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Centrosomal and acentrosomal microtubule nucleation during neuronal development

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Neurons rely on the microtubule cytoskeleton to create and maintain their sophisticated cellular architectures. Advances in cryogenic electron microscopy, expansion microscopy, live imaging, and gene editing have enabled novel insights into mechanisms of centrosomal and acentrosomal microtubule nucleation, the key process generating new microtubules. This has paved the way for the functional dissection of distinct microtubule networks that regulate various processes during neuronal development, including neuronal delamination, polarization, migration, maturation, and synapse function. We review recent progress in understanding the molecular concepts of microtubule nucleation, how these concepts underlie neurodevelopmental processes, and pinpoint the open questions. Since microtubules play a pivotal role in axon regeneration within the adult central nervous system, understanding the processes of microtubule nucleation could inform strategies to enhance the regenerative capabilities of neurons in the future.

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Introduction

Microtubules are cytoskeletal filaments that drive myriads of cellular processes, governing cell shape, motility, intracellular transport, cell division, and signalling [1–4]. Microtubules are essential especially in neurons, highly polarized cells with sophisticated morphology [4]. It enables them to form complex networks, giving rise to emergent properties that reach its pinnacle in human consciousness.

Yet, neurons are born as small, simple cells. They undergo complex morphological transformations, while growing and travelling to their destination in the brain [5–7]. Neurons make thousands of connections, typically via elaborate dendritic trees and axons [6], whose architecture relies on different microtubule networks. Some undergo massive expansion and become progressively stable, such as microtubule bundles in extending axons [8]. Others, such as microtubules in navigating growth cones, undergo cyclic remodelling [7,9,10]. As development progresses, microtubule networks gradually diversify. In axons, microtubules form a unipolar arrangement, whereas in neurites, they exhibit mixed polarity, varying by developmental stage and species [11,12]. This unique organization provides a basis for further regulatory mechanisms that coordinate cargo molecule sorting and are important for establishing neuronal polarity [11–14]. Microtubules also form a cage surrounding the nucleus during neuronal migration [7]. They are connected to the centrosome that is cyclically moving inside the cytoplasmic dilation [15], coupling the leading process and the nucleus in the neuronal rear [7]. Other microtubule networks form elaborate signalling structures, including dendritic trees, primary cilia and synapses [16,17].

Such massive expansion and remodeling requires highly regulated generation of new microtubules. They arise from severing of existing microtubules by microtubule severing enzymes, such as katanin, spastin or fidgetin, with microtubule remnants serving as polymerization seeds, or by *de novo* microtubule nucleation [4]. Microtubule nucleation has been implicated in many key events in neuronal lives and will be the main topic of this review.

First, we will discuss advances in the understanding of different modes of microtubule nucleation. Second, we will summarize when and where these modes of microtubule nucleation are employed, and how they are related to the diverse and dynamically changing microtubule networks during neuronal development. It will provide insights into how microtubule networks drive the form and function of neurons.

Modes of microtubule nucleation

Microtubule nucleation by γ-TuRC

Microtubules are stiff, 25 nm thick tubes. They form by unidirectional lateral association of typically 13 protofilaments, linear polymers of α- and β-tubulin heterodimers [1]. α-Tubulin is exposed on the minus end and β-tubulin on the plus end of each protofilament [4]. Minus and plus ends differ structurally and biochemically, which results in different kinetics of microtubule polymerization and depolymerization, with plus ends exhibiting much greater dynamicity [4]. This difference magnifies in cells, where specific proteins bind to the two different microtubule ends, modulating their dynamics [1,4].

In cells, microtubule nucleation is catalyzed primarily by the cone-shaped γ-Tubulin Ring Complex (γ-TuRC) [4], which contains seven main protein modules, each composed of a γ-tubulin complex protein (GCP) heterodimer and two γ-tubulins [18—20] (Figure 1a).

The assembled γ-TuRC is splayed. Thereby, it does not match the 13-protofilament microtubule, explaining its low nucleation activity [20—25]. Recent studies revealed that γ-TuRC can transition to a more closed ('early closed') conformation, which increases its microtubule nucleation efficiency [19—21,26] (Figure 1a).

γ-TuRC associates with many proteins that modulate its activity and subcellular targeting. For a more complete list of these proteins, we refer to recent excellent reviews [2,4,27]. An important class of γ-TuRC-interacting proteins are its stimulators. We will discuss CDK5 regulatory subunit-associated protein 2 (CDK5RAP2) [28,29], Targeting protein for Xklp2 (TPX2) [30], the augmin complex [31], AKNA [32], Cytoskeleton-associated protein 5 (CKAP5), and CLIP-associating protein 2 (CLASP2) [33].

Proteins stimulating γ-TuRC activity

The *bona fide* γ-TuRC activator is CDK5RAP2 (Centrosomin in *Drosophila*). It contains a conserved Centrosomin motif 1 (CM1, also termed γ-tubulin nucleation activator, γ-TuNA) that binds to the γ-TuRC via Mitotic-spindle organizing protein 2 (MZT2) and GCP2, changing γ-TuRC conformation to the 'early closed' state that better matches the 13-protofilament microtubules [28,29] (Figure 1b). Interestingly, only a fraction of γ-TuRCs interact with CDK5RAP2 in cells [15,34] and the interaction is likely transient [28]. CDK5RAP2 autoinhibition prevents uncontrolled γ-TuRC activation [35,36].

