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REVIEW

Canonical and noncanonical Wnt signaling in neural stem/progenitor cells

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Abstract The first mammalian Wnt to be discovered, Wnt-1, was found to be essential for the development of a large part of the mouse brain over 25 years ago. We have since learned that Wnt family secreted glycolipoproteins, of which there are nineteen, which activate a diverse network of signals that are particularly important during embryonic development and tissue regeneration. Wnt signals in the developing and adult brain can drive neural stem cell self-renewal, expansion, asymmetric cell division, maturation and differentiation. The molecular events taking place after a Wnt binds to its cell-surface receptors are complex and, at times, controversial. A deeper understanding of these events is anticipated to lead to improvements in the treatment of neurodegenerative diseases and stem cell-based replacement therapies. Here, we review the roles played by Wnts in neural stem cells in the developing mouse brain, at neurogenic sites of the adult mouse and in neural stem cell culture models.

Keywords Wnt signaling · Neural stem cells · Beta-catenin · AP-1 family transcription factors

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Wnt signaling

Wnt proteins play roles in many cellular and physiological processes, regulating cell proliferation, differentiation, migration and patterning during embryonic development and tissue homeostasis in the adult [1, 2]. The Wnt family in mammals comprises nineteen secreted glycolipoproteins that are able to bind to a wide variety of receptors and elicit a number of different responses in the cell [3]. These have classically been divided into canonical (β-catenin-dependent) and noncanonical (β-catenin-independent) Wnt signaling pathways.

Canonical Wnt signaling

In the canonical Wnt signaling pathway, β-catenin is actively degraded by a protein complex that includes Axin, glycogen synthase kinase-3 (GSK-3), casein kinase 1 (CK1) and adenomatous polyposis coli (APC). In the classical pathway, binding of a Wnt protein to receptors of the frizzled (FZD) and low-density lipoprotein receptor-related protein (LRP5/6) families leads to membrane recruitment of Axin and disheveled (DVL), thereby disrupting the function of the degradation complex. β-catenin is thus stabilized, enters the nucleus and activates genes in association with T cell factor/lymphoid enhancer factor-1 (TCF/LEF) family transcription factors [1]. However, a more recent version of the model (Fig. 1) posits that the destruction complex is not disrupted by Wnt activation and that changes in the levels of free and transcriptionally active β-catenin result from relocation of the complex to the membrane, which disrupts β-catenin ubiquitination rather than the complex itself, leading to β-catenin accumulation [4]. Other extracellular ligands have been shown to alter the output of the pathway. Members of the

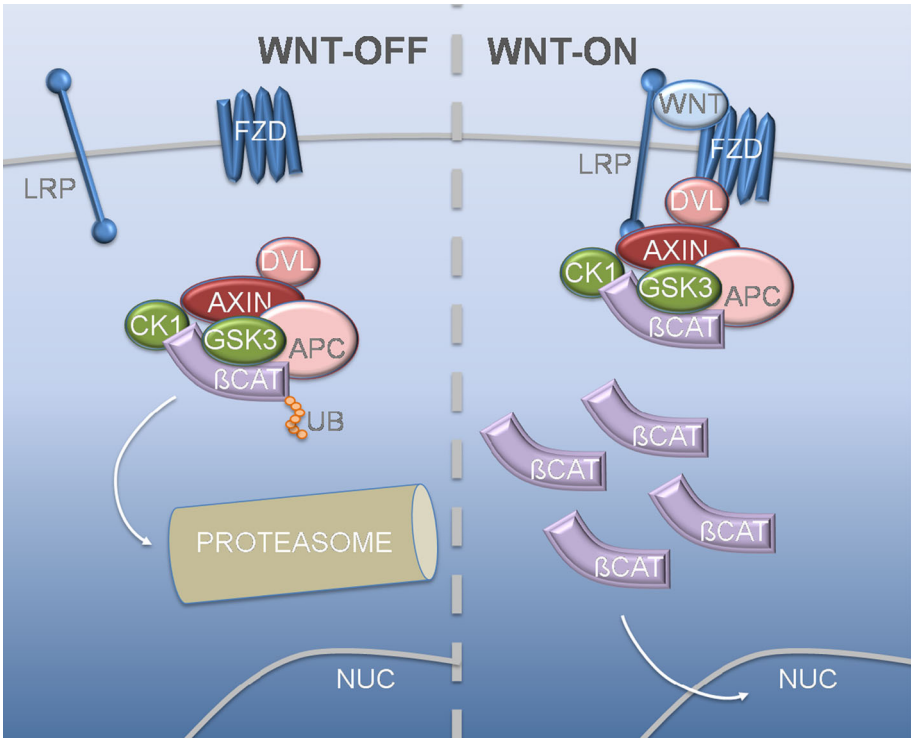

Fig. 1 Representation of canonical Wnt signaling, according to Li et al. [4]. Wnt binds to FZD receptors and LRP5/6 co-receptors, recruiting the destruction complex to the membrane, thereby preventing β-catenin (β-CAT) ubiquitination, leading to its accumulation and entry into the nucleus. *UB* ubiquitin, *NUC* nucleus

Dickkopf and sFRP families, and WIF1 and Cerberus can bind to Wnt receptors or Wnt ligands inhibiting or, in some instances, enhancing their effects [5, 6]. In addition, R-spondins (RSPOs) modulate the Wnt response at the cell membrane by binding to leucine-rich repeat containing G protein-coupled receptors (LGRs) [3].

The canonical pathway is implicated in many human diseases [2]. Perturbations in the levels of Axin, APC, β-catenin, LEF1 or TCF4, for example, contribute to the initiation and/or progression of several different types of cancer [7–12]. It is therefore not surprising that so much effort has gone into the development of new drugs based on our knowledge of Wnt signaling to treat disease. Among the commercially available drugs that have been developed to manipulate Wnt signaling are IWP-2 and Wnt-C59, potent porcupine inhibitors that block Wnt secretion [13, 14], IWR-1 and XAV939, which are tankyrase inhibitors that stabilize Axin and thereby inhibiting canonical Wnt signaling [13, 15], CHIR99021 (CHIR), a GSK-3 inhibitor that activates Wnt signaling [16], and iCRT-14, which inhibits the β-catenin/TCF complex [17]. These inhibitors, as well as several others not mentioned here, have been important for driving progress of research in this field, and the development of possible therapies for Wnt-related diseases.

