

## REVIEW

# Glycogen synthase kinase 3: more than a namesake

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Glycogen synthase kinase 3 (GSK3), a constitutively acting multi-functional serine threonine kinase is involved in diverse physiological pathways ranging from metabolism, cell cycle, gene expression, development and oncogenesis to neuroprotection. These diverse multiple functions attributed to GSK3 can be explained by variety of substrates like glycogen synthase,  $\tau$  protein and  $\beta$  catenin that are phosphorylated leading to their inactivation. GSK3 has been implicated in various diseases such as diabetes, inflammation, cancer, Alzheimer's and bipolar disorder. GSK3 negatively regulates insulin-mediated glycogen synthesis and glucose homeostasis, and increased expression and activity of GSK3 has been reported in type II diabetics and obese animal models. Consequently, inhibitors of GSK3 have been demonstrated to have anti-diabetic effects *in vitro* and in animal models. However, inhibition of GSK3 poses a challenge as achieving selectivity of an over achieving kinase involved in various pathways with multiple substrates may lead to side effects and toxicity. The primary concern is developing inhibitors of GSK3 that are anti-diabetic but do not lead to up-regulation of oncogenes. The focus of this review is the recent advances and the challenges surrounding GSK3 as an anti-diabetic therapeutic target.

*British Journal of Pharmacology* (2009) **156**, 885–898; doi:10.1111/j.1476-5381.2008.00085.x; published online 13 March 2009

**Keywords:** diabetes; pancreatic  $\beta$  cells;  $\beta$  catenin; Wnt signalling; inhibitors

**Abbreviations:** APC, adenomatous polyposis coli; BIO, 6-bromoindirubin-3'-oxime; CDK, cyclin-dependent kinase; CK, casein kinase; eIF2B, eukaryotic initiation factor 2B; GLUT4, glucose transporter 4; GS, glycogen synthase; GSK3, glycogen synthase kinase 3; IKK $\beta$ , inhibitor  $\kappa$ B kinase; IPF1/PDX1, insulin promoter factor or pancreatic/duodenal homeobox 1 transcription factor 1; IR, insulin receptor; IRS, insulin receptor substrates; JNK, c-Jun N terminal kinase; LEF/TCF, lymphoid enhancer factor/T cell factor; LRP, LDL receptor related protein; MAPK, mitogen-activated protein kinase; PKB, protein kinase B; PKC, protein kinase C; PP1, protein phosphatase 1; T2DM, type II diabetes mellitus

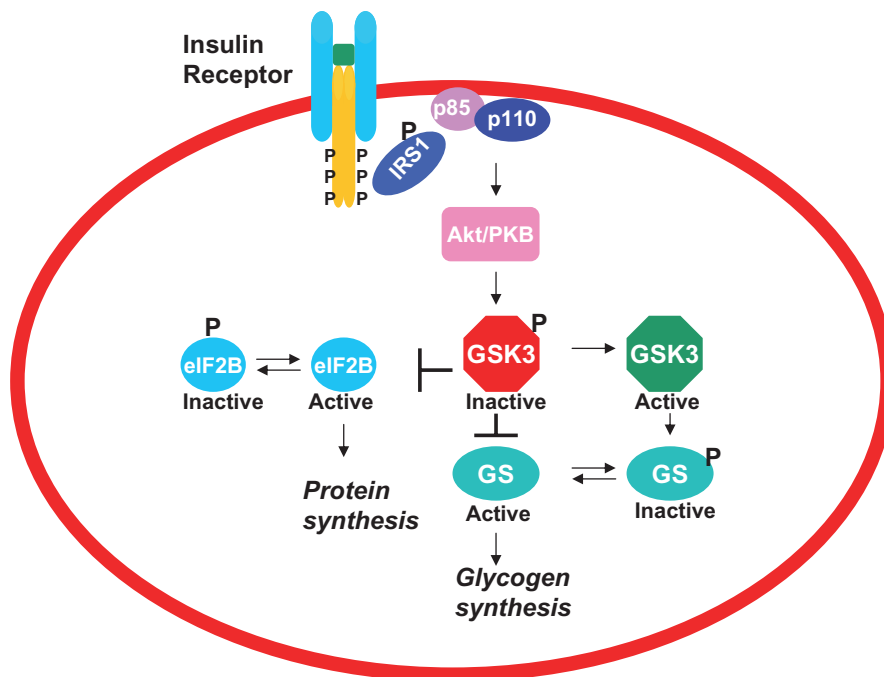
## Introduction

Glycogen synthase kinase 3 (GSK3), a Ser/Thr kinase derives its name from its substrate glycogen synthase (GS) a key enzyme involved in the glycogen synthesis (Embi *et al.*, 1980; Frame and Cohen, 2001). The name glycogen synthase kinase does not adequately describe the multitude of diverse substrates and functions attributed to GSK3. It is involved in a variety of cellular processes ranging from glycogen metabolism, insulin signalling, cell proliferation, neuronal function, oncogenesis to embryonic development. Of the many diverse substrates, some of the key molecules mediating GSK3 functions in regulation of several cellular processes are glycogen synthase (GS), tau protein and beta catenin proteins (Eldar-Finkelman, 2002; Jope and Johnson, 2004). Given the multiple processes regulated by GSK3, it is not surprising that GSK3 has been implicated in various diseases such as diabetes,

cancer, bipolar disorder and Alzheimer's to name a few, and GSK inhibitors are being actively developed as drugs for the treatment of the various disorders.

Glycogen synthase kinase 3 is ubiquitously expressed with high levels of expression in brain and is associated with a variety of neurological disorders like Alzheimer's, bipolar disorder, Huntington disease and other neurodegenerative disorders (Jope and Roh, 2006; Mazanetz and Fischer, 2007). GSK3-mediated hyper phosphorylation of  $\tau$  protein has been implicated in Alzheimer's disorder, and currently there are several GSK3 inhibitors being developed for Alzheimer's and bipolar disorder (Jope and Roh, 2006; Mazanetz and Fischer, 2007). This area is beyond the scope of this article, and readers are referred to other reviews in this area (Mazanetz and Fischer, 2007; Rowe *et al.*, 2007).

The focus of the present review is regulation of GSK3, its function in insulin signalling and its role in maintenance of glucose homeostasis. Further, the involvement of GSK3 in Wnt signalling and its implication for oncogenesis (Jope and Johnson, 2004; Patel *et al.*, 2004) and the impact of this on the development of GSK3 inhibitors for treatment of diabetes mellitus will be discussed.



**Figure 1** Schematic representation of insulin-signalling events and role of GSK3. Binding of insulin to its receptor leads to autophosphorylation, followed by binding of IRS that is phosphorylated leading to activation of PI3 kinase and Akt/PKB that phosphorylates GSK3. Inactive phosphorylated GSK3 relieves glycogen synthase inhibition and eIF2B phosphorylation, leading to active form of GS and eIF2B, which leads to increase in glycogen and protein synthesis. eIF2B, eukaryotic initiation factor 2B; GS, glycogen synthase; GSK3, glycogen synthase kinase 3; IRS, insulin receptor substrates; PKB, protein kinase B.

