVIEWS3001.1–3001.15

31 Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T. et al. (2003) Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature **423:** 448–452

32 Nusse R. (2003) Wnts and Hedgehogs: lipid-modified proteins and similarities in signaling mechanisms at the cell surface. Development **130:** 5297–5305

33 Strutt D. (2003) Frizzled signalling and cell polarisation in *Drosophila* and vertebrates. Development **130:** 4501–4513

34 Kuhl M., Sheldahl L. C., Park M., Miller J. R. and Moon R. T. (2000) The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. Trends Genet. **16:** 279–283

35 Adler P. N., Krasnow R. E. and Liu J. (1997) Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. Curr. Biol. **7:** 940–949

36 Bhano P., Brink M., Samos C. H., Hsieh J. C., Wang Y., Macke J. P. et al. (1996) A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature **382:** 225–230

37 Hey P. J., Twells R. C., Phillips M. S., Yusuke N., Brown S. D., Kawaguchi Y. et al. (1998) Cloning of a novel member of the low-density lipoprotein receptor family. Gene **216:** 103–111

38 Brown S. D., Twells R. C., Hey P. J., Cox R. D., Levy E. R., Soderman A. R. et al. (1998) Isolation and characterization of LRP6, a novel member of the low density lipoprotein receptor gene family. Biochem. Biophys. Res. Commun. **248:** 879–888

39 Carron C., Pascal A., Djiane A., Boucaut J. C., Shi D. L. and Umbhauer M. (2003) Frizzled receptor dimerization is sufficient to activate the Wnt/beta-catenin pathway. J. Cell Sci. **116:** 2541–2550

40 Gonzalez-Sancho J. M., Brennan K. R., Castelo-Soccio L. A. and Brown A. M. (2004) Wnt proteins induce dishevelled phosphorylation via an LRP5/6-independent mechanism, irrespective of their ability to stabilize beta-catenin. Mol. Cell. Biol. **24:** 4757–4768

41 Glinka A., Wu W., Delius H., Monaghan A. P., Blumenstock C. and Niehrs C. (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature **391:** 357–362

42 Hoang B., Moos M. Jr, Vukicevic S. and Luyten F. P. (1996) Primary structure and tissue distribution of FRZB, a novel protein related to *Drosophila* frizzled, suggest a role in skeletal morphogenesis. J. Biol. Chem. **271:** 26131–26137

43 Rattner A., Hsieh J. C., Smallwood P. M., Gilbert D. J., Copeland N. G., Jenkins, N. A. et al. (1997) A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc. Natl. Acad. Sci. USA **94:** 2859–2863

44 Zorn A. M. (2001) Wnt signalling: antagonistic Dickkopfs. Curr. Biol. **11:** R592–R595

45 Niida A., Hiroko T., Kasai M., Furukawa Y., Nakamura Y., Suzuki Y. et al. (2004) DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. Oncogene **23:** 8520–8526

46 Golan T., Yaniv A., Bafico A., Liu G. and Gazit A. (2004) The human Frizzled 6 (HFz6) acts as a negative regulator of the canonical Wnt beta-catenin signaling cascade. J. Biol. Chem. **279:** 14879–14888

47 Ozawa M., Baribault H. and Kemler R. (1989) The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J. **8:** 1711–1717

48 Ozawa M., Ringwald M. and Kemler R. (1990) Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. Proc. Natl. Acad. Sci. USA **87:** 4246–4250

49 Kintner C. (1992) Regulation of embryonic cell adhesion by the cadherin cytoplasmic domain. Cell **69:** 225–236

50 Gottardi C. J. and Gumbiner B. M. (2004) Distinct molecular forms of beta-catenin are targeted to adhesive or transcriptional complexes. J. Cell. Biol. **167:** 339–349

51 Aberle H., Bauer A., Stappert J., Kispert A. and Kemler R. (1997) Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. **16:** 3797–3804

52 Ikeda S., Kishida S., Yamamoto H., Murai H., Koyama S. and Kikuchi A. (1998) Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J **17:** 1371–1384