Noncanonical Wnt signaling

Noncanonical Wnt signals (Fig. 2) regulate DVL and other intracellular proteins to activate the planar cell polarity (PCP) pathway, the Wnt-calcium (Ca^{2+}) pathway and other β-catenin/TCF-independent events [18]. In the Wnt-PCP pathway, FZD receptors activate a signaling cascade that involves the small GTPases Rho and Rac and c-Jun N-terminal kinase (JNK), leading to changes in the cytoskeleton and activation of activator protein-1 (AP-1) family transcription factors [19]. Noncanonical Wnt stimuli induce association of DVL-associated activator of morphogenesis (DAAM) proteins with FZD, DVL and GTP-bound Rho, which is then able to activate Rho-associated, coiled-coil containing protein kinase (ROCK) and even JNK. Although JNK is generally associated with phosphorylation and activation of c-Jun, it phosphorylates many other proteins, including activating transcription factor 2 (ATF2) and cyclic AMP response element-binding protein (CREB), which heterodimerize with c-Jun and other AP-1 family members to alter gene expression [20]. This pathway regulates cell polarity in several morphogenetic processes in vertebrates, including gastrulation, neural tube closure and orientation of stereocilia in the inner ear [19].

In the Wnt-Ca^{2+} pathway, Wnt binding to FZD receptors activates DVL, leading to activation of phospholipase C (PLC), producing 1,2 diacylglycerol (DAG), which activates protein kinase C (PKC), and inositol 1,4,5-triphosphate (IP3), which activates calcium release from the endoplasmic reticulum. Other events such as activation of ROCK have also been linked to this pathway [21]. Calcium release activates calcineurin (CNA) and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), which, respectively,

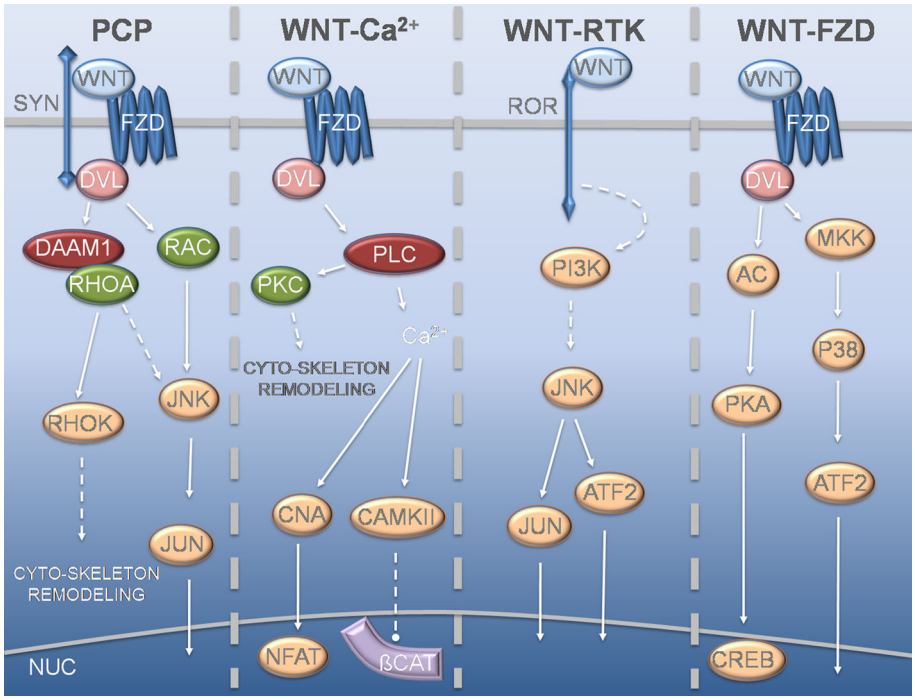

Fig. 2 Schematic representation of noncanonical Wnt signaling. Noncanonical Wnt signals activate several different pathways through distinct intracellular effectors. In the PCP pathway, Wnt activates Rho kinase (ROCK) and JNK, thereby eliciting changes in gene expression and remodeling of the cytoskeleton. In the Wnt-calcium pathway, DVL activation triggers activation of protein kinase C (PKC) and Ca^{2+} release. This then activates nuclear factor of activated T cells (NFAT)-dependent gene expression, with concomitant inhibition of β-CAT. Receptor tyrosine kinases (RTKs) activate phosphoinositide-3 kinase (PI3K), which can result in JNK activation. FZD receptors can activate several intracellular effectors, including protein kinase A (PKA) and p38 kinases. *SYN* Syndecan, *DAAM1* disheveled associated activator of morphogenesis 1, *CNA* calcineurin, *CAMKII* calmodulin kinase II, *PLC* phospholipase C, *AC* adenylate cyclase, *MKK* mitogen-activated protein kinase kinase

increase expression of nuclear factor of activated T cells (NFAT)-dependent genes and inhibit canonical Wnt signaling through nemo-like kinase (NLK) [22]. This pathway is involved in cancer, inflammation and neurodegeneration [3, 23].

