Emerging roles of Wnts in the adult nervous system

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Abstract | The roles of the Wnt signalling pathway in several developmental processes, including synaptic differentiation, are well characterized. The expression of Wnt ligands and Wnt signalling components in the mature mammalian CNS suggests that this pathway might also play a part in synaptic maintenance and function. In fact, Wnts have a crucial role in synaptic physiology, as they modulate the synaptic vesicle cycle, the trafficking of neurotransmitter receptors and the interaction of these receptors with scaffold proteins in postsynaptic regions. In addition, Wnts participate in adult neurogenesis and protect excitatory synaptic terminals from amyloid-β oligomer toxicity. Here, the latest insights into the function of Wnt signalling in the adult nervous system and therapeutic opportunities for neurodegenerative diseases such as Alzheimer's and Parkinson's disease are discussed.

Wnt proteins are important mediators of cell–cell communication and are involved in diverse cellular processes, including the development of the CNS[1,2]. They are thought to bind to receptors of the Frizzled (Fz) and low-density lipoprotein-related protein (LRP) families on the cell surface. Through several cytoplasmic relay components, including glycogen synthase kinase-3β (GSK3β) and adenomatous polyposis coli (APC), Wnts signal to β-catenin, which enters the nucleus and forms a complex with lymphoid enhancer-binding factor 1 (LEF1; also known as T cell factor) to activate the transcription of Wnt target genes (the Wnt–β-catenin or canonical Wnt signalling pathway)[3]. Wnt signalling is also executed independently of β-catenin, in what are referred to as the non-canonical pathways; these include the planar cell polarity pathway (PCP or Wnt–PCP pathway; also known as the Wnt–Jun N-terminal kinase (JNK) pathway) and the Wnt–Ca^{2+} pathway[4] (FIG. 1).

The neuronal expression of components of the Wnt signalling pathway has been described in the past few years. One of its key components, GSK3β, was first identified in 1993 as the enzyme that phosphorylates tau, a microtubule-associated protein[5]. In 1999, β-catenin, another key component of Wnt signalling, was found in the human brain[6]. More recently several Fz receptors and other Wnt signalling components have been described in the adult brain and spinal cord of rodents[7,8], and the expression of different Wnt ligands has been detected in the rat hippocampus of adult animals[9].

In addition to its well-described role in synaptic differentiation[10,11], the Wnt signalling pathway has been implicated in neurogenesis in adult mice[12] and in the modulation of synaptic plasticity: activity-regulated secretion of Wnt ligands induces long-term potentiation in adult mouse hippocampal slices[13] and dendrite arborization[14]; NMDAR (*N*-methyl-D-aspartate receptor) activation induces calpain-mediated β-catenin cleavage, which leads to LEF1-dependent gene transcription[15]; and physical exercise modulates the expression of Wnt signalling pathway components in aged mice[16]. Furthermore, overexpression of GSK3β, which is expressed in dendritic spines[17], causes a decrease in spatial learning, as evaluated in the Morris water maze[18], and prevents the induction of NMDAR-dependent long-term potentiation in CA3–CA1 hippocampal synapses of 2-week-old rats[19,20]. Recent studies indicated that Wnt signalling mediates the global regulation of synapse numbers in response to experience and age in the adult hippocampus[21]. These studies illustrate the emerging role of Wnt and/or β-catenin signalling in postnatal brain plasticity.

The persistent expression of Wnts in the adult brain, together with their role in the modulation of neurogenesis and synaptic plasticity, indicates that Wnt signalling plays a part in maintaining and protecting neuronal connections throughout the entire lifespan. This Review discusses how different Wnt ligands, acting through different signalling pathways, operate in pre- and postsynaptic regions to modulate synapse structure and function, as well as their role in neurogenesis in the developed nervous system. We also discuss evidence that the Wnt signalling pathway offers potential targets for

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FM-1-43
An amphiphatic dye that becomes intensely fluorescent when inserted into the cell membrane. It is used in a wide variety of studies involving the plasma membrane and vesiculation.

Miniature excitatory postsynaptic current
(mEPSC). The postsynaptic current that is evoked by release of a single vesicle of neurotransmitter from the presynaptic terminal into the synapse.

the treatment of neurodegenerative diseases that affect synaptic integrity, such as Alzheimer's disease (AD) and Parkinson's disease (PD).

Wnt signalling at the presynaptic sites

The finding that WNT7A increases the clustering of synapsin 1 in granule cells[2,10] was the first hint that Wnt signalling has a key role in presynaptic assembly during neural development. A similar function has been established for wingless, the prototypical *Drosophila spp.* Wnt, during synaptogenesis at the larval glutamatergic neuromuscular junction[22,23]. More recently, several Wnt ligands were shown to induce clustering of several presynaptic proteins and regulate the trafficking of the α_7 nicotinic acetylcholine receptor (α_7-nAChR) to the plasma membrane in mature hippocampal neuronal cultures[24]. The mechanism implicated in the presynaptic localization of α_7-nAChR involves APC and does not follow the classical canonical Wnt–β-catenin signalling pathway[24]. APC is expressed at high levels in the cytoplasm of hippocampal neurons[25] and it has been reported that β-catenin forms a cytoplasmic complex with APC in rat brain[26]. WNT7A induces the dissociation of APC from β-catenin, allowing APC to interact with the α_7-nAChR and induce the trafficking of the receptor to the presynaptic plasma membrane[24].

In addition, WNT7A has been shown to modulate the synaptic vesicle cycle and synaptic neurotransmission in mature hippocampal neurons[9]. Examination of vesicle recycling and exocytosis using FM-1-43 indicated that WNT7A stimulates the recycling of presynaptic vesicles in a fast and robust way. WNT3A has a moderate effect, whereas WNT1 and WNT5A do not affect the recycling of synaptic vesicles. Mature hippocampal neurons exposed to WNT7A temporarily increase their rate of synaptic vesicle exocytosis, suggesting that this Wnt ligand modulates neurotransmitter release at the presynaptic nerve terminal[9]. This was supported by electrophysiological studies carried out in hippocampal slices from adult rats, in which WNT7A decreased paired-pulse facilitation and increased the frequency of miniature excitatory postsynaptic currents (mEPSCs)[9], indicating an increase in neurotransmitter release in CA3–CA1 synapses. Analysis of a double-mutant mouse lacking both WNT7A and the downstream scaffold protein Dishevelled (DVL) showed a decrease in the mEPSC frequency, indicating a defect in the release of neurotransmitter[27]. Together these results indicate that WNT7A increases synaptic transmission through a presynaptic mechanism, probably involving an increase in neurotransmitter release (FIG. 2). Recently, the presynaptic distribution of the receptor Fz1 was determined in hippocampal neurons[28]. In addition, it was shown that the induction of presynaptic protein clustering and the increase in functional presynaptic recycling sites by WNT3A was mediated by this receptor. These results suggest that the synaptic effects of the Wnt signalling pathway could be modulated by local activation of synaptic Fz receptors.

Some authors have proposed that Wnt signalling regulates synapse formation by promoting neuronal maturation through gene transcription[29]. However, evidence suggests that the mechanism involved in synapse assembly is independent of gene transcription, at least in short-term studies. First, in cultured neurons, Wnt signalling increases the number and size of synaptic vesicle recycling sites without affecting synaptic protein expression[9]. Second, in the *Wnt7a*$^{-/-}$;*Dvl1*$^{-/-}$ mouse the localization, but not the levels, of synaptic proteins is affected[27]. Third, in conditional knockouts of β-catenin it has been observed that this protein is required for the proper localization of synaptic vesicles along the axon[30], and scribble (a member of the LAP (leucine-rich repeats and PDZ domains) family) functions downstream of β-catenin to cluster synaptic vesicles at developing synapses[31]. Thus, there seems to be a consensus that a β-catenin-dependent Wnt signalling pathway mediates

