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Tubulins and brain development – The origins of functional specification

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ABSTRACT

The development of the vertebrate central nervous system is reliant on a complex cascade of biological processes that include mitotic division, relocation of migrating neurons, and the extension of dendritic and axonal processes. Each of these cellular events requires the diverse functional repertoire of the microtubule cytoskeleton for the generation of forces, assembly of macromolecular complexes and transport of molecules and organelles. The tubulins are a multi-gene family that encode for the constituents of microtubules, and have been implicated in a spectrum of neurological disorders. Evidence is building that different tubulins tune the functional properties of the microtubule cytoskeleton dependent on the cell type, developmental profile and subcellular localisation. Here we review of the origins of the functional specification of the tubulin gene family in the developing brain at a transcriptional, translational, and post-transcriptional level. We remind the reader that tubulins are not just loading controls for your average Western blot.

Contents

1. Introduction

There are three cellular processes that are indispensable for the development of the vertebrate brain: neurogenesis; neuronal migration and neuronal differentiation (Kriegstein and Noctor, 2004; Gotz and Huttner, 2005; Ayala et al., 2007). This review begins by reflecting on the role the microtubule cytoskeleton plays in each of these developmental events, highlighting its diverse functional role. In the second part, we focus on the tubulin gene family and the molecular variation that underlies the functional specification of microtubules. We discuss this topic in light of transcriptional, translational, and post-translational regulation.

2. Microtubules and neurogenesis

The formation of the neural tube during gastrulation is the first step in the development of the brain. During neural tube closure

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microtubules are known to align along the apico-basal axis of the vertebral neural plate (Cearns et al., 2016). Neuroepithelial cells (NECs) then self-renew to expand the progenitor pool or give rise to a progenitor population called radial glial cells (RGCs) (Kriegstein and Gotz, 2003). While the cell body of RGCs resides close to the ventricular surface, processes of the cell are connected to both the basal and apical side, thereby spanning the entirety of the cortical anlage. As cortical development proceeds, RGCs are complemented by transitional progenitors including: (1) intermediate progenitors; (2) basal radial glial cells (bRGCs); and (3) transit-amplifying progenitors (TAPs) (Lui et al., 2011; Florio and Huttner, 2014). To propagate its genome from one cell to the next each progenitor must successfully duplicate and segregate its complement of chromosomes. This requires the formation of a bipolar mitotic spindle in prometaphase, followed by the generation of force to separate sister chromatids. Aided by kinetochores microtubules are charged with the task of searching and capturing chromosomes, before aligning them along the metaphase plate. Upon induction of the anaphase promoting complex microtubules rapidly translocate attached chromosomes to either end of the cell cortex, and play an active role in cytokinesis (Fig. 1D) (Glotzer, 2009). In addition radial glial cells and neuroepithelial cells exhibit a remarkable behaviour known as interkinetic nuclear migration (Tsai et al., 2010; Lee and Norden, 2013). Within the pseudostratified epithelium nuclei migrate basally during G1, undergo S phase, before migrating apically during G2, followed by the induction of M phase at the ventricular surface (Fig. 1A–D). This movement is microtubule dependent, and is driven by a suite of motor proteins (Messier, 1978). Kif1a drives basal nuclear migration, whereas dynein and Tpx2 are critical for return of the nucleus to the apical surface (Tischfield et al., 2010; Kosodo et al., 2011).

3. Microtubules and neuronal migration

Following their exit from the cell cycle, newly born neurons begin their journey, migrating tangentially or radially (Marin and

