WNT-Frizzled signalling and the many paths to neural development and adult brain homeostasis

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1. ABSTRACT

The regulation of brain development and function is the result of complex cell-restricted and temporal expression profiles directed by signaling networks constantly imposing exquisite regulatory control on many genes at any one moment within a cell. The ultimate outcome is a genetically controlled balancing act where expression profiles of these hundreds of genes result in cellular proliferation, differentiation and the ultimate choice between long-term survival and apoptosis. During embryonic development there is a massive expansion of neurons and glia, which is balanced with programmed cell death as the brain matures and remodels. As developing brain cells differentiate, they migrate toward the region where they will ultimately seek out interactions with other cells and perform their specialized tasks. Although a number of signaling pathways have been shown to contribute to various processes allowing the maintenance of normal neurogenesis, the precise signaling machinery necessary for modulating the maintenance of both the neuroblast and differentiated neuronal population, and regulating transition between the two, is still being solved. Not surprisingly, the Wnt signaling pathway is important in regulating neural development but also appears to be involved in adult neurogenesis and some brain disorders. Here, we review key findings showing the pivotal nature of Wnt-Frizzled (FZD) signaling in neurogenesis as revealed by a number of molecular genetic studies using mice and other model organisms. We also review the current literature on the role of the Wnt pathway in the generation of brain cancers, particularly the most common primitive neuroectodermal tumors in childhood, neuroblastomas, and in neurodegenerative diseases such as Alzheimer's disease.
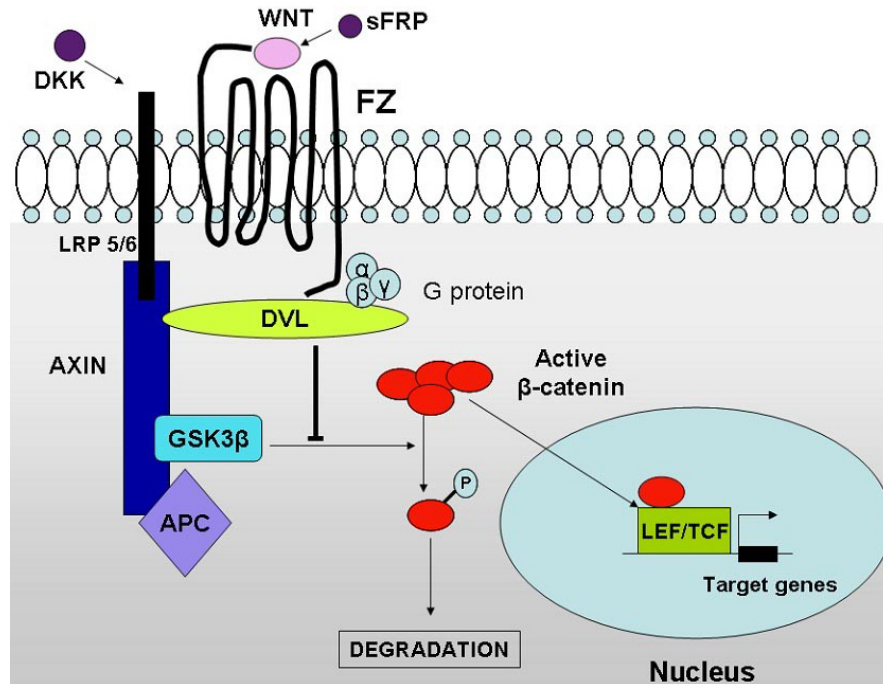
Figure 1. The canonical Wnt signaling pathway. In the canonical pathway, Wnt binding to the 7-transmembrane-domain protein Frizzled (FZD) and to its co-receptors, low-density lipoprotein receptor-related protein (LRP) 5/6 triggers activation of the cytoplasmic scaffold protein Dishevelled (DVL). FZD activation through Wnt requires the presence of heterotrimeric G protein. Activated DVL then interact with the AXIN/APC/GSK-3beta and lead to the inhibition of GSK3, which phosphorylates beta-catenin and promotes beta-catenin degradation throughout the proteasome under non-stimulated conditions. Inhibition of GSK-3beta results in the accumulation of stabilized beta-catenin in the cytoplasm and to its translocation to the nucleus. In the nucleus, active beta-catenin interacts with the LEF/TCF transcriptional complex leading to the transcriptional activation of target genes involved in cell fate and/or proliferation.

2. WNT SIGNALING PATHWAWS

Most genetic studies on Wnt signaling have focused on the so-called 'canonical' pathway, which is reliant on the dynamic balance between beta-catenin degradation and beta-catenin stabilization and subsequent nuclear translocation. Once in the nucleus, the interaction of beta-catenin with T-cell factor (TCF) and lymphoid enhancer-binding protein (LEF) transcription factors triggers transcriptional activation of genes involved in cell proliferation and cell fate. FZD can also act through beta-catenin independent pathways including: i) the Wnt/calcium pathway (1) leading to activation of phospholipase C (PLC) (2) and phosphodiesterase (PDE) (3), ii) the planar cell polarity pathway that regulates *Drosophila* development and gastrulation in mammals through the activation of Rho GTPases (4) and iii) a recently described pathway in mouse myogenesis involving adenylyl cyclase signaling via PKA and activation of the important neural transcription factor, cAMP Response Element Binding protein (CREB) (5).

