

# Wnt Signal Pathways and Neural Stem Cell Differentiation

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## Key Words

Neural progenitors · Neural development · Signal pathways · Wnt molecules ·  $\beta$ -Catenin

## Abstract

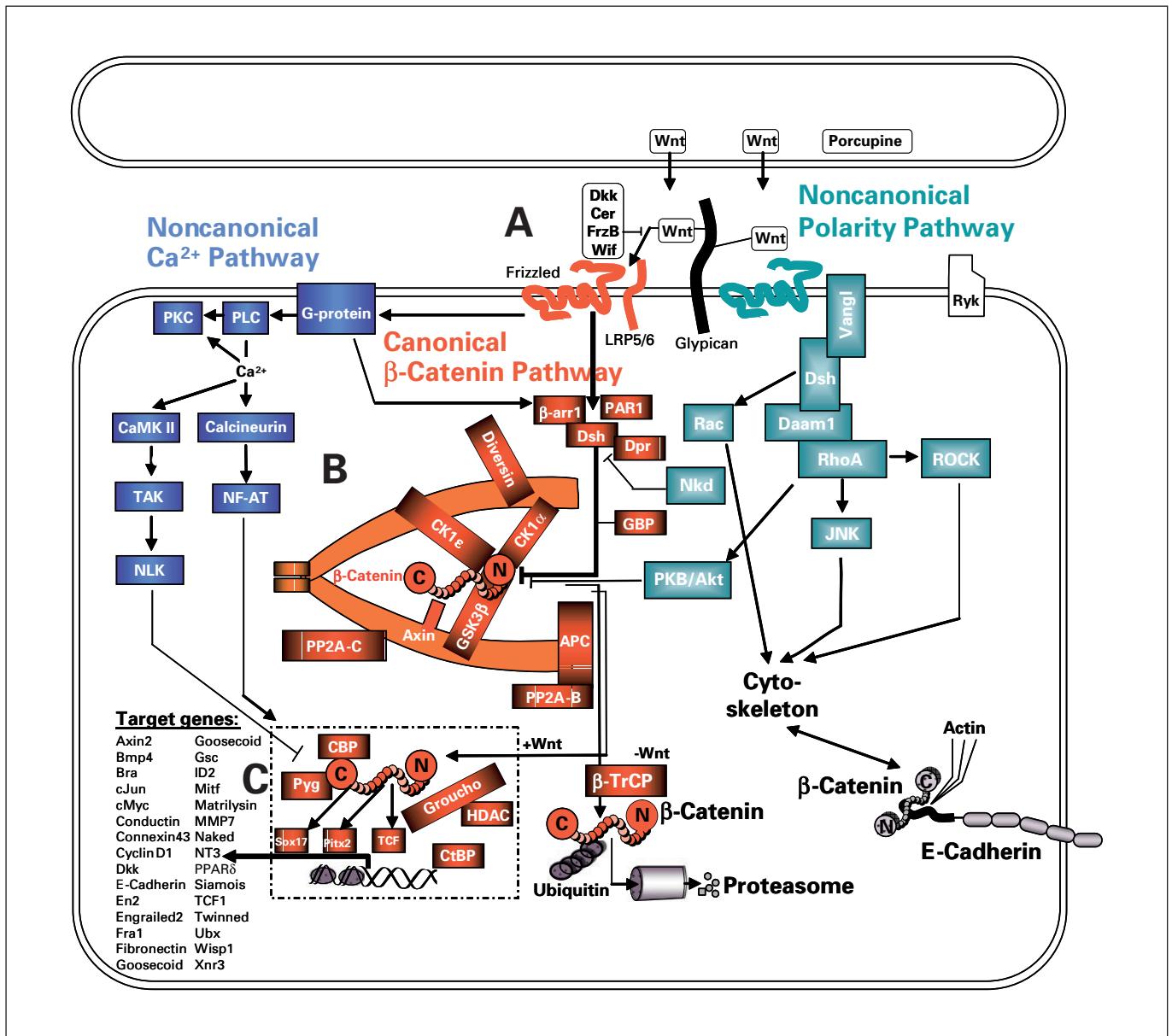
Self-renewal, migration and differentiation of neural progenitor cells are controlled by a variety of pleiotropic signal molecules. Members of the morphogen family of Wnt molecules play a crucial role for developmental and repair mechanisms in the embryonic and adult nervous system. A strategy of disclosure of the role of different canonical (glycogen synthase kinase-3 $\beta$ / $\beta$ -catenin-dependent) and noncanonical (Ca<sup>2+</sup>- and JNK-dependent) signal pathways for progenitor cell expansion and differentiations is illustrated at the example of the rat striatal progenitor cell line ST14A that is immortalized by stable retroviral transfection with a temperature-sensitive mutant of the SV40 large T antigen. A shift from permissive 33°C to nonpermissive 39°C leads to proliferation stop and start of differentiation into glial and neuronal cells. Investigation of expression of Wnts, Wnt receptors and Wnt-dependent signal pathway assay point to a stage-dependent involvement of canonical and noncanonical signaling in proliferation and differentiation of ST14A cells, whereby a mutual suppression of pathway activities is likely. Canonical Wnt molecules are not de-

