

## Appendix A14.15

### The Wnt Signaling Pathway: An Overview

The Wnt signaling pathway is highly conserved, and plays a critical role in normal development in diverse species from *Drosophila* to human. In humans, the Wnt protein family is composed of at least 15 secreted glycoproteins (Wodarz and Nusse, 1998). These proteins bind to the frizzled family of extracellular receptors, resulting in a signal that is transduced via an intracellular protein, disheveled. Signaling through disheveled results in inhibition of the enzyme glycogen synthase kinase (GSK), which is also evolutionarily conserved, is found in species ranging in diversity from *Dictyostelium* to humans, and is found in two forms:  $3\alpha$  and  $3\beta$ . Both GSK isozymes were originally identified based on their ability to phosphorylate—and thereby inactivate—glycogen synthase, resulting in stimulation of the formation of glycogen. While most research has focused on the  $3\beta$  isoform, available evidence suggests that the two forms may have very similar—though not identical—biological properties (Plyte and others, 1992; Ali and others, 2001).

GSK- $3\beta$  is a serine/threonine kinase that is believed to be, in general, constitutively active in cells. Phosphorylation of GSK- $3\beta$  by other kinases (including PKC and Akt) generally results in a decrease in its activity. Thus, the activity of GSK- $3\beta$  can be regulated by the action of a variety of other cellular signaling mechanisms, which may serve to fine-tune this constitutively active enzyme's activity (Woodgett, 2001). One of GSK- $3\beta$ 's major substrates is  $\beta$ -catenin, which is found in two functional reservoirs within the cell—a cytoplasmic and a membrane associated pool. The cytoplasmic pool is involved in transmitting the Wnt signal to the nucleus, while the membrane-associated pool interacts with cadherin to provide structural support in cell adhesions. Phosphorylation of  $\beta$ -catenin by GSK- $3\beta$  is regulated by a complex that includes axin, adenomatous polyposis coli (APC), frequently rearranged in T-cell lymphoma (FRAT1), disheveled, casein kinase I $\epsilon$ , and protein phosphatase-2A (PP2A) (Peifer and Polakis, 2000).

Phosphorylation of  $\beta$ -catenin by GSK- $3\beta$  results in its destruction in an ubiquitin-dependent manner, thereby terminating/inhibiting the actions of this important mediator of the Wnt signaling pathway (Peifer and Polakis, 2000; Sharpe and others, 2001).  $\beta$ -catenin binds with the T-cell factor/lymphoid enhancer factor (tcf/lef) transcription factor that translocates to the nucleus and acts as a transcription factor at lef/tcf sites in the promoter of a number of genes (Novak and Dedhar, 1999; Harwood, 2001). Activation of the Wnt pathway in early development modulates organizers, which are groups of cells that provide instructions for early

development and organization of the surrounding tissue, and thereby regulates cell fate determination and body patterning (Cadigan and Nusse, 1997; Wodarz and Nusse, 1998).

While this linear description of Wnt signaling—the canonical pathway—has received the most attention, recent evidence suggests that Wnt signal may interact with other signaling pathways. For example, at least in certain systems, phosphoinositol and  $\text{Ca}^{2+}$  signaling appear to be activated by Wnt-stimulation of the frizzled family of receptors (Kuhl and others, 2000; Patapoutian and Reichardt, 2000).

### **Wnt Signaling in the Mature CNS**

GSK-3 $\beta$  is highly expressed in the adult brain (Ali et al., 2001), and is known to phosphorylate a number of important cytoskeletal proteins, including three major microtubule-associated proteins: Tau, MAP-1B and MAP-2 (Salinas and Hall, 1999). Recent studies have suggested that changes in GSK-3 $\beta$ -mediated MAP-1B phosphorylation are associated with the loss and/or unbundling of stable axonal microtubules (Lucas and others, 1998). Furthermore, GSK-3 $\beta$  inhibition results in the accumulation of synapsin I, a protein involved in synaptic vesicle docking and release, at growth cone-like areas (Lucas and Salinas, 1997). Wnt-7a may mimic many of these actions, as this protein has recently been shown to induce axon and growth cone remodeling (Hall and others, 2000). Although additional research is clearly required, these data suggest that Wnt signaling through GSK-3 $\beta$  plays important roles in axonal remodeling and regulation of synaptic connectivity.