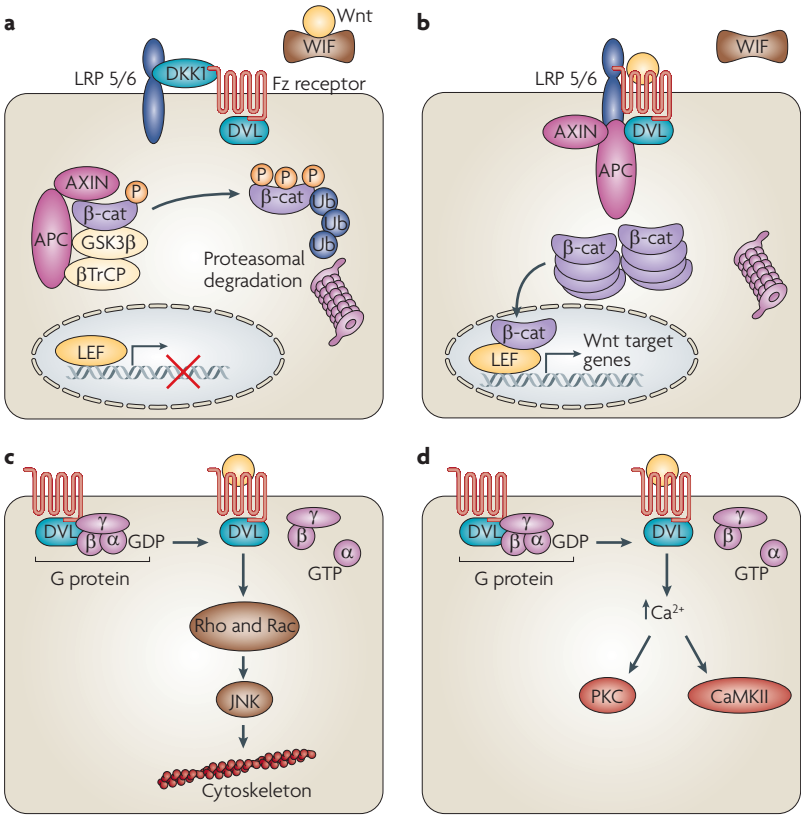

Figure 1 | **The Wnt signalling pathways. a** | Canonical Wnt signalling is inhibited in the presence of DKK1. Under these conditions glycogen synthase kinase 3β (GSK3β) is activated and β-catenin (β-cat) phosphorylated and eventually degraded in the proteasome. **b** | When canonical Wnt signalling is activated, the Wnt ligand interacts with Frizzled (Fz) receptors and the co-receptor low-density lipoprotein receptor-related protein 5 (LRP5)/LRP6. Under these conditions GSK3β is blocked and β-catenin accumulates in the cytoplasm before moving into the nucleus, where it activates the transcription of Wnt target genes. **c** | In non-canonical Wnt–Jun N-terminal kinase (JNK) signalling the activation of Fz receptors, Dishevelled (DVL) and the monomeric GTPases Rho and Rac activates JNK. This facilitates the interaction of JNK with the cytoskeleton, or activates transcription through AP-1 (not shown). **d** | In non-canonical Wnt–Ca^{2+} signalling activation of Fz and DVL increases the intracellular Ca^{2+} concentration, which in turn activates both protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII); these kinases can then modify different signalling components, including postsynaptic receptors. In both cases of non-canonical Wnt signalling, evidence suggests that G proteins are probably involved in the transduction of the Wnt signal. βTrCP, transducin repeat-containing protein; APC, adenomatous polyposis coli; LEF, lymphoid enhancer-binding factor (also known as T cell factor); Ub, ubiquitin; WIF, Wnt inhibitory factor.

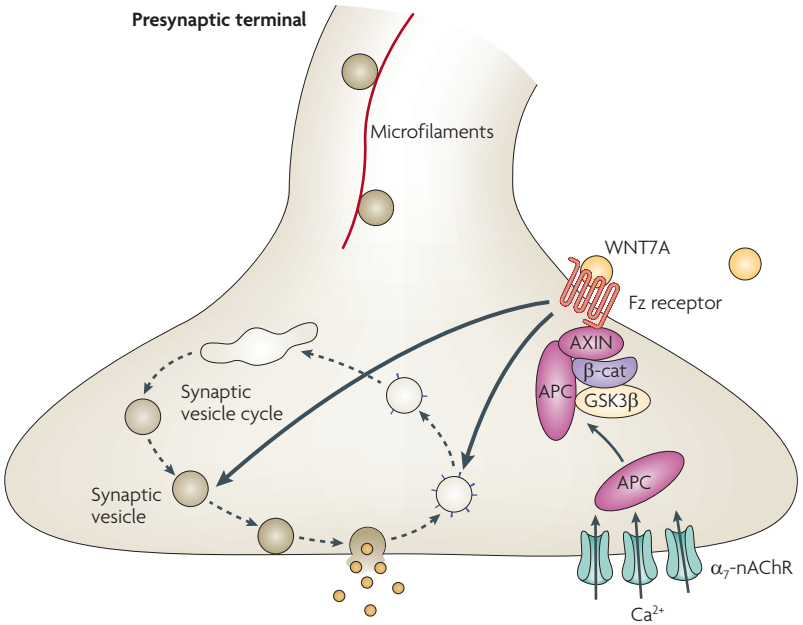

Figure 2 | **Canonical Wnt signalling regulates the presynaptic component of mature central synapses.** Wnt ligands, probably released from the postsynaptic site, modulate the synaptic vesicle cycle in the presynaptic nerve terminal, which includes the regulation of exocytosis (glutamate release) and endocytosis. Whether Wnt ligands regulate the reserve pool of synaptic vesicles is uncertain. In addition, Wnt ligands regulate the targeting and trafficking of the α_7 nicotinic acetylcholine receptor (α_7-nAChR) through adenomatous polyposis coli (APC), a member of the cytoplasmic destruction complex. β-cat, β-catenin; Fz, Frizzled; GSK3β, glycogen synthase kinase 3β.

this presynaptic function, without requiring gene transcription. However, the mechanism by which this non-conventional canonical Wnt pathway regulates the presynaptic region and whether a similar mechanism operates in mature neurons to regulate neurotransmitter release are still under investigation.

Wnt signalling at the postsynaptic sites

Glutamate receptors, such as NMDARs and AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors), are located at the postsynaptic membrane of excitatory synapses, with the NMDARs at the centre, directly in front of the active zone, and the AMPARs more peripherally distributed. The scaffold protein post-synaptic density protein 95 (PSD95; also known as Disks large homolog 4) forms membrane-perpendicular and roughly equally spaced filamentous structures, with its amino terminus attached to the membrane[32].

So far, few synaptogenic factors have been reported to regulate the postsynaptic region of central synapses. In the vertebrate cholinergic neuromuscular junction, agrin, a heparan sulphate proteoglycan secreted by motor neurons, induces aggregation of AChRs at the postsynaptic membrane[33,34]. Recent studies have demonstrated that WNT3 functions as a modulator of postsynaptic differentiation at the vertebrate peripheral neuromuscular synapses by collaborating with agrin[34]. In the CNS, electrophysiological studies in rat hippocampal slices indicate that WNT5A increases field EPSP (fEPSP) amplitude without affecting synaptic facilitation[35]. This effect was reversible and antagonized by a WNT5A-specific antibody. Patch-clamp analysis at different holding potentials (–90 mV and +40 mV) in CA1 pyramidal neurons revealed that the potentiation induced by WNT5A is due to the postsynaptic modulation of NMDAR- and AMPAR-mediated currents[36]. In addition, WNT5A induces rapid insertion of NMDARs in the postsynaptic region of hippocampal neurons[36] and has been shown to increase the number of PSD95 clusters in dendritic spines within 30 minutes[35]. Interestingly, WNT5A induces fast and transient phosphorylation of calcium/calmodulin-dependent protein kinase II after 15 minutes, followed by JNK phosphorylation, which peaks after 30 minutes of treatment and lasts for at least 2 hours[35]. These results indicate that WNT5A is acting as a non-canonical ligand, activating both the Wnt–Ca^{2+} and the Wnt–JNK signalling pathways without stabilizing β-catenin. The mechanism whereby WNT5A induces synaptic PSD95 clustering may be related to the phosphorylation of Ser295 of PSD95 by JNK[37]. Together, these findings indicate that WNT5A regulates the assembly and function of the excitatory postsynaptic region in the mature CNS (FIG. 3).

Wnt signalling and adult neurogenesis

Adult neurogenesis in the mammalian brain is generally considered an active process encompassing the proliferation and cell fate specification of adult neural progenitors, and their subsequent differentiation, maturation, navigation and functional integration into the existing neuronal circuitry[38]. In the intact adult mammalian CNS, active neurogenesis occurs in two discrete 'neurogenic' regions: the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles in the forebrain[39,40]. Accumulating evidence suggests that these new neurons are essential for brain functions, such as learning, memory, olfaction and mood modulation[38]. They are thought to originate from multipotent adult neural stem cells, but their exact identity is still subject to debate and their multipotency at the clonal level *in vivo* has not been universally demonstrated. It seems that astrocyte-like cells function as neural stem cells in both the SVZ and the SGZ[38–44].

Stem cell differentiation is controlled by both intrinsic and extrinsic regulators. Wnt ligands are among the extracellular factors that regulate this process[45,46]. During development Wnts act on CNS progenitor cells, and the activation of β-catenin leads to the proliferation of the neural progenitor pool, resulting in the expansion of the entire neural tube[47]. In addition, a GSK3β inhibitor was found to induce the selective differentiation of stem cells into neurons[48], and WNT7A promoted the maturation of neural precursor cells into neurons[49]. More recent studies provide evidence that Wnt signalling enhances the proliferation of neural stem cells derived from the adult CNS[50–52]. WNT3 is expressed by adult hippocampal stem or progenitor cells and has been found to act as an intrinsic regulator of hippocampal neurogenesis by modulating the generation of newborn neurons in the adult dentate gyrus[52]. In addition, Wnts secreted by adult

Active zone
The portion of the presynaptic membrane located opposite the postsynaptic density. It is the site of synaptic vesicle docking and neurotransmitter release.