2.1. The canonical Wnt pathway

First identified as developmental morphogens in *Drosophila* Wnt proteins are comprised of a family of at least 19 members in mammals (6, 7). These ligands are secreted cysteine-rich glycoproteins that can signal through the activation of their receptors, the FZDs . FZDs are 7-transmembrane spanning domain proteins, typical of G protein coupled receptors, and contain an extracellular cysteine-rich domain that binds Wnt. The canonical Wnt signaling pathway has been extensively studied and is implicated in many cellular processes in stem cells [reviewed in (8)]. In the canonical pathway (Figure 1), Wnt binding to its receptor FZD leads to activation of Dishevelled (DVL), -and subsequent inactivation of a degradation complex, composed of adenomatosis polyposis coli (APC), AXIN and GSK-3beta. Under non-stimulated conditions, GSK-3beta induces phosphorylation of beta-catenin and its subsequent ubiquitylation promotes beta-catenin degradation in proteasomes. Inhibition of GSK-3beta leads to the stabilization and the accumulation of beta-catenin in the cytoplasm, which is characteristic of the canonical pathway. Activated beta-catenin translocates to the nucleus and binds to TCF/LEF transcription factor family members, leading to the transcription of target genes. In the brain and central nervous system target include proteins involved in neural patterning, such as the caudal-type homeodomain transcription factors 1 (CDX1) (9) and engrailed-1 (10) during mid-brain development and EMX2 dorsal telencephalic development (11). Target genes in neural tissues may also include proliferation-promoting genes such as c-myc and cyclin D1, as in other tissues (12-15).

Activation of FZD also requires interaction with its co-receptor LDL receptor related proteins, LRP5 and 6, to which Wnts can also bind. LRP5/6 also activates the canonical pathway by inducing the degradation of AXIN. Complex regulation occurs at the cell membrane with the extracellular binding of antagonist molecules such as Dickkopf (DKK) and FZD-related protein (sFRP), that inhibit the Wnt pathway(16, 17). Inhibition of the pathway occurs through the binding of DKK to LRP5/6 or through the binding of sFRP directly to Wnt. Separate components of other pathways involving Notch, cadherin or TGFalpha, all important for brain development, can also participate in the modulation of the canonical pathway. As an example, the Wnt pathway can also interact with the Notch-Delta signaling pathways and LRP6 is required for this cross-talk during somitogenesis (18).

2.2. The non-canonical Wnt pathways

The Wnt/calcium pathway had been identified in both *Xenopus* and Zebrafish. Components of the Wnt/calcium pathway are represented in Figure 2A. In the Wnt/calcium pathway, FZD activates PDE and PLC promoting the mobilization of free intracellular calcium. The increased level of intracellular calcium triggers the activation of downstream target molecules including the calcium-calmodulin-dependant kinase (CamKII), calcineurin and PKC. Activation of the phosphatase calcineurin leads to the dephosphorylation of the transcription factor NF-AT and its accumulation in the nucleus (19, 20). However few target genes of this pathway have been thus far characterized. Other intracellular calcium sensors such as cyclic nucleotide-gated ion channels, guanylylcyclases and protein kinase G are also likely to be activated in response to increased intracellular calcium; however, their role in Wnt/calcium signaling is still under investigation. Wnt5a can activate the Wnt/calcium pathway, regulating dorso-ventral axis patterning (21). Activation of the Wnt/calcium-dependant cascade is DVL dependant in *Xenopus* embryos (22), implying that DVL might be a common component of all Wnt pathways.

In the planar cell pathway (Figure 2B), Wnt binding to FZD leads to activation of Jun-N-terminal kinase (JNK) pathway. This pathway is known to branch downstream of DVL which interacts with Daam1 to activate the small GTPase, Rho and Rho-associated kinase (ROCK). During early development, the planar cell pathway regulates cytoskeletal organization and cell polarization. In the retina, modulation of the PCP pathway occurs through competition of Diego, an ankyrin-repeat protein, with the FZD PCP antagonist Prickle, for the binding to DVL (23).

Recent work has revealed another non-canonical Wnt signaling pathway involving Wnt1 and Wnt7a which activate adenylyl cyclase, which in turn phosphorylates CREB (5) (Figure 3). CREB then activates transcription of target genes through binding to cAMP Response Elements (CREs) on their promoters. This hitherto unrecognized pathway is especially interesting in the context of neural function, as CREB is one of the best studied neuronal transcription factors with many important functions, including proliferation and differentiation [for review see (24)]. Moreover, CREB is critical for neuronal survival and is implicated in neural stem cell function and a number of neurodegenerative diseases (25, 26).

3. THE WNT PATHWAY IN NEUROGENESIS

3.1. Wnts and neural stem cells

Stem cells from many tissue compartments require Wnt pathway activation to maintain a number of cellular characteristics, including one very important property which distinguishes stem cells form all other cells: the capacity for self-renewal. Indeed many of the genes regulating stem cell self-renewal appear to be shared amongst stem cells of various tissue origins, as revealed by gene profiling experiments (27). Conversely, many studies have since shown that factors within the Wnt pathway have a number of different roles in stem cells depending on the time during development and in which cell type they are activated [reviewed in (8)].

Neurogenesis is the term used to describe the birth of new nerve cells that are derived from stem and progenitor cells. Until recently this was believed to occur only during embryonic development and in some vertebrate species through to early post-natal periods; it is now known that neurogenesis continues throughout life in many species studied, including man. In mice, cells destined to become the forebrain, midbrain and hindbrain is apparent as early as E8.5. At this stage there is intense neurogenic activity occurring, with almost all cells in the neuroepithelium participating in the cell cycle. During late gestation and early postnatal periods, there is still significant neurogenesis, with about 4% of nerve cells dividing (28). Although embryonic neurogenesis in vertebrates has been well studied in recent decades, it was not clear whether neurogenesis was commonplace in the adult brain amongst various vertebrate species. Indeed, the adult mammalian brain was long thought to harbor only fully differentiated post-mitotic cells (neurons and glia), which would only be lost through the process of ageing. The turning point in this discovery was the identification of a small number of proliferating cells embedded in two zones of the adult mammalian brain (see Figure 4A). In rodents and man this activity occurs in a restricted area in the hippocampus called the dentate gyrus and in the sub-ventricular zone of the lateral ventricles, with fewer than 0.001% of cells having the capacity to proliferate (28, 29). The functional significance of adult neurogenesis is still unclear but seems to result in the generation of fully integrated and functional neurons and glia.

