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·Review·

Microtubule dynamics in axon guidance

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Precise modulation of the cytoskeleton is involved in a variety of cellular processes including cell division, migration, polarity, and adhesion. In developing post-mitotic neurons, extracellular guidance cues not only trigger signaling cascades that act at a distance to indirectly regulate microtubule distribution, and assembly and disassembly in the growth cone, but also directly modulate microtubule stability and dynamics through coupling of guidance receptors with microtubules to control growth-cone turning. Microtubule-associated proteins including classical microtubule-associated proteins and microtubule plus-end tracking proteins are required for modulating microtubule dynamics to influence growth-cone steering. Multiple key signaling components, such as calcium, small GTPases, glycogen synthase kinase-3β, and c-Jun N-terminal kinase, link upstream signal cascades to microtubule stability and dynamics in the growth cone to control axon outgrowth and projection. Understanding the functions and regulation of microtubule dynamics in the growth cone provides new insights into the molecular mechanisms of axon guidance.

Keywords: axon guidance; growth cone; microtubule dynamics; signal transduction

Introduction

In the developing nervous system, proper axon outgrowth and pathfinding are essential for neurons to reach their final destination and establish precise neuronal circuits. Extracellular guidance signals including guidance cues, growth factors, and cell adhesion molecules, are responsible for directing the navigation of the growth cone (GC) of an extending axon through the modulation of cytoskeleton dynamics including filamentous (F) actin and microtubules (MTs), fundamental cytoskeleton components of GC motility[1-7]. Research in the past two decades has gained significant knowledge of the functional importance of actin dynamics in axon guidance, which has been the focus of several excellent reviews[3-6]. Here, we review recent studies examining direct modulation of MT dynamics in axon outgrowth and guidance.

MTs in the GC

The GC is the specialized, highly-motile tip of an extending axon, probing extracellular guidance signals and leading axon projection along specific pathways in the developing nervous system[1-3]. The GC has two general regions: the central (C) and peripheral (P) regions, and forms two kinds of protrusions: filopodia, finger-like projections, and lamellipodia, flat sheet-like protrusions[3-5, 8] (Fig. 1). These regions and protrusions of the GC are dynamic and persistently undergo shape changes *in vivo*, depending on both actin and MT dynamics in the GC. MTs are polarized hollow polymers of tubulins assembled by the lateral interaction of 11–15 protofilaments, in which α/β tubulin heterodimers hold together in a head-to-tail fashion. In general, MTs are bundled together in the axon shaft, whereas some are defasciculated crossing the C region of the GC as single MTs[9] (Fig. 1). In the C region of the GC, MTs may be relatively straight or form prominent loops, while some occasionally invade the P region as well as filopodia[9]. Individual MTs in the GC are pioneered by their plus ends, the fast-growing ends that favor polymerization compared to the minus ends[5]. MTs in the

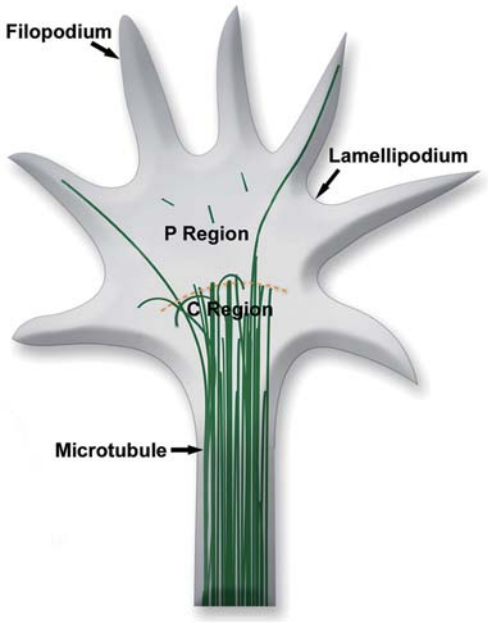

Fig. 1. Organization of microtubules in the growth cone (GC). The GC, an expanded tip of the axon, includes two regions: the C and P regions (delineated by an orange dotted line) with veil-like lamellipodia and finger-like filopodia. The P region of the GC contains unpolymerized tubulins and 'pioneer polymerized MTs', while the C region consists of stable, bundled MTs. MTs are shown in green.

GC spontaneously switch between phases of growth and shortening, a behavior termed dynamic instability, which may function as a direct sensor to control GC steering[10].

Role of α- and β-Tubulin in Axon Guidance

The importance of tubulin isotypes in axon guidance has emerged from recent discoveries of patients carrying mutations in genes encoding α- and β-tubulin (e.g. *TUBA1A*, *TUBB2B*, *TUBB3*, and *TUBA8*)[11-14]. In addition to classic lissencephaly and hypoplasia of the hippocampus, cerebellum, and brainstem, brain malformations in patients harboring *TUBA1A* mutations include partial or complete absence of the corpus callosum and commissural fiber tracts, as well as hypoplasia of the internal capsule and corticospinal tract associated with dysmorphic basal ganglia[15, 16]. Mutations of *TUBB2B or TUBA1A* are associated with both lissencephaly and polymicrogyria[16, 17] which have in common axon-guidance defects including partial or complete agenesis of the corpus callosum and the internal capsule[12, 16]. Patients with homozygous deletions in *TUBA8* have extensive polymicrogyria, callosal anomalies, and optic nerve hypoplasia[18]. Missense mutations in *TUBB3*, encoding the neuron-specific β-tubulin isotype III, result in various neurological disorders, such as ocular motility disorder, congenital fibrosis of the extraocular muscle type 3, facial paralysis, intellectual and behavioral impairments, and axonal sensorimotor polyneuropathy[13, 14]. Fetopsy and imaging studies have demonstrated that *TUBB3* mutations cause a spectrum of axonal-projection defects such as agenesis or hypoplasia of the commissural axon tracts, the corticospinal tract, the anterior commissure, and oculomotor nerves[13, 14]. The *TUBB3*$^{R262C/R262C}$ knock-in mouse model reveals axon-guidance defects in commissural axons and cranial nerves[14], in which the anterior commissure is thinned and/or absent and the corpus callosum is composed of stalled commissural axons adjacent to the midline compared to the wild-type mouse[14]. In addition, knockdown of TUBB3 inhibits spinal cord commissural axon outgrowth and causes their misguidance, suggesting that TUBB3 is specifically involved in commissural axon projection[19]. All tubulin isotype mutations (e.g. *TUBA1A*, *TUBB2B*, *TUBB3*, and *TUBA8*) commonly cause a generalized defect in axon guidance (Table 1), indicating that MTs play an essential role in controlling axon outgrowth and projection during brain development. The disease-associated tubulin isotype mutations impair tubulin heterodimer formation and alter MT instability[11, 13, 14], further suggesting that modulation of MT dynamics is required for proper axon guidance.

Modulation of MT Dynamics during GC Steering

Although the major function of MTs has been thought to be to consolidate and provide mechanical support to GC steering initiated by actin dynamics, an increasing number of studies suggest that they play an essential and instructive role in GC behavior[4, 30-33]. For example, dynamic MTs are oriented and stabilized preferentially in the direction of the GC turn, and distal dynamic MT ends in the P region of the GC are required for GC repulsion at substrate borders[32-34]. During adhesion-mediated GC steering of *Aplysia* bag-cell neurons, dynamic MTs in the P region explore adhesion sites prior to changes

Table 1. Summary of tubulin-related deficits in axon guidance and brain development

