Glycogen synthase kinase 3β and the regulation of axon growth

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Abstract

One of the earliest hallmarks that distinguish growing axons from dendrites is their growth rate; axons grow faster than dendrites. In vertebrates, where axons are required to grow for considerable distances, particularly in the peripheral nervous system, a fast axon growth rate is a requisite property. In neurons that respond to the neurotrophin growth factor/nerve growth factor with increased axon growth rates, two distinct intracellular signalling pathways are recruited: the MAPK (mitogen-activated protein kinase) pathway and the phosphatidylinositol-3 kinase pathway. The activation of either pathway leads to changes in microtubule dynamics within growing axons and growth cones and these underlie fast axon growth rates. Microtubule dynamics is regulated by microtubule-associated proteins and in the MAPK pathway this function is subserved by microtubule-associated protein 1B, whereas in the phosphatidylinositol-3 kinase pathway, adenomatous polyposis coli is the regulating microtubule-associated protein.

Introduction

A critical event in the differentiation of neurons during embryogenesis is the initiation and extension of cellular processes (neurites) that will eventually form axons and dendrites. One of the first hallmarks distinguishing growing axons from dendrites is their growth rate. Embryonic neurons differentiating in culture initially extend several equipotential neurites, then, apparently randomly, one neurite starts to grow faster than the other neurites [1]. The fast growing neurite is destined to become an axon and the others will eventually become dendrites. Dendritic growth is restricted to the local region of the neuronal cell body, whereas axons are often required to grow considerable distances from the cell body to reach their synaptic targets, particularly in vertebrates. A fast growth rate is, therefore, a requisite property of extending axons. The intracellular mechanisms that maintain and regulate axon growth are not well understood, although it is clear that individual axon growth rates are not uniform during the period of axon extension and that extracellular factors, including members of the neurotrophin family [2], can modulate axon growth rates. The archetypal neurotrophin, NGF (nerve growth factor), is required for the proper innervation of sympathetic neuron targets in the peripheral nervous system [3]. NGF can act directly on axons and growth cones to enhance growth and can even re-orient growth cones when applied locally in vitro [2]. It is well established that NGF enhances axon growth by activating two, distinct, intracellular signalling pathways: the MAPK (mitogen-activated protein kinase) and the PI3K (phosphatidylinositol-3 kinase) pathway (Figure 1; [4]). To influence axon growth, these pathways must ultimately regulate the cytoskeleton and exactly how this is achieved is slowly revealed by recent experiments ([6], and R.G. Goold and P.R. Gordon-Weeks, unpublished work).

Glycogen synthase kinase 3β is a key downstream regulator of axon growth

We have recently shown that NGF drives enhanced neurite growth rates in PC12 cells and in the axons of cultured superior cervical ganglion and dorsal root ganglion neurons by activating the serine/threonine kinase GSK3β (glycogen synthase kinase 3β [7–9], reviewed in [10], and R.G. Goold and P.R. Gordon-Weeks, unpublished work). GSK3β is a highly conserved kinase located in several important signalling pathways, such as those regulating glycogen metabolism, apoptosis and cell fate determination during embryogenesis [11]. In vertebrates, there are two isoforms, GSK3α and GSK3β, encoded by separate genes. Although GSK3α is the dominant form in most vertebrate tissues, GSK3β is particularly abundant in neural tissue [12], where it is neuron-specific [13,14]. In the central nervous system, GSK3β is developmentally regulated, with peak levels of expression during axogenesis. It is present in growing axons but completely excluded from them at the end of axogenesis, being restricted in the adult to neuronal cell bodies and dendrites [14]. This suggests that GSK3β has a role in axogenesis, an idea supported by pharmacological and cell biological studies. GSK3β is inhibited by lithium ions and these have profound morphological effects on developing neurons and neuroblastoma cells in culture [7,9,15–18]. It reversibly reduces axon growth rate, enlarges axonal growth cones and induces spread areas along neurite shafts.
NGF stimulates axon growth rates by regulating microtubule dynamics through simultaneous activation of the MAPK and PI3K pathways