53 Fagotto F., Gluck U. and Gumbiner B. M. (1998) Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin. Curr. Biol. **8:** 181–190

54 Yokoya F., Imamoto N., Tachibana T. and Yoneda Y. (1999) Beta-catenin can be transported into the nucleus in a Ran-unassisted manner. Mol. Biol. Cell **10:** 1119–1131

55 Pawlowski J. E., Ertel J. R., Allen M. P., Xu M., Butler C., Wilson E. M. et al. (2002) Liganded androgen receptor interaction with beta-catenin: nuclear co-localization and modulation of transcriptional activity in neuronal cells. J. Biol. Chem. **277:** 20702–20710

56 Nusse R. (1999) WNT targets: repression and activation. Trends Genet. **15:** 1–3

57 Tago K., Nakamura T., Nishita M., Hyodo J., Nagai S., Murata Y. et al. (2000) Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. Genes Dev. **14:** 1741–1749

58 Daniels D. L. and Weis W. I. (2002) ICAT inhibits beta-catenin binding to Tcf/Lef-family transcription factors and the general coactivator p300 using independent structural modules. Mol. Cell **10:** 573–584

59 Gottardi C. J. and Gumbiner B. M. (2004) Role for ICAT in beta-catenin-dependent nuclear signaling and cadherin functions. Am. J. Physiol. Cell Physiol. **286:** C747–C756

60 Ishitani T., Ninomiya-Tsuji J. and Matsumoto K. (2003) Regulation of lymphoid enhancer factor 1/T-cell factor by mitogen-activated protein kinase-related Nemo-like kinase-dependent phosphorylation in Wnt/beta-catenin signaling. Mol. Cell. Biol. **23:** 1379–1389

61 Thorpe C. J. and Moon R. T. (2004) Nemo-like kinase is an essential co-activator of Wnt signaling during early zebrafish development. Development **131:** 2899–2909

62 Kioussi C., Briata P., Baek S. H., Rose D. W., Hamblet N. S., Herman T. et al. (2002) Identification of a Wnt/Dvl/beta-catenin → Pitx2 pathway mediating cell-type-specific proliferation during development. Cell **111:** 673–685

63 Sommer L. and Rao M. (2002) Neural stem cells and regulation of cell number. Prog. Neurobiol. **66:** 1–18

64 Dickinson M. E., Krumlauf R. and McMahon A. P. (1994) Evidence for a mitogenic effect of Wnt-1 in the developing mammalian central nervous system. Development **120:** 1453–1471

65 Megason S. G. and McMahon A. P. (2002) A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. Development **129:** 2087–2098

66 Zechner D., Fujita Y., Hulsken J., Muller T., Walther I., Taketo M. M. et al. (2003) Beta-catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev. Biol. **258:** 406–418

67 Lee S. M., Danielian P. S., Fritzsch B. and McMahon A. P. (1997) Evidence that FGF8 signalling from the midbrain-hindbrain junction regulates growth and polarity in the developing midbrain. Development **124:** 959–969

68 Panhuysen M., Vogt Weisenhorn D. M., Blanquet V., Brodski C., Heinzmann U., Beisker W. et al. (2004) Effects of Wnt-1 signaling on proliferation in the developing mid-/hindbrain region. Mol. Cell. Neurosci. **26:** 101–111

69 Lee S. M., Tole S., Grove E. and McMahon A. P. (2000) A local Wnt-3a signal is required for development of the mammalian hippocampus. Development **127:** 457–467

70 Chenn A. and Walsh C. A. (2002) Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science **297:** 365–369

71 Panchision D. M., Pickel J. M., Studer L., Lee S. H., Turner P. A., Hazel T. G. et al. (2001) Sequential actions of BMP receptors control neural precursor cell production and fate. Genes Dev. **15:** 2094–2110

72 Chesnutt C., Burrus L. W., Brown A. M. and Niswander L. (2004) Coordinate regulation of neural tube patterning and proliferation by TGFbeta and WNT activity. Dev. Biol. **274:** 334–347

