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The glamour and gloom of glycogen synthase kinase-3

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Glycogen synthase kinase-3 (GSK3) is now recognized as a key component of a surprisingly large number of cellular processes and diseases. Several mechanisms play a part in controlling the actions of GSK3, including phosphorylation, protein complex formation, and subcellular distribution. These are used to control and direct the far-reaching influences of GSK3 on cellular structure, growth, motility and apoptosis. Dysregulation of GSK3 is linked to several prevalent pathological conditions, such as diabetes and/or insulin resistance, and Alzheimer's disease. Therefore, much effort is currently directed towards understanding the functions and control of GSK3, and identifying methods capable of diminishing the deleterious impact of GSK3 in pathological conditions.

For many years, glycogen synthase kinase-3 (GSK3) suffered from an identity crisis owing to a name that is inexorably linked to glycogen metabolism. However, increasing knowledge has changed the image of GSK3 to that of a broadly influential enzyme that is a crucial regulator of many cellular functions. This fascinating enzyme has several intriguing regulatory characteristics, it is centrally involved in regulating cellular structure, function and survival, and it is linked to several prevalent diseases, such as diabetes and Alzheimer's disease.

Multiple mechanisms contribute to the regulation of GSK3

GSK3 refers to two isoforms – GSK3α and GSK3β – as well as a recently identified splice variant of GSK3β [1]. The two isoforms are encoded by different genes and share nearly identical sequences in their kinase domains. Outside of the kinase domain, their sequences differ substantially, but little is known about isoform-specific functions.

Few enzymes exert as broad a regulatory influence on cellular function as GSK3. More than 40 proteins have been reported to be phosphorylated by GSK3, including over a dozen transcription factors (Table 1). Although most of these proteins have not yet met all of the criteria set out by Frame and Cohen [2] necessary to prove that a protein is an *in vivo* substrate of GSK3, this large number of putative substrates illustrates the great potential of GSK3 to affect many cellular functions. This suggests that the activity of GSK3 must be carefully regulated by mechanisms individually tailored for each substrate to avoid indiscriminate phosphorylation by GSK3 of its many substrates. Although the mechanisms regulating GSK3 are not fully understood, precise control appears to be achieved by a combination of phosphorylation, localization, and interactions with GSK3-binding proteins.

Regulation by phosphorylation

GSK3 activity is significantly reduced by phosphorylation of an N-terminal serine, Ser9 in GSK3β and Ser21 in GSK3α (Figure 1). Several kinases can phosphorylate these serines, including Akt, protein kinase A (PKA), protein kinase C and p90Rsk, among others (Figure 1). Therefore, many signaling pathways contribute to the control of GSK3. However, it must be kept in mind that each kinase probably affects only a specific pool of the GSK3 present in cells because of the subcellular distribution of both GSK3 and each regulatory kinase. Scaffolding proteins provide one mechanism to restrict interactions between the kinases. For example, PKA-anchoring protein 220 binds GSK3 to facilitate its phosphorylation by PKA [3] (Figure 2). In opposition to inhibitory regulation by serine phosphorylation, GSK3 activity is facilitated by phosphorylation of Tyr216 in GSK3β and Tyr279 in GSK3α. This might occur by autophosphorylation or by other tyrosine kinases but little is known about regulation of the processes that modulate tyrosine phosphorylation of GSK3 [2,4].

The actions of GSK3 are also often regulated by the phosphorylation state of its substrates. This is because most substrates of GSK3 must be pre-phosphorylated (primed) to be phosphorylated by GSK3 (Figure 1). Thus, two sets of phosphorylation-based signaling systems are integrated to regulate the actions of GSK3. Upstream of GSK3, signaling systems transmit regulatory information to GSK3 via kinases that directly phosphorylate GSK3 to control its activity (Figure 1, top portion). Downstream of GSK3, another set of signaling systems transmit information by pre-phosphorylating substrates of GSK3 (Figure 1, bottom portion). The timing and location of these signals combine to contribute to the necessary individualized control of the actions of GSK3 towards each substrate.

Regulation of intracellular localization

In addition to regulation by phosphorylation, mechanisms that regulate the intracellular localization of GSK3 control

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Table 1. Putative substrates of glycogen synthase kinase-3[a]

Metabolic and signaling proteins	Structural proteins	Transcription factors
AcetylCoA carboxylase	DF3/MUC1	AP-1 (Jun family)
Amyloid precursor protein	Dynamin-like protein	β-catenin
APC	Kinesin light chain	C/EBP
ATP-citrate lyase	MAP1B	CREB
Axin	MAP2	GATA4
Cubitus interruptus	Neural cell-adhesion Protein (NCAM)	Glucocorticoid receptor (rat)
Cyclic-AMP-dependent protein kinase	Neurofilaments	HIF-1
Cyclin D1	Ninein	HSF-1
Cyclin E	tau	Mash1
eIF2B	Telokin (KRP)	MITF
Glycogen synthase		c-Myb
hnRNP		c-Myc
Insulin receptor substrate-1		NeuroD
Myelin basic protein		NFAT
NGF receptor		NF-κB (p65 and p105)
Nucleoporin p62		Notch
P21		p53
Presenilin-1		TCF
Protein kinase A (RII subunit)		
Protein phosphatase 1		
Protein phosphatase inhibitor-2		
Pyruvate dehydrogenase		