### GSK3 a unique multi-tasking kinase

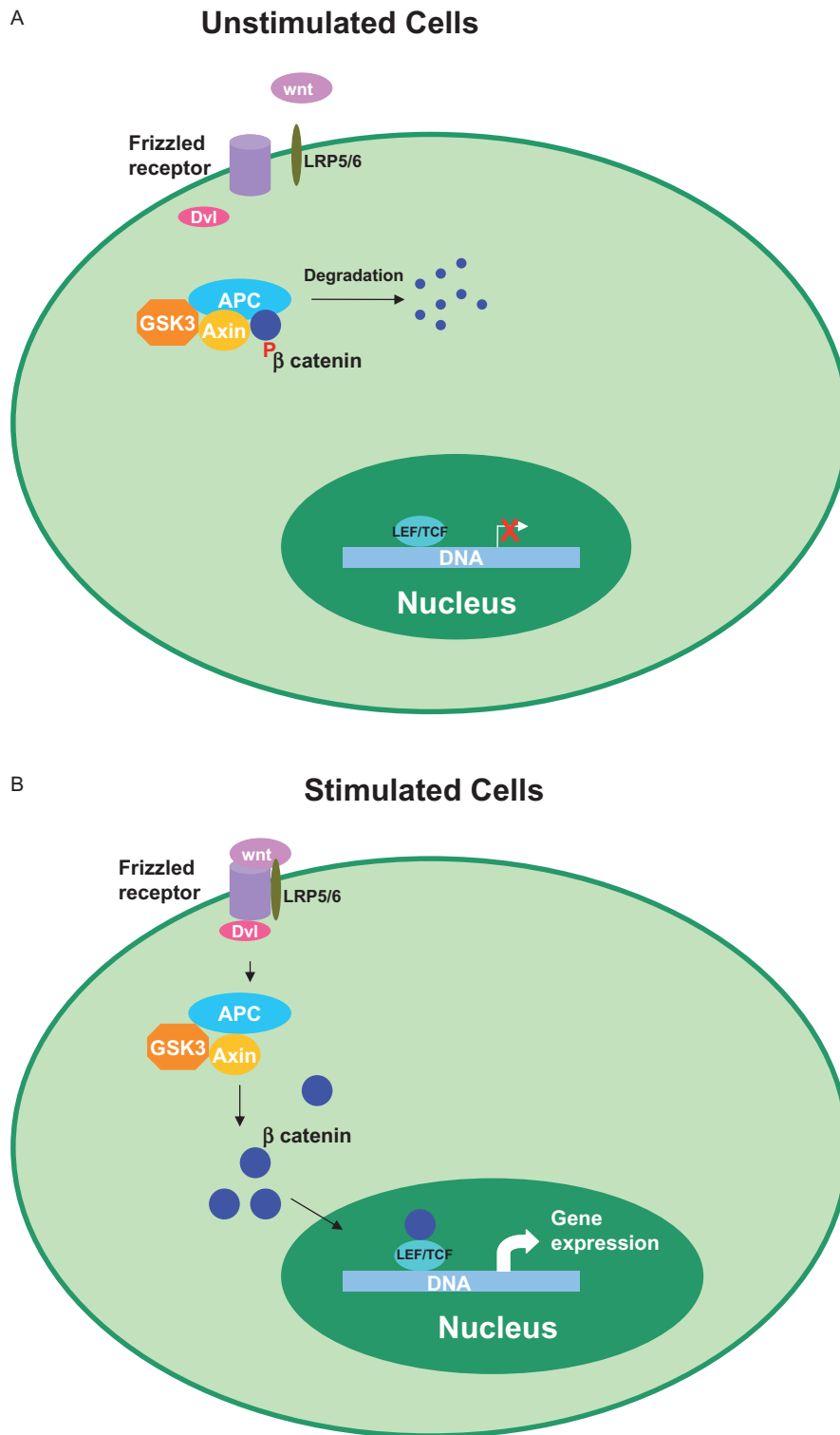
Apart from its diverse substrates and several pathophysiological roles, GSK3 is a unique kinase for several other reasons. First, unlike other kinases the enzyme is constitutively active and is inactivated in response to cellular signals (Harwood, 2001; Doble and Woodgett, 2003). Second, phosphorylation of its substrates generally leads to their inactivation. Further, it has unique substrate specificity wherein many of its substrates need a 'priming phosphate' (which is a phosphorylated Ser/Thr residue) located at four amino acids C terminal to the site of phosphorylation (Frame and Cohen, 2001; Harwood, 2001; Doble and Woodgett 2003). Several protein kinases have been identified, which can phosphorylate the 'priming site' with their identity depending on the substrate in question. The consensus recognition sequence is Ser/Thr-(X-X-X)-pSer/pThr with X being any amino acid and pSer/pThr being the phosphorylated serine/threonine, which serves as a priming phosphate (Frame and Cohen, 2001; Harwood, 2001; Doble and Woodgett 2003). Multiple phosphorylations by GSK3, is carried out in a 'relay' fashion with the 'primed' phosphate serving to initiate the process of phosphorylation. Subsequently, the phosphorylated residue itself serves as a 'priming' phosphate to the subsequent residue to be phosphorylated. This relay mechanism may continue further ranging from two (transcription factor c-Jun) to five residues (human GS) (Frame and Cohen, 2001; Frame *et al.*, 2001). The mechanism underlying the requirement of 'priming phosphate' was revealed subsequently, with the identification of a specific binding site for the priming phosphate on GSK3 (Frame and Cohen, 2001; Frame *et al.*, 2001).

Interestingly, GSK3 itself is subject to regulation by phosphorylation and is phosphorylated at Ser/Thr and also at Try residues at N and C terminus respectively (Wang *et al.*, 1994).

Glycogen synthase kinase 3 is involved in several diverse pathways, and several substrates have been identified, which are phosphorylated *in vitro*. However, the physiological relevance and function of many of the substrates has not been determined so far. Of the several pathways in which GSK3 is implicated, its role in insulin signalling and Wnt signalling has been studied in detail (Figure 1). GSK3 regulates insulin signalling (Figure 1) via inhibition of GS leading to decreased glycogen synthesis and hence inhibition of GSK3 leads to increase in glycogen synthesis and increase in insulin sensitivity (Henriksen and Dokken, 2006; Lee and Kim, 2007). In the Wnt pathway (Figure 2), GSK3 exists in a multimeric complex comprising of adenomatous polyposis coli (APC) protein, scaffolding protein axin and  $\beta$  catenin (Figure 2) (Rubinfeld *et al.*, 1996; Seidensticker and Behrens, 2000). Activation of Wnt signalling pathway through its receptors Frizzled and LDL receptor related protein (LRP)5/6 co-receptors and intracellular protein Dishevelled, leads to inactivation of GSK3 (Cadigan and Liu, 2006; Zeng *et al.*, 2008). GSK3-mediated phosphorylation of the N terminal Ser/Thr of  $\beta$  catenin leads to its degradation and regulates the levels, cellular localization and function of  $\beta$  catenin (Figure 2) (Cadigan and Liu, 2006).

### GSK3 $\alpha/\beta$

Glycogen synthase kinase 3 has two isoforms encoded by two different genes with GSK3 $\alpha$  having a higher molecular weight



**Figure 2** Schematic illustration of role of GSK3 in Wnt signalling. In the unstimulated cells  $\beta$  catenin is present in a multi-protein complex with GSK3, Axin and APC. Phosphorylation of  $\beta$  catenin by GSK3 targets  $\beta$  catenin to proteasome-mediated degradation. In stimulated cells binding of Wnt to its receptor Frizzled and its co-receptor LRP5/6 leads to activation of Dishevelled (Dvl) leading to inhibition of phosphorylation of  $\beta$  catenin by the multimeric complex and its stabilization and accumulation in the cytosol. Stabilized  $\beta$  catenin translocates to the nucleus and in association with transcription factors LEF/TCF leads to increase in gene expression of target genes such as c-myc. APC, adenomatous polyposis coli; GSK3, glycogen synthase kinase 3; LEF/TCF, lymphoid enhancer factor/T cell factor; LRP, LDL receptor related protein.