tected in proliferating and differentiating ST14A cells except Wnt2. The noncanonical Wnt molecules Wnt4, Wnt5a and Wnt11 are expressed in proliferating cells and increase during differentiation, whereas cellular  $\beta$ -catenin decreases in the early phase and is restored in the late phase of differentiation. Accumulation of  $\beta$ -catenin at the membrane in undifferentiated proliferating cells and its nuclear localization in nondividing undifferentiated cells under differentiation conditions argues for a distinct spatially regulated role of the molecule in the proliferation and early differentiation phase. Ca<sup>2+</sup>-dependent and JNK-dependent noncanonical Wnt signaling is not detected during differentiation of ST14A cells. Complete exploration of the role of Wnt pathways, for differentiation of the neural progenitor cells ST14A will require Wnt overexpression and exposure of ST14A cells to exogenous Wnts either with purified Wnts or by co-cultures with Wnt producers.

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## Introduction

During recent years, a variety of signal molecules and pathways have been identified that ensure the maintenance of neural stem cells and control the development of neural tissues and progenitor cells. In particular, mem-



**Fig. 1.** Schematic representation of canonical and noncanonical Wnt pathways with indication of methodical approaches for pathway analysis in ST14A cells: detection of gene expression of Wnt and frizzled molecules by RT-PCR (A), detection of  $\beta$ -catenin by immunofluorescence and immunoblotting (B), and assessment of the  $\beta$ -catenin-dependent luciferase activity (C).

bers of the Wnt morphogen family have been found to play a key role in the regulatory networks controlling the self-renewal, migration and differentiation of the progenitor cells (table 1). Principally, canonical (glycogen synthase kinase-3 $\beta$ , GSK-3 $\beta$ / $\beta$ -catenin-dependent) and non-canonical pathways (mediated mainly via Ca<sup>2+</sup>/PKC or Rho GTPase/JNK) are induced by Wnt molecules divid-

ing the Wnt family into two major groups (fig. 1), whereby Wnt4, Wnt5a, Wnt6 and Wnt11 comprise the non-canonical Wnts and Wnt1, Wnt2, Wnt3a, Wnt7a and Wnt8a belong to the canonical ones [1]. The complete Wnt protein family consists of 19 members and not every member has been thoroughly tested for its signaling properties so far. Wnt molecules are glycoproteins that require lipid

**Table 1.** Overview on investigations of Wnt signalling in neural development

Tissue	Species	Time	Environment	Wnt	Pathway	Function	References
Blastocyst	mouse	<E5	in vitro	Wnt1	canonical	suppression of neuronal differentiation	Aubert et al., 2002
Blastocyst	mouse	<E5	in vitro in vivo		canonical ( $\beta$ -catenin)	suppression of neuronal differentiation	Hägele et al., 2003
Blastocyst	mouse	<E5	in vivo	Wnt3	canonical	differentiation of embryonic stem cells into dorsal interneurons	Murashov et al., 2005
Neural tube	chicken	E8.5, E9.5, E11.5	in vivo	Wnt1 Wnt3a	canonical	proliferation of neural precursor cells at dorsal midline	Megason and McMahon, 2002
Neural tube	mouse	E9.5, E10.5	in vivo in vitro	Wnt1	canonical	instruction of all neural crest stem cells to become sensory neurons on the expense of sympathetic progenitor cells	Lee et al., 2004
Neural tube	<i>Xenopus laevis</i>	stage 9–26	in vivo in vitro	Wnt11	noncanonical ( $Ca^{2+}$ , PCP)	neural crest cell delamination and migration	De Calisto et al., 2005
Neural tube	zebrafish	gastrula	in vivo	Wnt8	canonical	posteriorization	Erter et al., 2001
Ventral nerve cord	<i>Drosophila melanogaster</i>	stage 12 stage 14 stage 16	in vivo	Wnt5a	derailed receptor mediated	guidance of anterior commissure axons	Yoshikawa et al., 2003
Telencephalon	mouse	E11.5	in vitro	Wnt3a	canonical	differentiation of neurosphere cells into neurons and astrocytes	Muroyama et al., 2004
Telencephalon	mouse	E8.5, E9.5, E10.5, E11.5, E14.5, E16.5	in vivo		canonical ( $\beta$ -catenin)	dorsalization	Backman et al., 2005
Cortex	mouse	(E8.5) E12.5, E14.5, E15.5, E16.5, E17.5	(in vitro) in vivo		canonical ( $\beta$ -catenin)	proliferation of neural precursors (ventricular zone progenitors)	Chenn and Walsh, 2002
Cortex	mouse	E10.5, E11.5, E13, E16	in vitro in vivo	Wnt7a Wnt7b	canonical	proliferation and neurosphere formation of stem cells, maturation of ventricular to subventricular zone progenitors	Viti et al., 2003
Cortex	mouse	E10.5, E11.5, E13.5	in vivo in vitro	Wnt7a	canonical	differentiation of neural precursors	Hirabayashi et al., 2004
Hippocampus	rat		in vitro	no	$\beta$ -catenin/ cadherin	dendritic arborization and increase in axonal complexity	Yu and Malenka, 2004
Hippocampus	mouse		in vivo in vitro	no	$\beta$ -catenin/ cadherin	presynaptic assembly of synaptic vesicles	Bamji et al., 2003
Hippocampus	rat	E18	in vitro	no	GSK-3 $\beta$ inhibition	promotion of axon formation (establishing of neuronal polarity)	Jiang et al., 2005, Yoshimura et al., 2005
Cerebellum and pons	mouse	>P0	in vivo	Wnt7a	canonical	axonal remodeling and synapse formation between cerebellar granule cells and pontine mossy fibres	Hall et al., 2000

