A conceptual view at microtubule plus end dynamics in neuronal axons

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Abstract

Axons are the cable-like protrusions of neurons which wire up the nervous system. Polar bundles of microtubules (MTs) constitute their structural backbones and are highways for life-sustaining transport between proximal cell bodies and distal synapses. Any morphogenetic changes of axons during development, plastic rearrangement, regeneration or degeneration depend on dynamic changes of these MT bundles. A key mechanism for implementing such changes is the coordinated polymerisation and depolymerisation at the plus ends of MTs within these bundles. To gain an understanding of how such regulation can be achieved at the cellular level, we provide here an integrated overview of the extensive knowledge we have about the molecular mechanisms regulating MT de/polymerisation. We first summarise insights gained from work *in vitro*, then describe the machinery which supplies the essential tubulin building blocks, the protein complexes associating with MT plus ends, and MT shaft-based mechanisms that influence plus end dynamics. We briefly summarise the contribution of MT plus end dynamics to important cellular functions in axons, and conclude by discussing the challenges and potential strategies of integrating the existing molecular knowledge into conceptual understanding at the level of axons.

1. Introduction

Axons are the slender, up-to-a-metre long processes of neurons which form the cables that wire the nervous system. These delicate structures often need to survive for a lifetime, i.e. many decades in humans. Parallel polar, predominantly plus end out oriented, bundles of microtubules (MTs) run all along axons to form their structural backbones and highways for life-sustaining transport (Baas and Lin, 2011). These MT bundles are so densely packed,with MTs being separated by less than 100nm, that they can only be reliably resolved through electron microscopy (Mikhaylova et al., 2015).

MTs are important at every life stage and for virtually all morphogenetic changes of axons (Penazzi et al., 2016; Prokop, 2013) (Fig. 1). Alterations in MT properties are often linked to developmental and/or intellectual brain disorders, and the precocious decay of MT bundles is seen as an important cause for axon degeneration (Adalbert and Coleman, 2012; Neumann and Hilliard, 2014). MTs are therefore viewed as attractive targets for drug therapies (Baas and Ahmad, 2013; Benitez-King et al., 2004; Eira et al., 2016).

In vitro and *in vivo*, MTs are dynamic, characterised by cycles of endothermic polymerisation, and exothermic depolymerisation (Brouhard, 2015) (section 3). However, *in vivo*, these dynamics are not left to chance but are controlled by different classes of MT binding proteins (MTBPs) and the proteins they recruit and associate with. Such MT binding and associating proteins regulate polymerisation, severing, depolymerisation, stability, transport and force production, as well as cross-linkage and interaction with other organelles or cell structures (Penazzi et al., 2016; Prokop et al., 2013).

Many of these MTBPs and associated proteins have acknowledged links to brain disorders or other human diseases (Prokop et al., 2013). Although the molecular function of these proteins is often known, this knowledge is usually not sufficient to explain their associated diseases. One key challenge is posed by the fact that MT regulators form complex networks that establish robust and redundant machinery which often responds with surprisingly subtle changes upon mutation of single components. Furthermore, the cytoskeleton is required for virtually every cell function including cell division, dynamics and shape, adhesion and signalling, intracellular trafficking, and organelle biology (Alberts et al., 2014). Therefore, mutations of cytoskeletal regulator genes can be expected to have pleiotropic effects, further complicating their study and requiring a broader spectrum of functional readouts.

To shed light into these complex networks and to find explanations for disease phenotypes, it seems a promising strategy to dissect cytoskeletal machinery into its conceptual sub-machineries and then study how these sub-machineries interface with each other. Along these lines, a previous review was dedicated to the intricate relationship of motor proteins and MTs (Prokop, 2013). Here we provide a conceptual overview of the sub-machinery (or sub-machineries) that governs the polymerisation and depolymerisation of MTs in axons.
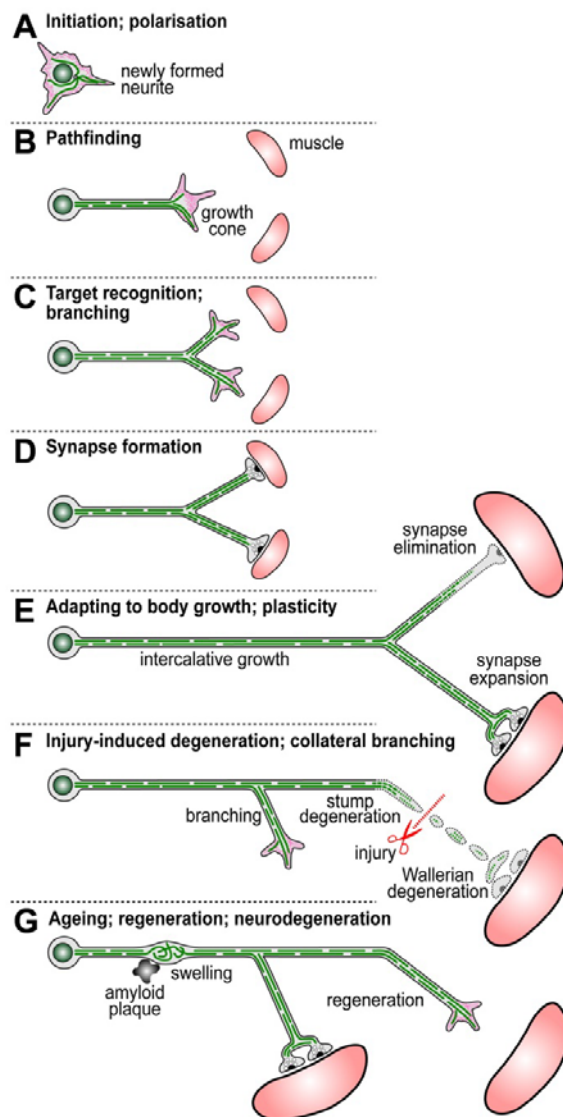
Figure 1: Life stages of axons. Images show one neuron during its various life stages, with the cell body on the left and axon on the right; MTs are shown as green interrupted lines, actin in magenta and muscles as pink round shapes. The different life stages are indicated in the headings, and the various biological processes associated with those stages are annotated in each figure.

2. The importance of MT dynamics in axons

In axons, MTs primarily grow in the anterograde direction with a certain bias towards the more distal axon segment, but retrograde polymerisation events have also been reported when performing live imaging with typical plus end markers (Kollins et al., 2009; Sánchez-Soriano et al., 2010; Stepanova et al., 2003; Yau et al., 2016). These may reflect either plus end polymerisation of antiparallel MTs or minus end polymerisation of parallel MTs, which can be observed *in vitro* (Akhmanova and Hoogenraad, 2015; Akhmanova and Steinmetz, 2015; Jiang et al., 2014).

The abundance of MT polymerisation along the axon shaft could mean that axonal MT mass is generated in the shaft, rather than in growth cones (Fig.1C) where polymerisation gives rise to rather short-lived MTs similar to those in non-neuronal cells (Prokop, 2013; Prokop et al., 2013). It has been proposed from studies in developing vertebrate and *Drosophila* neurons that the MT mass generated in the axonal shaft gradually shifts anterogradely (Miller and Sheetz, 2006; Roossien et al., 2013). In such a scenario, the growth potential would originate from MT bundles along the axon and growth cones would be the intelligent units which can control this extension and its directionality.

This model of shaft-based growth is further supported by the fact that significant axon growth occurs at post growth cone stages (Fig. 1E). Thus, many growth cones reach their target destination and transform into synapses already in the embryo. Since the organism continues to grow towards its much larger adult dimension, significant further axon elongation has to occur through intercalative shaft growth (Bray, 1984).

Even in adulthood, MT polymerisation does not seize. Firstly, it is required for plastic growth and branching as well as for regenerative growth (Fig. 1F,G) (Bilimoria and Bonni, 2013; Bradke et al., 2012; Kalil and Dent, 2014; Lewis et al., 2013). Secondly, most axons have to be sustained for the lifetime of an organism, i.e. up to a century in humans, and continued MT turn-over is needed to prevent senescence through mechanical wear (Dumont et al., 2015) and remove irreversible posttranslational modifications (Janke and Kneussel, 2010). Accordingly, continued MT polymerisation has been demonstrated in adult *Drosophila* brains (Medioni et al., 2015; Nguyen et al., 2011; Rolls et al., 2007; Soares et al., 2014) and similar results can be expected in vertebrates. Thirdly, the MT volume of many axons seems to be a well-regulated property. This is suggested by neuron type-specific axon diameters and MT densities (Wortman et al., 2014), and the fact that artificially slimmed axons (achieved through pulling) reconstitute their original diameters (Bray, 1984). How axon diameters are sensed and MT volume (i.e. number and density) maintained within, remains largely unresolved. Intermediate filaments may play a role in regulating axon diameter and MT volume (Kriz et al., 2000) (and references therein). However, other mechanisms must be in place, because *Drosophila* also has defined axon diameters but lacks intermediate filament genes (Weber et al., 1991).

In any case, it seems a reasonable assumption to propose that axons "know" and continuously adapt their MT volume, and that the MT de/polymerisation machinery has to be one key element of this regulation. This poses the fundamental question of what the molecular nature of this machinery is.

3. Properties of MTs and MT dynamics as revealed by *in vitro* studies

Before delving into the molecular complexity observed at the cellular level, it is important to provide a brief overview of our knowledge about the physical and biochemical properties of tubulin and MT dynamics gained from 5 decades of intense *in vitro* studies.

MTs are the stiffest of the three cytoskeletal polymers with a persistence length of ~5 mm, as compared to ~12 μm measured for actin filament (Fletcher and Mullins, 2010; Howard, 2001). The building blocks of MTs are α- and ß-tubulin heterodimers which will polymerise into MTs even *in vitro*. They form strong longitudinal head-to-tail bonds which make up the protofilaments. As detailed elsewhere (Chretien and Fuller, 2000; Howard, 2001), between 8 and 19 protofilaments arrange in parallel into a sheet by forming lateral bonds between homologous (α-α, ß-ß; B-type lattice) or heterologous subunits (α-ß; A-type). To form a tube, the edges of this sheet join, forming a seam at which the joining protofilaments are out of register by

2-4 tubulins (referred to as "start helical arrangement"; Fig. 2). In consequence, the lateral bonds of MTs take on a helical structure where the angle of the helical rise (as a function of start helical arrangement and protofilament number) can cause a skew of lateral bonds of up to 2°; such skew angles were proposed to be energetically unfavourable (Chretien and Fuller, 2000). Accordingly, the majority of MTs contain 13-protofilaments with a 3-start helical arrangement, where the skew angle is almost 0° and the outer diameter is 25nm. However, MTs with different protofilament numbers occur *in vivo*, but the functional relevance of this is not clear (Chrétien and Wade, 1991) (and references therein).

MTs are polar, with a plus end (with exposed ß-tubulin) and a minus end (with exposed α-tubulin). *In vitro*, both ends have very different kinetics of MT polymerisation or depolymerisation; as an extreme example, conditions can be set so that MTs undergo treadmilling similar to actin filaments, polymerising at plus ends and depolymerising at minus ends (Margolis and Wilson, 1998; Panda et al., 1999).