than GSK3 $\beta$  owing to an extension at the N terminus (Woodgett, 1990; 1991). The two isoforms share extensive homology, especially at the kinase domain with 97% homology between the two. Perhaps, not surprisingly, they both have similar functions. However, despite this the two are not functionally redundant as demonstrated by the gene knockout studies. Deletion of GSK3 $\beta$  leads to embryonic lethality at E16 due to hepatocyte apoptosis thought to be mediated by regulation of nuclear factor  $\kappa$ B by GSK3 $\beta$  (Hoeflich *et al.*, 2000). This suggests that the function of GSK3 $\beta$  is not compensated by GSK3 $\alpha$ , which is intriguing as both the isoforms display similar substrate specificity and functions. In this context knockout of shaggy, a *Drosophila* GSK3 homolog is rescued by over-expression of human GSK3 $\beta$  but not GSK3 $\alpha$  suggesting that they might have different physiological functions (Ruel *et al.*, 1993). It has been demonstrated that GSK3 $\alpha$  and GSK3 $\beta$  have distinct role during zebrafish cardiogenesis with GSK3 $\alpha$  being necessary for cardiomyocyte survival and GSK3 $\beta$  modulating left right symmetry and affecting heart positioning (Lee *et al.*, 2007; Markou *et al.*, 2008). However, in certain pathways both the isoforms are implicated, as in axon formation in hippocampal neurons (Garrido *et al.*, 2007). Although most of the research so far has primarily been on GSK3 $\beta$ , recent advances on the understanding of the function of GSK3 $\alpha$  in Alzheimer's disease (Phiel *et al.*, 2003) and glucose metabolism (MacAulay *et al.*, 2007) may change the course.

Another variant of GSK3 $\beta$  called as GSK3 $\beta$ 2 that contains a 13-amino acid residue insert within the kinase domain has recently been discovered (Mukai *et al.*, 2002). Although its physiological functions are not well characterized, GSK3 $\beta$ 2 has been recently shown to be localized in neuronal cell bodies and not in neuronal processes too unlike GSK3 $\beta$ , which is localized in both. Further, GSK3 $\beta$ 2 has reduced activity towards  $\tau$  protein as compared with GSK3 $\beta$  whose significance yet needs to be understood.

## Regulation of GSK3

As GSK3 is involved in diverse processes, regulation of its activity is critical to ensure that the pathways are insulated and yet could be appropriately coordinated. To achieve this GSK3 is subject to multiple levels of regulation mediated by phosphorylation, cellular localization and protein-protein interactions. GSK3 $\beta$  can phosphorylate at both primed substrates like GS, eukaryotic initiation factor 2B (eIF2B),  $\beta$  catenin and unprimed substrates like cyclin D3, axin and APC although it phosphorylates the unprimed substrates less efficiently (Frame and Cohen, 2001; Frame and Zheleva, 2006). The mechanistic details for the requirement of the priming site have been well understood, and the phosphorylated priming site of the substrate interacts with three positive residues in GSK3 $\beta$ , Arg 96, Arg 180 and Lys 205, which form a binding pocket for the priming phosphate (Dajani *et al.*, 2001; Frame *et al.*, 2001; ter Haar, 2001). Mutation of Arg 96 to Ala prevents the phosphorylation of primed substrates by GSK3 (Frame *et al.*, 2001). Apart from the requirement of priming phosphate, substrate recognition requires GSK3 $\beta$  residues, Phe 67, Gln 89 and Asn 95, which facilitates precise

positioning of the substrate within the substrate binding pocket and provides an insight into the substrate binding and specificity (Ilouz *et al.*, 2006). GSK3 protein itself undergoes multiple phosphorylation events, which impact its activity depending on the amino acid being modified (Hughes *et al.*, 1993; Wang *et al.*, 1994). Phosphorylation of the N terminal Ser 21 for GSK3 $\alpha$  and Ser 9 for GSK3 $\beta$  has an inhibitory effect and plays an important role in regulation of GSK3 function during insulin signalling (Stambolic and Woodgett, 1994; Cross *et al.*, 1995). The Ser/Thr-phosphorylated N terminus serves as a pseudo substrate and inhibits its interaction with the binding of the priming phosphate resulting in inactivation of GSK3. These Ser residues are phosphorylated by Akt/PKB (protein kinase B) during insulin signalling leading to the inactivation of the enzyme and resulting in activation of GS and increased glycogen synthesis. Ser 21/9 could also be phosphorylated by several other kinases like AGC kinase, p70 ribosomal S6 kinase, p90 ribosomal S6 kinase (Frame and Cohen, 2001). Phosphorylation at Tyr in the C terminus at 279 for  $\alpha$  and 216 for  $\beta$  activates the enzyme, and its mutation decreases the activity of the enzyme. Kinases phosphorylating GSK3 at this site are not well characterized (Hughes *et al.*, 1993; Wang *et al.*, 1994). In a recent development, phosphorylation at C terminus of GSK3 by p38 mitogen-activated protein kinase (MAPK) leading to its inactivation has been reported (Thornton *et al.*, 2008). Significantly, p38 MAPK phosphorylates only GSK3 $\beta$  on Thr 390 and not GSK3 $\alpha$  residue leading to an increased accumulation of  $\beta$  catenin (Thornton *et al.*, 2008). In addition, Thr 390 residue is not conserved between the two isoforms (Thornton *et al.*, 2008). This suggests that apart from AKT/PKB-mediated inactivation of GSK3, p38 MAPK could be an alternative mechanism for regulation of GSK3 activity especially in brain (Thornton *et al.*, 2008).

Within the Wnt pathway, it is intriguing that GSK3 activates/inactivates Wnt signalling and functions as an on/off switch of Wnt signalling pathway by its ability to phosphorylate primed and unprimed substrates. Unlike most of the phosphorylation events mediated by GSK3 $\beta$  that leads to inactivation, phosphorylation of LRP6 by GSK3 leads to activation and promotes the engagement of LRP6 with scaffolding protein axin (Zeng *et al.*, 2005). LRP6 is an unprimed substrate, and phosphorylation of LRP6 promotes axin recruitment and Wnt activation. On the other hand phosphorylation of  $\beta$  catenin a primed substrate by GSK3 leads to inactivation of Wnt signalling (Zeng *et al.*, 2005). It is also likely that different pools of GSK3 have different functions where cytosolic GSK3 phosphorylates  $\beta$  catenin and antagonizes Wnt signalling and the plasma membrane bound GSK3 phosphorylates LRP6 and activates Wnt/ $\beta$  catenin signalling (Zeng *et al.*, 2005).

Glycogen synthase kinase 3 $\beta$  can phosphorylate  $\tau$  protein, implicated in Alzheimer's disease, at both primed and unprimed substrates with phosphorylation at primed sites (pThr 231) being important for the function of  $\tau$  than phosphorylation at unprimed substrates (pSer 396/404) (Cho and Johnson, 2004). Presenilin-1 is another unprimed substrate of GSK3 involved in Alzheimer's disease (Twomey and McCarthy, 2006). Significantly, role of different GSK3 substrates in regulating nervous system development and specially axon

morphology has been examined (Kim *et al.*, 2006). By using GSK3 R96A mutant that inhibits GSK3 activity towards primed substrates, it was demonstrated that differential regulation of primed substrates is associated with defects in axon branching (Kim *et al.*, 2006). It has been suggested that partial inhibition due to low concentrations of inhibitors or inhibition of primed substrates produces an intermediate condition and suggests the importance of dosage effects and also spatial regulation of GSK3 substrates. Another new form of regulation of GSK3 involving N terminal cleavage by calpain, which removes the N terminal inhibitory domain and activates its kinase activity has also been recently described (Goñi-Oliver *et al.*, 2007).