[a]Abbreviations: AP-1, activator protein-1; APC, adenomatous polyposis coli gene product; C/EBP, CCAAT/enhancer-binding protein; CREB, cyclic AMP response element-binding protein; DF3/MUC1, high molecular weight mucin-like glycoprotein; eIF2B, Eukaryotic initiation factor 2B; HIF-1, hypoxia-inducible factor-1; hnRNP, heterogeneous nuclear ribonucleoprotein; HSF-1, heat shock factor-1; KRP, kinase-related protein; MAP, microtubule-associated protein; Mash1, mammalian achaete-scute homolog 1; MITF, microphthalmia-associated transcription factor; NFAT, nuclear factor of activated T cells; NF-kB, nuclear factor kB; NGF, nerve growth factor; TCF, T cell factor.

its access to substrates. Although GSK3 is traditionally considered a cytosolic protein (where it is predominantly located), it is also present in nuclei and mitochondria, where it is highly activated compared with cytosolic GSK3 [5]. Nuclear GSK3 is particularly interesting because of the many transcription factors that it regulates (Table 1), enabling GSK3 to influence many signaling pathways that converge on these transcription factors, thereby regulating the expression of many genes. The nuclear level of GSK3 is not static but changes dynamically in response to intracellular cues. Nuclear GSK3 levels fluctuate during the cell cycle, being highest in the S-phase, which facilitates phosphorylation of nuclear cyclin D1 by GSK3 [6]. Levels of GSK3 in the nucleus can rapidly increase early in the process of apoptosis, enabling GSK3 to modulate gene expression through its regulation of transcription factors [7]. Regulation of the nuclear localization of GSK3 has only recently begun to be examined, so it is probable that other conditions also modulate its level and activity in the nucleus. Even less is known about mitochondrial GSK3. However, it was recently shown that, in opposition to the nuclear level of GSK3, which is decreased by activated Akt [7], activated Akt is imported into mitochondria where it phosphorylates Ser9 of GSK3β to inhibit its activity, without changing the mitochondrial level of GSK3β [8].

Regulation by binding proteins

Protein complexes that contain GSK3 are of major importance in regulating its actions. The best characterized of these is the canonical wnt pathway (Figure 2), where GSK3-binding proteins control access to its substrate, β-catenin, thus generating a high degree of specificity in regulating the actions of GSK3 [2,9]. In the absence of a stimulus, the scaffold protein axin binds GSK3, casein kinase I, β-catenin and other proteins. This enables casein kinase I to phosphorylate Ser45 on β-catenin, creating a primed site for GSK3 to phosphorylate Thr41 and, subsequently, Ser37 and Ser33, modifications that promote β-catenin degradation. Wnt stimulation activates disheveled (dvl), which, in concert with the GSK3-binding protein Frat, facilitates disruption of the axin-based complex. This decreases the phosphorylation of β-catenin, which results in β-catenin accumulation and activation. This is a classical example of how GSK3 binding proteins can regulate the action of GSK3 towards individual substrates.

A question that still remains unanswered is whether multiprotein complexes such as those used in the wnt system are used to regulate the specificity of the actions of GSK3 in other pathways. For example, intriguing similarities to wnt signaling have been noted in the Hedgehog signaling system (Figure 2), in which several proteins, including GSK3, interact to inactivate *cubitus interruptus* [10,11]. The integrated interactions of these proteins suggest that a scaffolding protein might facilitate the process and ensure specificity, although such a scaffold has not yet been identified.

Protein complexes also regulate the actions of GSK3 within subcellular organelles. Binding of Frat-1 to GSK3 facilitates its nuclear export [12]. The presence of other proteins of the wnt signaling pathway in the nucleus, such as axin, adenomatous polyposis coli protein, and disheveled, suggests that these proteins might regulate nuclear GSK3, although this remains to be investigated. The tumor suppressor p53, which is mutated in many human cancers, directly binds nuclear GSK3. This association activates GSK3, and GSK3 promotes the transcriptional and apoptotic actions of p53, although the mechanism involved has not yet been elucidated [13]. The viral tumor-associated latent nuclear antigen also binds GSK3 and sequesters it in the nucleus [14]. In the mitochondria, p53