73 Alonso L. and Fuchs E. (2003) Stem cells of the skin epithelium. Proc. Natl. Acad. Sci. USA **100 (suppl 1):** 11830–11835

74 Pinto D., Gregorieff A., Begthel H. and Clevers H. (2003) Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev. **17:** 1709–1713

75 Cheng C. W., Smith S. K. and Charnock-Jones D. S. (2003) Wnt-1 signaling inhibits human umbilical vein endothelial cell proliferation and alters cell morphology. Exp. Cell Res. **291:** 415–425

76 Torres M. A., Yang-Snyder J. A., Purcell S. M., DeMarais A. A., McGrew L. L. and Moon R. T. (1996) Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. J. Cell Biol. **133:** 1123–1137

77 Olson D. J. and Gibo D. M. (1998) Antisense wnt-5a mimics wnt-1-mediated C57MG mammary epithelial cell transformation. Exp. Cell Res. **241:** 134–141

78 Topol L., Jiang X., Choi H., Garrett-Beal L., Carolan P. J. and Yang Y. (2003) Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. J. Cell Biol. **162:** 899–908

79 Chen S., Guttridge D. C., You Z., Zhang Z., Fribley A., Mayo M. W. et al. (2001) Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. J. Cell Biol. **152:** 87–96

80 Longo K. A., Kennell J. A., Ochocinska M. J., Ross S. E., Wright W. S. and MacDougald O. A. (2002) Wnt signaling protects 3T3-L1 preadipocytes from apoptosis through induction of insulin-like growth factors. J. Biol. Chem. **277:** 38239–38244

81 You Z., Saims D., Chen S., Zhang Z., Guttridge D. C., Guan K. L. et al. (2002) Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. J. Cell Biol. **157:** 429–440

82 Kanei-Ishii C., Ninomiya-Tsuji J., Tanikawa J., Nomura T., Ishitani T., Kishida S. et al. (2004) Wnt-1 signal induces phosphorylation and degradation of c-Myb protein via TAK1, HIPK2, and NLK. Genes Dev. **18:** 816–829

83 You L., He B., Uematsu K., Xu Z., Mazieres J., Lee A. et al. (2004) Inhibition of Wnt-1 signaling induces apoptosis in beta-catenin-deficient mesothelioma cells. Cancer Res **64:** 3474–3478

84 Hasegawa S., Sato T., Akazawa H., Okada H., Maeno A., Ito M. et al. (2002) Apoptosis in neural crest cells by functional loss of APC tumor suppressor gene. Proc. Natl. Acad. Sci. USA **99:** 297–302

85 Kleber M. and Sommer L. (2004) Wnt signaling and the regulation of stem cell function. Curr. Opin. Cell Biol. **16:** 681–687

86 Sato N., Meijer L., Skaltsounis L., Greengard P. and Brivanlou A. H. (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat. Med. **10:** 55–63

87 Reya T., Duncan A. W., Ailles L., Domen J., Scherer D. C., Willert K. et al. (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature **423:** 409–414

88 Cobas M., Wilson A., Ernst B., Mancini S. J., MacDonald H. R., Kemler R. et al. (2004) Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. J. Exp. Med. **199:** 221–229

89 Haegele L., Ingold B., Naumann H., Tabatabai G., Ledermann B. and Brandner S. (2003) Wnt signalling inhibits neural differentiation of embryonic stem cells by controlling bone morphogenetic protein expression. Mol. Cell. Neurosci. **24:** 696–708

90 Maretto S., Cordenonsi M., Dupont S., Braghetta P., Broccoli V., Hassan A. B. et al. (2003) Mapping Wnt/beta-catenin signaling during mouse development and in colorectal tumors. Proc. Natl. Acad. Sci. USA **100:** 3299–3304