In vitro, new MTs are either nucleated via stabilised MT fragments anchored to the glass surface as powerful 'seeds' to form MTs (Akhmanova and Steinmetz, 2015) or polymerisation is facilitated by additional factors such as the MTBP tau (Akhmanova and Steinmetz, 2015; Brandt and Lee, 1993a, b) This resembles the situation in cells, where new MTs originate from nucleation complexes; these complexes contain a ring of □-tubulin as the key component onto which tubulin heterodimers are polymerised, and which subsequently anchors the minus ends of the newly formed, elongating MT. Nucleation complexes containing □-tubulin can be found on centrosomes, the kinetochore of chromosomes, other MTs, the nuclear envelope, the Golgi surface or the cell cortex (Petry and Vale, 2015; Teixido-Travesa et al., 2012). Accordingly, □-tubulin is also found in axons and, together with the augmin complex, forms a plus end-out directed nucleation machinery that can uphold MT bundle polarity (Nguyen et al., 2014; Sanchez-Huertas et al., 2016; Stiess et al., 2010). As a further mechanism, MT fragments were suggested to act as sites for re-polymerisation in axons (Baas et al., 2016). Such MT fragments could potentially be bound and directionally anchored to the axonal cortex via gigantic actin-MT linker molecules called spectraplakins (Baas et al., 2016; Nashchekin et al., 2016) (section 5).

Subsequent polymerisation *in vitro* can also be achieved in the absence of MTBPs, but polymerisation speeds are significantly lower than observed *in vivo* or when adding the right cocktail of MTBPs (Zanic et al., 2013). In MTBP-free *in vitro* assays, the essential parameters determining the polymerisation speed include the concentrations of tubulin, guanosine triphosphate (GTP), Mg^{2+} (influencing the GTP-tubulin interaction), calcium chelators, as well as an appropriate temperature to drive the endothermic polymerisation reaction (usually 37°C); also the tubulin isotype has an impact, with β3-tubulin de/polymerising considerably slower than other β-tubulins (Grover and Hamel, 1994; Lu and Luduena, 1994b).

The addition of GTP is important because α- and β-tubulins bind GTP (referred to as GTP-tubulin). Under standard conditions, GTP-tubulin is effectively incorporated into MTs, whereas GDP-tubulin incorporation *in vitro* requires non-physiological conditions (Lin and Hamel, 1987) (and references therein). Following incorporation into MTs, the GTP of β-tubulin (but not α-tubulin) gets hydrolysed (these heterodimers are referred to as GDP-tubulin) (Akhmanova and Steinmetz, 2015). This hydrolysis occurs with a certain delay, so that GTP-tubulin forms a zone of several hundred nm from the polymerising MT tip which is often referred to as the GTP cap (Fig. 2). As detailed elsewhere (Akhmanova and Steinmetz, 2015), *in vitro* experiments have demonstrated that the GTP cap has two important functions. Firstly, it provides the substrate to which EB proteins bind which, in turn, recruit a huge number of further proteins to the MT plus end (see section 4 for details). Secondly, the GTP cap directly stabilises MT plus ends as a consequence of its structural configuration which is favourable for the alignment into straight protofilaments. However, the hydrolysis to GDP-tubulin introduces a

compaction within the tubulin molecule which seems to generate longitudinal strain and hence stored elastic energy (Brouhard and Rice, 2014). This has no immediate consequence, for as long as lateral and longitudinal bonds hold the tubulin in its straight confirmation. But if the GTP cap shrinks, GDP-tubulin protofilaments may break their lateral bonds and release their stored energy by peeling off like banana skin, thus very rapidly depolymerising the MT plus end. These dynamic changes at MT plus ends (termed dynamic instability) occur *in vitro* and in cells, and are referred to as pause (de/polymerisation comes to a halt), catastrophe (polymerisation/pause turns into depolymerisation) and rescue (depolymerisation turns into pause or re-polymerisation).

Finally, MTs *in vitro* do not only de/polymerise at their plus ends, but also at their minus ends (though with far slower kinetics) (Akhmanova and Hoogenraad, 2015; Hendershott and Vale, 2014), and it has been shown that polymerisation can occur even along the MT lattice to repair lateral damage (Schaedel et al., 2015).
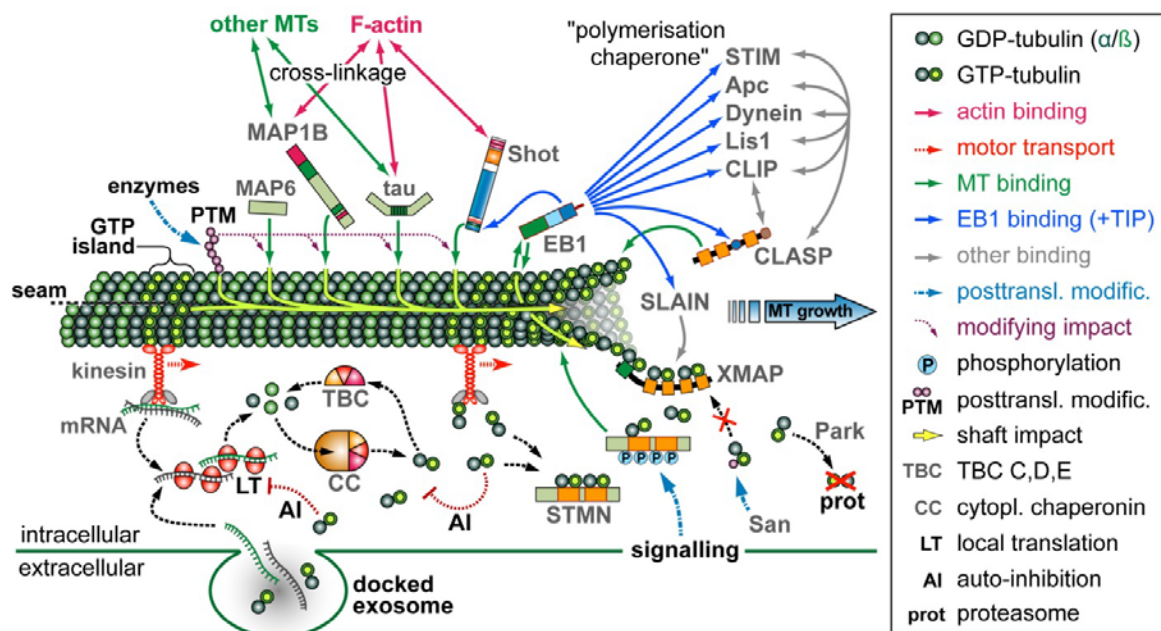

Figure 2: Integrated view of the various mechanisms regulating MT plus end dynamics. The image shows a single MT surrounded by regulating factors (gene/protein names in grey). Symbols are explained in the box on the right and further aspects are annotated in the figure, with details provided in the main text.

4. Regulating tubulin availability in axons

As has become clear from *in vitro* studies, the concentrations of tubulin dimers at plus and minus ends of MTs are essential parameters determining de/polymerisation (section 3). This raises the important question of how tubulin pools are controlled in axons.

In neurons, tubulin is under tight transcriptional control (Blackmore, 2012) and synthesis of specific isotypes can be favoured by stabilisation of their mRNAs (Rogers et al., 1993). Once produced, tubulin proteins tend to be rather stable, with half-lives ranging between 15-50 hrs depending on the cellular model (Caron et al., 1985a; Mooney et al., 1994; Semotok and Lipshitz, 2007). However, this half-life is not absolute but subject to tissue type (Burow et al., 2015), culture substrate (Mooney et al., 1994) and cell-cycle state (Ducommun et al., 1990). Furthermore, tubulins can be recycled for new rounds of polymerisation (Nogales and Wang,

2006), as illustrated also by *in vitro* studies with purified brain tubulin which can be repeatedly polymerised and depolymerised (e.g.) (Chretien and Fuller, 2000).

How the pools of tubulin are supplied and regulated in axons, especially during times of high net demand (e.g. during axon growth), remains little understood. One mode is motor protein-mediated transport of tubulin mRNAs, monomers, dimers or even MT fragments travelling at fairly low speeds of ~1mm per day (Fig. 2) (Frühbeis et al., 2013; Galbraith and Gallant, 2000; Tang et al., 2013) For a more immediate supply, axons contain the machinery to perform local tubulin biosynthesis from mRNAs, although cell culture studies have suggested that not more than 5% of axonal tubulin are generated this way (Eng et al., 1999; Giuditta et al., 2002b; Giuditta et al., 2008; Jung et al., 2012; Lee and Hollenbeck, 2003). However, it might be expected that local provision is far more essential in long axons *in vivo*, where also glia-derived supply of tubulin mRNA and/or protein, for example via exosomes, may play important roles (Fig. 2) (Frühbeis et al., 2013; Giuditta et al., 2002a). As a further advantage, local biogenesis would provide flexible means to fine-tune tubulin levels, and posttranscriptional cytoplasmic auto-regulation of tubulin has been reported (Theodorakis and Cleveland, 1992) (and references therein). For example, artificially raising the levels of tubulin dimers in de-nucleated fibroblasts (a situation similar to axons) caused a down-regulation of tubulin biosynthesis, reducing both mRNA and protein half-life (Fig. 2) (Caron et al., 1985b).

Tubulin levels in neurons can also be regulated through the ubiquitin ligase parkin and proteasome-mediated degradation (Fig. 2) (Ren et al., 2003), and through components of the chaperone machinery (Fig. 2). The biogenesis of GTP-tubulin is a multi-step process where native tubulins first encounter prefoldin, then enter the large ATP-dependent cytoplasmic chaperonin complex where they acquire their shape and GTP-bound status, after which the chaperonin cofactors A-E (TBCA-E) take over to catalyse dimerisation and cytoplasmic release (Bhamidipati et al., 2000; Tian et al., 1996). However, TBCC, -D and -E can also act outside this machinery by binding and cleaving heterodimers (Fig. 2) (Bhamidipati et al., 2000; Serna et al., 2015). This fine balance between MT promoting and destabilising roles of these TBCs may explain the contradictory results obtained from different gain- and loss-of-function experiments with TBCs, suggesting them to be either promoters or inhibitors (Bhamidipati et al., 2000; Bömmel et al., 2002; Jin et al., 2009) (and references therein).

It appears therefore that free cytoplasmic tubulin heterodimers would not have a long half-life and require stabilising factors. Such stabilisers could be stathmins which contain two tubulin binding regions (four in some isoforms of *Drosophila* stathmin), each binding one tubulin heterodimer (Fig. 2) (Chauvin and Sobel, 2015; Lachkar et al., 2010). Whilst stathmins are traditionally considered sequestering factors for tubulin heterodimers and inhibitors of MT polymerisation, their reported mutant or over-expression phenotypes suggest a complicated mix of MT promoting and inhibiting roles (Chauvin and Sobel, 2015; Manna et al., 2007; Manna et al., 2009; Riederer et al., 1997), as similarly observed for TBCs. These findings are likely due to the opposing roles that stathmins play (Chauvin and Sobel, 2015; Gupta et al., 2013; Nouar et al., 2016) (and references therein). On the one hand, stathmins are likely to maintain pools of tubulin heterodimers, protecting them from degradation and withholding them from autocatalytic down-regulation of their own biosynthesis, as was explained above. On the other hand, they induce catastrophes through active binding to protofilaments at MT plus ends and withdrawing tubulin heterodimers from the polymerisation machinery. These various functions are controlled by four phosphorylation sites in the Stathmin Like Domain (SLD) which are targeted by various signalling pathways (Fig. 2). A key question is therefore how phosphorylation and heterodimer release of stathmins is spatiotemporally controlled in axons, and how this is coordinated with polymerase activity (for example whether there is close functional collaboration as is the case for Enabled and profilin during actin polymerisation) (Bear and Gertler, 2009).