Field EPSP
(fEPSP). The extracellular signal recorded from a population of neurons when they all receive synaptic inputs from afferent axonic fibres. It is possible to make field recordings only in those areas of the brain, such as the hippocampus, in which the neurons are arranged in such a way that they all receive synaptic inputs from the same afferent.

hippocampal progenitors self-stimulate canonical Wnt signalling, and inhibition of this autocrine Wnt pathway increases the number of neurons formed and leads to a loss of the multipotency of the progenitors[53]. Inhibition of Wnt signalling by lentiviral expression of a dominant-negative Wnt in the dentate gyrus reduces neurogenesis in the hippocampus[52] and decreases long-term retention of spatial and object recognition memory in adult rats[54]. Further pointing to a role for GSK3β–β-catenin signalling in neurogenesis is the finding that suppression of expression of Disrupted in schizophrenia 1 (DISC1), a protein encoded by a gene that is implicated in schizophrenia susceptibility and which directly interacts with GSK3β, decreased the proliferation of adult hippocampal progenitors *in vitro* and *in vivo* through the GSK3β–β-catenin pathway[55].

Recently, NEUROD1, a pro-neurogenic transcription factor in the adult brain that is selectively expressed in dividing neural progenitors and in immature granule neurons in the adult dentate gyrus, was identified as a downstream effector of Wnts in adult neurogenesis[56,57]. WNT3A treatment induced the expression of NEUROD1 in adult neural progenitors *in vitro*, and β-catenin was directly associated with the NEUROD1 gene promoter during the course of neurogenesis[56]. Deletion of NEUROD1 in stem cells prevented neurogenesis *in vivo*[57], and Wnt treatment of these cells did not stimulate neurogenesis[56].

Wnt signalling has also been shown to modulate neurogenesis in the SVZ. WNT3A and WNT5A increase the proliferation of cultured progenitor cells isolated from postnatal and adult mouse SVZ and promote their neuronal differentiation[58]. In addition, retrovirus-mediated expression of stabilized β-catenin or treatment with an inhibitor of GSK3β were shown to promote the proliferation of progenitor cells in the SVZ, inhibit their differentiation into neuroblasts and increase the number of new neurons in the olfactory bulb[59]. Conversely, expression of the Wnt antagonist DKK1 reduced the proliferation of progenitor cells[59]. These studies indicate that activation of Wnt signalling regulates adult neurogenesis in the SVZ by regulating progenitor cell proliferation.

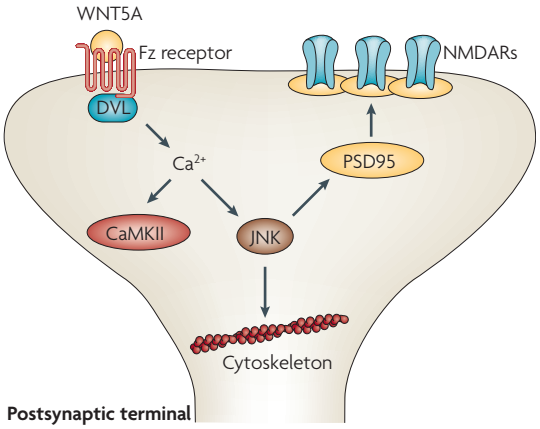

Figure 3 | **Non-canonical Wnt signalling regulates the postsynaptic component of mature central synapses.** WNT5A modulates rapid changes in the traffic of glutamatergic receptors, including both NMDARs (*N*-methyl-D-aspartate receptors) and AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) (not shown), through changes in calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) (not shown) activity (within the first 20 min) and then by activating Jun N-terminal kinase (JNK) (1 h) in the dendritic spines. The stabilization of glutamatergic receptors is defined by the eventual aggregation of postsynaptic density protein 95 (PSD95). WNT5A also induces an increase in the clustering of PSD95 (within 1–2 h) through changes in JNK activity, which also modulate the formation of dendritic spines. These results indicate that excitatory synapses are modulated by the non-canonical Wnt signalling pathway. Whether different types of Wnt receptors (Frizzled (Fz) receptors and receptor tyrosine kinase-like orphan receptor 2 (ROR2)) modulate different aspects of dendritic spine plasticity requires further investigation.

Wnt signalling in non-neurogenic regions

The finding that new neurons functionally integrate into adult brain circuits[60,61] has opened up the possibility that stimulating adult neurogenesis could become a therapeutic strategy to replace neurons lost by disease[38]. Müller glia in the adult mammalian retina may behave as stem cells and support retinal regeneration *in vivo*. Interestingly, Müller glia-derived progenitors can differentiate into multiple lineages of retinal cells under the control of intrinsic or extrinsic factors[62,63]. Recently, the activation of Wnt signalling has been shown to promote the proliferation of Müller glia-derived retinal progenitors and neural regeneration after damage or during degeneration in adult mammals[64]. WNT3A treatment increased the proliferation of de-differentiated Müller glial cells 20-fold in the photoreceptor-damaged retina compared with the control retina. Supplementation with an inhibitor of GSK3β induces differentiation of these cells primarily into cone or rod homeobox-positive and rhodopsin-positive photoreceptors. Conversely, inhibition of Wnt signalling with DKK1 attenuated retinal regeneration. Injury induced nuclear accumulation of β-catenin, an upregulation of cyclin D1 and Wnt/β-catenin reporter activity. This WNT3A-mediated regeneration of retinal cells also occurs in *rd* mice, a model of retinal degeneration[64], indicating that in the retina a Wnt-mediated repair process exists *in vivo* and that, under pathological conditions, application of Wnt or GSK3β inhibitors could promote regeneration.

Other pathological stimuli, such as stroke, have long been thought to be unable to activate neurogenesis outside the two neurogenic regions of the adult brain[38]. However, recent findings indicate that neurogenesis in the forebrain may play a significant part in repair and functional recovery after stroke[65]. These findings suggest that compounds that improve the efficiency of neurogenesis and/or enhance the survival and functional integration of newly produced neurons in the adult brain, such as Wnts, could become useful therapeutic agents.

Wnts may also find an application in neurodegenerative disorders, such as AD, PD and Huntington's disease, which are characterized by the slow progressive loss of specific neuronal populations in the brain. So far, only symptomatic therapeutic approaches are available, making these diseases potential candidates for restorative therapeutic approaches. In the following sections, the role of Wnt signalling in PD and AD is examined and the potential therapeutic use of Wnt-modifying treatments is discussed.

Wnt signalling in midbrain DA neurons

Damage-induced adult neurogenesis would particularly benefit the midbrain nigrostriatal dopaminergic (DA) system, as degeneration of these cells causes the main symptoms of PD[66]. Although some reports have found evidence of adult DA neurogenesis in the striatum of rodents following lesions in this area, whether such neurogenesis occurs in the substantia nigra remains controversial (see REF. 67 for a review and REFS 68–70 for more detail). Research in this area has focused on understanding midbrain DA neurogenesis during development, with the hope of stimulating the underlying mechanisms in either endogenous or reprogrammed adult stem cells *in vivo* or *ex-vivo* for use in transplantation. Interestingly, Wnt signalling, through the Wnt–β-catenin pathway (WNT1 or WNT3A) or the Wnt–PCP pathway (WNT5A), is crucial for several aspects of midbrain DA neuron development (FIG. 4). WNT5A promotes ventral midbrain morphogenesis, reduces DA progenitor proliferation and neurogenesis in loss-of-function experiments *in vivo,* enhances DA precursor differentiation both *in vitro* and *in vivo*[71,72] and possibly increases survival by regulating the glia-derived neurotrophic factor receptor, RET[71]. Finally, gain-of-function experiments *in vitro* showed increased neurogenesis (indicated by the presence of Nurr1+ and BrdU+ cells)[71], whereas loss-of-function studies *in vivo* showed increased Nurr1+ cells in the absence of cell death[72], suggesting that a negative regulation of DA neurogenesis occurs during development.

