Neurite outgrowth involves adenomatous polyposis coli protein and β-catenin

Violet Votin*, W. James Nelson and Angela I. M. Barth‡
Department of Biological Sciences, and Department of Molecular and Cellular Physiology, Stanford University, Beckman Center B121, Stanford, CA 94305, USA
*Present address: Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720-3202, USA
‡Author for correspondence (e-mail: angieb@stanford.edu)

Accepted 8 September 2005
Journal of Cell Science 118, 5699-5708 Published by The Company of Biologists 2005
doi:10.1242/jcs.02679

Summary

Neuronal morphogenesis involves the initial formation of neurites and then differentiation of neurites into axons and dendrites. The mechanisms underlying neurite formation are poorly understood. A candidate protein for controlling neurite extension is the adenomatous polyposis coli (APC) protein, which regulates membrane extensions, microtubules and β-catenin-mediated transcription downstream of Wnt signaling. APC is enriched at the tip of several neurites of unpolarized hippocampal neurons and the tip of only the long axon in polarized hippocampal neurons. Significantly, APC localized to the tip of only one neurite, marked by dephospho-tau as the future axon, before that neurite had grown considerably longer than other neurites. To determine whether neurite outgrowth was affected by β-catenin accumulation and signaling, a stabilized β-catenin mutant was expressed in PC12 cells, and neurite formation was measured. Stabilized β-catenin mutants accumulated in APC clusters and inhibited neurite formation and growth. Importantly, these effects were also observed was independently of the gene transcriptional activity of β-catenin. These results indicate that APC is involved in both early neurite outgrowth and increased growth of the future axon, and that β-catenin has a structural role in inhibiting APC function in neurite growth.

Key words: Adenomatous polyposis coli, β-Catenin, Axon, Microtubule, Neurite, Neuronal polarity

Introduction

During morphogenesis, neurons undergo dramatic shape changes that are unmatched by any other cell type in the body. Typically, neurons extend long membrane processes, termed neurites, one of which will outgrow the others to form the axon. The growth cone at the end of the axon eventually forms synapses with target cells. The role of the cytoskeleton during these morphological changes has been studied intensively for many years. Axon formation is affected by several microtubule-associated proteins (e.g. dephospho-tau, MAP1B, CRMP-2) as well as microtubule drugs and actin-microtubule interactions (Baas, 2002; Dent and Gertler, 2003). Although axon specification requires increased neurite outgrowth and may use a similar mechanism to initial neurite outgrowth, little is known about initial neurite outgrowth, specifically how signaling pathways regulate cytoskeletal networks and how these in turn are involved in the necessary membrane changes.

Adenomatous polyposis coli (APC) protein and its binding partners are good candidates to link the cytoskeletal dynamics with membrane extension. APC is specifically enriched at the tips of neurites in neurons (Shi et al., 2004; Morrison et al., 1997) and is required to form neurites in PC12 pheochromocytoma cells (Dobashi et al., 2000). APC localization to the axon tip is required for Par3 localization to the axon tip, where Par3 and its binding partner Par6 are required for neuronal polarity (Shi et al., 2004; Shi et al., 2003). APC is a large multi-domain protein that localizes with bundles of microtubules at the tips of membrane extensions in many cell types (Nathke et al., 1996; Etiene-Manneville and Hall, 2003; Zhou et al., 2004). APC binds microtubules directly and indirectly through EB1, a microtubule plus-end-binding protein. APC promotes microtubule assembly and bundling in vitro (Zumbrunn et al., 2001; Munemitsu et al., 1994), and APC association with microtubules in the growth cone is important during axon outgrowth induced by nerve growth factor (NGF) (Zhou et al., 2004). APC also regulates β-catenin levels (Logan and Nusse, 2004). APC facilitates phosphorylation of β-catenin by GSK3β and targeting of β-catenin for ubiquitin-mediated degradation. Wnt signals inhibit GSK3β, leading to accumulation of β-catenin that can then mediate transcription by the Tcf/Lef family of transcription factors. Significantly, stabilized β-catenin inhibits membrane extension in epithelial cells (Pollack et al., 1997), but it is unknown how APC and β-catenin are involved in more complex patterns of membrane extension during neuronal morphogenesis.