3.2. Wnts in neural development

Elucidation of a role for the Wnt pathway has come from studying various components of the canonical pathway, including the Wnt ligands, the inhibitors of the pathway (30, 31) and the transcriptional endpoint of the canonical pathway, namely beta-catenin. Wnt1 is expressed in the caudal midbrain and mutant mouse studies showed that in conjunction with FGF8, Wnt1 acts as a mid-
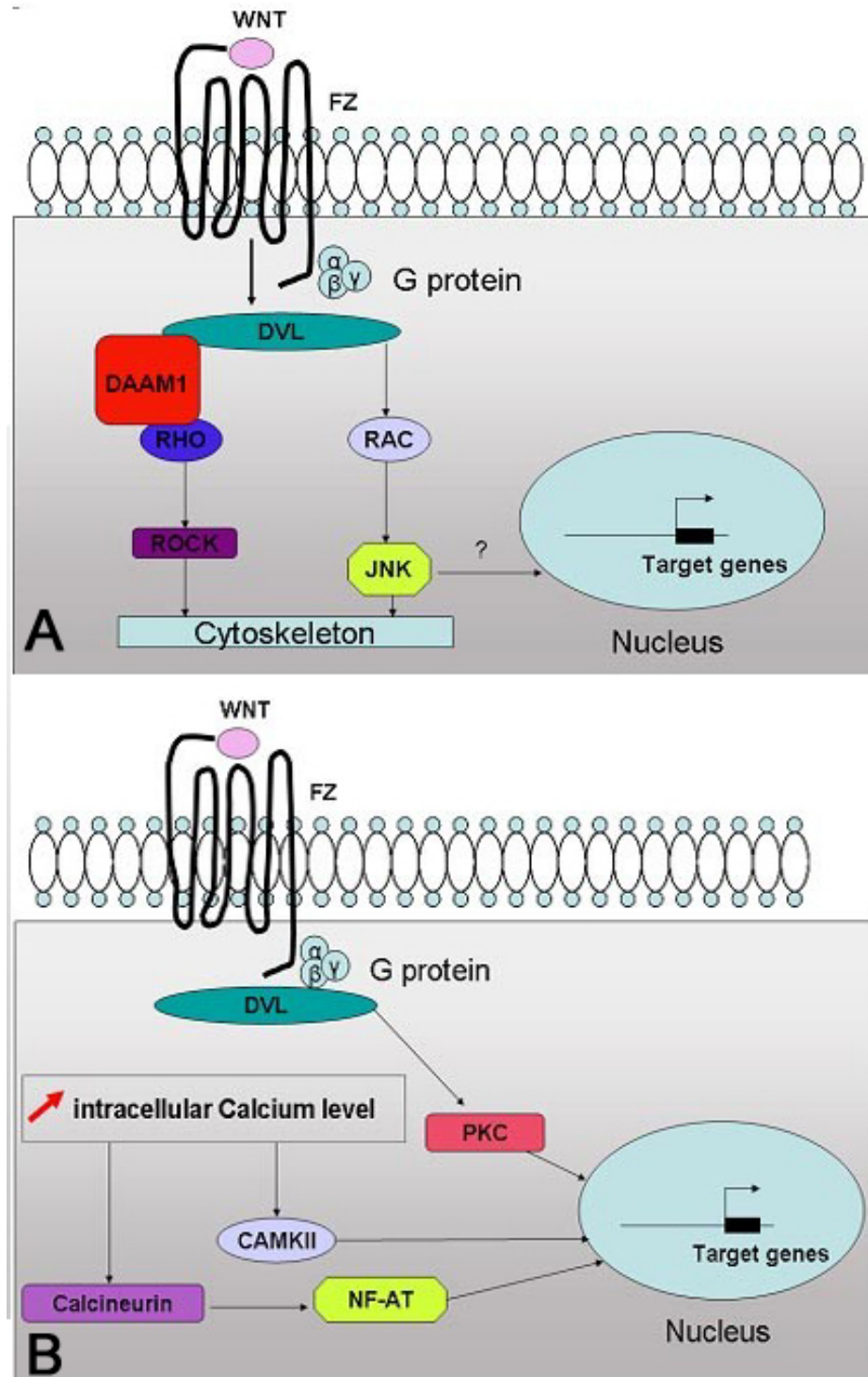
Figure 2. The non-canonical pathways. In the planar cell polarity pathway (PCP) (A), activation of DVL, which is mediated by Wnt binding to FZD, leads to the activation of small G protein Rho through interaction with DAAM1 and/or Rac. Rho and Rac in turn activates the ROCK and JNK pathways, respectively. Activation of this pathway influences the cytoskeleton and affects cell polarity, and is likely to be involved in neurite morphogenesis. In the Wnt/Calcium pathway (B), activated DVL leads to intracellular calcium mobilization that triggers the activation of calcium-sensitive molecules such as PKC, CAMKII and calcineurin. These events then influence expression of genes involved in cell fate and cell movement through activation of transcription factors such as NF-AT.