91 Hirabayashi Y., Itoh Y., Tabata H., Nakajima K., Akiyama T., Masuyama N. et al. (2004) The Wnt/beta-catenin pathway

directs neuronal differentiation of cortical neural precursor cells. Development **131:** 2791–2801

92 Muroyama Y., Kondoh H. and Takada S. (2004) Wnt proteins promote neuronal differentiation in neural stem cell culture. Biochem. Biophys. Res. Commun. **313:** 915–921

93 Otero J. J., Fu W., Kan L., Cuadra A. E. and Kessler J. A. (2004) Beta-catenin signaling is required for neural differentiation of embryonic stem cells. Development **131:** 3545–3557

94 Le Douarin N. M. and Dupin E. (2003) Multipotentiality of the neural crest. Curr. Opin. Genet. Dev. **13:** 529–536

95 Chang C. and Hemmati-Brivanlou A. (1998) Neural crest induction by Xwnt7B in *Xenopus*. Dev. Biol. **194:** 129–134

96 Garcia-Castro M. I., Marcelle C. and Bronner-Fraser M. (2002) Ectodermal Wnt function as a neural crest inducer. Science **297:** 848–851

97 Hari L., Brault V., Kleber M., Lee H. Y., Ille F., Leimeroth R. et al. (2002) Lineage-specific requirements of beta-catenin in neural crest development. J. Cell Biol. **159:** 867–880

98 Lee H. Y., Kleber M., Hari L., Brault V., Suter U., Taketo M. M. et al. (2004) Instructive role of Wnt/beta-catenin in sensory fate specification in neural crest stem cells. Science **303:** 1020–1023

99 Galceran J., Miyashita-Lin E. M., Devaney E., Rubenstein J. L. and Grosschedl R. (2000) Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. Development **127:** 469–482

100 Huelsken J., Vogel R., Erdmann B., Cotsarelis G. and Birchmeier W. (2001) Beta-catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell **105:** 533–545

101 Merrill B. J., Gat U., DasGupta R. and Fuchs E. (2001) Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. Genes Dev. **15:** 1688–1705

102 Cauthen C. A., Berdougo E., Sandler J. and Burrus L. W. (2001) Comparative analysis of the expression patterns of Wnts and Frizzleds during early myogenesis in chick embryos. Mech Dev **104:** 133–138

103 Muroyama Y., Fujihara M., Ikeya M., Kondoh H. and Takada S. (2002) Wnt signaling plays an essential role in neuronal specification of the dorsal spinal cord. Genes Dev. **16:** 548–553

104 Lyuksyutova A. I., Lu C. C., Milanesio N., King L. A., Guo N., Wang Y. et al. (2003) Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. Science **302:** 1984–1988

105 Bonkowsky J. L., Yoshikawa S., O'Keefe D. D., Scully A. L. and Thomas J. B. (1999) Axon routing across the midline controlled by the *Drosophila* Derailed receptor. Nature **402:** 540–544

106 Yoshikawa S., McKinnon R. D., Kokel M. and Thomas J. B. (2003) Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. Nature **422:** 583–588

107 Yoshikawa S. and Thomas J. B. (2004) Secreted cell signaling molecules in axon guidance. Curr. Opin. Neurobiol. **14:** 45–50

108 Lu W., Yamamoto V., Ortega B. and Baltimore D. (2004) Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. Cell **119:** 97–108

109 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

110 Lucas F. R., Goold R. G., Gordon-Weeks P. R. and Salinas P. C. (1998) Inhibition of GSK-3beta 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

111 Ciani L., Krylova O., Smalley M. J., Dale T. C. and Salinas P. C. (2004) A divergent canonical WNT-signaling pathway regulates microtubule dynamics: dishevelled signals locally to stabilize microtubules. J. Cell. Biol. **164:** 243–253

112 Orme M. H., Giannini A. L., Vivanco M. D. and Kypta R. M. (2003) Glycogen synthase kinase-3 and Axin function in a beta-catenin-independent pathway that regulates neurite outgrowth in neuroblastoma cells. Mol. Cell. Neurosci. **24:** 673–686