Apart from tubulin availability in the axon, also the type of tubulins needs consideration, because different tubulins have significantly different polymerisation dynamics (section 3), suggesting neuron type-specific deviations. Furthermore, the acetyltransferase Nat13/San was shown to mediate acetylation of cytosolic tubulin heterodimers at K252 of ß-tubulin, thus inhibiting their incorporation into MTs (Fig. 2) (Chu et al., 2011).

Taken together, a cocktail of mechanisms regulates the availability of tubulins, thus influencing MT de/polymerisation in neurons. The importance of this machinery is clearly illustrated by the prominent links that components of this machinery have to brain disorders.

5. The MT de/polymerisation machinery at MT plus ends

The number of proteins associating with MT plus ends (referred to as +TIPs) is steadily increasing, and many have been reported to influence MT stability or polymerisation (Fig. 2) (Akhmanova and Steinmetz, 2010; Akhmanova and Steinmetz, 2015; van de Willige et al., 2016). Strikingly, no reports so far seem to have pinpointed a single master +TIP of polymerisation, the absence of which would cause the total or next to complete loss of MTs. As one explanation, the "polymerisation chaperone hypothesis" was put forward (Fig. 2) (Gupta et al., 2014). This hypothesis states that the many known functional interactions between the various +TIPs construct molecular networks that culminate in a protein superstructure protecting and stabilising MT plus ends and promoting their polymerisation. Loss of one or few types of +TIPs from such superstructures would have little impact due to functional redundancy.

Alternatively, there could be two or more central players which can partly compensate for one another. The other +TIPs would have less significant functions such as fine tuning plus end polymerisation dynamics and adapting +TIP dynamics to different cellular contexts (see also section 7). As will be discussed, central players could be EB proteins and XMAP215, and potentially CLASP. In support of this notion, *in vitro* studies demonstrated a strong synergy between EB1 and XMAP215, significantly speeding up MT polymerisation to *in vivo*-like levels (Zanic et al., 2013).

As summarised elsewhere (Akhmanova and Steinmetz, 2015), up to a few hundred EB molecules can bind to a MT plus end with a very short dwell time, i.e. in a highly dynamic manner with constant turn-over. EB localisation at polymerising MT plus ends closely correlates with the GTP cap, mediated by the N-terminal calponin homology domain which simultaneously binds to four GTPs, linking together tubulin heterodimers across two parallel protofilaments (Akhmanova and Hoogenraad, 2015). In this way, EBs might stabilise lateral and longitudinal tubulin bonds. At the same time, EBs also promote GTP hydrolysis so that, at high concentration, they may cause catastrophes - representing a further set of factors juggling a balance between promotion and inhibition at MT plus ends (Akhmanova and Hoogenraad, 2015; Brouhard and Rice, 2014; Maurer et al., 2014).

The importance of EBs for neuronal microtubules was demonstrated in *Drosophila,* where EB depletion causes severe MT disorganisation and severe axon shortening (Alves-Silva et al., 2012). Short axons are likely to reflect a significant reduction in MT volume due to reduced net-polymerisation. This could either be explained through the direct effects of EB proteins on the MT lattice, or through the role of EBs as scaffold proteins recruiting numerous +TIPs. Thus, the C-terminal coiled-coil and EB homology domains of EB proteins mediate homo- and hetero-dimerisation as well as interaction with other +TIPs, allowing those proteins to "hitchhike" on MT plus ends (Fig. 2) (Akhmanova and Steinmetz, 2015).

These MT plus end hitchhikers often have a certain ability to bind MTs directly (a property that, combined with EB binding, could enable them to "surf" along the growing MT plus

ends) (Gupta et al., 2014). They also form numerous links with one another, which has led to the before mentioned chaperone hypothesis (Gupta et al., 2014). However, their localisation at MT plus ends is essentially determined by direct interaction with the C-terminus of EB proteins: either via a CAP-Gly domain binding to the EEY tail (e.g. CLIPs and p150), or via positively charged protein stretches containing SxIP motifs which bind to the EB homology domain (e.g. APC, spectraplakins, CLASP, KIF2C, KIF11, NAVs and SLAIN) (Akhmanova and Steinmetz, 2015; Honnappa et al., 2009). The C-terminus of EBs is very short so that binding of SxIP motifs and CAP-Gly domains to EB proteins is considered mutually exclusive and hence competitive (Duellberg et al., 2014). Binding to these specific domains seems to occur in hierarchical order of affinity which can also be modified through signalling events (Akhmanova and Steinmetz, 2015).

The contributions of individual hitchhikers to MT plus end dynamics remain little understood (Akhmanova and Steinmetz, 2010; Akhmanova and Steinmetz, 2015; van de Willige et al., 2016). Reported mutant phenotypes tend to be subtle and often inconsistent, and their impact on MT dynamics may be indirectly influenced by additional cellular functions of these proteins (for example, APC acts as an actin nucleator) (Okada et al., 2010). In addition, significant differences were reported between +TIP behaviours in neuronal axons and non-neuronal cells, and careful validation is required before adapting the enormous body of knowledge acquired in non-neuronal cells to axonal contexts (Beaven et al., 2015).

XMAP215/CKAP5/CHTOG does not require EB proteins but can bind to MT plus ends via its N-terminal TOG domain (Fig. 2) (Al-Bassam and Chang, 2011). In this position, it acts as a processive microtubule polymerase by binding soluble tubulin heterodimers via its C-terminal TOG domains and promoting their polymerisation (Fig. 2) (Akhmanova and Steinmetz, 2015; Brouhard and Rice, 2014); this polymerisation activity can be regulated through mechanical stretch (Trushko et al., 2013). XMAP215 clearly plays a role in axons, for example during axon initiation and during growth cone guidance, and does so in close collaboration with the transforming acidic coiled-coil protein TACC3 (Bearce et al., 2015; Lowery et al., 2013; Nwagbara et al., 2014). Our own studies with depletion of Mini spindles (Msps), the *Drosophila* homologue of XMAP215, reveal a very severe reduction in axon growth (unpublished data); like in the case of EB1 depletion, this suggests a potential major role in MT polymerisation in axons.

As mentioned earlier, *in vitro* polymerisation assays have revealed a strong synergy between EB proteins and XMAP215 (Zanic et al., 2013). This seems surprising because observations *in vitro* and in HeLa cells suggest that both proteins are spatially separated (Fig. 2): XMAP215 binds preferentially to curved protofilaments occurring at the very tip, whereas the bulk of EB proteins binds up to several hundred nm away from the tip (Akhmanova and Steinmetz, 2015; Maurer et al., 2014). It has been proposed that EBs could contribute to the straightening of protofilaments at the MT plus end, thus helping XMAP215 to shift anteriorly to curved protofilaments and engage in its next polymerisation cycle (Brouhard and Rice, 2014; Zanic et al., 2013). Alternatively, it was reported in HeLa cells and cultured neurons that the protein Slain2 (with SxIP-like motifs) links EB1 to XMAP215 thus promoting MT polymerisation (van der Vaart et al., 2012; van der Vaart et al., 2011). A similar mechanism was suggested for the non-related Sentin protein in *Drosophila* which likewise contains SxIP-like motifs and links EB1 and Msps (Li et al., 2011; Li et al., 2012).

The MT plus end polymerisation machinery might contain additional factors redundant to XMAP215. For example, the EB-binding CLASP proteins contain TOG-like domains (Fig. 2); they regulate MT catastrophes and promote re-polymerisation via tubulin recruitment (Al-Bassam et al., 2010; Brouhard and Rice, 2014). In axons, CLASP function can switch between growth inhibition and promotion, crucially dependent on cellular contexts and GSK-3β signalling (Bearce et al., 2015). In a recent report, the reduction of axon growth observed upon CLASP

knock-down in regenerating neurons was so strong (Hur et al., 2011) that CLASP might well act as a polymerase in this context. Furthermore, also stathmins might contribute as redundant factors during polymerisation because they bind tubulin heterodimers and can associate with MT plus ends (Fig. 2; see section 4).

Finally, it needs to be mentioned that some +TIPs primarily disassemble MTs (Homma et al., 2003). These include members of the kinesin-13 family (e.g. Kif2C/MCAK) which use their ATP-dependent motor domain to actively remove tubulins. They also include members of the kinesin-8 family which walk along MTs towards the plus tip and take tubulins with them when falling off the tip (Brouhard and Rice, 2014). Such mechanisms seem relevant in axons, as shown for the kinesin-13 member Kif2A which inhibits collateral branching (Homma et al., 2003).

6. Influence of lattice-based mechanisms on MT de/polymerisation

Axons are known to contain subpopulations of stabilised MTs (Baas et al., 2016). In our view the term stabilisation is imprecise and at least five modes of MT stabilisation need to be distinguished: (1) stabilising MTs through sustaining their plus end polymerisation (e.g. maintaining polymerase active; see previous section) (Qu et al., 2016); (2) stabilising MT lattices against depolymerisation (e.g. turning depolymerisation into pause or recovery); (3) stabilising MTs against disassembly through severing proteins (Yu et al., 2005); (4) stabilising MT minus ends through proteins such as CAMSAP/Patronin (Akhmanova and Hoogenraad, 2015); (5) stabilising MTs against mechanic deformation through bundling or other forms of cross-linkage.

In line with the topic of this review, we will focus here on a number of lattice-based mechanisms which affect plus end dynamics, either by preventing depolymerisation or through promoting polymerisation (see yellow arrow in Fig. 2). First, it has been reported that axonal MTs maintain high levels of GTP tubulin (Nakata et al., 2011) which, based on observations *in vitro*, should have stabilising functions. Thus, GTP tubulin at MT plus ends prevents catastrophes (section 3) and islands of GTP-bound tubulin within the MT lattice trigger rescue events (Dimitrov et al., 2008; Tropini et al., 2012).

Second, posttranslational modifications (PTM) of MTs are often taken as indicators for MT stability. In particular detyrosination and acetylation were shown to correlate well with stable MT fractions when analysed at the ultrastructural level (Baas et al., 2016). Commonly used indicators of acetylation are antibodies against acetylated K40 of α-tubulin. This residue is positioned on the luminal side of MTs (Howes et al., 2014; Li and Yang, 2015; Soppina et al., 2012); whether K40 itself mediates MT stabilisation, or other lysine residues potentially acetylated in parallel (Choudhary et al., 2009), will need further investigation. How PTMs stabilise MTs remains poorly understood: they could directly stabilise tubulin bonds, or they could modify MT interactions with stabilising MAPs or destabilising kinesins (Baas et al., 2016; Janke and Bulinski, 2011). Exploring these mechanisms is complicated by the pleiotropic functions of PTM-mediating enzymes; for example, the acetyltransferase α-TAT1 (responsible for K40 acetylation of α-tubulin) (Akella et al., 2010; Friedmann et al., 2012; Howes et al., 2014) regulates MT stability through direct MT interaction which is independent of its acetylation function (Kalebic et al., 2013).