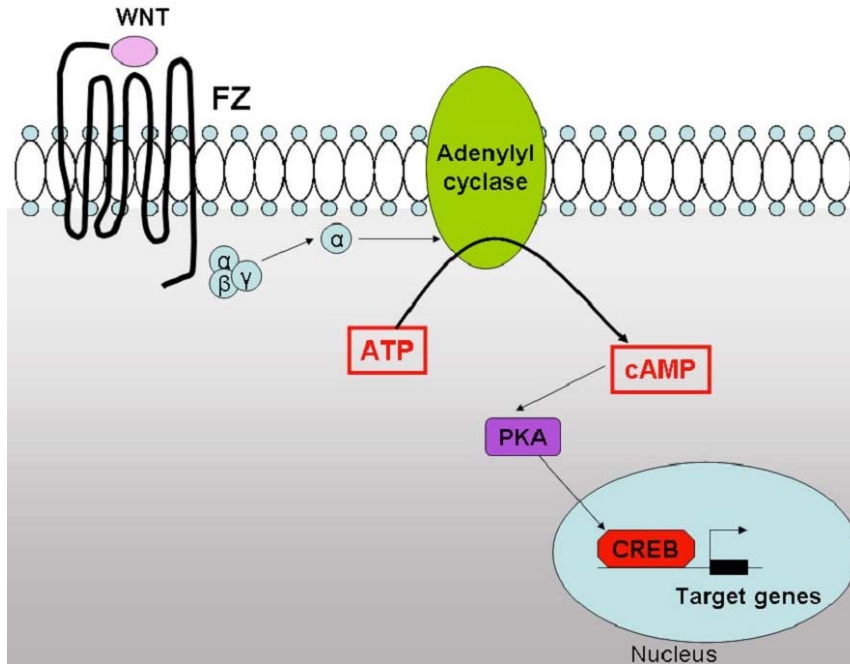
Figure 3. Wnt/CREB pathway. Wnt binding to its receptor FZD triggers the activation of adenylyl cyclase, which increases the level of intracellular cAMP, resulting in nuclear translocation of PKA. In the nucleus, PKA phosphorylates the transcription factor CREB resulting in transcriptional activation of CREB target genes.
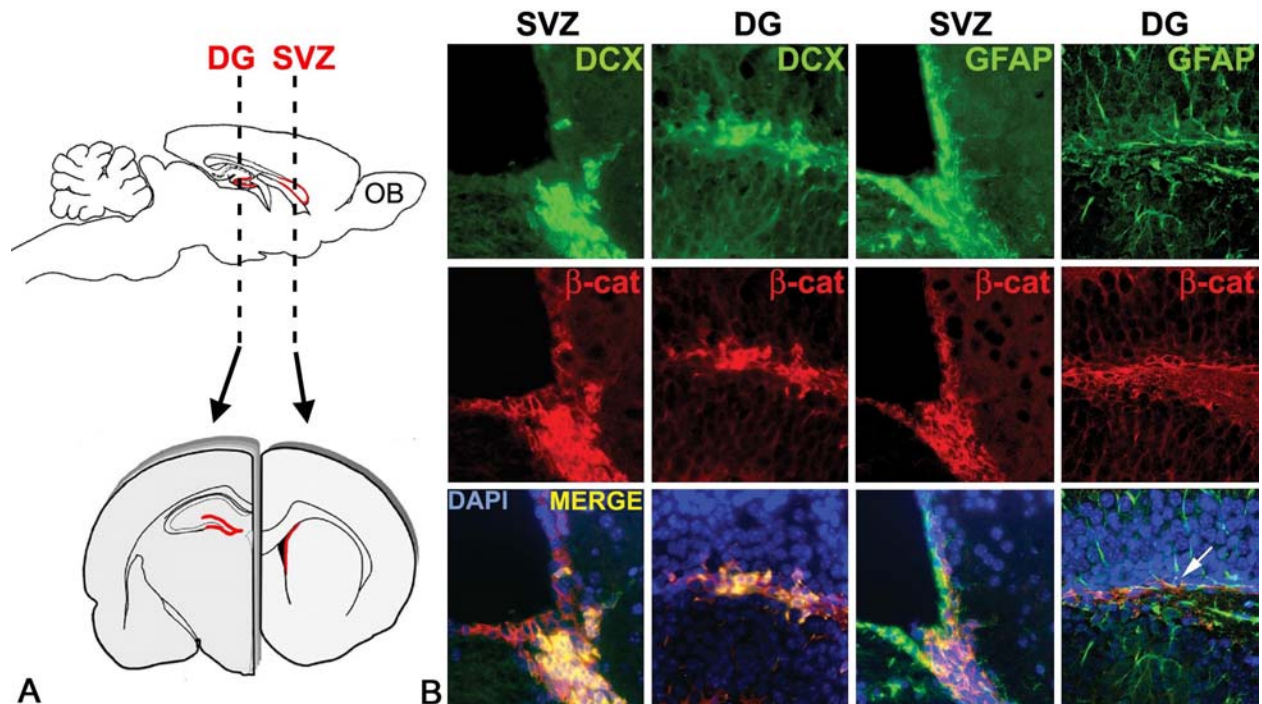

Figure 4. A. A sagital section (TOP) and cross-section (BOTTOM) showing the location of the major neurogenic regions (in RED) in adult mouse brain. The olfactory bulb (OB) is shown. B. Expression of beta-catenin in the neurogenic zones of adult mouse brain. Most beta-catenin-positive cells are co-labeled by the migrating neuroblast marker, DCX, while there is also some overlap with the neural stem cell marker, GFAP. The immunofluorescence was performed by incubating "antigen-retrieved" 6 micron paraffin-embedded brain sections overnight at 4°C, in a mixture of goat anti-DCX antibody (1:100, Santa Cruz) or rabbit anti-GFAP (1:100, Dako) and mouse anti-beta catenin antibody (1:100, Transduction Laboratories). Fluorescent detection of primary antibodies was performed using species-specific secondary antibodies.