In addition to its apparent role in regulating synapse formation and axonal growth in developing systems, there has been considerable recent excitement regarding the role of GSK-3 $\beta$  in regulating cell death in mature neuronal tissue, and for the development of GSK-3 $\beta$  inhibitors as novel therapeutic agents for neurodegenerative diseases. Although it was initially reported in 1993 that GSK-3 $\beta$  activity was required for  $\beta$ -amyloid induced neurotoxicity in primary hippocampal neurons (Takashima and others, 1993), these observations were not followed up until very recently. Indeed, it is quite likely that the demonstration in 1996 that GSK-3 $\beta$  is a target for lithium's actions (Klein and Melton, 1996; Phiel and Klein, 2001) has greatly contributed to the resurgence in interest of GSK-3 $\beta$  as a potential therapeutic target. Recent studies have demonstrated that GSK-3 $\beta$  may regulate cell death beyond its role in  $\beta$ -amyloid induced toxicity. For example, GSK-3 $\beta$  overexpression was found to induce apoptosis in Rat-1 and PC-12 cells; additionally, dominant negative GSK-3 $\beta$  mutants prevented apoptosis following inhibition of PI 3-kinase in these cells (Pap and Cooper, 1998). Furthermore, the expression of

FRAT-1, a protein that is thought to interact with the  $\beta$ -catenin/axin/GSK-3 $\beta$  complex and inhibit GSK-3 $\beta$  in a substrate specific manner, also rescues primary sympathetic neurons from PI 3-kinase inhibition induced cell death (Crowder and Freeman, 2000). A number of endogenous growth factors (e.g. nerve growth factor and brain derived neurotrophic factor [BDNF]) utilize the PI 3-kinase signaling cascade as a major effector system. Thus, growth factors may bring about many of their neurotrophic/neuroprotective effects, at least in part, by GSK-3 $\beta$  inhibition. Consistent with such a contention, serum deprivation or PI 3-kinase induced apoptosis is attenuated by either a dominant negative form of GSK-3 $\beta$  or an inhibitory GSK-3 $\beta$  binding protein (Hetman and others, 2000). Neuronal apoptosis in all three of these experiments was decreased following exposure to either a dominant negative form of GSK-3 $\beta$  or an inhibitory GSK-3 $\beta$  binding protein. In this regard, as discussed later in this review, lithium is neuroprotective in many preclinical models (Manji and others, 1999b).

Although the study of the effects of selective small molecule GSK-3 $\beta$  inhibitors is still in its infancy, the available data suggest that pharmacological inhibition of GSK-3 $\beta$  also exerts neuroprotective effects. Two novel inhibitors of GSK-3 $\beta$  have been demonstrated to protect primary sensory and granule neurons from potassium deprivation or phosphatidylinositol 3-kinase induced cell death (Cross and others, 2001).

As discussed already, GSK-3 $\beta$  is the target of multiple signaling systems, and the demonstration of GSK-3 $\beta$ 's role in neuronal survival and apoptosis does not necessarily implicate the canonical Wnt signaling cascade per se in mediating these effects. In an attempt to address the specific role of Wnt in the regulation of apoptosis, a recent study reported that Rat-1 cell lines stable expressing Wnt-1 were resistant to vincristine- and vinblastine-regulated apoptosis and that these findings appeared to be mediated by Tcf mediated transcription (Chen and others, 2001). By contrast, another study suggested pro-apoptotic effects of up-regulation of components of the Wnt pathway (van Gijn and others, 2001). Although additional studies are clearly needed, these findings suggest that the precise manner in which the Wnt signaling pathway regulates cell survival/cell death may be both development stage and cell line/cell type specific.

Perhaps the strongest evidence suggesting that the Wnt signaling pathway may play a major role in cell survival comes from the observations that mutations in Wnt pathway genes are associated with a large number of tumors. For example, over 80 percent of colorectal carcinomas have mutations in the APC or  $\beta$ -catenin genes (Miyoshi and others, 1992; Polakis, 2000). APC and  $\beta$ -catenin mutations are also found in medulloblastomas and neuroectodermal tumors, albeit at a much lower percentage (Huang and others, 2000; Koch and others, 2001). Finally, Turcot's

syndrome, characterized clinically by the co-occurrence of primary brain tumors and multiple colorectal adenomas, appears to be caused in most cases by defects in the APC gene (Hamilton and others, 1995). At this point, it should be emphasized that these tumors arise from mutations in the Wnt signaling pathway which dramatically alter its functioning. There is no data to suggest that subtle, reversible modulation of the pathway pharmacologically is associated with tumor formation.

Table A14.15a

GSK-3 Inhibition is Neuroprotective

- GSK-3 $\beta$  activity required for  $\beta$ -amyloid induced neurotoxicity in primary hippocampal cultures (Takashima et al., 1993)
- GSK-3 $\beta$  overexpression induces apoptosis in Rat-1 and PC-12 cells (Pap and Cooper, 1998)
- Dominant negative GSK-3 $\beta$  prevents apoptosis following inhibition of PI3K (Pap and Cooper, 1998)
- FRAT-1 (a protein that interacts with the b-catenin/axin/GSK-3 $\beta$  complex) rescues primary sympathetic neurons from PI3K inhibition induced cell death (Crowder and Freeman, 2000)
- Dominant negative form of GSK-3 $\beta$  or an inhibitory GSK-3 $\beta$  binding protein attenuates serum deprivation of PI3K induced apoptosis (Hetman et al., 2000)
- Synthetic GSK-3 $\beta$  inhibitors protect primary sensory and granule neurons from potassium deprivation of PI3K induced cell death (Cross et al., 2001)
- Rat-1 cells stable overexpressing Wnt-1 are resistant to vincristine and vinblastine apoptosis (Chen et al., 2001)

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