Tubulin amination catalysed by transglutaminases, is a PTM essentially contributing to cold-stable MTs in brain extracts (Song et al., 2013). It has been suggested that amination could protect fragments of MTs from disassembly which could then be used as initiation points for *de novo* polymerisation, thus helping to maintain MT populations in axons (see Section 3) (Baas et al., 2016; Song et al., 2013). Apart from amination, cold protection is conferred also by

MAP6/STOP (stable tubule only peptide; Fig. 2). MAP6 has been shown to bind and stabilise MTs preferentially at lower temperatures induced by conformational changes in its MT-binding Mc domain (Delphin et al., 2012; Poulain and Sobel, 2010). However, MAP6 seems to play roles also at normal temperatures, as suggested by progressive developmental brain dysfunction observed in MAP6 knock-out animals (Volle et al., 2013) (and references therein) - although these functions might work through mechanisms independent of MT binding (Deloulme et al., 2015).

Third, classical MAPs including MAP1B and tau have long been known as stabilisers against drug-induced de-polymerisation in axons (Takemura et al., 1992). They are likely to stabilise tubulin-tubulin bonds and/or compete for tubulins with destabilising kinesins or drugs. However, the mechanisms through which MAPs act remain little understood. For example, tau has been suggested to be co-assembled into MT lattices, whereas others suggest dynamic interaction with very brief dwell times on MTs (Amos and Schlieper, 2005; Janning et al., 2014; Kadavath et al., 2015); clearly, these two modes would be associated with very different mechanisms of MT stabilisation.

Further challenges for the understanding of classical MAPs are posed by the staggering inconsistency of phenotypes observed in different knock-out models of tau and MAP1B (Chilton and Gordon-Weeks, 2007; Morris et al., 2011; Villarroel-Campos and Gonzalez-Billault, 2014), as well as the enormous breadth of cellular functions they are suggested to perform, apart from stabilising MTs against depolymerisation. Such functions include MT bundling, protection against MT severing proteins, regulation of axonal transport, MT nucleation, actin regulation and MT-actin cross-linkage, the regulation of signalling processes and of MT plus end polymerisation (Elie et al., 2015; Morris et al., 2011; Rosenberg et al., 2008; Villarroel-Campos and Gonzalez-Billault, 2014).

Of these functions, potential roles in MT plus end polymerisation are of particular interest for this review, and several potential mechanisms have been proposed. First, MAP1B has been suggested to impact negatively on MT plus end polymerisation through sequestering EB1/3 in the cytoplasm (Tortosa et al., 2013). However, this report deviates from other reports showing that MAP1B promotes axon growth and MT polymerisation (Tymanskyj et al., 2012; Villarroel-Campos and Gonzalez-Billault, 2014). An alternative model can be deduced from work on the centrosomal protein TPX2 which localises along MT shafts up to their tips, where it directly binds and stabilises XMAP215 (Roostalu et al., 2015). Similarly, shaft-based tau was suggested to bind and stabilise EB1 (Sayas et al., 2015), and the same might be true also for MAP1B. Finally, *in vitro* work has shown that classical MAPs directly promote the incorporation of tubulins, potentially by neutralising or even attracting their negatively charged C-terminal domains (Lu and Luduena, 1994a) (and references therein).

A high degree of functional redundancy has been suggested for classical MAPs. Accordingly, the combined loss of different MAPs was shown to cause enhanced phenotypes including stronger reduction in neurite growth (Takei et al., 2000; Teng et al., 2001). More recently, proteins of the spectraplakin family, in particular ACF7/MACF1 and BPAG1/dystonin, were suggested as factors redundant to classical MAPs (Voelzmann et al., 2016). Like tau and MAP1B, spectraplakins are potent MT lattice binders and stabilisers, and they can also influence plus end polymerisation dynamics in axons (Alves-Silva et al., 2012; Qu et al., 2016; Sun et al., 2001). Unlike tau and MAP1B, spectraplakin deficiency causes very strong MT phenotypes correlating with neuro-developmental disorders as well as severe neurodegeneration in mouse and humans (Edvardson et al., 2012; Ferrier et al., 2013; Goryunov et al., 2010). Spectraplakins are therefore very likely candidates for masking key functions of tau and MAP1B and should be considered in future studies.

7. Other cellular functions of MT plus ends in axons

So far the focus of this review lay on the role of plus end de/polymerisation in the context of net growth and MT volume regulation. However, MT plus end dynamics have a greater scope of functions in axons. Analogous to the role that growth cones play for axons, plus ends can be considered as the steering units of MTs which can interact with their environment and determine the directionality and fate of MTs, thus turning MTs into important tools contributing to cellular processes. Below, we will depict some examples:

MT plus ends interact with proteins at the plasma membrane. Non-favourable membrane compartments contain factors repelling MTs by inducing their collapse. Such mechanism could be important in growth cones where MTs pointing away from the direction of future growth tend to retract (Bearce et al., 2015; Prokop et al., 2013). Some suggested factors mediating MT collapse include cortical katanin (Zhang et al., 2011) as well as Efa6 (the *C. elegans* homologue of PSD/pleckstrin and sec7 domains-containing protein) which is an inhibitor of axon regeneration (Chen et al., 2015; Chen et al., 2011; O'Rourke et al., 2010).

Vice versa, molecular networks associated with the cellular cortex can positively guide MT extension in neurons, for example by establishing links of plus ends to sub-membranous actin networks via spectraplakins (Alves-Silva et al., 2012) or drebrin-EB3 complexes (Geraldo et al., 2008). Furthermore, the MT plus end binding protein navigator1 localises to the periphery of growth cones and mediates axonal guidance (Martinez-Lopez et al., 2005).

MTs can also be captured at specialised membrane compartments, for example to deliver signals or materials via MT-based transport to cell junctions. Capture mechanisms were proposed to include the MT plus end binding proteins CLIP, APC, CLASP (Komarova et al., 2002; Schmidt et al., 2012; Swiech et al., 2011; Temburni et al., 2004), links from dynein to the NCAM adhesion receptor (Perlson et al., 2013), direct interaction of β3-tubulin with Netrin receptors (Qu et al., 2013), or palmitoylation of α-tubulin (Caron et al., 2001). As a further mode of mutual interaction, it has recently been described that cortical actin networks of axons act as powerful promoters of MT plus end polymerisation (Qu et al., 2016).

MTs are essential for the structure of endoplasmic reticulum (ER) which forms uninterrupted tubular networks all along axons that have been referred to as "neuron-within-neuron" (Berridge, 1998). The importance of axonal ER is illustrated by its close links to motor neuron disease (Blackstone et al., 2011; Yalçýn and O'Kane, 2013) and is explained by its many functions: ER regulates levels of intracellular free calcium in the axonal cytoplasm, calcium homeostasis, fission and fusion of mitochondria, and it has been suggested to mediate local translation of membrane-associated or releasable proteins as well as lipid biogenesis contributing to axonal membranes (Berridge, 1998; Friedman and Nunnari, 2014; Gonzalez and Couve, 2014; Korobova et al., 2013; Wojnacki Fonseca and Galli, 2016). The SxIP protein STIM1 can link ER to MT plus ends to mediate directed ER elongation (Grigoriev et al., 2008). Since STIM1 can also regulate calcium influx at the plasma membrane (Cahalan, 2009), it might be a crucial mediator between ER and axonal surfaces to control levels of intracellular free calcium, and MTs might play important roles during this process. This latter notion would be in line with the general idea that MTs, and in particular also MT plus ends, are important hubs for signalling events in cells (Dent and Baas, 2013).

8. Conclusions and future prospects

Fig. 2 illustrates the complexity of the various regulatory networks coordinating MT plus end dynamics in axons. To make sense of these dynamics at the cellular level, we need to

integrate the detailed knowledge we have gathered, into conceptual systemic understanding. For example, as was explained above, a number of factors including EB1, stathmins, XMAP215, CLASPs, TBCs and tubulins themselves have the ability either to promote or to inhibit MT plus end polymerisation. This cocktail of bi-directional regulators could form an effective buffer against unwanted changes in polymerisation processes. On the other hand, intentional changes induced by signals need to achieve a systemic reversal of the activity status of many or even all of these regulators. Furthermore, a number of proteins mentioned in this review are not restricted to one function, but can act through very different mechanisms. Extreme examples are the classical MAPs (section 5) and the multi-functional cylindromatosis protein (CYLD), which directly interacts with MTs, binds to MT plus ends through EB proteins, regulates MT de-acetylation and acts as a de-ubiquitinase (Yang and Zhou, 2016). Therefore, a reductionist approach by studying single factors in isolation seems insufficient to explain MT behaviours in cellular environments.

A systemic view of the problem of MT dynamics can be of benefit also by facilitating the discovery of commonalities and fundamental principles. For example, the different stages of a neuron's life, as depicted in Fig. 1, illustrate the continuum of the same MT bundle from the birth of the axon through to its degeneration. As already explained in section 2, MT de/polymerisation occurs throughout all these stages, and it seems reasonable to assume that the same fundamental machinery of MT de/polymerisation underlies processes of growth, branching, degeneration and regeneration. Accordingly, factors like tau, spectraplakins or CLASP to name but a few, play important roles during development as well as during the adult life of axons (Hur et al., 2011; Prokop et al., 2013). Mechanistic understanding gained from work on one stage or functional context might therefore apply more widely to studies of axonal biology.

Building on this idea, we started to assess whether MT regulating mechanisms known from axon development might play similar roles during axonal ageing. This work led us to propose the model of local homeostasis of axons (Fig. 3) (Prokop, 2016). In this model, we propose that MTs of mature axons are constantly renewed through steady state de/polymerisation processes (see also section 2). However, MTs do not necessarily align into bundles: the force environment in axons, which is rich in kinesins and dyneins, causes MTs to curl, and excessive looping of MTs is the most common phenotype we observe upon manipulations of different classes of MT regulators (Fig. 3) (Beaven et al., 2015; Sánchez-Soriano et al., 2009). These loops and their diameters are comparable to loops observed in gliding assays *in vitro* where MTs are kept on kinesin carpets (Liu et al., 2011). Models explaining this phenomenon have been proposed (Ziebert et al., 2015). The tendency of axonal MTs to go into disarray requires that different classes of MT regulators impose order by arranging axonal MTs into bundles and maintaining this condition for decades (details in Fig. 3). We predict that mutations of single regulators do not eliminate but weaken this machinery, increasing the probability of accidental MT disorganisation. This could explain axonal swellings observed in neurodegenerative disorders associated with various MT regulators (as well as in normal ageing). These swellings are thought to form traps for organelles and initiation points of axon degeneration (Adalbert and Coleman, 2012; Prokop et al., 2013).