hindbrain organizer (6, 32). A recent study designed to ectopically express Wnt1 during mouse development showed that the major role of Wnt1 is to regulate the proliferation of precursor populations in the developing mid-/hindbrain region (33). Work in other vertebrate models such as zebrafish (34) indicates that Wnt1 activity in brain is conserved across many ancient species. Ccd1 (Coiled-coil-DIX1), which can form a heteromeric complex with AXIN and DVL, has recently been shown to regulate neural patterning in zebrafish embryos through the activation of the Wnt pathway (35). It is clear that very specific Wnt ligand expression occurs in distinct regions of the developing human brain. For example, Wnt7b is expressed in cerebral cortical and diencephalic progenitor cells during early human development (36), remarkably similar to the expression profiles in developing mouse brain, indicating functional evolutionary conservation of specific Wnt ligand activity in brain development. This is important in unraveling complex neuropsychiatric disorders that are thought to underlie some neuro-developmental defects. Wnt3a appears to have a very specific role in the development of the hippocampus, a structure involved in integrating many of the higher order tasks, such as memory and learning. In mice lacking Wnt3a there is such a profound reduction in cortical progenitor cell number during early development that by mid-gestation the hippocampus is absent (37).

The outcome of beta-catenin inactivation or overexpression in the brain is dependent on the specific cells affected and the time at which expression is altered. Using conditional knockout mice has allowed specific cells or regions of the developing nervous system to be targeted. An elegant study where beta-catenin was specifically deleted only in cells normally expressing Wnt1 showed that beta-catenin loss resulted in a severe anterior truncation, characterized by the absence of part of the midbrain and all the cerebellum (38). This and other similar studies utilizing conditional knockout and transgenic mice overexpressing beta-catenin have shown that beta-catenin has an important role in neuronal progenitor proliferation and neural crest cell survival and differentiation (39, 40). A recent study shows that beta-catenin in the E9.5 telencephalon is highly enriched at the apical end of the neural precursor cells and colocalizes with N-cadherin at adherens junctions, implying that the main role of beta-catenin at this stage of telencephalic specification, is to promote neuroepithelial adhesion (41).

Other canonical Wnt pathway-related factors are also implicated in a number of aspects of brain development. GSK-3beta and the beta-catenin transcriptional partners LEF1 and TCF4 are expressed during brain development in mouse (12). In mice, LEF1 has also been shown to be required for hippocampal development and granule cell generation in the dentate gyrus (42).

3.3. Wnts in adult neurogenesis
Wnt function in neurogenesis has been well characterized during neural development in invertebrate and vertebrate species. In contrast, an understanding of Wnt function in adult brain is less well understood. Although Wnts promote retinal neurogenesis and hindbrain neurogenesis during zebrafish development (43), Wnt pathways have only recently been investigated in the context of adult neurogenesis. While key components of Wnt pathways are expressed in the adult brain, little is known about the diversity of Wnt function. Given the broad dependence of neural stem cells on Wnt signaling during embryogenesis, it is likely that there is a similar role for Wnt in adult neural stem cells. Indeed, as already described in the previous section, the Wnt signaling pathway can promote neural crest stem cell renewal or neuronal differentiation in a context-dependant manner and progenitors appear to differentially respond according to their developmental stage.

In this section, we argue that the collective literature support a role for the Wnt pathway in adult neurogenesis. Evidence of expression of Wnt pathway components in adult germinal regions as well as in other areas provides a fundamental basis to further study the role of Wnt pathways in the adult brain. Birth of new neurons in the adult brain, referred to as secondary neurogenesis in contrast to the embryonic neurogenesis, is now a well established phenomenon occurring in both invertebrate and vertebrate species including humans [see review (44, 45)]. The two major neurogenic areas, harboring stem cells and progenitor cells in adult vertebrate brains, including humans, have been characterized: the subgranular cell layer of the adult hippocampal dentate gyrus and in the subventricular zone of the lateral wall in the forebrain which in rodents gives rise to olfactory bulb (OB) interneurons. Recent investigations on the expression pattern of Wnt pathway components have led to their identification in the adult brain. Indeed the Wnt antagonist Dkk3 is expressed in the lateral ventricular zone in the adult forebrain (46). In mouse, Ccd1 is a positive regulator of Wnt/ beta-catenin pathway and is expressed in the embryonic and adult brain suggesting a role in adult neural plasticity (47). Mutant mouse studies have shown that impaired Wnt signaling lead to abnormalities in hippocampal development due to hypoproliferation of neural progenitors (37, 42, 48, 49) whereas enforced expression of beta-catenin triggers an increase of the progenitor pool size (50). In humans, Wnt7A mRNA expression has been shown in different brain regions including the hippocampus, a site of ongoing neurogenesis (51). An overview of Wnt pathway expression in the adult brain has been listed in Table 1, highlighting the supporting evidence for a role of Wnt pathway components in brain. Recent studies provide a detailed analysis of Wnt signaling gene expression in the postnatal mouse brain, showing that adult hippocampal brain and progenitor cells express all components of the canonical Wnt pathway, including Wnt3, FZD-1, GSK3-beta, DVL-1, Axin and LEF1 (52, 53). Our recent research shows that beta-catenin expression is localized to the neurogenic zones of adult mouse brain, and is mainly expressed in doublecortin (DCX) positive neuroblasts (Figure 4). This observation is in line with the