We examined the role of APC clusters in axon and neurite outgrowth in hippocampal neurons and PC12 cells, respectively. Detailed analysis of APC localization, particularly at an intermediate stage of neuronal polarization, signified its role in microtubule-dependent neurite outgrowth.
We defined the localization of APC relative to β-catenin in hippocampal neurons and PC12 cells. Significantly, expression of a stabilized β-catenin mutant that separated structural from transcriptional roles of β-catenin inhibited neurite extension.

Materials and Methods

Cell culture and transfection

E18 embryonic rat hippocampi were trypsinized and plated onto poly-L-lysine-coated coverslips, which were suspended above a dish of glial feeder cells, as described (Goslin et al., 1998). Hippocampal neurons were imaged after 1.5 or 2 days in vitro. Animals were handled ethically, according to standard protocols. Rat PC12 pheochromocytoma cells were cultured on tissue culture plates in high-glucose Dulbecco’s modified Eagle’s medium plus 10% horse serum plus 5% calf serum. Cells were transfected with Lipofectamine 2000 according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA).

Tcf/Lef-mediated transcription was measured in uninduced, transfected PC12 cells by the TOPFLASH assay as described (Barth et al., 1999). Transfection efficiency of pTOPFLASH or pTOPFLASH luciferase reporters (kind gift from M. van de Wetering and H. Clevers, Center for Biomedical Genetics, Utrecht, The Netherlands) (Korinek et al., 1997) and β-catenin constructs were normalized by activity of co-transfected β-galactosidase. Luciferase/β-galactosidase ratios were then normalized within each experiment to controls co-transfected with pTOPFLASH and β-galactosidase.

For counting neurite extensions, cells were split 1 day after transfection at 1:2 or 1:4 onto collagen-coated coverslips. To induce extensions, 20 ng/ml nerve growth factor (NGF 2.5S) (Upstate, Lake Placid, NY) was added to fresh complete medium for 3 days. To quantify cells with extensions, tubulin-stained, non-contacting, non-mitotic, mononucleate cells at 40× magnification were counted blind to which construct was transfected.

Myc-tagged β-catenin was a kind gift from P. McCrea at University of Texas, Houston, TX (Montross et al., 2000; Tepera et al., 2003). Stabilized β-catenin mutants, indicated by *, were generated by mutating four GSK3β phosphorylation sites (Ser33, Ser37, Thr41 and Ser35) to alanine and cloning into the ‘tet-off’ expression vector pHDH10-3, as described for ΔGSK-β-catenin (Barth et al., 1999). ΔGSKΔC-β-catenin, a vector for the expression of stabilized β-catenin without the C-terminal transcriptional transactivation domain (amino acids 696-781 of mouse β-catenin), was obtained by replacing the 3′-terminal BglII/XbaI in ΔGSK-β-catenin with the corresponding fragment from ΔNΔC-β-catenin as described (Barth et al., 1997). These KT3-tagged constructs were expressed in ‘tet-off’ PC12 cells in Fig. 4A,B (ΔGSK-β-catenin represented by *β-catenin and ΔGSKΔC-β-catenin, *β-catenin, *β-catenin). For all other experiments, stabilized *β-catenin mutant proteins were expressed as fusion proteins with green fluorescent protein (GFP) using expression vector pEGFP-C1 (BD Biosciences Clontech, Palo Alto, CA). For *β-catenin/pEGFP-C1 the SacII/BamHI fragment encoding ΔGSK-β-catenin was removed from pHDH10-3 and cloned into pEGFP-C1, and the endogenous β-catenin stop codon was restored. The C-terminal SpeI/BamHI fragment in *β-catenin/pEGFP-C1 was replaced with the corresponding KT3-tagged fragment of ΔGSKΔC to obtain *β-catenin.