This model represents an attempt to explain axon biology through integrating our knowledge about regulatory mechanisms of MTs. It provides a hypothesis that can be experimentally tested and refined. Such experiments require cellular systems in which the various MT regulators and their interactors can be functionally manipulated in parallel, in order to assess functional redundancies and study the interfaces between different regulatory mechanisms. As has been detailed elsewhere (Prokop et al., 2013), neurons of the fruitfly *Drosophila* provide the means to carry out such studies. *Drosophila* has been used for decades to unravel biological complexity because it provides access to a powerful combination of highly

efficient genetic and experimental approaches (Hahn et al., 2016). Importantly, many of the lessons learned in the fly can be translated into mammalian systems, and this clearly applies to cytoskeletal studies in axons (Beaven et al., 2015; Prokop et al., 2013; Sánchez-Soriano et al., 2009).
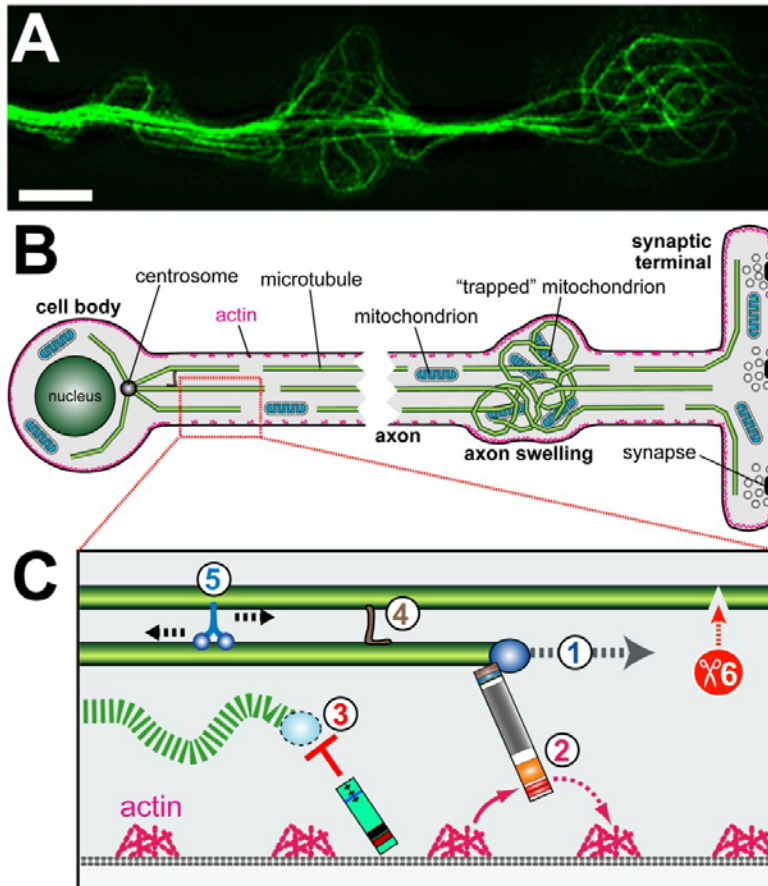

Figure 3: The model of local homeostasis in axons. **A**) MT looping in axons of primary neurons is a common phenotype observed upon mutation of MT regulators (scale bar 1μm). **B**) MT looping observed in primary neurons might reflect the same pathomechanisms that give rise to axon swellings during ageing or neurodegeneration. **C**) The model of local homeostasis proposes that continued polymerisation of MTs **(1)** poses a constant risk of MT disorganisation, and that different MT regulators contribute in different ways to maintain MT bundles and prevent axon swellings: spectraplakins **(2)** bind MT plus ends and guide them along cortical F-actin to lay them out into parallel bundles (Alves-Silva et al., 2012); cortical collapse factors **(3)** act as check points, capturing off-track MTs and eliminating them (unpublished results); bundling mechanisms **(4)** are likely to stabilise MT bundles from within; MT sliding **(5)** could ensure even MT densities along the axon shaft; **(6)** MT severing could provide an important mechanisms to ensure MT turn-over in order to prevent senescence.

9. Acknowledgement

10. Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

11. References

Adalbert, R., and Coleman, M.P. (2012). Axon pathology in age-related neurodegenerative disorders. Neuropathol Appl Neurobiol *39*, 90–108.

Akella, J.S., Wloga, D., Kim, J., Starostina, N.G., Lyons-Abbott, S., Morrissette, N.S., Dougan, S.T., Kipreos, E.T., and Gaertig, J. (2010). MEC-17 is an alpha-tubulin acetyltransferase. Nature *467*, 218-222.

Akhmanova, A., and Hoogenraad, Casper C. (2015). Microtubule Minus-End-Targeting Proteins. Current Biology *25*, R162-R171.

Akhmanova, A., and Steinmetz, M.O. (2010). Microtubule +TIPs at a glance. J Cell Sci *123*, 3415-3419.

Akhmanova, A., and Steinmetz, M.O. (2015). Control of microtubule organization and dynamics: two ends in the limelight. Nat Rev Mol Cell Biol *16*, 711–726.

Al-Bassam, J., and Chang, F. (2011). Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. Trends Cell Biol *21*, 604-614.

Al-Bassam, J., Kim, H., Brouhard, G., van Oijen, A., Harrison, S.C., and Chang, F. (2010). CLASP promotes microtubule rescue by recruiting tubulin dimers to the microtubule. Dev Cell *19*, 245-258.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., and Walter, P. (2014). Molecular biology of the cell (6th edition), 6 edn (Garland Science).

Alves-Silva, J., Sánchez-Soriano, N., Beaven, R., Klein, M., Parkin, J., Millard, T., Bellen, H., Venken, K.J.T., Ballestrem, C., Kammerer, R.A.*, et al.* (2012). Spectraplakins promote microtubule-mediated axonal growth by functioning as structural microtubule-associated proteins and EB1-dependent +TIPs (Tip Interacting Proteins). J Neurosci *32*, 9143-9158.

Amos, L.A., and Schlieper, D. (2005). Microtubules and MAPs. Adv Protein Chem *71*, 257-298.

Baas, P.W., and Ahmad, F.J. (2013). Beyond taxol: microtubule-based treatment of disease and injury of the nervous system. Brain.

Baas, P.W., and Lin, S. (2011). Hooks and comets: The story of microtubule polarity orientation in the neuron. Dev Neurobiol *71*, 403-418.

Baas, P.W., Rao, A.N., Matamoros, A.J., and Leo, L. (2016). Stability properties of neuronal microtubules. Cytoskeleton (Hoboken).

Bear, J.E., and Gertler, F.B. (2009). ENA/VASP: towards resolving a pointed controversy at the barbed end. J Cell Sci *122*, 1947-1953.

Bearce, E.A., Erdogan, B., and Lowery, L.A. (2015). TIPsy tour guides: how microtubule plus-end tracking proteins (+TIPs) facilitate axon guidance. Front Cell Neurosci *9*, 241.

Beaven, R., Dzhindzhev, N.S., Qu, Y., Hahn, I., Dajas-Bailador, F., Ohkura, H., and Prokop, A. (2015). *Drosophila* CLIP-190 and mammalian CLIP-170 display reduced microtubule plus end association in the nervous system. Mol Biol Cell *26*, 1491-1508.

Benitez-King, G., Ramirez-Rodriguez, G., Ortiz, L., and Meza, I. (2004). The neuronal cytoskeleton as a potential therapeutical target in neurodegenerative diseases and schizophrenia. Curr Drug Targets CNS Neurol Disord *3*, 515-533.

Berridge, M.J. (1998). Neuronal calcium signaling. Neuron *21*, 13-26.

Bhamidipati, A., Lewis, S.A., and Cowan, N.J. (2000). ADP ribosylation factor-like protein 2 (Arl2) regulates the interaction of tubulin-folding cofactor D with native tubulin. J Cell Biol *149*, 1087-1096.

Bilimoria, P.M., and Bonni, A. (2013). Molecular control of axon branching. Neuroscientist *19*, 16-24.

Blackmore, M.G. (2012). Molecular control of axon growth: insights from comparative gene profiling and high-throughput screening. Int Rev Neurobiol *105*, 39-70.

Blackstone, C., O'Kane, C.J., and Reid, E. (2011). Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat Rev Neurosci *12*, 31-42.

Bömmel, H., Xie, G., Rossoll, W., Wiese, S., Jablonka, S., Boehm, T., and Sendtner, M. (2002). Missense mutation in the tubulin-specific chaperone E (Tbce) gene in the mouse mutant progressive motor neuronopathy, a model of human motoneuron disease. J Cell Biol *159*, 563-569.

Bradke, F., Fawcett, J.W., and Spira, M.E. (2012). Assembly of a new growth cone after axotomy: the precursor to axon regeneration. Nat Rev Neurosci *13*, 183-193.

Brandt, R., and Lee, G. (1993a). The balance between tau protein's microtubule growth and nucleation activities: implications for the formation of axonal microtubules. J Neurochem *61*, 997-1005.

Brandt, R., and Lee, G. (1993b). Functional organization of microtubule-associated protein tau. Identification of regions which affect microtubule growth, nucleation, and bundle formation *in vitro*. J Biol Chem *268*, 3414-3419.

Bray, D. (1984). Axonal growth in response to experimentally applied mechanical tension. Dev Biol *102*, 379-389.

Brouhard, G.J. (2015). Dynamic instability 30 years later: complexities in microtubule growth and catastrophe. Mol Biol Cell *26*, 1207-1210.

Brouhard, G.J., and Rice, L.M. (2014). The contribution of alphabeta-tubulin curvature to microtubule dynamics. J Cell Biol *207*, 323-334.

Burow, D.A., Umeh-Garcia, M.C., True, M.B., Bakhaj, C.D., Ardell, D.H., and Cleary, M.D. (2015). Dynamic regulation of mRNA decay during neural development. Neural Dev *10*, 11.

Cahalan, M.D. (2009). STIMulating store-operated Ca(2+) entry. Nat Cell Biol *11*, 669-677.

Caron, J.M., Jones, A.L., and Kirschner, M.W. (1985a). Autoregulation of tubulin synthesis in hepatocytes and fibroblasts. J Cell Biol *101*, 1763-1772.

Caron, J.M., Jones, A.L., Rall, L.B., and Kirschner, M.W. (1985b). Autoregulation of tubulin synthesis in enucleated cells. Nature *317*, 648-651.

Caron, J.M., Vega, L.R., Fleming, J., Bishop, R., and Solomon, F. (2001). Single site alpha-tubulin mutation affects astral microtubules and nuclear positioning during anaphase in *Saccharomyces cerevisiae*: possible role for palmitoylation of alpha-tubulin. Mol Biol Cell *12*, 2672-2687.

Chauvin, S., and Sobel, A. (2015). Neuronal stathmins: A family of phosphoproteins cooperating for neuronal development, plasticity and regeneration. Prog Neurobiol.

Chen, L., Chuang, M., Koorman, T., Boxem, M., Jin, Y., and Chisholm, A.D. (2015). Axon injury triggers EFA-6 mediated destabilization of axonal microtubules via TACC and doublecortin like kinase.

Chen, L., Wang, Z., Ghosh-Roy, A., Hubert, T., Yan, D., O'Rourke, S., Bowerman, B., Wu, Z., Jin, Y., and Chisholm, A.D. (2011). Axon regeneration pathways identified by systematic genetic screening in *C. elegans*. Neuron *71*, 1043-1057.