Table 1. Wnt pathway expression in the adult brain

Wnt pathway genes	Species	Localization	References
Wnt, FZD, sFRP, TCF/LEF	Mouse	Hippocampus, olfactory related areas, neocortex	52
Wnt1	Human	Hippocampus	106
	Mouse	Hippocampus	107
	Mouse		108
Wnt2	Rat	Hippocampus	55
Wnt3	Mouse	Cerebellum	109
	Mouse		110
	Mouse		111
Wnt7A	Mouse		112
Wnt10A	Mouse		113
Wnt13	Human		114
Wnt14B	Human	Occipital lobe	115
	Mouse		116
Wnt16B	Human		117
Wnt7A	Human	Temporal, occipital and parietal lobes, paracentral gyrus of cerebral cortex, hippocampus, putamen, medulla oblongata	51, 116
Beta-catenin	Rat	Subventricular zone	118
	Rat	Dentate gyrus	55
	Mouse	Dentate gyrus	56
	Human	Hippocampus	119
	Human	Prefrontal cortex	120
Beta-catenin	Mouse	Brain	121
	Human	Brain	122
GSK-3beta	Human	Prefrontal cortex	120
	Rat		123
DVL2	Human	Prefrontal cortex	120
FZD3	Human	Cerebellum	124
FZD7			125
FZD9	Mouse		126
FZD10	Mouse		127
	Human	Cerebellum	128
MRFP membrane type FZD related protein	Human	Hippocampus, medulla oblongata, corpus callosum	129
FAT protocadherin with homology to the beta-catenin binding region	Mouse	Subventricular zone, dentate gyrus	130
CCD1	Mouse		47
DKK3	Mouse	Lateral ventricular zone, hippocampus, cortex	46
Strabismus1 negative regulator of canonical pathway	Human	Cerebellum, corpus callosum, amygdala, medulla oblongata	131

view that the canonical Wnt pathway contributes to neural stem and progenitor cell function (53).

Different internal molecular cues such as hormones and growth factors, as well as environmental factors can influence adult neurogenesis. Growth factors such as BMP (member of the TGF-beta superfamily of secreted signaling molecules), TGF-alpha and FGF are known to play a crucial role in neural stem cell proliferation and are likely to cross talk with Wnt pathway. For example, FGF/Wnt cross-talk has emerged as a crucial network regulating a variety of biological processes (54). Direct administration of TGF-alpha triggers an increase in cell proliferation in the sub-ventricular zone (SVZ), correlating with an increased accumulation of cytoplasmic beta-catenin (REF). Moreover, chronic administration of electroconvulsive seizures in adult rats results in an increase of cell proliferation in the dentate gyrus and a consistent increase of beta-catenin immunoreactivity in newborn neurons (55), suggesting a role for beta-catenin in neural cell division and neuronal differentiation and recovery following seizures. Some of the most compelling evidence for a role of Wnt function in neurogenesis comes from a transgenic mouse model of Alzheimer's disease carrying a presenilin 1 A246E mutation where beta-catenin stabilization is increased and correlates with enhanced cell proliferation in the adult dentate gyrus (56).

Other intracellular pathways involved in promoting neural progenitor proliferation may cooperate with the Wnt pathway, allowing neural stem/progenitor cells to respond to various complex/simultaneous stimuli. Three sets of data from our laboratory indicate that the Wnt pathway may cooperate with the transcription factor, c-Myb. Firstly, reporter assays in cell lines show that activated beta-catenin transactivates mouse c-Myb expression (Ciznadija *et al*., unpublished data), consistent with a study identifying paneth cell beta-catenin/TCF4 target genes (57). Secondly, c-Myb cooperates with beta-catenin to allow the efficient expression of proliferation-associated genes (eg. c-myc) (unpublished data). Finally, we have shown that c-Myb is expressed in the neurogenic zones where beta-catenin is also expressed (Figure 4) and have generated neural progenitor cell-specific c-Myb knockout mice to show that c-Myb regulates neurogenesis in the adult mouse brain (Mantamadiotis *et al*., unpublished data). Other recent work has shown that c-Myb regulates neural crest cell (NCC) migration and differentiation in chick embryos (58), similar to that seen in mouse NCCs (39).

4. WNTs: BRAIN PATHOLOGY AND THERAPEUTIC TARGETING

Aberrant activation of Wnt signaling is believed to play a crucial role in triggering the development and

Table 2. Wnt pathway components involved in brain cancers

WNT pathway component	Cancer type	References
Wnt5a, Wnt10B, Wnt13	Brain tumor	132
Wnt2B	Blioblastoma cell lines	133
Wnt8A, 8B	Brain tumor cell lines	134
Wnt10A	Brain tumor cell lines	135
Wnt14	Brain tumor cell lines	115
FZD10	Glioblastoma cell lines	136
Beta-catenin	Adamantinomatous craniopharyngioma	137
	Pituitary adenoma	138
	Glioblastoma	139
	Medulloblastoma	73, 76
APC	Medulloblastoma	72
	Turcot syndrome medulloblastoma and colon cancer	66, 74
	Medulloblastoma and glioma	140
AXIN1	Medulloblastoma	141
	Medulloblastoma	71
SUFU	Medulloblastoma	80
TCF4	Pituitary adenoma, tumor brain metastasis	142

Table 3. Wnt pathway components involved in brain disorders

Wnt pathway component	Disorder	References
Wnt1	Schizophrenia	106
FZD3	Schizophrenia	143
DKK1	Alzheimer's	101
Wnt1, beta-catenin	Hemimegalencephaly	144
GSK-3beta	Bipolar Disorder	145

progression of several brain pathologies, particularly cancers. Data are emerging that provide clues to understanding the role of aberrant Wnt signaling in the development of neurodegenerative diseases such as Alzheimer's disease and neuropsychological disorders such as bipolar disorder.