Tubulin isotype	TUBA1A	TUBB2B	TUBB3	TUBA8
Number of reported mutations	32[15, 20-27]	15[12, 16, 17, 27-29]	14[13, 14]	1[18]
Mutation site(s)	p.I5L, E55K, L70S, L92V, V137D, Y161H, I188L, Y210C, D218Y, V235L, I238V, P263T, R264C, A270T, L286F, V303G, N329S, A333V, G366R, M377V, A387V, R390C, R390H, L397P, R402L, R402C, R402H, S419L, R422C, R422H, M425K, G436R	p. G98R, L117P, G140A, S172P, L207P, I210T, L228P, N256S, F265L, T312M, R380S, R380C, D417N, E421K, c.1080-1084 deletion	p. R62Q, G82R, T178M, E205K, R262C, R262H, A302V, A302T, M323V, R380C, M388V, E410K, D417H, D417N	14-bp deletion in intron 1
Cortex	Lissencephaly, pachygyria, and/or PMG	Lissencephaly, pachygyria, and/or PMG	MCD (microgyria, gyral disorganization and PMG)	Lissencephaly and PMG
Basal ganglia	Dysmorphisms and hypoplasia	Dysmorphisms	Dysmorphisms	N/A
Corpus callosum	Dysgenesis, Probst bundles (bundles of stalled axons)	Dysgenesis and dysmorphisms	Dysgenesis, Probst bundles adjacent to the midline	Dysgenesis
Anterior commissure	Hypoplasia in 1 patient, N/A in most cases	Hypoplasia in 1 patient, N/A in most cases	Dysgenesis, tortuous and aberrant axon projections at the midline in a knock-in mouse model	N/A
Internal capsule	Hypoplasia	Hypoplasia	Dysgenesis	N/A
Corticospinal tracts	Hypoplasia	N/A	Dysgenesis	N/A
Cerebellum	Hypoplasia	Hypoplasia	Hypoplasia	N/A
Brainstem	Hypoplasia	Dysmorphisms and hypoplasia	Dysmorphisms	Dysmorphisms
Cranial nerves	Hypoplasia in II	Hypoplasia in II and III	Hypoplasia of I, III, IV, VI, VII and X, axon projection defects of IV and V in a knock-in mouse model	Hypoplasia in II
Guidance signaling	N/A	N/A	Netrin/DCC	N/A

MCD, malformations of cortical development; N/A, not available; PMG, polymicrogyria.

in GC behavior and retrograde actin flow[35]. Laminin/integrin signaling promotes directional MT assembly and stabilization in axon development[36]. Combination of L1, laminin, and EphB alters the MT organization and distribution in paused retinal GCs, with increased numbers of MTs that extend into the P region of the GC and filopodia[7]. Disruption of MT dynamics in the GC by MT-stabilizing or destabilizing drugs completely abolishes both the GC attraction and repulsion induced by diffusible cues, such as Netrin-1[30]. The local stabilization of MTs in one side of the GC, using the focal pipette application approach or direct focal photoactivated release of the MT-stabilizing drug taxol, induces GC attraction toward the side of application[37]. Disruption of MT stabilization on one side of

a GC, using the MT-disrupting drug nocodazole, is sufficient to induce GC repulsion (turning away from that side)[30]. Intriguingly, the application of low concentrations of taxol promotes MT polymerization at plus ends and enhances axon outgrowth *in vitro* and *in vivo via* MT stabilization[38]. These studies suggest that intrinsically polarized MT dynamics in the GC may initiate and instruct the axon projection in response to extracellular guidance cues. Bath incubation with Semaphorin 3A (Sema3A) decreases MT exploratory behaviors in the GC and collapses MTs into the MT loop, whereas Netrin-1 causes opposite changes in MTs, increasing their splaying in the GC and axon shaft[31]. Wnt3a rapidly reduces the rate of axonal extension and subsequently increases GC enlargement and pausing *in vitro* through changes in the organization of MTs[39]. Time-lapse imaging reveals that Wnt3a regulates MT directionality and increases MT looping in the remodeled GC[39]. Thus, guidance cue-mediated GC navigation occurs in a MT dynamics-dependent manner.

However, whether MT dynamics are directly or indirectly regulated by guidance cues is still unclear. A recent study from our lab suggests that Netrin-1 directly regulates MT dynamics through the coupling of its receptor deleted in colorectal cancer (DCC) to TUBB3 during axon attraction[19]. TUBB3 co-localizes with DCC in the P region of developing spinal cord commissural and cortical neuron GCs, including both lamellipodia and filopodia[19]. Biochemical assays indicate that TUBB3 interacts directly with DCC and that Netrin-1 induces this interaction in primary neurons[19]. The Netrin-1-induced interaction of TUBB3 with DCC is dependent on MT dynamics because the disruption of MT dynamics either with taxol or nocodazole abolishes this interaction[19]. Results from an MT co-sedimentation assay demonstrate that Netrin-1 induces MT polymerization in dissociated neurons with more polymerized TUBB3 in the pellet *versus* the supernatant fraction, suggesting that Netrin-1 directly modulates MT dynamics[19]. Remarkably, DCC co-sediments with polymerized MTs and Netrin-1 further increases the co-sedimentation of DCC with stabilized MTs[19]. In addition, TUBB3 knockdown blocks both Netrin-1-induced spinal commissural axon outgrowth and attraction *in vitro* and causes defects in commissural axon projection *in vivo*[19], suggesting that TUBB3 is specifically involved in Netrin-1-promoted attraction. These results lead to a simple functional model that Netrin-1 signaling directly regulates MT dynamics through the coupling of its receptor DCC to TUBB3 (Fig. 2). Netrin-1-dependent initial local stabilization of MTs within the DCC complex on one side of the GC could lead to a differential increase in MT growth and a higher number of MT plus-ends on this side, which might influence actin dynamics and enable MTs to differentially protrude into this side of the GC and further promote GC protrusion on that side. At the same time, lamellipodia and filopodia on the other site of the GC collapse and the GC eventually turns towards the Netrin-1 source. In this model, in response to Netrin-1, the 'capture' of dynamic MTs by DCC in the GC is a critical step, which could stabilize filopodia against retraction and promote axon outgrowth and turning (Fig. 2). Interestingly, Src family kinase-dependent TUBB3 phosphorylation appears to be required for the subsequent interaction of TUBB3 with DCC and modulation of MT dynamics, suggesting that DCC serves as a signaling platform for the recruitment of a multiprotein complex, including TUBB3, Src family kinases, and other key signaling molecules to modulate MT dynamics in Netrin-1-induced axon outgrowth and turning[19].

Intriguingly, TUBB3 missense mutations lead to specific axon projection defects in commissural axon midline crossing (the corpus callosum and anterior commissure), considering it is widely expressed in the developing brain. The role of TUBB3 in Netrin-1 signaling fits well, albeit not exclusively, in the dysgenesis and organization of these axonal tracts in patients with TUBB3 mutations. Future studies are required to determine how TUBB3 mutations affect Netrin-1-mediated MT dynamics and axon guidance in the developing nervous system. In addition to Netrin functions, other guidance cues and cell-adhesion molecules are implicated in commissural axon guidance, such as bone morphogenetic proteins[40, 41], sonic hedgehog[42], Slits[43], Wnts[44, 45], Draxin[46, 47], axonin-1, and NrCAM[48]. It would be interesting to determine whether TUBB3 is involved in signal transduction cascades downstream of these guidance molecules. Since mutations of TUBB2B, TUBA1A, and TUBA8 share similar commissural axon projection defects in midline crossing, it is plausible that these tubulin isotypes also play a differential role in the aforementioned guidance signaling.