### Role of GSK3 in insulin resistance in type II diabetes mellitus

Homeostasis of blood glucose levels is controlled by efficient absorption of glucose and storage of excess glucose as glycogen. Insulin resistance is one of the main causes underlying the initiation and progression of type II diabetes mellitus (T2DM) (Bouché *et al.*, 2004). Insulin resistance is caused by the inability of the insulin-sensitive tissues to respond to insulin and efficiently clear the blood glucose by decreasing the hepatic glucose output and reduced glucose uptake by peripheral tissues (Bouché *et al.*, 2004). The various steps involved in insulin signal transduction have been well elucidated, and a general mechanism has emerged. Stimulation of insulin receptor (IR) upon binding of insulin leads to autophosphorylation of several tyrosine residues on the IR. This phosphorylation event triggers a cascade of phosphorylation events, where the insulin receptor substrates 1 and 2 (IRS1, IRS2) recognize and bind to the phosphorylated IR and are themselves phosphorylated. Subsequently, the activation of phosphoinositol 3 kinase converts phosphatidylinositol biphosphate to phosphatidylinositol triphosphate, which in turn stimulates and activates PKB. The next step in the signalling event involves the activated PKB to phosphorylate GSK3 leading to its inactivation (Figure 1) (Cross *et al.*, 1995; Eldar-Finkelman and Ilouz, 2003). This inactivation leads to a decrease in phosphorylation of GS resulting in its activation. Apart from GS, eIF2B is also activated in a similar fashion leading to an increase in glycogen synthesis and protein synthesis. Increased expression and activity of GSK3 has been reported in the skeletal muscle of T2DM patients (Nikoulina *et al.*, 2000) and in mice having diabetes (Eldar-Finkelman *et al.*, 1999) suggesting that dysregulation of GSK3 $\beta$  might result in impaired insulin signalling and hence diabetes. Evidence for this has been obtained by using pharmacological inhibitors of GSK3, which leads to improved insulin signalling and glucose levels in animal models of diabetes (Meijer *et al.*, 2004).

### GSK3 as a regulator of glycogen synthase activity

Glycogen synthase a key enzyme mediating the conversion of glucose to glycogen is kept in an inactive state by phosphorylation of a cluster of three serine residues at the C terminus termed site 3. The kinase chiefly responsible for phosphory-

lating the site 3 has been identified as GSK3. Insulin activates GS by inactivation of GSK3 and hence promoting dephosphorylation of serine residues Ser 641, Ser 645, Ser 549 and Ser 653 at C terminus of GS. Regulation of GS by insulin is multifactorial, and insulin activates GS by inducing glycogen-associated form of protein phosphatase 1 (PP1) that dephosphorylates the residues phosphorylated by GSK3 (Suzuki, *et al.*, 2001, Delibegovic *et al.*, 2003). Apart from site 3, site 2 in GS has also been implicated in regulation of GS activity, and PP1 and PP2 can dephosphorylate site 2 and site 3. Also, non-GSK3-mediated insulin-dependent GS activation has also been proposed (Semiz and McNeill, 2002). Glycogen synthesis is also stimulated in an insulin-independent manner after exercise when glycogen levels are depleted (Halse *et al.*, 2001; Nielsen and Wojtaszewski, 2004). However, data suggest that primarily the activation of GS by insulin is mediated by inactivation of GSK3. It has been proposed that hepatic GS activation by glucose is through stimulation of a protein phosphatase rather than Ser 21/Ser 9-mediated inactivation of GSK3 isoforms (McManus *et al.*, 2005). However, GSK3 inhibitors stimulate liver GS and are responsible for the blood glucose lowering effects of GSK3 inhibition observed in animal models of T2DM (Cline *et al.*, 2002).

The activity of GS is regulated by GSK3 $\alpha$  and  $\beta$  and insulin in both the skeletal muscle and liver. GS is a primed substrate, and casein kinase 2 (CK2) has been proposed to be the priming kinase that phosphorylates Ser 657 (Hanashiro and Roach, 2002; Semiz and McNeill, 2002). By using pharmacological inhibitors of GSK3, it has been demonstrated that inactivation of GS in muscle is mediated by GSK3, although the relative importance of the two isoforms to this process is not known. By using selective cell permeable inhibitors of GSK3, CT98014 and CHIR98023, acute treatment of human skeletal muscle cells, activation of GS was observed with an increase in glycogen synthesis. Significantly, increase in glucose uptake was observed only upon prolonged or chronic exposure of the inhibitors, and an increase in IRS1 levels was also observed (Nikoulina *et al.*, 2002).

Role for GSK3 in regulation of GS in muscle has been proposed (MacAulay *et al.*, 2005), and over-expression of GSK3 $\beta$  in muscle results in reduced muscle glycogen deposition, which suggests that GSK3 $\beta$  is the primary kinase regulating GS in muscle (Pearce *et al.*, 2004). By using GSK3 $\beta$ 9A/9A knock in mice, GSK3 $\beta$  has been proposed to be the principal isoform regulating GS in muscle (McManus *et al.*, 2005), and about threefold to fourfold higher level of expression of GSK3 $\beta$  has been reported in humans and mice, as compared with GSK3 $\alpha$ . However, down-regulation of GSK3 $\alpha$  by using antisense oligonucleotides in muscle cells leads to an increase in insulin-stimulated GS and glucose uptake in the muscle cells (Ciaraldi *et al.*, 2007). Further, GSK3 $\alpha$  over-expression in muscle cells impaired insulin-stimulated glycogen synthesis and glucose uptake but not basal glycogen synthesis and glucose uptake levels, implying that GSK3 $\alpha$  also may have a functional role in insulin action in muscle (Ciaraldi *et al.*, 2007). In contrast to this, recently MacAulay *et al.* (2007) demonstrated that GSK3 $\alpha$  regulates glycogen metabolism in liver and not in muscle using GSK3 $\alpha$  knockout mice (MacAulay *et al.*, 2007). Knockout animals are viable and show increased glucose tolerance and insulin sen-

sitivity with a significant reduction in total body fat mass as compared with wild type. In the knockout mice, liver GS was more active, accompanied by increased levels of glycogen and IRS1 in contrast to the GS and IRS1 of muscle from knockout mice. No significant increase in muscle or liver glucose transport was detected in the knockout animals (MacAulay *et al.*, 2007). Insulin-stimulated glycogen synthesis has been demonstrated in skeletal muscle in absence of GSK3 phosphorylation of GS by using GSK3 knockin mice with wild-type GSK3 $\alpha$  and GSK3 $\beta$  replaced with insulin-non-responsive mutant forms (S21A/S21A/S9A/S9A) suggesting that other mechanisms like allosteric regulation of GS by glucose-6-phosphate may be involved (Bouskila *et al.*, 2008).

Glycogen synthase kinase 3 also plays an important role in glucose metabolism in adipocytes apart from muscle and contributes to insulin regulation of glycogen synthesis but not in glucose transport and glucose transporter 4 (GLUT4) translocation (Summers *et al.*, 1999). However, some reports suggest that in 3T3L-1 adipocytes, insulin can stimulate GS despite low or lack of expression of GSK3 $\beta$  (Ueki *et al.*, 1998). Also, decrease in GSK3 $\beta$  expression has been reported during adipocyte differentiation (Brady *et al.*, 1998). Obese type II diabetic patients treated with troglitazone for 3–4 months displayed increased glucose disposal accompanied by decreased GSK3 $\alpha$  and GSK3 $\beta$  levels in skeletal muscle but with no changes in adipocytes (Ciaraldi *et al.*, 2006). This suggests that in humans GSK3 plays a role in skeletal muscle, while its role in adipose tissue is not clear (Ciaraldi *et al.*, 2006).

