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GSK-3: functional insights from cell biology and animal models

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Glycogen synthase kinase-3 (GSK-3) is a widely expressed and highly conserved serine/threonine protein kinase encoded in mammals by two genes that generate two related proteins: GSK-3α and GSK-3β. GSK-3 is active in cells under resting conditions and is primarily regulated through inhibition or diversion of its activity. While GSK-3 is one of the few protein kinases that can be inactivated by phosphorylation, the mechanisms of GSK-3 regulation are more varied and not fully understood. Precise control appears to be achieved by a combination of phosphorylation, localization, and sequestration by a number of GSK-3-binding proteins. GSK-3 lies downstream of several major signaling pathways including the phosphatidylinositol 3′ kinase pathway, the Wnt pathway, Hedgehog signaling and Notch. Specific pools of GSK-3, which differ in intracellular localization, binding partner affinity, and relative amount are differentially sensitized to several distinct signaling pathways and these sequestration mechanisms contribute to pathway insulation and signal specificity. Dysregulation of signaling pathways involving GSK-3 is associated with the pathogenesis of numerous neurological and psychiatric disorders and there are data suggesting GSK-3 isoform-selective roles in several of these. Here, we review the current knowledge of GSK-3 regulation and targets and discuss the various animal models that have been employed to dissect the functions of GSK-3 in brain development and function through the use of conventional or conditional knockout mice as well as transgenic mice. These studies have revealed fundamental roles for these protein kinases in memory, behavior, and neuronal fate determination and provide insights into possible therapeutic interventions.

Keywords: GSK-3, signal transduction, animal models, behavior

CHARACTERIZATION OF GSK-3

Glycogen synthase kinase-3 (ATP:protein phosphotransferase, E.C. 2.7.1.37) is a serine/threonine protein kinase, belonging to the CMCG family of proline-directed kinases (Cyclin-dependent kinases (CDKs), Mitogen-activated protein kinases (MAPKs), Glycogen synthase kinases (GSKs), and CDK-like kinases (CLKs). GSK-3 is a monomeric, second messenger-independent protein kinase that was first discovered through its ability to activate the ATP–Mg-dependent form of type-1 protein phosphatase ("Factor A") and to phosphorylate the key rate-limiting metabolic enzyme that catalyzes the last step of glycogen synthesis, glycogen synthase (GS; Embi et al., 1980; Rylatt et al., 1980; Vandenheede et al., 1980).

GSK-3 is a highly conserved protein kinase and has orthologs in plants, fungi, worms, flies, sea squirts, and vertebrates: isoenzymes from species as distant as flies and humans display more than 90% sequence similarity within the protein kinase domain (reviewed in Ali et al., 2001). GSK-3/F_A has been purified by several groups (Hemmings et al., 1982; Woodgett and Cohen, 1984; Tung and Reed, 1989), and was molecularly cloned based on partial peptide sequencing (Woodgett, 1990). Two rat brain cDNAs encoding GSK-3 were isolated that corresponded to isoenzymes designated GSK-3 alpha(α) and GSK-3 beta(β), with apparent M_r of 51,000 Da (483 aa in humans) and 47,000 Da (433 aa), respectively (Woodgett, 1990). Genes encoding GSK-3α and β are located on mouse chromosome 7/human chromosome 19 and on mouse chromosome 16/human chromosome 3, respectively (see **Figure 1**; based on http://genome.ucsc.edu). Mammalian GSK-3 is therefore represented by two paralogous proteins. Overall, GSK-3α and β share 85% overall sequence homology, including 98% amino acid sequence identity within their kinase domains (Woodgett, 1990). This latter fact makes the likelihood of development of small molecule inhibitors that can discriminate the two forms very unlikely. Despite their catalytic domain similarity, GSK-3 isoenzymes diverge in their N- and C-termini. For example, the two gene products share only 36% identity in the last 76 C-terminal residues (Woodgett, 1990). GSK-3α has an extended glycine-rich N-terminal region that has been proposed to function as a pseudosubstrate (Dajani et al., 2001). Interestingly, while many species such as fish, amphibians, and lizards harbor both isoforms, birds have only GSK-3β and appear to have selectively lost GSK-3α (Alon et al., 2011).

GSK-3 is expressed ubiquitously and both gene products are found in virtually all mammalian tissues. The kinase is highly expressed in the brain (Woodgett, 1990; Yao et al., 2002; Perez-Costas et al., 2010), both in neurons and glia (Ferrer et al., 2002). In the developing brain, the presence of GSK-3 is high at E18 and

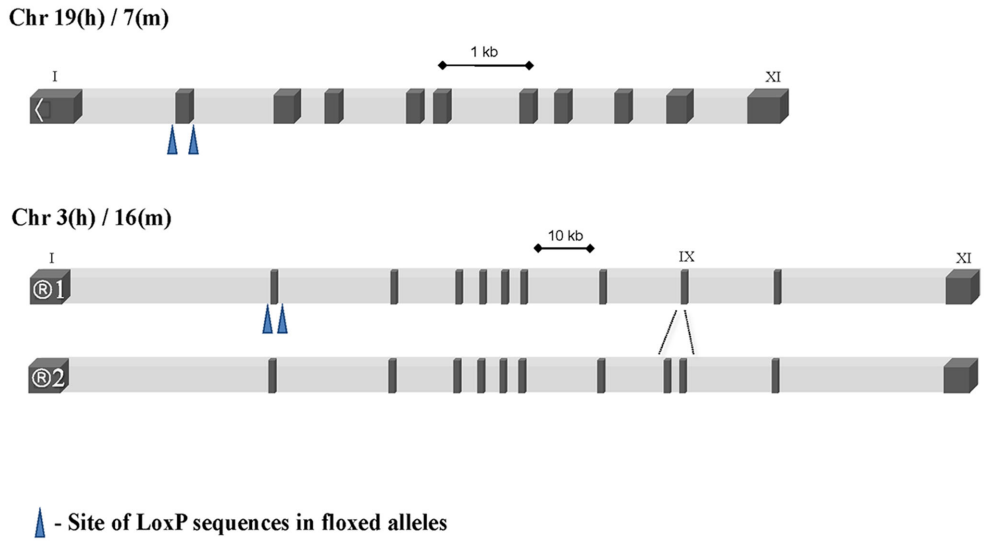

FIGURE 1 | Intron/exon structure of the two mammalian GSK-3 genes indicating the differential splice of GSK-3β and location of LoxP recombination sites in conditional alleles.

peaks at P8, decreasing somewhat after that period (Takahashi et al., 1994). In adult brain, GSK-3α is especially abundant in the hippocampus, cerebral cortex, striatum, and cerebellum (based on Allen Brain Atlas). GSK-3β is expressed in nearly all brain regions, although there are marked regional differences of GSK-3β mRNA levels in the human brain (Pandey et al., 2009). As a caution, the glycine-rich (and hence purine-rich) region of GSK-3α may distort comparative analysis of RNA expression between it and GSK-3β.

In certain cell types of the brain, alternative splicing between exon 8 and 9 of GSK-3β leads to the generation of an additional "long" form containing a 13 amino acid insert within the catalytic domain (GSK-3β2; see **Figure 1**). This insert is located between residues 303 and 304 of GSK-3β, and is flanked by two proximal α-helices of kinase subdomains X and XI (Hanks and Hunter, 1995; Mukai et al., 2002). This alternatively spliced isoform of GSK-3β in rodents (Mukai et al., 2002; Yao et al., 2002) and in human (Lau et al., 1999; Schaffer et al., 2003; Kwok et al., 2005) has been implicated in neuronal-specific functions. The short form of GSK-3β is ubiquitously expressed in the body, including the developing and adult nervous system (Takahashi et al., 1994; Leroy and Brion, 1999). By contrast, GSK-3β2 is predominantly expressed in the neural tissues, with highest levels in the developing brain and persistence into adulthood (Mukai et al., 2002; Wood-Kaczmar et al., 2009).

GSK-3 REGULATION

An unusual feature of GSK-3 is that the kinase displays high activity in cells under resting/unstimulated conditions (Sutherland et al., 1993; Stambolic and Woodgett, 1994; Woodgett, 1994) and is one of few protein kinases that is inhibited by extracellular signals that induce a rapid and reversible increase in serine phosphorylation of GSK-3 causing a decrease in enzymatic activity. For example, growth factor, insulin, or serum treatment decreases GSK-3 activity by 30–70% within 10 min (Sutherland et al., 1993; Welsh and Proud, 1993; Saito et al., 1994; Stambolic and Woodgett, 1994; Sutherland and Cohen, 1994; Cross et al., 1995; Eldar-Finkelman et al., 1995). The mechanisms of GSK-3 regulation are varied and not yet fully understood; precise control appears to be achieved by a combination of phosphorylation, localization, and sequestration by a number of GSK-3-binding proteins (reviewed in Frame and Cohen, 2001; Doble and Woodgett, 2003; Jope and Johnson, 2004; Kockeritz et al., 2006).

REGULATION THROUGH PHOSPHORYLATION

GSK-3 is dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation. The activity of GSK-3 is positively regulated by phosphorylation on a "T loop" tyrosine residue within subdomain VIII (Tyr279 for GSK-3α and Tyr216 for GSK-3β; Hughes et al., 1993; Lochhead et al., 2006). The kinase is negatively regulated by N-terminal phosphorylation of serine residues of the enzyme (Ser21 for GSK-3α and Ser9 for GSK-3β; Sutherland et al., 1993; Sutherland and Cohen, 1994). p38 MAPK can also inactivate GSK-3β via phosphorylation within its C-terminal region at Ser389 and Thr390 (Thornton et al., 2008). p38 MAPK-mediated phosphorylation of GSK-3β occurs primarily in the brain and thymocytes (Thornton et al., 2008). In addition, Thr43 of GSK-3β may be phosphorylated by Erk, resulting in GSK-3 inhibition (Ding et al., 2005).

From the crystal structure, it has been proposed that unphosphorylated Tyr276/Tyr216 act to block the access of primed substrates (as discussed below). Indeed, the structure of phosphorylated GSK-3β (Bax et al., 2001) shows that phosphorylated Tyr216 undergoes a conformational change that allows substrates to bind the enzyme. Previous studies, however, led to conflicting conclusions as to whether tyrosine phosphorylation of GSK-3 is catalyzed by GSK-3 itself (autophosphorylation) or by a distinct tyrosine kinase (Hughes et al., 1993; Kim et al., 1999; Lesort et al., 1999;

Wang et al., 2003; Cole et al., 2004a). In support of the autophosphorylation model, at least in mammals, Lochhead et al. (2006) showed that newly synthesized GSK-3β autophosphorylated itself on tyrosine and that this event could be prevented by exposure to GSK-3 inhibitors.

In contrast to tyrosine phosphorylation, regulation of N-terminal serine phosphorylation is only conserved in GSK-3 homologs from mammals, *Xenopus*, and *Drosophila*, but not in yeast, higher plants, *Dictyostelium*, or *Caenorhabditis elegans*. The phosphorylation state of serine residues of both isoenzymes is dynamic, involving phosphorylation by several protein kinases and dephosphorylation by protein phosphatase-1 (PP-1; Sutherland et al., 1993; Saito et al., 1994; Stambolic and Woodgett, 1994; Cross et al., 1995; Eldar-Finkelman et al., 1995; Welsh et al., 1998; Zhang et al., 2003). N-terminal domain serine phosphorylation of GSK-3α and GSK-3β leads to inhibition of its activity (Sutherland et al., 1993; Saito et al., 1994; Stambolic and Woodgett, 1994; Cross et al., 1995; Eldar-Finkelman et al., 1995; Welsh et al., 1998; Grimes and Jope, 2001; Zhang et al., 2003). Phosphorylation of GSK-3 within its N-terminal region creates a "pseudosubstrate" which intramolecularly binds to a "phosphoprotein binding pocket" within the active site of the kinase, suppressing activity by occluding primed substrate access to the binding pocket (Frame et al., 2001).

This inhibitory mechanism is induced by agonists such as neurotrophins and growth factors that activate protein kinases that act on the N-terminal domain of GSK-3 such as PKB/Akt, p90rsk, cyclic-AMP-dependent protein kinase, p70 S6 kinase, as well as regulators of phosphatase-1 (Sutherland et al., 1993; Stambolic and Woodgett, 1994; Alessi et al., 1996; Li et al., 2000; Svenningsson et al., 2003; see **Figure 2**). For example, insulin leads to inhibition of GSK-3 via insulin receptor substrate-1-dependent induction of phosphatidylinositol 3′ kinase (PI3K), which then stimulates PKB/Akt (Cross et al., 1995). GSK-3 has previously been shown to catalyze serine phosphorylation of IRS-1 and IRS-2, interfering with receptor-mediated tyrosine phosphorylation by the insulin receptor, effectively attenuating insulin receptor signaling via a negative feedback loop (Eldar-Finkelman and Krebs, 1997; Sharfi and Eldar-Finkelman, 2008; **Figure 2**). Moreover, recent studies have revealed novel bi-directionality in the interaction of PKB/Akt and GSK-3 whereby genetic ablation of GSK-3 significantly suppresses PKB/Akt phosphorylation (Lu et al., 2011), indicating a possible novel feedback loop in PKB/Akt/MAPK network (**Figure 2**).

Growth factors, such as EGF and PDGF can also inhibit GSK-3 activity through the phosphatidylinositol 3′ kinase (PI3K) pathway (Stambolic and Woodgett, 1994; Shaw and Cohen, 1999), as well as through induction of the MAPK cascade (Saito et al., 1994; Brady et al., 1998). Ser9/21 phosphorylation of GSK-3 can be modified by amino acid deprivation through mammalian target of rapamycin (mTOR; Armstrong et al., 2001; Krause et al., 2002; Terruzzi et al., 2002) or in response to agonists that elevate the intracellular levels of cAMP through cyclic-AMP-dependent protein kinase (PKA; Fang et al., 2000; Li et al., 2000; **Figure 2**). The PKA-anchoring

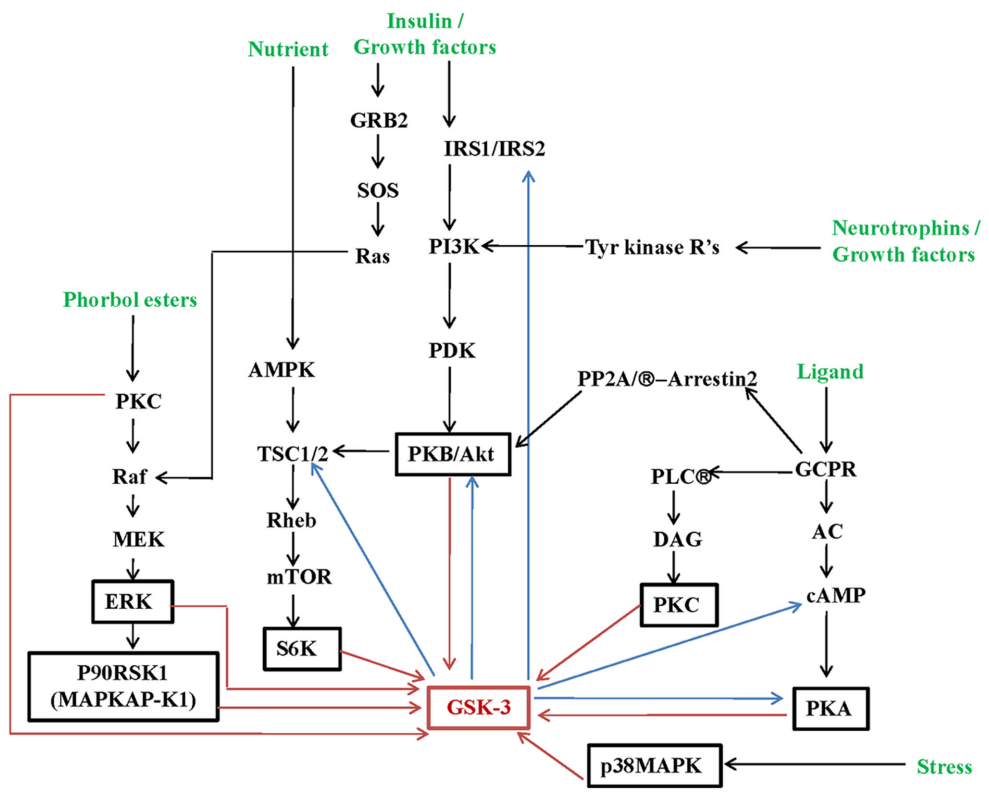

FIGURE 2 | Summary of regulatory signaling inputs into GSK-3.

protein 220 binds both GSK-3 and PKA and hence facilitates GSK-3 phosphorylation by this protein kinase (Tanji et al., 2002). PKC agonists can also regulate GSK-3 (Ballou et al., 2001; Fang et al., 2002), however certain PKCs may preferentially regulate GSK-3β but not GSK-3α (Goode et al., 1992).

REGULATION THROUGH COMPLEX FORMATION

Wnts are secreted glycolipoproteins that activate canonical and non-canonical (β-catenin independent) Wnt signaling cascades, which are essential for early embryonic patterning, cell fate, cellular polarity, cell movement, cell proliferation as well as adult homeostasis in both vertebrates and invertebrates (Logan and Nusse, 2004; Moon et al., 2004; Salinas, 2005; MacDonald et al., 2009). The canonical Wnt signaling pathway employs a distinct mechanism for regulating GSK-3 that is independent of N-terminal domain serine phosphorylation or tyrosine phosphorylation and, instead, relies on protein:protein interactions and intracellular sequestration. Thus, the canonical Wnt pathway comprises phylogenetically conserved proteins: the Wnt receptor, Frizzled; co-receptor, low-density lipoprotein receptor-related protein (LRP) 5/6; scaffolding proteins, Disheveled (Dvl), Axin, Adenomatous polyposis coli (APC) and GSK-3, β-catenin, and casein kinase-1 (CK1; see **Figure 3**).

In cells a small fraction (<5–10%) of cellular GSK-3 is associated with a scaffolding protein termed Axin (Lee et al., 2003; Benchabane et al., 2008). These molecules are joined by others to create a "destruction complex" comprising Axin, APC, CK1, GSK-3, and β-catenin (Zeng et al., 1997; Hart et al., 1998; Ikeda et al., 1998). Within this machine, CK1 phosphorylates Ser45 of β-catenin, which generates a priming site for subsequent GSK-3 phosphorylation on Thr41 (Amit et al., 2002; Hagen and Vidal-Puig, 2002; Hagen et al., 2002; Liu et al., 2002; Sakanaka, 2002; Yanagawa et al., 2002) and subsequently Ser37 and Ser33; resulting in β-catenin recognition by β-TrCP (an E3 ubiquitin ligase subunit), and subsequent ubiquitin-mediated proteasomal degradation of β-catenin (Aberle et al., 1997; Amit et al., 2002; Liu et al., 2002; He et al., 2004). This results in the maintenance of very low levels of β-catenin within the cytoplasm and nucleus of cells (significant amounts of β-catenin are associated, in epithelial cells, with the cadherin adhesion molecules but this fraction is effectively sequestered and does not play a role in Wnt signaling). In addition to β-catenin, both Axin and APC are phosphorylated by GSK-3. Phosphorylation of Axin by GSK-3 increases its stability and binding to β-catenin (Ikeda et al., 1998; Jho et al., 1999; Yamamoto et al., 1999). Phosphorylation of APC increases its affinity to β-catenin (Rubinfeld et al., 1996). Both events promote β-catenin phosphorylation and degradation complex stability.

Wnt ligand induces binding of the seven-pass transmembrane receptor Frizzled and the LRP5/6 co-receptor which leads to the recruitment of Dvl and induction of LRP 5/6 phosphorylation by GSK-3 and CK1. This creates a high affinity binding site for Axin (He et al., 2004; Zeng et al., 2005; Mi et al., 2006; Niehrs and Shen, 2010). Recruitment of Axin to the receptor proteins results in functional dissolution of the destruction complex allowing the stabilization and accumulation of β-catenin. The now stable β-catenin translocates to the nucleus where it binds with members of the TCF/LEF family of DNA-binding proteins, resulting in transcriptional activation of certain targets genes.

β-catenin is dephosphorylated primarily by protein phosphatase (PP) 2A (Su et al., 2008). APC may also act to interfere with PP2A dephosphorylation of β-catenin (Su et al., 2008). APC also facilitates Axin degradation (Lee et al., 2003; Takacs et al., 2008). PP-1 activity leads to dephosphorylation of Axin, antagonizing CK1 phosphorylation as well as negatively regulating GSK-3-Axin binding, promoting complex disassembly (Luo et al., 2007).

Several molecular mechanisms have been proposed to explain how canonical Wnt signaling may interfere with GSK-3-dependent phosphorylation of β-catenin (reviewed in Kimelman and Xu, 2006). For example, the intracellular domain of LRP6 may act as a direct inhibitor of GSK-3 (Mi et al., 2006; Cselenyi et al., 2008; Piao et al., 2008; Wu et al., 2009). A recent study suggested an important role of multi-vesicular endosomes in the canonical Wnt pathway (Taelman et al., 2010). In cells harboring a constitutively activated mutant of LRP6, sequestration of GSK-3 into these membrane-bound organelles was observed, leading to insulation of GSK-3 from other components of the pathway (Taelman et al., 2010). This trafficking machinery required β-catenin which formed a feed-forward loop by facilitating GSK-3 sequestration (Taelman et al., 2010). Whether this mechanism plays a role in physiological Wnt signaling has yet to be determined.

Both mammalian isoforms of GSK-3 function equivalently in Wnt signaling and are entirely redundant (Doble et al., 2007). Indeed, retention of just one of the four GSK-3 alleles is sufficient to maintain low levels of β-catenin in the absence of Wnt, reiterating the fact that only a small fraction of GSK-3 is tightly associated with Axin and therefore relevant to Wnt signaling.

GSK-3 can also associate with other proteins, e.g., GSK-3-binding protein (GBP or FRAT; Li et al., 1999; Fraser et al., 2002) and GSKIP (Chou et al., 2006); however, the roles of FRAT and GSKIP in GSK-3 biology have yet to be defined. GSK-3 is also a part of interacting complex of proteins involved in Hedgehog (Hh) pathway, regulating a key transcription factor of Hh signaling – cubitus interruptus (Ci; Price and Kalderon, 2002).

REGULATION THOUGH INTRACELLULAR LOCALIZATION

In addition to binding proteins in the cytoplasm, there are differences in patterns of subcellular localization of the GSK-3 isozymes (Hoshi et al., 1995; Franca-Koh et al., 2002; Bijur and Jope, 2003). GSK-3 is largely considered as a cytoplasmic protein, but the kinase can also be detected in the nucleus and mitochondria where it is more active compared with the larger cytoplasmic fraction (Bijur and Jope, 2003). Nuclear localization of GSK-3 is dynamic and dependent on the cell cycle (being highest during S-phase; Diehl et al., 1998). Activity is also rapidly increased during apoptosis (Bijur and Jope, 2001). The mechanisms governing intracellular localization of GSK-3 are not fully elucidated. Activated PKB/Akt has been reported to decrease nuclear levels of GSK-3 (Bijur and Jope, 2001). Binding of FRAT 1 to GSK-3 facilitates nuclear export (Franca-Koh et al., 2002). The viral tumor-associated latent nuclear antigen binds GSK-3 and acts to enrich it in the nuclear fraction (Fujimuro et al., 2003). As mentioned previously, GSK-3α has an N-terminal extension compared to GSK-3β. One role of this extra domain may be to provide a level of regulation to

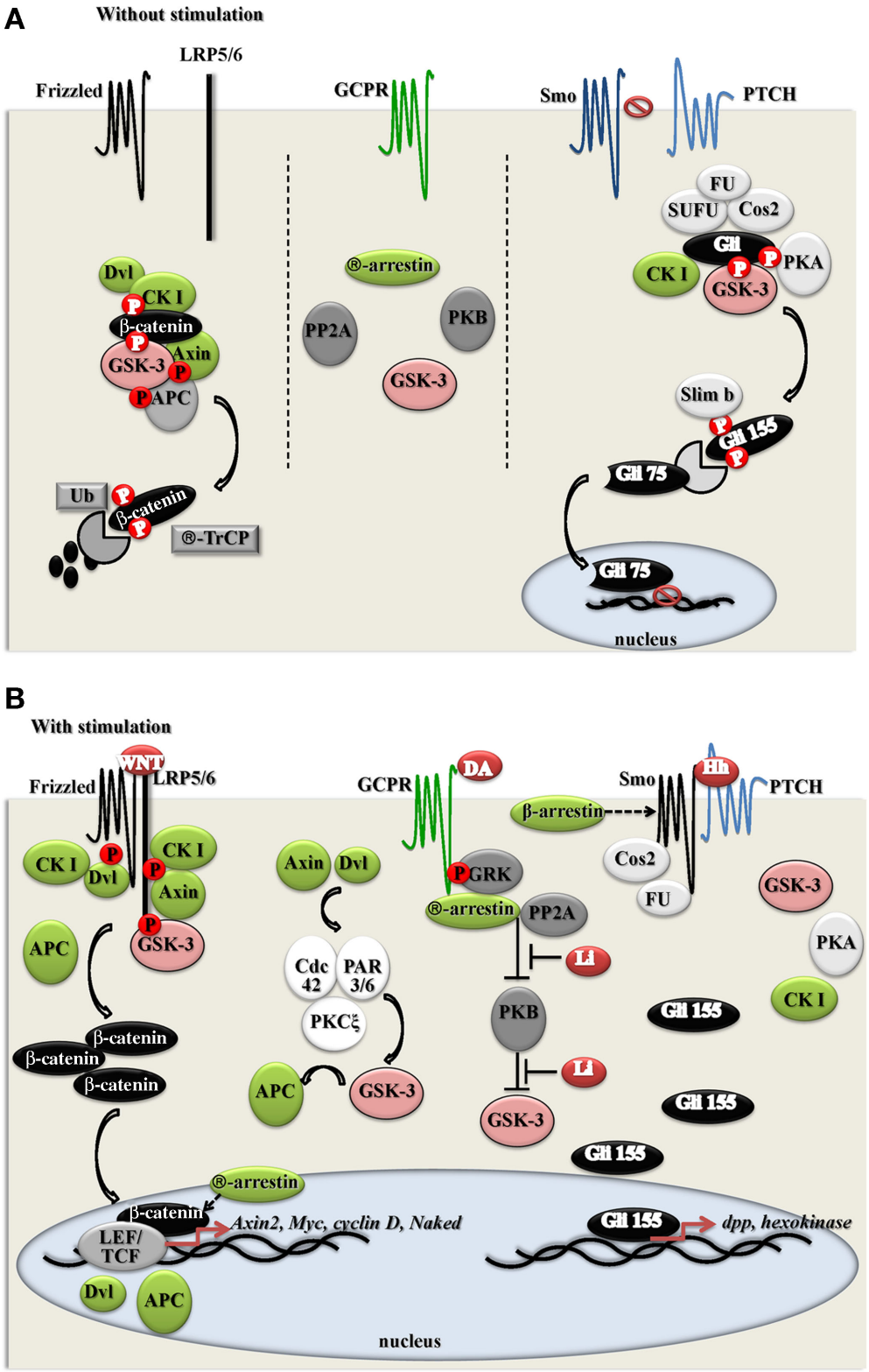

FIGURE 3 | Interaction between different intracellular pools of GSK-3 and protein complexes, involved into Wnt, Hedgehog (Hh), GCPR, and PAR3/6–Cdc42–PKC pathways. (A); resting conditions. (B); activated conditions.

nuclear transport of this isoform (Azoulay-Alfaguter et al., 2011). Notably, deletion of N-terminus of GSK-3 beta reduces its nucleus accumulation (Meares and Jope, 2007). These studies indicate that at least a fraction of GSK-3 may be regulated by intracellular compartmental shuttling.

The finding that GSK-3 acts downstream of multiple signaling pathways that have distinct effects on cells and tissues presents a conundrum. How might signal selectivity be achieved if a protein common to multiple pathways was a required intermediary? The elegant cellular solution to this is to fractionate GSK-3 between scaffolding proteins or other structures such that each system has its own population of GSK-3 molecules "assigned" to it. This effectively insulates the signals and requires that the GSK-3 subpopulations do not intermingle or exchange. It is still an open question why so many important pathways evolved with a common component, a subject of speculative commentary (McNeill and Woodgett, 2010).

GSK-3 SUBSTRATES

The determination of the crystal structure of GSK-3β provided further insight into the molecular nature of the regulation of GSK-3 and its predilection for primed, pre-phosphorylated, substrates (Dajani et al., 2001; ter Haar et al., 2001). GSK-3 shares common features with other protein kinases and has a small N-terminal lobe mostly consisting of β-sheets and a large C-terminal lobe essentially formed of α-helices (Noble et al., 2005). The ATP-binding pocket is located between the two lobes and is so highly conserved between the two isoforms that discrimination between the two protein kinases by an ATP analog-based inhibitor is highly unlikely (Bain et al., 2007).

GSK-3 is one of only a handful of the over 500 known protein kinases that has a strong (500- to 1000-fold) preference for substrates that are already primed by phosphorylation at a proximal serine/threonine to the GSK-3 target residue (Thomas et al., 1999). The phosphorylated residue within the presumptive substrate slots into to a "phosphate-binding" pocket that comprises three crucial basic residues – Lys205, Arg96, and Arg180 (Bax et al., 2001; Dajani et al., 2001; ter Haar et al., 2001). These three residues are conserved in all GSK-3 homologs identified to date, suggesting conservation of the priming phosphate-binding site and the substrate specificity of GSK-3 in all organisms. Binding of the priming phosphate of the substrate to this pocket on GSK-3 induces a conformational change, aligning the substrate for subsequent phosphorylation.

The majority of GSK-3 substrates exhibit an absolute requirement for prior phosphorylation by another kinase at a "priming" residue located C-terminal to the site of subsequent phosphorylation by GSK-3 (Fiol et al., 1987). GSK-3-catalyzed phosphorylation of these substrates occurs at the fourth (Fiol et al., 1990) or fifth (Cole et al., 2006) serine or threonine residue N-terminal to the primed site ($pS/T_1XXXpS/T_2$), where the first pS/T_1 (Ser or Thr) is the target residue, X is any amino acid (but often Pro), and the last pS/T_2 is the site for priming phosphorylation. Thus, the primed Ser/Thr is recognized by the positively charged "binding pocket" on GSK-3 which facilitates the correct orientation of the substrate within the active site of the kinase. Several protein kinases have been shown to act as priming enzymes for GSK-3 phosphorylation, including CDK-5 (Sengupta et al., 1997; Noble et al., 2003; Li et al., 2006), PAR-1 (Nishimura et al., 2004), casein kinase-1 (Amit et al., 2002), casein kinase-2 (Picton et al., 1982; DePaoli-Roach et al., 1983), PKA (Singh et al., 1995), and PKC (Liu et al., 2003). In the case of several substrates, the residue phosphorylated by GSK-3 acts to prime an additional Ser/Thr residue N-terminal to it. This can lead to a zippering effect where multiple residues become phosphorylated by GSK-3. Certain substrates apparently dodge the requirement for prior phosphorylation including c-Jun (Boyle et al., 1991), c-Myc (Saksela et al., 1992), histone H1.5 (Happel et al., 2009), and MARK2/PAR-1 (Kosuga et al., 2005; Timm et al., 2008). In these cases, acidic residues or peptide conformations may substitute for the effect of the priming phosphate.

To prove that an *in vitro* identified protein is an *in vivo* physiological substrate of GSK-3 the target has to meet several criteria (Frame and Cohen, 2001). These include phosphorylation of the protein at the appropriate residues by the protein kinase *in vitro* and under conditions known to modulate that kinase *in vivo* and selective reduction in those phosphorylation sites upon treatment with a specific inhibitor of the protein kinase (or via gene knockout/RNAi). To date, over 100 cytoplasmic and nuclear proteins have been identified as substrates of GSK-3 although not all of these meet the Frame and Cohen criteria as *bona fide* targets (reviewed in Doble and Woodgett, 2003; Jope and Johnson, 2004; Kockeritz et al., 2006; Sutherland, 2011; see **Table 1**).

With respect to biological processes, GSK-3 substrates may be classified into several groups of proteins/transcriptional factors/regulatory enzymes that have roles in processes such as metabolism, cellular architecture, gene expression, neurobiological processes, synaptogenesis, neurodevelopment, axonal growth and polarity, immune response, circadian rhythms, and neuronal/cellular survival (reviewed in Frame and Cohen, 2001; Doble and Woodgett, 2003; Jope and Johnson, 2004; Kockeritz et al., 2006; Sutherland, 2011; see **Table 1**).

GSK-3 SUBSTRATES RELATED TO CIRCADIAN RHYTHMS

Circadian (from the Latin *circa diem* meaning "about a day") rhythms occur with a periodicity of about 24 h and enable organisms to adapt and anticipate environmental changes. Circadian control provides an evolutionary advantage to organisms in adapting their behavior and physiology to the appropriate time of day (reviewed in Wijnen and Young, 2006; Sahar and Sassone-Corsi, 2009). Feeding behavior, sleep-wake cycles, hormonal levels, and body temperature are just a few examples of physiological circadian rhythms. Dysregulation of the cycle is associated with the onset and development of numerous human diseases, including sleep disorders, depression, and dementia.

From a molecular standpoint, circadian rhythms are regulated by transcriptional and post-translational feedback loops generated by a set of interplaying "clock" proteins. The positive limb of the mammalian clock machinery is comprised of CLOCK and BMAL1, which are transcription factors that heterodimerize through their PAS domains and induce the expression of clock-controlled genes by binding to their promoters at E-boxes. Cryptochromes (Cry 1, Cry2) and Period genes (Per1, Per2, Per3) are clock-controlled genes that encode proteins that form the

Table 1 | GSK-3 substrates.

Substrate name	GSK-3's phosphorylation site (in bold) S/TxxxS/T	Biological process/relevance to disease	Reference
METABOLIC ENZYMES AND SIGNALING PROTEINS			
ATP-citrate lyase	**T446**xxx**S450**xxxS454	Fatty acid biosynthesis	Hughes et al. (1992), Benjamin et al. (1994)
Glycogen synthase	**S640**xxx**S644** xxx**S648**xxx **S652**xxxS656	Glycogen metabolism, diabetes	Rylatt et al. (1980), Parker et al. (1983), Dent et al. (1989)
Pyruvate dehydrogenase	**. . .?...**	Metabolism	Hoshi et al. (1996)
Phosphocholine cytidylyltransferase (CTP)	C-terminus . . .?...	CDP-choline pathway	Cornell et al. (1995)
κ-casein	. . .?...	Milk protein	Donella-Deana et al. (1983)
Protein phosphatase-1 G-subunit	**S38**xxx**S42**xxxS46	Signaling/protein dephosphorylation	Dent et al. (1989)
Protein phosphatase inhibitor-2	T72xxxS86	Signaling/protein dephosphorylation	Aitken et al. (1984), DePaoli-Roach (1984)
Axin	**S322**xxx**S326**xxxS330	Wnt pathway, development, cancer	Ikeda et al. (1998), Jho et al. (1999), Yamamoto et al. (1999)
Axil	. . .**?...**	Member of axin family	Yamamoto et al. (1998)
Adenomatous polyposis coli (APC)	**S1501**xxxS1505 **S1503**xxxS1507	Wnt pathway, development, cancer	Rubinfeld et al. (1996), Ikeda et al. (2000), Ferrarese et al. (2007)
Cubitus interruptus/Gli	**S852**xxxS856 **S884**xxx**S888**xxxS892	Hedgehog pathway, development, cancer	Jia et al. (2002), Price and Kalderon (2002)
eIF2B (Eukaryotic initiation factor 2B)	**S535**xxxS539	Growth, cancer	Welsh et al. (1998), Wang et al. (2001), Woods et al. (2001)
Amyloid precursor protein (APP)	**T743**xxx? **T668**xxx?	Neuronal function, Alzheimer's disease	Aplin et al. (1996)
Presenilin-1	**S397**xxx**S401xxx?** **S353**xxx**S357xxx?**	Alzheimer's disease	Kirschenbaum et al. (2001), Twomey and McCarthy (2006)
Heterogeneous nuclear ribonucleoprotein D	**S83**xxxS87	Transcriptional regulator	Tolnay et al. (2002)
Phosphatase interactor targeting protein K (PITK)	**S1013**xxxS1017	PP-1 targeting subunit, modulates phosphorylation of hnRNP K	Kwiek et al. (2007)
p21 CIP1	**T57**xxx?	Cell cycle, apoptosis	Rössig et al. (2002)
Insulin receptor substrate 1	**S332**xxxS336	Diabetes, growth, cancer	Eldar-Finkelman and Krebs (1997), Liberman and Eldar-Finkelman (2005)
Insulin receptor substrate 2	**S484**xxxS488	Diabetes	Sharfi and Eldar-Finkelman (2008)
P75 NGF receptor	. . .?...	Neurotrophin signaling	Taniuchi et al. (1986)
Mcl-1	**S140**xxxT144	Growth, apoptosis, cancer	Maurer et al. (2006), Ding et al. (2007)
Cyclic-AMP-dependent protein kinase – RII subunit	**S44**xx**S47**xxx?	cAMP pathway, hormonal responses	Hemmings et al. (1982)
Cyclin D1	**T286**xxx?	Cell cycle, cancer	Diehl et al. (1998)
Cyclin E	**T380**xxx?	Cell cycle	Welcker et al. (2003)
Myelin basic protein	**T97**xxx?	Myelination of nerves in CNS	Yu and Yang (1994)
Cry2	**S553**xxxS557	Circadian rhythm	Harada et al. (2005), Kurabayashi et al. (2010)
Per2	. . .?...	Circadian rhythm	Kaladchibachi et al. (2007)
Nucleoporin p62	C-terminus . . .?...	Cell division	Miller et al. (1999)
PTEN (phosphatase and tensin homolog)	**S362**xxx**S366**xxxS370	Cell proliferation, migration and growth, cell survival	Al-Khouri et al. (2005)

(Continued)

Table 1 | Continued

Substrate name	GSK-3's phosphorylation site (in bold) S/TxxxS/T	Biological process/relevance to disease	Reference
Lipoprotein receptor-related protein 6	**S1490**xxx? **S1590**xxx? **T1572**xxx?	Wnt pathway, development., cancer	Zeng et al. (2005); Mi et al. (2006); Niehrs and Shen (2010)
TSC2 (tuberous sclerosis 2)/Tuberin	**S1337**xxx **S1341**xxxS1345	Tumor suppressor	Inoki et al. (2006)
CDC25 (cell division cycle)	**S76**xxxT80	Cell cycle, cancer	Kang et al. (2008)
RBL2/p130	**S948**xxxS952 **S962**xxxS966 **S982**xxxT986	Growth, cell cycle, cancer	Litovchick et al. (2004)
Voltage dependent anion channel	**T51**xxxT55	Apoptosis, cancer	Pastorino et al. (2005)
CTPS (cytidine triphosphate synthetase	**S571**xxxS575	Cell growth, cancer	Higgins et al. (2007)
Mdm2 (murine double minute 2)	**S240**xxxS244 **S254**xxxS258	Growth	Kulikov et al. (2005)
Calcipressin/RCN1 (regulators of calcineurin)	**S113**xxxS117	Neuronal regulator of calcineurin, growth, Alzheimer's disease, down syndrome	Hilioti et al. (2004)
Gephyrin	**S270**xxx?	GABA transmission, neuronal functions	Tyagarajan et al. (2011)
Mixed lineage kinase-3	**S789**xxx **S793**xxx?	c-Jun and p38 MAPK pathways, apoptosis, neurodegenerative disease	Mishra et al. (2007)
OMA-1	**T339**xxxT239	Oocyte maturation	Nishi and Lin (2005)
p27Kip1	**...?...**	Cell cycle regulator	Surjit and Lal (2007)
Polypyrimidine track-binding protein-associated-splicing factor (PSF)	**T687**	Alternative splicing, T cell function/activation	Heyd and Lynch (2010)
Zinc finger CCHC domain-containing protein 8 (Zcchc8)	**T492**xxx**?**	RNA metabolism	Gustafson et al. (2005)
SC35	**...?...**	Alternative splicing	Hernandez et al. (2004)
STRUCTURAL PROTEINS			
DF3/MUC1 (high molecular weight mucin-like glycoprotein)	**S40**xxxS44	Wnt pathway, β-catenin, and E-cadherin complex	Li et al. (1998)
Dynamin I	**T774**xxxT778	Endocytosis, neuronal function	Hong et al. (1998), Clayton et al. (2010)
Kinesin light chains (KLCs)	**S611**xxx**S615**xxx?	Axonal transport, mitosis, meiosis	Morfini et al. (2002)
Microtubule-associated protein 1B	**S1260**xxx? **T1265**xxx? **S1388**xxxS1392	Neuronal functions	Lucas et al. (1998), Trivedi et al. (2005), Scales et al. (2009)
Microtubule-associated protein 2C	**T1620**xxx? **T1623**xxx?	Neuronal functions	Sánchez et al. (2000)
Tau	**S208**xxxT212 **T231**xxx**T235**xxx? **S396**xxx? **S404**xxx?	Microtubule stabilization, neuronal functions, Alzheimer's disease	Hanger et al. (1992), Yang et al. (1993), Woods et al. (2001), Cho and Johnson (2004)
Paxillin	**S126**xxxS130	Cell adhesion and migration	Cai et al. (2006)
Collapsin response mediator protein 2	**T509**xxx**T514**xxx **S518**xxxS522	Neuronal functions, axonal growth, neuronal polarity, Alzheimer's disease	Cole et al. (2004b), Yoshimura et al. (2005)
Collapsin response mediator protein 4	**T509**xxx**T514**xxx **S518**xxxS522	Neuronal function, axonal growth	Cole et al. (2004b), Alabed et al. (2010)

(Continued)

Table 1 | Continued

Substrate name	GSK-3′s phosphorylation site (in bold) S/TxxxS/T	Biological process/relevance to disease	Reference
Neural cell adhesion protein (NCAM)	. . .?...	Cell–cell adhesion, neurite outgrowth, synaptic plasticity, learning, and memory	Mackie et al. (1989)
Neurofilament L	**S502**xxx**S506**xxx? **S603**xxx?	Cell cytoskeleton, axonal growth, axonal diameter	Guan et al. (1991); Yang et al. (1995), Sasaki et al. (2002)
Neurofilament M	**S666**xxx?		
Neurofilament H	**S493**xxx?		
Ninein	. . .?...	Centrosomal functions, brain development, tumorigenesis	Hong et al. (2000), Howng et al. (2004)
Telokin kinase-related protein	**S15**xxx?	Stabilization of smooth muscle myosin filaments	Krymsky et al. (2001)
CLIP-associated protein 1 (CLASP 1)	. . .**594–614**. . ..	Neuronal functions	Wittmann and Waterman-Storer (2005), Kumar et al. (2009)
CLASP 2 (CLIP-associated protein 2)	**S533**xxx**S537**xxxS541	Cell migration, neuronal function	Watanabe et al. (2009)
Focal adhesion kinase	**S722**xxxS726	Cell cycle, survival, migration, cancer	Bianchi et al. (2005)
Microtubule affinity-regulating kinase-2/PAR-1	**S212**xxx?	Neuronal function, axonal growth	Kosuga et al. (2005), Timm et al. (2008)
Polycystin 2	**S76**xxxS80	Growth, survival, polycystic kidney disease	Streets et al. (2006)
Dystrophin	. . .**?...**	Cytoskeleton of muscle fibers	Michalak et al. (1996)
Stathmin/oncoprotein 18 (STMN1)	**S31**xxx?	Microtubule polymerization and dynamics	Moreno and Avila (1998)
von Hippel–Lindau (VHL)	**S68**xxxS72	Oxygen sensor, tumor of CNS, kidney, eyes	Hergovich et al. (2006)
TRANSCRIPTION FACTORS			
β-catenin	**S33**xxx**S37**xxx **T41**xxx**S45**	Wnt pathway, development, cancer	Yost et al. (1996), Ikeda et al. (1998)
δ-catenin	**T1078**	Cell adhesion	Oh et al. (2009)
CCAAT/enhancer-binding protein αC/EBPα)	**T222**xxx**T226**xxx	Cell proliferation, growth, differentiation	Ross et al. (1999), Liu et al. (2006)
C/EBPβ (CCAAT/enhancer-binding protein β)	**T179**xxx**S184**xxxT188 **S177**xxx**S181**xxx **S185**xxx**T189**xxx	Cell proliferation, growth, differentiation	Tang et al. (2005), Zhao et al. (2005)
Cyclic AMP response element-binding protein (CREB)	**S129**xxxS133	Metabolism, neuronal function, memory formation, diabetes	Fiol et al. (1994), Bullock and Habener (1998)
GATA4	. . .2–116. . . . . .2–205. . .	Embryogenesis, myocardial differentiation and function	Morisco et al. (2001)
Hypoxia-inducible factor-1	**S551**xxx**T555**xxx? **S589**xxx?	Growth, cancer	Mottet et al. (2003), Flugel et al. (2007)
Heat shock factor-1 (HSF1)	**S303**xxxS307	Stress (heat) response	Chu et al. (1996)
c-Myc	**S62**xxx?	Growth, cancer, oncogenes	Saksela et al. (1992), Sears et al. (2000)
L-myc	**T58**xxxS62		
N-myc downstream regulated gene 1	**T342**xxxT346 **T352**xxxT356 **T362**xxxT366	Stress and hormone response, cell growth and differentiation, cancer	Murray et al. (2004)
c-Jun, Jun B, Jun D	**T239**xxxT243	Growth and cancer	Boyle et al. (1991), Nikolakaki et al. (1993), Woodgett et al. (1993), Morton et al. (2003)
c-Myb	**T572**xxx?	Hematopoiesis, tumorigenesis.	Kitagawa et al. (2009)
Nuclear factor of activated T cells c (NFATc)	SP2 domain SP3 domain	Immune system response	Beals et al. (1997), Neal and Clipstone (2001)

(Continued)

Table 1 | Continued

Substrate name	GSK-3's phosphorylation site (in bold) S/TxxxS/T	Biological process/relevance to disease	Reference
Nuclear factor κB (NF-κB) p65subunit precursorp105 of p50 subunit	**S468**xxx? **S903**xxx**S907**xxx?	Stress response, immune response, synaptic plasticity and memory, cancer, inflammation, autoimmune diseases	Demarchi et al. (2003), Buss et al. (2004)
Notch 1C	…?…	Development, cell–cell communication, cancer.	Foltz et al. (2002), Espinosa et al. (2003)
p53	**S33**xxxS37 **S315**xxx? **S376**xxx?	Cell cycle regulator, cancer.	Turenne and Price (2001), Qu et al. (2004)
Snail	**S97**xxx **S101** **S108**xxx **S112**xxx **S116**xxx**S120**xxx?	Epithelial to mesenchymal transition regulator	Zhou et al. (2004)
Activator protein 1 (AP-1)	…?…	Differentiation, proliferation, apoptosis	de Groot et al. (1993)
Glucocorticoid receptor	**T171**xxx?	Stress and immune response, development, metabolism	Rogatsky et al. (1998)
Microphthalmia-associated transcription factor	**S298xxx**	Melanocyte and osteoclast development	Takeda et al. (2000)
NeuroD	…?…	Central nervous system development	Moore et al. (2002)
BCL-3	**S394**xxxS398	Growth and cancer	Viatour et al. (2004)
Bmal1	**S17**xxx**T21**xxx?	Circadian rhythm	Sahar et al. (2010)
Rev-erb α	**S55**xxx**S59**xxx?	Circadian rhythm	Yin et al. (2006)
Timeless	…?…	Circadian rhythm	Martinek et al. (2001)
Clock	**S427**xxxS431	Circadian rhythm	Spengler et al. (2009)
SMAD1	…?…	Embryonic pattern formation, TGFβ signaling	Fuentealba et al. (2007)
SMAD3	**T66**xxx?	TGFβ signaling, development, cancer	Guo et al. (2008)
Neurogenin 2 (Ngn2)	**S231**xxx? **S234**xxx?	Neuronal function, motor neurons, development	Ma et al. (2008)
BCLAF1 (Bcl-2 interacting transcriptional repressor)	**S531**xxx?	Apoptosis, cancer	Linding et al. (2007)
Myocardin	**S455**xxx**S459**xxx **S463**xxx**S467**xxx? **S624**xxx**S628**xxx **S632**xxx**S636**xxx?	Development, cardiac hypertrophy	Badorff et al. (2005)
Histone H1.5	**T10**	Chromosome condensation	Happel et al. (2009)
Nascent polypeptide associated complex	**T159**	Transcriptional coactivator, bone development.	Quelo et al. (2004)
Nuclear factor E2-related factor 2	**…?…**	Antioxidant response, cell survival	Salazar et al. (2006), Rada et al. (2011)
SKN-1	**S393**xxxS397	Oxidative stress, detoxification	An et al. (2005)
Sterol regulatory element-binding protein	**T426**xxx**S430**xxx?	Lipid and cholesterol metabolism	Sundqvist et al. (2005)
MafA/RIPE3β1	**…?…**	Regulates insulin gene expression in β cells of pancreas, pancreatic development	Han et al. (2007)

negative limb of the circadian machinery. PER and CRY proteins are classically thought to translocate into the nucleus to inhibit CLOCK:BMAL1 mediated transcription, thereby closing the negative feedback loop (reviewed in Sahar and Sassone-Corsi, 2009).

GSK-3 is expressed in the primary center of circadian rhythm regulation – the suprachiasmatic nucleus (SCN) of hypothalamus (Iitaka et al., 2005). GSK-3αmRNA is found at higher levels in the mouse SCN than GSK-3β (Iwahana et al., 2004). The expression of both GSK-3αprotein and the phosphorylated form of GSK-3 have a daily rhythm on the SCN, with peak expression of GSK-3α at ZT5 (Iwahana et al., 2004). Lithium treatment reduces the expression of GSK-3α in the SCN at CT5 and CT 11 (Iwahana et al., 2004).

Lithium has been shown to lengthen the period of circadian rhythms in a wide range of experimental systems, including unicellular organisms, insects, mice, and humans (Abe et al., 2000; Iwahana et al., 2004; reviewed in Engelmann, 1988). The GSK-3 ortholog in *Drosophila*, Shaggy (Sgg), plays a central role in determining circadian period length in flies (Martinek et al., 2001). For example, mutation of GSK-3 in *Drosophila* causes period lengthening (Martinek et al., 2001). Sgg (GSK-3) phosphorylates Timeless and regulates nuclear translocation of the Period/Timeless heterodimer (Martinek et al., 2001).

GSK-3 has also been demonstrated to phosphorylate and regulate the stability of "core" circadian rhythm genes in mammals. GSK-3 together with another serine kinase, DYRK1A, phosphorylates CRY2 at Ser 557 and 553 (respectively) resulting in degradation of CRY2 (Harada et al., 2005; Kurabayashi et al., 2010). GSK-3 phosphorylates BMAL1 (Ser17/Thr21) and these events control the stability of the proteins and the amplitude of circadian oscillation (Sahar et al., 2010). Moreover, GSK-3 has been found to phosphorylate Rev-erbα (Yin et al., 2006), as well as Clock (Spengler et al., 2009). GSK-3 interacts with Per2 *in vitro* and *in vivo*, phosphorylates Per2 *in vitro* and promotes nuclear translocation of Per2 (Iitaka et al., 2005; Kaladchibachi et al., 2007). Overexpression of GSK-3 caused a ∼2 h advance in the phase of mPER2 (Iitaka et al., 2005). Genetic depletion of two alleles of GSK-3β in combination with deletion of one allele of GSK-3α in synchronized oscillating mouse embryonic fibroblasts (3/4 GSK-3α/β KO MEFs) resulted in a significant delay in the period of endogenous clock mechanism, particularly in the cycling period of Per 2 (Kaladchibachi et al., 2007). In contrast, one study revealed that siRNA knockdown of GSK-3β or treatment with GSK-3 inhibitors (CHIR 99021 and 1-azakenpaullone) shortened the circadian rhythm (Hirota et al., 2008); however, the same study observed prominent period lengthening by lithium in another experimental system (Hirota et al., 2008). Nevertheless, pharmacological inactivation of GSK-3 by a related molecule (kenpaullone) induced a phase delay in Per2 transcription (Kaladchibachi et al., 2007).

ANIMAL MODELS OF GSK-3

Several genetic approaches have been used to generate mutant mice for GSK-3: conventional knockouts and knock-ins (all tissues), conditional knockouts (tissue-specific), and transgenic mice (**Table 2**). Use of mice harboring genetic inactivation or overexpression of one or both of the GSK-3 genes has proven a powerful means to study GSK-3 function in brain development, morphology, neurogenesis, memory and learning, sensorimotor function, sociability, emotionality as well as depressive-like animal behaviors. The listings below are not exhaustive as they focus on publications describing findings relevant to brain functions. There are many more investigating the role of GSK-3 in other tissues (including mammary gland, liver, heart, etc.).

Table 2 | Animal models of GSK-3.

Type of approach	Mouse design	Mouse name	Characterized by (reference)
CONVENTIONAL			
Knockout	Deletion of exon 2 (ATP-binding loop) of GSK-3α	GSK-3α KO	MacAulay et al. (2007), Kaidanovich-Beilin et al. (2009), Lee et al. (2011), Lipina et al. (2011)
Knockout	Deletion of exon 2 (ATP-binding loop) of GSK-3β	GSK-3β KO	Hoeflich et al. (2000)
Knockout	Deletion of exon 2 (ATP-binding loop) of GSK-3β	GSK-3β HET	Hoeflich et al. (2000), Beaulieu et al. (2004), O'Brien et al. (2004), Beaulieu et al. (2008), Bersudsky et al. (2008), Kimura et al. (2008)
TRANSGENIC			
Knock-in	Mutations GSK-3 α^{S21A}, β^{S9A}	GSK-3α, β [S21A,S9A] KI	McManus et al. (2005), Eom and Jope (2009), Ackermann et al. (2010), Mines et al. (2010), Polter et al. (2010)
CONDITIONAL			
Double shRNA knockdown	GSK-3α/β shRNA (shGSK-3-dh$^{+/flox}$) × Nestin-Cre	Nestin-Cre/shGSK-3-dh$^{+/\Delta}$	Steuber-Buchberger et al. (2008)
Conditional knockout	GSK-3α/GSK-3β $^{flox/flox}$ × Nestin-Cre	Nestin-GSK-3α + β KO	Kim et al. (2009)
Double shRNA knockdown	GSK-3β shRNA	DG–GSK-3β knockdown (shRNA)	Omata et al. (2011)
TRANSGENIC			
Dominant-negative (DN) GSK-3β	K85RGSK-3β × CamkII-tTA-Cre	DN-GSK-3β	Gomez-Sintes et al. (2007)
Overexpression of GSK-3β	TetO GSK-3β × CamkII-tTA-Cre	Tet/GSK-3β	Lucas et al. (2001), Hernandez et al. (2002), Engel et al. (2006), Hooper et al. (2007)
Overexpression of constitutively active GSK-3β [S9A]	S9AGSK-3β in Thy-1 gene vector	GSK-3β [S9A]	Spittaels et al. (2000), Spittaels et al. (2002), Prickaerts et al. (2006)
Overexpression of GSK-3β	*Xenopus* GSK-3β × mouse prion promoter MoPrP.Xho	PrpGSK-3β^{L56} and PrpGSK-3β^{L64}	O'Brien et al. (2011)

CONVENTIONAL KO MICE

The first GSK-3 gene to be knocked out was GSK-3β (Hoeflich et al., 2000). These animals die late in development either due to hepatic apoptosis (Hoeflich et al., 2000) or a cardiac patterning defect (double outlet, right ventricle; Kerkela et al., 2008).

GSK-3β heterozygous (HET) mice are viable, morphologically normal and have been tested extensively. These animals exhibit a lithium-mimetic, anti-depressant-like state (Beaulieu et al., 2004; O'Brien et al., 2004). Notably, the anti-depressant-like behavior in GSK-3β HET mice effectively normalizes the depressive behavior caused by serotonin deficiency (Beaulieu et al., 2008). Exploratory activity in these animals is reduced although general locomotion remains normal (O'Brien et al., 2004). GSK-3β HET animals show reduced responsiveness to amphetamine treatment (Beaulieu et al., 2004; O'Brien et al., 2004), but have increased morphine-induced locomotion (Urs et al., 2011). Sensorimotor function as well as coordination and balance are normal in GSK-3β HET mice (O'Brien et al., 2004; Bersudsky et al., 2008). GSK-3β HET mice demonstrate increased anxiety (Bersudsky et al., 2008) and reduced aggressive behavior (Beaulieu et al., 2008). Recent studies by Kimura et al. (2008) have revealed the importance of GSK-3β in memory reconsolidation in adult brain. Mice heterozygous for GSK-3β exhibit retrograde amnesia (Kimura et al., 2008). These animals have reduced memory reconsolidation but normal memory acquisition, suggesting that they might be impaired in their ability to form long-term memories.

In contrast to GSK-3β null mice, animals lacking GSK-3α are viable and exhibit improved insulin sensitivity and hepatic glycogen accumulation on the ICR background (MacAulay et al., 2007). However, these anti-diabetic properties are not significant on the C57BL6 background (Patel et al., 2011). Similar to GSK-3βHET mice (Beaulieu et al., 2004, 2008; O'Brien et al., 2004), GSK-3α mutants have decreased exploratory activity, decreased immobility time, and anti-aggression behavior (Kaidanovich-Beilin et al., 2009). GSK-3α KO animals also have abnormal behavioral features that are unique to mice lacking the GSK-3α gene, such as decreased locomotion, increased sensitivity to environmental cues, decreased social motivation, and novelty; impaired sensorimotor gating, associative memory, and coordination (Kaidanovich-Beilin et al., 2009). GSK-3α KO mice also exhibit decreased numbers of Purkinje cells in the cerebellum (Kaidanovich-Beilin et al., 2009), as well as decreased dendrite length and surface, but show no changes in spine density in the frontal cortex (Lee et al., 2011).

CONDITIONAL KNOCKDOWN MODELS

Two studies have employed shRNA knockdown to suppress expression of the two GSK-3 genes in mouse brain. Nestin-Cre was employed to drive shRNA expression in the brain progenitor compartment by excising LoxP flanked transcriptional stop sites. This approach resulted in partial reduction of GSK-3α and β protein levels (60 and 50%, respectively) in whole brain lysate (Steuber-Buchberger et al., 2008). These mice have partial embryonic or neonatal lethality (50% of expected offspring). The surviving double-shGSK-3α and β knockdown animals exhibited ∼50% of the body weight of littermate controls (Steuber-Buchberger et al., 2008).

In an alternative approach, lentivirus-expressing short-hairpin RNAs targeting GSK-3β were injected bilaterally into the hippocampus to inactivate GSK-3β in the dentate gyrus (Omata et al., 2011). These DG–GSK-3β knockdown mice showed decreased immobility time in both forced swim and tail suspension tests (TST), while the locomotor activity of these animals was unchanged (Omata et al., 2011). This technique achieved 30% suppression of GSK-3β in the hippocampus, sufficient to yield an anti-depressant-like behavior in the mice (Omata et al., 2011).

DOMINANT-NEGATIVE MUTANTS

Dominant-negatively acting mutants interfere with the endogenous proteins by soaking up downstream targets or upstream regulators. This approach has been used to generate conditional transgenic expression of a dominant-negative (DN) form of GSK-3β in the brain (Gomez-Sintes et al., 2007). Mutation of a critical residue involved in ATP-binding, Lys85, to Arg inactivates the protein kinase activity of GSK-3β (Dominguez et al., 1995). Double transgenic mice were generated that expressed dominant-negative GSK-3β in a tetracycline-repressible manner under control of a promoter that is active in the postnatal forebrain (CamKIIα-tTA × K85RGSK-3β). These DN–GSK-3β mice grew normally and showed no evidence of tumor formation (Gomez-Sintes et al., 2007). However, these animals exhibited increased levels of apoptosis in the brain regions involved in motor control as well as showing behavioral deficits in motor coordination (Gomez-Sintes et al., 2007). Suppression of the DN–GSK-3 transgene by doxycycline administration restored normal GSK-3 activity and resulted full reversal of the motor and of the neuronal apoptosis phenotypes (Gomez-Sintes et al., 2007).

OVEREXPRESSION OF GSK-3β

Overexpression of GSK-3β has been postulated to be embryonic lethal as viable transgenic animals show only modest levels of the exogenously engineered gene (Brownlees et al., 1997). Mice overexpressing GSK-3β in the forebrain have been generated by placing the transgene under the control of a tetracycline response element that is induced by administration of doxycycline (Lucas et al., 2001). These Tet/GSK-3β mice have decreased levels of nuclear β-catenin, increased phosphorylation of tau in Alzheimer's disease-relevant epitopes (correlated with somatodendritic accumulation of microtubule-unbound tau in hippocampal neurons), increased neuronal cell death, and reactive astrocytosis and microgliosis (Lucas et al., 2001). Behavioral characterization of Tet/GSK-3β mice revealed that these animals have impaired acquisition of reference memory in a novel object recognition task (Engel et al., 2006) and impaired spatial learning (Hernandez et al., 2002). Moreover, Tet/GSK-3β mice have reduced LTP induction, a deficit that was rescued by chronic treatment with lithium (Hooper et al., 2007). Thus, mice with conditional overexpression of GSK-3 in forebrain neurons (Tet/GSK-3β) recapitulate aspects of Alzheimer's disease neuropathology such as tau hyperphosphorylation, apoptotic neuronal death, and reactive astrocytosis, as well as spatial learning deficits. Moreover, these sequelae can be completely reverted by restoration of GSK-3 activity by silencing of transgene expression indicating that these biological defects, at

least, may be responsive to therapeutic intervention (Engel et al., 2006).

Transgenic mice have also been generated that overexpress a mutant form of GSK-3β in which the inhibitory N-terminal phosphorylation site is mutated [S9A] (Spittaels et al., 2000, 2002). This form of the kinase cannot be inhibited by agonists of pathways that promote phosphorylation of this site (such as PI3K, cAMP, etc.). These mice are characterized by microcephaly and higher neuronal density due to reduction of the volume of the somatodendritic compartment (dendrites and cell bodies) of pyramidal neurons in the cortex (Spittaels et al., 2002). The levels of MAP2 were also significantly decreased in the brain and spinal cord of GSK-3β [S9A] mice (Spittaels et al., 2002). However, postnatal overexpression of this non-inhibitable form of GSK-3β in neurons did not alter behaviors of the mice in terms of general cognition and aging and they showed only a minor decline in psychomotor capability (Spittaels et al., 2002). Subsequent characterization revealed that mice with constitutive overexpression of GSK-3β [S9A] showed hypophagia, reduced water consumption, increased general locomotor activity, and increased ASR (acoustic startle response; Prickaerts et al., 2006). GSK-3β [S9A] mice showed reduced immobility times in the forced swim test (FST) but this is likely related to the hyperactivity of these animals (Prickaerts et al., 2006). There were no differences in baseline and stress-induced increases of plasma adrenocorticotrophic hormone and corticosterone levels in GSK-3β [S9A] mice (Prickaerts et al., 2006). Biochemical examination in these animals revealed upregulation of Akt1 together with downregulation of PPP2R3A (regulatory subunit of PP2A) and GSK-3α in the striatum, as well as increased brain-derived neurotrophic factor (BDNF) in the hippocampus (Prickaerts et al., 2006). In summary, mice overexpressing active GSK-3β [S9A] represent a model for studying hyperactivity, hyperreactivity, and disturbed eating patterns; aspects which recapitulate some of the symptoms observed in the manic phase of bipolar disorder patients, ADHD, and schizophrenia.

GSK-3α, β MUTANT KNOCK-IN MICE

As mentioned above, mutation of the N-terminal phosphorylation sites of GSK-3 renders the protein kinase insensitive to inhibition by that mode of regulation (although the kinase remains sensitive to Wnt regulation, for example). Mice have been generated in which the phosphorylation sites of the endogenous alleles have been replaced by non-phosphorylatable alanine (GSK-αS21A, βS9A). Since serine phosphorylation of GSK-3 is increased by lithium, anti-psychotic drugs, anti-depressants, etc., this model is attractive to use for studying the mechanism of action of aforementioned drugs and related pathological conditions. GSK-αS21A, βS9A knock-in mice have normal development and growth, with no signs of metabolic abnormalities/insulin resistance (McManus et al., 2005). However, these animals have a drastic (40%) impairment in neurogenesis, which is not increased/rescued by co-administration of fluoxetine and lithium (Eom and Jope, 2009). Expression of vascular endothelial growth factor (VEGF), but not BDNF, was reduced in GSK-αS21A, βS9A knock-in mice, suggesting that a deficiency in external support for neural precursor cells might contribute to impaired neurogenesis (Eom and Jope, 2009).

GSK-αS21A, βS9A knock-in mice exhibited increased susceptibility to hyperactivity and a heightened response to a novel environment (Polter et al., 2010). Moreover, these animals revealed increased susceptibility to amphetamine-induced hyperactivity, which was partially reversed by chronic lithium administration (Polter et al., 2010). Besides being sensitive to hyperactivity, these knock-in mutant mice displayed mild anxiety, had increased immobility time in FST and TST and were highly susceptible to stress-induced depressive-like behavior (Polter et al., 2010). In contrast, studies by another group revealed decreased immobility time in FST, indicating a phenotype less prone to depression (Ackermann et al., 2010). LTD in the mutants was found to be abnormal and emotion-associated memory was impaired (Polter et al., 2010). The knock-in animals also demonstrated impaired social preference (Mines et al., 2010). Additional studies revealed increased curiosity in these animals, associated with less sensitivity to application of chronic mild stress as well as decreased HPA axis activity (Ackermann et al., 2010).

CONDITIONAL KNOCKOUT MOUSE MODELS

Alleles of GSK-3α and β have been generated in which exon 2 (containing essential residues for ATP-binding) are flanked by LoxP (flox) sites (**Figure 1**). Tissue-specific expression of Cre recombinase allows selective excision of the critical exon and inactivation of the allele(s). Combination of a floxed GSK-3β gene on a GSK-3α null background has been employed to generate total GSK-3 nullizygous cells in the developing nervous system via nestin-Cre mediated excision (Nestin-GSK-3α, β KO; Kim et al., 2009). Selective deletion of both GSK-3α and β in neural precursor cells results in dramatic hyperproliferation of neuronal progenitors along the entire rostrocaudal extent of the neuraxis. The progenitor expansion was at the expense of neurogenesis, intermediate neural progenitors, and post-mitotic neurons indicating effective inhibition of the differentiation process (Kim et al., 2009). The morphological abnormalities were accompanied by dysregulation of β-catenin, Hedgehog and Notch signaling as well as loss of polarity of cell division (Kim et al., 2009).

LESSONS LEARNED FROM COMPARING BETWEEN GSK-3 ANIMAL MODELS

Ten different GSK-3 animal models have been described to date (**Table 2**). All of them display some kind of neuroanatomical and behavioral abnormalities (**Tables 3–6**). However some of them have not been fully characterized yet and require further examinations. Overall, analysis of different animal models supports *in vivo* role of GSK-3 in the regulation of fundamental brain functions (emotionality, sociability, learning and memory, and neurogenesis, etc.).

The differences in behavioral results between different GSK-3 animal models may be accounted for by the alternative design of the models, and/or by strain and gender differences, varying methodology, and animal house-keeping environment.

Well described and presented is effect of genetic GSK-3 manipulations on depressive-like phenotype in mice (**Table 3**). In all three models, inactivation of GSK-3α or β genes (**Table 3**) revealed anti-depressive phenotypes, supporting studies with GSK-3 inhibitors. However, a similar effect was found in mice

Table 3 | Emotionality behaviors of GSK-3 mutant mice.

Behavioral test	Type of test	GSK-3α KO	GSK-3α HET	GSK-3β HET	GSK-3αβ knock-in	GSK-3β [S9A] overexpression	DG–GSK-3β knockdown (shRNA)
Anxiety	EPM	▲ In females only (1)		▲ (2, 3)	Mild ▲ (6)		
	O-maze				▼ (11)		
	Light–dark box	≠ In males (1)					
Emotionality	Open field 5 min	▲ (Both genders) (1)					
Locomotor activity	Open field 30 min	▼ (1)	≠ (10)	≠ (4, 5, 2)	▲ (6, 11)	▲ (8)	≠ (12)
Exploratory activity/curiosity	Open field 30 min	▼ (1)		▼ (2, 5)	≠ (6)	▲ (8)	
	Light–dark box				▲(11)		
Depression-like behavior	Learned helplessness				Stress-induced ▲ (6)		
	FST	▼ (1)		▼ (2, 4, 5)	▲ (6), ▼ (11)	▼ (8)	▼ (12)
	TST	▼ (1)		▼(2, 4, 5)	▲ (6)		▼ (12)
Stress reactivity		Stress response▲ (1), ▼corticosteroids (1)			General ▼ (11) ≠ (6), stress response ▲(6), ▲body weight, food, and fluid intake (11), ▼ (11) ≠ (6), corticosteroids, ▼ACTH (11)	≠ Corticosteroids (8)	

▲, *Increased;* ▼, *decreased;* ≠, *same/no changes.*

References for the comparison tables are: 1. Kaidanovich-Beilin et al. (2009), 2. Bersudsky et al. (2008), 3. Beaulieu et al. (2008), 4. Beaulieu et al. (2004), 5. O'Brien et al. (2004), 6. Polter et al. (2010), 8. Prickaerts et al. (2006), 10. Lipina et al. (2011), 11. Ackermann et al. (2010), 12. Omata et al. (2011).

Table 4 | Sociability behaviors of GSK-3 mutant mice.

Type of behavior	Type of the test	GSK-3α KO	GSK-3β HET	GSK-3αβ KI
Sociability and social novelty	Social interaction test	▼ (1)		Impaired social preference to Str2 (7)
Aggression	Resident intruder	▼ (1)	▼ (Beaulieu et al., 2008)	

▼, *Decreased.*

References for the comparison tables are: 1. Kaidanovich-Beilin et al. (2009), 7. Mines et al. (2010).

overexpressing GSK-3β (GSK-3β S9A mice; **Table 3**). The contradictory result in GSK-3β S9A mice was explained by increased locomotor activity in these animals, which may affect performance in the FST and its interpretation.

It is important to mention that different GSK-3 animal models have employed different "Cre" promoters. Activation of specific "Cre" recombinases may happen at different stages of embryogenesis (or after birth), thus may affect specific neuronal populations (post-mitotic or precursors), which may affect structure and function of adult brain. For example, dominant-negative GSK-3β and Tet/GSK-3β mice have been generated by using CamkIIα-Cre, compare to GSK-3β S9A mice which have been created by using Thy-1 gene promoter (**Table 2**).

Moreover, there are different approaches have been used to generate mice with overexpression of GSK-3β gene. In all three models with overexpression of GSK-3β, different constructs for GSK-3β gene itself were used (**Table 2**): intact GSK-3β in Tet/GSK-3β mice versus point mutated form of GSK-3β (S9A) in GSK-3β[S9A] animals. GSK-3 has complex mechanisms of regulation, thus, is it likely that overexpression of wild type protein has different effects on specific brain functions than Serine 9 mutated forms of the protein, depending on the relative importance of "phosphorylation" as the regulatory mechanism in specific brain process/stimulation and structure.

Comparison between GSK-3α KO and GSK-3α + β serine phosphorylation site KI mice revealed similar impaired sociability in both models, despite different genetic approaches being used. These data indicate that both the protein level of GSK-3α as well as serine phosphorylation of GSK-3 are important aspects for neuronal circuits responsible for social interaction.

Moreover, studying and analyzing genetic animal models may be used to make predictions about long-term usage of GSK-3

Table 5 | Memory, informational process, pharmacology, coordination behaviors of GSK-3 mutant mice.

Type of behavior	Type of the test	GSK-3α KO	GSK-3α HET	GSK-3β HET	GSK-3αβ KI	GSK-3β [S9A] overexpression	Tet/GSK-3β	DN–GSK-3β
Amphetamine response	OF + amp	▲		▼ (4)	▲ (6)			▼ (16)
Response to morphine	OF + morph			▲ (23)				
Information processing	PPI/ASR	▲ PPI (1)	≠ (10)	≠ (2, 5)		▲ASR (8)		
	LI	▲ NPE in KO (1)	≠ (10)					
Long-term memory	FC	▼ (1)		Impaired memory reconsolidation (9)	▲ (6), Enhanced emotion-associated memory			
	Passive avoidance	≠ (1)						
	LTP/LTD				abnormal LTD in the ventral hippocampus (6)		▼ (20)	
Spatial memory							Impaired (22)	
Coordination, balance	Rotarod	▼ (1)		≠ (2, 5)				▼ (16)
Motor learning	Rotarod	≠ (1)		≠ (2, 5)				

▲, *Increased;* ▼, *decreased;* ≠, *same/no changes.*

References for the comparison tables are: 1. Kaidanovich-Beilin et al. (2009), 2. Bersudsky et al. (2008), 4. Beaulieu et al. (2004), 5. O'Brien et al. (2004), 6. Polter et al. (2010), 8. Prickaerts et al. (2006), 9. Kimura et al. (2008), 10. Lipina et al. (2011), 16. Gomez-Sintes et al. (2007), 20. Hooper et al. (2007), 22. Hernandez et al. (2002).

inhibitors (as therapeutic agents). For example, the well characterized GSK-3 inhibitor – lithium – has a diverse spectrum of effects after long-term treatment of patients, including tremor and death of Purkinje cells. Of note, similar changes in cerebellar structure and function were observed in GSK-3α KO and dominant-negative GSK-3β mice (**Tables 5** and **6**).

Thus, comparative analysis of different animal models may be very informative, however critical and combinatory approach needs to be used to make correct interpretation and right conclusions.

PATHOGENESIS OF NEUROLOGICAL DISORDERS THOUGH CROSSBREEDING OF GSK-3 MUTANT MICE AND OTHER NEUROLOGICAL MUTANTS

To study the role of GSK-3β in the pathogenesis of Alzheimer's disease, particularly with respect to the mechanism of tauopathy, double transgenic mice have been generated by inter-breeding mice overexpressing GSK-3β [S9A] with transgenic mice that overexpress the longest isoform of human protein tau (Spittaels et al., 1999, 2000). Biochemical examination of brain and spinal cord extracts from double transgenic mice revealed that the amount of protein tau associated with microtubules was reduced compared to mice harboring only hTau40 (Spittaels et al., 2000). Moreover, unbound tau protein was hyperphosphorylated suggesting that additional phosphorylation of tau in the double transgenic mice reduces its binding capacity to microtubules, but fails to cause tau filament formation (Spittaels et al., 2000). Importantly, virtually all of the pathological defects observed in the hTau40 transgenic mice (Spittaels et al., 1999) were rescued by the mild overexpression of GSK-3β, such as the reduction by about an order of magnitude of the number of axonal dilations in the brain and spinal cord, the reduction in axonal degeneration and muscular atrophy, as well as the alleviation of most motoric complication (Spittaels et al., 2000).

DISC1 (*Disrupted-in-Schizophrenia-1)* is one of the best characterized genetic risk factors for schizophrenia (reviewed in Harrison and Weinberger, 2005; Chubb et al., 2008; Brandon et al., 2009; Jaaro-Peled et al., 2009). One breakpoint of a chromosomal *t*(1;11)(q42.1;q14.3) translocation has been identified within DISC1 gene, which co-segregates in a Scottish family with major mental illness, including schizophrenia, bipolar disorder, and major depression (Millar et al., 2000; Blackwood et al., 2001). DISC1 has been demonstrated to play a role in essential brain functions from embryonic development through to adulthood (reviewed in Jaaro-Peled et al., 2009), such as neurogenesis, neuronal migration, neurite outgrowth, spine development, neurotransmitter signaling, cytoskeletal organization, cell cycle, signal transduction, intracellular transport/exocytosis, etc.(reviewed in Chubb et al., 2008; Brandon et al., 2009; Jaaro-Peled et al., 2009). DISC1 appears to act as a coordinating hub or scaffold protein and has multiple intracellular interacting proteins including GSK-3 (Camargo et al., 2007; Mao et al., 2009; Lipina et al., 2011).

GSK-3 acts as an important downstream component in the etiology of schizophrenia (reviewed in more detail elsewhere in this

Table 6 | Neurogenesis, anatomical changes, biochemical, histological, and molecular characterizations of GSK-3 mutant mice.

Type of analysis	Type of test	GSK-3α KO	Tet/GSK-3β	GSK-3αβ KI	GSK-3β [S9A] overexpression	Nestin-GSK-3αβ KO	DN–GSK-3β
Neuro genesis	BrdU [3H] thymidine incorporation			▼ Proliferation (14)	≠ Proliferation (8)	▲ Proliferation (15), ▼ differentiation (15)	
Apoptosis necrosis	TUNEL, caspase IHC		▲ Apoptosis (17)	≠ Apoptosis (14)	≠ Apoptosis (18), ≠ necrosis (18)		▲ Apoptosis (16)
Neuro anatomical changes	MRI	▲ Cerebellum (1)			▼ Cerebellum, cerebrum, hippocampus, cortex (18)		
	Histology	▼ Dendrite length and surface area in the frontal cortex (12), ≠ in spine density (12), ≠ dendritic arborization (12), ▼Purkinje cells (1)	▲ Microgliosis (17), ≠ tau fibrils (17, 22)		▲ Neuronal density in cortex (18), ≠ number of cortical neurons (18), ▼ caliber of the proximal and distal part of the apical dendrites (18), ▼ size of the cell body of pyramidal neurons (18)	▼ Tuj1, MAP2, SMI32, NeuN (15), ▲Nestin, Pax6 (15)	
	Brain weight	▲ Brain (1)			▼ Brain and spinal cord (18, 8)		
Biochemical molecular	Western blot RTPCR		▲ pTau (17), ▼ nuclear β-catenin (17)	▼ VEGF (14), ≠ BDNF (14)	▼ MAP2 (18), ▲ pTau in old mice (19), ▼GSK-3α, ▼PPP2R3A and ▲Akt1 in striatum (8), ▲BDNF in hippocampus (8)	▲ β-catenin, Axin, c-jun, Gli1, Gli2, Patched, Hes1, Hes5, NICD, c-myc, N-myc (15)	▼pTau (16), ≠ β-catenin (16)

▲, *Increased;* ▼, *decreased;* ≠, *same/no changes.*

References for the comparison tables are: 1. Kaidanovich-Beilin et al. (2009), 8. Prickaerts et al. (2006), 12. Omata et al. (2011), 14. Eom and Jope (2009), 15. Kim et al. (2009), 16. Gomez-Sintes et al. (2007), 17. Lucas et al. (2001), 18. Spittaels et al. (2002), 19. Spittaels et al. (2000), 22. Hernandez et al. (2002).

Special Topic series). There are several lines of evidence supporting the involvement of GSK-3 in the pathogenesis of schizophrenia. Polymorphisms in GSK-3 genes have been associated with schizophrenia (Souza et al., 2008; Benedetti et al., 2010). Dysregulation of the PKB/Akt/GSK-3 signaling pathway has been found in subjects with schizophrenia (reviewed in Koros and Dorner-Ciossek, 2007; Lovestone et al., 2007; Beaulieu et al., 2009; Freyberg et al., 2009). For example, phosphorylation of GSK-3β Ser9 is reduced in the peripheral lymphocytes and brains of schizophrenia patients (Emamian et al., 2004). Drugs that influence the DA and 5-HT systems indirectly affect the activity of GSK-3 (this topic is reviewed in greater detail in another chapter in this Special Topic series). In the dopaminergic system, anti-psychotic, and psychotomimetic drugs alter GSK-3 function (Mai et al., 2002; Svenningsson et al., 2003; Beaulieu et al., 2004; Emamian et al., 2004; Li et al., 2007). Lithium is used to augment anti-psychotic treatment in schizophrenia patients (Kang et al., 2004; Gould, 2006). Moreover, GSK-3 inhibitors can rescue schizophrenia-like behaviors in mice (Beaulieu et al., 2004; Lipina et al., 2011).

Several mouse models for Disc1 have been described (reviewed in Jaaro-Peled et al., 2009). For example, overexpression of the N-terminal portion of Disc1 in the dentate gyrus causes interaction with GSK-3, suppressing activity, and perturbing ability to down-regulate the Wnt/β-catenin pathway resulting in proliferation of neuronal progenitors (Mao et al., 2009). Treatment with a GSK-3 inhibitor, SB216763 rescued the behavioral effects of lentivirally induced DISC1 suppression in the adult dentate gyrus (Mao et al., 2009).

An ENU-induced mutant of Disc1, Disc1-L100P, exhibits schizophrenia-related behaviors in mice (Clapcote et al., 2007). Pharmacological as well as genetic inactivation of one allele of GSK-3α reverses pre-pulse inhibition (PPI) and latent inhibition (LI) deficits as well as normalizing the hyperactivity of Disc1-L100P mutants (Lipina et al., 2011). In parallel to these observations, interaction between DISC1 and GSK-3α and β is reduced in Disc1-L100P mutants (Lipina et al., 2011). At the histological level, genetic inactivation of GSK-3α partially corrected neurite outgrowth and spine development abnormalities in the frontal cortex induced by the Disc1-L100p mutation (Lee et al., 2011).

SUMMARY

The emergence of sophisticated animal models with tissue and developmentally selective expression of GSK-3 has allowed direct assessment of the roles of this protein kinase in a variety of neurological processes and conditions. Clearly, the complexity of brain development and disease pathogenesis requires the use of animal models to examine the biological role of candidate components and with the numbers of candidate genes for neurological illness increasing, allows relatively rapid assessment of genetic interactions through inter-breeding of variant alleles.

While GSK-3 was first implicated in a neurological disorder in 1992 through its capacity to phosphorylate residues on Tau that are associated with neurofibrillary tangles in AD, the potential importance of this kinase in brain function and disease took off with the identification by Klein and Melton of GSK-3 as a direct target of lithium (Klein and Melton, 1996; Stambolic et al., 1996). Since that time, there has been enormous expansion of understanding of this protein kinase with respect to regulation, roles in normal development, and in pathophysiology. However, despite this tsunami of knowledge, there are many remaining questions including the therapeutic reality of modulating GSK-3 in these disorders. Since the beneficial effects of lithium on stabilization of bipolar disorder is achieved at serum levels that reduce GSK-3 levels by only 25% (noting that there are likely several other targets of this drug) and the behavioral phenotypes of Disc1L110P mutations are alleviated by knocking out only one allele of GSK-3α (Lipina et al., 2011), subtle drug modulation of GSK-3 may be sufficient for therapeutic benefit in humans.

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