WNT1 serves a broader array of functions, some of which are complementary to the functions of WNT5A *in vivo*, such as patterning the midbrain region[73,74] and specifying DA progenitors or regulating survival[75]. WNT1 can also oppose the action of WNT5, for example by expanding the DA progenitor pool *in vitro* or *in vivo*[71,76], or have synergistic functions such as in the regulation of neurogenesis, cell survival and neuritogenesis *in vitro*[71]. Similarly, it has recently been reported that β-catenin regulates midbrain DA neurogenesis *in vivo*[77,78], providing evidence for the involvement of canonical Wnt signalling in DA neurogenesis. However, it remains to be determined which of the several Wnts expressed in the developing ventral midbrain[79,80] regulate this process *in vivo*. In line with the idea that activation of the Wnt–β-catenin pathway contributes to increased DA neurogenesis during development, treatment of prenatal DA progenitors with GSK3β inhibitors increased the generation of DA neurons[81]. Moreover, transplantation of rodent fetal neural stem cells treated

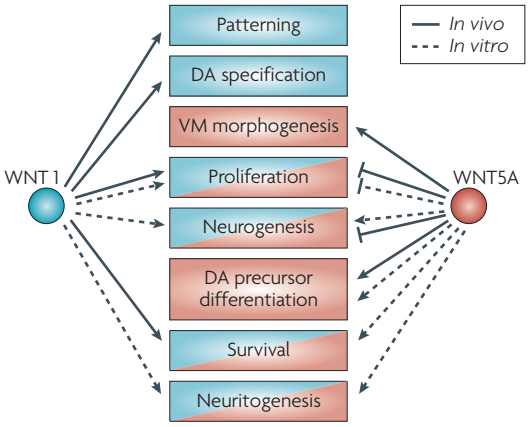

Figure 4 | **Function of WNT1 and WNT5A in midbrain dopaminergic neuron development.** Midbrain development depends on the sequential regulation of multiple functions by cell-intrinsic and cell-extrinsic factors, such as Wnts. The delicate balance between activation of distinct Wnt signalling pathways by ligands such as WNT1 (which signals through the Wnt–β-catenin pathway) and WNT5A (which signals through the Wnt–planar cell polarity pathway) controls midbrain dopaminergic (DA) neuron development. Loss- and gain-of-function experiments *in vivo* (continuous arrows) and *in vitro* (discontinuous arrows) have contributed to our understanding of this balance. Arrowheads indicate activation; blunt-ended arrows indicate inhibition. Blue shading indicates functions controlled by WNT1; red shading indicates functions controlled by WNT5A. VM, ventral midbrain.

with WNT5A resulted in enhanced survival, differentiation and functional integration in the absence of tumour formation in animal models of PD[82]. Similar results have recently been obtained for embryonic stem cells (E.A., C. Parish and I. Liste, unpublished observations). Thus, current evidence suggests that Wnts may find a therapeutic application in the preparation of stem cells for cell replacement therapy in PD.

However, it still remains to be determined whether progenitors actually exist in the adult midbrain and whether Wnt signalling has a role in promoting adult DA neurogenesis *in vitro*. One strategy to overcome an eventual absence of adult ventral midbrain progenitors could involve the reprogramming of adult somatic cells in this region. Adult cortical astrocytes can be reprogrammed to undergo neurogenesis by overexpression of PAX6 *in vitro*[83]. Interestingly, MYC, one of the four transcription factors needed to transform somatic cells into pluripotent stem cells[84], is a direct target of Wnt–β-catenin signalling[85], suggesting a role for Wnt signalling in somatic cell reprogramming. However, Wnt signalling is tightly regulated and a delicate balance between activation and inactivation of canonical and non-canonical signalling is maintained both during development and in the adult brain[86,87] (FIG. 4). A deregulation of such mechanisms, either at a genetic or an epigenetic level, leading to excessive Wnt–β-catenin signalling has been linked to oncogenesis in many different tissues[88]. The involvement of Wnt signalling in PD should also be examined more closely, in order to

determine whether cells will be capable of transducing the Wnt signal and whether Wnt signalling is impaired in DA neurons affected by PD (FIG. 5). As the aetiology of PD is largely unknown, it is difficult to establish whether there is a general pathophysiological mechanism. To date several genes have been identified as causative or as susceptibility factors (see REF. 89 for a review) and have provided clues as to the involvement of oxidative stress, mitochondrial dysfunction, the ubiquitin–proteasome system and autophagic–lysosomal systems. Evidence for a direct interaction between the PD-associated protein parkin and β-catenin has recently been published[90]; increased levels of total and active (dephosphorylated) β-catenin were found in mice lacking parkin. This increase in Wnt–β-catenin signalling resulted in an increase in DA neuron proliferation and death, suggesting that a decrease in the degradation of β-catenin may lead to loss of DA neurons as they try to re-enter the cell cycle[90]. This result is in contrast with the positive role of Wnt–β-catenin signalling during midbrain DA neuron development and in stem cells and suggests that diseased adult DA neurons may need lower levels of Wnt–β-catenin signalling, whereas DA progenitors may benefit from increased activation of Wnt–β-catenin signalling. These findings underline the need to characterize the impact of disease mechanism on Wnt signalling and emphasize the requirement for drugs that restore Wnt signalling to the correct level. Finally, they also underscore the importance of targeting such drugs to the desired cells. Taking these factors into consideration will avoid the perils of abnormally low or high levels of Wnt signalling.

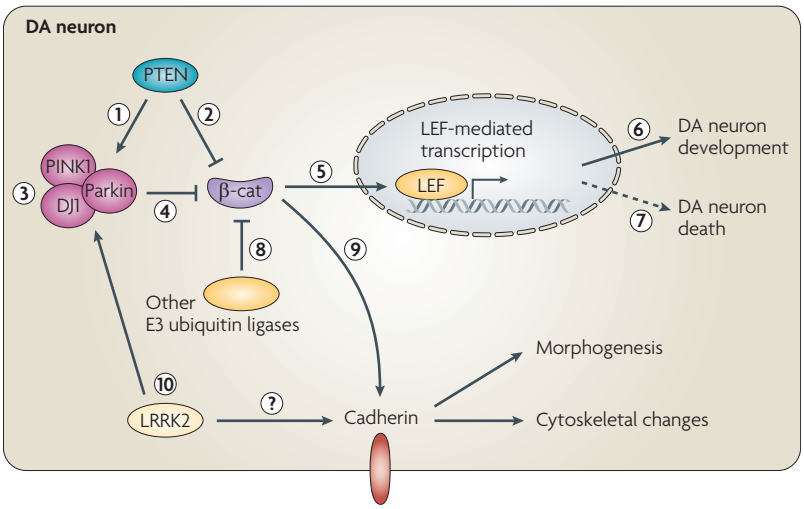

Figure 5 | **Hypothetical mechanism by which PD-related proteins may regulate Wnt signalling and the function of DA neurons.** Phosphatase and tensin homolog (PTEN) induces the expression of PINK1 (step 1) and inhibits Wnt–β-catenin (β-cat) signalling (step 2)[123]. Moreover, PINK1 and DJ1 (also known as PARK7) form a complex with parkin and regulate its E3 ubiquitin ligase activity (step 3)[124]. Parkin interacts with β-catenin and promotes its degradation (step 4)[90]. β-Catenin regulates the transcription of target genes through lymphoid enhancer-binding factor (LEF) (also known as T cell factor) (step 5). Activation of Wnt–β-catenin signalling promotes dopaminergic (DA) neuron development in DA progenitors[71–76] (step 6), whereas excessive Wnt–β-catenin signalling in postmitotic DA neurons leads to cell cycle re-entry and cell death (step 7)[90]. Other E3 ubiquitin ligases such as transducin repeat-containing protein (βTrCP), Siah and Jade-1 also promote β-catenin degradation (step 8)[125–127] and may partially compensate for a loss of parkin function. β-Catenin has an armadillo repeat that allows it to interact with cadherins and thus become involved in the regulation of morphogenesis[128] (step 9). Interestingly, the Parkinson's disease (PD)-related protein leucine-rich repeat serine/threonine protein kinase 2 (LRRK2) also has an armadillo repeat and was recently found to genetically interact with PINK1, DJ1 and Parkin in *Drosophila melanogaster*[129]. However, it might interfere with Wnt signalling by competing with β-catenin for the interaction with cadherin (step 10).

Wnt signalling and AD

Studies in humans indicate that Wnt signalling is directly related to neurogenesis and is altered or involved in the pathophysiology of AD. One example is the reduced renewal capacity of glial-like progenitor cells isolated from the temporal cortex of patients with AD — which correlated with elevated levels of GSK3β activity and increased phosphorylation of β-catenin — compared with that of cells from healthy controls[91]. Moreover, treating glial precursor cells from healthy controls with amyloid-β peptide (Aβ) also led to increased β-catenin phosphorylation and reduced neurogenesis. Conversely, β-catenin transfection led to restoration of neurogenesis[91]. These studies suggest that Wnt signalling is required for human cortical neurogenesis and that impaired Wnt signalling reduced the capacity of progenitors to undergo neurogenesis and contribute to repair.

Almost a decade ago a relationship between loss of Wnt signalling and Aβ-induced neurotoxicity was proposed[92,93]. The change in Wnt signalling was suggested to be the triggering factor for Aβ production and tau hyperphosphorylation, which induce synapse and neuron loss[93,94] (FIG. 6). Since then, other studies have shown that several Wnt signalling components are altered in AD[94–97]. Aβ directly binds to the Fz5 cysteine-rich domain at or in close proximity to the Wnt-binding site, inhibiting the canonical Wnt signalling pathway[98]. In addition, genetic studies show a link between Wnt signalling and AD. The apolipoprotein E ε4 allele, which is associated with an increased risk of developing AD[99], inhibits canonical Wnt signalling on stimulation with WNT7A[100], and recent studies have identified genetic polymorphisms in Wnt signalling components, which are also implicated in AD (BOX 1).