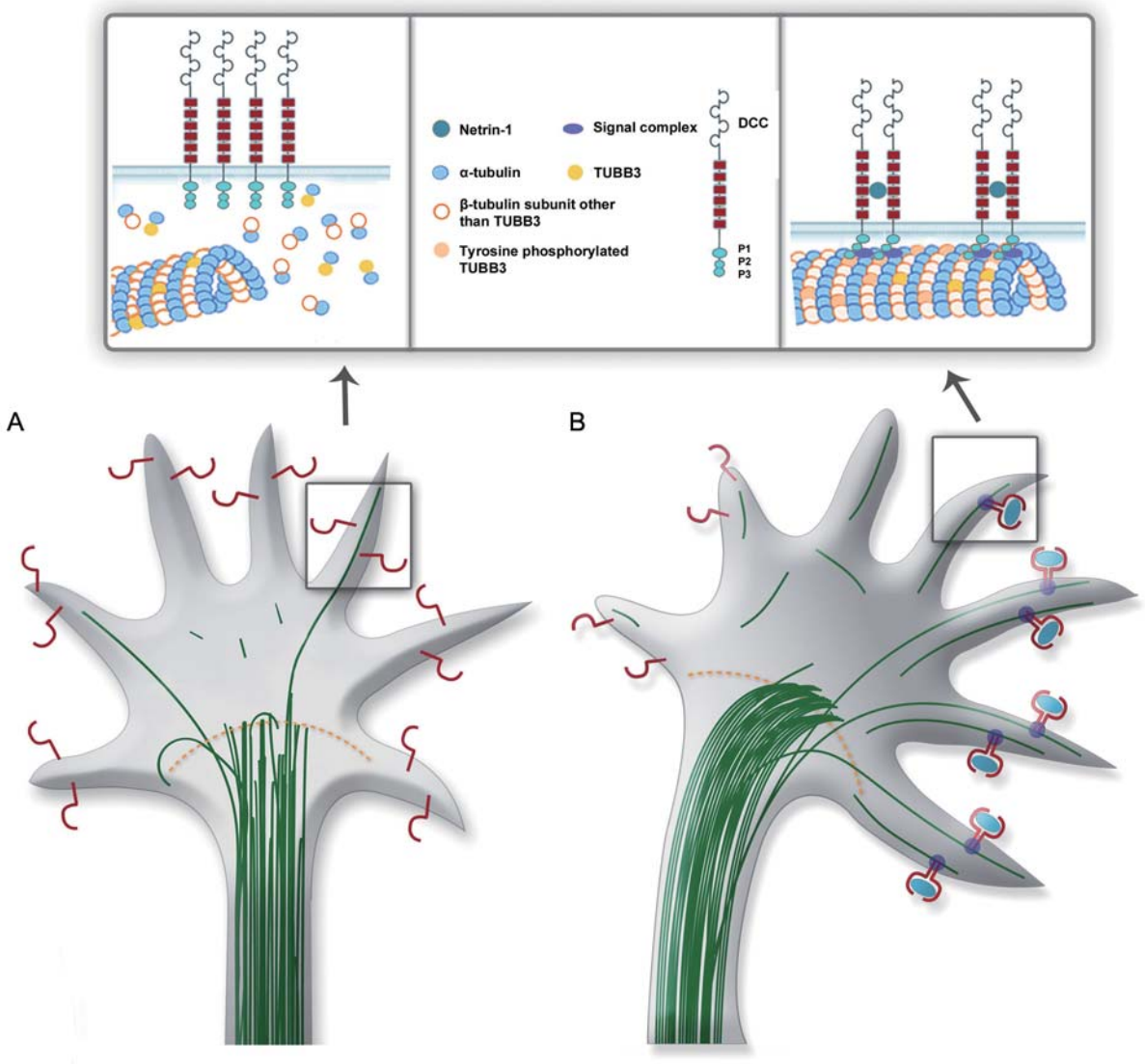

Fig. 2. Generalized model of direct involvement of MT dynamics in Netrin-1-promoted GC turning. Monomer DCC is evenly present on the GC (A) with unpolymerized tubulins in the P region (top left inset) in the absence of Netrin-1. Binding of Netrin-1 to DCC results in DCC homodimerization (B) and the recruitment of TUBB3, Src family kinases, and other key signaling molecules to form a 'molecular clutch' on the side of the GC close to the Netrin-1 gradient (top right inset). Netrin-1-induced MT polymerization/ stabilization occurs in the clutch site to polarize the GC and further maneuver GC steering (B). Actin dynamics in the GC is not included in this simplified model.

Microtubule-Associated Proteins (MAPs) in Axon Guidance

MT dynamics is regulated by various MAPs including the classical MAPs, which bind MTs along their entire length, and MT plus-end tracking proteins (+TIPs), which localize to the ends of growing MTs[3-5, 49]. MAP1B, an MT-stabilizing MAP, is highly expressed in the developing nervous system[50] and plays an important role in axon outgrowth and pathfinding[3, 4, 49]. Homozygous *MAP1B* mutants display a striking axon projection defect in the brain, the selective absence of the corpus callosum[51]. A follow-up study using microscale chromophore-assisted laser inactivation revealed that MAP1B is directly involved

in GC turning, suggesting that this is an axon guidance defect[52]. MAP1B knockdown by RNA interference in cultured cortical neurons alters the speed of MT growth in axons, resulting in axon outgrowth inhibition[53]. MAP1B binds mainly to dynamic MTs and promotes MT nucleation, polymerization, and stabilization *in vitro* and *in vivo*[54, 55]. In developing neurons, MAP1B is prominently located at the distal part of an extending axon and the GC, where the proportion of dynamic MTs is very high[56]. Primary neurons from hypomorphous *MAP1B* mutant mice present a reduced proportion of dynamic MTs in the distal part of the axon[57]. Phosphorylated isoforms of MAP1B are present at the highest concentrations in the distal axon and the GC of chick retinal ganglion cells and phospho-MAP1B inactivation on one side of the GC changes GC motility, morphology, and growth direction[52]. These results indicate that MAP1B involvement in GC steering relies on its function as a regulator of MT stability and dynamics.

Interestingly, the lack of MAP1B *in vivo* leads to dramatic abnormalities in the pontine nuclei and major axonal tracts such as the corpus callosum, the hippocampal commissure, the anterior commissure, and the reciprocal corticothalamic pathway. Most of these deficits are similar to the phenotypes of *Netrin-1*- and *DCC*-deficient mice[58-61], suggesting that MAP1B plays a role in Netrin-1-mediated axonal guidance. In addition, Netrin-1 stimulation of hippocampal and dorsal spinal cord explants from *MAP1B*-null embryos fails to induce axon outgrowth and attraction[61]. Netrin-1-directed axon outgrowth of developing neurons requires MAP1B phosphorylation through the activation of serine/threonine kinases cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 (GSK3)[61]. Thus, it is likely that MAP1B plays an essential role in promoting MT dynamics preferentially on one side of the GC during Netrin-1-mediated chemoattractive turning. We propose that, in the developing GC, asymmetrical assembly of a signaling complex including DCC, TUBB3, Src family kinases, and MAP1B in response to a Netrin-1 gradient leads to a polarized increase in MT growth and stability on one side of the GC, which in turn promotes GC protrusion on that side and eventually controls GC turning towards the Netrin-1 source. While the defects in some axonal tracts, such as the anterior commissure and the entorhinal-hippocampal pathway, are almost identical to those in *Netrin-1*- and *DCC*-mouse mutants, other connections such as the reciprocal corticothalamic projections are much more severely affected in *MAP1B* mutants than in *Netrin-1*- and *DCC*-mutants[59], suggesting that MAP1B is involved in several signaling cascades governing axon projections in the developing nervous system. Consistent with this, canonical Wnt signaling modulates MT dynamics through a Dishevelled-dependent inhibition of GSK3β with the consequent regulation of MAP1B phosphorylation[62, 63].

Other classic MAPs, such as collapsin response mediator proteins (CRMPs), MAP2, and Tau, are also involved in axon guidance and neurite outgrowth. For example, CRMPs, cytosolic phosphoproteins highly expressed in developing neurons, are involved in the regulation of MT dynamics and axon outgrowth[64]. CRMP1–5 associate with tubulin[65, 66] and CRMP2 promotes axon growth through direct binding to tubulin and modulating MT dynamics[65, 67]. Hyperphosphorylation of CRMP2 disrupts MT assembly in neurites and is implicated in Alzheimer's disease[68]. CRMP2 regulates the transport of soluble tubulin to the distal parts of growing axons through binding to the kinesin-1 light chain[69]. In addition, CRMP2 is required for Sema3A-mediated repulsive signaling *via* the induction of GC collapse[68, 70]. Interestingly, CRMP5 forms a ternary complex with MAP2 and tubulins (α- and β-tubulin) which antagonizes CRMP2-induced axon outgrowth through a tubulin-based mechanism[66]. Thus, the interaction of CRMP5 with tubulin and MAP2 inhibits the tubulin polymerization and neurite outgrowth induced by CRMP2[66]. While MAP2 is involved in regulating neurite outgrowth through the association with MTs and other cytoskeleton elements[71], the role of MAP2 in axon guidance is unclear.

+TIPs bind to the rapidly-growing (+) ends of dynamic MTs, which are concentrated in the P region of the GC, and form comet-like assemblies along the ends of polymerizing MTs[3-5, 9, 72]. Many of these +TIPs are involved in a wide range of guidance signaling. For example, the cytoplasmic linker protein-associated protein CIs/Orbit/MAST/CLASP promotes MT rescue (the change from MT depolymerization to polymerization) and stabilization in the GC and is one of the first +TIP proteins implicated in Slit/Robo-mediated axon guidance *via* the non-receptor tyrosine kinase Abl[73, 74]. In the GC, CLASP associates with

both MT plus-ends and MT lattices with opposite functions: the plus-end-binding activity promotes axon outgrowth *via* MT stabilization, whereas the lattice-binding activity inhibits GC navigation *via* suppression of GSK3 activity[75]. The adenomatous polyposis coli (APC) protein, another +TIP, is a well-characterized signaling molecule that mediates the canonical Wnt/β-catenin signaling pathway. APC is highly expressed in the developing brain and concentrated in the GCs of dissociated neurons, where it binds to a subset of MTs to direct GC steering[76]. Local disruption of the interaction of APC and extending MTs abolishes GC turning behavior, including both attraction and repulsion[76]. Wnt3a increases APC loss from MT-plus ends and induces MT looping in the GC, resulting in a guidance defect[39]. Although APC2, the second APC family member, does not contain the APC domain associated with MT or +TIP binding, it is preferentially expressed in the nervous system and has been shown to stabilize MTs and play a role in the ephrin-A2-mediated guidance of retinotectal neurons[77]. APC associates with plasma membranes[78], so it is plausible that APC interacts with guidance receptors in GCs to regulate MT dynamics and stability in axon guidance.

