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Role of the Microtubule Destabilizing Proteins SCG10 and Stathmin in Neuronal Growth

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ABSTRACT: The related proteins SCG10 and stathmin are highly expressed in the developing nervous system. Recently it was discovered that they are potent microtubule destabilizing factors. While stathmin is expressed in a variety of cell types and shows a cytosolic distribution, SCG10 is neuron-specific and membrane-associated. It contains an N-terminal targeting sequence that mediates its transport to the growing tips of axons and dendrites. SCG10 accumulates in the central domain of the growth cone, a region that also contains highly dynamic microtubules. These dynamic microtubules are known to be important for growth cone advance and responses to guidance cues. Because overexpression of SCG10 strongly enhances neurite outgrowth, SCG10 appears to be an important factor for the dynamic assembly and disassembly of growth cone microtubules during axonal elongation. Phosphorylation negatively regulates the microtubule destabilizing activity of SCG10 and stathmin, suggesting that these proteins may link extracellular signals to the rearrangement of the neuronal cytoskeleton. A role for these proteins in axonal elongation is also supported by their growth-associated expression pattern in nervous system development as well as during neuronal regeneration. © 2003 Wiley Periodicals, Inc. J Neurobiol 58: 60–69, 2004

Keywords: **SCG10; stathmin; microtubule dynamics; growth cone; neurite outgrowth**

INTRODUCTION

During the development of the nervous system, axons are guided to their targets by extracellular cues in the environment. The growth cone at the tip of the axon is capable of responding to a variety of attractive or repulsive signals thereby changing its behavior. For example, it can change from forward elongation to pauses or retraction, or it can change its direction. The combination of multiple inputs is believed to be important for the formation of precise axon pathways (Tessier Lavigne and Goodman, 1996). While a large number of axon guidance molecules have been identified, relatively little is known about the intrinsic mechanisms by which the extracellular signals are transduced into cytoskeletal dynamics.

An important component of the neuronal cytoskeleton is the microtubules. They act as tracks for organelle transport, to maintain neurite shape, and to participate in the regulation of axonal elongation and growth cone steering. Microtubules are polar structures with one end termed the "plus" end and the other end termed the "minus" end. They are composed of heterodimers of α- and β-tubulin which self-assemble to form polymers. Assembly preferentially occurs at the plus end. In the axon and the distal region of the dendrite, the plus-ends of microtubules are oriented distally (Heidemann et al., 1981; Baas et al., 1987, 1988). Microtubules form dense arrays within the

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neurite, while they are less stable in the growth cones (Ahmad et al., 1993). As microtubules enter the growth cone from the neurite shaft, the bundled microtubules separate from each other and extend into the organelle-rich central domain (C-domain) of the growth cone in the form of individual microtubules (Tanaka and Kirschner, 1991). Growth cones also contain a large pool of soluble, assembly competent tubulin (Letourneau and Ressler, 1984). The majority of growth cone microtubules is restricted to the C-domain but due to their highly dynamic behavior, called "dynamic instability", individual microtubules can also extend deep into the actin-rich peripheral domain (Gordon-Weeks, 1991; Tanaka and Kirschner, 1991). The importance of microtubule dynamics in axonal growth and guidance has been demonstrated by pharmacological inhibition of dynamics without affecting microtubule assembly. Low concentrations of drugs such as nocodazole, vinblastine, and taxol not only reduce axonal elongation but also prevent growth cone turning (Rochlin et al., 1996; Williamson et al., 1996; Challacombe et al., 1997).