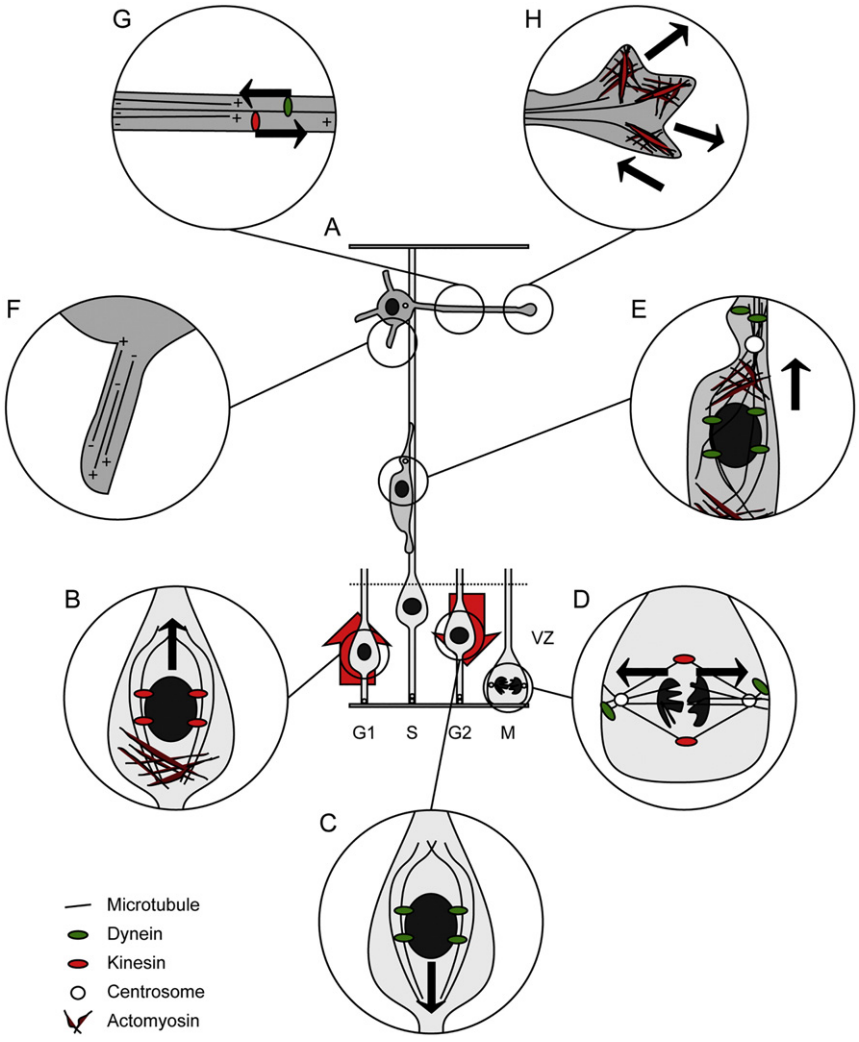

Fig. 1. The role of microtubules during neurodevelopment. (A) Schematic of the developing cortex showing radial glial cells (RGCs) in the ventricular zone (VZ) undergoing the four phases of cell cycle (G1, S, G2 and M). In addition a migrating and a differentiating neuron are depicted. (B) Magnification of a G1-phase RGC: basal movement is achieved by the exertion of direct force onto the nucleus by kinesin motor proteins as well as by apically located actomyosin contractility. (C) Magnification of a G2-phase RGC: apical movement is achieved by the exertion of direct force onto the nucleus by dynein motor proteins. (D) Magnification of an M-phase RGC: the sister chromatids are separated by the mitotic spindle that is organized by the centrosomes. Force is generated by the dynamic microtubule ends and by dynein and kinesin motor proteins that connect the plasma membrane to the spindle and the two halves of the spindle, respectively. (E) Magnification of a migrating neuron: the salutatory, step-wise migration of neurons is achieved by a microtubule cage surrounding the nucleus. Direct and indirect force is exerted by dynein motors associated with the nuclear envelope and those that are located basally at the centrosome. In addition, actomyosin contractility is required for proper nucleokinesis. (F) Magnification of a dendrite: microtubules are not uniformly arranged and both plus- (+) and minus-ends (−) can be found at the distal tip of the neurite. (G) Magnification of an axon: microtubules are uniformly arranged with the plus-end directed towards the distal end of the neurite. This arrangement results in dynein motor proteins transporting cargo towards the soma, whereas plus-end directed kinesin family proteins are responsible for transport towards the developing growth cone during development (and the axonal bouton following differentiation). (H) Magnification of a growth cone: propulsive and retractive forces are mainly generated by actomyosin. Actin bundles dominate in the filopodia and lamellipodia, which are fortified by microtubules at the base of the growth cone.

Rubenstein, 2003). Excitatory pyramidal neurons, which are born at the ventricular surface of the dorsal telencephalon, migrate radially (perpendicular to the ventricular surface) into the developing cortex and hippocampus (Rakic, 1972; Altman and Bayer, 1990) (Fig. 1A). In contrast, inhibitory cells which are born at the proliferative ganglionic eminences in the ventral telencephalon migrate tangentially (parallel to the ventricular surface) to reach their position in the dorsal forebrain (Kriegstein and Noctor, 2004). As excitatory neurons migrate they undergo a series of morphological transitions. Early after birth, they adopt a multipolar shape, an overall round appearance with multiple dynamic extensions that make contact with adjacent radial glial fibers (Tabata and Nakajima, 2003). Upon entry into the developing cortical plate, cells assume a bipolar shape with a pronounced leading and trailing process, as they attach and migrate along a radial glia fiber (Fig. 1E). As the leading process of a migrating cell touches the basal membrane, the nucleus is moved into the final position in a final mode of migration termed somal translocation (Nadarajah et al., 2001). Microtubules are indispensable at multiple stages on this cellular journey. The transition from multipolar to bipolar morphology in the intermediate zone involves a complex signaling cascade involving Rnd2, RhoA, GSK-3, Cdk5, Rac1 and Pak-1 which converge on the actin and microtubule cytoskeleton facilitating a co-ordinated change in cell shape (Heng et al., 2009). To enable nucleokinesis microtubules form a cage around the nucleus by extending from the basally located microtubule organising centre (Fig. 1E). Force generated by microtubule motors and the actomyosin cytoskeleton then moves the nucleus in a stepwise fashion into the cortical plate (Tsai and Gleeson, 2005; He et al., 2010; Kuijpers and Hoogenraad, 2011). It has been shown that this movement requires microtubule associated proteins such as Dcx, Lis1, Kif1a, Kif2a, as well as various members of the tubulin gene family (Breuss and Keays, 2014). It has been further demonstrated that neuronal migration is affected by post-translational modifications of microtubules, such as acetylation, which influences the stability of the structure and the cohort of proteins that bind to it (Reed et al., 2006; Creppe et al., 2009).

4. Microtubules and neuronal differentiation

As a neuron settles in the forebrain the cellular program of differentiation is started, which allows for the generation of functional circuits. Excitatory neurons build large dendritic trees that receive inputs from thousands of other cells (Gulledge et al., 2005). Extending from the primary neurite axons are formed, some of which are destined to cross the cerebral hemispheres. The morphology of a developing axon is similar to the processes formed during migration: the neurite itself is stabilized by parallel microtubule bundles while the growth cone is made up of actin-rich lamellipodia and filopodia that sense the local environment (Fig. 1G–H) (Namba et al., 2015). This growing axon is receptive to a wide range of guidance cues including ephrins, semaphorins and slit proteins which facilitate its navigation to the correct target (Dickson, 2002). In axons microtubules are organized in a unipolar fashion forming molecular highways, such that the plus-ends are orientated towards the growing tip (Baas, Deitch et al., 1988). As a consequence the kinesins, which are primarily plus end motors, move cellular freight in an anterograde direction, whereas the minus end directed dyneins move cargo in a retrograde direction (Kevenaar and Hoogenraad, 2015). The uniform polarity of microtubules in axons contrasts with those in dendrites that are arranged in a more haphazard fashion (Fig. 1F). Within dendrites microtubules have also been implicated in the formation and plasticity of spines. For instance, the treatment of hippocampal neurons with low doses of the microtubule destabilizing drug Nocodazole impairs BDNF induced dendritic spine formation (Gu et al., 2008).