Most +TIP proteins associate with end-binding (EB) proteins and require these 'core' +TIPs for plus-end tracking on growing MTs[49]. There are three EB proteins, EB1, EB2, and EB3. EB1 was first identified as an APC-binding protein and is required for the recruitment of APC to MT plus-ends[79]. In neuroblastoma cells, EB1 is localized to MT plus ends in neurites and GCs and plays an essential role in determining neurite length by regulating MT growth rate, growth distance, and duration[80]. Similarly, EB3 is preferentially expressed in brain, particularly in neuronal GCs, and involved in neuritogenesis *via* the coordination of dynamic F-actin-MT interactions[81]. Interestingly, MAP1B sequesters EB1 and EB3 in the cytosol of developing neurons through direct interactions, which do not require MT integrity[82]. The binding of MAP1B to EB3 is regulated by phosphorylation mediated by proline-directed kinases such as GSK-3 and CDK5, but not non-proline-directed kinase casein kinase 2 (CK2)[82]. Overexpression of MAP1B in N1E-115 cells inhibits EB protein binding to MT plus-ends, whereas MAP1B knockdown increases EB binding to MT growing-ends and to the MT lattice[82]. The interaction of EB3 with MTs is also enhanced in the GCs of primary MAP1B-knockdown neurons[82]. The excessive EB3 binding to MTs and induction of MT looping in the GC of *MAP1B*-deficient neurons likely lead to changes in MT dynamics, in particular overstabilization, which impairs GC navigation and affects axon outgrowth[82]. Therefore, too much or too little MAP1B disrupts EB protein-dependent MT growth and stability in the GC and further blocks axon projection. These results suggest that MAP1B functions as a direct regulator of EBs to modulate MT dynamics during neurite and axon extension. Although these studies have shown that +TIPs are essential for the regulation of axon outgrowth from developing neurons, results from a yeast two-hybrid screen and a GST pull-down assay reveal that all three EB protein members interact with plexin-A2, B1, and B3[83] and these interactions play an important role in regulating neurite growth of Neuro 2A cells[83], suggesting that they play a role in ephrin/Eph-mediated axon projection. Further studies are required to determine whether other guidance receptors also associate with EBs and whether the functional importance of +TIPs in GC turning is mediated by multiple guidance cues.

MT-regulating kinesins belong to the unconventional kinesin family which modulates MT assembly and/or disassembly rather than functioning as a molecular cargo transporter. These regulatory kinesins act as either MT elongases, pause factors, or depolymerases to regulate MT organization and dynamics[84]. Non-motile kinesin-13 family members, such as KIF2A, KIF2B and KIF2C/MCAK (mitotic centromere-associated kinesin), can also identify and stabilize curved protofilaments at MT ends to promote MT depolymerization[85]. KIF2A is highly expressed in developing neurons and *KIF2A*-knockout mice exhibit neuronal migration defects, abnormally-elongated collateral branches of axons, and severe sensory axon target hyperinnervation[86, 87], suggesting that KIF2s play an important role in axonal branching and pruning during brain development. Kinesin-4 KIF21A, a cortical MT growth inhibitor, strongly accumulates in the axonal GC[88]. Heterozygous missense mutations in *KIF21A* cause CFEOM1, a dominant neurodevelopmental disorder associated with axon-guidance defects[88, 89]. Expression of wild-type or mutant KIF21A in primary neurons increases the accumulation of KIF21A in the GC, and reduces the proportion of the GC with a fan-like morphology and

GC motility, as well as suppressing the responsiveness to Sema3F, a repulsive guidance cue[88]. Although the formation of shorter and branched axons induced by increased KIF21A levels is believed to cause alterations in axonal target innervation, the exact signaling mechanism underlying KIF21A-mediated Sema3F repulsion remains elusive.

Signaling Pathways That Regulate MT Dynamics in Axon Outgrowth and Guidance

Intracellular signal transduction pathways initiated by different guidance cues likely engage in cooperative crosstalk during axon guidance, which eventually converges on the modulation of MT stability and actin dynamics. Several key regulators appear to regulate MT dynamics directly in GCs[39, 73, 90]. For example, GSK3 is involved in multiple guidance pathways including the Wnt, Netrin-1[61], Sema3A[91], Slit2[92], and neurotrophin pathways, and it is known to regulate MT dynamics and assembly by phosphorylating multiple MAPs including APC, MAP1B, CRMPs, CLASPs, Tau, MAP2, and stathmins[4, 93, 94]. In general, inhibition of GSK3β-dependent phosphorylation of these MAPs directly modulates MT behavior which affects axon outgrowth and guidance. In the axonal GC, GSK3β-mediated MAP1B phosphorylation is required for maintaining MTs in a dynamic state and axon outgrowth and pathfinding[95]. Netrin-1 regulates mode I MAP1B phosphorylation and MAP1B activity through GSK3 and CDK5 both *in vivo* and *in vitro*[61]. MAP1B is required for Netrin-1-mediated chemoattraction *in vitro* and *in vivo*. Slit2 induces GSK3β phosphorylation and inhibits neurite outgrowth in adult dorsal root ganglion neurons[92]. In addition, the sequential phosphorylation of CRMP2 by CDK5 and GSK3β is necessary for Sema3A-induced GC collapse through MT reorganization[68, 70]. Inactivation of GSK3β by Wnts results in a significant decrease in the phosphorylation of MAP1B[63, 96], which leads to an increase in MT stability affecting axon outgrowth. The effects of Wnts on MT dynamics and GC behavior could be achieved through inhibition of phosphorylation of other MAPs induced by GSK3β[97]. Inactivation of GSK3β reduces CRMP2 phosphorylation, increasing its ability to bind tubulin and promoting MT assembly, whereas APC phosphorylation by GSK3β reduces the binding of APC to MT plus-ends[90, 98, 99]. Interestingly, NGF-induced axon growth is dependent on local inactivation of GSK3β at the distal axon, which results in the accumulation of dephosphorylated APC at MT plus ends and the promotion of MT assembly[90]. However, suppression of GSK3 activity to a greater extent inhibits axon growth in embryonic cortical neurons, suggesting that a precise balance of GSK3 activation and inactivation is required for efficient axon outgrowth in the developing nervous system[75, 100]. This dual function of GSK3 on axon growth is mediated by its physiological substrate CLASP2[75].

Mitogen-activated protein kinases (MAPKs) control the phosphorylation status and activity of several MAPs and are implicated in the regulation of axon outgrowth, guidance, and regeneration. c-Jun N-terminal kinases (JNKs) are strongly expressed in the developing nervous system and play an important role in axon outgrowth and guidance *in vitro* and *in vivo*[101, 102]. *JNK1*-deficient mice reveal defects in anterior commissure formation and axonal MT integrity[101]. JNK1 has recently been shown to play an essential role in Netrin-1-mediated axon outgrowth and attraction[102]. Activated JNK is strongly expressed in spinal cord commissural axons before and as they cross the floor plate[102]. Bath incubation with Netrin-1 dramatically increases the level of endogenous phospho-JNK in commissural axon GCs[102]. Netrin-1 increases JNK1 activity in the presence of DCC or Down syndrome cell adhesion molecule (DSCAM), two Netrin receptors, and the expression of both receptors further enhances Netrin-1-induced JNK1 activity[102]. Netrin-1-induced JNK1 activity is blocked by inactivation of the JNK pathway both *in vitro* and *in vivo*[102]. DCC collaborates with DSCAM to regulate JNK activity in Netrin signaling[102]. Netrin-1-induced axon outgrowth and attraction is inhibited either by JNK1 knockdown or a JNK inhibitor[102]. Expression of JNK1 shRNA *in ovo* causes defects in spinal cord commissural axon projection and pathfinding[102]. These studies indicate that JNK1 is specifically involved in the coordination of DCC and DSCAM in Netrin-1-mediated attractive signaling. Furthermore, JNK-deficient mice exhibit hypophosphorylation of MAP1B[101], suggesting that the JNK1 pathway is specifically involved in axon guidance *via* regulation of MAP-mediated MT dynamics in the GC.