Even at the cell surface, noncanonical Wnt signaling is highly variable and complex, with Wnt ligands interacting not only with FZD but also with numerous other receptors, including receptor tyrosine kinase-like orphan receptor (ROR1/2), receptor-like tyrosine kinase (RYK), protein tyrosine kinase 7 (PTK7) and van gogh-like (VANGL1/2) [24]. Adding to this complexity, the Wnt response can differ depending on cell context and on the repertoire of Wnt receptors expressed [25, 26], further underlining the importance of determining the "Wnt status" of cells. ROR2, for example, has been shown to activate phosphatidylinositol-3 kinase (PI3 K), which in turn activates JNK and its associated transcription factors c-Jun and ATF2 in *Xenopus* [27, 28]. Similarly, ROR1 activates the same signaling cascade to increase CREB phosphorylation in human breast cancer cells [29]. FZDs have been found to activate a number of additional intracellular effectors, including adenylate cyclase (AC), protein kinase A (PKA) and CREB [19], p38 and ATF2 [20] and Fyn and STAT3 [21]. In addition, a new β-catenin-independent aspect of Wnt signaling was recently reported in proliferating cells: Wnt signaling was found to peak at the G2/M phase of the cell cycle to produce so-called Wnt-dependent stabilization of proteins (Wnt/STOP) [30]. This appears to be a dominant mode of Wnt signaling in several cancer cell lines, where it is required for cell growth.

Wnts and their receptors

Although several Wnts preferentially activate either β-catenin-dependent (Wnt-1/3a) or β-catenin-independent (Wnt-5a/11) pathways, the activity of many Wnts is influenced by cellular context and the receptors available (LRPs versus RORs, for example) [31]. Traditionally, FZD-LRP receptor–co-receptor combinations have been considered to be canonical, while RORs, RYK, PTK7 and VANGLs alone or in combination with FZDs have been associated with noncanonical Wnt signaling [31]. However, there are more than 15 different Wnt receptors and co-receptors, and the particular combination of these, together with a given Wnt, can affect subsequent signaling

events [3]. Thus, Wnts and their receptors cannot be rigorously subdivided according to the pathway they induce in standard cell culture models.

Wnt proteins also compete with one another to give rise to different effects. Wnt-5a, for example, preferentially activates PCP signaling and competes with Wnt-3a for binding to FZD2, thereby suppressing the β-catenin-dependent pathway [32]. An explanation for this promiscuity came from structural studies: the crystal structure of a complex between *Xenopus* Wnt-8 and the FZD8 cysteine-rich domain (CRD) reveals that the CRD directly binds Wnt-8 at two sites, one is the amino-terminal palmitoleic lipid that is present in all Wnt proteins, and the other is a conserved hydrophobic region in the Wnt carboxyl terminal domain. Although the palmitoleic anchorage site is also found in other FZD receptors, the second anchorage point is variable in sequence. As a result, different Wnt proteins may preferentially bind different FZD receptors [33]. Thus, it becomes clear that a Wnt protein has the potential to elicit many different cellular responses dictated by the availability of a panoply of receptors and intracellular effectors.

Neural stem cells and their differentiation

Neuronal differentiation is the process that neural stem cells (NSCs) undergo to become neurons. This process has been extensively studied both in developmental biology and stem cell biology. During the development of the nervous system, primitive cells act as a source of various types of specialized cells that make up the functioning brain. In addition, NSCs are important for adult neurogenesis, a process that, in mammals, takes place in the subventricular zone (SVZ) and in the subgranular zone (SGZ) of the hippocampus [34]. Although embryonic neurogenesis is likely to be less restricted anatomically, the maintenance, proliferation and neuronal fate commitment of local stem cell populations is regulated by signals from the microenvironment both in adults and embryos [35]. While much effort has been devoted to understanding the development of the central nervous system (CNS) in both the adult and embryonic settings, our understanding of the signals regulating differentiation remains incomplete. Many models have been developed to study these signaling cues. Embryonic stem (ES) cells, induced pluripotent stem (iPS) cells and even dental pulp stem cells [36] are being used to recapitulate differentiation. Those efforts serve not only to increase our understanding of the processes involved, but also may lead to new therapeutic applications. Cortical development has been extensively studied in the mouse embryo. In addition, adult neurogenesis, which was only accepted a decade ago [37], also provides a fantastic neurogenic model. The most important findings related to Wnt ligands and receptors in these models are summarized in Tables 1 and 2, respectively, but we will nevertheless discuss the findings of each model individually.

Wnt signaling in the mouse brain

All neurons of the mammalian neocortex ultimately originate from neuroepithelial cells (NECs), which are the cells that initially form the columnar monolayer epithelium, constituting the neural plate, and, subsequently, the pseudostratified epithelium that constitutes the early neural tube [38]. NECs initially undergo symmetric proliferative divisions to expand the population. After this initial amplification, cortical neurogenesis begins with single NECs switching to asymmetric differentiative cell division [39]. The asymmetric daughter cells then either continue dividing as apical or basal progenitors or further undergo differentiation to become postmitotic neurons [38].

Through these symmetric and asymmetric expansions and differentiation, the cortex is shaped into a structure with a wide variety of neurons and glia, with highly stereotypical laminar arrangements and unique patterns of connectivity and function [40]. However, this is only a simplistic summary of a very complex process, of which only the stem cell aspect will be discussed here (see [41] for more details on developmental aspects).

Wnt signaling in the mouse brain: Wnt ligands and receptors

There is no general consensus on the roles played by Wnt proteins during neurogenesis in the mouse brain, with different studies reporting different roles for the same Wnt ligand. In the nervous system, Wnt-3a knockout (KO) mice exhibit under-development of the hippocampus, as a result of a reduction in proliferation [42]. In contrast to this, ectopic expression of Wnt-3a induces the differentiation of intermediate cortical progenitors during mid- and late-cortical neurogenesis [43]. These contradictory findings show that one Wnt ligand can have different outputs depending in the cellular context: given that these molecules can compete with each other, other agonists and antagonists, and that they can interact with a wide range of receptors and co-receptors, it is not surprising that they can exert such different effects on developing cortical and hippocampal precursors.