4.1. The Wnt pathway in brain cancers

Wnt was identified as Drosophila *wingless* (59) and the murine homologue and oncogene *int-1* (60-62), hence the term <u>Wnt</u> (<u>w</u>ingless and <u>int</u>-1). The oncogenic potential of the Wnt signaling pathway in various tissues such as the haematopoietic system and colon epithelium are well recognized (63, 64). The first evidence for Wnt pathway involvement in brain tumors came from the recognition that a subset of colon cancer patients carrying APC germline mutations, developed a neurological disorder called Turcot's syndrome (65). Medulloblastomas (MB), as well as other forms of brain cancer including glioblastomas (66) or ependymomas (67-69), were found in Turcot's patients. MBs are malignant neuroectodermal tumors of the cerebellum occurring in childhood, with an incidence of five per million (70). These neoplasms are characterized by the presence of primitive neural cells with immature progenitor cell features. Alterations of various Wnt pathway genes have also been recently identified in subsets of sporadic MBs in humans. Alteration of Wnt signaling in sporadic cases includes mutations in APC, beta-catenin, AXIN, GSK-3beta genes that can lead to activation of beta-catenin and downstream transcriptional activation of target genes including cyclin-D1 and c-Myc which is a crucial gene in MB (71-76). Wnt pathway genes altered in brain cancers are shown in Table 2.

Although Wnt pathway alterations appear to be crucial in the generation of brain cancer, enforced expression of beta-catenin by itself in neurons does not result in malignancy, suggesting that elevated expression of beta-catenin alone is not sufficient to induce brain tumors (77). Wnt pathway deregulation is thus likely to occur in combination with other mutations leading to the transformation of neural cells. For example, the neural stem/progenitor cell and embryonic morphogen, Sonic hedgehog (Shh), is involved in MB development. Mutations in the Suppressor-of-Fused (SUFU) gene which encodes a negative regulator of the Hedgehog and Wnt pathway, has also been identified in a subset of MB indicating that deregulation of both Shh and Wnt pathways may operate simultaneously to induce MB (78-80). Moreover SUFU mutants lose the ability to inhibit beta-catenin export from the nucleus, leading to increased transcriptional activation (79).

Polyomaviruses are implicated in the development of human cancers and a strong correlation has been established between the activity of these viruses and the development of brain tumors. Indeed, some cases of MB, astrocytoma and glioblastoma have been linked to the human neurotrophic JC virus known to cause progressive multifocal leukoencephalopathy and demyelinating diseases of the central nervous system. In murine MB, induced by human neurotrophic JC virus (JCV), the ability of the JCV T-antigen to stimulate c-Myc promoter activity may result from the deregulation of the Wnt pathway leading to increased expression of beta-catenin and upregulation of c-Myc abundance (81).

Emerging data supporting a role for Wnt signaling in stem cell maintenance, progenitor pool size regulation and cell fate in the developing nervous system [see (8) for review], characteristics important to the proposed "cancer stem cells" in some types of brain tumors. Indeed, cells with the ability to self renew have been isolated from glioblastomas using the stem cell
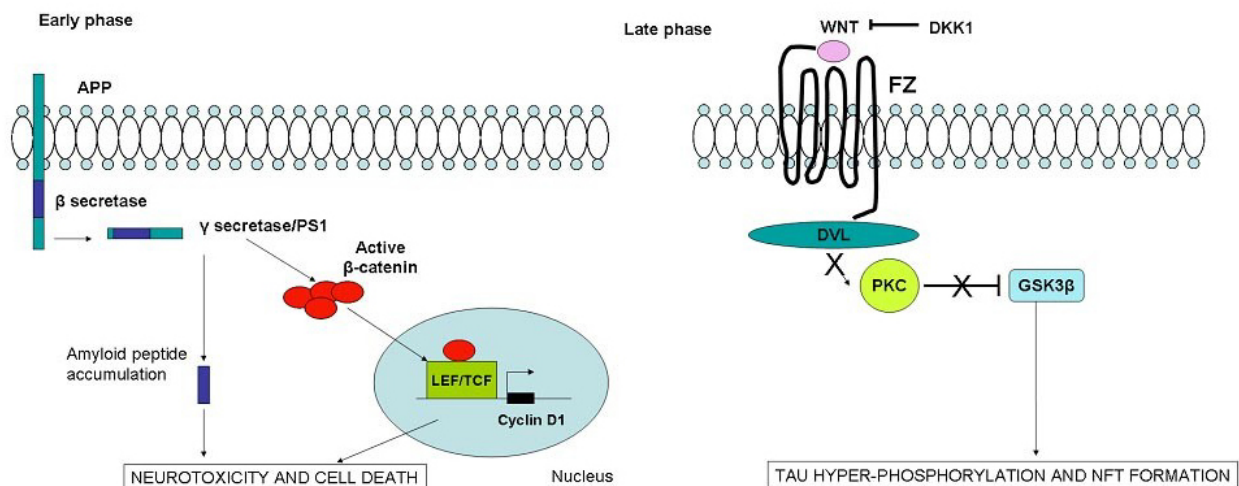
Figure 5. Alzheimer's disease and the Wnt hypothesis. Wnt may act in a biphasic manner in the Alzeihmer's disease neurodegenerative process. In an early phase, upregulation of the Wnt signaling pathway might increase Amyloid peptide toxicity by scheduling inappropriate cell cycle entry in postmitotic neurons. This process may involve presenilin 1 (PS1), a component of the gamma secretase complex responsible for the amyloid precursor protein APP cleavage, and increased abundance of nuclear beta-catenin, resulting in aberrant activation of cell cycle molecule such as cyclin D1. In a later phase, a decrease in Wnt signaling may be responsible for the occurrence of NFT and tau hyperphosphorylation mediated by increased GSK-3beta activity.