**Table 1** (continued)

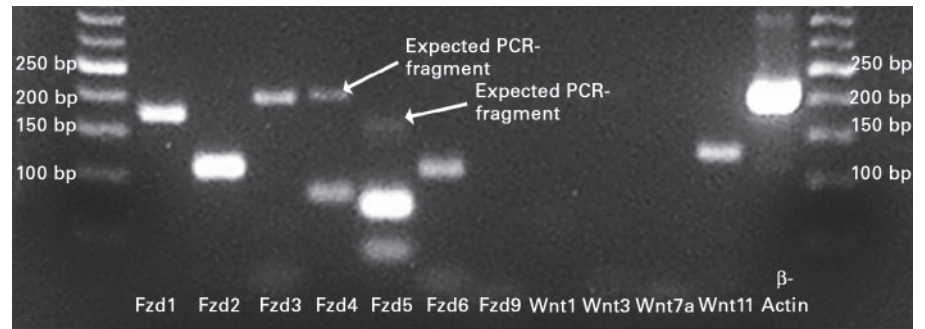
Tissue	Species	Time	Environment	Wnt	Pathway	Function	References
Ventral midbrain	mouse rat	E10.5 E14.5	in vivo in vitro	Wnt1 Wnt3a Wnt5a	canonical noncanonical	proliferation of neural progenitor cells differentiation of neural progenitor cells into dopaminergic neurons	Castelo-Branco et al., 2003
Ventral midbrain	rat mouse	E13.5 E14.5	in vitro		canonical (GSK-3 $\beta$ inhibition)	differentiation of neural progenitor cells into dopaminergic neurons	Castelo-Branco et al., 2004
Ventral midbrain	mouse rat	E13.5 E14.5	in vitro	Wnt3a Wnt5a	(canonical) noncanonical	differentiation of neural progenitor cells into dopaminergic neurons	Schulte et al., 2005
Striatum	mouse	E14.5	in vitro		canonical ( $\beta$ -catenin)	differentiation into neurons, promotion of astroglial and inhibition of oligodendroglial differentiation via BMP	Kasai et al., 2005
Neuroblastoma (N2A)	mouse	3 days	in vitro		noncanonical	differentiation into bipolar neurons	Fan et al., 2004

modification for tethering to the membrane proteoglycans and, therefore, for their full biological activity [2, 3]. They act mainly as intercellular short-range regulators, but they can also exert long-range activity via tissue-specific gradients [4]. Inactivation of Wnt molecules is induced by soluble receptor components of the Wnt-frizzled LPR5/6 ternary complex such as soluble frizzled-related proteins and some competitive and noncompetitive antagonists of the ternary complex such as Dickkopf [5]. Distinct Wnt molecules and their concentration gradients are involved in the embryonic neural development and probably also in adult repair and regeneration of damaged nervous tissue by inducing stem cell expansion as well as by controlling dorsalization [4, 6, 7] and posteriorization [8] of the neural tube, dendrite morphogenesis and axon guidance (establishing neuronal polarity), and neurite outgrowth and synaptogenesis. However, the function of single Wnts seems to depend on the type and developmental stage of the target cells rather than on the Wnt molecule itself, thereby ascribing multiple roles at multiple times to the same Wnt in different regions of the nervous system [1, 9]. Table 1 illustrates this statement by listing selective examples.