TPX2 stimulates γ-TuRC-dependent microtubule nucleation in several ways. It recruits γ-TuRC, soluble tubulin, and augmin to microtubules, enhancing branching microtubule nucleation [2,27] (Figure 1c). However, TPX2 is dispensable for augmin-dependent branching microtubule nucleation in some systems [31].

Augmin is a heterooctameric complex of proteins HAUS1-8 that organizes branching microtubule nucleation [27,37]. Augmin facilitates γ-TuRC-dependent unidirectional microtubule nucleation from a template microtubule (Figure 1c). Augmin binds to γ-TuRC through an adaptor protein Neural precursor cell expressed developmentally down-regulated protein 1 (NEDD1) [31,38] (Figure 1c).

TPX2 is able to condense on microtubules and concentrate augmin, γ-TuRC and tubulin in patches, which serve as microtubule nucleation spots [30] (Figure 1c). Notably, TPX2's simultaneous binding across longitudinal and lateral tubulin interfaces may be sufficient to overcome the nucleation energy barrier, even in the absence of its liquid—liquid phase separation (LLPS) [39,40].

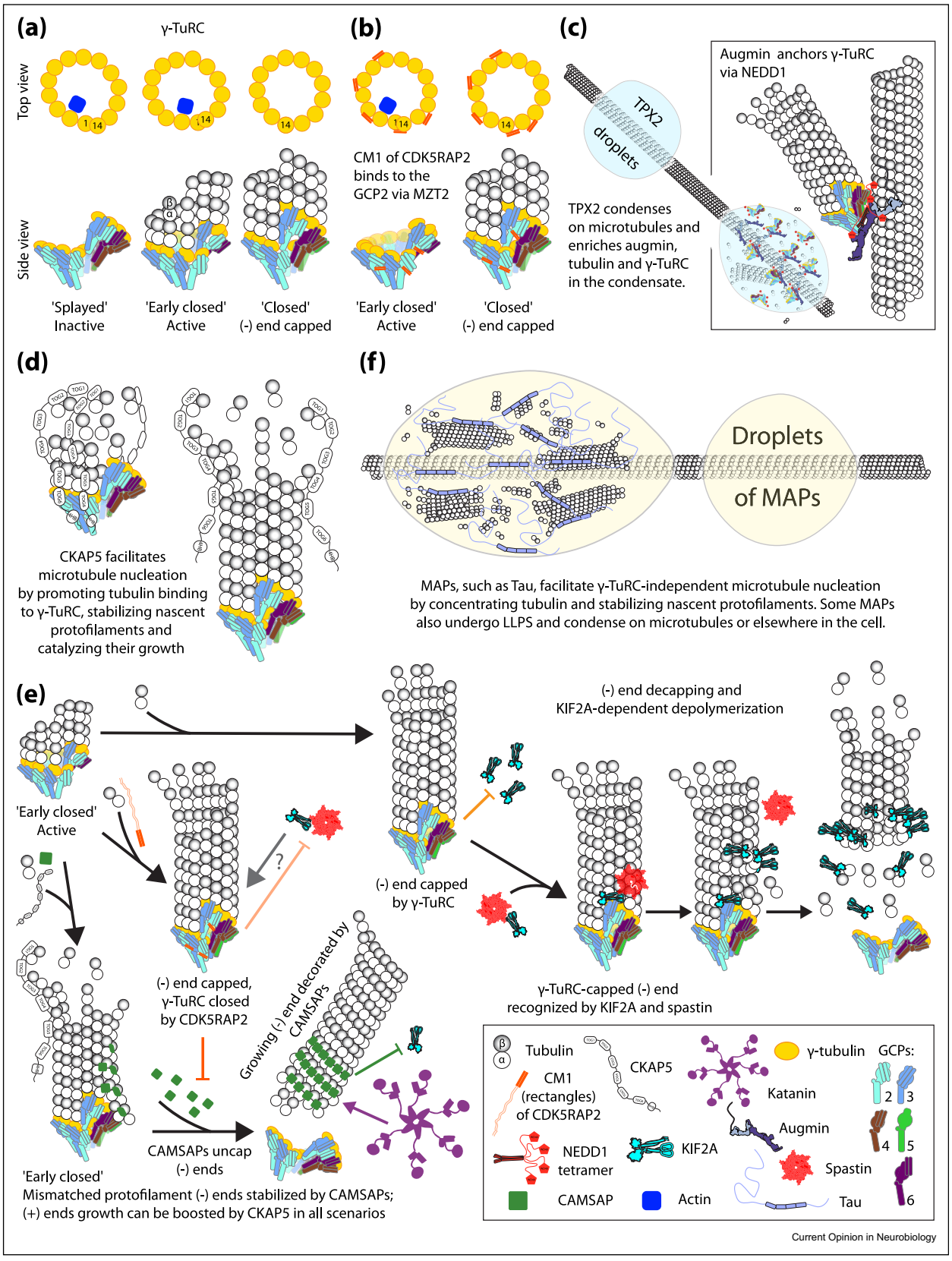
AKNA was recently identified as a γ-TuRC-interacting protein and a potent activator of centrosomal microtubule nucleation in neuronal progenitors and in normal murine mammary gland (NMuMG) epithelial cells [32]. Moreover, we have shown its very high potency to boost centrosomal microtubule nucleation in developing rodent neurons [15]. Nevertheless, the molecular mechanism of its action is still unclear.