Wnt-5a KO results in defects in dopaminergic neurogenesis and neurite development [44, 45], and Wnt-7a KO impairs maturation of dopaminergic and other neuronal populations [46–48] (Table 1). While Wnt-3a has been described as canonical, Wnt-5a is generally associated with

Table 1 Wnt ligand effects on neural mammalian models

Ligand	Neural phenotype in mammalian models	References
Wnt-1 KO	Altered central and peripheral neuronal development during initial axonogenesis	[54]
Wnt-1 KO	Impaired midbrain development	[55]
Wnt-1 dominant negative	Impaired hippocampal neurogenesis and spatial and object recognition memory	[56, 57]
Wnt-1 overexpression	Reduced neural differentiation of mESCs (also by treatment with lithium chloride)	[58]
Wnt-1 KO	Increased differentiation into DA neurons in KO mESCs	[59]
Wnt-2 KO	Decreased progenitor proliferation and neurogenesis in the ventral midbrain	[60]
Wnt-2 overexpression	Induced dendritic arborization in hippocampal progenitors	[61]
Wnt-3 overexpression	Increased differentiation of cortical intermediate progenitors	[43]
Wnt-3 overexpression	Induced differentiation through cleavage of RYK in cortical progenitors	[62]
Wnt-3a KO	Loss of the hippocampus	[42]
Recombinant Wnt-3a	Induced GABAergic neuronal differentiation through RYK, reduced oligodendrogenesis	[63]
Recombinant Wnt-3a	Induced differentiation of hESCs	[64]
Recombinant/purified Wnt-3a	Induced proliferation of hESCs/mNSCs	[65, 66]
Recombinant Wnt-3a	Induced proliferation and differentiation of hESCs	[67]
Wnt-4 silencing	Impaired early differentiation in hECCs	[68]
Wnt-5a KO	Impaired neurite development in the olfactory bulb (OB)	[44]
Wnt-1 and Wnt-5a DKO	Impaired neurogenesis of midbrain dopaminergic neurons	[52]
Wnt-5a KO	Impaired axon growth and guidance of dopaminergic neurons	[45]
Wnt-5a CM	Increased synaptogenesis and maturation of hippocampal progenitors	[69, 70]
Wnt-5a overexpression	Induced axonal differentiation in hippocampal cultures	[71]
Wnt-7a KO	Delayed morphological maturation of glomerular rosettes and synapsin I accumulation	[46]
Wnt-7a KO	Impaired ventral midbrain neurogenesis	[47]
Wnt-7a and Dvl DKO	Defective spine morphogenesis and mossy fiber-CA3 synaptic transmission	[48]
Wnt-7a	Proposed as a key element in the regulation of NSC self-renewal/differentiation; altered spindle-size asymmetry during corticogenesis	[72]
Recombinant Wnt-7a	Increased maturation and synaptogenesis of hippocampal progenitors	[48]
Recombinant Wnt-7b	Induced dendritic development in hippocampal progenitors	[51]
Wnt-11 overexpression	Maintenance of hEC-derived neural progenitors	[68]

DKO double-knockout, *CM* conditioned medium

noncanonical Wnt signaling [49], and Wnt-7a is frequently found to play both canonical and noncanonical roles [50, 51]. Bearing in mind that all these Wnts are required for the correct formation of the nervous system, and also that they often cooperate with one another [52], fine-tuning of canonical and noncanonical Wnt signals is likely to be necessary for neuronal development. In keeping with this, Wnt1/5a double KO (DKO) mice show exacerbated loss of dopaminergic neurons, when compared to Wnt5a KO mice, as described by Andersson et al. [52]. This elegant study also showed that mouse stem cells treated with both Wnt-3a and Wnt-5a produced more dopaminergic neurons, than cells treated with a single Wnt, providing further evidence for cooperation between noncanonical and canonical Wnts during dopaminergic differentiation, as suggested in an earlier study by the same group [53].

Canonical Wnt receptors are also important for correct neural development (Table 2): FZD3 KO mice show impaired axonal guidance [73] while LRP6 KO mice present cortical defects [74]. Also, FZD1 has been shown to be the receptor for canonical Wnt-1 in mouse tyrosine hydroxylase positive neurons, which activates β-catenin-dependent signaling promoting neuroprotection in dopaminergic neurons [75].

Knocking out noncanonical receptors such as RYK, RORs and VANGLs results in defective axonal guidance and branching and neural tube defects [76–78]. Furthermore, cleavage of RYK is required for the effects of Wnt-3 on differentiation [62], resulting in an increase in the numbers of GABAergic neurons and inhibition of oligodendrogenesis [63]. It is interesting that a receptor such as RYK is able to bind Wnt-3, which has classically been described as a canonical Wnt that enhances stemness and proliferation. This highlights the promiscuous behavior of Wnt ligands and their ability to play multiple roles that likely depends on the availability of receptors and the intracellular machinery required to transduce different signals.

Table 2 Wnt receptor effects on neural mammalian models

Receptor	Neural phenotype in mammalian models	References
FZD1	Implicated in DAergic neuron survival	[80]
	Implicated in synaptic organization	[81]
FZD2	Mediates downregulation of differentiation in mouse SVZ NSCs and gliomas by PLAGL2	[82]
FZD3	Defective neural axon guidance in KO	[73]
	Neural tube closure defect in FZD3/6 DKO	[83]
	Severe midbrain morphogenesis defects in FZD3/6 DKO	[84]
FZD4	Expressed in embryoid bodies and down-regulated upon differentiation of hESC	[85]
	Required for glioma stem cell stemness and invasion	[86]
FZD5	Expressed and down-regulated with differentiation in mouse ES cells	[87]
	Down-regulated upon neural differentiation of hESC	[88]
	Down-regulated upon differentiation of iPS cells	[89]
	FZD5 CRD promotes neuroectodermal differentiation	[90]
FZD6	Severe midbrain morphogenesis defects in FZD3/6 DKO	[84]
	Neural tube closure defect in FZD3/6 DKO	[83]
	Labels rare, highly tumorigenic stem-like cells in neuroblastoma	[91]
FZD7	Implicated in neural crest cell migration	[92]
	Required for hEC cell proliferation	[93]
	Expression under control of Klf4/TCF/SOX2	[94]
	FZD7 CRD promotes neuroectodermal differentiation	[90]
FZD8	FZD8 CRD promotes neuroectodermal differentiation	[90]
FZD9	KO mice show large increases in apoptotic cell death in the developing dentate gyrus	[95]
VANGL1	Neural tube defects in KO mice	[96]
	Neural tube defects in VANGL1/2 DKO mice	[78]
VANGL2	Neural tube defects in KO mice	[97]
	Neural tube defects in VANGL1/2 DKO mice	[78]
	Causes precocious differentiation of neural progenitors into early-born neurons	[79]
	Regulates asymmetric division in mouse SVZ	[72]
ROR1/2	Implicated in neurite extension	[98]
	Implicated in synapse formation	[99]
	Regulates differentiation in primary mouse neural progenitors	[100]
	Axon branching defect in ROR1/2 DKO mice	[77]
RYK	Axon guidance defects in KO mice	[76]
	Cleavage regulates neuronal differentiation	[62]
	Required for induction of GABAergic neurons & inhibition of oligodendrogenesis	[63]
PTK7	Implicated in neural tube closure and stereociliary bundle orientation	[101]
LRP4	Required during the earliest events in the postsynaptic neuromuscular junction	[102]
	Required for neuromuscular synapse formation	[103]
LRP6	Neural tube closure defects and mid/hindbrain deficiencies in KO mice	[102]
	Disrupted production of dentate granule neurons and radial glial scaffolding in KO mice	[104]
	Cortical defects in KO mice	[74]
	Increased differentiation into DA neurons in KO mESCs	[59]
	Delayed DA neuron differentiation in KO mice	[105]