The term "dynamic instability" of microtubules describes an intrinsic property of microtubules that allows them to switch abruptly between phases of elongation and rapid shortening (Mitchison and Kirschner, 1984; Desai and Mitchison, 1997). The transition from the state of growth to a state of shrinkage is called "catastrophe", and the transition from a state of shrinkage to a state of growth is called "rescue". Proteins that regulate microtubule dynamic instability fall into two main categories: proteins that stabilize microtubules and proteins that destabilize microtubules. The first category, known as structural microtubule-associated proteins (MAPs), includes molecules that promote microtubule assembly by increasing the frequency of rescues and/or by stabilizing microtubules by binding to the lattice of the microtubule polymer. MAPs are also thought to crosslink microtubules and thus to form bundles, to regulate the spacing between microtubules, and to regulate the interaction of microtubules with actin filaments (Matus, 1988; Hirokawa, 1994). Some of the MAPs, such MAP1B and tau, are present in growth cones and there is both *in vitro* and *in vivo* evidence that MAP1B and tau are important for neurite outgrowth (DiTella et al., 1996; Takei et al., 1997, 2000; Gonzales-Billault et al., 2002). Proteins that act as potent destabilizers of microtubules have been identified more recently (Belmont and Mitchison, 1996; Riederer et al., 1997; Desai et al., 1999). Among these are stathmin and SCG10, which are members of the same gene family. Stathmin, also referred to as Op18, is a ubiquitously expressed protein originally identified because it is highly expressed in diverse tumoral cells and is phosphorylated in response to numerous extracellular stimuli that regulate proliferation and differentiation in different cell types (Sobel, 1991). Stathmin is also expressed in neurons, where it is developmentally regulated. SCG10 was initially identified as neuronal marker of the neural crest and as a gene rapidly up-regulated during neurite outgrowth of PC12 cells (Stein et al., 1988). The stathmin gene family includes two additional members that are, like SCG10, specifically expressed in the nervous system (Ozon et al., 1999). In this review we will summarize our recent studies on the function and regulation of SCG10 and stathmin and discuss the role of SCG10 in relation to our current understanding of microtubule behavior in growth cones.

SCG10 AND STATHMIN MESSENGER RNA AND PROTEIN EXPRESSION ARE ASSOCIATED WITH AXONAL GROWTH DURING DEVELOPMENT AND REGENERATION

SCG10 and stathmin may be classified as neuronal growth-associated proteins because their expression closely correlates with neurite outgrowth. We have isolated stathmin in a subtractive cloning approach based on differential gene expression during neurite outgrowth and synapse formation. Stathmin appears to be highly expressed in the chick retina during the period of extensive axonal growth, and the same pattern is found for SCG10. Also, in cortical neurons developing in culture, high levels of stathmin and SCG10 are expressed during neurite outgrowth, while their levels are strongly down-regulated after synapse formation (Di Paolo et al., 1997b).

In the rat olfactory system where olfactory receptor neurons of the epithelium constantly regenerate and reinnervate the olfactory bulb throughout animal life span, SCG10 is restricted to immature olfactory receptor neurons, indicating that SCG10 is down-regulated as the olfactory receptor neurons reach the mature state. A similar observation has been made for stathmin, except that stathmin is additionally expressed in the proliferating basal cells. Following injury, both stathmin and SCG10 are highly up-regulated during olfactory axon regeneration (Pellier-Monnin et al., 2001). SCG10 expression also appears to be up-regulated during axonal regeneration in the peripheral and central nervous systems, and down-regulation in peripheral neurons correlates with target reinnervation (Mason et al., 2002). Altogether these studies suggest that SCG10 and stathmin play impor-

tant roles in axonal outgrowth, both during development and during axonal regeneration in the adult nervous system.

SCG10 IS TARGETED TO GROWTH CONES: ROLE OF PALMITOYLATION

Although highly related in structure and coexpressed in neurons, stathmin and SCG10 differ considerably in their subcellular distribution. In cortical neurons in culture, stathmin has a cytosolic distribution, is particularly enriched in the cell body and in proximal processes, and appears preferentially expressed in the thicker dendritic processes (Di Paolo et al., 1997b). In the olfactory system, stathmin is also strongly expressed in the axons of olfactory receptor neurons (Pellier-Monnin et al., 2001). Figure 1(A–C) shows the typical subcellular distribution of SCG10 in cultured neurons. It is found in the area of the Golgi complex, in a punctate pattern along the processes, and highly enriched in growth cones. Previous studies demonstrated that growth cones of both axons and dendrites are positive for SCG10 (Di Paolo et al., 1997b).

The N-terminal domain of SCG10, consisting of 35 aa and absent in stathmin, is responsible for Golgi localization and growth cone targeting (Di Paolo et al., 1997c; Lutjens et al., 2000). This N-terminal domain contains two cysteine residues at positions 22 and 24 that are sites for palmitoylation, a dynamic type of acetylation [Fig. 1(D)]. Site-directed mutagenesis of the cysteine residues indicates that palmitoylation is only partly critical for membrane binding but is absolutely essential for the specific subcellular localization of SCG10 in neurons. When palmitoylation is prevented, SCG10 is no longer found in growth cones. Indeed, the N-terminal domain containing both palmitoylation sites is sufficient to target stathmin or unrelated proteins to the growth cone. Because palmitoylation is a post-translational modification that may be dynamically regulated, one may speculate that palmitoylation/depalmitoylation may play a regulatory role in SCG10 transport to the tips of growing neurites.