Chilton, J., and Gordon-Weeks, P. (2007). Role of microtubules and MAPs during neuritogenesis. In Intracellular mechanisms for neuritogenesis, I. de Curtis, ed. (Springer US), pp. 57-88.

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., and Mann, M. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science *325*, 834-840.

Chretien, D., and Fuller, S.D. (2000). Microtubules switch occasionally into unfavorable configurations during elongation. J Mol Biol *298*, 663-676.

Chrétien, D., and Wade, R.H. (1991). New data on the microtubule surface lattice. Biol Cell *71*, 161-174.

Chu, C.W., Hou, F., Zhang, J., Phu, L., Loktev, A.V., Kirkpatrick, D.S., Jackson, P.K., Zhao, Y., and Zou, H. (2011). A novel acetylation of beta-tubulin by San modulates microtubule polymerization via down-regulating tubulin incorporation. Mol Biol Cell *22*, 448-456.

Deloulme, J.-C., Gory-Faure, S., Mauconduit, F., Chauvet, S., Jonckheere, J., Boulan, B., Mire, E., Xue, J., Jany, M., Maucler, C., *et al.* (2015). Microtubule-associated protein 6 mediates neuronal connectivity through Semaphorin 3E-dependent signalling for axonal growth. Nat Commun *6*.

Delphin, C., Bouvier, D., Seggio, M., Couriol, E., Saoudi, Y., Denarier, E., Bosc, C., Valiron, O., Bisbal, M., Arnal, I.*, et al.* (2012). MAP6-F is a temperature sensor that directly binds to and protects microtubules from cold-induced depolymerization. J Biol Chem *287*, 35127-35138.

Dent, E.W., and Baas, P.W. (2013). Microtubules in neurons as information carriers. J Neurochem.

Dimitrov, A., Quesnoit, M., Moutel, S., Cantaloube, I., Pous, C., and Perez, F. (2008). Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. Science *322*, 1353-1356.

Ducommun, B., Cance, J., and Wright, M. (1990). Regulation of tubulin synthesis during the cell cycle in the synchronous plasmodia of Physarum polycephalum. J Cell Physiol *145*, 120-128.

Duellberg, C., Trokter, M., Jha, R., Sen, I., Steinmetz, M.O., and Surrey, T. (2014). Reconstitution of a hierarchical +TIP interaction network controlling microtubule end tracking of dynein. Nat Cell Biol *16*, 804-811.

Dumont, E., L. P., Do, C., and Hess, H. (2015). Molecular wear of microtubules propelled by surface-adhered kinesins. Nat Nano *10*, 166-169.

Edvardson, S., Cinnamon, Y., Jalas, C., Shaag, A., Maayan, C., Axelrod, F.B., and Elpeleg, O. (2012). Hereditary sensory autonomic neuropathy caused by a mutation in dystonin. Ann Neurol *71*, 569-572.

Eira, J., Silva, C.S., Sousa, M.M., and Liz, M.A. (2016). The cytoskeleton as a novel therapeutic target for old neurodegenerative disorders. Progress in Neurobiology.

Elie, A., Prezel, E., Guerin, C., Denarier, E., Ramirez-Rios, S., Serre, L., Andrieux, A., Fourest-Lieuvin, A., Blanchoin, L., and Arnal, I. (2015). Tau co-organizes dynamic microtubule and actin networks. Sci Rep *5*, 9964.

Eng, H., Lund, K., and Campenot, R.B. (1999). Synthesis of beta-tubulin, actin, and other proteins in axons of sympathetic neurons in compartmented cultures. J Neurosci *19*, 1-9.

Ferrier, A., Boyer, J.G., and Kothary, R. (2013). Cellular and molecular biology of neuronal Dystonin. Int Rev Cell Mol Biol *300*, 85-120.

Fletcher, D.A., and Mullins, R.D. (2010). Cell mechanics and the cytoskeleton. Nature *463*, 485-492.

Friedman, J.R., and Nunnari, J. (2014). Mitochondrial form and function. Nature *505*, 335-343.

Friedmann, D.R., Aguilar, A., Fan, J., Nachury, M.V., and Marmorstein, R. (2012). Structure of the alpha-tubulin acetyltransferase, alphaTAT1, and implications for tubulin-specific acetylation. Proc Natl Acad Sci U S A *109*, 19655-19660.

Frühbeis, C., Fröhlich, D., Kuo, W.P., Amphornrat, J., Thilemann, S., Saab, A.S., Kirchhoff, F., Möbius, W., Goebbels, S., Nave, K.A.*, et al.* (2013). Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. PLoS Biol *11*, e1001604.

Galbraith, J.A., and Gallant, P.E. (2000). Axonal transport of tubulin and actin. J Neurocytol *29*, 889-911.

Geraldo, S., Khanzada, U.K., Parsons, M., Chilton, J.K., and Gordon-Weeks, P.R. (2008). Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. Nat Cell Biol *10*, 1181-1189.

Giuditta, A., Eyman, M., and Kaplan, B.B. (2002a). Gene expression in the squid giant axon: neurotransmitter modulation of RNA transfer from periaxonal glia to the axon. Biol Bull *203*, 189-190.

Giuditta, A., Kaplan, B.B., van Minnen, J., Alvarez, J., and Koenig, E. (2002b). Axonal and presynaptic protein synthesis: new insights into the biology of the neuron. Trends Neurosci *25*, 400-404.

Giuditta, A., Tai Chun, J., Eyman, M., Cefaliello, C., Bruno, A.P., and Crispino, M. (2008). Local Gene Expression in Axons and Nerve Endings: The Glia-Neuron Unit. Physiological Reviews *88*, 515-555.

Gonzalez, C., and Couve, A. (2014). The axonal endoplasmic reticulum and protein trafficking: Cellular bootlegging south of the soma. Semin Cell Dev Biol *27*, 23-31.

Goryunov, D., He, C.Z., Lin, C.S., Leung, C.L., and Liem, R.K. (2010). Nervous-tissue-specific elimination of microtubule-actin crosslinking factor 1a results in multiple developmental defects in the mouse brain. Mol Cell Neurosci *44*, 1-14.

Grigoriev, I., Gouveia, S.M., van der Vaart, B., Demmers, J., Smyth, J.T., Honnappa, S., Splinter, D., Steinmetz, M.O., Putney, J.W., Jr., Hoogenraad, C.C.*, et al.* (2008). STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. Curr Biol *18*, 177-182.

Grover, S., and Hamel, E. (1994). The magnesium-GTP interaction in microtubule assembly. Eur J Biochem *222*, 163-172.

Gupta, K.K., Alberico, E.O., Nathke, I.S., and Goodson, H.V. (2014). Promoting microtubule assembly: A hypothesis for the functional significance of the +TIP network. Bioessays *36*, 818-826.

Gupta, K.K., Li, C., Duan, A., Alberico, E.O., Kim, O.V., Alber, M.S., and Goodson, H.V. (2013). Mechanism for the catastrophe-promoting activity of the microtubule destabilizer Op18/stathmin. Proc Natl Acad Sci U S A *110*, 20449-20454.

Hahn, I., Ronshaugen, M., Sánchez-Soriano, N., and Prokop, A. (2016). Functional and genetic analysis of spectraplakins in *Drosophila*. Methods Enzymol *569*, 373-405.

Hendershott, M.C., and Vale, R.D. (2014). Regulation of microtubule minus-end dynamics by CAMSAPs and Patronin. Proc Natl Acad Sci U S A.

Homma, N., Takei, Y., Tanaka, Y., Nakata, T., Terada, S., Kikkawa, M., Noda, Y., and Hirokawa, N. (2003). Kinesin superfamily protein 2A (KIF2A) functions in suppression of collateral branch extension. Cell *114*, 229-239.

Honnappa, S., Gouveia, S.M., Weisbrich, A., Damberger, F.F., Bhavesh, N.S., Jawhari, H., Grigoriev, I., van Rijssel, F.J., Buey, R.M., Lawera, A.*, et al.* (2009). An EB1-binding motif acts as a microtubule tip localization signal. Cell *138*, 366-376.

Howard, J. (2001). Mechanics of motorproteins and the cytoskeleton (Sunderland: Sinauer Assoc.).

Howes, S.C., Alushin, G.M., Shida, T., Nachury, M.V., and Nogales, E. (2014). Effects of tubulin acetylation and tubulin acetyltransferase binding on microtubule structure. Mol Biol Cell *25*, 257-266.

Hur, E.M., Saijilafu, Lee, B.D., Kim, S.J., Xu, W.L., and Zhou, F.Q. (2011). GSK3 controls axon growth via CLASP-mediated regulation of growth cone microtubules. Genes Dev *25*, 1968-1981.

Janke, C., and Bulinski, J.C. (2011). Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. Nat Rev Mol Cell Biol *12*, 773-786.

Janke, C., and Kneussel, M. (2010). Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. Trends Neurosci *33*, 362-372.

Janning, D., Igaev, M., Sundermann, F., Bruhmann, J., Beutel, O., Heinisch, J.J., Bakota, L., Piehler, J., Junge, W., and Brandt, R. (2014). Single-molecule tracking of tau reveals fast kiss-and-hop interaction with microtubules in living neurons. Mol Biol Cell *25*, 3541-3551.

Jiang, K., Hua, S., Mohan, R., Grigoriev, I., Yau, K.W., Liu, Q., Katrukha, E.A., Altelaar, A.F.M., Heck, A.J.R., Hoogenraad, C.C.*, et al.* (2014). Microtubule minus-end stabilization by polymerization-driven CAMSAP deposition. Dev Cell *28*, 295-309.

Jin, S., Pan, L., Liu, Z., Wang, Q., Xu, Z., and Zhang, Y.Q. (2009). *Drosophila* Tubulin-specific chaperone E functions at neuromuscular synapses and is required for microtubule network formation. Development *136*, 1571-1581.

Jung, H., Yoon, B.C., and Holt, C.E. (2012). Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. Nat Rev Neurosci *13*, 308-324.

Kadavath, H., Hofele, R.V., Biernat, J., Kumar, S., Tepper, K., Urlaub, H., Mandelkow, E., and Zweckstetter, M. (2015). Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. Proceedings of the National Academy of Sciences *112*, 7501-7506.

Kalebic, N., Martinez, C., Perlas, E., Hublitz, P., Bilbao-Cortes, D., Fiedorczuk, K., Andolfo, A., and Heppenstall, P.A. (2013). Tubulin acetyltransferase alphaTAT1 destabilizes microtubules independently of its acetylation activity. Mol Cell Biol *33*, 1114-1123.

Kalil, K., and Dent, E.W. (2014). Branch management: mechanisms of axon branching in the developing vertebrate CNS. Nat Rev Neurosci *15*, 7-18.

Kollins, K.M., Bell, R.L., Butts, M., and Withers, G.S. (2009). Dendrites differ from axons in patterns of microtubule stability and polymerization during development. Neural Dev *4*, 26.

Komarova, Y.A., Vorobjev, I.A., and Borisy, G.G. (2002). Life cycle of MTs: persistent growth in the cell interior, asymmetric transition frequencies and effects of the cell boundary. J Cell Sci *115*, 3527-3539.