DA dopaminergic, *CRD* cysteine-rich domain, *mESC* mouse embryonic stem cell

Finally, the noncanonical PCP pathway also plays a critical role in cortical development, since Wnt-7a and Vangl2 control spindle-size asymmetry during corticogenesis and are thus proposed to be key elements in the regulation of NSC self-renewal and differentiation [72, 79]. This is only natural if we take into account that the PCP pathway plays a very important role in asymmetry, and that asymmetric divisions are essential for stem and progenitor cells to ultimately shape the developing brain.

Wnt signaling in the mouse brain: intracellular components

Several other transgenic mouse models have revealed clues about Wnt pathway components that are important for mouse brain development, many of them centered on the role of β-catenin-dependent canonical signaling. Ectopic expression of a β-catenin/LEF1 fusion protein, for example, activates canonical Wnt signaling in the developing cortex, promoting self-renewal and delaying expression of paired box 6 (PAX6), neurogenin 2 (NGN2) and eomesodermin (Tbr2) and subsequent neurogenesis. Several other reports showed that overexpression or activation of β-catenin expands the neuronal progenitor pool in the developing brain [106, 107], and expression of constitutively active β-catenin under the control of the GFAP promoter results in enlarged ventricles and an initial expansion of the PAX6-positive ventricular zone that is subsequently lost. Loss of PAX6 expression is not followed by expression of Tbr2, indicating that differentiation is impaired [108]. In keeping with these findings, conditional ablation of β-catenin accelerates expression of the previously mentioned neurogenic genes in a different study [109], and stabilization of Axin by the tankyrase inhibitor IWR-1 (which prevents β-catenin from signaling) reduces NSC proliferation in cortical neurospheres [110]. Thus, the importance of Wnt/β-catenin signaling may lie in its role in maintaining neural stem/progenitor cell proliferation. While these studies support a role for β-catenin-dependent canonical Wnt signaling in stemness and proliferation, others support a role for β-catenin in differentiation: Hirabayashi et al. found that ectopic expression of β-catenin was able to drive the differentiation of mouse cortical progenitors, whereas the inhibition of canonical Wnt signaling prevented differentiation in the mouse neocortex [111]. This was confirmed by Munji et al., who showed that ectopic Wnt-3a activates β-catenin in the mouse neocortex and leads to the differentiation of intermediate progenitors [43]. Interestingly, while the presence of cytoplasmic Axin is associated with proliferation of cortical intermediate progenitors, its phosphorylation leads to nuclear localization and β-catenin activation, which is required for differentiation in the mouse cortex [112]. While these studies support a role for β-catenin during differentiation, a very interesting study in cerebellar precursors by Pei et al. points out that while β-catenin overexpression induces cerebellar neural progenitor cell proliferation, it does not affect granule progenitor cells of embryonic and postnatal cerebellar origin [113]. These apparently contradictory findings suggest that β-catenin, like Wnts, can play multiple roles, in this case possibly through interaction with other transcription factors. Indeed, Israsena et al. have shown that overexpression of β-catenin in the presence of basic fibroblast growth factor 2 (bFGF) activates proliferation in mouse neural stem cells, while in its absence, β-catenin drives differentiation in the same cells [114]. Since bFGF promotes neural stem cell proliferation through MAP kinase signaling [115, 116], it is possible that interactions between intracellular components of these pathways result in different outcomes.

Several recent studies have focused on another interesting aspect of neural development related to canonical Wnt signaling, namely the role of the extracellular matrix. Targeted disruption of the gene encoding the Wnt co-receptor syndecan-1, for example, reduces β-catenin signaling and proliferation in neural progenitor cells [117]. Similar results were obtained in an unrelated study using N-cadherin mutant mice, where loss of N-cadherin reduced β-catenin signaling and induced migration from the niche and differentiation [118]. An analogous situation was reported in the SVZ, where MT5-MMP was found to be the metalloproteinase that controls N-cadherin cleavage and subsequent activation of NSCs [119]. These reports highlight the potential importance of β-catenin signaling in regulating cell interactions in the stem cell niche and linking them to proliferation and stemness. Interactions with other cells or with the niche itself might also therefore influence the output of β-catenin signaling.

Disruption of noncanonical components, such as the transcription factors ATF2 and CREB, has also been associated with effects on cortical development [120, 121]. ATF2-deficient mice were shown to carry severe neurological abnormalities, with up to 50 % of neuronal loss in the cerebellum [120]. Years later, Ackermann et al. were able to produce mice with a neuronal-specific ATF2 deletion that enabled its study in the CNS. Neuron-specific inactivation of ATF2 led to a significant loss of motor neurons in the brainstem; these developed normally but were unable to survive undergoing apoptosis. In this study, it was proposed that ATF2 is required for correct motor neuron differentiation, and that it might achieve this by limiting the activity of stress kinases [122]. DKO mice for CREB and cyclic AMP response element modulatory protein (CREM) also show extensive neuronal loss as a result of increased apoptosis during neuronal development [121]. Together, these findings support a role for AP-1 family members during neuronal maturation.