5. Microtubules and tubulin isoforms

It is clear that microtubules are essential for an array of cellular tasks in mitosis, migration and differentiation. This functional diversity reflects the molecular diversity of the constituents of microtubules: the tubulins. Tubulin heterodimers are formed by the stereotypic folding and assembly of exactly one α- and one β-tubulin, which polymerize into hollow microtubules in a head-to-tail juxtapositional arrangement (Fig. 2A) (Lewis et al., 1996; Lewis et al., 1997; Tian et al., 1997; Feng et al., 2016). Microtubules typically have 13 protofilaments, resulting in an outer diameter of 25 nm, however, the protofilament number can vary between 9 and 16 (Chretien et al., 1992; Wade and Chretien, 1993; Kwiatkowska et al., 2006). In a cellular environment microtubules undergo cycles of growth and shrinkage by transitioning through catastrophe and rescue. This property, which is referred to as dynamic instability, enables a cell to quickly reorganise its shape (Mitchison and Kirschner, 1984).

The tubulin gene family has been subject to evolutionary expansion (Cleveland et al., 1980; McKean et al., 2001). In yeast there are 2 α-tubulin and 1 β-tubulin genes, in fruit flies there are 4 α-tubulin and 4 β-tubulin genes, in mice there are 7 α- and 8 β-tubulin genes, whereas in humans there are 8 α- and 9 β-tubulin genes. It should be noted that it is currently unclear whether different α-tubulin isoforms have any preference with respect to their β-tubulin partner or whether the pairing is determined stochastically (Fig. 2B–C). This is largely because the generation of specific antibodies for individual isoforms has been problematic. In vertebrates each of the tubulin genes is located at a different genetic locus, and encodes for a protein that is approximately 400 amino acids in length. Despite the expansion of this family these proteins show a remarkable degree of homology, with more than 88% of sequence identity when comparing all the human α-tubulins. The notable exception is the negatively charged C terminal domain, which is the most variant, lies on the surface of the structure, and subject to extensive post translational modification. So what is the evidence supporting functional specification of the tubulins?

6. Evidence of functional specification – the tubulinopathies

Early genetic studies in *C. elegans* and *Drosophila* provided data supporting the hypothesis that different tubulins have different functions (Chalfie and Thomson, 1982; Hutchens et al., 1997). The most elegant of these were conducted by Raff and colleagues who showed that replacement of a testes specific tubulin isoform with a developmental variant failed to rescue a spermatogenesis defect, which was associated with a change in the microtubule architecture and protofilament number (Kemphues et al., 1982; Kimble et al., 1989; Raff et al., 1997). This theme of functional specification within the tubulin gene family has been further advanced by the discovery and characterisation of the tubulinopathies; a term that is used to describe a broad spectrum of disease states caused by mutations in tubulin genes. These pathogenic variants reveal an interesting phenotypic pattern, dependent on the tubulin gene and residue mutated (Fig. 3) (Chakraborti et al., 2016). In the case of *TUBB1*, *TUBB8*, and *TUBA4A* the diseases are unambiguously distinct. Mutations in *TUBB1* cause macrothrombocytopenia, a blood disorder that affects the formation of platelets (Kunishima et al., 2009), mutations in *TUBB8* cause the arrest of meiotic oocytes resulting in female sterility (Feng et al., 2016), whereas variants in *TUBA4A* cause amyotrophic lateral sclerosis, a late onset neurological disease that is characterised by the degeneration of motor neurons (Smith et al., 2014). In contrast mutations in *TUBA1A*, *TUBB2B*, and *TUBB3* result in overlapping phenotypes. Mutations in *TUBA1A*, were first described in patients with lissencephaly, cerebellar malformations and basal ganglia defects (Keays et al., 2007; Poirier et al., 2007; Bahi-Buisson et al., 2008; Morris-Rosendahl et al., 2008; Kumar et al., 2010). In time *TUBA1A* mutations were also identified in individuals with polymicrogyria, a phenotype that has also been reported in patients with *TUBB2B* and *TUBB3*