Where precisely is SCG10 localized in the growth cone? Following subcellular fractionation of growth cone particles (Pfenninger et al., 1983), SCG10 is found highly enriched in the growth cone vesicle fraction (Igarashi et al., 1997; Lutjens et al., 2000). This is consistent with ultrastructural analysis in which SCG10 is shown to be mainly associated with vesicular structures. SCG10-bound growth cone vesicles appear to be associated with the labile microtubules in the growth cone because a disassembly of these microtubules with low doses of nocodazole causes a dispersal of SCG10 immunofluorescence into punctate structures (Di Paolo et al., 1997b). Because SCG10 copurifies with the microtubules isolated by cycles of assembly and disassembly (Riederer et al., 1997), it might also be able to bind directly to microtubules.

STATHMIN AND SCG10 ARE MICROTUBULE DESTABILIZING FACTORS

The first evidence that stathmin is a potent microtubule-destabilizing factor comes from a study by Belmont and Mitchison (1996) that identified stathmin as a cellular factor that promotes microtubule depolymerization by increasing the rate of catastrophes. The role of stathmin has been mainly investigated in non-neuronal cells, where it plays a role in spindle microtubule regulation during the cell cycle (see Mistry and Atweh, 2002 for review). At a mechanistic level, stathmin has several actions [Fig. 2(A)]. It binds to tubulin dimers, forming a ternary complex consisting of one molecule of stathmin and two dimers of tubulin. This can cause microtubule destabilization by tubulin sequestering mechanisms (Jourdain et al., 1997). Because the free tubulin concentration is an important parameter for microtubule assembly, tubulin sequestration, which lowers the amount of soluble tubulin, causes reduced elongation at both microtubule ends and also increased catastrophes (Walker et al., 1988). Stathmin can also stimulate catastrophes independent of tubulin sequestering by acting at plus ends of microtubules (Belmont and Mitchison, 1996; Howell et al., 1999).

Our own studies have shown that SCG10 is as efficient as stathmin in destabilizing microtubules. In an *in vitro* assay that measures microtubule assembly by light scattering in a temperature-controlled spectrophotometer, SCG10 inhibits microtubule polymerization in a dose-dependent manner [Fig. 2(B)]. It can also induce microtubule disassembly and counteract the stabilizing effect of taxol or MAPs (Riederer et al., 1997 and unpublished results). The microtubule-destabilizing effect of SCG10 can also be observed in tissue culture cells that overexpress this molecule [Fig. 2(C)]. Like stathmin, SCG10 sequesters tubulin dimers forming a ternary complex (Fleury et al., 2000; Charbaut et al., 2001). Whether it can also directly promote catastrophes is currently under investigation.