To determine whether activation of Wnt signalling could protect hippocampal neurons from the Aβ toxicity, the effects of activating other signalling pathways that crosstalk with the Wnt pathway was examined[101]. As cholinergic dysfunction has been observed in patients with AD, treatment of rodents with an M1 muscarinic AChR receptor agonist[102] or nicotine[101] was examined. As shown in FIG. 6, activation of Wnt signalling through cholinergic activation seems to be a neuroprotective mechanism against Aβ. In fact, it is well known that M1 agonists increase the non-amyloidogenic processing of the amyloid precursor protein (APP), reducing Aβ production and tau phosphorylation[4]. In addition, cholinergic activation by the specific M1 agonist induces the phosphorylation (and therefore inactivation) of GSK3β in neuronal cultures from transgenic mice that overexpress GSK3β[102]. Ser9 phosphorylation of GSK3β

by cholinergic stimulation is probably mediated by a mechanism involving protein kinase C (PKC), as it was blocked by a PKC inhibitor[102]. The protection observed *in vitro* has been confirmed *in vivo*, as chronic treatment with the specific M1 agonist improved the spatial memory and reduced the Aβ load in the hippocampus of a triple-transgenic mouse model of AD[4]. These findings indicate that cholinergic activation interacts with the Wnt signalling pathway, leading to potential neuroprotection against Aβ toxicity.

Recent evidence suggests that lithium is neuroprotective in various neurodegenerative conditions, and it is noteworthy that lithium reduces the prevalence of AD in elderly patients with bipolar disorders[103]. In addition, studies in a mouse model of AD indicated that lithium reduces the size of the amyloid burden, including the Aβ oligomers, and prevents the behavioural disturbances of the animals[104]. Under these conditions lithium activates Wnt signalling, as demonstrated by the inhibition of GSK3β and the increase in β-catenin[104]. These studies are consistent with the idea that a loss of Wnt signalling is involved in Aβ-dependent neurodegeneration, and that activation of the canonical pathway by lithium protects against the synaptic changes triggered in AD.

Aβ oligomer-induced alterations in synapse composition, shape and density provide a molecular basis for loss of connectivity in AD[101,105]. Recent electrophysiological studies in rat hippocampal slices show that WNT5A augments the glutamatergic transmission mainly through a postsynaptic mechanism, increasing both NMDA and AMPA currents. Conversely, Aβ oligomers impair synaptic transmission by decreasing the NMDA currents and, to smaller degree, the AMPA currents[36]. Treatment of hippocampal slices with Aβ oligomers decreased the EPSC amplitude by around 60% at a holding potential of –80 mV, but in the presence of WNT5A the change in EPSC amplitude was not observed, indicating that WNT5A exerts a protective effect[36]. Aβ oligomers have also been shown to reduce the surface expression of glutamate receptors in hippocampal neurons[106]. Incubation of Aβ oligomers with WNT5A showed that the distribution of the NMDARs was similar to that in control neurons; however, co-treatment with the Wnt antagonist secreted Frizzled receptor protein 1 (sFRP-1) abolished the increase of NMDARs triggered by the Wnt ligand[36]. Together, these results indicate that activation of non-canonical Wnt signalling by WNT5A could also protect neurons from Aβ oligomer-induced toxicity, further pointing to the therapeutic potential of this signalling pathway in the treatment of AD.

Wnt signalling and other CNS-related diseases

To function properly, the brain must be wired correctly during crucial periods in development[1,107]. Wnt signalling is involved in the development of the brain and spinal cord and in the extension of numerous subpopulations of sensory and motor neurons. However, some CNS-related diseases in adulthood have also been associated with components of the Wnt signalling pathway, highlighting the fundamental role of this pathway in the proper functioning of the mature CNS.

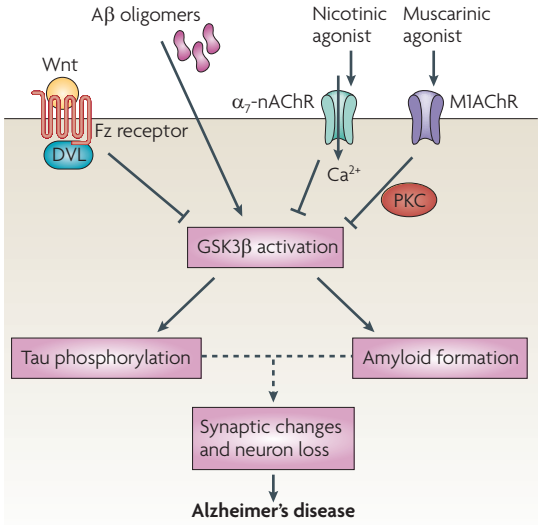

Figure 6 | **Activation of Wnt signalling protects from amyloid toxicity.** Under normal conditions, Wnt ligands inhibit glycogen synthase kinase 3β (GSK3β) activation (canonical Wnt signalling). In the presence of amyloid-β (Aβ) oligomers, GSK3β is activated and increases β-catenin and tau phosphorylation. At the same time, GSK3β activates γ-secretase, leading to intracellular Aβ formation. The increase in both Alzheimer's disease (AD) hallmarks (phosphorylated tau and amyloid build-up) triggers synaptic changes and neuron loss, which eventually lead to the clinical and cognitive alterations of AD. Activation of Wnt signalling leads to neuroprotection in hippocampal neurons both in culture and in transgenic AD models[4,104]. Activation of other receptors, such as the α_7 nicotinic acetylcholine receptor (α_7-AChR) and M1 muscarinic AChR receptors protects hippocampal neurons from Aβ oligomer-induced toxicity[101,102]. DVL, Dishevelled; Fz, Frizzled; PKC, protein kinase C.

Schizophrenia. Recently, *DISC1* was found to regulate neuronal progenitor proliferation by modulating GSK3β–β-catenin signalling[55]. DISC1 regulates β-catenin abundance and is required for WNT3A-induced cell proliferation and activation of downstream transcription factors of the LEF family in cultured adult neural progenitor cells. The phenotype of neural progenitor cells lacking DISC1 can be rescued by overexpressing degradation-resistant β-catenin *in vivo*. Furthermore, DISC1 interacts with GSK3β, blocking its activity, and a GSK3β inhibitor rescued the defect in neural progenitor cell proliferation induced by DISC1 suppression in mouse embryonic cortex and adult dentate gyrus[55]. Importantly, mice lacking DISC1 in the dentate gyrus exhibited schizophrenia-like and depression-like behaviour that could be normalized by treatment with a GSK3β inhibitor. In the adult brain, GSK3β mediates several aspects of synaptic plasticity[108], and changes in GSK3β regulation might underlie some of the cognitive deficits present in schizophrenia[108].

Box 1 | **LRP6, GSK3β and catenin genes are implicated in AD**

Genome-wide screens in humans have identified several regions with significant linkage to Alzheimer's disease (AD), in particular one region on chromosome 12 in the vicinity of the Wnt co-receptor gene low-density lipoprotein receptor-related protein 6 (*LRP6*)[118]. Indeed, subsequent studies have revealed an association between a highly conserved polymorphism in the coding sequence of *LRP6* (Ile1062Val) and the risk of late-onset AD (LOAD) in carriers of the apolipoprotein E ε4 allele. Interestingly, the Val1062 variant of *LRP6* causes reduced activation of a β-catenin-responsive reporter gene in HEK293T/STF recombinant cells, suggesting that reduced signalling through the canonical Wnt pathway may predispose people to AD. Glycogen synthase kinase 3β (GSK3β) was also found to be associated with LOAD, as active GSK3β associates with neurofibrillary tangles in the human brain[119]. Overexpression of GSK3β in mice caused both tau hyperphosphorylation and hippocampal dysfunction[120]. An intronic polymorphism in GSK3β has been found to occur at more than twice the normal frequency in patients with AD (14.6%) and patients with frontotemporal dementia (10.8%). This is the first evidence that a gene known to be involved in tau phosphorylation is associated with primary neurodegenerative dementias[121]. In addition, a genetic variation in αT-catenin, a protein involved in cadherin adhesive complex signalling and that is related to Wnt signalling, has also been linked to LOAD[122].

Bipolar disorders. Genetic linkage studies have implicated mutations in *DISC1* as a general risk factor for major affective disorders, including bipolar disorders[109]. As discussed above, DISC1 regulates the proliferation of adult neural progenitor cells through GSK3β–β-catenin signalling[55]. A possible association between a Wnt target gene and susceptibility to a familial bipolar disorder[110] has been described. Mutations in a member of the peroxisome proliferator-activated receptor (PPAR) family, PPARδ, have been associated with bipolar disorders. As PPARδ is expressed at high levels in the murine entorhinal cortex and hippocampus, as well as in the corpus callosum, and as its agonists are neuroprotective in several rat models of stroke and neurodegenerative disease[109], it is an interesting candidate for further studies.