Immunostaining

Cells were fixed with either 100% methanol at –20°C for 5 minutes (e.g. Fig. 3B) or, to preserve growth cone morphology or dephospho-

Fig. 1. APC in neurite tips is along microtubules and changes during neuronal polarization. (A) An axonal growth cone of a hippocampal neuron immunostained for tubulin and APC. Projection of deconvolved planes is shown. (Note that deconvolution resolves APC enrichment into puncta.) (B) Immunostaining for the axon marker dephospho-tau (Tau) and APC show APC enrichment at several neurite tips in an unpolarized neuron and only at the tip of the axon (arrowhead), which is marked by dephospho-tau staining (arrow), in a polarized neuron. Magnified images below also show phase-contrast images of neurite tips N3 and N4 from the unpolarized neuron and the axon tip from the polarized neuron. A, axon; D, dendrite; N, neurite. (C) Line scans quantifying fluorescence intensity of APC (red) and dephospho-tau (Tau, green) along corresponding neurites from B. (D) From line scans of polarized neurons (including that in C), we calculated the ratio of maximum APC intensity at the neurite tip to that of equivalent length of the neurite shaft. Shown is the mean±s.e.m. of the axon and the longest dendrite of 13 neurons. ***P<0.001 compared to APC enrichment in axons by Student’s t-test. Bar, 2 μm (A); 20 μm (whole-cell images in B); 2.5 μm (magnified images in B).
underestimation of the APC tip enrichment observed qualitatively. (Lee and Hollenbeck, 2003), this effect would only lead to however, because axonal growth cones are thinner than axon shafts cytoplasmic volume would affect fluorescence quantification; line was measured in an unsectioned image, so differences in the longest dendrite) of the same neuron. Total fluorescence along the enrichment at the growing tip (Fig. 1D). In contrast, in axons, maximum APC intensity in the tip to that in an equivalent length of the shaft. In dendrites, APC was only 1.5 times as intense as APC staining in the shaft, representing minimal APC enrichment at the growing tip (Fig. 1D). In contrast, in axons, APC staining was 3.4 times (range, 2-6 times) as intense as APC staining in the shaft. Given that axonal growth cones are usually flatter than the axon shaft (Lee and Hollenbeck, 2003), the actual APC enrichment per volume in the axonal growth cone is probably even greater. On average, axons showed 2.2-fold greater APC enrichment at the tip compared to the longest dendrites (Fig. 1D). Therefore, APC enrichment correlated with sites of increased neurite growth in polarized neurons.

APC enrichment in axonal growth cone precedes increased axonal elongation

We reasoned that if APC enrichment were required for the increased growth that differentiates the future axon from other neurites, it would be enriched early in axonal outgrowth and exclusively at the tip of the future axon. In a polarized neuron, the axon is defined as the neurite that meets both length and tau criteria, i.e. is exclusively enriched for dephospho-tau staining and is longer than twice the next-longest neurite. Of these two criteria for axon identification, dephospho-tau enrichment was more likely to first mark the future axon, because specification of a single future axon only by dephospho-tau enrichment was four times more prevalent than specification only by two-fold increased length (113 cells polarized only by tau criterion, compared to 31 cells polarized only by length criterion, from the experiments in Fig. 2). Therefore, we defined polarizing neurons as those with a ‘future axon’ specified by exclusive enrichment of dephospho-tau without twofold length increase over other neurites.

The distribution of APC among neurite tips was examined
Endogenous β-catenin partially colocalizes with APC

Neurite growth mediated by nerve growth factor (NGF) involves local inhibition of GSK3β, which phosphorylates APC and modifies its binding to β-catenin and microtubules (Zhou et al., 2004; Rubinfeld et al., 1996; Zumbrunn et al., 2001). These data indicate that β-catenin could regulate APC during neurite growth.