Korobova, F., Ramabhadran, V., and Higgs, H.N. (2013). An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science *339*, 464-467.

Kriz, J., Zhu, Q., Julien, J.P., and Padjen, A.L. (2000). Electrophysiological properties of axons in mice lacking neurofilament subunit genes: disparity between conduction velocity and axon diameter in absence of NF-H. Brain Res *885*, 32-44.

Lachkar, S., Lebois, M., Steinmetz, M.O., Guichet, A., Lal, N., Curmi, P.A., Sobel, A., and Ozon, S. (2010). *Drosophila* stathmins bind tubulin heterodimers with high and variable stoichiometries. J Biol Chem *285*, 11667-11680.

Lee, S.-K., and Hollenbeck, P.J. (2003). Organization and translation of mRNA in sympathetic axons. J Cell Sci *116*, 4467-4478.

Lewis, T.L., Jr., Courchet, J., and Polleux, F. (2013). Cell biology in neuroscience: Cellular and molecular mechanisms underlying axon formation, growth, and branching. J Cell Biol *202*, 837-848.

Li, L., and Yang, X.J. (2015). Tubulin acetylation: responsible enzymes, biological functions and human diseases. Cell Mol Life Sci *72*, 4237-4255.

Li, W., Miki, T., Watanabe, T., Kakeno, M., Sugiyama, I., Kaibuchi, K., and Goshima, G. (2011). EB1 promotes microtubule dynamics by recruiting Sentin in *Drosophila* cells. J Cell Biol *193*, 973-983.

Li, W., Moriwaki, T., Tani, T., Watanabe, T., Kaibuchi, K., and Goshima, G. (2012). Reconstitution of dynamic microtubules with *Drosophila* XMAP215, EB1, and Sentin. J Cell Biol *199*, 849-862.

Lin, C.M., and Hamel, E. (1987). Interrelationships of tubulin-GDP and tubulin-GTP in microtubule assembly. Biochemistry *26*, 7173-7182.

Liu, L., Tuzel, E., and Ross, J.L. (2011). Loop formation of microtubules during gliding at high density. J Phys Condens Matter *23*, 374104.

Lowery, L.A., Stout, A., Faris, A.E., Ding, L., Baird, M.A., Davidson, M.W., Danuser, G., and Van Vactor, D. (2013). Growth cone-specific functions of XMAP215 in restricting microtubule dynamics and promoting axonal outgrowth. Neural Dev *8*, 22.

Lu, Q., and Luduena, R.F. (1994a). In vitro analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of alpha beta II, alpha beta III, and alpha beta IV tubulin dimers in the absence of microtubule-associated proteins. J Biol Chem *269*, 2041-2047.

Lu, Q., and Luduena, R.F. (1994b). *In vitro* analysis of microtubule assembly of isotypically pure tubulin dimers. Intrinsic differences in the assembly properties of alpha beta II, alpha beta III, and alpha beta IV tubulin dimers in the absence of microtubule-associated proteins. J Biol Chem *269*, 2041-2047.

Manna, T., Grenningloh, G., Miller, H.P., and Wilson, L. (2007). Stathmin family protein SCG10 differentially regulates the plus and minus end dynamics of microtubules at steady state in vitro: implications for its role in neurite outgrowth. Biochemistry *46*, 3543-3552.

Manna, T., Thrower, D.A., Honnappa, S., Steinmetz, M.O., and Wilson, L. (2009). Regulation of microtubule dynamic instability *in vitro* by differentially phosphorylated stathmin. J Biol Chem *284*, 15640-15649.

Margolis, R.L., and Wilson, L. (1998). Microtubule treadmilling: what goes around comes around. Bioessays *20*, 830-836.

Martinez-Lopez, M.J., Alcantara, S., Mascaro, C., Perez-Branguli, F., Ruiz-Lozano, P., Maes, T., Soriano, E., and Buesa, C. (2005). Mouse neuron navigator 1, a novel microtubule-associated protein involved in neuronal migration. Mol Cell Neurosci *28*, 599-612.

Maurer, S.P., Cade, N.I., Bohner, G., Gustafsson, N., Boutant, E., and Surrey, T. (2014). EB1 accelerates two conformational transitions important for microtubule maturation and dynamics. Curr Biol *24*, 372-384.

Medioni, C., Ephrussi, A., and Besse, F. (2015). Live imaging of axonal transport in Drosophila pupal brain explants. Nat Protoc *10*, 574-584.

Mikhaylova, M., Cloin, B.M., Finan, K., van den Berg, R., Teeuw, J., Kijanka, M.M., Sokolowski, M., Katrukha, E.A., Maidorn, M., Opazo, F., *et al.* (2015). Resolving bundled microtubules using anti-tubulin nanobodies. Nat Commun *6*, 7933.

Miller, K.E., and Sheetz, M.P. (2006). Direct evidence for coherent low velocity axonal transport of mitochondria. J Cell Biol *173*, 373-381.

Mooney, D.J., Hansen, L.K., Langer, R., Vacanti, J.P., and Ingber, D.E. (1994). Extracellular matrix controls tubulin monomer levels in hepatocytes by regulating protein turnover. Mol Biol Cell *5*, 1281-1288.

Morris, M., Maeda, S., Vossel, K., and Mucke, L. (2011). The many faces of tau. Neuron *70*, 410-426.

Nakata, T., Niwa, S., Okada, Y., Perez, F., and Hirokawa, N. (2011). Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport. J Cell Biol *194*, 245-255.

Nashchekin, D., Fernandes, A.R., and St Johnston, D. (2016). Patronin/Shot Cortical Foci Assemble the Noncentrosomal Microtubule Array that Specifies the Drosophila Anterior-Posterior Axis. Dev Cell *38*, 61-72.

Neumann, B., and Hilliard, M.A. (2014). Loss of MEC-17 leads to microtubule instability and axonal degeneration. Cell Rep *6*, 93-103.

Nguyen, M.M., McCracken, C.J., Milner, E.S., Goetschius, D.J., Weiner, A.T., Long, M.K., Michael, N.L., Munro, S., and Rolls, M.M. (2014). Gamma-tubulin controls neuronal microtubule polarity independently of Golgi outposts. Mol Biol Cell *25*, 2039-2050.

Nguyen, M.M., Stone, M.C., and Rolls, M.M. (2011). Microtubules are organized independently of the centrosome in Drosophila neurons. Neural Dev *6*, 38.

Nogales, E., and Wang, H.W. (2006). Structural mechanisms underlying nucleotide-dependent self-assembly of tubulin and its relatives. Curr Opin Struct Biol *16*, 221-229.

Nouar, R., Breuzard, G., Bastonero, S., Gorokhova, S., Barbier, P., Devred, F., Kovacic, H., and Peyrot, V. (2016). Direct evidence for the interaction of stathmin along the length and the plus-end of microtubules in cells. FASEB J, 10.1096/fj.201500125R.

Nwagbara, B.U., Faris, A.E., Bearce, E.A., Erdogan, B., Ebbert, P.T., Evans, M.F., Rutherford, E.L., Enzenbacher, T.B., and Lowery, L.A. (2014). TACC3 is a microtubule plus-end tracking protein that promotes axon elongation and also regulates microtubule plus-end dynamics in multiple embryonic cell types. Mol Biol Cell.

O'Rourke, S.M., Christensen, S.N., and Bowerman, B. (2010). *Caenorhabditis elegans* EFA-6 limits microtubule growth at the cell cortex. Nat Cell Biol *12*, 1235-1241.

Okada, K., Bartolini, F., Deaconescu, A.M., Moseley, J.B., Dogic, Z., Grigorieff, N., Gundersen, G.G., and Goode, B.L. (2010). Adenomatous polyposis coli protein nucleates actin assembly and synergizes with the formin mDia1. J Cell Biol *189*, 1087-1096.

Panda, D., Miller, H.P., and Wilson, L. (1999). Rapid treadmilling of brain microtubules free of microtubule-associated proteins *in vitro* and its suppression by tau. Proc Natl Acad Sci U S A *96*, 12459-12464.

Penazzi, L., Bakota, L., and Brandt, R. (2016). Microtubule dynamics in neuronal development, plasticity, and neurodegeneration. Int Rev Cell Mol Biol *321*, 89-169.

Perlson, E., Hendricks, A.G., Lazarus, J.E., Ben-Yaakov, K., Gradus, T., Tokito, M., and Holzbaur, E.L. (2013). Dynein interacts with the neural cell adhesion molecule (NCAM180) to tether dynamic microtubules and maintain synaptic density in cortical neurons. J Biol Chem *288*, 27812-27824.

Petry, S., and Vale, R.D. (2015). Microtubule nucleation at the centrosome and beyond. Nat Cell Biol *17*, 1089-1093.

Poulain, F.E., and Sobel, A. (2010). The microtubule network and neuronal morphogenesis: Dynamic and coordinated orchestration through multiple players. Mol Cell Neurosci *43*, 15-32.

Prokop, A. (2013). The intricate relationship between microtubules and their associated motor proteins during axon growth and maintenance. Neur Dev *8*, 17.

Prokop, A. (2016). Fruit flies in biological research. Biological Sciences Review *28*, 10-14.

Prokop, A., Beaven, R., Qu, Y., and Sánchez-Soriano, N. (2013). Using fly genetics to dissect the cytoskeletal machinery of neurons during axonal growth and maintenance. J Cell Sci *126*, 2331-2341.

Qu, C., Dwyer, T., Shao, Q., Yang, T., Huang, H., and Liu, G. (2013). Direct binding of TUBB3 with DCC couples netrin-1 signaling to intracellular microtubule dynamics in axon outgrowth and guidance. J Cell Sci *126*, 3070-3081.

Qu, Y., Hahn, I., Webb, S., and Prokop, A. (2016). Periodic actin structures in neuronal axons are required to maintain microtubules. bioRxiv, dx.doi.org/10.1101/049379.

Ren, Y., Zhao, J., and Feng, J. (2003). Parkin binds to alpha/beta tubulin and increases their ubiquitination and degradation. J Neurosci *23*, 3316-3324.

Riederer, B.M., Pellier, V., Antonsson, B., Di Paolo, G., Stimpson, S.A., Lutjens, R., Catsicas, S., and Grenningloh, G. (1997). Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10. Proc Natl Acad Sci U S A *94*, 741-745.

Rogers, L.C., de Boer, I., Junier, M.P., and Ojeda, S.R. (1993). Estradiol Increases Neural-Specific Class II-beta-Tubulin mRNA Levels in the Developing Female Hypothalamus by Regulating mRNA Stability. Mol Cell Neurosci *4*, 424-431.

Rolls, M.M., Satoh, D., Clyne, P.J., Henner, A.L., Uemura, T., and Doe, C.Q. (2007). Polarity and intracellular compartmentalization of Drosophila neurons. Neural Dev *2*, 7.

Roossien, D.H., Lamoureux, P., Van Vactor, D., and Miller, K.E. (2013). *Drosophila* growth cones advance by forward translocation of the neuronal cytoskeletal meshwork *in vivo*. PLoS One *8*, e80136.