Progressive myoclonus epilepsy-ataxia syndrome. Several forms of progressive myoclonus epilepsy have been described. This syndrome is characterized by myoclonic seizures, generalized convulsive seizures, ataxia and dementia. Recent studies have shown that a mutation in PRICKLE1, a protein that is part of the non-canonical Wnt–PCP signalling pathway, disrupts its interaction with RE1-silencing transcription factor *in vitro* and alters its normal function in an *in vivo* zebrafish overexpression system[111]. This protein is expressed in brain regions implicated in epilepsy and ataxia in mice and humans, and is the first molecule in a non-canonical Wnt signalling pathway to be directly implicated in human epilepsy[112].

Retinitis pigmentosa. Elevated levels of sFRP-1 have been reported in the retinas of patients affected by retinitis pigmentosa, an inherited disease characterized by the progressive loss of photoreceptors[113]. Abnormal expression of sFRPs and other components of the Wnt signalling pathways has been detected in several mouse models of the disease[113], supporting the possibility that alterations in the Wnt signalling pathway are involved in the progression of photoreceptor degeneration. Alternatively, elevated sFRP expression might represent an attempt by the tissue to promote the generation of photoreceptors, as seen during the development of the chick retina[114].

Bardet–Biedl syndrome. Bardet–Biedl syndrome is a rare pleiotropic disorder characterized by a multitude of symptoms, including retinal degeneration, obesity and nephropathy. The orientation of cochlear cells in the inner ear was found to be determined by Wnt signalling through the Wnt–PCP pathway. Mice with mutations in genes related to Bardet–Biedl syndrome and mice with Wnt–PCP pathway mutations have some common phenotypes, including open eyelids, neural tube defects and disrupted cochlear stereociliary bundles[115].

Conclusions and future directions

Although significant progress has already been made in deciphering the roles of Wnt signalling during neural development, little is known about the roles of Wnts in the adult nervous system. As we have discussed throughout this Review, evidence indicates that Wnt pathways modulate fundamental aspects of the adult CNS, such as adult neurogenesis and synaptic stability and plasticity in some brain regions. However, much remains to be investigated with regard to other fundamental biological functions and the importance of Wnt signalling in other regions of the adult brain. The use of genetically modified animals and *in vivo* imaging with two-photon microscopy will contribute to developments in this area. Moreover, advanced imaging techniques, such as stimulated emission depletion (STED) far-field fluorescence nanoscopy[116] and techniques that allow the functional assessment of genetically labelled neural circuits in freely moving animals, such as optogenetics combined with solid-state optics[117], are likely to lead to significant advances in this field.

The emerging idea that Wnts are synaptogenic trophic factors should be investigated further. At a physiological level, it would be of great interest to understand whether Wnts are implicated in the effects of exercise, sensory deprivation and ageing on the CNS. More complex endeavours, like determining whether Wnts are involved in the processing and coding of information at the higher levels of the cerebral cortex, are also worth attempting.

The known functions of Wnt signalling in the adult brain suggest that disruptions to or impairments of the Wnt pathways could have dramatic consequences. Indeed, growing evidence implicates Wnt signalling in the pathogenesis of several neurodegenerative and neurological diseases. In the future it will therefore be important to further explore the molecular mechanisms that link these pathways to disease, in particular to the pathogenesis of AD and PD.

The emerging roles of Wnts in adult neurogenesis, neuronal differentiation, synaptogenesis and survival suggest that targeting Wnt signalling pathways could offer therapeutic benefits. Drugs capable of modulating Wnt signalling may become tools for regenerative or neuroprotective medicine, for example against diseases associated with neuron loss.

