NO and it has been speculated that these non-symbiotic haemoglobins might detoxify NO [15], thereby possibly affecting the observed NO-induced effects on cyclic nucleotide production that influences induction of defence reactions in plants [16]. It remains to be established if any of the symbiotic leghaemoglobins play such a regulatory role during the normal development of symbiotic nitrogen fixation in legume nodules.

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DOI: 10.1016/j.cub.2005.03.007

Neuronal Polarity: Until GSK-3 Do Us Part

Specification of the axon and dendrites is a critical step in the development of a neuron. Two new studies have shed light on the molecular pathway that controls the establishment of neuronal polarity.

Rong Li

Axons and dendrites are structurally and functionally distinct processes that extend from the cell body of neurons. A mature neuron usually has multiple dendrites and a single long axon. The specification of axons and dendrites, often referred to as neuronal polarity, is a critical step in neuronal differentiation [1,2]. Two new studies [3,4] have now uncovered a pathway, involving the multi-functional kinase GSK-3β, that plays a pivotal role in regulating this process.

The new studies [3,4] took advantage of isolated embryonic hippocampal neurons that are able to form distinct axonal and dendritic structures in culture (Figure 1) [5]. This process begins with the formation of lamellipodia (stage 1), followed by extension of multiple, highly dynamic protrusions from the cell body (stage 2). At some point, one of the protrusions undergoes a sharp transition to rapid growth to form a long process that soon acquires axonal characteristics (stage 3). The rest of the processes grow much more slowly and become dendrites (stage 4).

Utilizing this robust *in vitro* differentiation assay, a number of groups have explored the role of molecules already known to regulate cell polarity in other cell types. Both of the new studies [3,4] converged on GSK-3β, a protein kinase that is involved in a number of signaling pathways and was recently implicated in astrocyte polarization and migration [6]. GSK-3β is an unusual signaling kinase: its basal activity is normally high, but is downregulated by upstream pathways through inhibitory phosphorylation [7].

Jiang *et al*. [3] found that, whereas GSK-3β is present in all neurites at stage 2 and 3, the phosphorylated, hence inactive, form of GSK-3β is most enriched at the tip of the axons in polarized stage 3 neurons. Expression of a constitutively active mutant form of GSK-3β lacking the inhibitory phosphorylation site (Ser9) blocked the establishment of neuronal polarity, whereas inhibition of GSK-3β with pharmacological and peptide inhibitors and short hairpin RNA led to the formation of multiple axon-like processes. Interestingly, not only is GSK-3β essential for the establishment of neuronal polarity, treatment with a

GSK-3β inhibitor during a later stage (stage 3) led to conversion of existing dendrites to axons, suggesting that GSK-3β activity is continuously required for repressing the axon forming potential of dendrites.

Jiang *et al*. [3] also shed light on the upstream regulators of GSK-3β. Akt, a kinase involved in many signaling pathways [8], localizes specifically to axons and phosphorylates residue Ser9 of GSK-3β. A pharmacological inhibitor of phosphoinositide 3-kinase (PI3-kinase), which acts upstream of Akt, blocked the inhibitory phosphorylation on GSK-3β, whereas expression of a constitutively active form of Akt led to multiple axon formation. These observations suggest a sequential order of action from PI3-kinase to Akt activation, to GSK-3β inhibition. Consistent with an involvement of PI3-kinase activity, inhibition of PTEN, the lipid phosphatase that breaks down the product of PI3-kinase, resulted in multiple axon formation, whereas PTEN overexpression blocked the establishment of neuronal polarity.

On the downstream side, Yoshimura *et al*. [4] demonstrated that collapsin response mediator protein-2 (CRMP-2) is an important target of GSK-3β in the establishment of neuronal polarity. These authors previously showed that CRMP-2 is an axonal protein and its inhibition prevented axon formation [9]. In the new study, they demonstrated that CRMP-2 is a substrate of GSK-3β, and that non-phosphorylated CRMP-2 is enriched in the growth cone of the axon, compared to the shaft. Transfection of cells with a construct encoding a non-phosphorylatable form of CRMP-2 led to formation of multiple axons and rescued the axon formation defect caused by the constitutively active GSK-3β. These results establish CRMP-2 as a functional substrate of GSK-3β in axon formation. CRMP-2 interacts with tubulin, and this interaction is weakened upon phosphorylation of CRMP-2. Yoshimura *et al*. [4] speculate that CRMP-2 promotes the elongation of axonal microtubules through its tubulin binding activity, and that GSK-3β inhibits rapid axonal growth by phosphorylating CRMP-2.

