Signal transduction: **Life, the universe and … development**

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Glycogen synthase kinase 3 (GSK-3) is a key component of the Wnt signalling pathway, among others, and is known to be regulated by inhibition. Now a novel, dual specificity protein kinase known as Zaphod kinase has been discovered that activates GSK-3 by tyrosine phosphorylation.

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The *Hitchhiker's Guide to the Galaxy* [1] introduced Zaphod Beeblebrox, an exotic alien with an ego so large that he needs two heads. His name has now been adopted for a novel protein kinase that contains two functional kinase domains [2]. As with its two-headed namesake, Zaphod kinase (ZAK1) promises to provide an alternative perspective of the world. It has already cast new light on the regulation of glycogen synthase kinase 3 (GSK-3), a multi-function and virtually universal protein kinase. GSK-3 is now perhaps best known as a key component of

new type of protein kinase, the recent work on ZAK1 [2] has shown that GSK-1 can be regulated by activating tyrosine phosphorylation, as well as the better known inhibitory mechanism.

Inhibition of GSK-3 by insulin and Wnt-1

To understand the significance of ZAK1, it is necessary first to consider the regulation of GSK-3. GSK-3 was discovered as one of the serine/threonine protein kinases that phosphorylate glycogen synthase and thereby regulate glycogen synthesis. These enzymes were, for obvious reasons, named glycogen synthase kinases, or GSKs. Most of these kinases have, however, also been identified in different contexts, and are usually known by other names. For example, GSK-1 is cAMP-dependent protein kinase (PKA), whereas GSK-5 is commonly known as casein kinase II. Despite the specificity inferred by its name, GSK-3 has in fact turned out to be an important regulatory kinase with a number of significant cellular targets.

Phosphorylation of glycogen synthase by GSK-3 inhibits its activity in a manner that is reversed by insulin stimulation [3]. Initially, direct regulation of GSK-3 by insulin went undetected; this is not surprising, as it only causes a 50% reduction in GSK-3 activity. But coupled to the action of the protein phosphatase PP1, this relatively small change in the activity level of GSK-3 is sufficient to control glycogen synthesis. It is now known that insulin stimulates a phosphatidylinositide 3-kinase (PI 3-kinase) to generate phosphatidylinositide 3,4,5-trisphosphate (PIP_3). This in turn activates protein kinase B (PKB), which phosphorylates GSK-3 at a serine residue close to its amino terminus This phosphorylation causes a reduction in GSK-3 activity and leads to increased glycogen synthase activity (Figure 1a).

Much of the present interest in GSK-3 is due to its role in the Wnt signalling pathway (Figure 1b). The Wnt family of protein ligands control pattern formation during metazoan development, and have also been linked to oncogenesis. Central to this pathway is the degradation of β-catenin, a process that is stimulated by direct phosphorylation through GSK-3. In the presence of Wnt, β-catenin is stabilised and as a result the protein accumulates, enters the nucleus and regulates transcription. This response occurs through inhibition of GSK-3 in a complex that also contains the proteins Axin, Dishevelled and FRAT. GSK-3 binds to Axin, where it may phosphorylate β-catenin, FRAT proteins, including their *Xenopus* homo-

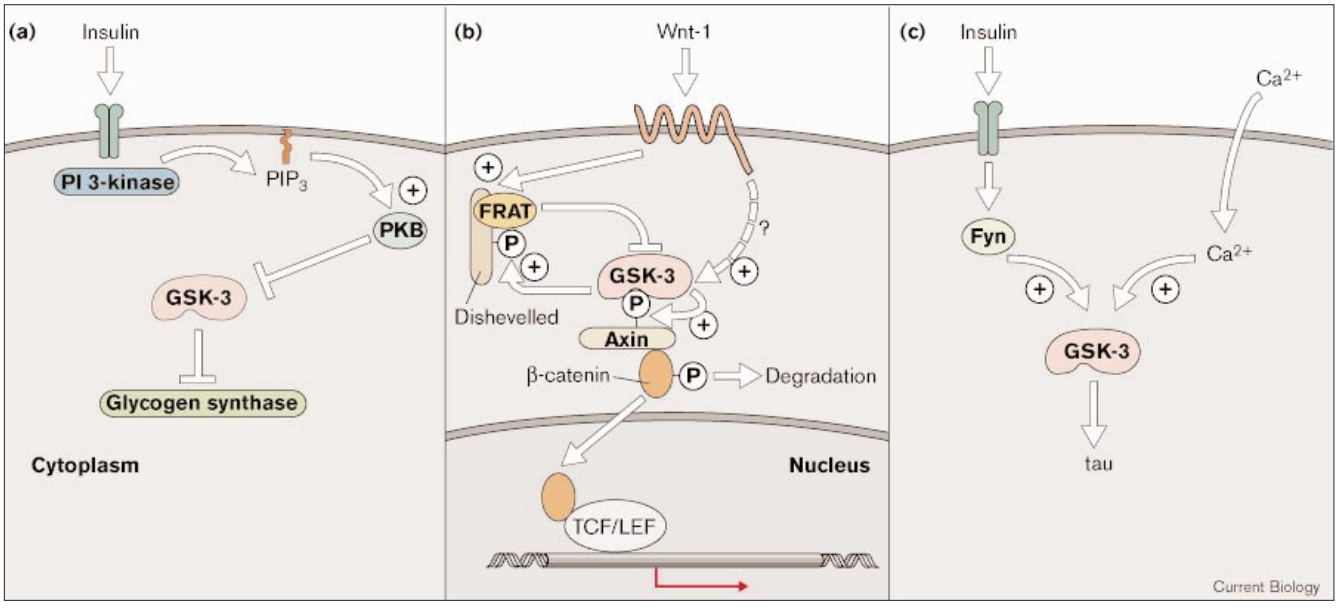
of Dishevelled into the Axin complex, and hence FRAT inhibition of GSK-3 [4].