To probe the role of APC/β-catenin during early neurite growth, we took advantage of the ability to induce neurite formation, and to express exogenous proteins prior to inducing neurite formation, in the well-studied PC12 pheochromocytoma cell line (Greene et al., 1998). PC12 cells induced with NGF are a good model for APC cluster function in hippocampal neurons because, as in neurons (Fig. 3A, Fig. 1A) (Shi et al., 2004), PC12 cells accumulated APC clusters at the tips of neurites (Fig. 3B).

Endogenous β-catenin and APC occasionally overlapped at the tips of neurites in hippocampal neurons (Fig. 3A) and in PC12 cells (Fig. 3B). Some neurite tips had no enrichment of β-catenin (Fig. 3A, arrow), whereas some had β-catenin enriched in the entire growth cone (Fig. 3A, solid arrowhead) or in a part of the growth cone just proximal to and partly overlapping with enriched APC (Fig. 3A, hollow arrowheads). Partial overlap of APC and β-catenin was most common in both hippocampal neurons and PC12 cells (Fig. 3A,B).

Stabilized β-catenin strongly co-clusters with APC

We asked whether β-catenin that cannot be degraded upon binding to APC colocalized more strongly with APC than endogenous β-catenin and thereby would amplify any effect of endogenous β-catenin on APC function. We expressed ‘stabilized’ β-catenin, termed *β-catenin, which lacks GSK3β phosphorylation sites (see Materials and Methods) and thus cannot be targeted for ubiquitin-proteasome degradation. Note that in PC12 cells, Wnt-induced β-catenin accumulation also activates β-catenin-mediated transcription (Chou et al., 2000) and prevents NGF-induced neurite outgrowth (Shackleford et al., 1993). Thus, to distinguish between transcriptional and other effects of β-catenin on neurite outgrowth, we expressed *β-cadAC-term, a stabilized β-catenin mutant lacking the transcriptional transactivation domain, and a non-stabilized chimeric protein, termed β-catenin, in which the transcriptional transactivation domain was replaced with an active transcriptional repression domain from the Drosophila engrailed gene (Montross et al., 2000).

Each of these three β-catenin mutants often co-clustered with APC at the distal ends of microtubules at the neurite tip in PC12 cells (Fig. 4). In hippocampal neurons, *β-catenin was enriched at the tips of neurite extensions (Yu and Malenka, 2003), and each β-catenin mutant co-clustered with APC (V.V., unpublished observations), similar to the localization in PC12 cells. This result indicates that transient overexpression of non-stabilized β-catenin (β-cadeng) is sufficient to induce its accumulation in the APC complex.

We then confirmed the activity of β-catenin mutants in Tcf/Lef-mediated transcription. β-catenin stabilized by Wnt binds to and coactivates transcription with Tcf/Lef (van de Wetering et al., 1997). To test transcriptional activity of β-catenin mutants, we coexpressed a luciferase reporter with Tcf/Lef-binding sites. As expected, full-length stabilized β-
catenin (*β-catenin) activated Tcf/Lef-mediated transcription of the reporter, in a manner dependent on functional Tcf/Lef-responsive elements in the reporter (Fig. 5A). Neither *β-catenin nor β-cat-eng activated Tcf/Lef-dependent transcription (Fig. 5A).

Stabilized β-catenin inhibits neurite growth

We tested whether stabilized β-catenin, which accumulated with APC clusters at neurite tips independent of Tcf-mediated transcriptional activity, affected formation of neurite extensions. In PC12 cells, expression of stabilized β-catenin, compared with GFP as a control, significantly decreased the proportion of cells that formed neurites in response to NGF (*β-catenin in Fig. 5B). To test the effect of stabilized β-catenin on neurite elongation, we quantified the proportion of cells with long neurites, defined as neurites at least twice the length of the cell body width (Fig. 5C). Expression of stabilized β-catenin also decreased the proportion of cells with long neurites (Fig. 5C). Thus, stabilized β-catenin inhibits neurite initiation as well as elongation (Table 1).