1. Moon, R. T., Kohn, A. D., De Ferrari, G. V. & Kaykas, A. WNT and β-catenin signalling: diseases and therapies. *Nature Rev. Genet.* **5**, 691–701 (2004).
A comprehensive review that summarizes disease-associated alterations in Wnt signalling and Wnt signalling components, suggesting that modulation of the Wnt pathways could be a useful therapeutic strategy.
2. Ciani, L. & Salinas, P. C. WNTs in the vertebrate nervous system: from patterning to neuronal connectivity. *Nature Rev. Neurosci.* **6**, 351–362 (2005).
3. Gordon, M. D. & Nusse, R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *J. Biol. Chem.* **281**, 22429–22433 (2006).
4. Toledo, E. M., Colombres, M. & Inestrosa, N. C. Wnt signaling in neuroprotection and stem cell differentiation. *Prog. Neurobiol.* **86**, 281–296 (2008).
5. Takashima, A., Noguchi, K., Sato, K., Hoshino, T. & Imahori, K. Tau protein kinase I is essential for amyloid β-protein-induced neurotoxicity. *Proc. Natl Acad. Sci. USA* **90**, 7789–7793 (1993).
6. Zhang, Z. *et al.* Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. *Nature* **395**, 698–702 (1998).
7. Chacon, M. A., Varela-Nallar, L. & Inestrosa, N. C. Frizzled-1 is involved in the neuroprotective effect of Wnt3a against Aβ oligomers. *J. Cell Physiol.* **217**, 215–227 (2008).
8. Shimogori, T., VanSant, J., Paik, E. & Grove, E. A. Members of the Wnt, Fz, and Frp gene families expressed in postnatal mouse cerebral cortex. *J. Comp. Neurol.* **473**, 496–510 (2004).
9. Cerpa, W. *et al.* WNT-7a modulates the synaptic vesicle cycle and synaptic transmission in hippocampal neurons. *J. Biol. Chem.* **283**, 5918–5927 (2008).
Electrophysiological analysis of adult rat hippocampal slices indicates that WNT7A increases neurotransmitter release in CA3–CA1 synapses, suggesting that Wnt signalling modulates presynaptic function.
10. Salinas, P. C. & Zou, Y. Wnt signaling in neural circuit assembly. *Annu. Rev. Neurosci.* **31**, 339–358 (2008).
11. Arikkath, J. & Reichardt, L. F. Cadherins and catenins at synapses: roles in synaptogenesis and synaptic plasticity. *Trends Neurosci.* **31**, 487–494 (2008).
12. Li, G. & Pleasure, S. J. Morphogenesis of the dentate gyrus: what we are learning from mouse mutants. *Dev. Neurosci.* **27**, 93–99 (2005).
13. Chen, J., Park, C. S. & Tang, S.-J. Activity-dependent synaptic Wnt release regulates hippocampal long term potentiation. *J. Biol. Chem.* **281**, 11910–11916 (2006).
This paper shows that Wnt signalling modulates long-term potentiation, revealing a role in synaptic plasticity.
14. Wayman, G. A. *et al.* Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron* **50**, 897–909 (2006).
15. Abe, K. & Takeichi, M. NMDA-receptor activation induces calpain-mediated β-catenin cleavages for triggering gene expression. *Neuron* **53**, 387–397 (2007).
16. Stranahan, A. M. *et al.* Hippocampal gene expression patterns underlying the enhancement of memory by running in aged mice. *Neurobiol. Aging* 11 Dec 2008 (doi:10.1016/j.neurobiolaging.2008.10.016).
17. Peineau, S. *et al.* The role of GSK-3 in synaptic plasticity. *Br. J. Pharmacol.* **153** (Suppl. 1), S428–S437 (2008).
18. Hernandez, F., Borrell, J., Guaza, C., Avila, J. & Lucas, J. J. Spatial learning deficit in transgenic mice that conditionally over-express GSK-3β in the brain but do not form tau filaments. *J. Neurochem.* **83**, 1529–1533 (2002).
19. Hooper, C. *et al.* Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. *Eur. J. Neurosci.* **25**, 81–86 (2007).
20. Peineau, S. *et al.* LTP inhibits LTD in the hippocampus via regulation of GSK3β. *Neuron* **53**, 703–717 (2007).
21. Gogolla, N., Galimberti, I., Deguchi, Y. & Caroni, P. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. *Neuron* **62**, 510–525 (2009).
This paper demonstrates that behavioural experience specifically regulates adult global stratum lucidum synapse numbers and hippocampal network structure through Wnt signalling.
22. Packard, M. *et al.* The *Drosophila* Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. *Cell* **111**, 319–330 (2002).
23. Speese, S. D. & Budnik, V. Wnts: up-and-coming at the synapse. *Trends Neurosci.* **30**, 268–275 (2007).
24. Farias, G. G. *et al.* Wnt-7a induces presynaptic colocalization of α 7-nicotinic acetylcholine receptors and adenomatous polyposis coli in hippocampal neurons. *J. Neurosci.* **27**, 5313–5325 (2007).
25. Brakeman, J. S., Gu, S. H., Wang, X. B., Dolin, G. & Baraban, J. M. Neuronal localization of the adenomatous polyposis coli tumor suppressor protein. *Neuroscience* **91**, 661–672 (1999).
26. Matsumine, A. *et al.* Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science* **272**, 1020–1023 (1996).
27. Ahmad-Annuar, A. *et al.* Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *J. Cell Biol.* **174**, 127–139 (2006).
This manuscript shows that Wnt–Dishevelled signalling in presynaptic terminals regulates synaptic assembly and neurotransmitter release.
28. Varela-Nallar, L., Grabowski, C. P., Alfaro, I. E., Alvarez, A. R. & Inestrosa, N. C. Role of the Wnt receptor Frizzled-1 in presynaptic differentiation and function. *Neural Dev.* **4**, 41 (2009).
29. Waites, C. L., Craig, A. M. & Garner, C. C. Mechanisms of vertebrate synaptogenesis. *Annu. Rev. Neurosci.* **28**, 251–274 (2005).
30. Bamji, S. X. *et al.* Role of β-catenin in synaptic vesicle localization and presynaptic assembly. *Neuron* **40**, 719–731 (2003).
31. Sun, Y., Aiga, M., Yoshida, E., Humbert, P. O. & Bamji, S. X. Scribble interacts with β-catenin to localize synaptic vesicles to synapses. *Mol. Biol. Cell* **20**, 3390–3400 (2009).
32. Feng, W. & Zhang, M. Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. *Nature Rev. Neurosci.* **10**, 87–99 (2009).
33. Sanes, J. R. & Lichtman, J. W. Induction, assembly, maturation and maintenance of a postsynaptic apparatus. *Nature Rev. Neurosci.* **2**, 791–805 (2001).
34. Henriquez, J. P. *et al.* Wnt signaling promotes AChR aggregation at the neuromuscular synapse in collaboration with agrin. *Proc. Natl Acad. Sci. USA* **105**, 18812–18817 (2008).
35. Farias, G. G. *et al.* WNT-5a/ JNK signaling promotes the clustering of PSD-95 in hippocampal neurons. *J. Biol. Chem.* **284**, 15857–15866 (2009).
This paper shows that there is a postsynaptic role for WNT5A in directing the clustering of the scaffold protein PSD95 through the non-canonical Wnt–JNK signalling pathway.
36. Cerpa, W., Farías, G. G., Fuenzalida, M., Bonansco, C. & Inestrosa, N. C. Wnt-5a occludes Aβ oligomer-induced depression of glutamatergic transmision in CA1 pyramidal neurons from hippocampal slices. *Mol. Neurodegener.* (in the press).
37. Kim, M. J. *et al.* Synaptic accumulation of PSD-95 and synaptic function regulated by phosphorylation of serine-295 of PSD-95. *Neuron* **56**, 488–502 (2007).
38. Duan, X., Kang, E., Liu, C. Y., Ming, G. L. & Song, H. Development of neural stem cell in the adult brain. *Curr. Opin. Neurobiol.* **18**, 108–115 (2008).
39. Alvarez-Buylla, A. & Lim, D. A. For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683–686 (2004).
40. Lie, D. C., Song, H., Colamarino, S. A., Ming, G. L. & Gage, F. H. Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Annu. Rev. Pharmacol. Toxicol.* **44**, 399–421 (2004).
Provides strong evidence that Wnt signalling is essential for adult hippocampal neurogenesis.
41. Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703–716 (1999).
42. Laywell, E. D., Rakic, P., Kukekov, V. G., Holland, E. C. & Steindler, D. A. Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc. Natl Acad. Sci. USA* **97**, 13883–13888 (2000).
43. Seri, B., Garcia-Verdugo, J. M., McEwen, B. S. & Alvarez-Buylla, A. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* **21**, 7153–7160 (2001).
44. Imura, T., Kornblum, H. I. & Sofroniew, M. V. The predominant neural stem cell isolated from postnatal and adult forebrain but not early embryonic forebrain expresses GFAP. *J. Neurosci.* **23**, 2824–2832 (2003).
45. Ming, G. L. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250 (2005).
46. Nusse, R. Wnt signaling and stem cell control. *Cell Res.* **18**, 523–527 (2008).
47. Chenn, A. & Walsh, C. A. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* **297**, 365–369 (2002).
48. Ding, S. *et al.* Synthetic small molecules that control stem cell fate. *Proc. Natl Acad. Sci. USA* **100**, 7632–7637 (2003).
49. Hirabayashi, Y. *et al.* The Wnt/β-catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development* **131**, 2791–2801 (2004).
50. Michaelidis, T. M. & Lie, D. C. Wnt signaling and neural stem cells: caught in the Wnt web. *Cell Tissue Res.* **331**, 193–210 (2008).
51. Lie, D. C. *et al.* Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370–1375 (2005).
52. Zhou, C.-J., Zhao, C. & Pleasure, S. J. Wnt signaling mutants have decreased dentate granule cell production and radial glial scaffolding abnormalities. *J. Neurosci.* **24**, 121–126 (2004).
53. Wexler, E. M., Paucer, A., Kornblum, H. I., Plamer, T. D. & Geschwind, D. H. Endogenous Wnt signaling maintains neural progenitor cell potency. *Stem Cells* **27**, 1130–1141 (2009).
54. Jessberger, S. *et al.* Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn. Mem.* **16**, 147–154 (2009).
55. Mao, Y. *et al.* Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3β/β-catenin signaling. *Cell* **136**, 1017–1031 (2009).
56. Kuwabara, T. *et al.* Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. *Nature Neurosci.* **12**, 1097–1105 (2009).
57. Gao, Z. *et al. Neurod1* is essential for the survival and maturation of adult-born neurons. *Nature Neurosci.* **12**, 1090–1092 (2009).
58. Yu, J. M., Kim, J. H., Song, G. S. & Jung, J. S. Increase in proliferation and differentiation of neural progenitor cells isolated from postnatal and adult mice brain by Wnt-3a and Wnt-5a. *Mol. Cell Biochem.* **288**, 17–28 (2006).
59. Adachi, K. *et al.* β-catenin signaling promotes proliferation of progenitor cells in the adult mouse subventricular zone. *Stem Cells* **25**, 2827–2836 (2007).
60. Ramirez-Amaya, V., Marrone, D. F., Gage, F. H., Worley, P. F. & Barnes, C. A. Integration of new neurons into functional neural networks. *J. Neurosci.* **26**, 12237–12241 (2006).
61. Toni, N. *et al.* Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature Neurosci.* **11**, 901–907 (2008).
62. Ooto, S. *et al.* Potential for neural regeneration after neurotoxic injury in the adult mammalian retina. *Proc. Natl Acad. Sci. USA* **101**, 13654–13659 (2004).
63. Das, A. V. *et al.* Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev. Biol.* **299**, 283–302 (2006).
64. Osakada, F. *et al.* Wnt signaling promotes regeneration in the retina of adult mammals. *J. Neurosci.* **27**, 4210–4219 (2007).
65. Carlen, M. *et al.* Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. *Nature Neurosci.* **12**, 259–267 (2009).
66. Lees, A. J., Hardy, J. & Revesz, T. Parkinson's disease. *Lancet* **373**, 2055–2066 (2009).
67. Deierborg, T., Soulet, D., Roybon, L., Hall, V. & Brundin, P. Emerging restorative treatments for Parkinson's disease. *Prog. Neurobiol.* **85**, 407–432 (2008).
68. Zhao, M. *et al.* Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **100**, 7925–7930 (2003).
69. Lie, D. C. *et al.* The adult substantia nigra contains progenitor cells with neurogenic potential. *J. Neurosci.* **22**, 6639–6649 (2002).
70. Frielingsdorf, H., Schwarz, K., Brundin, P. & Mohapel, P. No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **101**, 10177–10182 (2004).