I have outlined the two well characterised regulatory pathways for GSK-3. As both pathways when active inhibit GSK-3, this has led to the belief that inhibition may be the only means to control GSK-3 activity. Implicit in this idea is the notion that all cells possess a high basal activity of GSK-3, which is attenuated in response to cell stimulation. This is an unusual situation, as most other signal transduction pathways operate through cascades of kinase activation. Is this generalisation true, or has GSK-3 activation been overlooked?

Anomalous regulation of GSK-3

A number of recent observations are not easily explained by a purely inhibitory mechanism for the control of GSK-3. In the nematode *Caenorhabditis elegans*, in contrast to the fruitfly *Drosophila* or vertebrates, removal of GSK-3 activity has the same effect as loss of the Wnt homologue (Mom-2) [5]. This suggests that Mom-2 has a positive effect on GSK-3 activity in nematodes, rather than inhibiting its activity. An important consideration here is that no close Axin homologue has been identified in the nematode genome, and therefore the model of Wnt signalling described above may not apply. A further curious feature

Figure 1



(a) Insulin inhibits GSK-3 activity through the generation of phosphatidylinositide (3,4,5) trisphosphate (PIP_3) by the stimulation of phosphatidylinositide 3-kinase (PI 3-kinase). This leads to the activation of protein kinase B (PKB), which phosphorylates and inhibits GSK-3 to alleviate inhibition of glycogen synthase. **(b)** Wnt-1 stimulation stabilises β-catenin by inhibiting the activity of GSK-3. This occurs in a complex with Axin. Wnt stimulation may promote binding of Dishevelled and FRAT to the complex, inhibiting GSK-3. These interactions all require GSK-3 activity, raising the possibility that activation of GSK-3 may lead to its own inhibition. β-catenin regulates gene expression by interacting with the transcription factors TCF and LEF. **(c)** GSK-3 phosphorylation of tau may proceed through activation of GSK-3. This may be stimulated by insulin via the tyrosine kinase Fyn, or through a transient increase of cytosolic Ca^{2+}.

observed in vertebrate systems is that GSK-3 activity is required for its own interaction with Axin and also for FRAT's interaction with Dishevelled — in effect, GSK-3 activity is required for its own inhibition. Could GSK-3 be activated by Wnt stimulation? If this were the case, perhaps the net effect of stimulation in *Drosophila* and vertebrates is for GSK-3 to bring about its own inhibition by Dishevelled, whereas in nematodes, in the absence of Axin, only GSK-3 activation is observed.

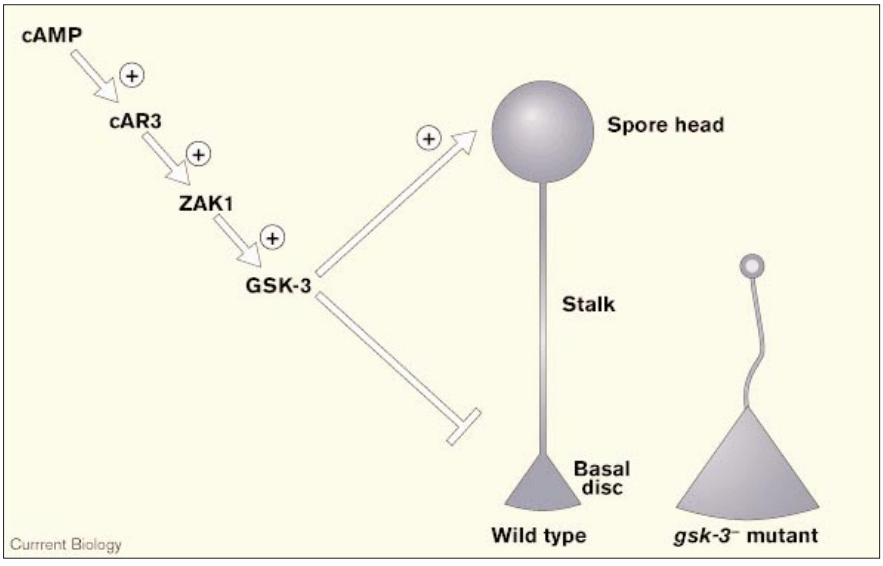
Two recent biochemical studies [6,7] have pointed to pathways which increase GSK-3 activity (Figure 1c). These both concern the phosphorylation of the neural microtubule-binding protein, tau. This is of clinical interest, as hyper-phosphorylation of tau by GSK-3 leads to the formation of the paired helical filaments that are associated with Alzheimer's disease. Hartigan and Johnson [6] found that a transient rise in cytosolic calcium led to an increase in tau phosphorylation through elevation of GSK-3 kinase activity. This was accompanied by increased tyrosine phosphorylation of GSK-3. Lesort *et al.* [7] found that insulin stimulation led to a transient increase in tau phosphorylation, before becoming inhibitory over the long term. This dynamic response proceeded through a transient increase of GSK-3 activity and was also associated with increased tyrosine phosphorylation of GSK-3; the authors suggested that the tyrosine kinase Fyn may be responsible for this phosphorylation.