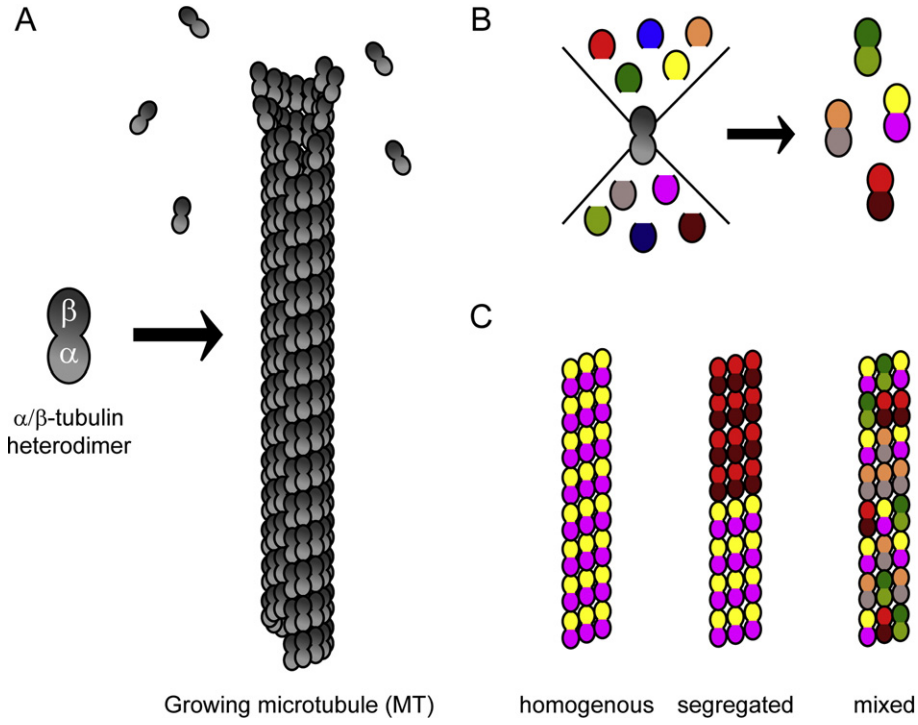

Fig. 2. Tubulin heterodimers polymerize into microtubules. (A) Each tubulin heterodimer consists of an α- and a β-tubulin subunit. Once assembled they polymerize into the hollow microtubules in a justapositional arrangement. (B) Tubulins are a multi-gene family, which in humans consists of 8 α- and 9 β-tubulin genes. Potentially tubulins could form a combinatorial code if different α-tubulin isoforms preferentially assemble with specific β-tubulins at different time points. (C) We present three hypothetical models for the arrangement of different tubulin isotypes. First, if only a single α tubulin and a single β tubulin were expressed in a particular cell type you would expect the formation of homogeneous microtubules. Second, if the expression of tubulin genes were to switch during cellular maturation segregated microtubules might result. Thirdly, it is possible that microtubules are assembled stochastically from different α- and 9 β-tubulins resulting in a mixed distribution of heterodimers.

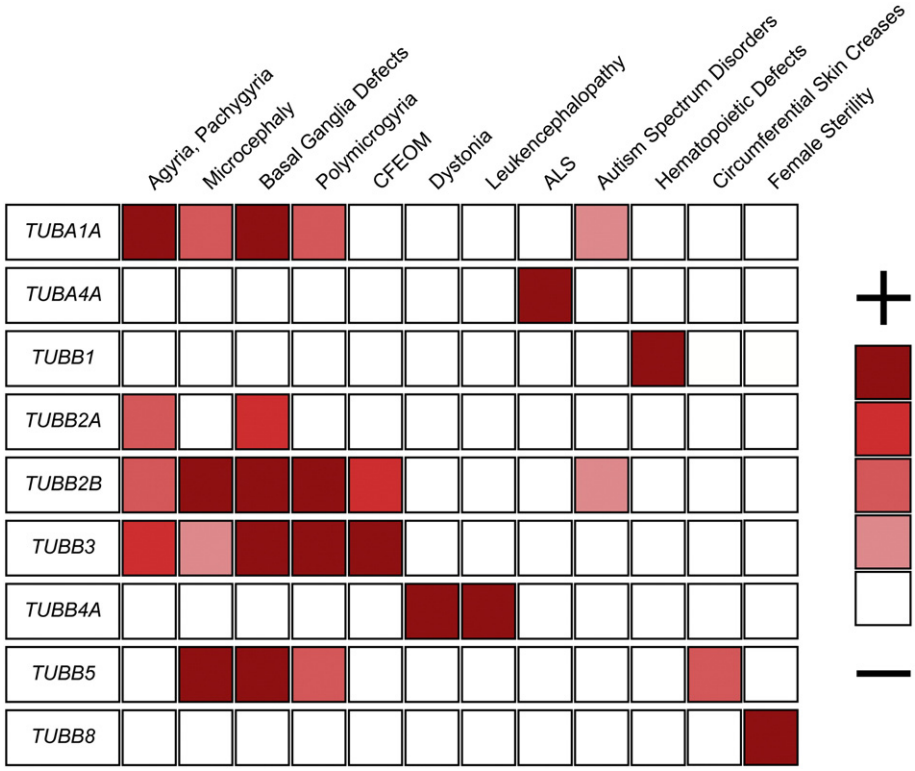

Fig. 3. Tubulin gene mutations result in distinct as well as convergent phenotypes. Heatmap for the tubulinopathies highlighting the association between mutations in a particular tubulin gene with various phenotypes. It can be observed that mutations in some tubulin genes result in highly specific disease states (*e.g.* TUBB8, TUBA4A and TUBB1), whereas mutations in other isoforms result in overlapping phenotypes (*e.g.* TUBA1A, TUBB2B, and TUBB3). CFEOM: congenital fibrosis of the extra-ocular muscle; ALS: amyotrophic lateral sclerosis. The darkness of the red colour indicates an increased prevalence of a particular phenotype with a given tubulin gene mutation.

mutations (Jaglin et al., 2009; Poirier et al., 2010; Jansen et al., 2011; Poirier et al., 2012; Cushion et al., 2013). Moreover, *TUBB2B* and *TUBB3* mutations have been associated with axon guidance disorders, specifically congenital fibrosis of the extra-ocular muscles (CFEOM) (Tischfield et al., 2010; Cederquist et al., 2012). Despite this convergence there are distinctions with respect to genotypic-phenotypic association. For instance, *TUBA1A* mutations invariably result in cortical dyslamination, whereas *TUBB3* variants can result in CFEOM without affecting the cortical structure or size (Tischfield et al., 2010; Bahi-Buisson et al., 2014).