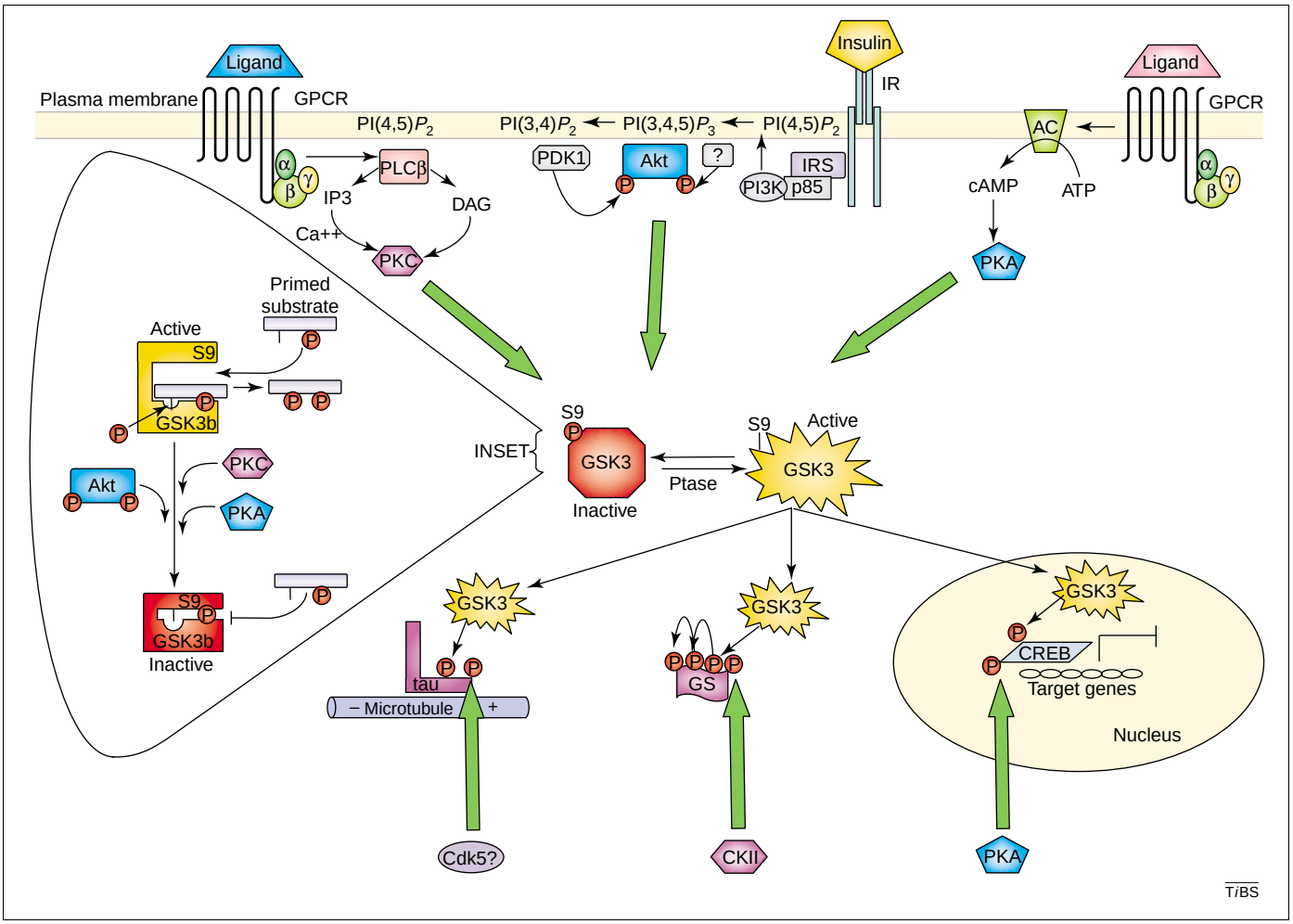

Figure 1. Regulation of glycogen synthase kinase-3 (GSK3) by phosphorylation. Several signaling cascades activate kinases that directly inhibit GSK3 by phosphorylating a regulatory N-terminal serine of GSK3 [shown as Ser9 (S9) of GSK3β]. Activation of G-protein-coupled receptors (GPCRs) linked to heterotrimeric G proteins (α-, β- and γ-subunits) that activate phospholipase-Cβ (PLCβ) causes the hydrolysis of phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] to two second messengers, inositol trisphosphate (IP3), which increases intracellular calcium levels, and diacylglycerol (DAG). These messengers induce activation of protein kinase C (PKC), which is capable of phosphorylating Ser9 of GSK3β. Similarly, ligand binding of GPCRs coupled to G proteins that activate adenylyl cyclase (AC) to produce cyclic-AMP, leads to activation of protein kinase A (PKA), which can also phosphorylate Ser9 of GSK3β. The most well-described signaling pathway that results in serine phosphorylation of GSK3 is initiated by the activation of tyrosine kinase receptors, such as the insulin receptor (IR) (IRS, insulin-receptor substrate). This results in sequential activation of phosphoinositide 3-kinase (PI3K), 3′-phosphoinositide-dependent kinase 1 (PDK1), and the protein kinase, Akt, which phosphorylates GSK3 on Ser9. Reactivation of GSK3 is mediated by specific protein phosphatases (Ptase). Many substrates of GSK3 must be primed, which means they are pre-phosphorylated at a serine or threonine, four residues removed from the serine or threonine that is phosphorylated by GSK3. Thus, the consensus site for phosphorylation of primed substrates by GSK3 is [S/T]xxx[S/T](*P*). This provides an additional regulatory mechanism controlling the action of GSK3 because signaling pathways phosphorylating its substrates must be active before GSK3 can phosphorylate such substrates. For example, pre-phosphorylation of the microtubule-associated protein tau by other kinases (which might include Cdk5) facilitates phosphorylation of tau by GSK3, which decreases the ability of tau to bind and stabilize microtubules [62]. Glycogen synthase (GS) must be pre-phosphorylated by casein kinase II (CKII) for GSK3 to phosphorylate GS efficiently and downregulate its activity. The transcription factor cyclic-AMP-response element binding protein (CREB) must first be phosphorylated by PKA, which enables subsequent phosphorylation of CREB by GSK3. How the cell integrates these dual regulatory phosphorylation mechanisms, phosphorylation of serine on GSK3, and priming phosphorylation of its substrates, has been clarified by structural studies of GSK3 [63,64]. The preference of GSK3 for phosphorylating substrates that have been pre-phosphorylated (or primed), and the inhibition of GSK3 activity by N-terminal serine phosphorylation, are both caused by the presence of a phosphate-binding pocket in GSK3 (inset). The phosphate of the primed substrate sits in this pocket and positions the phosphate acceptor site to enable efficient phosphorylation by GSK3. However, if GSK3 is phosphorylated at the N-terminal serine (Ser9 of GSK3β), this acts as a pseudo-substrate, inserting into the phosphate-binding pocket, blocking the active catalytic site and inhibiting the phosphorylation of substrates.