Microtubule polymerase and plus-end tracking protein CKAP5 (also known as Xmap215 or chTOG) is another key protein that enhances γ-TuRC-dependent microtubule nucleation [33,41] (Figure 1d). Although CKAP5 can stimulate microtubule nucleation even without γ-TuRC [42,43], the kinetics of such nucleation reaction and, remarkably, the properties of the nucleated microtubules then differ from the canonical γ-TuRC-dependent pathway [43]. As CKAP5 plays key roles in neuronal development [44,45], it will be important to distinguish its functions at the microtubule plus and minus ends. Moreover, CKAP5 bundles actin filaments and links them to microtubules [46], which is crucial for microtubule advancement in neuronal growth cones and axonal growth [44].

The interplay of microtubule minus-end proteins in regulating γ-TuRC minus-end capping

CLIP-associating protein 2 (CLASP2) stimulates microtubule nucleation from purified γ-TuRCs [33]. Interestingly, γ-TuRCs are released from the nucleated microtubules by Calmodulin-regulated spectrin-associated protein 2 (CAMSAP2) and CAMSAP3 [33] (Figure 1e). CAMSAPs bind to microtubule minus ends, stabilize them [47] and facilitate their growth [33]. Katanin recognizes longer stretches of CAMSAPs and severs them [48]. CAMSAP-driven minus-end growth of

Figure 1


(a)
γ-TuRC
Top view
Side view
'Splayed'
Inactive
'Early closed'
Active
'Closed'
(-) end capped
(b)
CM1 of CDK5RAP2
binds to the
GCP2 via MZT2
'Early closed'
Active
'Closed'
(-) end capped
(c)
TPX2
droplets
TPX2 condenses
on microtubules and
enriches augmin,
tubulin and γ-TuRC
in the condensate.
Augmin anchors γ-TuRC
via NEDD1
(d)
CKAP5 facilitates
microtubule nucleation
by promoting tubulin binding
to γ-TuRC, stabilizing nascent
protofilaments and
catalyzing their growth
(f)
Droplets
of MAPs
MAPs, such as Tau, facilitate γ-TuRC-independent microtubule nucleation
by concentrating tubulin and stabilizing nascent protofilaments. Some MAPs
also undergo LLPS and condense on microtubules or elsewhere in the cell.
(e)
'Early closed'
Active
(-) end capped,
γ-TuRC closed
by CDK5RAP2
(-) end capped
by γ-TuRC
(-) end decapping and
KIF2A-dependent depolymerization
γ-TuRC-capped (-) end
recognized by KIF2A and spastin
Growing (-) end decorated by
CAMSAPs
CAMSAPs uncap
(-) ends
'Early closed'
Mismatched protofilament (-) ends stabilized by CAMSAPs;
(+) ends growth can be boosted by CKAP5 in all scenarios
Tubulin
CM1
(rectangles)
of CDK5RAP2
NEDD1
tetramer
CAMSAP
CKAP5
KIF2A
Actin
γ-tubulin
GCPs:
Katanin
Augmin
Spastin
Tau
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a subset of protofilaments on the γ-TuRC-attached microtubule leads to γ-TuRC removal and minus-end decapping [33]. Of note, CAMSAPs can also nucleate microtubules independently of γ-TuRC through LLPS [49].

Intriguingly, CDK5RAP2, but neither CKAP5 nor CLASP2, prevents CAMSAP2 and CAMSAP3 binding to the γ-TuRC:microtubule interface and subsequent microtubule decapping (Figure 1e) [33]. Thus, we speculate that CDK5RAP2 might, paradoxically, decrease cellular microtubule mass by preventing minus end decapping and subsequent stabilization by CAMSAPs and also by keeping γ-TuRCs locked on microtubules, making them unavailable for next rounds of microtubule nucleation.

Microtubule nucleation is also influenced by microtubule depolymerases [50,51]. Remarkably, KIF2A is a specific minus-end depolymerase [51]. Its affinity for the plus end is low, allowing unperturbed γ-TuRC-dependent nucleation in its presence. γ-TuRC-capped minus ends are protected from both KIF2C and KIF2A [51]. However, when KIF2A is combined with spastin, they recognize the γ-TuRC:microtubule interface and synergistically remove the γ-TuRC from the minus end, which is then further depolymerized by KIF2A [51] (Figure 1e).

Collectively, described mechanisms of γ-TuRC regulation at microtubule minus ends open new research perspectives on how microtubule networks might be regulated in neurons.

Microtubule nucleation by microtubule-associated proteins (MAPs)

Unexpectedly, microtubule nucleation activity resides in dozens of MAPs [52,53]. In addition, many of the MAPs undergo LLPS, which appears to be a dominant mechanism of their microtubule nucleation activity [52,54] (Figure 1f). In general, LLPS has emerged as an important mechanism of cellular homeostasis and organization [54,55], however, further studies are needed to specify the contributions of LLPS to regulation of microtubule nucleation *in vivo*, both generally and in neurons specifically.