Initially, the majority of findings were consistent with the view that canonical Wnt signaling predominates in stem cell proliferation and neural progenitor cell expansion [10–15] and inhibits neural differentiation [16] to ensure maintenance of stem cell pluripotency, whereas neural migration [17] and neuronal differentiation is

driven by a block of canonical Wnt signaling [18, 19] and induction of noncanonical Wnt signaling [11, 20–22]. However, increasing evidence demonstrates opposite effects, e.g. it has been shown that canonical Wnts and  $\beta$ -catenin (1) are involved in development of murine cortical and hippocampal neuroepithelium [23], in instruction of murine neural crest stem cells to adopt the fate of sensory neurons [24] and in all-trans-retinoic acid-induced neuronal differentiation of human embryonal NT2/NTera2 tumor cells [25], (2) promote neuronal differentiation in neurosphere stem cells derived from mouse telencephalon [26], (3) induce differentiation of mouse embryonic stem cells into dorsal interneurons [6] and telencephalic precursors [27], (4) contribute to dopaminergic differentiation of rat midbrain progenitor cells [28], (5) direct neuronal differentiation of mouse cortical neural precursor cells [29], and (6) promote growth cone differentiation, axonal remodeling and synaptogenesis in the mouse cerebellum [30, 31]. Moreover, the  $\beta$ -catenin/cadherin complex enhances dendritic arborization as well as axonal length and complexity in developing rat hippocampal neurons [32] and presynaptic assembly of synaptic vesicles in mouse hippocampal neurons [33]. Inhibition of glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) promoted polarization and axon formation in rat hippocampal neurons, however in a  $\beta$ -catenin-independent manner [34, 35], pointing to regulation of alternative signal pathways by essential components of the canonical Wnt pathway. Of interest, an important interplay also seems to exist

**Fig. 2.** Gene expression of Wnt and frizzled molecules in proliferating neural progenitor cells, ST14A cells, as detected by RT-PCR.



between Wnt signaling and other signal pathways, such as those activated by bone morphogenetic proteins (BMPs) [6, 36, 37], sonic hedgehog [6, 38] and Notch [31, 39] during neural development. For example, in mouse embryonic stem cells,  $\beta$ -catenin and BMP activation was accompanied by inhibition of neural differentiation [16], whereas in mouse striatal progenitor cells,  $\beta$ -catenin induced neuronal differentiation and BMP expression, which in turn led to the induction of astroglial differentiation [40]. However, with regard to the development of dopaminergic neurons, Wnt pathways have recently attracted main attention. According to new findings, especially of the group of Arenas [11, 22, 28], both canonical and noncanonical Wnt molecules are key regulators of proliferation and differentiation of progenitors of mid-brain dopaminergic neurons [41]. In view of recent progress in experimental and clinical cell replacement therapy [42] it is important to unravel in detail the regulatory mechanisms of neural stem and progenitor cell expansion and differentiation in model systems of progenitor cell lines that are available for repeated disposal. Since Wnt-dependent signal pathways are expected to play a major role within these regulatory mechanisms, we have analyzed the expression of Wnts, Wnt receptors and Wnt-dependent signal pathway components on the transcriptional and protein level in the prototype progenitor cell line ST14A derived from embryonic rat striatum.

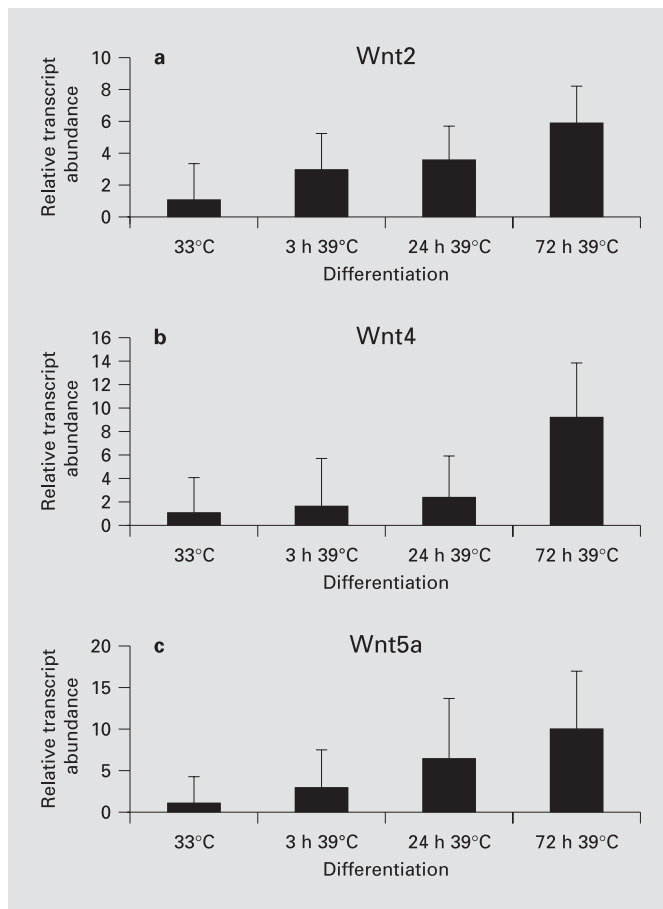
### ST14A Cells

Transplantation of embryonic stem cells is a promising strategy to restore functional deficits in neurodegenerative diseases like Parkinson's or Huntington's disease. Suitable cells for this cell replacement strategy should fulfill certain criteria such as (1) unlimited availability, (2) long-term stability in vitro, (3) standardized quality, (4)