levels increase during certain forms of apoptosis, and p53 binds GSK3β within the mitochondria [15]. The functional outcome of this mitochondrial interaction is unknown, but it might facilitate apoptosis because inhibition of GSK3 attenuates both p53-induced cytochrome *c* release from the mitochondria, and caspase activation [15]. Further studies will undoubtedly identify additional GSK3-binding proteins in the nucleus, mitochondria and cytosol, and clarify how regulatory mechanisms are integrated to achieve substrate-specific regulation of GSK3 activity.

GSK3 regulates cellular architecture and motility

Several key effects of GSK3 on cell biology are depicted in Figure 2. Neurite retraction and extension, which are crucial processes in nervous system development and remodeling, are regulated by GSK3. For correct axonal growth and targeting, extension and retraction events must be temporally and spatially coordinated. GSK3 at the leading edge of extending growth cones is maintained in its inactive form, suggesting that active GSK3 inhibits growth cone extension [16,17]. In accordance with this, experimental inactivation of GSK3 in cerebellar granule

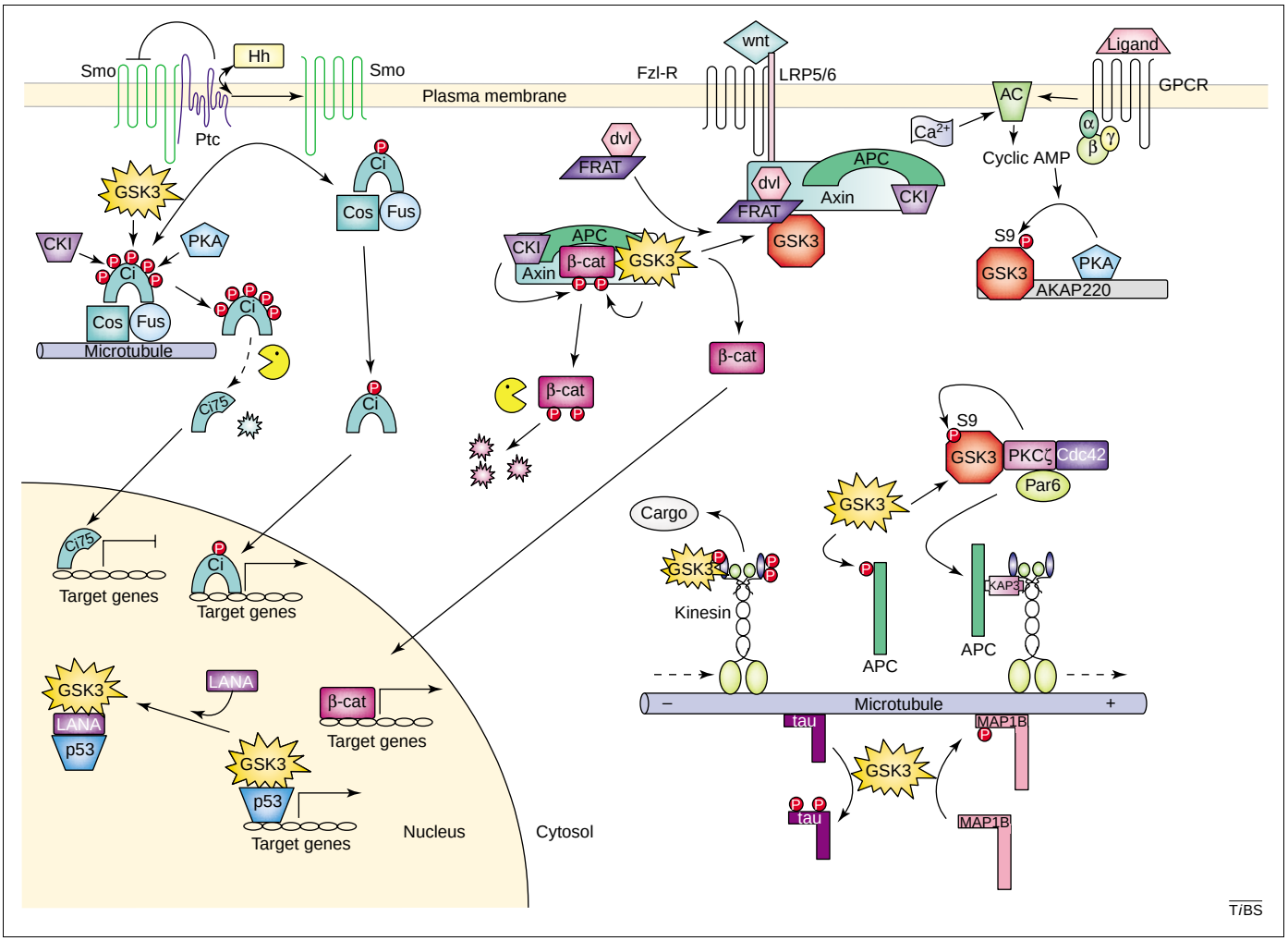

Figure 2. Interactions between glycogen synthase kinase-3 (GSK3) and protein complexes. Shown are representatives of different types of protein complexes that interact with GSK3 to regulate and direct its actions. The central portion of the figure shows the most well-characterized protein complex system that regulates GSK3: the wnt signaling pathway. In the absence of the wnt ligand, GSK3 is bound to axin in a complex with β-catenin (β-cat), casein kinase I (CKI) and adenomatous polyposis coli protein (APC). CKI phosphorylates β-catenin to prime it for phosphorylation by GSK3, resulting in proteosomal degradation of β-catenin. Stimulation by wnt of the two associated receptors, frizzled receptor (Fzl-R) and the low-density lipoprotein (LDL)-related protein 5/6 (LRP5/6) receptor, results in the recruitment of FRAT (frequently arranged in T cell lymphomas) and disheveled (dvl) into the GSK3 complex. This prevents the phosphorylation of β-catenin by GSK3, enabling β-catenin to accumulate and translocate to the nucleus where it is a co-transcriptional activator of T cell factor/lymphocyte-enhancer-binding factor (TCF/LEF), facilitating gene expression. The role of GSK3 in the developmentally important Hedgehog (Hh) signaling is, in some respects, similar to wnt signaling [10,11]. The Hedgehog receptor patched (Ptc) inhibits the activity of the smoothened (Smo) receptor in the absence of Hedgehog ligand. This enables *cubitus interruptus* (Ci) – a zinc finger transcription factor – to form a complex with costal2 (Cos) and fused (Fus) bound to microtubules. In this complex, Ci is phosphorylated by protein kinase A (PKA), CKI and GSK3, which results in the proteolysis of Ci to Ci75, a transcriptional repressor. Stimulation of Ptc by Hedgehog activates Smo. This causes dissociation of the Cos–Fus–Ci complex from the microtubules, and decreases phosphorylation of Ci, which increases its stability, enabling it to accumulate in the nucleus where it functions as a transcriptional activator. Signaling pathways regulating the kinases that phosphorylate GSK3 can