Wnt signaling and adult neurogenesis

The discovery of neural stem/progenitor cells in the subventricular (SV) and subgranular (SG) zones of the adult CNS has changed our view of the brain from a static tissue to one that is dynamic and adaptive. Again, a complex process involving asymmetric division, expansion and differentiation of neural progenitors is necessary for correct

hippocampal function [123], and so, as in the developing mouse brain, many studies have centered on studying the role of Wnt signaling in the hippocampus.

Wnt signaling and adult neurogenesis: Wnt ligands and receptors

The phenotype of Wnt-3a mutant mice (Table 1) highlights the essential role of Wnt signaling in the growth of the hippocampus. Wnt proteins secreted by hippocampal astrocytes promote proliferation in the hilus below the SGZ, a property that is lost in aging mice [56, 124]. Further evidence that Wnt-mediated neurogenesis contributes to adult hippocampal function comes from studies in which lentiviral expression of a dominant-negative form of Wnt-1 (dnWnt-1) was found to reduce neurogenesis, resulting in impaired long-term retention of spatial and object recognition memory [56, 57]. Lie et al. reported that overexpression of Wnt3 is sufficient to induce differentiation from adult hippocampal progenitors in vitro and in vivo [56]. By contrast, blockade of canonical Wnt signaling using dnWnt-1 reduces differentiation in vitro and abolishes neurogenesis almost completely in vivo. These examples provide evidence that canonical Wnt ligands are essential for adult neurogenesis.

Noncanonical Wnt signaling has also been studied in hippocampal mouse models. In cultured hippocampal neurons, Wnt-5a activates a signaling cascade leading to activation of AP-1 [69] and increases dendritic spine morphogenesis [70]. Wnt-7a similarly increases dendritic spine density and maturity, albeit through a CAMKII-dependent mechanism [48]. On the other hand, knockout of the gene encoding Wnt-7a results in a decrease in the numbers of newborn neurons in the SGZ and impairs their maturation, linking Wnt-7a both to self-renewal and differentiation [125]. The fact that canonical and noncanonical Wnt ligands, such as Wnt-7a, are required for correct neuronal production, suggests roles for both stimulation and repression of Wnt/β-catenin signaling during neurogenesis. It is important to bear in mind, the coexistence of quiescent neural progenitors, amplifying neural progenitors, early differentiating neuroblasts, maturing neurons and granule cells in the hippocampus [126] can make it difficult to interpret results at the population level, since different cells may respond differently to proliferative and differentiative stimuli. The identity of the Wnt proteins involved in the SVZ is less clear; Wnt-7a is secreted by glial cells and promotes SVZ and olfactory bulb progenitor cell proliferation, and this has also been shown to be through a β-catenin-independent mechanism [127].

The roles of Wnt receptors in the hippocampus have also been investigated using mouse models. Knockout of FZD9 results in increased apoptosis in the developing dentate gyrus [95], and knockout of the Wnt co-receptor LRP6 disrupts production of dentate granule neurons and radial glial scaffolding [104]. Moreover, FZD5 transduces a noncanonical signal that establishes neuronal polarity [128], and ROR1 and ROR2 modulate synaptogenesis in hippocampal neurons [99].

The importance of secreted Wnt antagonists in neurogenesis should not be overlooked. Loss of Dickkopf-1 (Dkk-1), which normally inhibits Wnt/β-catenin signaling by binding to LRP5/6, increases the number of neural progenitors in the hippocampus [129]. In addition, lentivirus-mediated knockdown of the Wnt antagonist secreted frizzled related protein 3 (sFRP3 or FRZB) in the dentate gyrus increases canonical Wnt signaling, neural progenitor proliferation and neuronal development [130]. These observations suggest that secreted Wnt antagonists promote functional homeostasis in the niche during adult neurogenesis. Abnormal activation of the stem cell niche leads to NSC depletion, so soluble Wnt antagonists could be among the factors that prevent excessive activation of the stem cell population, which would have detrimental effects.

Wnt signaling and adult neurogenesis: intracellular components

Many different approaches have been used to study the effects of altering canonical Wnt signaling in the mouse hippocampus. The FZD8 CRD, which inhibits Wnt signaling, increases the numbers of neurons and leads to a concomitant depletion of the multipotent progenitor cell population [131], while the GSK-3β inhibitor lithium chloride (LiCl), which stabilizes and activates β-catenin, induces adult hippocampal progenitor cell proliferation [132]. LiCl treatment also stimulates cell proliferation and neuronal fate specification in a mouse model of Alzheimer's disease [133]. Conditional knockout of APC in GFAP-expressing cells of mice activates β-catenin, reduces neurogenesis and impairs neuronal differentiation [134]. However, it is worth noting that while LiCl and APC gene deletion both activate β-catenin signaling, these treatments have unrelated β-catenin-independent effects [135–138]. APC, for example, also plays a role in neuronal migration by binding the 3′ UTR of β2B-tubulin mRNA [139]. Nevertheless, studies generally point towards a role for Wnt/β-catenin signaling in stemness and proliferation in the hippocampus.

Numerous studies have also reported roles for Wnt signaling in the SVZ. β-catenin-responsive cells exist in the SVZ throughout the development of the CNS [140], and their activation promotes progenitor cell proliferation [141]. Activation of β-catenin signaling increases the

numbers of oligodendrocytes derived from this neurogenic site, while inhibition seems to reduce the glial cell number [142]. In another study, β-catenin activation via GSK-3 inhibition increased cell proliferation in the SVZ, and this was accompanied by increased numbers of oligodendrocytes [143], confirming an earlier study that highlighted oligodendrocytic genes as targets of β-catenin [144]. Thus, Wnt/β-catenin signaling may also play important roles in the proliferation of progenitors and in oligodendrocytic development in the SVZ.