The neurotrophin NGF binds to TrkA (tropomyosin-related kinase A) receptors in sympathetic and primary sensory neurons. This leads to simultaneous activation of the MAPK and PI3K pathways. The MAPK pathway activates GSK3β that phosphorylates the microtubule-associated protein MAP1B, which maintains the population of unstable microtubules in growing axons. Conversely, the PI3K pathway inhibits GSK3β by phosphorylating it at Ser-9. This leads to an inhibition of the phosphorylation of primed substrates such as APC and to an increase in stable microtubules. Axon growth depends on an appropriate balance between stable and unstable microtubules.

Specific, small molecule, inhibitors of GSK3β that have an independent mechanism of action from lithium, mimic the effects of lithium on differentiating neurons [9]. Significantly, Wnt proteins, the vertebrate orthologues of the Drosophila signalling protein wingless, which act by inhibiting GSK3β, produce a similar phenotype, corroborating the involvement of GSK3β [16,19]. These experiments strongly implicate GSK3β in the regulation of axon growth rate and growth cone structure and dynamics but the precise role of the molecule has been elusive until recently. The first clue to the function of MAP1B came from our experiments in which we showed that MAP1B influences microtubule dynamics by controlling microtubule stability and that this function is regulated by GSK3β phosphorylation of MAP1B [17]. In these experiments, we showed that expression of GSK3β-phosphorylated MAP1B in COS-7 or CHO cells increased the population of unstable microtubules at the expense of the stable microtubules. These findings predicted that a reduction in MAP1B expression or an inhibition of GSK3β would lead to an increase in stable microtubules in differentiating neurons. Both predictions have been borne out. We have shown that inhibition of GSK3β causes a striking increase in the population of stable microtubules in growing axons and growth cones and that this is associated with a reduction in axon growth and a significant increase in growth cone size [9,17]. These findings have been independently confirmed [19,21,22]. Neurons cultured from MAP1B knock-out mice also show striking increases in stable microtubules associated with a reduction in axon growth and an increase in growth cone size [23,24]. Furthermore, GSK3β-phosphorylated MAP1B is required to maintain unstable microtubules in the leading extensions of migrating cortical neurons [25]. The significance of these findings is that maintaining a population of unstable microtubules in growth cones and growing axons is an important requirement for axon growth and growth cone turning during pathfinding [10]. Recent experiments showing that MAP1B is required for the signalling of the guidance molecule netrin-1 during axon guidance confirm that MAP1B plays a role in both axon growth and growth cone pathfinding [26].

MAP1B is a major phosphorylation target of GSK3β in growing axons

MAP1B is a developmentally regulated phosphoprotein expressed at high levels in differentiating neurons and in regions of the adult nervous system that show neuronal plasticity or regenerate after injury [20]. A considerable body of indirect evidence strongly suggests that MAP1B plays an important role in regulating axon growth rate and growth cone structure and dynamics but the precise role of the molecule has been elusive until recently. The first clue to the function of MAP1B came from our experiments in which we showed that MAP1B influences microtubule dynamics by controlling microtubule stability and that this function is regulated by GSK3β phosphorylation of MAP1B [17]. In these experiments, we showed that expression of GSK3β-phosphorylated MAP1B in COS-7 or CHO cells increased the population of unstable microtubules at the expense of the stable microtubules. These findings predicted that a reduction in MAP1B expression or an inhibition of GSK3β would lead to an increase in stable microtubules in differentiating neurons. Both predictions have been borne out. We have shown that inhibition of GSK3β causes a striking increase in the population of stable microtubules in growing axons and growth cones and that this is associated with a reduction in axon growth and a significant increase in growth cone size [9,17]. These findings have been independently confirmed [19,21,22]. Neurons cultured from MAP1B knock-out mice also show striking increases in stable microtubules associated with a reduction in axon growth and an increase in growth cone size [23,24]. Furthermore, GSK3β-phosphorylated MAP1B is required to maintain unstable microtubules in the leading extensions of migrating cortical neurons [25]. The significance of these findings is that maintaining a population of unstable microtubules in growth cones and growing axons is an important requirement for axon growth and growth cone turning during pathfinding [10]. Recent experiments showing that MAP1B is required for the signalling of the guidance molecule netrin-1 during axon guidance confirm that MAP1B plays a role in both axon growth and growth cone pathfinding [26].