71. Castelo-Branco, G. *et al.* Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. *Proc. Natl Acad. Sci. USA* **100**, 12747–12752 (2003).
72. Andersson, E. R. *et al.* Wnt5a regulates ventral midbrain morphogenesis and the development of A9-A10 dopaminergic cells *in vivo*. *PLoS ONE* **3**, e3517 (2008).
Describes WNT5A regulation of Wnt–PCP signalling in the DA neuron lineage and provides evidence for multiple functions of WNT5A in the ventral midbrain *in vivo*.
73. McMahon, A. P. & Bradley, A. The *Wnt*-1 (*int*-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* **62**, 1073–1085 (1990).
74. Thomas, K. R. & Capecchi, M. R. Targeted disruption of the murine *int-1* proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* **346**, 847–850 (1990).
75. Prakash, N. *et al.* A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors *in vivo*. *Development* **133**, 89–98 (2006).
76. Panhuysen, M. *et al.* Effects of Wnt1 signaling on proliferation in the developing mid-/hindbrain region. *Mol. Cell Neurosci.* **26**, 101–111 (2004).
77. Joksimovic, M. *et al.* Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. *Nature Neurosci.* **12**, 125–131 (2009).
78. Tang, M., Miyamoto, Y. & Huang, E. J. Multiple roles of β-catenin in controlling the neurogenic niche for midbrain dopamine neurons. *Development* **136**, 2027–2038 (2009).
79. Rawal, N. *et al.* Dynamic temporal and cell type-specific expression of Wnt signaling components in the developing midbrain. *Exp. Cell Res.* **312**, 1626–1636 (2006).
80. Fischer, T., Guimera, J., Wurst, W. & Prakash, N. Distinct but redundant expression of the Frizzled Wnt receptor genes at signaling centers of the developing mouse brain. *Neuroscience* **147**, 693–711 (2007).
81. Castelo-Branco, G., Rawal, N. & Arenas, E. GSK-3β inhibition/β-catenin stabilization in ventral midbrain precursors increases differentiation into dopamine neurons. *J. Cell Sci.* **117**, 5731–5737 (2004).
82. Parish, C. L. *et al.* Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in Parkinsonian mice. *J. Clin. Invest.* **118**, 149–160 (2008).
83. Heins, N. *et al.* Glial cells generate neurons: the role of the transcription factor Pax6. *Nature Neurosci.* **5**, 308–315 (2002).
84. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006).
85. He, T. C. *et al.* Identification of c-MYC as a target of the APC pathway. *Science* **281**, 1509–1512 (1998).
86. Chien, A. J., Conrad, W. H. & Moon, R. T. A Wnt survival guide: from flies to human disease. *J. Invest. Dermatol.* **129**, 1614–1627 (2009).
87. Kawano, Y. & Kypta, R. Secreted antagonists of the Wnt signalling pathway. *J. Cell Sci.* **116**, 2627–2634 (2003).
88. Polakis, P. The many ways of Wnt in cancer. *Curr. Opin. Genet. Dev.* **17**, 45–51 (2007).
89. Lesage, S. & Brice, A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum. Mol. Genet.* **18**, R48–R59 (2009).
90. Rawal, N. *et al.* Parkin protects dopaminergic neurons from excessive Wnt/β-catenin signaling. *Biochem. Biophys. Res. Commun.* **388**, 473–478 (2009).
Demonstrates a novel regulation of Wnt signalling by parkin and suggests that parkin protects DA neurons against excessive Wnt signalling and β-catenin-induced cell death.
91. He, P. & Shen, Y. Interruption of β-catenin signaling reduces neurogenesis in Alzheimer's disease. *J. Neurosci.* **29**, 6545– 6557 (2009).
92. De Ferrari, G. V. & Inestrosa, N. C. Wnt signaling function in Alzheimer's disease. *Brain Res. Brain Res. Rev.* **33**, 1–12 (2000).
93. De Ferrari, G. V. *et al.* Activation of Wnt signaling rescues neurodegeneration and behavioral impairments induced by β-amyloid fibrils. *Mol. Psychiatry* **8**, 195–208 (2003).
This manuscript revealed that Aβ-dependent neurotoxicity induces a loss of function of Wnt signalling components and revealed that activation of this signalling pathway may be used for treating patients with AD.
94. Mudher, A. & Lovestone, S. Alzheimer's disease-do tauists and baptists finally shake hands? *Trends Neurosci.* **25**, 22–26 (2002).
95. Caricasole, A. *et al.* The Wnt pathway, cell-cycle activation and β-amyloid: novel therapeutic strategies in Alzheimer's disease? *Trends Pharmacol. Sci.* **24**, 233–238 (2003).
96. Small, S. A. & Duff, K. Linking Aβ and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron* **60**, 534–542 (2008).
97. Boonen, R. A., van Tijn, P. & Zivkovic, D. Wnt signaling in Alzheimer's disease: up or down, that is the question. *Ageing Res. Rev.* **8**, 71–82 (2009).
98. Magdesian, M. H. *et al.* Amyloid-β binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/β-catenin signaling. *J. Biol. Chem.* **283**, 9359–9368 (2008).
99. Bu, G. Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. *Nature Rev. Neurosci.* **10**, 333–344 (2009).
100. Caruso, A. *et al.* Inhibition of the canonical Wnt signaling pathway by apolipoprotein E4 in PC12 cells. *J. Neurochem.* **98**, 364–371 (2006).
101. Inestrosa, N. C. & Toledo, E. M. The role of Wnt signaling in neuronal dysfunction in Alzheimer's disease. *Mol. Neurodegener.* **3**, 9 (2008).
102. Farias, G. G. *et al.* M1 muscarinic receptor activation protects neurons from β-amyloid toxicity. A role for Wnt signaling pathway. *Neurobiol. Dis.* **17**, 337–348 (2004).
103. Nunes, P. V., Forlenza, O. V. & Gattaz, W. F. Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. *Br. J. Psychiatry* **190**, 359–360 (2007).
104. Toledo, E. M. & Inestrosa, N. C. Activation of Wnt signaling by lithium and rosiglitazone reduced spatial memory impairment and neurodegeneration in brains of APPswe/PSEN1ΔE9 mouse model of Alzheimer's disease. *Mol. Psychiatry* 21 Jul 2009 (doi:10.1038/mp.2009.72).
105. Lacor, P. N. *et al.* Aβ oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J. Neurosci.* **27**, 796–807 (2007).
106. Shankar, G. M. *et al.* Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Med.* **14**, 837–842 (2008).
107. De Ferrari, G. V. & Moon, R. T. The ups and downs of Wnt signaling in prevalent neurological disorders. *Oncogene* **25**, 7545–7553 (2006).
108. Lovestone, S., Killick, R., Di Forti, M. & Murray, R. Schizophrenia as a GSK-3 dysregulation disorder. *Trends Neurosci.* **30**, 142–149 (2007).
109. Chubb, J. E., Bradshaw, N. J., Soares, D. C., Porteous, D. J. & Millar, J. K. The DISC locus in psychiatric illness. *Mol. Psychiatry* **13**, 36–64 (2008).
110. Zandi, P. P. *et al.* Association study of Wnt signaling pathway genes in bipolar disorder. *Arch. Gen. Psychiatry* **65**, 785–793 (2008).
111. Veeman, M. T., Slusarski, D. C., Kaykas, A., Louie, S. H. & Moon, R. T. Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr. Biol.* **13**, 680–685 (2003).
112. Bassuk, A. G. *et al.* A homozygous mutation in human PRICKLE1 causes an autosomal-recessive progressive myoclonus epilepsy-ataxia syndrome. *Am. J. Hum. Genet.* **83**, 572–581 (2008).
113. Hackam, A. S. *et al.* Identification of gene expression changes associated with the progression of retinal degeneration in the rd1 mouse. *Invest. Ophthalmol. Vis. Sci.* **45**, 2929–2942 (2004).
114. Esteve, P., Trousse, F., Rodriguez, J. & Bovolenta, P. SFRP1 modulates retina cell differentiation through a β-catenin-independent mechanism. *J. Cell Sci.* **116**, 2471–2481 (2003).
115. Ross, A. J. *et al.* Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nature Genet.* **37**, 1135–1140 (2005).
116. Eggeling, C. *et al.* Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature* **457**, 1159–1162 (2009).
117. Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M. & Deisseroth, K. Optical deconstruction of parkinsonian neural circuitry. *Science* **324**, 354–359 (2009).
118. De Ferrari, G. V. *et al.* Common genetic variation within the low-density lipoprotein receptor-related protein 6 and late-onset Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **104**, 9434–9439 (2007).
119. Pei, J. J. *et al.* Distribution of active glycogen synthase kinase 3β (GSK-3β) in brains staged for Alzheimer disease neurofibrillary changes. *J. Neuropathol. Exp. Neurol.* **58**, 1010–1019 (1999).
120. Lucas, J. J. *et al.* Decreased nuclear β-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3β conditional transgenic mice. *EMBO J.* **20**, 27–39 (2001).
121. Schaffer, B. A. *et al.* Association of *GSK3B* with Alzheimer disease and frontotemporal dementia. *Arch. Neurol.* **65**, 1368–1374 (2008).
122. Bertram, L. *et al.* Is α-T catenin (*VR22*) an Alzheimer's disease risk gene? *J. Med. Genet.* **44**, e63 (2007).
123. Fournier, M. V., Fata, J. E., Martin, K. J., Yaswen, P. & Bissell, M. J. Interaction of E-cadherin and PTEN regulates morphogenesis and growth arrest in human mammary epithelial cells. *Cancer Res.* **69**, 4545–4552 (2009).
124. Xiong, H. *et al.* Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *J. Clin. Invest.* **119**, 650–660 (2009).
125. Chitalia, V. C. *et al.* Jade-1 inhibits Wnt signalling by ubiquitylating β-catenin and mediates Wnt pathway inhibition by pVHL. *Nature Cell Biol.* **10**, 1208–1216 (2008).
126. Matsuzawa, S. I. & Reed, J. C. Siah-1, SIP, and Ebi collaborate in a novel pathway for β-catenin degradation linked to p53 responses. *Mol. Cell* **7**, 915–926 (2001).
127. Kitagawa, M. *et al.* An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of β-catenin. *EMBO J.* **18**, 2401–2410 (1999).
128. Brembeck, F. H., Rosario, M. & Birchmeier, W. Balancing cell adhesion and Wnt signaling, the key role of β-catenin. *Curr. Opin. Genet. Dev.* **16**, 51–59 (2006).
129. Venderova, K. *et al.* Leucine-rich repeat kinase interacts with Parkin, DJ-1 and PINK-1 in a *Drosophila melanogaster* model of Parkinson's disease. *Hum. Mol. Genet.* **18**, 4390–4404 (2009).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
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