depend on specific protein complexes, as represented by the cyclic-AMP second messenger system. Protein kinase A (PKA) is activated in response to a signal, such as binding of a ligand to a G-protein-coupled receptor (GPCR), resulting in adenylyl cyclase (AC) activation and increased cyclic-AMP production. The PKA-anchoring protein 220 (AKAP220) binds both PKA and GSK3 to bring GSK3 into close proximity with PKA, which phosphorylates GSK3 to block its activity [3]. This enables localized inhibition of GSK3 by PKA. GSK3 phosphorylates microtubule-associated proteins (MAPs), regulating their interactions with microtubules and thus modulating microtubule dynamics. Phosphorylation of tau by GSK3 decreases its affinity for microtubules, whereas phosphorylation of MAP-1B increases its association with microtubules. GSK3 also phosphorylates the light chains of kinesin, a motor protein that moves proteins towards the plus-end of microtubules, promoting release of cargos (e.g. protein and vesicles). This is an essential function because it is important that the cargos be unloaded at appropriate destinations. APC is also associated with microtubules bound to kinesin-associated protein 3 (KAP3), which interacts with kinesin [65]. APC is phosphorylated by GSK3, which decreases the association of APC with microtubules. The positioning of APC at the plus-end of microtubules is essential for maintaining the appropriate direction of cell migration. To avoid disruption of this function of APC – which would occur if it were phosphorylated by GSK3 – the activity of GSK3 is inhibited in growth cones during cell migration by serine phosphorylation. This is accomplished by activation of the small GTPase Cdc42, which translocates the Par6–protein kinase Cζ (PKCζ)–GSK3 complex to the leading edge of the cell. PKCζ phosphorylates and inactivates GSK3, reducing the phosphorylation of APC. This enables increased binding of APC at the plus-ends of microtubules, which appears to be essential for maintaining the proper orientation of astrocytic protrusions [16]. In the nucleus, the transcription factor p53 binds and activates GSK3, and GSK3, in turn, promotes the transcriptional and apoptotic actions of p53 [13]. In Kaposi's sarcoma-associated herpes virus-associated tumors, GSK3 is sequestered in the nucleus by the latent nuclear antigen (LANA) – a nuclear viral oncoprotein expressed in these tumors – thus enabling accumulation and activation of β-catenin [14]. Additionally, LANA binds p53 and inhibits its actions, which GSK3 would otherwise promote [13]. Thus, proteins binding to GSK3 in the nucleus can activate (p53) or inhibit (LANA) GSK3.

neurons caused growth cone expansion, axonal spreading and increased branching [18,19]. Inhibition of GSK3 is also essential for appropriate astrocytic growth-cone extension and motility, and this is mediated by a complex of Cdc42, Par6 and protein kinase Cζ [16] (Figure 2). Conversely, both semaphorin 3A [17] and lysophosphatidic acid [20] activate GSK3, and this causes growth cone collapse and neurite retraction. These studies demonstrate that inactivation and activation of GSK3 promote outgrowth and collapse, respectively, the key processes that regulate

the dynamics of neuronal and astrocytic growth and remodeling events.