Outstanding questions

Discussed molecular mechanisms enable investigation of microtubule organization and regulation in neurons on a new level. What proportion of the new minus ends are capped by γ-TuRC vs. by CAMSAPs? What is the interplay between these systems? Are there different neuronal microtubule populations, some uncapped and some capped by various types of γ-TuRC complexes? How do these microtubule populations differ in terms of biophysical properties, abundance, subcellular localization, and among neuronal types? What is the contribution of γ-TuRC-independent microtubule nucleation by diverse MAPs to neuronal morphogenesis and migration *in vivo*? These questions will be central to future research.

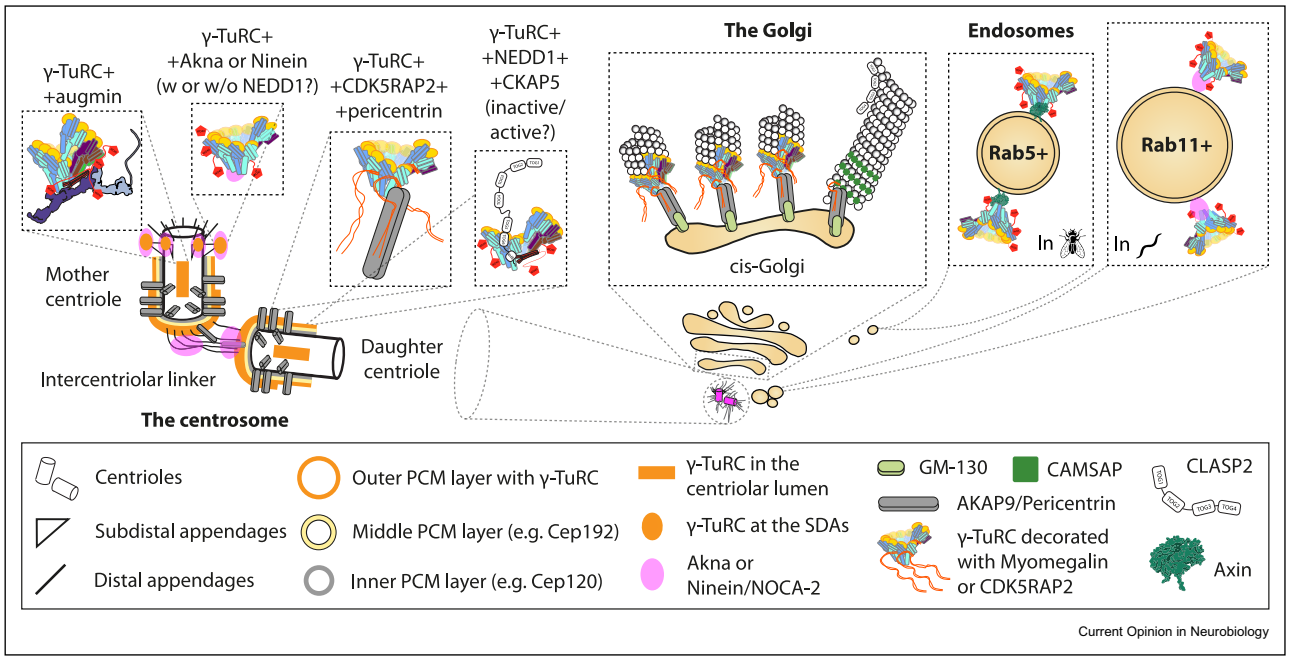
Microtubule organizing centers (MTOCs)

In nascent neurons, the dominant MTOC is the centrosome [15] (Figure 2), composed of two microtubule-based centrioles, which are surrounded by intricate protein matrix called the pericentriolar material (PCM) [56,57]. The older, *mother* centriole, bears additional specific proteinaceous structures at its distal end — the subdistal (SDA) [58] and the distal (DA) appendages [59] (Figure 2). Both types of appendages play key roles in ciliogenesis and cilia positioning [58,59], with SDA mediating also microtubule anchoring [58].

The centrosome contains several pools of γ-TuRC (Figure 2), including at the base of the centrioles [56], in the centriolar lumen - anchored via augmin [56,60], in the outer layer of the PCM - anchored via Centrosomal protein of 192 kDa (Cep192)-NEDD1 or A-kinase anchor protein 9 (AKAP9)/Pericentrin-CDK5RAP2 modules [58,60], and at the SDA [58,60]. Remarkably, CKAP5 is necessary for γ-TuRC localization to the PCM and to the SDA [61]. At SDA, γ-TuRC interacts with ninein, important for microtubule anchoring [58], and with AKNA [32] (Figure 2).

Diverse Modes of Microtubule Generation.
(a) The core of the γ-TuRC is formed by GCP2-GCP3, GCP4-GCP5, GCP4-GCP6 pairs and MZT1, which are subsequently joined by actin, four other GCP2-GCP3 γ-Tubulin Small Complexes (γ-TuSCs), and MZT2 [18,28,29]. The γ-TuRC 'splayed' conformation correlates with negligible microtubule nucleation activity, while its partial closure increases it [19–21,26]. **(b)** CM1 domain dimers of CDK5RAP2 bind to the outer parts of the γ-TuRC via MZT2:GCP2 structural modules and push γ-TuRC towards closed conformations, while simultaneously destabilizing actin in the luminal bridge [28,29]. MZT2 not shown in the schematic. **(c)** Branching microtubule nucleation depends on augmin and γ-TuRC and can be enhanced by TPX2, which condenses on microtubules and concentrates components of the branching microtubule nucleation pathway [30,37]. NEDD1 seems to be required for augmin:γ-TuRC interaction [31]. **(d)** CKAP5 acts at both the plus and minus end of microtubules. It binds and stimulates γ-TuRC [41], likely by holding together γ-TuRC and the first tubulin layer, and by stabilization of nascent protofilaments [41]. **(e)** Interplay of novel mechanisms of microtubule minus end regulation. Microtubule minus ends can be timely protected by CAMSAPs or capped by various types of γ-TuRCs. They either protect or destabilize the minus ends by attracting or repulsing microtubule severases or depolymerizing kinesins, such as spastin, katanin or KIF2A, respectively. **(f)** Multivalent MAPs bundle microtubules, concentrate tubulin and many have the ability to undergo LLPS on microtubules or elsewhere [52,53], thus likely facilitating γ-TuRC-independent microtubule nucleation.