It is further apparent that the residue that is mutated within the tubulin molecule is critical in determining the phenotypic outcome. We have previously shown that mutations in *TUBB5*, such as E401K, M299V, and V353I, cause microcephaly with severe mental retardation that is accompanied by structural brain malformations such as polymicrogyria and dysmorphic basal ganglia (Breuss et al., 2012). More recently, in collaboration with the Van Esch laboratory we have demonstrated that mutations in the same gene (Q15K, Y222F) can also cause the "Michelin Baby Syndrome" which is characterised by circumferential skin folding and facial abnormalities (Isrie et al., 2015) (Fig. 3). While these patients also present with a reduction in brain size, the phenotypes are strikingly different. Taken together, the range of human diseases associated with tubulin mutations strongly suggests that the various isoforms, perhaps even individual residues, have distinct functional properties. In our next section, we will explore the possible mechanisms that drive these differences.

7. Sources of functional specification

7.1. Transcription - spatiotemporal expression of the tubulin genes

Transcriptional processes determine whether or not a gene is expressed and at what level. The activity of the promoter, the action of enhancers (and repressors), as well as the overall epigenetic landscape

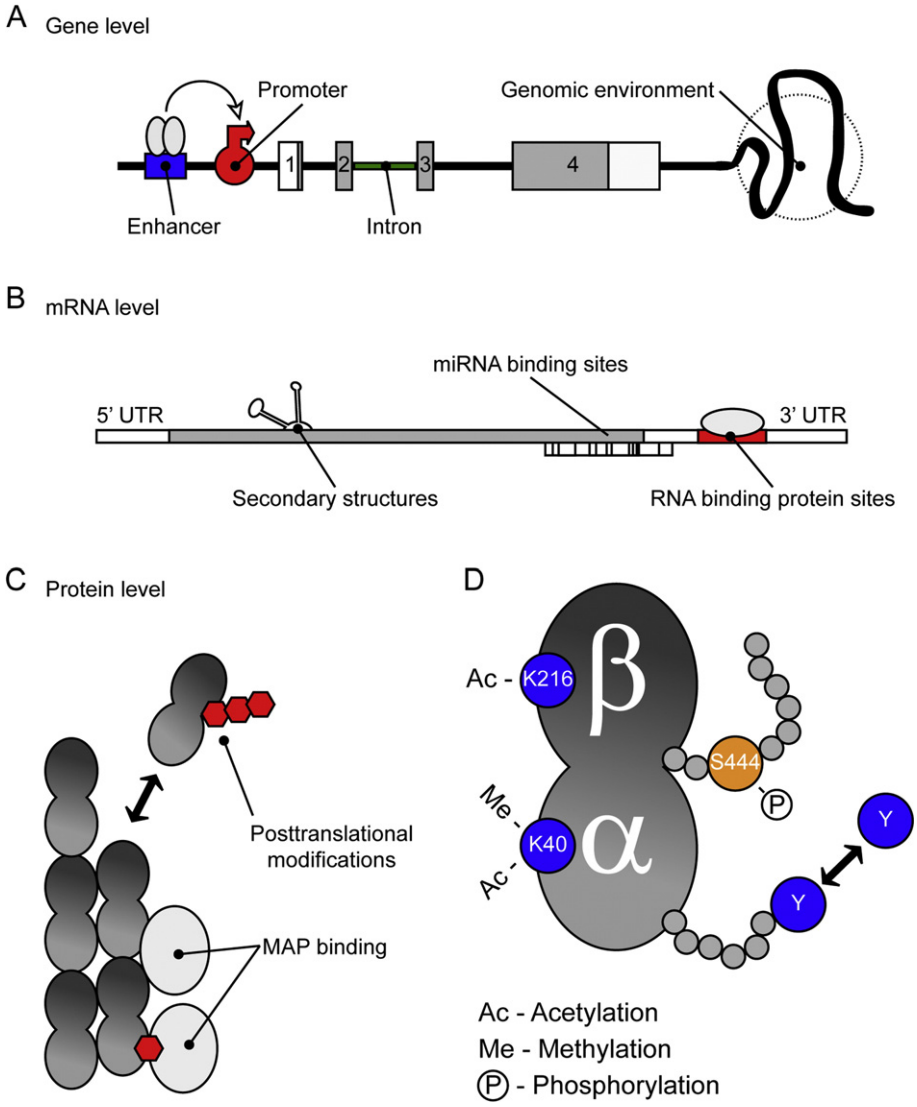

Fig. 4. Sources of functional specification for the tubulins. Functional specification for the tubulins can arise at a transcriptional (A), translational (B) and post-translational level (C). (A) Transcriptional: enhancers, promoters, intronic sequences, and the general genomic environment differ between tubulin isoforms, influencing the expression of transcripts spatially and temporally. (B) Translational: mRNA stability, mRNA localization, secondary structure, miRNA binding sites and the interaction with RNA binding proteins all contribute to translational efficiency of tubulin genes. Note that many of these features are associated with the 5′- and 3′-UTRs, which are highly divergent when comparing different tubulins isoforms. (C) Post-translational: variation in the peptide sequence of the tubulin protein determines whether an isoform can be subject to specific post-translational modification. These modifications (depicted as red hexagons) influence the complement of microtubule associated proteins, as well as the stability and dynamism of the microtubule superstructure. (D) Schematic of a tubulin heterodimer showing examples of post-translational modifications affecting amino acids that vary between tubulin isoforms. Depicted are the: acetylation and methylation of lysine 40 (K40) of α-tubulin (which is absent in Tuba8); the (de)tyrosination of the terminal tyrosine (Y) of α-tubulin (which is absent in Tuba4a and Tuba8); the acetylation of lysine 216 (K216) of β-tubulin (which is absent in Tubb1); and the phosphorylation of serine 444 (S444) of β-tubulin (which is unique to Tubb3).

surrounding the gene are all involved in this regulation (Fig. 4A) (Orphanides and Reinberg, 2002; Gibney and Nolan, 2010). Following the discovery in the 1980s that there are multiple tubulin genes, it soon became apparent that their spatial and temporal expression was varied (Cowan et al., 1981; Kemphues et al., 1982; Wilde et al., 1982; Cowan and Dudley, 1983; Villasante et al., 1986; Wang et al., 1986; Burgoyne et al., 1988; Carpenter et al., 1992). These early studies primarily relied on analysis of RNA expression employing methods such as *in situ* hybridisation and Northern blotting. More recently the generation of transgenic mouse models expressing markers driven by the endogenous transcriptional machinery at a specific tubulin locus has permitted a more refined cellular analysis of tubulin gene expression.