marker CD133/prominin (82). A recent study has also revealed the existence of prominin-positive stem cells in the cerebellum (83), raising the possibility that deregulation of Wnt pathways in the cerebellum may participate in the emergence of cancer stem cell-derived MB. Taken together, these data suggest that the Wnt pathway might be a useful therapeutic target for brain cancer therapy and that by interfering with the Wnt pathway using small molecule inhibitors, siRNA or therapeutic antibodies directed against Wnts, it may be possible to inhibit cancer progression [see (84) for review].

4.2. The Wnt pathway in disorders of the brain

Aside from cancer, aberrant Wnt signaling has been identified in other brain disorders such as schizophrenia, epilepsy, , neurodegenerative diseases and bipolar disorder (Table 3). Alzheimer's disease (AD) is one of the most common age-related neurodegenerative disorders with progressive dementia accompanied by two main histological hallmarks: neurofibrillary tangles (NFT) composed of intracellular protein deposits in neuronal perikarya and extracellular amyloid beta deposits surrounded by dystrophic neurites forming senile plaques. Under normal condition, amyloid precursor protein (APP) undergoes two cleavages by beta and gamma-secretases leading to amyloid beta peptide formation. Study of the early onset familial autosomal dominant form of AD had led to the identification of mutations in presenilin, a component of the gamma-secretase complex, and mutations of APP itself that result in increased production of amyloid beta-1-40 or the longer form, amyloid beta-1-42 (85). Studies of inherited forms of AD support the amyloid beta cascade hypothesis according to which, amyloid beta deposits are a primary event in AD. Several studies have suggested that presenilin might be regulated by the Wnt

pathways (Figure 5). Mechanistically, it appears that presenilin is a scaffold protein which binds GSK-3beta and beta-catenin and modulates beta-catenin turnover (86-91). Indeed, mutations in PS1 do influence GSK-3beta activity based on studies suggesting that GSK-3beta could mediate amyloid beta induced neurotoxicity by decreasing Wnt pathway activation, whereas inhibition of GSK-3beta has a neuroprotective effect by reactivating Wnt signaling (92, 93). Conversely, over-expression of GSK-3beta in transgenic mice leads to neurodegeneration (94).

Whereas some presenilin mutations are associated with a decrease in Wnt pathway activation, characterized by a decrease of nuclear beta-catenin, exposure of cultured neurons to amyloid promote activation of Wnt signaling pathway and increase the abundance of cytoplasmic beta-catenin. For example, PS1 deficiency results in increased cytoplasmic beta-catenin, leading to an increase in cyclin D1 transcription (95). To reconcile these apparently conflicting data, Caricasole and collaborators (96), suggest a scenario in which amyloid beta toxicity acts in a bimodal manner. At an early stage, amyloid beta promotes neuronal cell death following inappropriate activation of Wnt target genes, allowing inappropriate cell cycle re-activation; later, downregulation of Wnt signaling by amyloid beta in surviving neurons can lead to NFT formation. Notably, GSK-3beta has been identified in NFTs (97) and inhibition of DVL-mediated PKC activity, might be responsible for increased GSK-3beta activity (98). Furthermore, observations that amyloid-beta-induced Tau hyper-phosphorylation, occurring through a GSK-3beta-dependant mechanism also support the view that Wnt signaling pathway deregulation might be responsible for NFT formation (99, 100). Dkk1 is expressed in NFT and dystrophic neurites (101) suggesting that inhibition of the

Wnt signaling pathway could contribute to the pathological cascade triggered by amyloid beta. These data suggest that the Wnt pathway may be a valuable therapeutic target in the treatment of AD. In an experimental animal model, the potential neuroprotective effect of Wnt signaling potentiation against amyloid beta induced neurotoxicity has been demonstrated (102-105). There are two potential thearapeutic approaches to target the Wnt pathway in neurodegenrative diseases. First, potentiation of Wnt signaling might be used to prevent neurotoxicity and rescue neurons from cell death. Alternatively, manipulating the Wnt pathway to direct embryonic or neural stem/progenitor cells toward a certain lineage may aid in promoting neural regeneration in an already damaged region of the brain.

5. CONCLUSIONS

The Wnt/FZD pathway plays fundamental roles in all crucial and well-regulated events that shape the brain during development including stem cell/progenitor cell proliferation, tissue patterning, cell fate, apoptosis, neuronal differentiation and dendrite morphogenesis. The expression of Wnt pathway components in the adult brain provided the circumstantial evidence that this pathway has important roles in adult brain function and homeostasis. These roles are now becoming increasingly apparent using experimental systems *in vitro* and *in vivo* and in human brain disorders. Furthermore, a deeper neurobiological understanding of canonical, non-canonical and novel Wnt pathways in normal brain will provide the basis for understanding pathological deregulation of the pathway in diseases of the brain. This knowledge will lead to therapeutic tinkering of the Wnt pathway, providing new hope for the development of approaches to better manage brain diseases such as tumorigenesis, neurodegeneration and some neuropsychiatric diseases.

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