### GSK3 and glucose homeostasis

Apart from its role in regulation of GS activity that is chiefly responsible for its function in glucose homeostasis it has also been implicated in glucose uptake. GSK3 has been proposed to phosphorylate light chains of kinesin motor proteins, which mediate the translocation of the GLUT4 glucose transporters (Morfini *et al.*, 2002). This phosphorylation of kinesin results in its inhibition, and inactivation of GSK3 by insulin may increase the glucose uptake. However, the data from knock in studies suggest no change in the insulin-stimulated glucose uptake in muscle (McManus *et al.*, 2005; MacAulay *et al.*, 2007). GSK3 can also phosphorylate the IRS (IRS1) and influence insulin action (Eldar-Finkelman and Krebs, 1997). Insulin mediates most of its actions through IRS1 and IRS2 that recruit src homology 2 proteins such as p85 regulatory subunit of phosphoinositol 3 kinase, src homology protein tyrosine phosphatase 2, growth factor receptor bound protein 2 (Eldar-Finkelman and Krebs, 1997). In unstimulated cells IRS1 is phosphorylated on Ser/Thr residues, and upon stimulation of cells with insulin, IRS1 is phosphorylated on Tyr residues (Greene and Garofalo, 2002). Hyper phosphorylation of IRS1 on Ser/Thr residues leads to a reduction of the ability of the IR to phosphorylate IRS1, and this has been implicated in insulin resistance (Hotamisligil *et al.*, 1996). GSK3-mediated phosphorylation of IRS1 on Ser residues has been reported (Eldar-Finkelman and Krebs, 1997), and over-expression of GSK3 leads to an increase in serine phosphorylation of IRS1 and decreased tyrosine phosphorylation of IRS1 and IR (Aguirre *et al.*, 2002; Liberman and Eldar-Finkelman,

2005). However, apart from GSK3 other kinases like CK2 have been implicated in IRS1 phosphorylation, and several other factors like tumour necrosis factor  $\alpha$  that induce insulin resistance also lead to serine phosphorylation of IRS1 by c-Jun N terminal kinase (JNK) and inhibitor  $\kappa$ B kinase (IKK $\beta$ ) (Eldar-Finkelman and Krebs, 1997; Liberman and Eldar-Finkelman, 2005). The serine residues phosphorylated by GSK3 have been identified as Ser 332 with Ser 336 serving as the 'priming' phosphate required for phosphorylation. Mutation of these sites to Ala increased the tyrosine phosphorylation of IR and GSK3 inhibitor lithium reduced Ser 322 phosphorylation and over-expression of GSK3 increased its phosphorylation (Liberman and Eldar-Finkelman, 2005). Hyper phosphorylation of IRS1 on serine residues induces IRS1 degradation in proteosomes, and hence inhibition of GSK3 could reduce its phosphorylation and its degradation. Increase in IRS1 protein expression in skeletal muscle could augment insulin action. GSK3 has been implicated in repression of gluconeogenic enzymes such as phosphoenolpyruvate carboxy kinase and glucose 6 phosphatase that reduces hepatic glucose production and helps in modulating glucose homeostasis (Lochhead *et al.*, 2001). However, there are contradicting reports regarding the regulation of gluconeogenic enzymes by GSK3. While some reports suggest that inhibition of GSK3 leads to the repression of the gluconeogenic enzymes, other reports suggest that the gluconeogenic enzymes can be repressed without inhibiting GSK3 (Lipina *et al.*, 2005).

The data so far suggest that apart from its primary effect on GS, GSK3 is also involved in upstream and downstream insulin-signalling events. GSK3 can suppress GS activity in short term and in long term negatively regulate glucose uptake and insulin signalling. Chronic effects of GSK3 inhibitors could also be mediated by regulation of protein expression and inactivation of eIF2B, can lead to up-regulation of protein translation.

### Role of GSK3 in pancreas

Imbalanced glucose homeostasis due to the combined effect of insulin resistance and  $\beta$  cell failure is a hallmark of progression of T2DM (Donath *et al.*, 2005; Bouché *et al.*, 2004). Progressive insulin resistance leads to increased insulin secretion by pancreas finally resulting in the failure of pancreatic  $\beta$  cells (Donath *et al.*, 2005; Bouché *et al.*, 2004). Hence, as the disease progresses, there is a reduction in  $\beta$  cells of pancreas and desired goal of anti-diabetic agents is to preserve and increase the  $\beta$  cell mass and restore their function. Recently developed glucagon like peptide 1 analogs and dipeptidyl peptidase IV inhibitors have been demonstrated to improve  $\beta$  cell function in preclinical models (Baggio and Drucker, 2006). The function of GSK3 in T2DM has been primarily focused on the insulin signal transduction in liver and skeletal muscle. Recent studies have provided novel insights into the function of GSK3 in pancreas.

It is likely that GSK3 has a function in pancreas as it is a substrate of PI3 kinase/Akt pathway, which previously has been shown to be a central regulator of  $\beta$  cell growth. GSK3 has also been implicated in endoplasmic reticulum stress in pancreatic  $\beta$  cells, and down-regulation of GSK3 can protect

the cells from death (Srinivasan *et al.*, 2005). Further, GSK3 has been reported to be inactivated by several ligands like glucagon like peptide 1, glucose-dependent insulintropic peptide, insulin-like growth factor-1, which promote  $\beta$  cell preservation and proliferation (Mussmann *et al.*, 2007).

Transcription factor IPF1/PDX1 (insulin promoter factor or pancreatic/duodenal homeobox 1 transcription factor 1) plays a crucial role in pancreas development and maintenance of  $\beta$  cell function as its deletion leads to diabetes in mice and reduced expression affects insulin expression and secretion (Boucher *et al.*, 2006). IPF1/PDX1 has been shown to be phosphorylated by GSK3 on Ser 61 and/or Ser 66 in  $\beta$  cells, which targets it for degradation by proteasomes (Boucher *et al.*, 2006). However, it is not clear if IPF1/PDX1 is indeed substrate of GSK3 or an indirect effect of oxidative stress observed in diabetic state, which increases this phosphorylation event and decreases the levels of IPF1/PDX1.

Apart from PDX1, it has been recently demonstrated that Maf A, a  $\beta$  cell transcription factor is constitutively phosphorylated by GSK3 at its amino terminus leading to its rapid degradation under low-glucose conditions and not in high-glucose conditions (Han *et al.*, 2007). Surprisingly, although the GSK3-mediated phosphorylation status of Maf A is not altered by glucose concentration it is required for its degradation (Han *et al.*, 2007).

Mussman *et al.*, have demonstrated that inhibition of GSK3 $\beta$  promotes replication and survival of pancreatic  $\beta$  cells against death induced by high glucose and fatty acid palmitate (Mussmann *et al.*, 2007). Interestingly, experiments by using siRNA demonstrate that inhibition of GSK3 $\alpha$  and GSK3 $\beta$  is required for stimulation of replication as inhibition of either of them alone did not stimulate the proliferation (Mussmann *et al.*, 2007). Transgenic mice over-expressing constitutively active form GSK3 $\beta$  (S9A) in  $\beta$  cells showed impaired glucose tolerance after 5 months of age. The  $\beta$  cell mass and proliferation were decreased along with a 50% decrease in the PDX1 levels (Liu *et al.*, 2008).

Recently, role of GSK3 $\beta$  in regulation of  $\beta$  cell mass (Liu *et al.*, 2008) has been examined in mice lacking one subunit of IR (Ir+/-) and in mice lacking two alleles of IRS (Irs2-/-) (Tanabe *et al.*, 2008). Although both the genotypes exhibit insulin resistance Ir+/- mice exhibit doubling of  $\beta$  cell mass, and Irs2-/- mice develop  $\beta$  cell loss and severe diabetes. Significantly, increased levels of GSK3 $\beta$  activity were reported in  $\beta$  cells of Irs2-/- mice, and these mice heterozygous for GSK3 $\beta$  have reduced insulin resistance and preserved  $\beta$  cell mass due to increase in proliferation and decreased apoptosis. Ir+/-Gsk3 $\beta$ +/- mice exhibit improved insulin sensitivity, reduced hyperinsulinemia and reduced  $\beta$  cell mass. GSK3 $\beta$  has been proposed to phosphorylate and stabilize cyclin-dependent kinase (CDK) inhibitor p27 kip1 levels and destabilize PDX1 levels. Although insulin resistance was reduced in Irs2-/-Gsk3 $\beta$ +/- mice, it was still insulin-resistant as compared with wild-type, suggesting that the beneficial effects of GSK3 $\beta$  deficiency on restoration of glucose homeostasis is not solely due to altered insulin sensitivity. Contribution of increased insulin sensitivity in tissues like muscle to the preservation of  $\beta$  cell mass has been ruled out by using  $\beta$  cell specific knock-out of GSK3 mice, which also prevented diabetes of Irs2-/- mice. These results demonstrate that GSK3 $\beta$  plays an impor-

tant role in  $\beta$  cell regulation and that inhibitors of GSK3 have a good therapeutic potential to preserve  $\beta$  cell function and delay or prevent diabetes progression (Tanabe *et al.*, 2008). GSK3 $\beta$  has also been implicated in improved  $\beta$  cell mass observed by inhibition of JNK kinase (Fornoni *et al.*, 2008).