Microtubules are cytoskeletal filaments that are crucial for maintaining cellular organization, structure and motility. Microtubule-associated proteins (MAPs) play a central role in regulating microtubule function. Several MAPs, such as tau and MAP1B are phosphorylated by GSK3, which regulates their binding to microtubules, thereby regulating microtubule dynamics (Figures 1 and 2). GSK3 also phosphorylates kinesin light chains of microtubule motors, which are responsible for transporting cargo (e.g. proteins, vesicles and organelles) along microtubules to specific destinations (Figure 2); phosphorylation facilitates the release of cargo at its destination [21]. Kinesin family motors also regulate microtubule events during mitosis, and inhibition of GSK3 results in mitotic spindle defects and inappropriate orientation of chromosomes, potentially owing to its regulatory influence on kinesin [22].

These studies show that localized inhibition of GSK3 is essential for the many important functions of microtubules and also supports appropriate neurite outgrowth and cell migration. Conversely, compartmentalized activation of GSK3 must occur for growth cone collapse and neurite retraction, events that are necessary for maintaining the appropriate direction and path taken by extending processes. Thus, regulation of GSK3 activity is an essential component of cellular architectural dynamics, transport mechanisms, and remodeling events.

GSK3 influences cell survival

GSK3 contributes to both cell death and cell survival. In 1998, overexpression of GSK3 was found to induce apoptosis [23]. Since then, activation of GSK3 has been shown to promote apoptosis in a remarkably wide variety of conditions, such as trophic factor withdrawal, phosphatidylinositol 3-kinase inhibition, and toxicity induced by Alzheimer's disease amyloid β-peptide (Aβ), ceramide, human immunodeficiency virus type 1 Tat protein, platelet activating factor, heat shock, and mitochondrial toxins (reviewed in [4]). Recently, GSK3 has been linked to apoptosis induced by p53 following DNA damage [13], hypoxia [24], prion toxicity [25], stress of the endoplasmic reticulum [26], and Huntington's disease-associated polyglutamine toxicity [27]. Activated GSK3 also facilitates death by anoikis, which results from disruption of cell–matrix interactions [28].

Discovery of the role of GSK3 in many apoptotic conditions coincided with a growing interest in neuroprotection provided by lithium, which inhibits GSK3 [29], as observed with stroke-induced ischemia [30]. Although lithium has many effects on cells, it was shown to provide protection from apoptosis caused by hyperactive GSK3 [31], suggesting that the protective capacity of lithium is, in part, owing to GSK3 inhibition [31,32]. Now, there is a growing appreciation for this relationship because numerous conditions that cause apoptosis in association with activation of GSK3 also are counteracted by lithium, and new inhibitors of GSK3 have a similar protective effect to lithium [33].

The broad spectrum of apoptotic conditions to which GSK3 contributes suggests that it plays a fundamental role in apoptotic signaling, although the precise mechanisms remain unclear. Inhibition of protein synthesis following eukaryotic initiation factor 2B (eIF2B) phosphorylation by GSK3 was found to be an important mechanism in facilitating apoptosis [34]. In addition, GSK3 facilitates activation of the c-Jun NH_2-terminal kinase pathway that often contributes to apoptotic signaling [35,36]. Furthermore, GSK3 probably promotes apoptosis by regulating transcription factors, both inhibiting survival-promoting transcription factors, such as CREB and heat shock factor-1 [4], and facilitating pro-apoptotic transcription factors, such as p53 [13] (Figure 2).

This brief summary of the pro-apoptotic actions of GSK3 raises the question of why there are so many different targets. One possibility is that, in different cell types and with different apoptotic insults, GSK3 is recruited to phosphorylate different targets that are selectively important for each condition. Alternatively, GSK3 might be a pluripotent facilitator of apoptosis, providing cells with a single enzyme that is capable of simultaneously blocking several anti-apoptotic actions and activating multiple processes that promote apoptotic signaling. Although at present we cannot distinguish between these two possibilities, it seems probable that GSK3 acts on several targets during apoptosis, but these might vary with cell type and insult.

Conversely, GSK3β is absolutely essential for survival. A landmark study showed that GSK3β-knockout mice developed normally to mid-gestation, but died around day 14 following massive tumor necrosis factor-α (TNFα)-induced hepatocyte apoptosis [37]. This finding was one of the first important indications of the isoform-specific functions of GSK3 because GSK3α was unable to compensate for the loss of GSK3β [37]. Nuclear factor κB (NF-κB) activation counteracts TNFα-induced apoptotic signaling to promote survival, and this was found to require GSK3β [37]. These findings built upon studies by Beyaert and colleagues demonstrating that inhibition of GSK3 increases TNFα-induced cytotoxicity [38]. Recently, components of the NF-κB system have been found that are directly phosphorylated by GSK3 to promote NF-κB activity [39,40]. Taken together, it is evident that maintenance of appropriate levels of GSK3 activity is crucial, because either too little or too much GSK3 activity can promote cell death in certain conditions. The importance of regulating GSK3 is further highlighted by recent results showing a strong regulatory influence of GSK3 on neuronal differentiation of stem cells [41,42] and angiogenesis [28], findings that raise new therapeutic possibilities for agents that modulate GSK3 activity.