In both of these cases, the changes observed in GSK-3 activity and phosphotyrosine content were small and hence difficult to detect. Furthermore, the data do not adequately distinguish between a direct activation of kinase activity and an indirect mechanism involving inhibition of an inhibitory pathway. But the recent work of Leung *et al.* [2], using as a model system the simple eukaryote *Dictyostelium*, has now provided both genetic and biochemical evidence that GSK-3 can be activated through the tyrosine kinase activity of the dual specificity kinase ZAK1 (Figure 2).

ZAK1 and GSK-3 in *Dictyostelium*

Dictyostelium cells grow as unicellular amoebae, but when starved they aggregate and undergo morphogenesis to form a multicellular structure. This multicellular unit contains three major structural elements: the spore head, the stalk and the basal disc. GSK-3 is required to establish the patterning of these elements. In the absence of GSK-3, an enlarged basal disc forms at the expense of the spore head [8]. GSK-3 activity peaks as *Dictyostelium* cells begin cell-type-specific differentiation, consistent with the kinase playing a role in pattern formation. This increase of GSK-3 activity is stimulated by extracellular cAMP, a

Figure 2



Dictyostelium cells develop to form a fruiting body. This comprises a spore head, a stalk and a basal disc. The proportion of spore and basal disc cells is regulated by GSK-3, so that in its absence an enlarged basal disc forms at the expense of the spore head. ZAK1 activates GSK-3 activity and is regulated by extracellular cAMP via the receptor cAR3.

signal that both induces spore precursor cells and represses formation of basal disc precursors. When cAMP is added to isolated *Dictyostelium* cells, GSK-3 activity rapidly increases by a process that requires at least one of the organism's four cAMP receptors, identified as cAR3 [9].

This behaviour suggests that, during *Dictyostelium* development, cAMP acts via cAR3 to increase GSK-3 activity, but this alone does not demonstrate direct activation. The discovery of ZAK1 has provided this evidence [2]. ZAK1 was isolated in a screen for *Dictyostelium* tyrosine kinases, and in fact has turned out to be an unusual dual specificity protein kinase, containing both a tyrosine kinase domain and a serine/threonine kinase domain. In that respect, it resembles members of the JAK kinase family, which phosphorylate the STAT transcription factors. Unlike the JAKs, both kinase domains of ZAK1 are active. Genetic disruption of ZAK1 caused a phenotype resembling that of loss of GSK-3 or cAR3, suggesting that it acts within the same activation pathway. Furthermore, cAMP was found to increase the tyrosine kinase activity of ZAK1 with similar kinetics to that observed for GSK-3, and again this increase requires cAR3.

Does ZAK1 regulate GSK-3 activity? Loss of ZAK1 had no effect on the GSK-3 protein level, but did cause a significant reduction of GSK-3 kinase activity during development. As seen with cAR3 mutants, in a ZAK1 mutant cAMP stimulation failed to fully stimulate GSK-3 activity. Significantly, recombinant ZAK1 protein will use GSK-3 as a substrate, and this phosphorylation leads to a doubling of GSK-3 activity, the same degree of activation observed in whole cells. Together, these results not only place ZAK1 between cAR3 and GSK-3 on the cAMP response pathway, they also demonstrate that ZAK1 interacts directly with GSK-3 and can activate it in the absence of other protein intermediates.

Although no ZAK1 homologues have yet been identified in other species, these results with *Dictyostelium* [2] establish that tyrosine phosphorylation can positively regulate GSK-3 activity. Having established the principle that activation of GSK-3 can occur, this possibility should now be considered when investigating regulation of GSK-3 in other systems. Indeed, comparing the observations made in *Dictyostelium* with those for GSK-3 phosphorylation of tau suggests the activation of GSK-3 may be widespread.

In the *Hitchhiker's Guide to the Galaxy*, Zaphod Beeblebrox and the other characters discover that they are on a quest to find the answer to "life, the universe and everything". Back on Earth, a complete understanding of GSK-3 regulation may not provide the answer to everything, but the discovery of ZAK1 may lead us into new territory for both the regulation of GSK-3 and signal transduction in developmental systems.

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