Using small molecule GSK3 inhibitors like 1-azapallones derived from 1-paullone, stimulation of  $\beta$  cell replication and also protection from cell death induced by glucolipototoxicity has been demonstrated in insulinoma cell line (Stukenbrock, *et al.*, 2008). In addition, Cazpaullone (9-cyano-1-azapallone) stimulated replication of primary  $\beta$  cells and increased expression of  $\beta$  cell transcription factor Pax4 involved in  $\beta$  cell growth and development (Stukenbrock, *et al.*, 2008). The evidence so far suggests that GSK3 has an important function in  $\beta$  cells of pancreas, which is mediated by modulating key factors like PDX1, Maf A and Pax4 that adds yet another feature to this multifaceted protein.

### GSK3 in Wnt signalling and oncogenesis

Apart from regulation of GS and insulin signalling, GSK3 also plays an important role in canonical Wnt signalling pathway, which is critical for embryonic development (Logan and Nusse, 2004). As discussed earlier, GSK3 exists in a multimeric complex with APC, axin and  $\beta$  catenin, where GSK3 phosphorylates the N terminal Ser/Thr of  $\beta$  catenin leading to its degradation mediated by ubiquitin/proteasomes (Figure 2) (Rubinfeld *et al.*, 1996; Chen *et al.*, 2000). Activation of Wnt signalling leads to GSK inactivation resulting in dephosphorylation and stabilization of  $\beta$  catenin protein in the cytosol. The increased levels of  $\beta$  catenin lead to its nuclear translocation, and its interaction with transcription factors LEF/TCF (lymphoid enhancer factor/T cell factor) activate the expression of target genes like c-myc and cyclin D1, leading to an increase in cell proliferation (Jope and Johnson, 2004; Patel *et al.*, 2004). Abnormal Wnt signalling (Figure 2) has been associated with several human diseases like cancers (Luo *et al.*, 2007). Role of  $\beta$  catenin was first implicated in colorectal cancers as mutations in either APC or GSK3 $\beta$  sites in  $\beta$  catenin lead to increase in levels of  $\beta$  catenin as observed in colon cancer (Luo *et al.*, 2007). Aberrant Wnt signalling has also been reported in wide range of cancers (Luo *et al.*, 2007). Further, GSK3 has been implicated in accurate chromosome segregation based on studies in cultured cells, suggesting that GSK3 inhibitors may induce chromosome instability (Tighe *et al.*, 2007), which might promote tumorigenesis. However, GSK3 inhibitors-induced chromosome instability needs to be examined in animal models and humans. Recently, GSK3 $\beta$  has also been implicated in skin tumorigenesis (Ma *et al.*, 2007). Apart from GSK3 $\beta$ , GSK3 $\alpha$  also regulates  $\beta$  catenin levels and  $\beta$  catenin-mediated transcription through Wnt pathway (Asuni *et al.*, 2006; Doble *et al.*, 2007).

Importantly, as inhibitors of GSK3 can improve insulin resistance and serve as therapeutic agents for diabetes, it is of concern that GSK3 inhibition via Wnt pathway leads to stabilization of  $\beta$  catenin that may promote tumorigenesis (Jope and Johnson, 2004; Patel *et al.*, 2004). Hence, it is important to address these issues, which would be aided by a detailed understanding of the mechanism of regulation of GSK3 in the

insulin and Wnt pathway. Significantly, although both Wnt and insulin decrease the GSK3 $\beta$  activity to a similar extent, research demonstrates that the downstream effect is specific to each of the pathways. In the Wnt pathway GSK3 activity is regulated by protein-protein interactions in multi-protein complexes. Insulin signalling does not regulate  $\beta$  catenin levels and regulates only GS suggesting that there are different pools of GSK3 $\beta$  complexes, which are differentially regulated (Patel *et al.*, 2004). Ser 9 of GSK3 $\beta$  is not regulated by Wnt or Dishevelled, and GSK3 $\beta$  in the complex is not phosphorylated on Ser 9 upon insulin stimulation (Ding *et al.*, 2000). Further, in GSK3 $\alpha/\beta$  21A/21A/9A/9A mice, Wnt induced inhibition of phosphorylation of  $\beta$  catenin on the sites targeted by GSK3, suggesting that Wnt3 $\alpha$ -mediated inactivation of GSK3 does not involve phosphorylation of GSK3 $\alpha$  and GSK3 $\beta$  at Ser 21 and Ser 9 (McManus *et al.*, 2005). It has been proposed that inactivation of GSK3 isoforms by Ser 21/Ser 9 phosphorylation does not play a major role in regulating development, cell growth and proliferation (McManus *et al.*, 2005). This elaborate mechanism of substrate specificity, different binding partners and compartmentalization ensures that there is little or no crosstalk between the two pathways (Ding *et al.*, 2000). In addition, protein kinase C (PKC) has also been shown to be involved in Wnt-induced inactivation of GSK3 $\beta$  (Chen *et al.*, 2000). It has been suggested that phosphorylation of  $\beta$  catenin by GSK3 does not require priming *in vitro*, in contrast to the phosphorylation of GS (Hagen *et al.*, 2006). However, recent reports provide evidence that phosphorylation of  $\beta$  catenin also requires priming phosphate (Hagen and Vidal-Puig, 2002), and it has been demonstrated that CK1 is the priming kinase for  $\beta$  catenin (Jope and Johnson 2004, Patel *et al.*, 2004; Bustos *et al.*, 2006). Ser 33, Ser 37, Thr 41 phosphorylation on  $\beta$  catenin requires prior phosphorylation on Ser 45, and the Arg 96 mutant is unable to induced degradation and indeed stabilizes  $\beta$  catenin (Jope and Johnson 2004, Patel *et al.*, 2004).

Another protein of the Wnt pathway regulating GSK3 is intracellular protein FRAT (frequently rearranged in advanced T cell lymphoma) for which two isoforms FRAT1 and FRAT2 have been identified in humans (van Amerongen *et al.*, 2004; Stoothoff *et al.*, 2005; Hagen *et al.*, 2006). FRAT1 and FRAT2 inhibit GSK3 activity towards  $\beta$  catenin by competing with axin for binding to GSK3 $\beta$  (Ferkey and Kimelman, 2002; Stoothoff *et al.*, 2005). Both FRAT1 and 2 have a conserved GSK3 $\beta$ -interacting domain, and it has been proposed that FRAT1 competes with axin for binding GSK3 $\beta$  as the binding sites on GSK3 $\beta$  for these two proteins overlap (Ferkey and Kimelman, 2002; Stoothoff *et al.*, 2005). FRATtide a 39 amino acid peptide derived from FRAT1 is sufficient to bind to GSK3 and inhibit axin association with GSK3 (Thomas *et al.*, 1999; Jope and Johnson 2004, Patel *et al.*, 2004). Significantly, inhibition of GSK3 activity by FRATtide is specific to axin and  $\beta$  catenin and does not inhibit its activity towards GS and eIF2B (Thomas *et al.*, 1999; van Amerongen and Berns, 2005). In addition, FRATtide inhibits GSK3 $\beta$ -mediated phosphorylation of unprimed sites on  $\tau$  and not the primed phosphorylation sites (van Amerongen and Berns, 2005; Stoothoff *et al.*, 2005). On the other hand, FRAT2 interacts with GSK3 $\beta$  directly, and it may enhance the GSK3 $\beta$  activity selectively directed towards primed substrates (Stoothoff *et al.*, 2005).