suitability for genetic modification, (5) reproducible differentiation into desired neuronal cell types [43]. For experimental approaches, the neural progenitor cells ST14A, which are derived from embryonic day 14 rat striatum by stable retroviral transfection of a temperature-sensitive mutant of the SV40 large T antigen, meet most of these requirements. The inhibition of the immortalizing SV40 large T antigen by shifting the temperature from permissive 33°C to nonpermissive 39°C stops proliferation and induces differentiation into glial and neuronal cells [44, 45]. ST14A cells have been extensively characterized for their functional properties by our group [46–52], differentiation capacity [53, 54] and response to genetic modification by transfection with constructs of the neurotrophic factors ciliary neurotrophic factor [48, 49] and glia cell line-derived neurotrophic factor [50]. To explore the involvement of Wnt-dependent signal pathways in proliferation and differentiation of ST14A cells, the following approaches have been applied (fig. 1): (1) gene expression analysis of the majority of known Wnt receptors and the Wnt isoforms with known signaling properties by semiquantitative RT-PCR, (2) analysis of the intracellular  $\beta$ -catenin amount by Western blot and immunofluorescence, (3) assessment of  $\beta$ -catenin-dependent transcriptional activity by a TCF-driven luciferase reporter gene assay, (4) PKC and JNK activity by immunoblotting with phosphorylation-specific antibodies.

### Wnts and Wnt Receptors

Semiquantitative RT-PCR using a custom prevalidated RT-PCR Kit (SuperArray, Frederick, Md., USA) revealed that the majority of Frizzleds (Fzd1, 2, 3, 4, 5, 6, and 9) and the noncanonical Wnt11 are expressed in proliferating ST14A cells, whereas the canonical Wnts Wnt1, 3, and 7a were not detected (fig. 2). Absence of their ex-



**Fig. 3.** Gene expression of Wnt2 (a), Wnt4 (b) and Wnt5a (c) in neural progenitor cells, ST14A cells, under proliferative (permissive 33°C) and differentiating (nonpermissive 39°C) conditions by quantitative real-time RT-PCR (LightCycler technology). T-bars indicate SD; n = 3.

pression in ST14A was confirmed with positive controls from embryonic rat brain. The transcription profile of the canonical Wnt2 and the noncanonical Wnt4 and Wnt5a was estimated during cell differentiation between up to 72 h by quantitative real-time RT-PCR (Light Cycler 2.0, Roche, Mannheim, Germany) using SYBR Green® (Roche) for DNA staining (fig. 3). For Wnt5a, primers were designed on the basis of a newly detected exon-intron organization [51]. The expression of Wnt2, 4 and 5a increased after the start of differentiation, but with a certain delay for Wnt4. However, after 72 h the induction of Wnt4 and Wnt5a expression was higher than that of Wnt2 expression. Wnt11 expression decreased during differentiation and the canonical Wnt molecules Wnt1, 3 and 7a were not detected, either during proliferation or

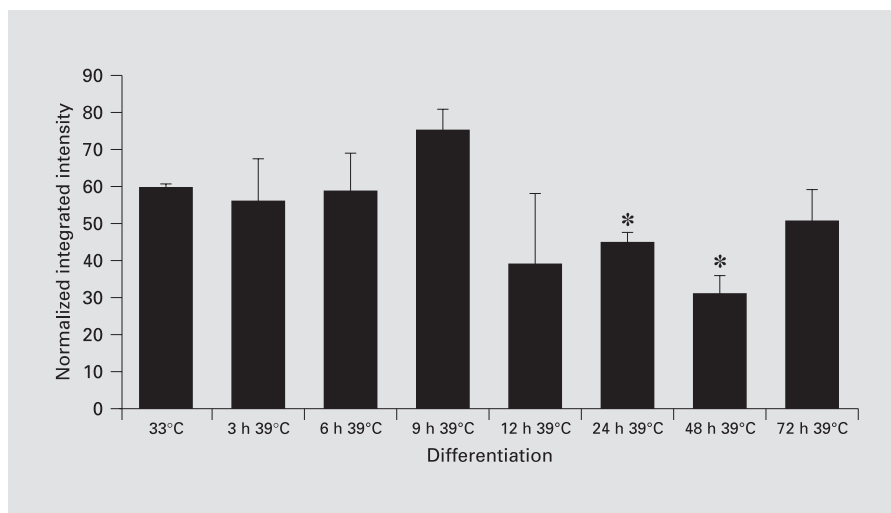
under differentiation conditions by semiquantitative RT-PCR (fig. 2 and data not shown). The obtained results show that various Wnts and the Wnt receptors of the Frizzled protein family are expressed at mRNA level under proliferation permissive as well as nonpermissive conditions. However, it is difficult to conclude solely from these data the functional activation of downstream signaling pathways, because it is not clear which Wnt can efficiently cooperate with which Frizzled to activate certain signaling events. In fact, it is still uncertain whether the Wnt isoform, the respective Frizzled isoform or the combination of both determines if canonical or noncanonical pathways are activated. Therefore, we analyzed the activation of known Wnt signaling events by endogenous Wnt signaling in ST14A cells in more detail.