Many JNK substrates are MAPs, such as doublecortin (DCX), superior cervical ganglion 10 (SCG10), and Tau, which control MT dynamics and stability in the GC. DCX, a MAP expressed in the developing nervous system, plays an important role in neuronal migration and axon guidance[103, 104]. DCX is enriched in axonal GCs[105] and stabilizes MTs in developing neurons[104, 106]. Although *DCX*-knockout mice display defects in axon tracts[104], there is no direct evidence to link DCX to specific guidance cues. Whether the effects of Netrin-1 on axon outgrowth and pathfinding might be mediated through the JNK-DCX-MT dynamic pathway needs further evaluation. SCG10, a MAP in axons, is also a JNK substrate and plays an important role in axonal outgrowth by modulating MT stability[107, 108]. In developing neurons, phospho-JNK1 and SCG10 are enriched in GCs[102, 109] and control the balance of MT assembly and disassembly *via* the sequestration of tubulin dimers or the severing of polymerized MTs[110, 111]. SCG10-mediated regulation of the GC MT cytoskeleton is also involved in EphB-mediated axon guidance[7]. Whether the JNK-SCG10 pathway is involved in Netrin signaling is not clear. Tau functions as a MAP and is differentially localized in the distal end of the axon[112]. Downregulation of Tau levels in neurons using antisense oligonucleotides inhibits axonal outgrowth[113]. Interestingly, *Tau*-deficient mice exhibit normal brain development[114, 115] due to possible compensatory increases in MAP1A[116]. Mice devoid of both *Tau* and *MAP1B* suffer premature death and manifest significant neuronal and axonal defects[117]. Tau stabilizes MTs through the regulation of tubulin-tubulin interactions along the protofilament[118] and promotes MT stability within the axon[118]. However, it remains unclear how Tau regulates MT dynamics in axon guidance. Tau actively interacts with various signaling partners, including Src-family kinases, phosphoinositides, and PLCγ[119]. Phosphorylation of Tau within its MT binding site at Ser262 by Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) is required for Wnt5a-mediated axon outgrowth and repulsion through modulation of dynamic MTs in the GC[120].

Localized cytosolic Ca^{2+} signals in the GC are known to mediate axon turning[121-125]. CaMKII and calcineurin (CaN) phosphatase, two frequency-dependent Ca^{2+} effectors, function as a switch to control the direction of GC steering: preferential activation of CaMKII by a relatively large local Ca^{2+} elevation promotes attraction, whereas activation of CaN by modest local Ca^{2+} levels induces repulsion[121]. CaMKII/CaN has been shown to mediate multiple guidance signaling, such as Netrin-1, Sema2a, and Wnts[126, 127], indicating that differential activation of CaMKII and CaN phosphatase is specifically involved in GC steering. It is well known that tubulin proteins and MAPs, such as MAP2 and Tau, can be phosphorylated and dephosphorylated by CaMKII and CaN-PP1, respectively, which affects MT assembly, stability, and dynamics in the GC[128-130]. Therefore, differential activation of the Ca^{2+}-dependent CaMKII/CaN pathway controls GC navigation depending on asymmetric local modification of MT stability and dynamics in the GC. Indeed, Wnt5a gradients can induce CaMKII-dependent asymmetric redistribution of dynamic MTs in the GC, which is required for Wnt5a-mediated axon repulsion[120].

The Ras superfamily of small GTPases, consisting of Rho, Ras, Rap, Arf, Sar, and Ran, also plays a crucial role in controlling axon turning *via* modulation of the F-actin and MT dynamics in the GC[4, 5, 131-133]. Local stimulation with guidance cues or extracellular adhesion molecules triggers asymmetric signaling of the Rho family GTPases RhoA, Rac1 ,and Cdc42, which in turn locally modulate actin and MT assembly, disassembly, and organization in the GC to orient axon outgrowth and projection[131, 134]. Several MAPs, Short Stop/ACF7, MAP1B, CLASPs, CRMPs, and APC, are regulated by multiple small GTPases[4, 5, 131, 135]. Therefore, the asymmetrical localization and redistribution of guidance receptors in the GC in response to guidance cues or adhesion molecules may lead to the assembly of an asymmetrical signal complex including small GTPases, MAPs, and MTs to polarize GC navigation[134].

Final Thoughts

A ‘search and capture’ model of MT regulation has been proposed for more than two decades[136]. In this model, exploring MTs in the cytoplasm are captured at specific cellular sites and transiently stabilized to initiate several biological processes, such as directing vesicle traffic and chromosome separation[136, 137]. GC turning is preceded by asymmetric enrichment of F-actin and MTs. The discovery of the direct interaction of DCC and TUBB3 as essential linking factors between MTs and Netrin signaling validates

this model at the molecular level in GC turning. Dynamic MTs 'captured' by attractive receptors on one side of the GC lead to a differential increase in MT growth and stabilization on this side, which could stabilize filopodia against retraction and promote axon outgrowth and attraction (Fig. 2). It is tempting to speculate that dissociation of dynamic MTs and repulsive receptors may result in the collapse of GC lamellipodia and filopodia inducing axon repulsion. Further studies are required to evaluate this model.

Clearly, many important questions about the regulation of MT dynamics in axon guidance remain to be answered. For example, where are MTs in the GC generated: the cytoplasm, axon shaft, or the C or P region of the GC during axon turning? How many MAPs or guidance receptors are specifically involved in directly regulating MT stability and dynamics in axon outgrowth and pathfinding? How do multiple signal cascades initiated by different guidance cues coordinate to regulate MT dynamics in GC steering? Since the coordination of F-actin and MT dynamics plays an essential role in axon guidance, it would be interesting to untangle the role of actin dynamics in these models as well: how MTs and F-actin work together to influence axon turning; how they interconvert; and how they regulate, or are regulated by MAPs and/or actin-binding proteins in axon guidance. The combination of super-resolution microscopy with genetics, biochemical assays, sophisticated axon turning assays, and fluorescence cytochemistry will allow us to better understand how guidance cues spatiotemporally modulate intracellular MT dynamics in the GC to control axon outgrowth and pathfinding.

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REFERENCES

[1] Guan KL, Rao Y. Signalling mechanisms mediating neuronal responses to guidance cues. Nat Rev Neurosci 2003, 4: 941–956.

[2] Kolodkin AL, Tessier-Lavigne M. Mechanisms and molecules of neuronal wiring: a primer. Cold Spring Harb Perspect Biol 2011, 3.

[3] Lowery LA, Van Vactor D. The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 2009, 10: 332–343.

[4] Dent EW, Gupton SL, Gertler FB. The growth cone cytoskeleton in axon outgrowth and guidance. Cold Spring Harb Perspect Biol 2011, 3.

[5] Vitriol EA, Zheng JQ. Growth cone travel in space and time: the cellular ensemble of cytoskeleton, adhesion, and membrane. Neuron 2012, 73: 1068–1081.

[6] Stoeckli E, Zou Y. How are neurons wired to form functional and plastic circuits? Meeting on Axon Guidance, Synaptogenesis & Neural Plasticity. EMBO Rep 2009, 10: 326–330.

[7] Suh LH, Oster SF, Soehrman SS, Grenningloh G, Sretavan DW. L1/Laminin modulation of growth cone response to EphB triggers growth pauses and regulates the microtubule destabilizing protein SCG10. J Neurosci 2004, 24: 1976–1986.

[8] Letourneau TMGaPC. Actin dynamics in growth cone motility and navigation. J Neurochem 2013: 14.

[9] Gordon-Weeks PR. Microtubules and growth cone function. J Neurobiol 2004, 58: 70-83.

[10] Conde C, Caceres A. Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci 2009, 10: 319–332.

[11] Tischfield MA, Cederquist GY, Gupta ML, Jr., Engle EC. Phenotypic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. Curr Opin Genet Dev 2011, 21: 286–294.