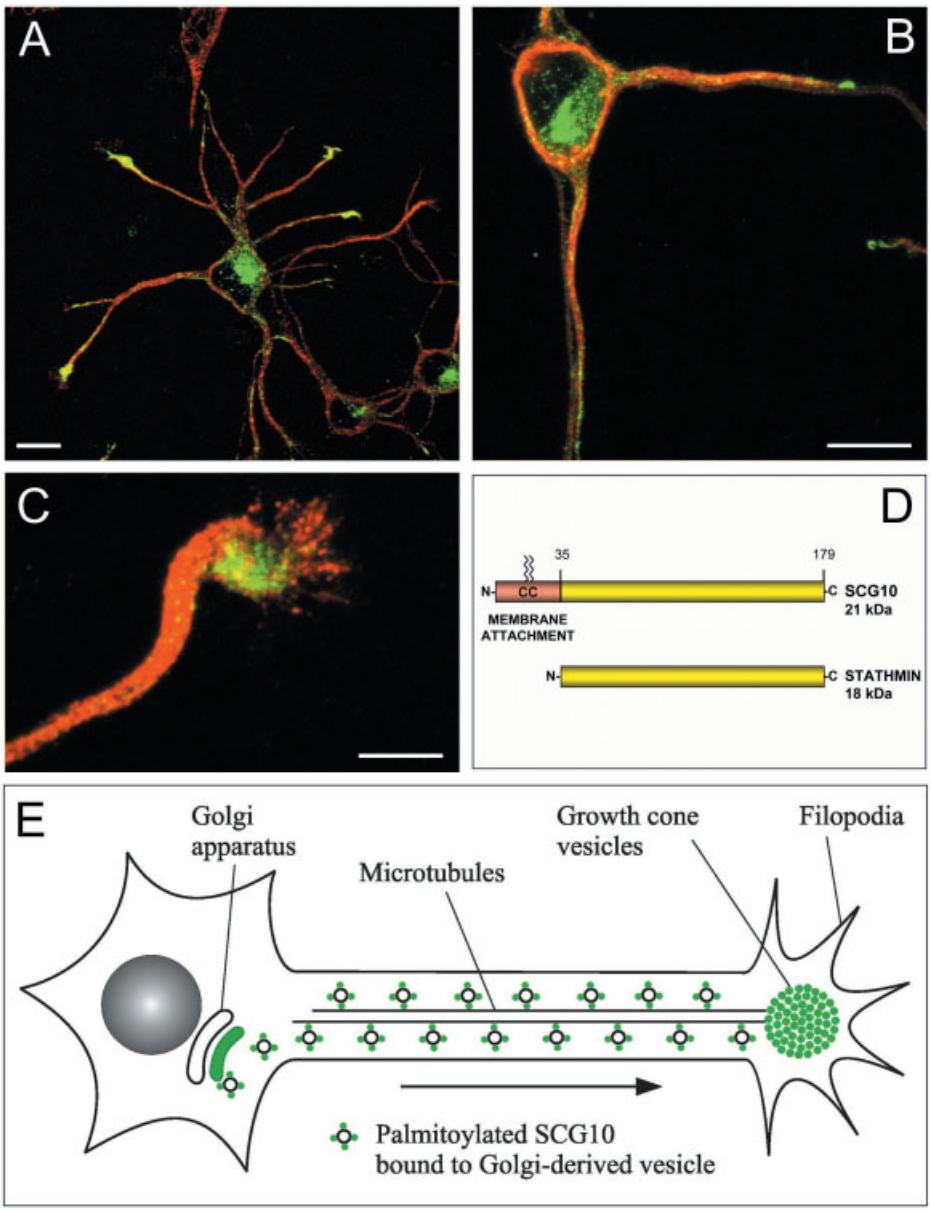

Figure 1 Localization and targeting of SCG10 in neurons. (A–C) Hippocampal neurons 2 days in culture stained with antibodies against SCG10 (green) and tyrosinated α-tubulin (red) by indirect immunofluorescence. (A) SCG10 is localized to the perinuclear region corresponding to the area of the Golgi complex and enriched in growth cones. Bar = 10 μm. (B) Higher magnification of a cell body and processes showing SCG10 in the Golgi and punctate structures along the processes. Bar = 10 μm. (C) Higher magnification of a growth cone showing splayed out microtubules and an accumulation of SCG10 in the central domain. Bar = 5 μm. (D) Schematic comparison of SCG10 and stathmin structure. SCG10 contains an N-terminal domain (aa 1-34) in which two cysteine residues are sites for palmitoylation. (E) Schematic illustration summarizing SCG10 localization and targeting in neurons. SCG10 is specifically localized to the trans face of the Golgi apparatus, where it is sorted into membranous vesicles that are targeted to the central domain of growth cones. The N-terminal domain containing two palmitoylation sites is essential for the targeting. When fused to other proteins this element can also mediate the transport of unrelated proteins to growth cones.