To determine whether inhibition of neurite extension required Tcf/Lef-mediated transcription, we compared the effects of different β-catenin mutants. Expression of transcriptionally inactive β-catenin (*β-cateninΔC-term or β-cat-eng) significantly reduced the percentage of cells with neurites (Fig. 5B), and the percentage of cells with neurites longer than twice the cell body width (Fig. 5C). Together these data indicate that accumulation of β-catenin at APC clusters inhibits neurite extension.

To clarify the contribution of structural and transcriptional functions of β-catenin to neurite inhibition, we calculated inhibition of neurite formation as the proportion of cells with neurites compared to GFP-expressing cells. Expression of *β-catenin and β-cat-eng proteins inhibits neurite formation by 59% and 25%, respectively (from Fig. 5B). Therefore, Tcf-mediated transcription, which is activated by *β-catenin but not β-cat-eng, accounts for about half of the inhibition of neurite formation by β-catenin, while nontranscriptional effects account for the other half. Expression of *β-catenin and β-cat-eng proteins inhibit neurite elongation (Fig. 5C) by 89% and 70%, respectively, indicating a much greater role for structural (non-transcriptional) effects of β-catenin in neurite elongation compared to neurite initiation. Furthermore, both transcriptionally active and transcriptionally inactive β-catenin mutants inhibited neurite growth in proportion to protein expression level (Fig. 5D,E). Cells expressing high levels of *β-catenin or *β-cateninΔC-term (i.e. an arbitrary fluorescence value greater than 50) formed significantly shorter neurites than cells expressing low amounts of stabilized β-catenin (Table 2). Note the similarity in distribution of expression levels and effects on neurite growth inhibition in cells expressing *β-catenin or *β-cateninΔC-term.
We conclude that stabilized β-catenin has structural effects, in addition to its transcriptional effects, that inhibit neurite growth.

Discussion

We propose that endogenous β-catenin is rapidly turned over at APC clusters in neurite tips and that this regulates APC function in neurite growth (see Fig. 6). APC clusters in neuronal extensions are very similar to the previously described APC clusters at microtubule ends in epithelial extensions (Nathke et al., 1996). In epithelial extensions, proteasome inhibition causes accumulation of endogenous β-catenin and axin, another component of the APC destruction complex (Hart et al., 1998; Ikeda et al., 1998) at APC clusters (A.B., unpublished results). This further indicates that APC clusters represent part of the destruction complex engaged in β-catenin turnover. Overexpression or stabilization of β-catenin causes accumulation of β-catenin in the APC clusters and may disrupt the normal function of APC clusters in promoting neurite outgrowth.
Table 2. Dose-dependent effect of stabilized β-catenin on neurite length

<table>
<thead>
<tr>
<th>Stabilized β-catenin</th>
<th>Expression level</th>
<th>Longest neurite (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-catenin ΔC-term</td>
<td>Low</td>
<td>16.6±2.0</td>
</tr>
<tr>
<td>β-catenin ΔC-term</td>
<td>High</td>
<td>4.3±2.0</td>
</tr>
<tr>
<td>β-catenin</td>
<td>Low</td>
<td>14.7±1.9</td>
</tr>
<tr>
<td>β-catenin</td>
<td>High</td>
<td>2.3±2.3</td>
</tr>
</tbody>
</table>

Data points from Fig. 5E were grouped by expression level of fluorescently tagged stabilized β-catenin. Low, <50 fluorescence intensity units; High, >50 fluorescence intensity units. Mean neurite length of the longest neurites of high-expressing cells was significantly shorter than that of low-expressing cells (t-test, P<0.01). This dose-dependence was significant for both transcriptionally inactive (β-catenin ΔC-term, n=12 cells total) and transcriationally active (β-catenin, n=11 cells total) stabilized β-catenin mutants. Data are the mean lengths of the longest neurites of the cells ± s.e.m.