[12] Jaglin XH, Poirier K, Saillour Y, Buhler E, Tian G, Bahi-Buisson N*, et al.* Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. Nat Genet 2009, 41: 746–752.

[13] Poirier K, Saillour Y, Bahi-Buisson N, Jaglin XH, Fallet-Bianco C, Nabbout R*, et al.* Mutations in the neuronal SS-tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. Hum Mol Genet 2010, 19: 4462–4473.

[14] Tischfield MA, Baris HN, Wu C, Rudolph G, Van Maldergem L, He W*, et al.* Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell 2010, 140: 74–87.

[15] Kumar RA, Pilz DT, Babatz TD, Cushion TD, Harvey K, Topf M*, et al.* TUBA1A mutations cause wide spectrum lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. Hum Mol Genet 2010, 19: 2817–2827.

[16] Cushion TD, Dobyns WB, Mullins JGL, Stoodley N, Chung

S-K, Fry AE*, et al.* Overlapping cortical malformations and mutations in TUBB2B and TUBA1A. Brain 2013, 136: 536–548.

[17] Guerrini R, Mei D, Cordelli DM, Pucatti D, Franzoni E, Parrini E. Symmetric polymicrogyria and pachygyria associated with TUBB2B gene mutations. Eur J Hum Genet 2012, 20: 995–998.

[18] Abdollahi MR, Morrison E, Sirey T, Molnar Z, Hayward BE, Carr IM*, et al.* Mutation of the variant alpha-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. Am J Hum Genet 2009, 85: 737–744.

[19] Qu C, Dwyer, T, Shao, Q, Yang, T, Huang, H, Liu, G. Direct binding of TUBB3 with DCC couples netrin-1 signaling to intracellular microtubule dynamics in axon outgrowth and guidance. J Cell Sci 2013, 126: 12.

[20] Sohal AP, Montgomery T, Mitra D, Ramesh V. TUBA1A mutation-associated lissencephaly: case report and review of the literature. Pediatr Neurol 2012, 46: 127–131.

[21] Fallet-Bianco C, Loeuillet L, Poirier K, Loget P, Chapon F, Pasquier L*, et al.* Neuropathological phenotype of a distinct form of lissencephaly associated with mutations in TUBA1A. Brain 2008, 131: 2304–2320.

[22] Lecourtois M, Poirier K, Friocourt G, Jaglin X, Goldenberg A, Saugier-Veber P*, et al.* Human lissencephaly with cerebellar hypoplasia due to mutations in TUBA1A: expansion of the foetal neuropathological phenotype. Acta Neuropathol 2010, 119: 779–789.

[23] Okumura A, Hayashi M, Tsurui H, Yamakawa Y, Abe S, Kudo T*, et al.* Lissencephaly with marked ventricular dilation, agenesis of corpus callosum, and cerebellar hypoplasia caused by TUBA1A mutation. Brain Dev 2013, 35: 274–279.

[24] Poirier K, Saillour Y, Fourniol F, Francis F, Souville I, Valence S*, et al.* Expanding the spectrum of TUBA1A-related cortical dysgenesis to Polymicrogyria. Eur J Hum Genet 2013, 21: 381–385.

[25] Zanni G, Colafati GS, Barresi S, Randisi F, Talamanca LF, Genovese E*, et al.* Description of a novel TUBA1A mutation in Arg-390 associated with asymmetrical polymicrogyria and mid-hindbrain dysgenesis. Eur J Paediatr Neurol 2013, 17: 361–365.

[26] Cushion TD, Dobyns WB, Mullins JG, Stoodley N, Chung SK, Fry AE*, et al.* Overlapping cortical malformations and mutations in TUBB2B and TUBA1A. Brain 2013, 136: 536–548.

[27] Romaniello R, Arrigoni F, Cavallini A, Tenderini E, Baschirotto C, Triulzi F*, et al.* Brain malformations and mutations in alpha- and beta-tubulin genes: a review of the literature and description of two new cases. Dev Med Child Neurol 2014, 56(4): 354–360.

[28] Cederquist GY, Luchniak A, Tischfield MA, Peeva M, Song Y, Menezes MP*, et al.* An inherited TUBB2B mutation alters a kinesin-binding site and causes polymicrogyria, CFEOM and axon dysinnervation. Hum Mol Genet 2012, 21: 5484–5499.

[29] Romaniello R, Tonelli A, Arrigoni F, Baschirotto C, Triulzi F, Bresolin N*, et al.* A novel mutation in the beta-tubulin gene TUBB2B associated with complex malformation of cortical development and deficits in axonal guidance. Dev Med Child Neurol 2012, 54: 765–769.

[30] Buck KB, Zheng JQ. Growth cone turning induced by direct local modification of microtubule dynamics. J Neurosci 2002, 22: 9358–9367.

[31] Dent EW, Barnes AM, Tang F, Kalil K. Netrin-1 and semaphorin 3A promote or inhibit cortical axon branching, respectively, by reorganization of the cytoskeleton. J Neurosci 2004, 24: 3002–3012.

[32] Challacombe JF, Snow DM, Letourneau PC. Dynamic microtubule ends are required for growth cone turning to avoid an inhibitory guidance cue. J Neurosci 1997, 17: 3085–3095.

[33] Tanaka E, Kirschner MW. The role of microtubules in growth cone turning at substrate boundaries. J Cell Biol 1995, 128: 127–137.

[34] Sabry JH, O'Connor TP, Evans L, Toroian-Raymond A, Kirschner M, Bentley D. Microtubule behavior during guidance of pioneer neuron growth cones in situ. J Cell Biol 1991, 115: 381–395.

[35] Lee AC, Suter DM. Quantitative analysis of microtubule dynamics during adhesion-mediated growth cone guidance. Dev Neurobiol 2008, 68: 1363–1377.

[36] Lei WL, Xing SG, Deng CY, Ju XC, Jiang XY, Luo ZG. Laminin/beta 1 integrin signal triggers axon formation by promoting microtubule assembly and stabilization. Cell Res 2012, 22: 954–972.

[37] Kalil K, Dent EW. Touch and go: guidance cues signal to the growth cone cytoskeleton. Curr Opin Neurobiol 2005, 15: 521–526.

[38] Sengottuvel V, Leibinger M, Pfreimer M, Andreadaki A, Fischer D. Taxol facilitates axon regeneration in the mature CNS. J Neurosci 2011, 31: 2688–2699.

[39] Purro SA, Ciani L, Hoyos-Flight M, Stamatakou E, Siomou E, Salinas PC. Wnt regulates axon behavior through changes in microtubule growth directionality: a new role for adenomatous polyposis coli. J Neurosci 2008, 28: 8644–8654.

[40] Augsburger A, Schuchardt A, Hoskins S, Dodd J, Butler S. BMPs as mediators of roof plate repulsion of commissural neurons. Neuron 1999, 24: 127–141.

[41] Butler SJ, Dodd J. A role for BMP heterodimers in roof plate-mediated repulsion of commissural axons. Neuron 2003, 38: 389–401.

[42] Charron F, Stein E, Jeong J, McMahon AP, Tessier-

Lavigne M. The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with netrin-1 in midline axon guidance. Cell 2003, 113: 11–23.

[43] Stein E, Tessier-Lavigne M. Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 2001, 291: 1928–1938.

[44] Lyuksyutova AI, Lu CC, Milanesio N, King LA, Guo N, Wang Y*, et al.* Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. Science 2003, 302: 1984–1988.

[45] Keeble TR, Halford MM, Seaman C, Kee N, Macheda M, Anderson RB*, et al.* The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. J Neurosci 2006, 26: 5840–5848.

[46] Ahmed G, Shinmyo Y, Ohta K, Islam SM, Hossain M, Naser IB*, et al.* Draxin inhibits axonal outgrowth through the netrin receptor DCC. J Neurosci 2011, 31: 14018–14023.

[47] Islam SM, Shinmyo Y, Okafuji T, Su Y, Naser IB, Ahmed G*, et al.* Draxin, a repulsive guidance protein for spinal cord and forebrain commissures. Science 2009, 323: 388–393.

[48] Stoeckli ET, Landmesser LT. Axonin-1, Nr-CAM, and Ng-CAM play different roles in the *in vivo* guidance of chick commissural neurons. Neuron 1995, 14: 1165–1179.

[49] Galjart N. Plus-end-tracking proteins and their interactions at microtubule ends. Curr Biol 2010, 20: R528–537.