However, there is also evidence that supports roles for noncanonical downstream effectors in this context. As noted earlier, AP-1 family members are regulated by JNK family protein kinases. Mice lacking JNK1, JNK2 or JNK3 perform less well than their wild-type littermates in several behavioral tasks, including the elevated plus maze, open field, novel object recognition memory test and Morris water maze [145]. Moreover, injection of the JNK inhibitor SP600125 into the mouse hippocampus reduces long-term memory [146]. On the other hand, expression of a dominant-negative form of CREB, which blocks the activity of all CREB heterodimers, disrupts hippocampus-dependent spatial memory [147]. Moreover, hippocampal granule cell proliferation is increased by activation of cAMP signaling and reduced by CREB inhibition [148], and combined disruption of CREB and CREM leads to neurodegeneration in the hippocampus and in the dorsolateral striatum [121]. In contrast, conditional knockout of c-Jun using the Nestin gene promoter does not affect hippocampal-dependent behavior or brain morphology [149], but greatly impairs axonal regeneration, supporting a role for this AP-1 family member in neuronal maturation. ATF2 is also found in the human hippocampus and its expression is reduced in patients with Parkinsońs and Alzheimeŕs disease [89]. These studies highlight the relevance of JNK and AP-1 family members for adult neurogenesis. Since the loss of neurons in the hippocampus is linked to several neurodegenerative diseases [90], further studies of Wnt/AP-1 signaling in this niche are warranted.

Human neural stem and progenitor cells

Unlike conventional cell lines, human ES- and iPS-derived neural stem cells are not transformed and resemble primary NSC cultures, thus providing good models for studying human NSC differentiation [115, 150, 151]. In addition, the unique developmental potential and replicative capacity of these cells offers an abundant source of specific somatic cell types that can be exploited for in vitro mechanistic studies and cell transplantation therapies. However, to obtain human neural progenitor (hNP) cells, hES cells and iPS cells need to be oriented towards the neural lineage. To do so, hES cells, normally cultured as embryoid bodies [115, 152], are placed in stringent serum-free culture conditions that selectively facilitate the survival and growth of neural cells [153]. CHIR99021 has been widely used to keep cells in an undifferentiated and proliferative state to initially increase neural progenitor cell number, and ultimately neuronal yield [16, 154–156]. When the cells enter the neural lineage, rosette-like structures appear. These structures resemble the cellular organization of the neural tube [157] and can be mechanically selected to increase their numbers. Growth factors, normally bFGF and leukemia inhibitory factor (LIF) are used to maintain these cells in a state of self-renewal [107], such that they retain the expression of NSC markers, such as Nestin and SOX2, and can be induced to differentiate into neurons upon withdrawal of bFGF.

Human neural stem and progenitor cells: Wnt ligands and receptors

Wnt signals can affect neuronal differentiation of hES- and iPS-derived NSCs [16, 131, 158], but there are conflicting reports in the literature on the signaling pathways involved. Canonical Wnt-3a has been shown to stimulate differentiation when added exogenously to hES cell cultures [67]. In keeping with these findings, differentiation of neural rosette progeny in the presence of Wnt-3a leads to the induction of markers compatible with ventral forebrain fate and the emergence of GABA+ neurons and cells expressing dorsal markers [159]. On the other hand, exogenous Wnt-3a was shown to support the expansion and maintenance of hES cells [65]. In mES cells, the secreted Wnt antagonist sFRP2 stimulates production of neural progenitors [58]. In the same study, activation by canonical Wnt-1 and LiCl blocked differentiation, supporting a model in which inhibition of canonical Wnt signaling is required for neuronal differentiation.

Less is known about the role of noncanonical Wnt signaling. Wnt-11, a noncanonical Wnt, promotes stem cell differentiation in several contexts [160]. For example, it induces hES cell exit from the pluripotent state, mesodermal/hematopoietic cell fate [161] and cardiac differentiation of mES cells [162]. In human embryonal carcinoma cells, Wnt-11 maintains neural progenitor cell proliferation but prevents further differentiation, which instead is driven by another noncanonical Wnt, Wnt-4 [68]. We recently found that noncanonical Wnt-3a signaling stimulates differentiation of hES cells and iPS cells, something that could account for some of the controversy found in the literature regarding the role of Wnt-3a [163]. The nature of the response to Wnt-3a, canonical versus noncanonical, is likely to be influenced by the relative levels of Wnt receptors, secreted Wnt antagonists and intracellular effectors expressed by target cells. Comprehensive approaches that take these factors into

account will be required for a complete understanding of how Wnt signals drive neurogenesis.

Human neural stem and progenitor cells: intracellular components

Again, controversial studies have been published on the role of canonical downstream components of Wnt signaling in this field. Many studies provide evidence that canonical Wnt signaling drives neuronal differentiation. Davidson et al. reported that β-catenin signaling is repressed by Oct4 in hES cells, and that activation of β-catenin promotes differentiation [64]. In another study, recombinant Wnt-3a stimulated both proliferation and differentiation of hES cells. Canonical β-catenin/Tcf-dependent transcriptional activity was found to be elevated in the differentiating cells, suggesting that canonical activity supports differentiation [67]. However, many other reports highlight the role of canonical Wnt signaling in stem/progenitor cell maintenance, rather than induction of differentiation. For example, Wexler et al. showed that baseline β-catenin signaling represses neuronal differentiation in human NSCs [131], and another study reported that inhibition of Wnt/β-catenin signaling using Dkk-1 or the tankyrase inhibitor XAV939 promotes neural precursor specification [164]. Moreover, GSK-3 inhibition, which induces stem cell renewal and sustains the expression of pluripotency markers [16], is a widely used tool to expand neural progenitors [154–156], and bFGF treatment of hES and iPS cells, which maintains the undifferentiated state, activates canonical Wnt signaling through inhibition of GSK-3 [165].