Roostalu, J., Cade, N.I., and Surrey, T. (2015). Complementary activities of TPX2 and chTOG constitute an efficient importin-regulated microtubule nucleation module [corrigendum: same issue, p.1512]. Nat Cell Biol *17*, 1422-1434.

Rosenberg, K.J., Ross, J.L., Feinstein, H.E., Feinstein, S.C., and Israelachvili, J. (2008). Complementary dimerization of microtubule-associated tau protein: Implications for microtubule bundling and tau-mediated pathogenesis. Proc Natl Acad Sci U S A *105*, 7445-7450.

Sanchez-Huertas, C., Freixo, F., Viais, R., Lacasa, C., Soriano, E., and Lüders, J. (2016). Non-centrosomal nucleation mediated by augmin organizes microtubules in post-mitotic neurons and controls axonal microtubule polarity. Nat Commun *7*.

Sánchez-Soriano, N., Gonçalves-Pimentel, C., Beaven, R., Haessler, U., Ofner, L., Ballestrem, C., and Prokop, A. (2010). *Drosophila* growth cones: a genetically tractable platform for the analysis of axonal growth dynamics. Dev Neurobiol *70*, 58-71.

Sánchez-Soriano, N., Travis, M., Dajas-Bailador, F., Goncalves-Pimentel, C., Whitmarsh, A.J., and Prokop, A. (2009). Mouse ACF7 and *Drosophila* Short stop modulate filopodia formation and microtubule organisation during neuronal growth. J Cell Sci *122*, 2534-2542.

Sayas, C.L., Tortosa, E., Bollati, F., Ramirez-Rios, S., Arnal, I., and Avila, J. (2015). Tau regulates the localization and function of End-binding proteins 1 and 3 in developing neuronal cells. J Neurochem *133*, 653-667.

Schaedel, L., John, K., Gaillard, J., Nachury, M.V., Blanchoin, L., and Thery, M. (2015). Microtubules self-repair in response to mechanical stress. Nat Mater *14*, 1156-1163.

Schmidt, N., Basu, S., Sladecek, S., Gatti, S., van Haren, J., Treves, S., Pielage, J., Galjart, N., and Brenner, H.R. (2012). Agrin regulates CLASP2-mediated capture of microtubules at the neuromuscular junction synaptic membrane. J Cell Biol.

Semotok, J.L., and Lipshitz, H.D. (2007). Regulation and function of maternal mRNA destabilization during early *Drosophila* development. Differentiation *75*, 482-506.

Serna, M., Carranza, G., Martin-Benito, J., Janowski, R., Canals, A., Coll, M., Zabala, J.C., and Valpuesta, J.M. (2015). The structure of the complex between alpha-tubulin, TBCE and TBCB reveals a tubulin dimer dissociation mechanism. J Cell Sci *128*, 1824-1834.

Soares, L., Parisi, M., and Bonini, N.M. (2014). Axon injury and regeneration in the adult *Drosophila*. Scientific reports *4*, 6199.

Song, Y., Kirkpatrick, L.L., Schilling, A.B., Helseth, D.L., Chabot, N., Keillor, J.W., Johnson, G.V., and Brady, S.T. (2013). Transglutaminase and polyamination of tubulin: posttranslational modification for stabilizing axonal microtubules. Neuron *78*, 109-123.

Soppina, V., Herbstman, J.F., Skiniotis, G., and Verhey, K.J. (2012). Luminal localization of alpha-tubulin K40 acetylation by cryo-EM analysis of fab-labeled microtubules. PLoS One *7*, e48204.

Stepanova, T., Slemmer, J., Hoogenraad, C.C., Lansbergen, G., Dortland, B., De Zeeuw, C.I., Grosveld, F., van Cappellen, G., Akhmanova, A., and Galjart, N. (2003). Visualization of

microtubule growth in cultured neurons via the use of EB3-GFP (End-Binding Protein 3-Green Fluorescent Protein). J Neurosci *23*, 2655-2537.

Stiess, M., Maghelli, N., Kapitein, L.C., Gomis-Ruth, S., Wilsch-Brauninger, M., Hoogenraad, C.C., Tolic-Norrelykke, I.M., and Bradke, F. (2010). Axon extension occurs independently of centrosomal microtubule nucleation. Science *327*, 704-707.

Sun, D., Leung, C.L., and Liem, R.K. (2001). Characterization of the microtubule binding domain of microtubule actin crosslinking factor (MACF): identification of a novel group of microtubule associated proteins. J Cell Sci *114*, 161-172.

Swiech, L., Blazejczyk, M., Urbanska, M., Pietruszka, P., Dortland, B.R., Malik, A.R., Wulf, P.S., Hoogenraad, C.C., and Jaworski, J. (2011). CLIP-170 and IQGAP1 cooperatively regulate dendrite morphology. J Neurosci *31*, 4555-4568.

Takei, Y., Teng, J., Harada, A., and Hirokawa, N. (2000). Defects in axonal elongation and neuronal migration in mice with disrupted *tau* and *map1b* genes. J Cell Biol *150*, 989-1000.

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

Tang, Y., Scott, D., Das, U., Gitler, D., Ganguly, A., and Roy, S. (2013). Fast vesicle transport is required for the slow axonal transport of synapsin. J Neurosci *33*, 15362-15375.

Teixido-Travesa, N., Roig, J., and Luders, J. (2012). The where, when and how of microtubule nucleation - one ring to rule them all. J Cell Sci *125*, 4445-4456.

Temburni, M.K., Rosenberg, M.M., Pathak, N., McConnell, R., and Jacob, M.H. (2004). Neuronal Nicotinic Synapse Assembly Requires the Adenomatous Polyposis Coli Tumor Suppressor Protein. J Neurosci *24*, 6776-6784.

Teng, J., Takei, Y., Harada, A., Nakata, T., Chen, J., and Hirokawa, N. (2001). Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic outgrowth, and microtubule organization. J Cell Biol *155*, 65-76.

Theodorakis, N.G., and Cleveland, D.W. (1992). Physical evidence for cotranslational regulation of beta-tubulin mRNA degradation. Mol Cell Biol *12*, 791-799.

Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C., and Cowan, N.J. (1996). Pathway leading to correctly folded beta-tubulin. Cell *86*, 287-296.

Tortosa, E., Galjart, N., Avila, J., and Sayas, C.L. (2013). MAP1B regulates microtubule dynamics by sequestering EB1/3 in the cytosol of developing neuronal cells. EMBO J *32*, 1293-1306.

Tropini, C., Roth, E.A., Zanic, M., Gardner, M.K., and Howard, J. (2012). Islands containing slowly hydrolyzable GTP analogs promote microtubule rescues. PLoS One *7*, e30103.

Trushko, A., Schaffer, E., and Howard, J. (2013). The growth speed of microtubules with XMAP215-coated beads coupled to their ends is increased by tensile force. Proc Natl Acad Sci U S A *110*, 14670-14675.

Tymanskyj, S.R., Scales, T.M., and Gordon-Weeks, P.R. (2012). MAP1B enhances microtubule assembly rates and axon extension rates in developing neurons. Mol Cell Neurosci *49*, 110-119.

van de Willige, D., Hoogenraad, C.C., and Akhmanova, A. (2016). Microtubule plus-end tracking proteins in neuronal development. Cell Mol Life Sci.

van der Vaart, B., Franker, M.A., Kuijpers, M., Hua, S., Bouchet, B.P., Jiang, K., Grigoriev, I., Hoogenraad, C.C., and Akhmanova, A. (2012). Microtubule Plus-End Tracking Proteins SLAIN1/2 and ch-TOG Promote Axonal Development. J Neurosci *32*, 14722-14728.

van der Vaart, B., Manatschal, C., Grigoriev, I., Olieric, V., Gouveia, S.M., Bjelic, S., Demmers, J., Vorobjev, I., Hoogenraad, C.C., Steinmetz, M.O*., et al.* (2011). SLAIN2 links microtubule plus end-tracking proteins and controls microtubule growth in interphase. J Cell Biol *193*, 1083-1099.

Villarroel-Campos, D., and Gonzalez-Billault, C. (2014). The MAP1B case: an old MAP that is new again. Dev Neurobiol *74*, 953-971.

Voelzmann, A., Okenve Ramos, P., Qu, Y., Chojnowska-Monga, M., del Caño-Espinel, M., Prokop, A., and Sánchez-Soriano, N. (2016). Tau and spectraplakins promote synapse formation and maintenance through JNK and neuronal trafficking. eLife, in press.

Volle, J., Brocard, J., Saoud, M., Gory-Faure, S., Brunelin, J., Andrieux, A., and Suaud-Chagny, M.F. (2013). Reduced expression of STOP/MAP6 in mice leads to cognitive deficits. Schizophr Bull *39*, 969-978.

Weber, K., Riemer, D., and Dodemont, H. (1991). Aspects of the evolution of the lamin/intermediate filament protein family: a current analysis of invertebrate intermediate filament proteins. Biochem Soc Trans *19*, 1021-1023.

Wojnacki Fonseca, J.I., and Galli, T. (2016). Membrane traffic during axon development. Dev Neurobiol.

Wortman, J.C., Shrestha, U.M., Barry, D.M., Garcia, M.L., Gross, S.P., and Yu, C.C. (2014). Axonal transport: how high microtubule density can compensate for boundary effects in small-caliber axons. Biophys J *106*, 813-823.

Yalçýn, B., and O'Kane, C.J. (2013). Maintaining long supply lines: axon degeneration and the function of hereditary spastic paraplegia genes in *Drosophila* (chapter 4). In *Drosophila melanogaster* models of motor neuron disease, R.J. Cauchi ed. (Nova Science Publishers), pp. 85-120

Yang, Y., and Zhou, J. (2016). CYLD – a deubiquitylase that acts to fine-tune microtubule properties and functions. J Cell Sci *129*, 2289-2295.

Yau, K.W., Schätzle, P., Tortosa, E., Pagès, S., Holtmaat, A., Kapitein, L.C., and Hoogenraad, C.C. (2016). Dendrites *in vitro* and *in vivo* contain microtubules of opposite polarity and axon formation correlates with uniform plus-end-out microtubule orientation. J Neurosci *36*, 1071-1085.

Yu, W., Solowska, J.M., Qiang, L., Karabay, A., Baird, D., and Baas, P.W. (2005). Regulation of microtubule severing by katanin subunits during neuronal development. J Neurosci *25*, 5573-5583.

Zanic, M., Widlund, P.O., Hyman, A.A., and Howard, J. (2013). Synergy between XMAP215 and EB1 increases microtubule growth rates to physiological levels. Nat Cell Biol *15*, 688–693.

Zhang, D., Grode, K.D., Stewman, S.F., Diaz-Valencia, J.D., Liebling, E., Rath, U., Riera, T., Currie, J.D., Buster, D.W., Asenjo, A.B.*, et al.* (2011). *Drosophila* katanin is a microtubule depolymerase that regulates cortical-microtubule plus-end interactions and cell migration. Nat Cell Biol *13*, 361-370.

Ziebert, F., Mohrbach, H., and Kulić, I.M. (2015). Why Microtubules Run in Circles: Mechanical Hysteresis of the Tubulin Lattice. Physical Review Letters *114*, 148101.