The utility of this approach was first demonstrated by Miller and colleagues who expressed a transgenic LacZ protein under the control of the *Tuba1a* promoter region to visualize cells expressing this isoform (Gloster et al., 1994; Bamji and Miller, 1996). They found that this major α-tubulin is expressed almost exclusively in post-mitotic neurons during development, consistent with this tubulin's pathogenic role in migration disorders, such as pachygyria or lissencephaly (Bahi-Buisson et al., 2014). To study the cellular expression of *Tubb5* and *Tubb2b* in the developing brain we have adopted a similar strategy creating BAC-transgenic mice that express GFP driven by the native promoter. These studies have demonstrated both the differences and the dynamism of tubulin gene expression. For instance, *Tubb5* is expressed

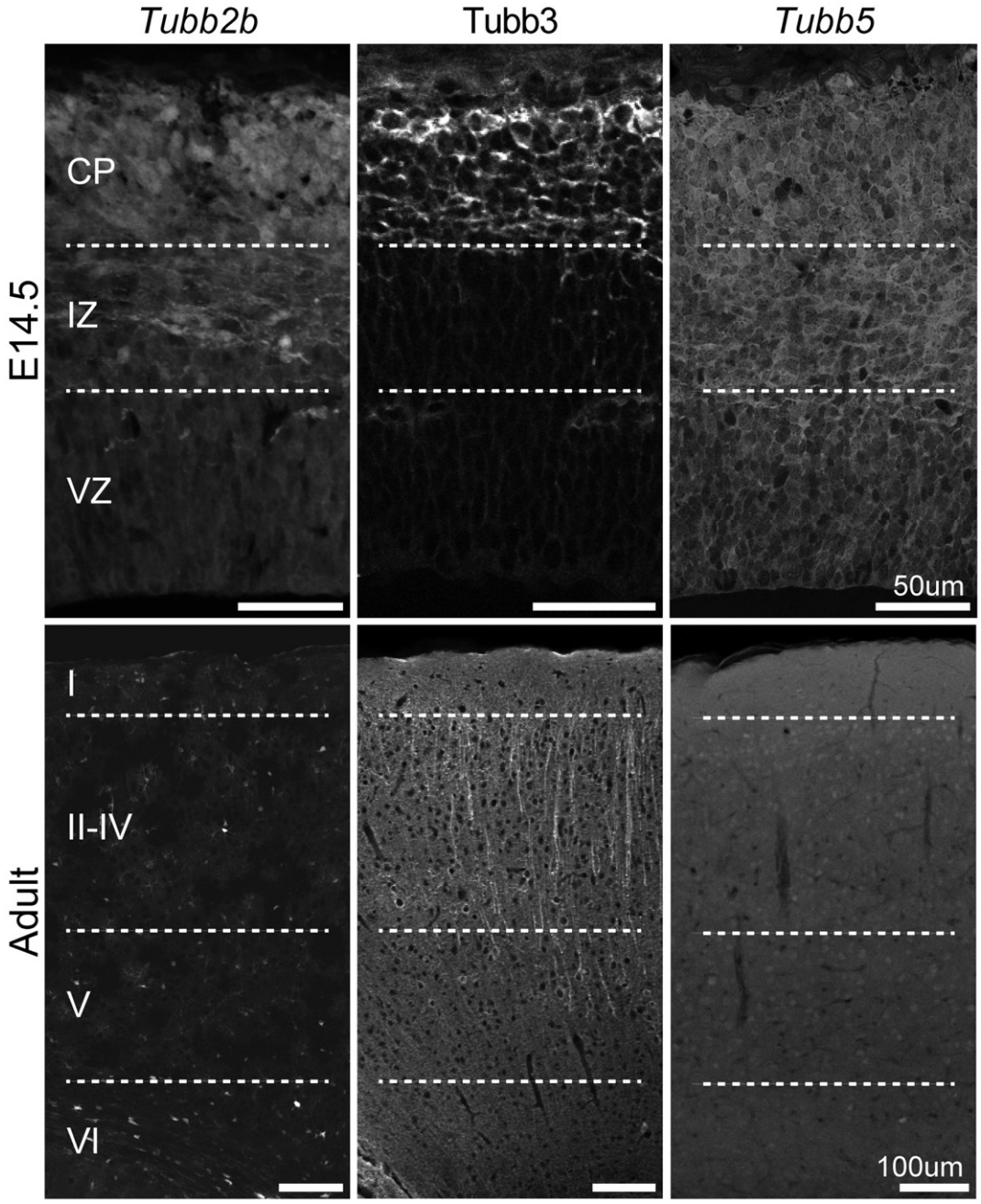

Fig. 5. Differential spatiotemporal expression of tubulin genes. Images show the expression of the three murine tubulin isoforms *Tubb2b*, Tubb3, and *Tubb5* at embryonic day (E) 14.5 and at 8 weeks (adult). Whereas *Tubb2b* and *Tubb5* are ubiquitously expressed during embryonic development at high levels, Tubb3 predominates in post-mitotic neurons. In adulthood this pattern changes dramatically, as *Tubb2b* is expressed only in glial cells, Tubb3 is restricted to a subset of neurons, and *Tubb5*, although still broadly expressed, is present at low levels. Expression was visualized by employing transgenic mouse lines for *Tubb2b* and *Tubb5* (*Tg*(*Tubb2b-eGFP*)*GlbDAK* and *Tg*(*Tubb5-eGFP*)) and by antibody staining with Tuj1 for Tubb3. Descriptions of the lines, staining methods, and antibody conditions were published previously (Breuss et al., 2012; Breuss et al., 2015). Scale bars show 50 μm at E14.5 and 100 μm for adult as indicated.