Figure 2



Neuronal MTOCs.
In nascent neurons, the centrosome is active as MTOC [15]. There are various γ-TuRC pools in and on the centrosome [60]. The Golgi is another important neuronal MTOC; perturbation of Golgi-dependent microtubule nucleation impairs axonal growth [15]. Studies in *Drosophila* and *C. elegans* implicated Rab5+ and Rab11+ endosomes, respectively, as MTOCs generating minus-end-out microtubules in dendrites [64–66]. Endosomal MTOCs still need to be shown in mammalian neurons.

Other neuronal MTOCs are the Golgi apparatus [62], probably some subsets of the Golgi outposts — although this is still unclear [63], endosomes [64—66] (Figure 2), and microtubules themselves — via augmin [67—70] (Figure 1c). The γ-TuRC anchoring and activating protein ensemble at the Golgi is partially different from the centrosomal machinery [71]. Golgi-associated γ-TuRC is bound and activated by the AKAP9-Myomegalin (MMG) protein complex, which is functionally related to Pericentrin-CDK5RAP2. Notably, AKAP9 also recruits CDK5RAP2 [71]. AKAP9 is anchored at the cis-Golgi membrane via Golgin subfamily A member 2 (GM130) [4,71]. Interestingly, only isoform 8 of MMG, but not CDK5RAP2, facilitates CAMSAP2 recruitment to the microtubules nucleated at the Golgi. Golgi-derived microtubules decorated by CAMSAP2 are further stabilized by CLASPs [71] (Figure 2).

Endosomes are a new class of neuronal MTOCs, recently discovered in *Drosophila* [65] and *Caenorhabditis elegans* [64] neurons. In *Drosophila*, Rab5-positive endosomes localize to the dendritic branch points and recruit γ-TuRC via Axin [65]. In *C. elegans*, γ-TuRC is anchored to Rab11-positive endosomes in the growth cone of outgrowing dendrites by a ninein homolog Non-centrosomal microtubule array protein 2 (NOCA-2) [66] (Figure 2). Microtubule nucleation at endosomes is important for ensuring proper minus-end-out orientation of invertebrate dendritic microtubules (Figure 3).

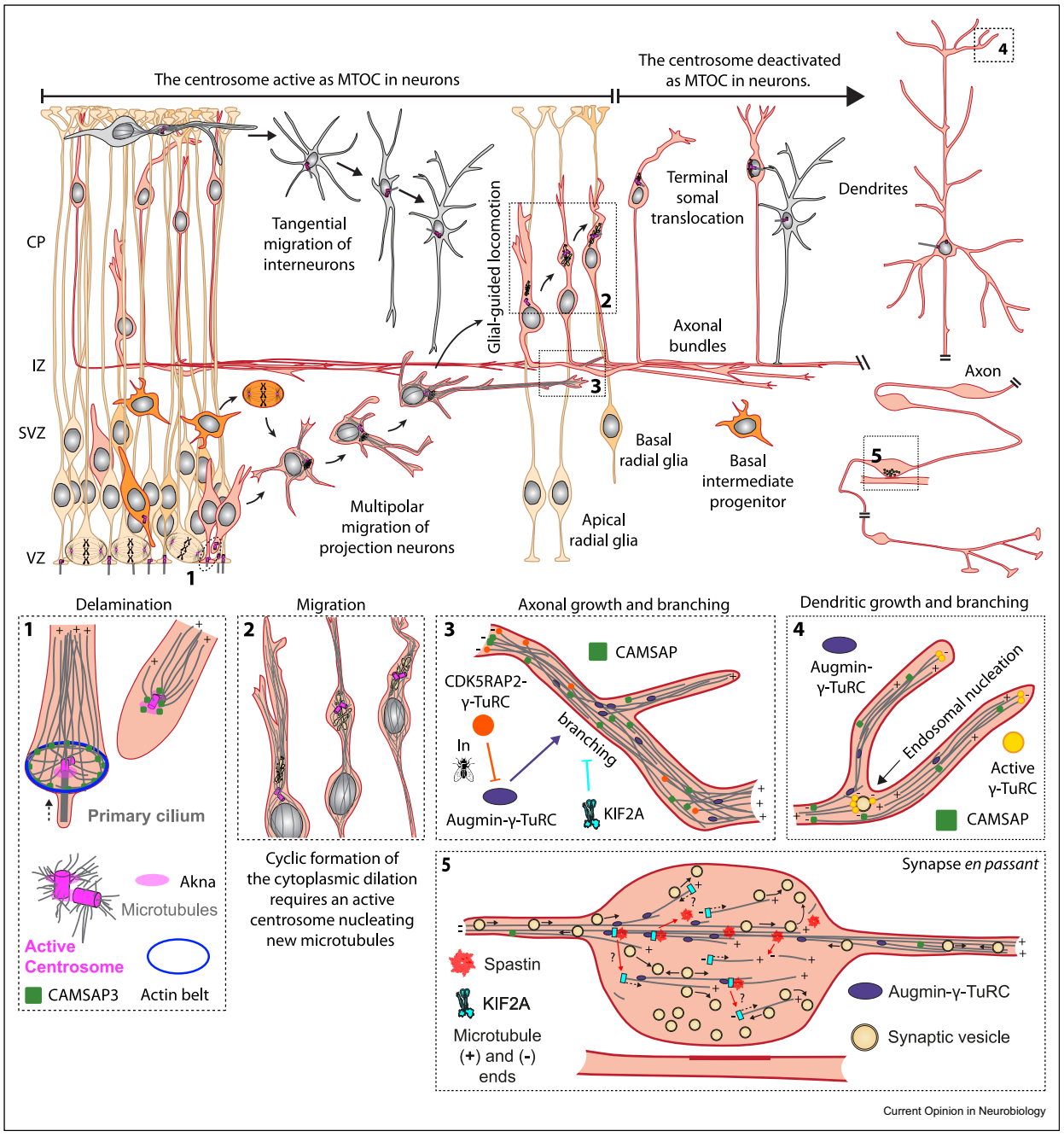
Augmin guides branching microtubule nucleation in both axons and dendrites [67—70] (Figure 3). Although the importance of augmin for microtubule polarity and density in axons and dendrites was shown [67—70], the direct visualization of branching microtubule nucleation via augmin and its molecular mechanism in neurons has remained undefined.