APC associated with microtubules marks the future axon

Axon growth is an example of neurite growth, and axon specification involves increased relative growth of one neurite. APC within axonal growth cones is at distal microtubule ends (Fig. 1A), consistent with recent work showing that microtubule-associated APC at axon tips functions in axon growth (Zhou et al., 2004). Overexpression of APC dominant-negative mutants disrupts axon specification and axon growth (Shi et al., 2004; Zhou et al., 2004), but it is unclear whether

β-catenin associates with APC

In the absence of Wnt signaling, APC forms a multi-protein ‘β-catenin degradation complex’ that includes GSK3β, axin and PP2A (Ikeda et al., 2000). In this complex, GSK3β phosphorylates β-catenin and APC. Of the components in this complex that could mediate APC cluster function in neurites, spatially regulated GSK3β activity is required for axon growth, morphology and differentiation (Jiang et al., 2005; Goold and Gordon-Weeks, 2004) (see also Fig. 6). However, little is known about the effect of β-catenin on initial neurite outgrowth. Both β-catenin and APC localize generally to neurite tips in primary mouse neuronal cultures (Morrison et al., 1997). Here we examined localization of β-catenin in APC clusters using deconvolution microscopy. We show varying degrees of colocalization of endogenous APC and β-catenin in hippocampal neurons and PC12 cells (Fig. 3, see also supplementary material Fig. S1). This variability may indicate transient interaction of APC and β-catenin as endogenous β-catenin is degraded or binds with lower affinity to unphosphorylated APC when NGF inhibits GSK3β (see Fig.
6). The model that β-catenin degradation and turnover in the APC complex regulate β-catenin/APC colocalization is supported by the enrichment of undegradable (stabilized) or overexpressed β-catenin mutants in APC clusters at the tips of neurites (Fig. 4). In epithelial cells, stabilized β-catenin similarly accumulates at APC clusters at extension tips (Barth et al., 1997; Nathke et al., 1996), and stabilized β-catenin that associates with APC has delayed turnover compared to normal β-catenin (Barth et al., 1999). APC co-immunoprecipitated a stabilized β-catenin mutant identical to *tβ-catenin (with the exception of the GFP tag) from stably-expressing MDCK epithelial cells, even 18 hours after expression of stabilized β-catenin was repressed by doxycycline (Barth et al., 1999), indicating a very stable association of this mutant β-catenin with APC.

Stabilized β-catenin inhibits neurite outgrowth

We show that expression of stabilized β-catenin decreased neurite initiation and elongation in NGF-treated PC12 cells (Fig. 5). Several mechanisms could explain how stabilized β-catenin inhibits neurite outgrowth in PC12 cells. When β-catenin is stabilized by Wnt signals it can promote cadherin-mediated cell-cell adhesion (Hinck et al., 1994) in addition to Tcf/Lef-mediated transcription. Experiments expressing stabilized β-catenin in whole animals or in neuronal cultures directly contacting glial cells may mask the role of β-catenin in the APC complex with its role in adhesion (Yu and Malenka, 2003/H9252; Tanaka et al., 1995). The direct role of APC in these processes could be inhibited by constitutive rather than cyclical binding to β-catenin (Ha et al., 2004). The β-catenin-binding region of APC overlaps with the GSK3β-phosphorylated region; phosphorylation of APC decreases its ability to bundle microtubules in vitro (Zumbrunn et al., 2001). Similar to phosphorylation by GSK3β, binding of stabilized β-catenin to this region of APC may decrease interaction of APC with microtubules (see also Fig. 6).