GSK3 is linked to a diverse array of diseases

GSK3 has been linked to a surprisingly large number of diseases. Recent reports discuss the association of GSK3 with muscle hypertrophy [43,44], cancer [9,45], bipolar mood disorder [29,46,47] and schizophrenia [48]. Owing to space restrictions, not all of the conditions linked to GSK3 can be discussed here; therefore, we focus specifically on diabetes and Alzheimer's disease.

Diabetes and insulin resistance

Insulin resistance is an early event in the development of type 2 diabetes, in which insulin production is normal but there are deficient cellular responses to insulin, such as stimulation of glucose uptake and glycogen synthesis. Insulin-induced inactivation of GSK3 normally contributes to these responses (Figure 1). The mechanisms contributing to insulin resistance and type 2 diabetes are multifactorial, but one factor is inadequate inhibitory control of GSK3 [49]. As a result, GSK3 activity is above normal in diabetic rodents [49] and in skeletal muscle from patients with type 2 diabetes [50]. GSK3 generally opposes the actions of insulin. Thus, GSK3 inhibits glycogen synthesis and glucose uptake, alters the expression of genes regulated by insulin [51], and inhibits the insulin-receptor-coupled protein, IRS-1, which probably exacerbates deficient insulin signaling [49]. Evidence that GSK3 is an important regulator – not just a secondary component – in insulin resistance comes from studies in which inhibitors of GSK3 enhanced responses to insulin in a variety of model systems [52–54]. For example, GSK3 inhibitors lowered blood glucose levels and stimulated glucose transport and glycogen synthesis in skeletal muscle from insulin-resistant Zucker rats [55,56], and increased IRS-1 expression and glucose uptake in human skeletal muscle [57]. These findings indicate that deficient inhibitory control of GSK3 is an important factor in type 2 diabetes and that inhibitors of GSK3 could be therapeutically beneficial.