throughout the developing brain in Pax6 positive radial glial cells, Tbr2 positive intermediate progenitors, DCX positive post-mitotic neurons and persists at low levels in NeuN positive adult neurons (Breuss et al., 2012) (Fig. 5). *Tubb2b* is likewise expressed in developmental progenitors and migrating neurons, but undergoes a distinctive development switch postnatally. In adulthood its expression is restricted to GFAP positive astrocytes and Olig-2 positive oligodendrocytes, as well as Bergmann glia in the cerebellum. Interestingly, *Tubb2b* is largely absent from proliferative progenitors in the retina but is expressed in post-mitotic migrating neurons, and is maintained in differentiated adult neurons in the ganglion cell layer and the granule cells of the inner nuclear layer (Breuss et al., 2015). The expression pattern of Tubb2b contrasts with Tubb3 (TuJ) which is considered to be a marker for post-mitotic neurons in development and adulthood (Fig. 5) (Fanarraga et al., 1999).

We have proposed that the expression pattern of a tubulin isoform might reflect the role of the microtubule cytoskeleton in that particular cell type at a given point in time. This notion is further supported by the differential expression patterns of other tubulin isoforms. For instance; *Tuba8* is predominantly expressed in the testes and skeletal muscle (Braun et al., n.d.); the expression of *Tubb1* is restricted to the hematopoietic system (Leandro-Garcia et al., n.d.); and the primate specific *TUBB8* is enriched in oocytes but is absent from mature sperm (Feng et al., 2016). Little is known of the transcriptional machinery that regulates these differential expression patterns, as well as the developmental transitions we have observed in the case of *Tubb2b.*

7.2. Translation - mRNA stability, efficiency and localization of tubulin transcripts

mRNAs not only encode the protein to be, but also contain sequences that can determine their stability, localization and expression (Gebauer and Hentze, 2004; Glisovic et al., 2008; Schoenberg and Maquat, 2012). Common mechanisms of regulation include folding of mRNA secondary structures, recognition by micro-RNAs and binding of various RNA binding proteins (Fig. 4B). Many of these functions are mediated by the 5′- and 3′-UTRs that are under less evolutionary constraint compared to the coding sequence, allowing much higher differentiation between isoforms that are closely related on the protein level. The latter is particularly event with respect to the tubulin genes, which exhibit highly variant 5′- and 3′-UTRs (Lewis et al., 1985, Villasante, Wang et al., 1986).

Micro-RNAs (miRNAs) are known to play a critical role during neural development and have emerged as an important element for post-transcriptional gene regulation (Cochella and Hobert, 2012) (Fig. 4B). This class of small, non-coding transcripts can act by decreasing the levels of target mRNAs or repressing translational efficiency (Kosik, 2006). In cell culture systems it has been shown that the 3′-UTR *TUBB3* is a direct target of miR-200c (Cochrane et al., 2009; Leskela et al., 2011). An additional factor which determines the stability, and therefore the abundance, of any given mRNA is its codon usage. For instance, it has been shown that the replacement of optimal codons with synonymous non-optimal codons has a dramatic effect on mRNA stability, whereas the inverse substitution results in increased stability of the mRNA molecule (Presnyak et al., 2015). Consistent with this finding Mishima and Tomari have reported that specific codon usage is necessary to ensure the stability of maternal mRNAs in zebrafish embryos (Mishima and Tomari, 2016). A comparison of the transcripts for the alpha tubulin genes in mice, reveals 70% identity of mRNA transcripts in comparison to 88% conservation of the protein sequence. The extent to which differential codon usage influences the stability of various tubulin isoforms is currently unknown; however, it may influence the half-life of different transcripts. Tubulin transcripts with a reduced half-life would enable more precise temporal control over the generation of specific isoforms, which may be beneficial for the generation of particular cellular structures.

The subcellular localisation of the mRNA transcript is an additional parameter that may determine the functional repertoire of different tubulin genes. Preitner and colleagues have shown that the RNA binding protein APC interacts with the mRNA of *Tubb2b*, ensuring its localization to the growth cone of extending neurons. They showed that impairment of this interaction results in aberrant growth cone shape, implying that a critical concentration of *Tubb2b* is required in this subcellular compartment (Preitner et al., 2014). We still lack a comprehensive understanding of the subcellular localisation of different tubulin mRNAs, the half-life of the transcripts, or the miRNAs that target them. All of these aspects, however, are likely to contribute to the functional specification of the tubulins.

7.3. Post translational modifications

Tubulin heterodimers in their depolymerized as well as polymerized forms undergo a host of posttranslational modifications (PTMs), including polyamination, S-nitrosylation, sumoylation, and polyglutamylation (Janke and Bulinski, 2011; Yu et al., 2015) (Fig. 4C–D). Excellent reviews of the effect of PTMs on microtubule function have been published elsewhere (Westermann and Weber, 2003). Here, we focus on those modifications that are associated with variations in the amino acids and therefore have the potential to influence the properties of different tubulin isotypes.