Microtubule nucleation in the neuronal development space-time

Neuronal delamination

Neurons are born after neurogenic divisions of neuronal progenitors [72]. Depending on the type of the progenitor, some newly born neurons delaminate — disassemble their adhesions to neighboring cells in the differentiating neuroepithelium and retract their centrosome-containing apical tip [73] (Figure 3). This process requires active centrosomal microtubule nucleation and actin contractility, and involves N-cadherin downregulation [73]. While centrosomal Akna directs delamination of neuronal progenitors [32], it might also be involved in the delamination of nascent neurons (Figure 3).

Figure 3



Microtubule Nucleation During Neuronal Development.
A schematic of developing rodent neocortex. Neurons can arise from radial glia progenitors or from intermediate progenitors [72]. There are more modes of axon formation than shown in the schematic. VZ-ventricular zone, SVZ-subventricular zone, IZ-intermediate zone, CP-cortical plate. **1.** A speculative model of neuronal delamination in developing rodent neocortex is shown based on data from different systems [32,73]. **2.** Active centrosomal microtubule nucleation is required for radial migration of projection neurons [7,15]. **3.** Augmin-γ-TuRC complex is key for axonal branching, growth and plus-end-out microtubule polarity in axons [67,68,79]. Augmin-γ-TuRC is inhibited by Centrosomin (CDK5RAP2 homolog) in *Drosophila* [88] and might be influenced by the action of KIF2A (may be in complex with spastin), since KIF2A reduces axonal branching in mice [83]. **4.** γ-TuRC complexes nucleate minus-end-out microtubules at the distal dendrites and at dendrite branching points. Endosomes nucleate minus-end-out microtubules in inverterbrates [64–66]. Dysregulation of augmin-γ-TuRC, γ-TuRC itself, and CAMSAPs leads to the reduction of dendritic arbor complexity, length and microtubule mass [68–70,79]. **5.** A speculative model of microtubule mass generation and accumulation of synaptic vesicles in *en passant* synapses by a combined action of augmin-γ-TuRC, spastin and KIF2A. Dashed arrows indicate the direction of depolymerization/degradation.

Neuronal differentiation in mice triggers drastic transcriptional downregulation of several players involved in microtubule nucleation, namely, NEDD1, TPX2 and important kinases such as Cyclin-dependent kinase 1 (CDK1), Serine/threonine-protein kinase (PLK1), and Aurora A [74]. However, AKAP9, MMG, CAMSAP2, and ninein are upregulated [74].

Neuronal alternative splicing generates a dominant non-centrosomal ninein isoform as well as variants of many other proteins, including AKAP9, CLASPs, and KIF2A [75]. While this uncovers that regulators of microtubule nucleation have a surprising diversity and richness in neurons, their functions are still unclear; this new territory is only beginning to be uncovered.

Neuronal polarization

The newly born neurons form dynamic neurites, quickly exploring their surroundings, while moving through the growing vertebrate neocortex [7] (Figure 3). This first step in neuronal polarization varies in different types of neurons, however, it relies on the reorganization of actin and microtubules [5,7]. The impact of microtubule nucleation on neurite formation has remained unclear. Since the centrosome is still active in nascent neurons [15], its localization was frequently studied in relation to the first neurite formation [5]. However, the observed patterns of centrosome behaviour before and during neurite outgrowth in different systems are variable. Moreover, the centrosome moves together with the Golgi, further complicating the interpretation of experiments [5]. Endogenous tagging of various regulators of microtubule nucleation with inducible degrons allows rapid and targeted protein depletion [42]. The employment of this technology in neurons might shed new light on the role of microtubule nucleation and respective MTOCs in the early developmental steps.