It was earlier proposed that as priming phosphate is not required for phosphorylation of  $\beta$  catenin by GSK3 $\beta$  it is feasible to develop drugs that selectively inhibit the phosphorylation of insulin-signalling proteins without affecting the unprimed substrates like  $\beta$  catenin thereby reducing the potential of the inhibitors being oncogenic (Frame *et al.*, 2001). However, although with the demonstration that  $\beta$  catenin is also a primed substrate, that approach might not be feasible, it is significant note that Gsk3 $\alpha$ -/- mice do not show increased stabilization of proto oncogene  $\beta$  catenin, which has been believed to lead to enhanced oncogenesis. Also, the knockout mice show no increase in the propensity for tumorigenesis. In another significant development it has been demonstrated that loss of at least three if not all the four alleles of GSK3 $\alpha$  and  $\beta$  is required for significant dysregulation of  $\beta$  catenin levels and its signalling. Deletion of either both the alleles of GSK3 $\alpha$  alone or  $\beta$  alone does not disrupt  $\beta$  catenin signalling and there is no compensatory up-regulation of the other isoform that might explain for the lack of tumorigenesis in the GSK3 $\alpha$  knockout mice. These findings suggest that despite the concern of GSK3 inhibitors leading to increased  $\beta$  catenin levels and enhanced oncogenesis, this might not hinder the development of GSK3 inhibitors for diabetes.

### GSK 3 inhibitors for type II diabetes

One of the best studied inhibitors of GSK3 is lithium, which is ATP-non-competitive inhibitor and competes with magnesium and inhibits GSK3 with a Ki of 2 mmol-L<sup>-1</sup> (Ryves and Harwood, 2001). Lithium has been used as a therapeutic agent for treatment of manic depression and bipolar disorders (O'Brien *et al.*, 2004). Lithium inhibition stimulates glucose uptake, glycogen synthesis and normalizes insulin sensitivity in diabetic rats (Rossetti, 1989). However, importantly lithium is not a selective inhibitor of GSK3 as it can inhibit several other enzymes like CK2, p38-regulated/activated kinase and MAPK-activated protein kinase-2 (Phiel and Klein, 2001). Lithium also inhibits polyphosphate 1-phosphatase and inositol monophosphate (Phiel and Klein, 2001). Hence, the phenotypes attributed to inhibition of lithium would be difficult to assign to sole inhibition of GSK3. In this regard it would be important to use specific inhibitors of GSK3.

Inhibitors of GSK3 have enormous potential as therapeutics for diabetes, bipolar disorder and Alzheimer's and several other neurological disorders. The anti-diabetic effect of GSK3 is exerted by enhancing glucose disposal and improving insulin resistance. Apart from this, the recently demonstrated beneficial effects on  $\beta$  cells provide a distinct advantage over other anti-diabetic therapies. GSK3 inhibitors could also have a beneficial effect for type I diabetes and also islet transplantation patients. Several potent GSK3 inhibitors have been identified and characterized in preclinical models for treatment against T2DM.

The inhibitors identified so far belong to diverse chemotypes described following and significantly, most are directed to the ATP-binding site and function as ATP-competitive inhibitors. Of these, maleimides were some of the earliest GSK3 inhibitors with SB-216763 and SB-415286



inhibiting GSK3 $\alpha$  and GSK3 $\beta$  with Ki of 9 and 31 nmol·L<sup>-1</sup> (Coghlan *et al.*, 2000; MacAulay *et al.*, 2003). These compounds stimulate glycogen synthesis in human liver cells and also induce expression of  $\beta$  catenin – LEF/TCF reporter gene (Coghlan *et al.*, 2000). Arylmaleimide (Smith *et al.*, 2001), pyrazolo pyridines (Witherington *et al.*, 2003; Engler *et al.*, 2005), pyrimidin amines (Tavares *et al.*, 2004), series of pyrazolo arylhydrazones (Peat *et al.*, 2004), thiazolo [5,4-f] quinazolin-9-ones (Testard *et al.*, 2006), 7-hydroxy-1H-benzoimidazole derivatives (Shin *et al.*, 2007), bis(indolyl)maleimide pyridinophanes (Zhang *et al.*, 2007) and pyrimidine derivatives (Miyazaki *et al.*, 2008) have all been reported as potent and selective GSK3 inhibitors with some reported to have cellular efficacy. Of these 2-cyanoethylalsterpaullones are most potent with IC50s in picomolar range (Kunick *et al.*, 2005). Significantly, as discussed above Cazpaullone a GSK3 inhibitor has been reported to stimulate replication and protection of pancreatic  $\beta$  cells (Stukenbrock *et al.*, 2008) extending the role of GSK3 to pancreatic function in T2DM.

Importantly, as most of the inhibitors are targeted to the ATP-binding site, achieving selectivity over other related kinases is challenging and is one of the key issues that need to be addressed. As discussed below, some of the inhibitors have been shown to be selective over a small panel of about 20–50 kinases. Also, importantly it has been difficult to obtain selectivity over CDKs, which are key regulators of cell cycle. Most potent GSK3 inhibitors also inhibit CDKs as the homology between the two ATP-binding sites is 86%. It is desirable to avoid inhibition of CDKs for the treatment of diabetes as it is a chronic disease needing long-term treatment although CDK inhibitors are reported to function as anti-cancer agents (Fischer, 2003). However, for neurodegenerative diseases it might be desirable to have dual GSK3/CDK inhibitors as CDKs especially CDK5 has been shown to phosphorylate similar substrates as GSK3 (Fischer, 2003). In this context several inhibitors have been reported that exhibit moderate to good selectivity over CDKs. SB-216763 and SB-415286 are reported to be selective over 25 Ser/Thr kinases (Coghlan *et al.*, 2000), macrocyclic bisindolylmaleimides that inhibit GSK3 $\beta$  and PKC $\beta$  (Zhang *et al.*, 2003); a series of pyrazolo pyridines are selective over the closely related CDK2 (Witherington *et al.*, 2003); azaindolylmaleimides are slightly selective over CDK2 with little or no inhibition over 62 protein kinases (Kuo *et al.*, 2003; Shen *et al.*, 2004). Moderate selectivity over PKC and CDK with good selectivity over 50 kinases has been reported for macrocyclic polyoxygenated bis-7-azaindolylmaleimides (Kuo *et al.*, 2003), and AR-A014418 was selective over CDK2 and CDK5 and 26 other kinases (Bhat *et al.*, 2003).

## Preclinical data

In acute experiments several GSK3 inhibitors like CT118637, CT98014, CHIR98023 have been demonstrated to enhance insulin signalling in skeletal muscle from diabetic animals (Nikoulina *et al.*, 2002; Henriksen *et al.*, 2003; Dokken *et al.*, 2005; Henriksen and Teachey, 2007). Acute inhibition with CT118637 in muscles of pre-diabetic obese Zucker rat enhances insulin signalling with increased basal GS, insulin

stimulated glucose transport only in insulin-resistant skeletal muscle (Dokken *et al.*, 2005). Up-regulation of IR and IRS1 tyrosine phosphorylation, reduction in the serine phosphorylation of IRS1 (Ser 307) along with increased GSK3 $\beta$  Ser 9 and AKt Ser 473 phosphorylation were reported when muscles from Zucker diabetic fatty rats were treated with CT98014 *in vitro* (Henriksen and Teachey, 2007). An increase in liver glycogen synthesis with little effect on muscle glycogen synthesis was reported with GSK3 inhibitors CHIR98023 and CHIR99021 in Zucker fa/fa rats (Cline *et al.*, 2002).