[50] Tucker RP, Garner CC, Matus A. In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain. Neuron 1989, 2: 1245–1256.

[51] Meixner A, Haverkamp S, Wassle H, Fuhrer S, Thalhammer J, Kropf N*, et al.* MAP1B is required for axon guidance and Is involved in the development of the central and peripheral nervous system. J Cell Biol 2000, 151: 1169–1178.

[52] Mack TG, Koester MP, Pollerberg GE. The microtubule-associated protein MAP1B is involved in local stabilization of turning growth cones. Mol Cell Neurosci 2000, 15: 51–65.

[53] Tymanskyj SR, Scales TME, Gordon-Weeks PR. MAP1B enhances microtubule assembly rates and axon extension rates in developing neurons. Mol Cell Neurosci 2012, 49: 110–119.

[54] Vandecandelaere A, Pedrotti B, Utton MA, Calvert RA, Bayley PM. Differences in the regulation of microtubule dynamics by microtubule-associated proteins MAP1B and MAP2. Cell Motil Cytoskeleton 1996, 35: 134–146.

[55] Takemura R, Okabe S, Umeyama T, Kanai Y, Cowan NJ, Hirokawa N. Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. J Cell Sci 1992, 103 (Pt 4): 953–964.

[56] Black MM, Slaughter T, Fischer I. Microtubule-associated protein 1b (MAP1b) is concentrated in the distal region of growing axons. J Neurosci 1994, 14: 857–870.

[57] Gonzalez-Billault C, Avila J, Caceres A. Evidence for the role of MAP1B in axon formation. Mol Biol Cell 2001, 12: 2087–2098.

[58] Serafini T, Colamarino SA, Leonardo ED, Wang H, Beddington R, Skarnes WC*, et al.* Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell 1996, 87: 1001–1014.

[59] Braisted JE, Catalano SM, Stimac R, Kennedy TE, Tessier-Lavigne M, Shatz CJ*, et al.* Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. J Neurosci 2000, 20: 5792–5801.

[60] Fazeli A, Dickinson SL, Hermiston ML, Tighe RV, Steen RG, Small CG*, et al.* Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. Nature 1997, 386: 796–804.

[61] Del Rio JA, Gonzalez-Billault C, Urena JM, Jimenez EM, Barallobre MJ, Pascual M*, et al.* MAP1B is required for Netrin 1 signaling in neuronal migration and axonal guidance. Curr Biol 2004, 14: 840–850.

[62] Hall AC, Lucas FR, Salinas PC. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell 2000, 100: 525–535.

[63] Ciani L, Krylova O, Smalley MJ, Dale TC, Salinas PC. A divergent canonical WNT-signaling pathway regulates microtubule dynamics: dishevelled signals locally to stabilize microtubules. J Cell Biol 2004, 164: 243–253.

[64] Arimura N, Kaibuchi K. Neuronal polarity: from extracellular signals to intracellular mechanisms. Nat Rev Neurosci 2007, 8: 194–205.

[65] Fukata Y, Itoh TJ, Kimura T, Menager C, Nishimura T, Shiromizu T*, et al.* CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat Cell Biol 2002, 4: 583–591.

[66] Brot S, Rogemond V, Perrot V, Chounlamountri N, Auger C, Honnorat J*, et al.* CRMP5 interacts with tubulin to inhibit neurite outgrowth, thereby modulating the function of CRMP2. J Neurosci 2010, 30: 10639–10654.

[67] Inagaki N, Chihara K, Arimura N, Menager C, Kawano Y, Matsuo N*, et al*. CRMP-2 induces axons in cultured hippocampal neurons. Nat Neurosci 2001, 4: 781–782.

[68] Uchida Y, Ohshima T, Sasaki Y, Suzuki H, Yanai S, Yamashita N*, et al.* Semaphorin3A signalling is mediated via sequential Cdk5 and GSK3beta phosphorylation of CRMP2: implication of common phosphorylating mechanism underlying axon guidance and Alzheimer's disease. Genes Cells 2005, 10: 165–179.

[69] Kimura T, Watanabe H, Iwamatsu A, Kaibuchi K. Tubulin and CRMP-2 complex is transported via Kinesin-1. J Neurochem 2005, 93: 1371–1382.

[70] Goshima Y, Nakamura F, Strittmatter P, Strittmatter SM.

Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. Nature 1995, 376: 509–514.

[71] Brugg B, Matus A. PC12 cells express juvenile microtubule-associated proteins during nerve growth factor-induced neurite outgrowth.J Cell Biol 1988, 107: 643–650.

[72] Schuyler SC, Pellman D. Microtubule "plus-end-tracking proteins": The end is just the beginning. Cell 2001, 105: 421–424.

[73] Lee H, Engel U, Rusch J, Scherrer S, Sheard K, Van Vactor D. The microtubule plus end tracking protein Orbit/MAST/CLASP acts downstream of the tyrosine kinase Abl in mediating axon guidance. Neuron 2004, 42: 913–926.

[74] Galjart N. CLIPs and CLASPs and cellular dynamics. Nat Rev Mol Cell Biol 2005, 6: 487–498.

[75] Hur E-M, Saijilafu, Lee BD, Kim S-J, Xu W-L, Zhou F-Q. GSK3 controls axon growth via CLASP-mediated regulation of growth cone microtubules. Genes Dev2011, 25: 1968–1981.

[76] Koester MP, Muller O, Pollerberg GE. Adenomatous polyposis coli is differentially distributed in growth cones and modulates their steering. J Neurosci 2007, 27: 12590–12600.

[77] Shintani T, Ihara M, Tani S, Sakuraba J, Sakuta H, Noda M. APC2 plays an essential role in axonal projections through the regulation of microtubule stability. J Neurosci 2009, 29: 11628–11640.

[78] Rosin-Arbesfeld R, Ihrke G, Bienz M. Actin-dependent membrane association of the APC tumour suppressor in polarized mammalian epithelial cells. EMBO J 2001, 20: 5929–5939.

[79] Su LK, Burrell M, Hill DE, Gyuris J, Brent R, Wiltshire R*, et al.* APC binds to the novel protein EB1. Cancer Res 1995, 55: 2972–2977.

[80] Morrison EE, Moncur PM, Askham JM. EB1 identifies sites of microtubule polymerisation during neurite development. Brain Res Mol Brain Res 2002, 98: 145–152.

[81] Nakagawa H, Koyama K, Murata Y, Morito M, Akiyama T, Nakamura Y. EB3, a novel member of the EB1 family preferentially expressed in the central nervous system, binds to a CNS-specific APC homologue. Oncogene 2000, 19: 210–216.

[82] Tortosa E, Galjart N, Avila J, Sayas CL. MAP1B regulates microtubule dynamics by sequestering EB1/3 in the cytosol of developing neuronal cells. EMBO J 2013, 32: 1293–1306.

[83] Laht P, Pill K, Haller E, Veske A. Plexin-B3 interacts with EB-family proteins through a conserved motif. Biochim Biophys Acta 2012, 1820: 888–893.

[84] Sturgill EG, Ohi R. Microtubule-regulating kinesins. Curr Biol 2013, 23: R946-948.

[85] Howard J, Hyman AA. Microtubule polymerases and depolymerases. Curr Opin Cell Biol 2007, 19: 31–35.

[86] Maor-Nof M, Homma N, Raanan C, Nof A, Hirokawa N, Yaron A. Axonal pruning is actively regulated by the microtubule-destabilizing protein kinesin superfamily protein 2A. Cell Rep 2013, 3: 971–977.

[87] Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. Neuron 2010, 68: 610–638.

[88] van der Vaart B, van Riel WE, Doodhi H, Kevenaar JT, Katrukha EA, Gumy L*, et al.* CFEOM1-associated kinesin KIF21A is a cortical microtubule growth inhibitor. Dev Cell 2013, 27: 145–160.

[89] Yamada K, Andrews C, Chan WM, McKeown CA, Magli A, de Berardinis T*, et al.* Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). Nat Genet 2003, 35: 318–321.

[90] Zhou FQ, Zhou J, Dedhar S, Wu YH, Snider WD. NGF-induced axon growth is mediated by localized inactivation of GSK-3beta and functions of the microtubule plus end binding protein APC. Neuron 2004, 42: 897–912.

[91] Hida T, Yamashita N, Usui H, Nakamura F, Sasaki Y, Kikuchi A*, et al.* GSK3beta/axin-1/beta-catenin complex is involved in semaphorin3A signaling. J Neurosci 2012, 32: 11905–11918.