Note that in our experiments, exogenous stabilized β-catenin prevents NGF-stimulated neurite growth, whereas β-catenin stabilization that is assumed to result from local inactivation of GSK3β after NGF stimulation allows neurite growth. Before NGF stimulation, active GSK3β phosphorylates APC, increasing its affinity for β-catenin (Rubinfeld et al., 1996) and inhibiting APC function in microtubule bundling (Zumbrunn et al., 2001). Turnover of β-catenin from this high-affinity APC/β-catenin complex is probably mediated by proteolytic degradation (Fig. 6A). Conversely, after NGF stimulation, inactive GSK3β cannot phosphorylate APC or β-catenin, and therefore the endogenous β-catenin that accumulates does not bind as strongly to unphosphorylated APC (Ha et al., 2004), allowing APC to dissociate and bundle microtubules (Fig. 6B). Accordingly, NGF stimulation increases the localization of APC to neurite tips but does not significantly increase the level of endogenous β-catenin in APC clusters (see supplementary material Fig. S1).

If stabilization of β-catenin at APC clusters interferes with APC function and inhibits extension formation, and transient interaction of endogenous β-catenin with APC clusters properly regulates extension formation, the absence of β-catenin at APC clusters should inappropriately increase microtubule growth and extension formation. Although transfection of β-catenin siRNA was not successful in PC12 cells, siRNA-induced β-catenin knockdown in HeLa cells did result, as expected, in increased cell extension. That is, the average cell elongation (measured as the major axis of the ellipse best fitted to the cell) of HeLa cells treated with a pool of siRNAs targeting β-catenin was 115±21.6 μm, whereas that of cells treated with a control siRNA against GFP was 66±5.1 μm (see Fig. S2 in supplementary material). Therefore, stabilized β-catenin or reduced APC (Dobashi et al., 2000) decreases cell extension, and reduced β-catenin increases cell extension. In HeLa cells depleted of β-catenin, APC clusters still form at extension tips (arrowheads in Fig. S2, supplementary material) and presumably are free to bundle microtubules.

APC is highly expressed in developing rat brain during neuronal maturation (Bhat et al., 1994). Brain development involves physiological signals that modulate the effects of GSK3-β and APC. For example, both Wnt and NGF signaling inactivate GSK3β (Logan and Nusse, 2004; Zhou et al., 2004), and local inactivation of GSK3β is required for APC instead, and specifically through microtubule-associated APC, transcriptionally active and inactive β-catenins colocalized with APC clusters at distal microtubule ends.

APC regulates microtubule growth, bundling and stability (Munemitsu et al., 1994; Zumbrunn et al., 2001). Neurite growth requires cycles of microtubule bundling and unbundling, and balance between microtubule stabilization and destabilization (Goold and Gordon-Weeks, 2004; Tanaka and Kirschner, 1991/Tanaka et al., 1995). The direct role of APC in these processes could be inhibited by constitutive rather than cyclical binding to β-catenin (Ha et al., 2004). The β-catenin-binding region of APC overlaps with the GSK3β-phosphorylated region; phosphorylation of APC decreases its ability to bundle microtubules in vitro (Zumbrunn et al., 2001). Similar to phosphorylation by GSK3β, binding of stabilized β-catenin to this region of APC may decrease interaction of APC with microtubules (see also Fig. 6).
localization to microtubule ends in extension tips in astrocytes and neurons (Etienne-Manneville and Hall, 2003; Zhou et al., 2004). Axons turn toward Wnt in a time frame suggestive of structural rather than transcriptional effects (Lyuksyutova et al., 2003). Other physiological signals inhibiting axonal growth, such as semaphorin, activate GSK3β (Eickholt et al., 2002). Furthermore, caderhins are involved in promoting axonal outgrowth on non-neuronal cells (Matsunaga et al., 1988). Future studies will determine how binding of β-catenin to APC clusters in growth cones responding to these diverse signals regulates microtubule changes necessary for neurite and axon outgrowth and potentially neuronal targeting and synaptogenesis.

We would like to thank the lab of Stephen J. Smith, especially Kristina D. Micheva, for preparation of hippocampal cultures, Elias Spiliotis and Bronwyn MacInnis for critical reading of the manuscript and the entire Nelson lab for helpful discussions. Supported by grant NIH GM35527.

References