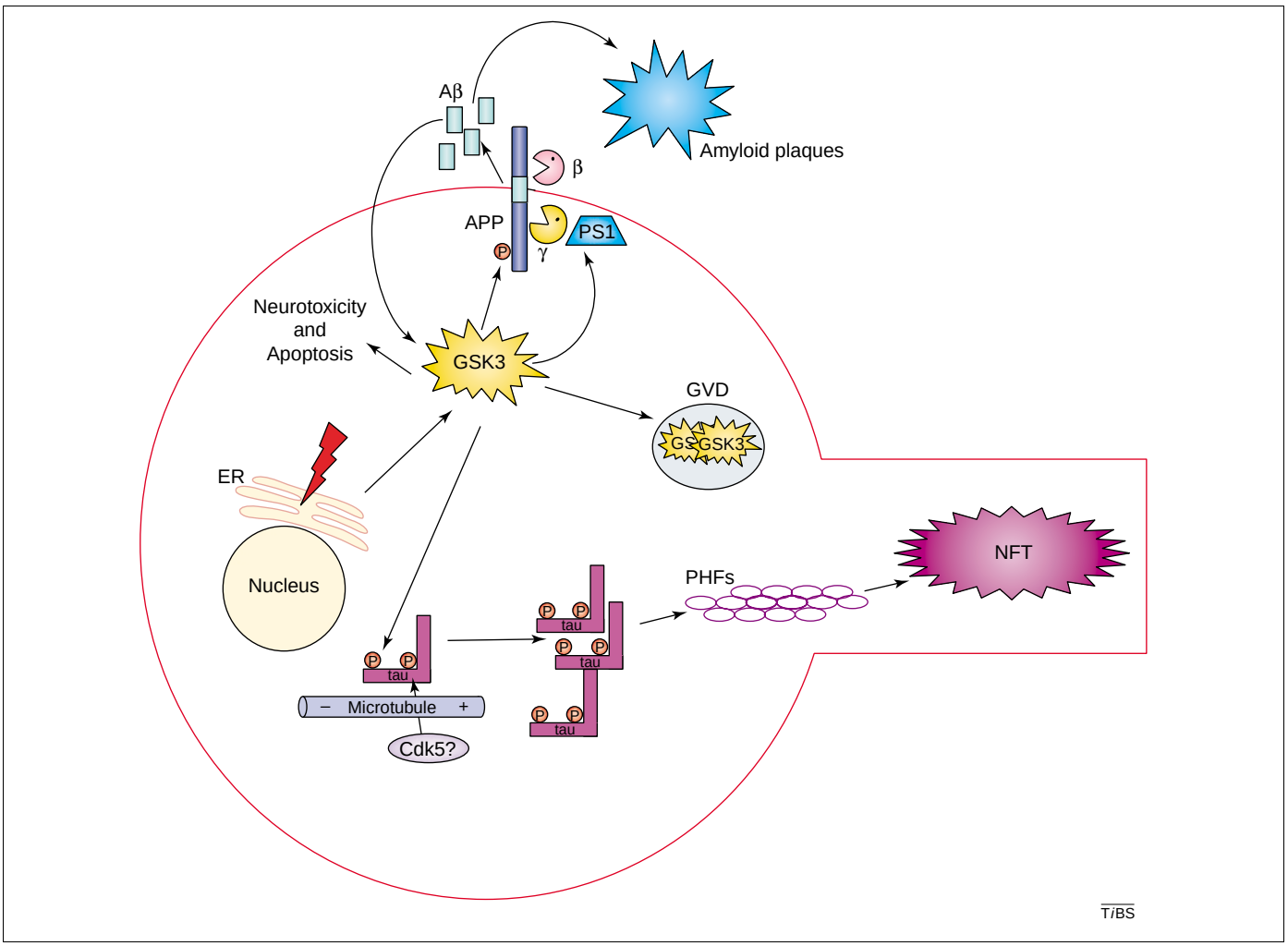

Figure 3. Glycogen synthase kinase-3 (GSK3) is associated with the neuropathology of Alzheimer's disease. Many links have been identified between GSK3 and neuropathological features of Alzheimer's disease. Tau is a widely recognized substrate of GSK3, and GSK3 is a prime candidate for contributing to the Alzheimer's disease-associated hyperphosphorylation of tau (especially at primed sites, possibly following phosphorylation of tau by Cdk5). GSK3 phosphorylation of tau results in decreased microtubule binding, perhaps enhancing paired helical filament (PHF) formation, a possible prelude to neurofibrillary tangle (NFT) formation [66]. GSK3 might also contribute to hyperphosphorylated tau deposits found in other neurodegenerative diseases [67]. Transgenic mouse models in which GSK3β expression is increased have increased tau phosphorylation and deficits in spatial learning [18,68,69]. GSK3 is intricately involved in amyloid-associated processes (reviewed in [4]). The core of amyloid plaques consists of Aβ, which is produced by proteolysis of a membrane-spanning protein called amyloid precursor protein (APP) by β-secretase (β) and γ-secretase (γ), and GSK3 facilitates the production of Aβ (as indicated by the reduction in Aβ production accompanying inhibition of GSK3) [58,70]. Perhaps most intriguing, selective inhibition of GSK3α – but not GSK3β – decreased Aβ accumulation in a transgenic mouse model of Alzheimer's disease that overproduces amyloid precursor protein [58]. Many reports have shown that Aβ is neurotoxic. Application of Aβ to neurons activates GSK3β, and inhibition of GSK3 provides protection from Aβ-induced neurotoxicity. GSK3β also phosphorylates APP, although the ramifications of this modification have yet to be clarified. Thus, GSK3 contributes to the neurotoxicity and production of Aβ, potentially influencing the production of amyloid plaques. Presenilin-1 (PS1), which is crucial for proteolytic processing of the amyloid precursor protein, directly binds GSK3β. This association is altered by mutations of presenilin-1 (PS1) that are associated with familial Alzheimer's disease, mutations that also alter the interaction of GSK3 with kinesin, resulting in impaired axonal transport [61]. There is considerable evidence that endoplasmic reticulum (ER) stress and the accumulation of misfolded proteins contributes to the neurotoxicity evident in Alzheimer's disease, and ER stress causes activation of GSK3β [26]. Active GSK3 was identified in neurons developing granulovacuolar degeneration (GVD) in Alzheimer's disease, which could represent an attempt to sequester active GSK3 [71].

Alzheimer's disease

Alzheimer's disease is a severe, progressive neurodegenerative disease with many links to GSK3 [4]. The two classical neuropathological features of Alzheimer's disease are neurofibrillary tangles – intraneuronal filamentous aggregates composed primarily of hyperphosphorylated tau – and amyloid plaques, which are extracellular deposits composed primarily of Aβ. GSK3 might contribute to both of these neuropathologies (Figure 3). For example, Aβ can activate GSK3, and inhibition of GSK3 attenuates Aβ-induced neurotoxicity [4]. Additionally, GSK3 promotes Aβ production, and, in a novel twist, this was found to be caused only by GSK3α and not GSK3β [58]. Presenilin-1, a protein that contributes to Aβ production, is mutated in one form of familial Alzheimer's disease. Presenilin-1 binds and can regulate both GSK3 and β-catenin, but the functional outcomes and the effects of mutations of presenilin-1 remain unclear [59,60]. Recent studies have suggested that Alzheimer's disease presenilin-1 mutations might compromise neuronal function by increasing GSK3 activity and impairing kinesin-based motility [61]. These and other examples of the involvement of GSK3 in a remarkably broad spectrum of Alzheimer's disease-associated events are illustrated in Figure 3. Along with its promotion of apoptosis, these interactions provide strong evidence that GSK3 is intricately involved in the neuronal dysfunction associated with Alzheimer's disease.

Concluding remarks

Given the large number of substrates and signaling pathways impacted by GSK3, it is evident that GSK3 must be exquisitely well regulated, and by mechanisms that are individually tailored for each substrate. This regulation encompasses integration of the coordinated phosphorylation of both GSK3 and its substrates, regulated subcellular distribution of GSK3, and the formation of distinct multiprotein complexes that control the activity of GSK3 towards individual substrates. These mechanisms provide the means for precise control of GSK3 while enabling its influence on cell function at nearly all levels, including cytoskeletal dynamics and cellular architecture, transcriptional and translational processes, development and death. However, the multiplicity of substrates and complexity of regulation might also represent a weak link in cell biology, providing the basis for emerging evidence that multiple diseases are linked to dysregulated GSK3. Thus, current research is focused on understanding the mechanisms that regulate GSK3, how these mechanisms are impaired in disease, and how pharmacological and molecular methods can be used to modulate the actions of GSK3.

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