There are also conflicting reports from studies using mES cells. Again, some emphasize the importance of canonical Wnt signaling for differentiation [166, 167], while others suggest the opposite [59, 168–170]. This could be partially explained by the fact that the culture conditions used vary, and that NSCs and neural progenitors are very general terms. Thorough characterization of each cellular model is therefore necessary to link any findings to their particular context. Whatever the conclusions drawn, studies to date highlight the importance of tight control of Wnt/β-catenin signaling during neuronal differentiation. It might very well be the case that canonical and noncanonical Wnt signaling are required at different time points and even cooperate to promote stem cell maintenance and/or differentiation.

More recently, mES cell differentiation was found to be accompanied by activation of noncanonical signaling via increased expression of Tcf3 [171], which is known to signal independently of β-catenin in several contexts [172]. This is consistent with our findings in human NSCs, where Wnt-3a promotes differentiation via JNK/ATF2 independently of β-catenin [163], and with a recent study in human iPS cells, which showed that Wnt-3 and Wnt-9B cooperate to promote dopaminergic differentiation, with canonical signaling maintaining proliferation and noncanonical signaling, involving JNK, driving differentiation [173]. These studies indicate that noncanonical signaling can play an important role during the differentiation of hES cells and warrant further studies of noncanonical Wnt signals to shed light on the process.

Wnt signals also interact with other pathways. Li et al. showed that endogenous Wnt signaling in hES cells

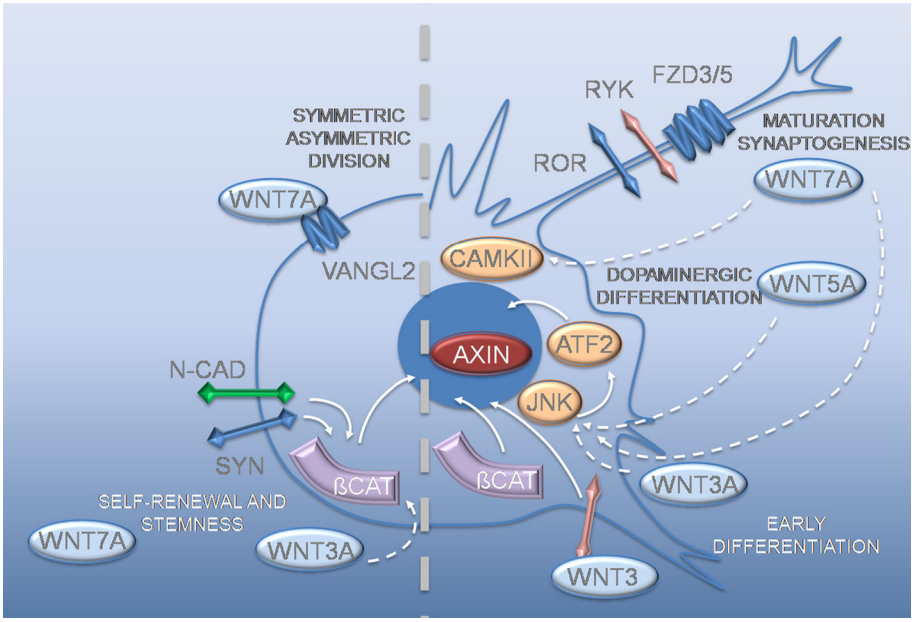

Fig. 3 Canonical and Noncanonical Wnt signaling in neural stem cells. Both aspects of Wnt signaling play important roles in NSC maintenance, differentiation and maturation. Wnt-3a and Wnt-7a are able to activate both canonical and noncanonical signals and so induce proliferation or differentiation and maturation, respectively; N-cadherin, N-CAD

upregulates the truncated form of GLI3, a repressor of sonic hedgehog (SHH), producing dorsal telencephalic neural progenitors. A high concentration of SHH, or the inhibition of Wnt by Dkk-1 together with a low concentration of SHH, almost completely converted primitive dorsal precursors to ventral progenitors. These dorsal and ventral telencephalic progenitors later differentiate to functional glutamatergic and GABAergic neurons, respectively [174]. Indeed, midbrain progenitors, which can express both floor and roof plate markers, are enriched when hES cells were treated with both SHH and Wnt activators [154]. Crosstalk between pathways such as these plays central roles in neuronal specification and so is critical for cell therapy-oriented studies. Given that individual Wnts are likely to have different impacts on other pathways and thus on self-renewal and differentiation (Fig. 3), comprehensive studies are required to clarify existing controversies in the field.

Conclusions

Previous studies highlight the critical role Wnt signaling plays in NSCs, but the nature of the Wnt signals involved remains unclear, with reports of increased and decreased Wnt signaling taking place during differentiation [68, 175] and disease [176, 177]. Neurogenesis in the hippocampus, where Wnt signaling plays important roles, is gradually lost as we age [178], and this loss is implicated in neurodegenerative diseases [123]. Furthermore, Wnt signaling has been implicated in other neurodegenerative events, such as impaired myelination and loss of dopaminergic neurons [177, 179]. It is also important to note that, given its complexity, Wnt signaling is likely to play many different roles that will depend on the identities of the ligands, receptors and effectors that expressed by the stem cells themselves and by cells in their niche. Global approaches will be required to identify and interrogate the functions of the key components that promote differentiation. This information can then be used to identify those changes that have an impact on disease and aging, and to optimize methods to generate neurons for stem cell-based therapies. In addition, further studies are warranted to determine the impact of Wnt signaling on cell physiology at the point of cell harvest and at cell implantation at sites of injury. While the results of these studies are anticipated to be important from a biomedical perspective, many basic key questions remain unanswered. What roles do Wnt signals play in the neural stem cell niche? Are Wnt proteins important for neuro-immune interactions during inflammation-directed brain repair? Do Wnt signals control glial/neuronal progenitor signaling crosstalk? How is Wnt ligand expression regulated? Recent studies are beginning to provide answers [80, 180–182]. Nevertheless, further work will be essential to understand how Wnt signals are coordinated during the generation, expansion and differentiation of neurons, and apply this knowledge to optimize stem cell-based therapies.

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