Axon specification comes as the next milestone in neuronal development. Here, one of the dynamic neurites acquires initial axonal characteristics, namely, faster and persistent growth and stabilization of microtubule bundles that become progressively unipolar with plus ends pointing towards the growth cone [76,77] (Figure 3). Axon specification depends on microtubule stabilization and bundling [78]. Furthermore, the centrosome has long been implicated in axon specification [11]. However, neither a specific centrosome position, nor nucleation activity is necessary for axon outgrowth, as was recently shown by two alternative approaches: by laser-ablating the centrosome in non-polarized projection neurons and by deactivating its microtubule nucleation capability by displacing centrosomal γ-TuRC with a dominant-negative-acting C-terminal part of NEDD1 [15].

Microtubule nucleation needed for the development of both axons and dendrites depends on the γ-TuRC and augmin (Figure 3). Their depletion reduces microtubule density [67,68,79]. Proper microtubule density in dendrites and axons is also maintained by CAMSAPs [79], which have a profound impact on neuronal polarity and maturation [48,79,80]. Studies in *C. elegans* indicate that microtubule bundles in neurites are maintained by two main pathways: 1) protein complexes containing γ-tubulin and Ninein (NOCA-2 in *C. elegans*) and 2) CAMSAPs (PTRN-1 in *C. elegans*) [66,81]. Either of these pathways can compensate for the impairment of the other [81]. However, in mammalian neurons, loss of γ-TuRC is accompanied by a simultaneous loss of CAMSAP2-decorated microtubules [79], indicating a tighter link between both pathways.

CAMSAP-driven microtubule minus end decoration may be accomplished by decapping γ-TuRC-capped minus ends [20]. Since there is no augmin in *C. elegans*, it would be interesting to investigate whether the vertebrate γ-TuRC-augmin complexes fullfill similar roles as γ-TuRC-NOCA-2 in *C. elegans*, or whether the γ-TuRC-ninein and γ-TuRC-augmin complexes co-exist and cooperate (or compete) in both developing and mature vertebrate neurons.

Intriguingly, although Aprea et al. reported drastic transcriptional downregulation of TPX2 already in nascent neurons [74], Chen et al. detected TPX2 protein in hippocampal neurons [82], localizing besides cytoplasm also to the centrosome and after three days in culture to undefined filamentous structures. The activity of TPX2 depends on RanGTP and importins and its downregulation decreased neurite length [82]. The presence of sufficient amounts of TPX2 might have major effects on branching microtubule nucleation, both with or without augmin. Hence, it would be exciting to investigate the behaviour of this protein in detail using endogenous tagging *in vivo*.

Neuronal migration

While neurons are polarizing and grow their axon, their cell bodies simultaneously migrate to their final destination in the central nervous system (CNS) [7] (Figure 3). Whereas, for example, interneurons are bipolar, cortical projection neurons undergo complex switching of migratory modes during their journey through the cortex [7]. Both radial and tangential migration require a functional centrosome [7,15].

Only recently, it was uncovered that properly regulated centrosomal microtubule nucleation is essential for directional migration of cortical projection neurons [15]. Modulation of the neuronal centrosome to either downregulate or upregulate centrosomal microtubule nucleation resulted in stalled migration and accumulation of neurons in the subventricular (SVZ) and intermediate zone (IZ) of the developing neocortex [15]. Neurons with dysregulated centrosomes failed to switch

from multipolar to bipolar morphology to start locomoting along the radial glia towards the cortical plate [15]. Both too strong and too weak centrosomal microtubule nucleation disrupts proper cycling of the cytoplasmic dilation in the leading process of the migrating neuron [15], which is essential for radial migration [7].

Strikingly, dysregulation of expression and function of the major neuronal microtubule-depolymerising kinesin-13 KIF2A leads to a similar migratory block [83]. Since KIF2A is present at the centrosome [84] and in light of recent findings linking KIF2A and γ-TuRC [51] (Figure 1e), it is tempting to speculate that KIF2A might primarily regulate microtubule minus ends. This might be important for the cyclic remodelling of the cytoplasmic dilation in migrating neurons. Interestingly, KIF2A together with Centrosomal protein of 170 kDa B (Cep170B) locally antagonize microtubule minus end stabilization by CAMSAPs in epithelial cells [85]. Cep170B interaction with microtubules, which attracts KIF2A and repels CAMSAPs, is regulated by phosphorylation [85]. These novel mechanisms of the crosstalk between different microtubule minus end stabilization/destabilization pathways might have a profound impact on neuronal microtubule networks and would be an exciting new avenue for further studies.

In contrast to the centrosome dysregulation, impairment of microtubule nucleation from the Golgi by specific γ-TuRC-displacement, using a GM130-binding domain of AKAP9, attenuated axon growth, while radial migration was unaffected [15]. It indicates that different microtubule networks (e.g. centrosomal vs. Golgi) in neurons are specialized for different tasks (migration vs. axon growth, respectively).

Downregulation of NEDD1, and upregulation of CAMSAPs, AKAP9, MMG, and the acentrosomal ninein isoform [74,75] likely contributes to the progressive loss of microtubule nucleating and anchoring capacity of the centrosome that occurs during neuronal development [15] (Figure 3). Microtubule nucleation then becomes acentrosomal, with AKAP9/MMG, CAMSAPs, augmin and likely ninein becoming the major regulators. Since the centrosome can limit MT nucleation at other MTOCs [86], its switch-off may be required to ensure the massive growth of axons and dendrites.