Acetylation of the lysine at position 40 (K40) of α-tubulin is the sole luminal amino acid that is the target of PTM (Fig. 4D). It is associated with increased stability of microtubules through indirect mechanisms that rely on the recruitment of other factors (Al-Bassam and Corbett, 2012). In *C. elegans*, this modification is crucial for the assembly of 15 protofilament microtubules that are required for touch receptivity (Fukushige et al., 1999; Cueva et al., 2012). This requires the presence of the K40 residue as a substrate, which in humans TUBA8 lacks (Stanchi et al., 2000). Moreover, It has been shown that K40 is also subject to methylation by SETD2 (Park et al., 2016). Similar to the histone code, methylation of α-tubulin K40 acts as a bistable switch. These mutually exclusive modifications impact microtubule function in opposite ways: whereas acetylation is correlated with stable microtubules, methylation is involved with destabilization during mitosis. Recently, it has been shown that K216 in the β-tubulin family is also subject to acetylation (see Table 1 in (Liu et al., 2015). While the functional relevance of this modification is not known, it is pertinent to note that Tubb1 lacks this lysine.

Most α-tubulin genes encode a tyrosine at the final amino acid position with the notable exception of Tuba4a and Tuba8 (Villasante et al., 1986) (Fig. 4D). Following integration, this tyrosine can be removed by tubulin tyrosine carboxypeptidase which has been shown to increase microtubule stability (Webster et al., 1987). Depolymerized heterodimers can later be targeted for tyrosination to re-start the cycle, by tubulin tyrosine ligase (Yu et al., 2015). It is known that detyrosinated microtubules are enriched in the axon, in contrast to the more dynamic growth cone where tyrosinated tubulin dominates (Marcos et al., 2009). Holzbaur and colleagues have reported that this influences intracellular transport by a CLIP-170 mediated mechanism (Nirschl et al., 2016). Most recently, formation of the metaphase plate during mitosis has been shown to require spatially regulated detyronsination of spindle microtubules. The depletion of detyrosinated tubulin, which is enriched in the vicinity of the spindle polls, results in misaligned chromosomes and delayed mitotic progression by influencing CENP binding (Barisic et al., 2015). In light of this work it is tempting to speculate that the expression of Tuba4a and Tuba8 might reflect the need to generate structures with more stable microtubules, such as the axenomes within sperm or the axons of neurons.

A final example of how PTMs tune the functional properties of the microtubule cytoskeleton relates to the phosphorylation of serine 444, a residue that is unique to Tubb3 (Fig. 4D). It has previously been shown that removal of this phosphate, inhibits MAP2 stimulated

microtubule assembly *in vitro* (Khan and Luduena, 1996). While Tubb3 is generally considered to be expressed exclusively in postmitotic neurons, a recent study by Cunto and colleagues report that it is present at low levels in murine cortical progenitors, most notably in the vicinity of the midbody (Sgro et al., 2016). They demonstrated that deletion of citron kinase (CIT-K) which promotes phosphorylation of the S444 residue, compromises neurogenic cytokinesis. Exploiting HeLa cells as a model system they further demonstrated that the expression of a S444A Tubb3 mutant increases the percentage of binucleated cells, which is not apparent in the case of the phospho-mimetic mutant S444D. These data support the hypothesis that the phosphorylation of Tubb3 at S444 is critical for midbody formation, and the progression of cytokinesis in the developing brain.

These are just three examples of how PTMs contribute to the functional diversity of the microtubule cytoskeleton. There are undoubtedly many more. Recently a proteomic study conducted by Liu and colleagues dramatically increased the repertoire of tubulin PTMs identifying more than 80 tubulin residues that are subject to at least one modification, 24 of which were previously unreported (Liu et al., 2015). Clearly, much remains to be discovered on this front.

8. Concluding remarks

Why do neurons need multiple tubulins? The current literature and the emergence of the tubulinopathies suggest that this evolutionary expansion was driven by the need for functional diversity. In this review we have highlighted that this specification results from transcriptional, translational and post-translation regulation. This tuning enables the microtubule cytoskeleton to perform a stunning array of tasks as a neuron is born, migrates to a different location and then forms functional circuits with distant synaptic partners. Without doubt, the tubulins are much more than a mere loading control for your average Western blot.

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References

Al-Bassam, J., Corbett, K.D., 2012. Alpha-tubulin acetylation from the inside out. Proc. Natl. Acad. Sci. U. S. A. 109 (48), 19515–19516.
Altman, J., Bayer, S.A., 1990. Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pyramidale. J. Comp. Neurol. 301 (3), 343–364.
Ayala, R., Shu, T., Tsai, L.H., 2007. Trekking across the brain: the journey of neuronal migration. Cell 128 (1), 29–43.
Baas, P.W., Deitch, J.S., Black, M.M., Banker, G.A., 1988. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc. Natl. Acad. Sci. U. S. A. 85 (21), 8335–8339.
Bahi-Buisson, N., Poirier, K., Boddaert, N., Saillour, Y., Castelnau, L., Philip, N., Buyse, G., Villard, L., Joriot, S., Marret, S., Bourgeois, M., Van Esch, H., Esch, H., Lagae, L., Amiel, J., Hertz-Pannier, L., Roubertie, A., Rivier, F., Pinard, J.M., Beldjord, C., Chelly, J., 2008. Refinement of cortical dysgeneses spectrum associated with TUBA1A mutations. J. Med. Genet. 45 (10), 647–653.
Bahi-Buisson, N., Poirier, K., Fourniol, F., Saillour, Y., Valence, S., Lebrun, N., Hully, M., Bianco, C.F., Boddaert, N., Elie, C., Lascelles, K., Souville, I., Consortium, L.I.-T., Beldjord, C., Chelly, J., 2014. The wide spectrum of tubulinopathies: what are the key features for the diagnosis? Brain 137 (Pt 6), 1676–1700.
Bamji, S.X., Miller, F.D., 1996. Comparison of the expression of a T alpha 1:nlacZ transgene and T alpha 1 alpha-tubulin mRNA in the mature central nervous system. J. