GSK3 as a sensor determining cell fate in the brain

Adam R. Cole*
Neurosignalling Group, Garvan Institute of Medical Research, Sydney, NSW, Australia

Glycogen synthase kinase 3 (GSK3) is an unusual serine/threonine kinase that controls many neuronal functions, including neurite outgrowth, synapse formation, neurotransmission, and neurogenesis. It mediates these functions by phosphorylating a wide range of substrates involved in gene transcription, metabolism, apoptosis, cytoskeletal dynamics, signal transduction, lipid membrane dynamics, and trafficking, amongst others. This complicated list of diverse substrates generally follow a more simple pattern: substrates negatively regulated by GSK3-mediated phosphorylation favor a proliferative/survival state, while substrates positively regulated by GSK3 favor a more differentiated/functional state. Accordingly, GSK3 activity is higher in differentiated cells than undifferentiated cells and physiologically (Wnt, growth factors) and pharmacological inhibitors of GSK3 promote the proliferative capacity of embryonic stem cells. In the brain, the level of GSK3 activity influences neural progenitor cell proliferation/differentiation in neuroplasticity and repair, as well as efficient neurotransmission in differentiated adult neurons. While defects in GSK3 activity are unlikely to be the primary cause of neurodegenerative diseases, therapeutic regulation of its activity to promote a proliferative/survival versus differentiated/mature functional environment in the brain could be a powerful strategy for treatment of neurodegenerative and other mental disorders.

Keywords: GSK3, phosphorylation, kinase, substrate, proliferation, differentiation, neural progenitor, Alzheimer’s disease

Abbreviations: AD, Alzheimer’s disease; ES, embryonic stem (cell); GSK3, glycogen synthase kinase 3; NPC, neural progenitor cell.
AD patients, nor any other types of neurodegenerative, developmental, or psychiatric disorders. Instead, a key function of GSK3 is to act as an "environmental sensor," by relaying signals from extracellular stimuli (e.g., growth factors, insulin, Wnt) to signaling and transcriptional machinery inside the cell to influence cell fate. This implies that pharmacological manipulation of GSK3 in the brain could be used to selectively promote survival, proliferation, differentiation, neurogenesis, or neuroplasticity in diseased brains. This type of therapy could be used to artificially create an environment in the brain that delays/prevents disease development, or promotes neurogenesis and neuroplasticity to compensate for specific insults. Indeed, encouraging data is now emerging showing chronic lithium treatment improves cognitive function in human patients and mouse models of neurodegeneration and ischemic stroke (for a review, see Chiu and Chuang, 2010). Although GSK3 is not the only in vivo target of lithium (e.g., phosphoinositide phosphatases), these effects are consistent with the known actions of GSK3. It remains to be seen what benefits more selective and potent GSK3 inhibitors might provide.

**GSK3 Substrates**

In order to fully understand the function of GSK3 in the brain, it is essential to characterize its substrates, since this is the primary function of a kinase and it is the substrates that mediate the functional effects directed by GSK3. Ultimately, all physiological substrates of GSK3 should be cataloged and assigned to particular functions regulated by GSK3 (e.g., neurogenesis, neurite outgrowth, neurotransmission, cytoskeletal regulation). This exercise would delineate the mechanisms by which GSK3 maintains healthy brain function. Importantly, it could identify new therapeutic targets downstream of GSK3 that could be exploited for the treatment of mental and neurodegenerative diseases. Theoretically, these could be more specific with less side effects than targeting GSK3, which is a pleiotropic kinase with many different substrates involved in diverse cellular functions.

So far, over 70 substrates have been identified for GSK3, although caution should be taken since many substrates have been reported with various levels of confidence/evidence (for a full review, see Sutherland, 2011). Reported substrates include a number of cytoskeletal, signaling, and DNA-binding proteins. Interestingly, a pattern emerges whereby many substrates that are negatively regulated by GSK3 are involved in proliferation/survival of cells, whereas substrates that are positively regulated by GSK3 are predominantly expressed and function in mature, differentiated cells. Key substrates that contribute to cellular proliferation, differentiation, and survival are listed in Tables 1 and 2 and discussed below.

**GSK3 and Proliferation**

For some time, it has been known that pharmacological inhibition of GSK3 activity maintains the proliferative state of embryonic stem (ES) cells (Sato et al., 2004; Ying et al., 2008). The GSK3 substrates c-myc (Hall et al., 2009) and Klf5 (Jiang et al., 2008) are among several transcription factors that have been used to induce pluripotency (iPS system). GSK3 has also been implicated as a key regulator of adult neurogenesis (generation and incorporation of new neurons into existing circuits of adult brains). Genetic (Eom and Jope, 2009; Kim et al., 2009; Mao et al., 2009) and pharmacological (Sato et al., 2004; Ying et al., 2008; Bone et al., 2009) inhibition of GSK3 activity increases proliferation of neural progenitor cells (NPC's), but decreases differentiation and incorporation of newborn neurons into the adult brain. Together, these observations demonstrate that low levels of GSK3 activity promote proliferation in ES cells and NPC's. This correlates with signaling pathways upstream of GSK3 that inhibit GSK3 activity and promote proliferation (e.g., Wnt, growth factors).

Several transcription factors are directly phosphorylated by GSK3 within an [ST]PPx[ST]P or [ST]PxL[ST]P motif. Following priming by another kinase (often a Cdk or MAPK), phosphorylation by GSK3 creates a binding site for E3 ubiquitin ligases that ubiquitinate the protein and target it for proteosome-mediated degradation. Many of these transcription factors have short half-lives, largely due to the actions of GSK3, which is highly active under basal conditions in differentiated cells, including postmitotic neurons. However, GSK3 activity levels are comparatively lower in ES cells and NPC's, induced by persistent growth factor and Wnt signaling to maintain the proliferative capacity of these cells (Cartwright et al., 2005). Here, phosphorylation and ubiquitination of transcription factors by GSK3 is reduced, thus stabilizing the proteins (prolonging their half-lives) and contributing to stem/precursor cell proliferation. Such GSK3 targets include well-known proliferative factors, such as c-myc, c-jun, β-catenin, cyclin E1, and Klf5 (Tables 1 and 2; Figure 1). Recent studies suggest that attenuating GSK3-mediated degradation of β-catenin, a key effector of the Wnt signaling pathway, is vital for maintaining ES proliferation and pluripotency (Mao et al., 2009; Kelly et al., 2011; Wray et al., 2011). Interestingly, a viral oncogenic form of c-jun (v-jun) is mutated at the GSK3 target site (Ser239). This prevents phosphorylation by GSK3 and subsequent ubiquitination, thus stabilizing the protein and driving uncontrolled proliferation in tumorigenesis (Wei et al., 2005). Similarly, the GSK3 phospho-(Thr58) is mutated in the viral oncogenic form of c-myc (v-myc; Pulverer et al., 1994). While it is established that low GSK3 activity levels are required for maintaining the proliferative capacity of ES cells and NPC's, there are many DNA-binding substrates of GSK3 implicated in this process and their precise roles and relative importance are only beginning to be clarified.

**GSK3 and Differentiation**

Not only does low GSK3 activity promote proliferation, it also prevents differentiation. GSK3α/β double knockout ES cells are severely compromised in their ability to differentiate, largely due to hyperactivation of the Wnt signaling pathway (Doble et al., 2007), while conditional deletion of both isoforms in NPC's in mice suppressed the generation of post-mitotic neurons (Kim et al., 2009). Also, expression of mutant GSK3 and RNAi-mediated knockdown impairs neuronal polarization in cultured primary neurons (Jiang et al., 2005; Yoshimura et al., 2005; Kim et al., 2009). GSK3 knockin mice expressing GSK3α/β (Ser21/9Ala) that are insensitive to growth factor-induced inhibition exhibited reduced neurogenesis and behavioral defects, despite normal NPC proliferation (Eom and Jope, 2009; Ackermann et al., 2010), suggesting defective differentiation/maturation of NPC's. In contrast, mice expressing mutant DISC1 (mutated in schizophrenia patients)
Table 1 | Substrates involved in proliferation/survival that are negatively regulated by GSK3.

| Substrate | Function | Effect of GSK3-mediated phosphorylation | Reference |
|-----------|----------|-----------------------------------------|-----------|
| c-myc     | Transcription factor and oncogene – promotes proliferation | Promotes degradation of the protein via Fbw7-mediated ubiquitination and the proteosome | Moberg et al. (2004), Wei et al. (2004), Yada et al. (2004) |
| c-jun     | Transcription factor and oncogene – promotes proliferation | Promotes degradation of the protein via Fbw7-mediated ubiquitination and the proteosome | Wei et al. (2005) |
| β-Catenin | Transcription factor and oncogene – promotes proliferation | Promotes degradation of the protein | Ikeda et al. (1998) |
| HIF1α     | Transcription factor induced by hypoxia. Activates transcription of genes promoting adaptation/survival | Promotes degradation of the protein | Mottet et al. (2003) |
| HSF1      | Transcription factor that promotes expression heat shock factors to protect cells from environmental stress | Reduces DNA-binding and transcriptional activity | Chu et al. (1998) |
| Klf5      | Transcription factor that promotes cell proliferation | Promotes degradation of the protein via Fbw7-mediated ubiquitination and the proteosome | Liu et al. (2010), Zhao et al. (2010) |
| CyclinE1  | Activating cofactor for Cdk2, promoting cell cycle progression | Promotes degradation of the protein via Fbw7-mediated ubiquitination and the proteosome | Welcker et al. (2003) |
| Mef2D     | Transcription factor that promotes survival and activity-dependent synapse formation | Inhibits its transcriptional activity, antagonizing neuronal survival but antagonizing neuronal differentiation. | Wang et al. (2009) |
| Gli3 (C155) | Target of the hedgehog signaling pathway that is important for patterning during development. Full-length Gli3 (C155) is a transcriptional activator, while the truncated form is a transcriptional repressor. | Promotes β-TrCP-mediated ubiquitination and proteolytic processing | Jia et al. (2002), Price and Kalderon (2002), Pan et al. (2006), Tempe et al. (2006), Wang and Li (2006) |
| Snail     | Transcription factor that regulates E-cadherin expression during epithelial–mesenchymal transitions (development) | Promotes β-TrCP-mediated ubiquitination and degradation, also promotes translocation from the nucleus to the cytoplasm | Zhou et al. (2004) |
| NDRG1     | Regulated by the cell cycle and cell differentiation, although cellular function is not yet clear | Unknown | Murray et al. (2004) |
| BCL3      | Transcription factor and oncoprotein that regulates NFκB signaling | Promotes ubiquitin and proteasome-mediated degradation | Viatour et al. (2004) |
| MCL1      | Pro-survival member of the Bcl2 family of proteins controlling apoptosis. Overexpressed in some cancer types. | Promotes degradation of the protein via Fbw7-mediated ubiquitination and the proteosome | Maurer et al. (2008) |
| RBL2      | Involved in heterochromatin formation and structure. A key regulator of entry into the cell cycle | Not yet clear | Litovchick et al. (2004) |
| Smad1     | Transcription factor and key mediator of BMP signaling in embryogenesis and tissue homeostasis | Promotes ubiquitination by Smurf1 and proteasome-mediated degradation | Fuentealba et al. (2007), Sapkota et al. (2007) |
| eIF2B     | Activates initiation of protein translation from mRNA transcripts | Phosphorylation inhibits eIF2B activity, reducing protein translation | Welsh and Proud (1993) |
| Myocardin | Muscle-specific transcription factor and SRF-dependent cofactor that promotes expression of contraction-related genes | Inhibits its transcriptional activity and promotes CHIP or UBR5-mediated ubiquitination and degradation by the proteasome | Badorff et al. (2005), Xie et al. (2009), Hu et al. (2010) |
| VDAC1     | Voltage-dependent anion channel in the mitochondrial outer membrane. Mediates cytochrome c release from mitochondria during apoptosis | Reduces binding to hexokinase 1, which is overexpressed in many transformed cells, thereby reducing aerobic glycolysis and ATP production in tumor cells | Pastorino et al. (2005) |
| IRS1      | Adaptor protein that mediates signaling downstream of insulin and growth factor receptors | Reduces tyrosine phosphorylation of IRS1, attenuating insulin, and growth factor signaling | Elder-Finkelman and Krebs (1997), Liberman and Elder-Finkelman (2005) |
Table 1 | Continued

| Substrate | Function | Effect of GSK3-mediated phosphorylation | Reference |
|-----------|---------|----------------------------------------|-----------|
| Bax       | Pro-apoptotic member of the Bcl2 family that oligomerizes at the mitochondrial outer membrane, forming a pore to release cytochrome c | Promotes translocation to the mitochondria to induce apoptosis | Linseman et al. (2004) |
| Sufu (exception to the pattern) | Negative regulator of sonic hedgehog pathway, which regulates animal development and cell fate determination. In adults, it maintains the proliferative state of stem cells | Stabilizes Sufu by preventing its degradation and promotes localization in the primary cilium | Chen et al. (2011) |
| PTEN (exception to the pattern) | Lipid phosphatase and commonly mutated tumor suppressor in human cancers | Phosphorylation stabilizes the protein by reducing degradation | Al-Khouri et al. (2005), Maccario et al. (2007) |

Table 2 | Substrates predominantly expressed and functional in mature differentiated cells that are positively regulated by GSK3.

| Substrate | Function | Effect of GSK3-mediated phosphorylation | Reference |
|-----------|---------|----------------------------------------|-----------|
| Polycystin-2 (PKD2) | Non-selective calcium permeable cation channel and part of the TRP channel family, which are broad cellular sensors for multiple stimuli | Promotes translocation to the cell membrane | Streets et al. (2006) |
| CRMP2 | Binds to tubulin heterodimers to promote polymerization of microtubules. Also involved in kinesin-mediated transport and receptor trafficking | Regulates neurite outgrowth and neuronal polarity | Brown et al. (2004), Cole et al. (2004b), Uchida et al. (2005), Yoshimura et al. (2005) |
| MAP1B | Cytoskeletal component of the developing nervous system with important functions in migrating and differentiating neurons | Unclear, but may destabilize microtubules, making them more dynamic | Goid et al. (1999) |
| MAP2C | Abundant cytoskeletal components predominantly expressed in neurons | Promotes dissociation from the cytoskeleton, destabilizing microtubules | Sanchez et al. (2000) |
| Tau | Tubulin-binding protein that stabilizes microtubule structures. Primary constituent of neurofibrillary tangles generated in brains of Alzheimer’s Disease and other dementia patients | Reduces binding to tubulin, destabilizing microtubules, making them more dynamic. Promotes aggregation of tau, forming neurofibrillary tangles | Hanger et al. (1992) |
| β-Adducin | Cytoskeletal-associated protein that links the actin and spectrin networks | Promotes neurite outgrowth | Farghaian et al. (2011) |
| Dynamin1 | GTPase protein that regulates vesicular trafficking processes. Contributes to efficient neurotransmitter release at the pre-synapse | Promotes activity-dependent bulk endocytosis at the pre-synapse, facilitating efficient neurotransmission | Clayton et al. (2010) |
| CLASP2 | Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules | Causes dissociation from the plus end of microtubules and other MT-associated proteins | Wittmann and Waterman-Storer (2005), Watanabe et al. (2009) |
| CaMKKβ | Calcium/CaM dependent protein kinase that regulates learning, memory, migration, neurite outgrowth, and synaptogenesis | Stabilizes newly synthesized protein, decreases calcium/CaM autonomous activity | Green et al. (2011) |
| Glycogen synthase (exception to the pattern) | Enzyme involved in converting glucose to glycogen for storage | Reduces its enzymatic activity, thus reducing glycogen synthesis and storage | Ryll et al. (1980) |
| FAK (exception to the pattern) | Plasma membrane protein and tyrosine kinase involved in cell–cell adhesion | Reduces FAK kinase activity, reducing cell migration | Bianchi et al. (2005) |
| pVHL (exception to the pattern) | Tumor suppressor that binds and stabilizes microtubules. Important in primary cilium. Component of an E3 ubiquitin ligase complex. Antagonizes cell cycle progression. | Phosphorylation negatively regulates stability (but not binding) of microtubules | Hergovich et al. (2006) |
caused increased GSK3 activity, inhibition of the Wnt signaling pathway, and decreased NPC proliferation (Mao et al., 2009). This suggests that inhibition of GSK3 by the Wnt signaling pathway promotes NPC proliferation, while inhibition of GSK3 by growth factor signaling promotes differentiation of NPCs into post-mitotic neurons.

Candidate substrates for promoting differentiation include the zinc-finger transcriptional regulator Gli3 (mammalian homolog of Ci155 in the fly), an effector of the hedgehog pathway that is critical for maintaining epithelial cell identity. In the absence of hedgehog, Gli3 is phosphorylated by GSK3 and CK1 (following priming by PKA), which targets it for ubiquitination and proteasome-mediated degradation. In summary, several substrates of GSK3 regulating cell differentiation have been identified, although mostly in non-neural cell types and neuron-specific differentiation factors await identification.

**GSK3 AND SURVIVAL**

Glycogen synthase kinase 3 promotes intrinsic apoptotic signaling in neurons, and overexpression of GSK3 is sufficient to induce apoptosis in cultured cells (Pap and Cooper, 1998; Bijur et al., 2000) and in mouse brain (Lucas et al., 2001). Deletion of the GSK3β isoform in mice causes severe liver degeneration during mid-gestation due to excessive tumor necrosis factor-induced apoptosis (Hoefflich et al., 2000). In contrast, numerous studies have demonstrated that genetic or pharmacologic inhibition of GSK3 protects neurons from a wide range of environmental stresses, including hypoxia and amyloid toxicity, which may be relevant for treatment of stroke and AD patients, respectively (for a review, see Mines et al., 2011).

Several GSK3 substrates have been implicated in regulation of apoptosis. Bax is a pro-apoptotic member of the Bcl2 family that oligomerizes at the mitochondrial outer membrane, forming a pore to release cytochrome c, inducing cell death. Phosphorylation of Bax at Ser163 by GSK3 promotes translocation to the mitochondrial outer membrane, where it enhances accumulation of Aβ in blood vessels (Bell et al., 2009). Myocardin is phosphorylated by GSK3, targeting it for ubiquitin, and proteasome-mediated degradation (Badorff et al., 2005; Xie et al., 2009; Hu et al., 2010), however it is not yet clear if upregulation of myocardin levels is due to reduced GSK3-mediated phosphorylation and degradation. In summary, several substrates of GSK3 regulating cell differentiation have been identified, although mostly in non-neural cell types and neuron-specific differentiation factors await identification.

**FIGURE 1 | Glycogen synthase kinase 3 as an enzymatic sensor for determining cell fate in the brain.** (A) Low levels of GSK3 activity in ES cells/NPC's are maintained by persistent growth factor/Wnt signaling to promote proliferation. Some pro-proliferation transcription factors that are direct targets of GSK3 are shown. (B) Low levels of GSK3 activity inhibit apoptosis and promote survival when cells are exposed to toxic stimuli, such as hypoxia and amyloid peptides. Some pro-survival substrates of GSK3 are shown. (C) Relatively high levels of GSK3 activity help to promote differentiation and efficient function of mature, post-mitotic neurons, including several cytoskeleton-associated proteins that maintain neuronal morphology and neurotransmission.

---

**cole**

GSK3 regulates cell fate in the brain

---

Cells during development and tumor metastasis, essentially a form of "de-differentiation." Snail suppresses the expression of E-cadherin, a cell–cell adhesion molecule that is critical for maintaining epithelial cell identity. Relatively high activity of GSK3 in epithelial cells promotes phosphorylation and ubiquitin/proteasome-mediated degradation of snail (Zhou et al., 2004). However, in fibroblast/mesenchymal-like cells of human breast tumors where GSK3 activity is lower, snail is stabilized and suppresses E-cadherin expression (Zhou et al., 2004; Yook et al., 2006). Pharmacological inhibition of GSK3 activity in epithelial cells reduces E-cadherin expression and induces a more-mesenchymal-like morphology via increased snail activity (Bachelder et al., 2005). These observations demonstrate that Snail is an example of a GSK3 substrate controlling cellular differentiation. It does not appear to regulate neuronal differentiation (Murray and Gridley, 2006), although it has been shown to regulate cell fate in glioblastoma cells (Han et al., 2011).

Myocardin is a transcription factor that is required for cardiac and skeletal muscle cell differentiation during development. Again, it is not expressed in neurons or glial cells, but interestingly, it is upregulated in vascular smooth muscle cells in the brains of AD patients, where it enhances accumulation of Aβ in blood vessels (Bell et al., 2009). Myocardin is phosphorylated by GSK3, targeting it for ubiquitin, and proteasome-mediated degradation (Badorff et al., 2005; Xie et al., 2009; Hu et al., 2010), however it is not yet clear if upregulation of myocardin levels is due to reduced GSK3-mediated phosphorylation and degradation. In summary, several substrates of GSK3 regulating cell differentiation have been identified, although mostly in non-neural cell types and neuron-specific differentiation factors await identification.
contrast, MCL1 is an anti-apoptotic, pro-survival member of the Bcl2 family, and phosphorylation by GSK3 targets it for degradation by the ubiquitin–proteasome-mediated pathway (Maurer et al., 2006). Thus, low GSK3 activity would reduce phosphorylation and degradation of MCL1, favoring cell survival. Several transcription factor substrates of GSK3 have also been implicated in the balance between apoptosis and cell survival by regulating transcription of pro-apoptotic or pro-survival genes, including the pro-survival factors HIF1α, HSF1, Me2D, and BCL3. GSK3 phosphorylation of each of these substrates targets them for ubiquitin and proteasome-mediated degradation. In summary, many apoptosis-related GSK3 substrates identified so far are pro-survival, and when GSK3 activity is low (e.g., undifferentiated or pharmacologically treated cells), reduced phosphorylation of substrates protects them against ubiquitin and proteasome-mediated degradation, promoting survival of the cell.

GSK3 AND NEURONAL MORPHOLOGY

GSK3 is an important regulator of neuronal morphology and synapse formation in mature, post-mitotic neurons. Pharmacologic inhibition of GSK3 activity reduces the rate of axon elongation in hippocampal neurons, increases the size of growth cones (Owen and Gordon-Weeks, 2003), and disturbs polarity, leading to the formation of multiple axon-like processes (Gartner et al., 2006; Garrido et al., 2007). Treatment of cerebellar granule cells with a GSK3 inhibitor increased the number of synapses on mossy fibers (Hall et al., 2000), whereas inactivation of the Drosophila homolog of GSK3, shaggy, promoted synapse formation at neuromuscular junctions by increasing the number of synaptic boutons (Franco et al., 2004). Assuming these interventions were selective, then taken together they demonstrate that GSK3 regulates synapse formation. Accordingly, neurotrophin and growth factor stimuli (e.g., BDNF, NGF, IGF–1) that inhibit GSK3 activity, promote neurite outgrowth, and synapse formation.

Several groups recently demonstrated that the actin-capping protein β-adducin is critical for synapse stability and turnover, underlying learning and memory in flies and mammals (Bednarek and Caroni, 2011; Pielage et al., 2011; Ruediger et al., 2011). In theory, high levels of phosphorylation of these substrates would promote their dissociation from microtubules, favoring dynamic remodeling of the cytoskeleton, and enhancing neuroplasticity, although this is yet to be proven in vivo.

GSK3 AND NEUROTRANSMISSION

A systematic screen of Ser/Thr kinases using a panel of pharmacologic inhibitors revealed that GSK3 was the only kinase among 58 Ser/Thr kinases that was required for induction of NMDA-induced long-term depression (LTD) in hippocampal CA1 pyramidal neurons (Peineau et al., 2009). LTD increases GSK3 activity via decreased phosphorylation of Ser219 at its N-terminus, while NMDA-induced long-term potentiation (LTP) reduces GSK3 activity by increasing Ser219 phosphorylation (Hooper et al., 2007; Peineau et al., 2007). Meanwhile, GSK3 inhibitors do not affect baseline synaptic transmission (Peineau et al., 2007; Zhu et al., 2007; Li et al., 2009). GSK3 regulates transmission at both the pre- and post-synapse. For example, high GSK3 activity reduces glutamate release from the pre-synapse, inhibiting LTP (Hooper et al., 2007; Zhu et al., 2007, 2010), while retrieval of synaptic vesicles at the pre-synapse by endocytosis requires GSK3 (Clayton et al., 2010). Dynamin 1 is a large GTPase that regulates vesicle endocytosis at the pre-synapse. Phosphorylation by GSK3 at Ser774 is required for re-uptake of neurotransmitters during times of elevated neuronal activity (Clayton et al., 2010). Thus, relatively high GSK3 activity in differentiated neurons would be expected to activate Dynamin 1 and facilitate efficient recycling of neurotransmitters at the synapse. At the post-synapse, pharmacologic inhibition of GSK3 decreases surface expression of NMDA and AMPA receptors (Chen et al., 2007; Wei et al., 2010). CRMP2 is a GSK3 substrate that has been implicated in trafficking of transmembrane proteins to the cell surface (Nishimura et al., 2003; Brittain et al., 2011), although the effect that phosphorylation has on this process has not yet been determined. Together, these observations demonstrate a clear requirement for GSK3 at the synapse, although the synaptic substrates that mediate these effects remain to be fully uncovered.

CONCLUSION

When analyzing the substrates of GSK3, a pattern emerges whereby those that are negatively regulated by GSK3 are commonly involved in promoting proliferation and/or survival, while substrates that are positively regulated by phosphorylation are predominantly expressed in differentiated post-mitotic neurons and are required for efficient function of mature neurons. The former substrates include pro-proliferation transcription factors or pro-survival proteins targeted for ubiquitin-mediated degradation by GSK3, while the latter are often cytoskeleton-associated proteins. Thus, low GSK3 activity levels are conducive to proliferative ES cells and NPC’s, while higher GSK3 activity is required for efficient function of differentiated neurons. This pattern implies that pharmacologic manipulation of GSK3 activity can be used to influence cell fate between proliferative/undifferentiated and mature/differentiated states, as has already been successfully demonstrated for ES cells. In the brain, inhibition of GSK3 would promote proliferation of NPC’s, while high levels of GSK3 would...
promote neuronal differentiation and efficient function of post-mitotic neurons. Also, it is possible that high GSK3 activity in post-mitotic neurons could promote neuroplasticity, learning, and memory via increased dynamics of the cytoskeleton. Manipulation of GSK3 activity may be of great therapeutic benefit for neurodegenerative and other mental disorders. In AD, the use of pharmacologic inhibitors of GSK3 has been proposed to decrease phosphorylation of Tau, reducing its aggregation and formation of neurofibrillary tangles. This strategy has shown some success in mouse models of AD (Perez et al., 2003; Nakashima et al., 2005; Noble et al., 2005; Leroy et al., 2010). In elderly humans and AD patients, chronic (but not acute) treatment with GSK3 inhibitors reduced decline in cognitive and memory function (Nunes et al., 2007; Chiu and Chuang, 2010; Kessing et al., 2010; Forlenza et al., 2011). These studies have been performed using lithium, a relatively weak and non-specific inhibitor of GSK3, so it is necessary to advance these studies using more potent and specific inhibitors of GSK3.

Another exciting potential therapeutic use of GSK3 inhibitors in the clinic is to maintain neuron survival under stressful conditions, including neurodegenerative diseases and acute injuries, such as stroke. Since GSK3 inhibitors are such effective inhibitors of neuronal apoptosis (at least in vitro), rapid administration of these drugs could help to prevent neuronal loss during the immediate period following injury. By keeping these neurons alive, one might expect an improved prognosis for functional recovery. It might also promote proliferation of NPC’s that could later be induced to differentiate into functional post-mitotic neurons to compensate for damages incurred at the site of injury. So far, several groups have elegantly demonstrated that lithium treatment effectively protects neurons and even promotes migration of stem cells to affected regions (Chiu and Chuang, 2010; Tsai et al., 2011).

Of course, there is the danger that inhibition of GSK3 activity could impede the basic function of post-mitotic neurons. However, it should be remembered that very few drugs inhibit kinases 100%, therefore any treatments are likely to reduce GSK3 activity, not completely inhibit it. Also, GSK3 substrates that are relatively resistant to phosphatases are beginning to be discovered (e.g., β-adducin (Farghaian et al., 2011), CRMP2 (Cole et al., 2008)) and moderate reduction of GSK3 activity is unlikely to affect the stoichiometry of phosphorylation of these substrates. This provides another good reason for identifying and characterizing each individual substrate of GSK3 in the brain. Importantly, downstream targets of GSK3 that are specifically involved in a particular neuronal process (e.g., neurogenesis, neurotransmission) may prove to be better therapeutic targets than GSK3, being more potent and selective with fewer side effects. Therefore, the full catalog of GSK3 substrates and their physiological functions needs to be completed.

ACKNOWLEDGMENTS

Thank you to Dr. Calum Sutherland and Dr. Ritchie Williamson, University of Dundee, Scotland for advice, and comments on this review. Adam R. Cole is supported by an Australian NHMRC Peter Doherty Fellowship (#45886).

REFERENCES

Ackermann, T. F., Kempe, D. S., Lang, E. and Lang, U. E. (2010). Hyperactivity and enhanced curiosity of mice expressing PKB/SGK-resistant glycogen synthase kinase-3 (GSK-3). Cell. Physiol. Biochem. 25, 775–786.

Al-Khouri, A. M., Ma, Y., Togo, S. H., Williams, S. and Mustelin, T. (2005). Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casin kinases and glycogen synthase kinase 3beta. J. Biol. Chem. 280, 35195–35202.

Aplin, A. E., Gibb, G. M., Jacobsen, J. S., Gallo, J. M., and Anderton, B. H. (1996). In vitro phosphorylation of the cytoplasmic domain of the aloyd precursor protein by glycogen synthase kinase-3beta. J. Neurochem. 67, 699–707.

Bachelder, R. E., Yoon, S. O., Franci, C., De Herreros, A. G., and Mercurio, A. M. (2005). Glycogen synthase kinase-3 is an endogenous inhibitor of snail transcription: implications for the epithelial-mesenchymal transition. J. Cell Biol. 168, 29–33.

Baderoff, C., Seeger, F. H., Zeiher, A. M., and Dimmeler, S. (2005). Glycogen synthase kinase 3β inhibits myocardin-dependent transcription and hypertrophy induction through site-specific phosphorylation. Circ. Res. 97, 645–654.

Bednarek, E., and Caroni, P. (2011). beta-Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. Neuron 69, 1132–1146.

Bell, R. D., Deane, R., Chow, N., Long, X., Sagar, A., Singh, I., Streb, I. W., Guo, H., Rubio, A., Van Nostrand, W., Miano, J. M., and Zlokovic, B. V. (2009). SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. Nat. Cell Biol. 11, 143–153.

Bianchi, M., De Lucchini, S., Marin, O., Turner, D. L., Hanks, S. K., and Villa-Moruzzi, E. (2005). Regulation of FAK Ser-722 phosphorylation and kinase activity by GSK3 and PK11 during cell spreading and migration. Biochem. J. 391, 359–370.

Bijur, G. N., De Sarno, P., and Hope, J. S. (2000). Glycogen synthase kinase-3β facilitates staurosporine- and heat shock-induced apoptosis. Protection by lithium. J. Biol. Chem. 275, 7583–7590.

Bone, H. K., Damiano, T., Bartlett, S., Perry, A., Letchford, J., Rippoll, Y. S., Nelson, A. S., and Welham, M. J. (2009). Involvement of GSK-3 in regulation of murine embryonic stem cell self-renewal revealed by a series of bisindolylmaleimides. Chem. Biol. 16, 15–27.

Brittain, J. M., Duarte, D. B., Wilson, M. Z., Zhu, W., Ballard, C., Johnson, P. L., Liu, N., Xiong, W., Ripsch, M. S., Wang, X., Fehrenbacher, J. C., Fita, S. D., Khanna, M., Park, C. K., Schmutzler, B. S., Cheon, B. M., Due, M. R., Brustovetsky, T., Ashpole, N. M., Hudson, A., Meroueh, S. O., Hingtgen, C. M., Brustovetsky, N., Ji, R. R., Hurley, J. H., Jin, X., Sheikh, A., Xu, X. M., Oxford, G. S., Vasko, M. R., White, F. A., and Khanna, R. (2011). Suppression of inflammatory and neuropathic pain by uncoupling CRMP-2 from the presynaptic Ca2+ channel complex. Nat. Med. 17, 822–829.

Brown, M., Jacobs, T., Eickholt, B., Ferrari, G., Teo, M., Monfries, C., Qi, R. Z., Leung, T., Lim, L., and Hall, C. (2004). Alpha2-chimaerin, cyclin-dependent Kinase 5p35, and its target collapsin response mediator protein-2 are essential components in semaphorin 3A-induced growth-cone collapse. J. Neurosci. 24, 8994–9004.

Cartwright, P., Mclean, C., Sheppard, A., Rivett, D., Jones, K., and Dalton, S. (2005). LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Dev. Dynamics 132, 885–896.

Chen, P., Gu, Z., Liu, W., and Yan, Z. (2007). Glycogen synthase kinase 3 regulates N-methyl-D-aspartate receptor channel trafficking and function in cortical neurons. Mol. Pharmacol. 72, 40–51.

Chen, Y., Yue, S., Xie, L., Pu, X. H., Jin, T., and Cheng, S. Y. (2011). Dual phosphorylation of suppressor of fused (Sufu) by PKA and GSK3beta regulates its stability and localization in the primary cilium. J. Biol. Chem. 286, 13502–13511.

Chiu, C. T., and Chuang, D. M. (2010). Molecular actions and therapeutic potential of lithium in preclinical and clinical studies of CNS disorders. Pharmacol. Ther. 128, 281–304.

Chu, B., Zhong, R., Soncin, F., Stevenson, M. A., and Calderwood, S. K. (1998). Transcriptional activity of heat shock factor 1 at 37 degrees C is repressed through phosphorylation on two distinct serine residues by glycogen synthase kinase 3 and protein kinases Calpha and Ceta. J. Biol. Chem. 273, 18640–18646.
Clayton, E. L., Sue, N., Smillie, K. J., O’Leary, T., Bache, N., Chung, G., Cole, A. R., Whylie, D. J., Sutherland, C., Robinson, P. J., and Cousin, M. A. (2010). Dynamin I phosphorylation by GSK3 controls activity-dependent bulk endocytosis of synaptic vesicles. Nat. Neurosci. 13, 845–851.

Cole, A. R., Knebel, A., Morrice, N. A., Robertson, L. A., Irving, A. J., Connolly, C. N., and Sutherland, C. (2004b). GSK-3 phosphorylation of the Alzheimer epitope within col- lapsin response mediator proteins regulates axon elongation in pri- mary neurons. J. Biol. Chem. 279, 50176–50180.

Cole, A. R., Noble, W., Van Aalten, L., Plattner, F., Meimaridou, R., Hogan, D., Taylor, M., Lafranchis, J., Gunn-Moore, F., Verkhratsky, A., Oddo, S., Lafera, F., Giese, K. P., Dineley, K. T., Duff, K., Richardson, J. C., Yan, S. D., Hanger, D. P., Allan, S. M., and Sutherland, C. (2007). Collapsin response mediator protein-2 hyper- phosphorylation is an early event in Alzheimer’s disease progression. J. Neurochem. 103, 1132–1144.

Cole, A. R., Souat, M. P., Rembouts, N., Van Aalten, L., Hastie, C. J., Melachlan, H., Peggie, M., Bal- astik, M., Lu, K. P., and Suther- land, C. (2008). Relative resistance of GSK3-phosphorylated CRMP2 to dephosphorylation. J. Biol. Chem. 283, 18227–18237.

Ding, V. W., Chen, R. H., and Cole, A. R., Soutar, M. P., Rembutsu, J., Alzari, M., Lu, K. P., and Suther- land, C., (2004a). Further evidence that the activity-dependent bulk endocytosis of GSK-3alpha and GSK-3beta in mammalian cells is an autophos- phorylation event. Biochem. J. 377, 249–255.

Forlenza, O. V., Dinzin, B. S., Radovanovic, M., Santos, F. T., Taib, L. L., and Gattaz, W. F. (2011). Disease- modifying properties of long-term lithium treatment for amnestic mild cognitive impairment: randomised controlled trial. Br. J. Psychiatry 198, 351–356.

Frame, S., Cohen, P., and Biondi, R. M. (2001). A common phosphate-bind- ing site explains the unique substrate specificity of GSK3 and its inactiva- tion by phosphorylation. Mol. Cell 7, 1321–1327.

Franco, B., Bogdalan, L., Robine- nec, Y., Debesc, A., Bockaert, J., Parmentier, M. L., and Grau, Y. (2004). Shaggy, the homolog of glycogen synthase kinase 3, controls neuronal muscular junction growth in Drosophila. J. Neurosci. 24, 6573–6577.

Fuentelba, L. C., Evers, I., Ikeda, A., Hurtado, C., Kuroda, H., Pera, E. M., and De Robertis, E. M. (2007). Integrating patterning sig- nals: Wnt/GSK3 regulates the dura- tion of the BMP/Smad signal. Cell 131, 980–993.

Garrido, J. J., Simon, D., Varea, O., and Wadensoll, F. (2007). GSK3 alpha and GSK3 beta are necessary for axon formation. FEBS Lett. 581, 1579–1586.

Gartner, A., Huang, X., and Hall, A. (2006). Neuronal polarity is regu- lated by glycogen synthase kinase-3 (GSK-3beta) independently of Akt/PRB serine phosphorylation. J. Cell Sci. 119, 3927–3934.

Goold, R. G., Owen, R., and Gordon-Weeks, P. R. (1999). Glycogen syn- thase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of micro- tubules in growth cones. J. Cell Sci. 112(Pt 19), 3373–3384.

Green, M. F., Scott, J. W., Steel, R., Oakhill, J. S., Kemp, B. E., and Means, A. R. (2011). Ca2+/calmodulin-depen- dent protein kinase kinase beta is regulated by maltolite phos- phorylation. J. Biol. Chem. 286, 28066–28079.

Hall, A. C., Lucas, F. R., and Salii- nas, P. C. (2000). Axon remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell 100, 525–535.

Hall, J., Gao, G., Wang, D. J., Eyres, L., Nichols, J., Grotewold, L., Mor- fpoulos, S., Humphreys, P., Mans- field, W., Walker, R., Tomlinson, S., and Smith, A. (2009). Oct4 and LIF/Stat3 additively induce Kruppel factors to sustain embryonic cell self-renewal. Cell Stem Cell 5, 597–609.

Han, S. P., Kim, J. H., Han, M. E., Sim, H. E., Kim, K. S., Yoon, S., Baek, S. Y., Kim, B. S., and Oh, S. O. (2011). SNAIL is involved in the pro- liferation and migration of globlas- toma cells. Cell. Mol. Neurobiol. 31, 489–496.

Hanger, D. P., Hughes, K., Woodgett, J. R., Brion, J. P., and Anderton, B. H. (1992). Glycogen synthase kinase-3 induces Alzheimer’s disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci. Lett. 147, 58–62.

Herberg, A., Lizarzuri, J., Thomas, C. R., Wirbelauer, C., Barry, R. E., and Krek, W. (2006). Priming-dependent phosphorylation and regulation of the tumor suppressor pVHL by glycogen synthase kinase 3. Mol. Cell. Biol. 26, 5784–5796.

Hoeft, K. P., Luo, J., Ruebig, R. E. A., Tsao, M. S., Jin, O., and Woodgett, J. R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. Nature 406, 86–90.

Hooper, C., Markevich, V., Plattner, F., Killick, R., Schofield, E., Engel, T., Hernandez, F., Anderton, B., Rosen- blum, K., Bliss, T., Cooke, S. F., Arila, J., Lucas, J. I., Giese, K. P., Stephenson, L. and Loveostone, S. (2007). Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. Eur. J. Neurosci. 25, 81–86.

Hu, G., Wang, X., Saunders, D. N., Hen- derson, M., Russell, A. J., Herring, B. P., and Zhou, J. (2010). Modulation of myocardin function by the ubiquitin E3 ligase UBR5. J. Biol. Chem. 285, 11800–11809.

Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, M. (2006). Phosphorylation of beta-catenin. EMBO J. 17, 1371–1384.

Jaworski, T., Dewachter, I., Lechat, B., Kockeritz, L. K., and Woodgett, J. R., (2004). Priming-dependent phosphorylation of beta-adducin as a disease marker. FEBS Lett. 571, 137–140.

Jia, J., Tong, G. Q., Lim, C. A., Rob- son, P., Zhang, S., and Ng, H. H. (2008). A core Ki circuitry regulates self-renewal of embryonic stem cells. Nat. Cell Biol. 10, 353–360.

Kelly, K. F., Ng, D. Y., Jayakumar, G., Wood, G. A., Koide, H., and Doble, B. W. (2011). beta-Catenin enhances Oct-4 activity and rein- forces pluripotency through a TCF- independent mechanism. Cell Stem Cell 8, 214–227.

Kesting, L. V., Forman, J. L., and Ander- sen, P. K. (2010). Does lithium pro- tect against dementia? Bipolar Dis- order, 12, 87–94.

Kim, W. Y., Wang, X., Wu, Y., Doble, B. W., Patel, S., Woodgett, J. R., and Snider, W. D. (2009). GSK-3 is a master regulator of neuronal progeni- tor homeostasis. Nat. Neurosci. 12, 1390–1397.

Leroy, A., Ando, K., Hераud, C., Yilm- az, Z., Autelet, M., Boeynams, J. M., Buee, L., De Decker, R., and Brion, J. P. (2010). Lithium treatment arressthe development of neurofibrillary tangles in mutant tau transgenic mice with advanced neu- rofibrillary pathology. J. Alzheimers Dis. 19, 705–719.

Li, B., Ryder, J., Su, Y., Zhou, Y., Liu, F., and Ni, B. (2003). FRAT1 pep- tide decreases Abeta production in swAPP(751) cells. FEBS Lett. 553, 347–350.

Li, Y. C., Xi, D., Romani, J., Huang, Y. Q., and Gao, W. J. (2009). Activita- tion of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibi- tion of synaptic NMDA recep- tor function in the rat pre- frontal cortex. J. Neurosci. 29, 15351–15363.

Limaron, Z. and Eldar-Finkelman, H. (2005). Serine 323 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenu- ates insulin signaling. J. Biol. Chem. 280, 4422–4428.
GSK3 regulates cell fate in the brain
GSK3 regulates cell fate in the brain

Williamson, R., Van Aalten, L., Mann, D. M., Platt, B., Plattner, F., Bedford, L., Mayer, J., Howlett, D., Usardi, A., Sutherland, C., and Cole, A. R. (2011). CRMP2 hyperphosphorylation is characteristic of Alzheimer’s disease and not a feature common to other neurodegenerative diseases. J. Alzheimers Dis. 27, 615–625.

Wray, J., Kalkan, T., Gomez-Lopez, S., Eckardt, D., Cook, A., Klemler, R., and Smith, A. (2011). Inhibition of glycinogen synthase kinase-3 alleviates Trf3 repression of the pluripotency network and increases embryonic stem cell resistance to differentiation. Nat. Cell Biol. 13, 838–845.

Xie, P., Fan, Y., Zhang, H., Zhang, Y., She, M., Gu, D., Patterson, C., and Li, H. (2009). CHIP represses myocardin-induced smooth muscle cell differentiation via ubiquitin-mediated protein degradation. Mol. Cell. Biol. 29, 2398–2408.

Yada, M., Hatakeyama, S., Kamura, T., Nishiyama, M., Tsunematsu, R., Imaki, H., Ishida, N., Okumura, F., Nakayama, K., and Nakayama, K. I. (2004). Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. EMBO J. 23, 2116–2125.

Ying, Q. L., Wray, J., Nichols, J., Battle-Morera, L., Doble, B., Woodgett, J., Cohen, P., and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. Nature 453, 519–523.

Yook, J. I., Li, X. Y., Ota, I., Hu, C., Kim, H. S., Kim, N. H., Cha, S. Y., Ryu, J. K., Choi, Y. J., Kim, J., Fearon, E. R., and Weiss, S. J. (2006). A Wnt/Axin2-GSK3beta cascade regulates Snail activity in breast cancer cells. Nat. Cell Biol. 8, 1398–1406.

Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A., and Kaibuchi, K. (2005). GSK-3beta modulates proteasomal degradation of CRMP2 and neuronal polarity. Cell 120, 137–149.

Zhao, D., Zheng, H. Q., Zhou, Z., and Chen, C. (2010). The Fbw7 tumor suppressor targets KLFS for ubiquitin-mediated degradation and suppresses breast cell proliferation. Cancer Res. 70, 4728–4738.

Zhou, B. P., Deng, J., Xia, W., Xu, J., Li, Y. M., Gunduz, M., and Hung, M. C. (2004). Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat. Cell Biol. 6, 931–940.

Zhu, L. Q., Liu, D., Hu, J., Cheng, J., Wang, S. H., Wang, Q., Fang, C. J., and Wang, J. Z. (2010). GSK-3 beta inhibits presynaptic vesicle exocytosis by phosphorylating P/Q-type calcium channel and interrupting SNARE complex formation. J. Neurosci. 30, 3624–3633.

Conflict of Interest Statement: The author declares that there is no conflict of interest.

Received: 03 November 2011; accepted: 10 January 2012; published online: 09 February 2012.

GSK3 as a putative GSK3 substrate? Int. J. Alzheimers Dis. 2011, 505607.

Tempe, D., Casas, M., Karaz, S., Blanchet-Tournier, M. E., and Cordett, J. P. (2006). Multisite protein kinase A and glycyogen synthase kinase 3beta phosphorylation leads to Gli3 ubiquitination by SCFbeta-TrCP. Mol. Cell. Biol. 26, 4316–4326.

Thomas, G. M., Frame, S., Goedert, M., Nathke, I., Polakis, P., and Cohen, P. (1999). A GSK3-binding peptide from FRAT selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. FEBS Lett. 458, 247–251.

Trivedi, N., Marsh, P., Goold, R. G., Wood-Kaczmar, A., and Gordon-Weeks, P. R. (2005). Glycogen synthase kinase-3beta phosphorylation modulates its degradation and facilitates recovery in a stroke primed with valproate and lithium. J. Neurosci. 25, 993–1005.

Takeda, N., Noritake, J., Kakeno, U., Bours, V., and Chariot, A. (2004). Gli3 is required for maintenance of neuronal apoptosis. J. Biol. Chem. 284, 32619–32626.

Uchida, Y., Ohshima, T., Sasaki, Y., Mikoshiba, K., Kolattukudy, P., Honnorat, J., and Goshima, Y. (2005). Semaphorin3A signalling is mediated via sequential Cdk5 and GSK3beta phosphorylation of CRMP2: implication of common phosphorylating mechanism underlying axon guidance and Alzheimer’s disease. Genes Cells 10, 165–179.

Viatour, P., Dejardin, E., Warnier, M., Lair, F., Claudio, B., Bureau, F., Marine, J. C., Merville, M. P., Mau- rer, U., Green, D., Piette, J., Sebbenlist, U., Bours, V., and Chariot, A. (2004). GSK3-mediated BCL-3 phosphorylation modulates its degradation and its oncoenergy. Mol. Cell 16, 35–45.

Wang, B., and Li, Y. (2006). Evidence for the direct involvement of (beta)TrCP in Gli3 protein processing. Proc. Natl. Acad. Sci. U.S.A. 103, 33–38.

Wang, H., Ge, G., Uchida, Y., Lau, B., and Ahn, S. (2011). Gli3 is required for maintenance and fate specification of cortical progenitors. J. Neurosci. 31, 6440–6448.

Wang, X., She, H., and Mao, Z. (2009). Phosphorylation of neuronal survival factor MEF2D by glycogen synthase kinase 3beta in neuronal apoptotic. J. Biol. Chem. 284, 32619–32626.

Watanaue, T., Noritake, J., Kakeno, M., Matsu, T., Harada, T., Wang, S., Itoh, N., Sato, K., Matsuuzawa, K., Iwamatsu, A., Galjart, N., and Kaibuchi, K. (2009). Phosphorylation of CLASP2 by GSK-3beta regulates its interaction with IQGAP1, EBI and microtubules. J. Cell Sci. 122, 2969–2979.

Wei, J., Liu, W., and Yan, Z. (2010). Regulation of AMPA receptor trafficking and function by glycogen synthase kinase 3. J. Biol. Chem. 285, 26369–26376.

Wei, W., Jin, J., Schlisio, S., Harper, J. W., and Kaelin, W. G. Jr. (2003). The v-Jun point mutation allows c-Jun to escape GSK3-dependent recognition and destruction by the Fbw7 ubiquitin ligase. Cancer Cell 8, 25–33.

Welcker, M., Oriam, A., Jin, J., Grim, J. E., Harper, J. W., Eisenman, R. N., and Clurman, B. E. (2004). The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc. Natl. Acad. Sci. U.S.A. 101, 9085–9090.

Welcker, M., Singer, J., Loeb, K. R., Grim, J., Blocher, A., Gurien-West, M., Clurman, B. E., and Roberts, J. M. (2003). Multisite phosphorylation by Cdk2 and GSK3 controls cyclin E degradation. Mol. Cell 12, 381–392.

Welsh, G. I., and Proud, C. G. (1993). Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. Biochem. J. 294 (Pt 3), 625–629.

Sutherland, C. (2011). What are the bona fide GSK3 substrates? Int. J. Alzheimers Dis. 2011, 505607.