### Canonical Wnt Signaling

After induction of cell differentiation by temperature shift from 33 to 39°C the total cellular  $\beta$ -catenin changed biphasically with an early increase (up to 9 h), intermediate decrease (until 48 h) and late recovery (until 72 h) (fig. 4), as detected by quantitative Western blot using a primary monoclonal antibody from mice against  $\beta$ -catenin (Santa Cruz, Heidelberg, Germany) together with a primary polyclonal rabbit anti- $\beta$ -actin antibody (Delta Biolabs, Gilroy, Calif., USA). The bound antibodies were detected with Alexafluor 680-coupled goat antimouse IgG antibody (Molecular Probes, Invitrogen, Karlsruhe, Germany) and IRDye800-coupled goat anti-rabbit IgG antibody (Molecular Probes), respectively, on the same blotting membrane. Visualization was done by the Odyssey Infrared Imaging System (LI-COR Biosciences, Bad Homburg, Germany). The diagram in figure 4b shows the  $\beta$ -catenin immunoreactivity normalized to the reference protein  $\beta$ -actin. The results show a decrease in the intracellular amount of  $\beta$ -catenin from 9 h at 39°C cultivation temperature to 24 h after temperature increase.

Immunofluorescence staining with the same primary monoclonal anti- $\beta$ -catenin antibody (Santa Cruz) as used for Western blot and the secondary polyclonal rabbit antimouse IgG antibody (Invitrogen) revealed that proliferating cells accumulated  $\beta$ -catenin at the plasma membrane (fig. 5a), connecting the actin cytoskeleton to cadherin in homophilic adherens junctions [55], and are released to the cytoplasm after the start of differentiation, giving rise to the increase in the early differentiation phase. Nuclear translocation of  $\beta$ -catenin did not occur in dividing cells at 33°C (fig. 5b) but in a subset of mor-

**Fig. 4.** Expression of  $\beta$ -catenin in proliferating (33°C) and differentiating (39°C) ST14A cells as detected by Western blot after staining of  $\beta$ -catenin and  $\beta$ -actin applying respective primary antibodies with near-infrared dye-conjugated secondary antibodies. Quantitative evaluation was performed by background subtraction and normalization to  $\beta$ -actin using Odyssey Infrared Imaging System (LI-COR). T-bars indicate SD;  $n = 2$ . Asterisks indicate significant differences ( $p = 0.020$  and  $p = 0.016$  for 9 and 24 h, respectively) in comparison with proliferating cells (33°C) as tested by Student's two-sided t test for independent samples with no significantly different SDs according to the F test.



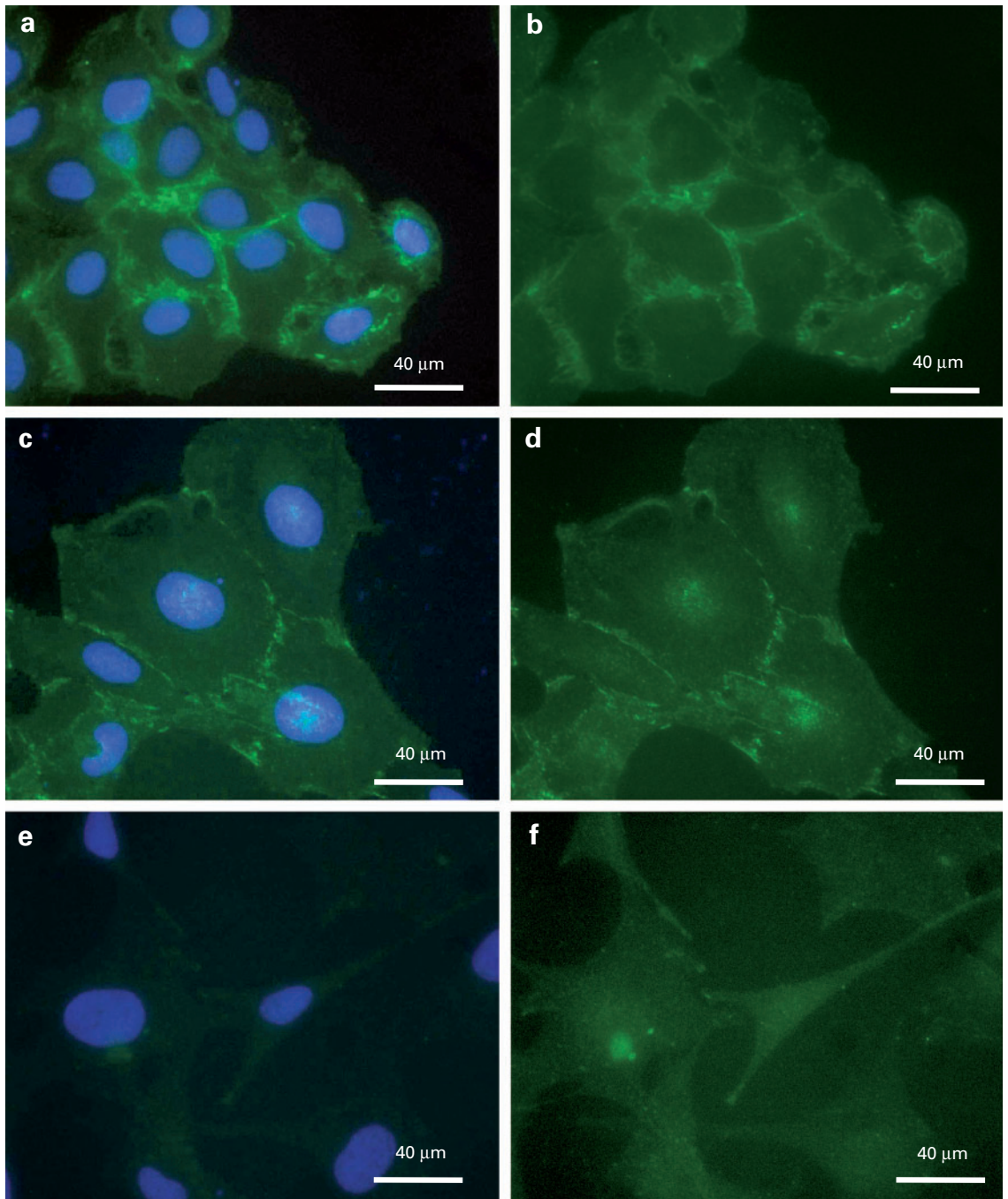
phologically undifferentiated cells after 3 days at 39°C (fig. 5c, d). In differentiated cells, no nuclear  $\beta$ -catenin accumulation was detectable (fig. 5e, f). With progression of the temperature-induced differentiation of the ST14A cell line, most of the cells remodel their morphology from flat cells with a broad cytoplasmic hem and extensive cell-cell contacts (fig. 5a, b) to small cells with extensions and much reduced contact between cells (fig. 5e, f). Additionally, the homogenic cell population at 33°C diversifies into glial and neuronal lineages. Both processes lead to disintegration of the cadherin-mediated homophilic adhesion junctions. This may lead to the loss of membrane-bound  $\beta$ -catenin (fig. 5e, f). The degradation of previously cadherin-bound  $\beta$ -catenin in the cytoplasm could also account for the observed loss of  $\beta$ -catenin as detected by Western blot (fig. 4).