GSK3β requires activation to phosphorylate MAP1B

GSK3β activity is regulated by phosphorylation; Ser-9 phosphorylation inhibits the kinase whereas Tyr-216 phosphorylation increases kinase activity [11]. In neurons, the kinase Akt in the PI3K pathway phosphorylates GSK3β at Ser-9, but Tyr-216 phosphorylation probably occurs through autophosphorylation [27]. NGF can activate GSK3β enabling it to phosphorylate MAP1B in primary neurons and neuronal cell lines that respond to NGF with enhanced axon growth, and, although the mechanism of activation is unknown, it does not seem to involve either de-phosphorylation at Ser-9 or increased phosphorylation at Tyr-216 [7,8]; and R.G. Goold and P.R. Gordon-Weeks, unpublished work). NGF binds either to the TrkA (tropomyosin-related...
kinase A) tyrosine kinase receptor or to the p75NRTR receptor in differentiating neurons [4] and it is engagement with the former that is responsible for the activation of GSK3β by NGF [8]. TrkA receptors signal through several, distinct intracellular signalling pathways including the MAPK and PI3K pathways [4]. We have recently found that it is the MAPK pathway, and not the PI3K pathway, that couples NGF engagement with the TrkA receptor to the activation of GSK3β and, therefore, to MAP1B phosphorylation (R.G. Goold and P.R. Gordon-Weeks, unpublished work). We showed this by comparing the effects of specific pharmacological inhibitors of the MAPK and PI3K pathways on axon growth and MAP1B phosphorylation in PC12 cells and sympathetic neurons in culture and by both in vitro kinase and GSK3β activation assays. Although the mechanism by which NGF activates GSK3β for MAP1B phosphorylation is unknown, indirect evidence suggests that it involves phosphorylation of a novel site(s) (R.G. Goold and P.R. Gordon-Weeks, unpublished work).

Our experiments show that NGF activates GSK3β thereby enabling the phosphorylation of MAP1B and the enhancement of axon growth. However, as explained in the Introduction, NGF also stimulates axon growth through the PI3K pathway [4]. Recent experiments from Snider’s laboratory [6] provide evidence that NGF activation of the PI3K pathway in dorsal root ganglion neurons also regulates activation of GSK3β. However, in the PI3K pathway, in contrast with the MAPK pathway, the phosphorylation of GSK3β at Ser-9 is increased. The effect of Ser-9 phosphorylation is to prevent GSK3β phosphorylating the so-called ‘primed’ substrates; those that require prior phosphorylation by another kinase [11] (Figure 1). This class of substrates includes APC, but not MAP1B ([7]; and R.G. Goold and P.R. Gordon-Weeks, unpublished work), and Snider and colleagues [6] show that APC is downstream of GSK3β. Inhibition of GSK3β by activation of the PI3K pathway is predicted to decrease APC phosphorylation, which increases APC binding to microtubules and leads to increased microtubule stability. Thus, NGF regulates axon growth by utilizing two, distinct, intracellular signalling pathways which converge on the regulation of microtubule dynamics by controlling microtubule-associated proteins. Axon growth requires a subtle balance between the levels of stable and unstable microtubules and simultaneous activation of the MAPK and PI3K pathways ensures this occurs.

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