Chronic treatment of GSK3 inhibitors like CT118637 (Dokken and Henriksen, 2006), aminopyrimidine derivatives CHIR 98014 and CHIR 99021 (Ring *et al.*, 2003) in Zucker rats enhanced glucose tolerance, activated GS, with improved insulin sensitivity and increased IRS1-dependent insulin signalling (Dokken and Henriksen, 2006). Other inhibitors like bisarylmaleimide that are >160- to >10 000-fold selective over CDK2/4 and PKC $\beta$ II demonstrated lowering of plasma glucose levels in Zucker diabetic fatty rats (Engler *et al.*, 2004).

Among the non-ATP-competitive GSK inhibitors, thienyl and phenyl alpha-halomethyl ketones (Conde *et al.*, 2003) and thiadiazolidinone derivatives have been reported (Castro *et al.*, 2008). Substrate-competitive peptide inhibitors have also been reported for GSK3 in contrast to most other inhibitors that are ATP-competitive (Plotkin *et al.*, 2003). They are selective for several related kinases like Cdc2 and increase GS activity and glucose uptake in cell-based systems and also improved glucose tolerance in insulin-resistant obese mice (Plotkin *et al.*, 2003). Further, chronic treatment in ob/ob mice, reduced blood glucose levels, improved glucose tolerance, suppressed hepatic phosphoenolpyruvate carboxy kinase, increased hepatic glycogen content and lead to up-regulation of GLUT4 in skeletal muscle (Kaidanovich-Beilin and Eldar-Finkelman, 2006). Also, in high fat fed C57BL/6J mice, it has been shown to improve hepatic and peripheral insulin resistance by increasing liver GS activity and hepatic glycogen synthesis (Rao *et al.*, 2007).

Many GSK3 inhibitors have been reported during the identification of inhibitors for CDKs with anti-tumour properties like paullones (Leost *et al.*, 2000) and indirubins that inhibit CDK5 and GSK3 $\beta$  (Leclerc *et al.*, 2001). Also, pyrazolo [3,4-b] quinoxalines (Ortega *et al.*, 2002) and aloisines (Mettey *et al.*, 2003) were found to inhibit both CDK5 and GSK3. Not, all CDK inhibitors inhibit GSK3 (Leclerc *et al.*, 2001) and 1-azakenpaullone has been reported to be selective for GSK3 $\beta$  over CDK1 (Kunick *et al.*, 2004). 9-oxo-thiazolo [5,4-f] quinazolin-2-carbonitrile derivatives have been reported as dual CDK1 and GSK3 inhibitors with potency in sub-micromolar range (Logé *et al.*, 2007). Significantly, CDK/GSK3 inhibitors have been proposed as therapy for proliferative renal disease, and efficacy has been demonstrated in preclinical models of mesangial proliferative glomerulonephritis (Soos *et al.*, 2006).

Glycogen synthase kinase 3 inhibitors have also been identified from natural sources, like hymenialdisine from marine sponge (Meijer *et al.*, 2000). Bisindole indirubin from a traditional Chinese medicine and other indirubins have been reported to be potent inhibitors of CDKs and GSK3 $\beta$  (Leclerc *et al.*, 2001). Subsequently, 6-bromo indirubin was identified as a potent and selective GSK3 inhibitor from 'tyrian purple'

dye of mollusks (Meijer *et al.*, 2003). By using a cell permeable derivative 6-bromindirubin-3'-oxime (BIO) it was shown that it inhibits phosphorylation of GSK3 $\alpha/\beta$  on Tyr276/216 and also reduces  $\beta$  catenin phosphorylation (Meijer *et al.*, 2003). Moreover, BIO was demonstrated to have >16-fold selectivity over CDK2 and CDK5 (Meijer *et al.*, 2003). Novel derivatives of indirubin like 5-substituted indirubins inhibit CDKs and GSK3 (Beauchard *et al.*, 2006) where as 7-bromindirubin-3'-oxime (7BIO) is less potent for CDK and GSK3 and has anti-tumour function (Ribas *et al.*, 2006). Recently, manzamine A and related derivatives from an Indonesian sponge have been reported as a new class of GSK3 inhibitors (Hamann *et al.*, 2007). Surprisingly, these inhibitors appear to inhibit specifically GSK3 $\beta$  and CDK5 and not CDK1, protein kinase A, MAPK, GSK3 $\alpha$  (Hamann *et al.*, 2007). Although several potent inhibitors belonging to different chemical classes have been reported to exhibit efficacy in various animal models of diabetes the critical translation of these results in human diabetic patients is awaited.

## Conclusion

Glycogen synthase kinase 3 is undoubtedly, a promising target for diabetes given its role in improving insulin resistance along with the added benefit of protecting pancreatic  $\beta$  cells. However, it has proven to be challenging given the complexity of its regulation, implication in several diverse pathways and the proposed side effect of GSK3 inhibitors leading to cancer. The fact that most of the GSK3 inhibitors are targeted at the ATP-binding site, obtaining selectivity over other kinases is a key issue and is important to achieve good selectivity encompassing a broad range of kinases. As GSK3 has been implicated in tumorigenesis it is important that the safety of GSK3 inhibitors be demonstrated in long-term treatment of chronic diseases like T2DM. Intriguingly, GSK3 has been implicated in cancer therapy, and recently GSK3 inhibitors have been proposed as a novel class of therapeutic agents for colon cancer (Shakoori *et al.*, 2007). Significantly, it has been suggested that inhibition of GSK3 alone in untransformed cells is not a primary event to increase levels of  $\beta$  catenin unless accompanied by other transformation event (Frame and Zheleva, 2006). Further, GSK3 $\beta$ -independent regulation of  $\beta$  catenin by other kinases like CK1, CK2, protein kinase A, IKK $\alpha$  and IKK $\beta$  has also been proposed, which might regulate the levels of  $\beta$  catenin. In this context recently, Cyclacel has reported GSK3 inhibitors that do not increase levels of  $\beta$  catenin although the mechanistic basis for this is not clear (Frame and Zheleva, 2006).

Long-term use of lithium the well studied GSK3 inhibitor for bipolar disorder has not been shown to be associated with an increased risk of cancer. Recent research has also demonstrated that inactivation of at least three alleles of GSK3 $\beta$  and GSK3 $\alpha$  is required for the increase of  $\beta$  catenin levels. Hence, it is possible that moderate inhibition of GSK3 might result in beneficial effects without up-regulation of  $\beta$  catenin. Further, a modest inhibition of GSK3 has been shown to be sufficient for stimulation of insulin action and hence an optimal level of inhibition of GSK3 can be achieved without affecting the  $\beta$  catenin levels as heterozygous mice are not cancer-prone.

Research so far has demonstrated that GSK3 $\alpha$  and  $\beta$  forms are similar yet functionally not redundant. Future research into the isoform specific functions and their implication for various disease in which GSK3 is implicated might facilitate development of isoform specific inhibitors. It would be interesting to identify the regions and interacting partners within GSK3 $\alpha$  and  $\beta$  contributing to the differential physiological function. Differential involvement of either isoform in specific pathways would provide a selective tool for drug discovery and enable development of inhibitors, which may not target the active site and achieve isoform selectivity and also selectivity over several other related kinases and minimize the side effects associated with them. Although at present it appears that most substrates of GSK3 are primed, use of inhibitors that might specifically affect primed versus unprimed might also facilitate the development of target specific GSK3 inhibitors. Although GSK3 inhibitors identified so far have demonstrated efficacy in preclinical models, it is important that efficacy and safety of GSK3 inhibitors be demonstrated in humans, which will aid in the development of novel drugs for T2DM and especially with a potential for protective and beneficial effects on pancreatic  $\beta$  cells, which further extends the scope of GSK3 inhibitors to T1DM.

## Conflict of interest

None.

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