TCF-dependent transcription was analyzed by the TOPFlash reporter gene assay [56]. Briefly, the cells were transfected with the TOPflash or a control vector (FOP-Flash) containing mutated TCF binding sites together with the pRL-TK vector that encodes constitutively expressed Renilla luciferase (Promega, Madison, Wisc., USA). After 24 h, the cells were either directly harvested or subjected to differentiation by temperature increase for various time periods or cultured at the permissive temperature of 33°C for the same time period as the differentiating cells. The activity of the firefly luciferase (TOPflash/FOPflash) and Renilla luciferase were measured using the luminometer LB 9508 (Berthold, Bad Wildbad, Germany) and the Dual Luciferase Assay Kit (Promega.) Analysis showed that TCF-dependent transcription was suppressed in undifferentiated ST14A cells

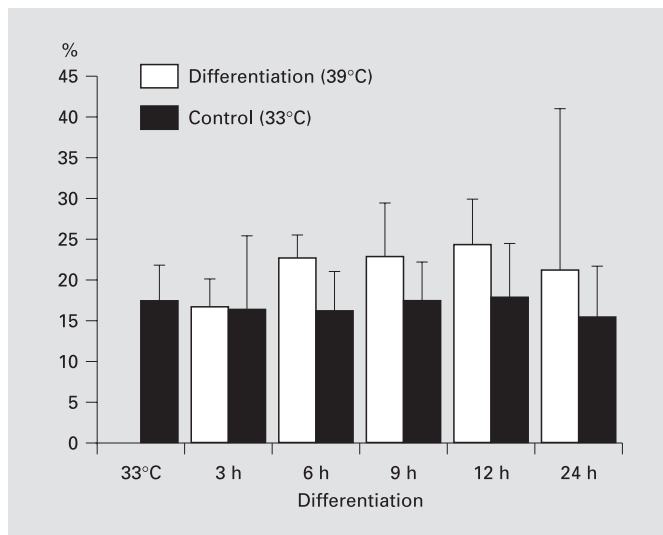
at 33°C and that it was not induced during early differentiation (within 1 day at 39°C) of the cells (fig. 6). Later phases of the differentiation could not be investigated with the applied TCF optimal promoter reporter construct (TOPFlash) due to the transient transfection of the reporter plasmid. However, the increased expression of Wnt2 between 24 and 72 h of differentiation (fig. 3b) could indicate that canonical Wnt signaling is involved in later phases of ST14A cell differentiation perhaps in a subpopulation of cells and after exceeding a threshold concentration of Wnt2 [25].