[92] Byun J, Kim BT, Kim YT, Jiao Z, Hur EM, Zhou FQ. Slit2 inactivates GSK3beta to signal neurite outgrowth inhibition. PLoS One 2012, 7: e51895.

[93] Hur EM, Zhou FQ. GSK3 signalling in neural development. Nat Rev Neurosci 2010, 11: 539-551.

[94] Zhou FQ, Snider WD. Cell biology. GSK-3beta and microtubule assembly in axons. Science 2005, 308: 211–214.

[95] Goold RG, Owen R, Gordon-Weeks PR. Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. J Cell Sci 1999, 112 (Pt 19): 3373–3384.

[96] Lucas FR, Goold RG, Gordon-Weeks PR, Salinas PC. Inhibition of GSK-3beta leading to the loss of phosphorylated MAP-1B is an early event in axonal remodelling induced by WNT-7a or lithium. J Cell Sci 1998, 111 (Pt 10): 1351–1361.

[97] Salinas PC. Modulation of the microtubule cytoskeleton: a role for a divergent canonical Wnt pathway. Trends Cell Biol 2007, 17: 333–342.

[98] Zumbrunn J, Kinoshita K, Hyman AA, Nathke IS. Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 beta phosphorylation. Curr Biol 2001, 11: 44–49.

[99] Fukata Y, Itoh TJ, Kimura T, Menager C, Nishimura T, Shiromizu T*, et al.* CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nat Cell Biol 2002, 4: 583–591.

[100] Kim WY, Zhou FQ, Zhou J, Yokota Y, Wang YM, Yoshimura

T*, et al*. Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. Neuron 2006, 52: 981–996.

[101] Chang L, Jones Y, Ellisman MH, Goldstein LSB, Karin M. JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. Dev Cell 2003, 4: 521–533.

[102] Qu C, Li W, Shao Q, Dwyer T, Huang H, Yang T*, et al.* c-Jun N-terminal Kinase 1 (JNK1) Is Required for Coordination of Netrin Signaling in Axon Guidance. J Biol Chem 2013, 288: 1883–1895.

[103] Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM*, et al.* LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. Hum Mol Genet 1998, 7: 2029–2037.

[104] Deuel TA, Liu JS, Corbo JC, Yoo SY, Rorke-Adams LB, Walsh CA. Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth. Neuron 2006, 49: 41–53.

[105] Tint I, Jean D, Baas PW, Black MM. Doublecortin associates with microtubules preferentially in regions of the axon displaying actin-rich protrusive structures. J Neurosci 2009, 29: 10995–11010.

[106] Bechstedt S, Brouhard GJ. Doublecortin recognizes the 13-protofilament microtubule cooperatively and tracks microtubule ends. Dev Cell 2012, 23: 181–192.

[107] Morii H, Shiraishi-Yamaguchi Y, Mori N. SCG10, a microtubule destabilizing factor, stimulates the neurite outgrowth by modulating microtubule dynamics in rat hippocampal primary cultured neurons. J Neurobiol 2006, 66: 1101–1114.

[108] Tararuk T, Ostman N, Li W, Bjorkblom B, Padzik A, Zdrojewska J*, et al*. JNK1 phosphorylation of SCG10 determines microtubule dynamics and axodendritic length. J Cell Biol 2006, 173: 265–277.

[109] Stein R, Mori N, Matthews K, Lo LC, Anderson DJ. The NGF-inducible SCG10 mRNA encodes a novel membrane-bound protein present in growth cones and abundant in developing neurons. Neuron 1988, 1: 463–476.

[110] Antonsson B, Kassel DB, Di Paolo G, Lutjens R, Riederer BM, Grenningloh G. Identification of *in vitro* phosphorylation sites in the growth cone protein SCG10. Effect Of phosphorylation site mutants on microtubule-destabilizing activity. J Biol Chem 1998, 273: 8439–8446.

[111] Riederer BM, Pellier V, Antonsson B, Di Paolo G, Stimpson SA, Lutjens R*, et al.* Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10. Proc Natl Acad Sci U S A 1997, 94: 741–745.

[112] Kempf M, Clement A, Faissner A, Lee G, Brandt R. Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. J Neurosci 1996, 16: 5583–5592.

[113] Caceres A, Kosik KS. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. Nature 1990, 343: 461–463.

[114] Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J Cell Sci 2001, 114: 1179–1187.

[115] Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T*, et al.* Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature 1994, 369: 488–491.

[116] Ke YD, Suchowerska AK, van der Hoven J, De Silva DM, Wu CW, van Eersel J*, et al.* Lessons from tau-deficient mice. Int J Alzheimers Dis 2012, 2012: 873270.

[117] Takei Y, Teng J, Harada A, Hirokawa N. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. J Cell Biol 2000, 150: 989–1000.

[118] Al-Bassam J, Ozer RS, Safer D, Halpain S, Milligan RA. MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. J Cell Biol 2002, 157: 1187–1196.

[119] Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. Neuron 2011, 70: 410–426.

[120] Li L, Fothergill T, Hutchins BI, Dent EW, Kalil K. Wnt5a evokes cortical axon outgrowth and repulsive guidance by tau mediated reorganization of dynamic microtubules. Dev Neurobiol 2013.

[121] Wen Z, Guirland C, Ming GL, Zheng JQ. A CaMKII/calcineurin switch controls the direction of Ca(2+)-dependent growth cone guidance. Neuron 2004, 43: 835–846.

[122] Wang GX, Poo MM. Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. Nature 2005, 434: 898–904.

[123] Li Y, Jia YC, Cui K, Li N, Zheng ZY, Wang YZ*, et al.* Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. Nature 2005, 434: 894–898.

[124] Henley J, Poo MM. Guiding neuronal growth cones using Ca2+ signals. Trends Cell Biol 2004, 14: 320–330.

[125] Gomez TM, Zheng JQ. The molecular basis for calcium-dependent axon pathfinding. Nat Rev Neurosci 2006, 7: 115–125.

[126] Carrillo RA, Olsen DP, Yoon KS, Keshishian H. Presynaptic activity and CaMKII modulate retrograde semaphorin signaling and synaptic refinement. Neuron 2010, 68: 32–44.

[127] Li L, Hutchins BI, Kalil K. Wnt5a induces simultaneous cortical axon outgrowth and repulsive axon guidance through distinct signaling mechanisms. J Neurosci 2009, 29: 5873–5883.

[128] Goto S, Yamamoto H, Fukunaga K, Iwasa T, Matsukado Y,

Miyamoto E. Dephosphorylation of microtubule-associated protein 2, tau factor, and tubulin by calcineurin. J Neurochem 1985, 45: 276–283.

[129] Mandelkow E, Mandelkow EM. Microtubules and microtubule-associated proteins. Curr Opin Cell Biol 1995, 7: 72–81.

[130] Yamamoto H, Fukunaga K, Goto S, Tanaka E, Miyamoto E. Ca2+, calmodulin-dependent regulation of microtubule formation via phosphorylation of microtubule-associated protein 2, tau factor, and tubulin, and comparison with the cyclic AMP-dependent phosphorylation. J Neurochem 1985, 44: 759–768.

[131] Hall A, Lalli G. Rho and Ras GTPases in axon growth, guidance, and branching. Cold Spring Harb Perspect Biol 2010, 2: a001818.

[132] Gomez TM, Letourneau PC. Actin dynamics in growth cone motility and navigation. J Neurochem 2014, 129(2): 221–234.

[133] Lowery LA, Van Vactor D. The trip of the tip: understanding the growth cone machinery. Nat Rev Mol Cell Biol 2009, 10: 332–343.

[134] Quinn CC, Wadsworth WG. Axon guidance: asymmetric signaling orients polarized outgrowth. Trends Cell Biol 2008, 18: 597–603.

[135] Namekata K, Watanabe H, Guo X, Kittaka D, Kawamura K, Kimura A*, et al.* Dock3 regulates BDNF-TrkB signaling for neurite outgrowth by forming a ternary complex with Elmo and RhoG. Genes Cells 2012, 17: 688–697.

[136] Kirschner M, Mitchison T. Beyond self-assembly: from microtubules to morphogenesis. Cell 1986, 45: 329–342.

[137] Gundersen GG. Evolutionary conservation of microtubule-capture mechanisms. Nat Rev Mol Cell Biol 2002, 3: 296–304.