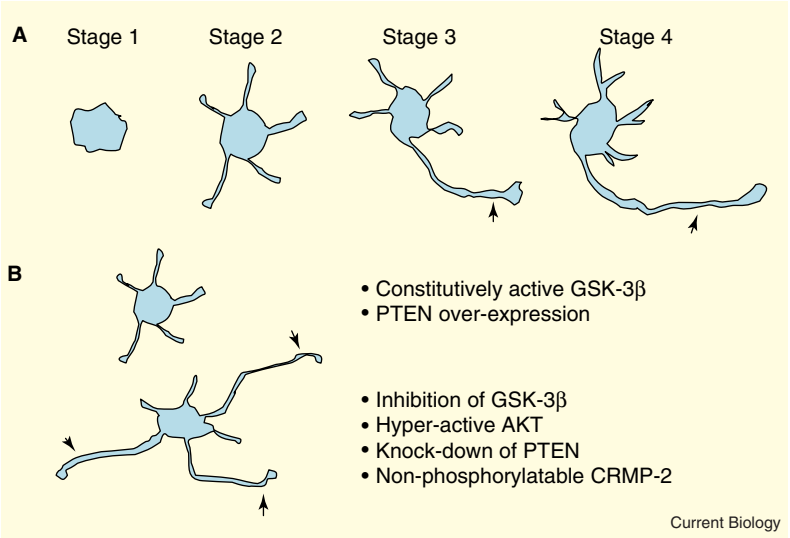

Figure 1. Polarization of hippocampal neurons.
(A) Stages of hippocampal neuron differentiation *in vitro.* Arrows point to axonal processes. (B) Some of the phenotypes described in the new studies [3,4] discussed in the text.

These and other recent results have begun to sketch out a signaling network that controls the establishment and maintenance of neuronal polarity, but there are still major gaps in the picture. For example, it is not clear how other proteins implicated in the regulation of neuronal polarity, such as the Par-3/Par-6/aPKC polarity complex or the small GTPases Rap1 and Cdc42 [10–12], interact with the Akt/GSK-3β/CRMP-2 pathway. Most recently, a Par-1/MARK family kinase, SAD-1, was found to be required for neuronal polarity in *Caenorhabditis elegans* and mouse, possibly acting through regulating the phosphorylation of the microtubule binding protein Tau [13,14].

While filling the gaps of the signaling network constitutes much of the ongoing research, a perhaps deeper question is how any resulting pathway model might explain the fascinating early findings on the stochastic nature of axon specification in the *in vitro* system and its relationship to neurite length [15]. All neurites have an equal probability to become the axon if their lengths are similar to each other; however, the one that happens to be >10 μm longer than the rest will inevitably become the axon. Thus, the underlying molecular pathway must have embedded in it an ability to measure length and a mechanism for spontaneous symmetry breaking.

A model put forward to explain spontaneous polarization in yeast involves a positive feedback loop in which a small GTPase stimulates formation of cytoskeletal polymers which direct transport of the same GTPase [16]. An analogous feedback loop might also underlie the establishment of neuronal polarity: it was reported that Par-3 may be transported along axonal microtubules by the kinesin KIF3 [11,17]. Other regulatory proteins that function at the tip of the axons could also take a ride along microtubules, and these proteins could in turn control the dynamics and functionality of the microtubule-based transport system.

A battle between anterograde transport and diffusion could provide a length measure required to explain the results of early axotomy experiments, as shown in a theoretical model by Samuels *et al.* [18]. This model assumed a positive feedback loop in which the transport rate of a limiting polarity determinant is proportional to the

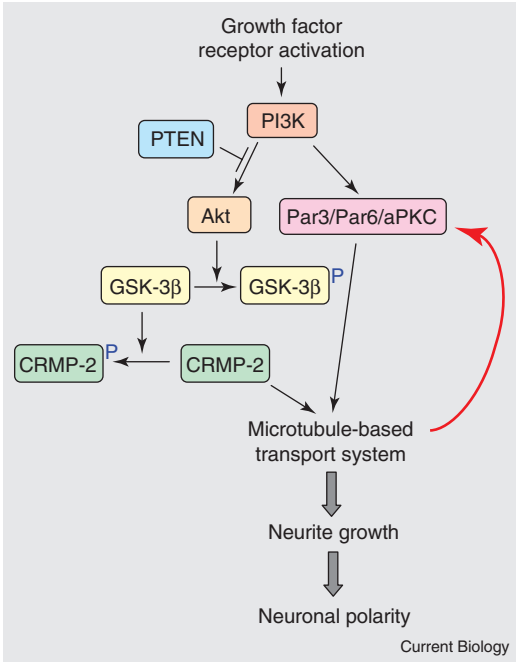

Figure 2. A speculative and simplified pathway model for axon specification.

The positive feedback loop from microtubule-based transport system to localization of signaling complexes to neurite tips is denoted with a red arrow.

concentration of this determinant. Combining theoretical formulation with the *in vitro* neuronal differentiation system should be a powerful approach for elucidating the mechanism governing neuronal polarity. Stay tuned!

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DOI: 10.1016/j.cub.2005.03.008

Homologous Recombination: Needing to Have My Say

Recent studies in budding and fission yeasts have revealed Mei5 and Sae3 as factors necessary for the proper function of the recombinases Dmc1 and Rad51 in DNA repair and meiotic recombination, providing new insights into how strand exchange proteins are directed along specific recombination pathways.

Takashi Okada and Scott Keeney

Strand exchange proteins are at the heart of homologous recombination reactions, where they carry out the search for homology, strand invasion and strand exchange. In eukaryotes these reactions are promoted by Rad51, homolog of the bacterial RecA protein. Dmc1 is another RecA homolog found in some but not all eukaryotes, acting specifically in recombination during meiosis [1]. These proteins bind to single-stranded DNA, forming a presynaptic nucleoprotein filament which promotes subsequent pairing and strand exchange reactions. Under appropriate conditions in the test tube, these recombinases are sufficient to carry out all of the steps of recombination.

The situation is more complicated *in vivo*, however, because many other proteins modulate the activities of RecA family members [2]. These factors impinge on the dynamics and targeting of filament assembly on single-stranded DNA, the choice of homologous partner for strand invasion, and the disassembly of