### Noncanonical Wnt Signaling

To investigate the  $\text{Ca}^{2+}$ -dependent Wnt pathway, we analyzed the activity of  $\text{Ca}^{2+}$ -dependent conventional PKC isozymes (cPKCs) by assessing specifically phosphorylated consensus motifs of cPKC substrates [57] by quantitative Western blot using a rabbit polyclonal antibody against a phosphorylated cPKC substrate consensus motif (Cell Signaling Technology, Beverly, Mass., USA) together with a murine monoclonal anti- $\beta$ -actin antibody (Sigma, St. Louis, USA). The bound antibodies were detected with Alexafluor 680-coupled goat antirabbit IgG antibody and IRDye800-coupled goat-anti mouse IgG antibodies (both from Molecular Probes), respectively, on the same blotting membrane. Visualization was done by the Odyssey Infrared Imaging System (LI-COR). Two phosphorylated substrates of 35 and 70 kDa were detected in proliferating ST14A cells. Phosphorylated 70-kDa substrates decreased from 12 h of differentiation onward



**Fig. 5.** Expression of  $\beta$ -catenin in ST14A cells as detected by immunofluorescence: proliferating undifferentiated cells at 33°C (**a, b**), nonproliferating undifferentiated cells after 3 days at 39°C (**c, d**), differentiated cells after 3 days at 39°C (**e, f**). Cells were stained with monoclonal anti- $\beta$ -catenin antibody (Santa Cruz, Heidelberg, Germany) and Alexafluor 488-conjugated rabbit antimouse-IgG (Invitrogen) and counterstained for nuclear DNA with DAPI (Vectashield Mounting Medium, Vector Laboratories, Burlingame, Calif., USA).



**Fig. 6.**  $\beta$ -Catenin-dependent TCF-driven transcriptional activity in proliferating and differentiating ST14A cells as detected by TOP-Flash luciferase reporter assay. T-bars indicate SD; n = 3.

and phosphorylated 35-kDa substrates did not change during differentiation (data not shown). JNK as a common downstream target of alternative noncanonical Wnt pathways was investigated for activation by detecting its phosphorylation at the Tyr-183 residue using the quantitative Western blot method. This analysis revealed no activated JNK in proliferating, and in differentiating cells (data not shown). In either case the activation profile of reported targets of noncanonical Wnts does not reflect the expression of noncanonical Wnts in our study. However, the stable overexpression of Wnt5a in the HEK-293 subclone Flp-In 293 (Invitrogen) confirmed the activation of cPKCs and JNK by Wnt5a using our methodology (data not shown). Since simultaneous activation of canonical and noncanonical Wnt pathways and different PKC isoforms may lead to antagonistic effects [58–60], final elucidation of Wnt functions in ST14A cell differentiation will require gain-of-function experiments by selective Wnt overexpression or supplementation of recombinant purified Wnts.

## Conclusions

We conclude that different Wnt molecules and Wnt-dependent signal pathways are involved in the different phases of self-renewal and differentiation of neural stem cells. Whereas the noncanonical Wnts Wnt4, Wnt5a and

Wnt11 are expressed in proliferating and differentiating ST14A cells, the canonical Wnt2 isoform is selectively and increasingly expressed in differentiating ST14A cells. Therefore, the activation of the canonical Wnt pathway seems to be characteristic for the *in vitro* differentiation of ST14A neural progenitor cells, especially for its late phase. Noncanonical Wnt pathways have obviously a role in both, the replication of ST14A cells as well as regulation of their differentiation. However, in ST14A cells the activation of reported downstream targets of noncanonical Wnts has not been detected in this study. Thus, either noncanonical Wnts are expressed and secreted by ST14A cells, but not functional, or the intracellular signaling cascades triggered by these Wnts in neural progenitor cells differ from those found in early embryos and standard cell lines such as HEK 293 and NIH3T3 cells [58, 59, 61–63]. We found that overexpression of Wnt5a in a HEK 293 subclone readily activated cPKC and JNK. Interestingly, in a recent study purified biologically active Wnt5a applied to midbrain-derived neural progenitor cells failed to activate PKC and JNK [22]. This is in accordance with the view of Nusse [1] that the outcome of Wnt signaling is mainly determined by the responding cell. Since Wnt5a has a proven role in the maturation of ventral midbrain progenitor cells to dopaminergic neurons [11, 64], the elucidation of signaling properties of noncanonical Wnts in neural progenitor cells is urgently required. To explore a possible role of noncanonical Wnts, especially Wnt5a, in the differentiation of ST14A cells into neuronal cells, Wnt overexpression and exposure of ST14A cells to exogenous Wnts will be required either with purified Wnts or by co-cultures with Wnt producers. In this approach, the activated signaling pathways will be identified by proteome analysis of enriched phosphoproteins in combination with screening for other activated signaling components. Further perspectives are the modulation of Wnt signaling pathways by small molecules with the final aim to enhance the differentiation capacity of donor neural progenitor cells and neighboring cells after transplantation in therapeutic trials of neurodegenerative diseases.

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