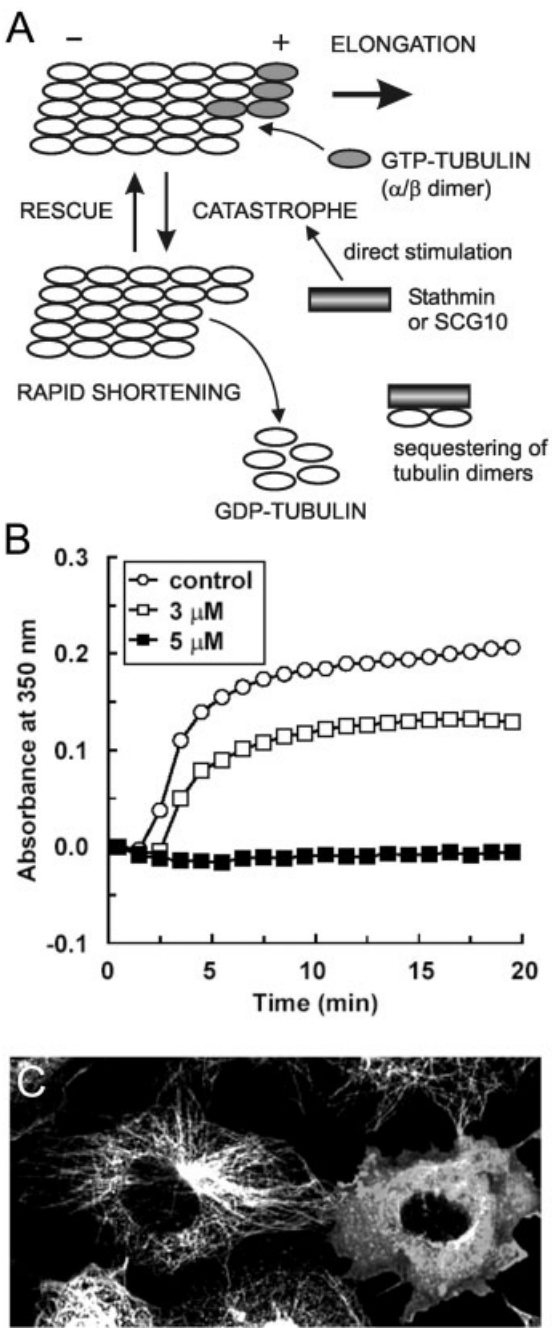

Figure 2 Microtubule destabilization by stathmin and SCG10. (A) Illustration of dynamic instability of microtubules. Microtubules are polar polymers composed of α/β tubulin heterodimers. GTP-bound tubulin is preferentially added to the plus ends of microtubules. Microtubules are dynamic polymers that switch between phases of growth and shrinkage. The transitions between the phases are called catastrophe or rescue. Stathmin and SCG10 increase the rate of catastrophes by dual activity: by direct stimulation at the plus end of microtubules or by binding to two molecules of tubulin, thereby depleting the pool of soluble tubulin available for polymerization. (B) Microtubule destabilizing activity of SCG10 measured by an *in vitro* assay of microtubule assembly. Tubulin (2 mg/mL) was incubated at 4°C in the absence of SCG10 (control), or in the presence of 3 or 5 μM SCG10 recombinant protein. The polymerization was induced by changing the temperature to 37°C at time zero. Tubulin polymerization was followed by measuring the absorbance at 350 nm. At a concentration of 5 μM SCG10, SCG10 blocks microtubule polymerization 100%. (C) Effect of SCG10 overexpression on cellular microtubules. The transfected cell on the right shows a completely disrupted microtubule network.

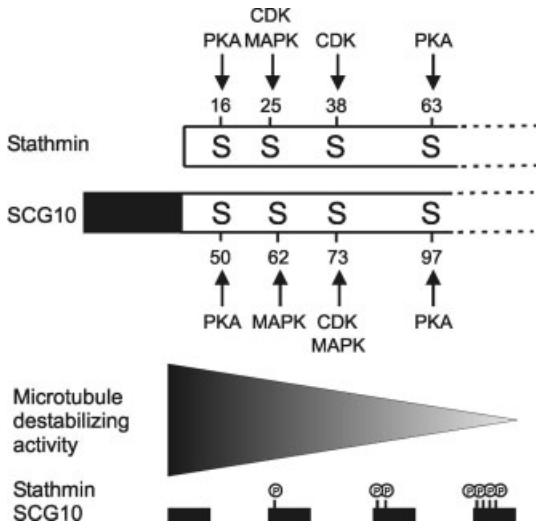

Figure 3 Schematic representation of the phosphorylation sites of stathmin and SCG10 and the effect of phosphorylation on the microtubule destabilizing activity of the proteins.

ACTIVITY OF SCG10 AND STATHMIN IS NEGATIVELY REGULATED BY PHOSPHORYLATION

Stathmin was initially identified as a protein phosphorylated in response to a number of extracellular signals (Sobel, 1991). We speculated as to whether this was the mechanism by which these powerful microtubule destabilizers could be controlled. Phosphorylation of stathmin occurs on four serine residues by different serine/threonine protein kinases (Fig. 3). Several recent reports suggest that phosphorylation turns off the microtubule destabilizing activity of stathmin. Phosphorylation on two serine residues (Ser 16 and 63) significantly reduces tubulin binding and inhibits the ability of stathmin to destabilize microtubules *in vitro* (Di Paolo et al., 1997a; Holmfeldt et al., 2001). This effect is further increased by multiple phosphorylation.

Is SCG10 also inactivated by phosphorylation? To answer this question, it was necessary to know the phosphorylation sites. Mass spectrometry analysis of *in vitro* phosphorylated recombinant SCG10 identified four phosphorylation sites (Antonsson et al., 1998). SCG10 is phosphorylated by protein kinase A (PKA) at Ser50 and Ser97, by three subclasses of mitogen-activated protein kinase (MAP kinase) at Ser62 and Ser73, and by cyclin-dependent kinases (CDKs p34cdc2 and CDK5) at Ser73 (Fig. 3). The effect of phosphorylation at these sites was addressed by expressing mutants in which the serine residues were replaced by phosphorylation mimicking aspartate residues in non-neuronal cells (Antonsson et al., 1998). Analysis of the microtubule network in the transfected cells shows that the microtubule destabilizing activity of SCG10 is efficiently regulated by phosphorylation. Quantification of the different phosphorylation combinations indicates that the relative importance of each site varies but that the activity decreases with increased phosphorylation (Fig. 3).

The identified kinases are good candidates to regulate SCG10 and stathmin *in vivo*. PKA, MAPK, and CDK5 are highly expressed in neurons and are also present in growth cones (Morishima-Kawashima and Kosik, 1996; Pigino et al., 1997). Many studies implicate in particular MAP kinases in neuronal differentiation (see Fukunaga and Miyamoto, 1998 for review). Moreover, both SCG10 and stathmin are found to be phosphorylated on all four serine residues in developing brain (Chneiweiss et al., 1989; Antonsson et al., 1998 and unpublished results). These observations indicate that SCG10 and stathmin may link cell signaling mechanisms to changes in microtubule dynamics.

ROLE OF SCG10 AND STATHMIN IN NEURITE OUTGROWTH

The dynamic state of growth cone microtubules has been shown to be important for neurite elongation and growth cone tuning. It is not clear, however, how dynamics are regulated by cellular factors. Most efforts have focused on MAPs that have a strong stabilizing activity. Some of the MAPs, like MAP1b and tau, are enriched in growth cones, but nevertheless, the microtubules are particularly labile in this region. Given the potent destabilizing activity of SCG10 and its localization in the C-domain of the growth cone, it might provide an antagonistic influence to MAPs and thus contribute to the regulation of microtubule dynamics in the growth cone.

Direct evidence that SCG10 plays a role in neurite outgrowth comes from experiments in PC12 cells stably transfected with SCG10 (Riederer et al., 1997). These lines, which overexpress SCG10 only about 10-fold in the nondifferentiated state, show strongly enhanced neurite outgrowth in the response to NGF. Not only is the number of cells with neurites higher, but also a remarkable increase in the lengths of the processes can be observed. This overexpression of SCG10 does not disrupt the microtubules as is observed in non-neuronal cells following strong overexpression [as seen in Fig. 2(C)]. Instead, it is consistent with acting on the catastrophe frequency. Increased microtubule instability provided by higher levels of SCG10 apparently enhances neurite elongation.

Stable PC12 cell lines that overexpress stathmin could not be generated (unpublished results), probably because these lines were not viable. In a different approach, stathmin function in PC12 cells was blocked by antisense oligonucleotides (Di Paolo et al., 1996). Antisense treated cells are apparently unable to respond to NGF. Morphological changes such as cell body flattening and extension of processes do not occur; instead the cells continue to proliferate. As a target of the MAP kinase pathway of NGF signaling, stathmin probably plays a role in the differentiation pathway. Given its high expression in developing and regenerating axons, it is likely that stathmin also plays a role during neurite elongation. However, the generation of knockout mice by Schubart and his colleagues (1996) gave no clues about its function during brain development. The stathmin null mice develop normally. Only aged animals show an obvious phenotype. They develop an axonopathy of the central and peripheral nervous systems, suggesting that stathmin plays a role in the maintenance of axonal integrity (Liedtke et al., 2002).

SCG10 AND STATHMIN PROTEIN INTERACTIONS

In addition to the interaction with tubulin, the yeast two-hybrid as well as biochemical approaches isolated several molecules that interact with stathmin or SCG10. Sobel and colleagues have identified a putative RNA binding kinase termed KIS (Maucuer et al., 1995) and members of the heat shock protein family Hsp70 (Maucuer et al., 1995; Manceau et al., 1999) as stathmin-interacting proteins. How these proteins regulate stathmin function is not known yet. The serine-threonine-directed protein kinase c-Jun N-terminal kinase-3 (JNK3), also termed stress-activated protein kinase-β, phosphorylates and binds SCG10. Although two other subclasses of MAP kinases, ERK and p38α,

phosphorylate SCG10, only JNK3 specifically and tightly binds SCG10. Phosphorylation occurs at Ser62 and Ser73, residues that result in reduced microtubule-destabilizing activity of SCG10. In sympathetic neurons in culture deprived from nerve growth factor, SCG10 also undergoes increased phosphorylation at times of JNK3 activation. Activation of JNK thus provides a pathway for phosphorylation of SCG10 and possibly for controlling growth cone microtubule formation following neuronal exposure to cellular stresses (Neidhart et al., 2001).

More recent studies point to a novel connection between G protein signaling and the regulation of the microtubule cytoskeleton. SCG10 was found to bind to RGSZ1, a member of the diverse family of RGS proteins (regulators of G protein signaling). These proteins play a major role in negatively regulating heterotrimeric G protein signal transduction. RGSZ1 is specifically expressed in the brain (Glick et al., 1998), and it interacts in biochemical assays, as well as in PC12 cells treated with nerve growth factor, with SCG10 (Nixon et al., 2002). RGSZ1 blocks the ability of SCG10 to induce microtubule depolymerization *in vitro*. Therefore, it is likely that the binding of RGSZ1 to SCG10 might offset the destabilizing effect of SCG10 in cells and promote stability of microtubules. One may speculate that this interaction could be important under conditions when microtubules need to be stabilized, for example during microtubule reorganization in a growth cone responding to an extrinsic guidance cue. Liu et al. (2002) also reported an interaction between an RGS protein (RGS6) and SCG10. RGS6, a member of a different RGS protein subfamily, contains a so-called GGL domain that is involved in this interaction. RGS6 seems to have the opposite effect on SCG10 activity than RGSZ1. They found that overexpression of both proteins in cells potentiates the microtubule disrupting activity of SCG10. Although SCG10's interactions with RGS proteins suggest a novel mechanism for the regulation of the microtubule cytoskeleton, there is still much to learn about how these proteins interact in neurons, which signals stimulate their interaction, and what are the functional consequences of their interactions in terms of microtubule organization, cellular responses, and neuronal development.

CONCLUSIONS AND FUTURE PROSPECTS

The correlation between neurite outgrowth and stathmin expression in several systems provides compelling evidence that stathmin is important in neurite outgrowth in development and regeneration. The lack of developmental abnormalities in the stathmin knockout mice developed by Schubart was therefore surprising. A possible explanation could be that SCG10 and/or other members of this gene family might be sufficient to provide the necessary plasticity of the microtubule cytoskeleton during development. SCG10 is concomitantly expressed with stathmin at high levels in developing neurons, and its membrane association is regulated by a dynamic type of acetylation. It is therefore conceivable that a soluble form of SCG10 could replace stathmin with respect to microtubule-directed activities.

The role of SCG10 appears more defined because of excellent studies that have addressed the behavior of growth cone microtubules (see Tanaka and Sabry, 1995; Letourneau, 1996 for review). Our data implicate this molecule as a regulator of microtubule dynamics in growth cones. SCG10 is specifically targeted to the growth cone where it accumulates in the C-domain. The presence of a potent microtubule destabilizer in this particular region provides an explanation for the dynamic behavior of growth cone microtubules. Whether by direct action on microtubule plus ends or by sequestering soluble tubulin, or both, SCG10 is able to promote dynamic instability. Because the activity of SCG10 is inhibited by phosphorylation, SCG10 may link extracellular signals to changes in microtubule dynamics. The presence of MAPs, such as MAP1B or tau, in growth cones suggests that counteracting activities of SCG10 and MAPs are probably required for the proper regulation of microtubules. Figure 4 summarizes a hypothetical model of the regulation of microtubule dynamics in the growth cone. Supported by experimental data (Riederer et al., 1997 and unpublished results), we can conclude that during NGF promoted neurite outgrowth, SCG10 is present mainly in its nonphosphorylated active form and that higher levels of SCG10 lead to increased microtubule dynamics and thus enhanced axonal elongation. We speculate that following inactivation of SCG10 by phosphorylation, or possibly by other mechanisms, the microtubules are prominently exposed to the activity of MAPs. This would lead to increased microtubule polymerization and stability, and consequently to decreased axonal elongation. Indeed, pharmacological treatment with drugs that promote microtubule polymerization or prevent dynamic instability inhibits axonal elongation (Letourneau and Ressler, 1984; Rochlin et al., 1996). Dynamic instability of microtubules is also essential for growth cone turning (Williamson et al., 1996; Challacombe et al., 1997). Thus it is possible that antagonistic influences of SCG10 and MAPs also

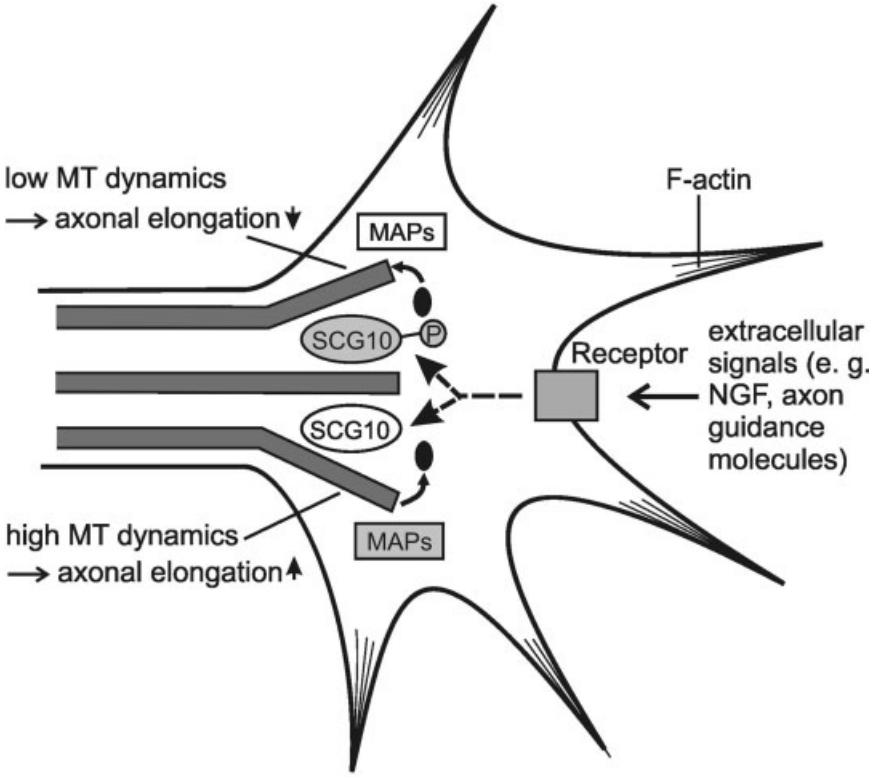

Figure 4 Hypothetical model illustrating the regulation of microtubule dynamics in growth cones by antagonistic influences of stabilizing and destabilizing factors in response to extracellular signals. When SCG10 is in its nonphosphorylated active form (white background) it stimulates catastrophes and thus provides highly dynamic microtubules. It excels the stabilizing effect of MAPs, which themselves might be in a less active form (gray background). Highly dynamic microtubules promote axonal elongation. In response to extracellular signals, SCG10 becomes phosphorylated and inactivated (gray background). MAPs in their active form (white background) can then stabilize microtubules, which then will lead to decreased microtubule dynamics and consequently to decreased axonal elongation. ⬬ = tubulin dimers.

provide a mechanism for the regulation of the microtubule cytoskeleton during growth cone turning. Extracellular guidance cues might locally change the balance between stabilizing and destabilizing factors in a growth cone undergoing turning.

Future studies we will be directed to test this hypothesis. We have developed phosphorylation site specific antibodies that should reveal whether SCG10 is locally phosphorylated in the growth cone. Introduction of SCG10 into neurons and high-resolution imaging might give insight into how it modulates microtubule behavior in living growth cones. The identification of axon guidance molecules that alter the function of SCG10 will be important to describe pathways by which environmental cues signal the cytoskeleton. A future task to better understand the role played by microtubule destabilizers in the living organism will be the analysis of mice mutant in the SCG10 gene.

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