Neuronal maturation, branching, synapse formation and regeneration

Microtubule nucleation in dendrites and axons is key not only for generation of microtubule mass, but also for orientation of microtubule bundles, and neurite branching [64]. In general, microtubule bundles in axons are uniformly plus-end-out, while they are of mixed polarity in mammalian dendrites and dominantly minus-end-out in longer branches of invertebrate dendrites [12,87]. Remarkably, minus-end-out microtubules are located centrally in dendrites, while plus-end-out microtubules reside on the dendrite periphery [12].

A major player in acentrosomal microtubule nucleation in growing axons and dendrites is augmin [67–70] (Figure 3). Its downregulation leads to the loss of microtubule plus-end-out uniformity in axons and to the loss of microtubule mass [67]. The growth of both axons and dendrites is impaired after augmin or γ-TuRC depletion [68]. The importance of augmin for microtubule branching in neurons with complicated dendritic trees was recently shown in two independent elegant studies on *Drosophila* larval class IV dendritic arborization (da) neurons [69,70]. In addition, microtubule nucleation from endosomes in both *Drosophila* [65] and *C. elegans* [64] is crucial for generation of dendritic minus-end-out microtubules. How all these systems (ninein/NOCA-2-dependent vs. CAMSAPs/Patronin/PTRN-1-dependent vs. augmin-dependent) work together to establish the proper microtubule density and orientation needed for building elaborate neuronal morphology remains to be investigated.

Interestingly, *Drosophila* Centrosomin attenuates dendrite branching by suppressing or competing with the augmin pathway [88]. Indeed, CDK5RAP2- or NEDD1-attached γ-TuRCs are distinguished as two different γ-TuRC pools in other systems [15,34]. As described above, CDK5RAP2/Centrosomin might lock the γ-TuRC at the microtubule minus ends and limit its availability for augmin and other pathways. Similarly, KIF2A suppresses excessive collateral axonal branching [83]. It might act together with spastin on the microtubules capped by augmin-γ-TuRC close to or at the neurite branching points and destabilize them (Figure 3).

Augmin-γ-TuRC regulates axonal *en passant* synapses in hippocampal neurons, where localized bouts of microtubule nucleation are crucial for proper inter-bouton synaptic vesicle transport and exocytosis [89]. A recent study on human neurons confirmed that *en passant* presynaptic sites are hotspots of new microtubule growth, necessary for proper synapse function, and identified spastin as a generator of new microtubules [90]. Whether there is a spastin-KIF2A-γ-TuRC-augmin module acting at these presynaptic sites remains to be investigated (Figure 3).

Coupling microtubule nucleation with microtubule severing seems to be a powerful way to increase microtubule mass and turnover. Spastin, possibly acting with KIF2A close to the γ-TuRC:microtubule interface, could recover γ-TuRCs for new rounds of directed nucleation, ensured by augmin. The newly formed microtubule minus ends might be orderly depolymerized by KIF2A [51], increasing the amount of treadmilling microtubules. Spastin might also act at the microtubule shaft, generating shorter microtubules [90]. Thus, molecular

motors carrying synaptic vesicles along microtubules would reach a microtubule end with increased probability, facilitating synaptic vesicles unloading at the presynaptic site.

There are several indications that microtubule nucleation is neuroprotective after neuronal injury, however, the underlying mechanisms seem complex and invite further research [6,91]. For axon regeneration, microtubules are central. Upon CNS injury, they depolymerize in rodents and human patients [92,93], while they remain intact and bundled after an injury in the peripheral nervous system (PNS) [92]. Microtubule stabilization leads to axon regeneration in the injured spinal cord [93–97]. Yet, it is still unclear where and how the microtubules are nucleated to replenish the lost microtubule mass [6]. Investigation of the intricate interplay between various microtubule nucleation modes and related microtubule networks in neurons might bring novel insights into how neurons develop and regenerate.

Conclusions and future perspectives

We have learned a great deal about centrosomal and acentrosomal pathways of microtubule nucleation in neurons. Yet, we still lack a more comprehensive models of where, when and how the various microtubule nucleation modules are localized, regulated, and how they compete or work together.

The big task ahead is to disentangle various neuronal MT networks and their regulators. Moreover, the intriguing discrepancy between transcriptional downregulation of TPX2 and NEDD1 and their reported (TPX2) or implied (Augmin requires NEDD1 for γ-TuRC binding) functioning later during neuronal development awaits reconciliation. The finding that the type of microtubule nucleation might influence microtubule properties [43] is interesting especially in relation to the different features of minus-end-out and plus-end-out dendritic microtubules in mammals [12]. Many important questions remain open for investigation, including dissecting the dominant minus-end protection and regulation mechanisms. Additionally, understanding the contributions of different microtubule nucleation modes—such as LLPS of MAPs, which requires clarification on its occurrence and role *in vivo*—is crucial for comprehending how microtubule mass is generated during neuronal development and maintained in neuronal homeostasis.

Addressing these questions will ultimately help us to enhance the regenerative potential of neurons.

Declaration of competing interest

The authors declare no conflicts of interest.

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Data availability

No data was used for the research described in the article.

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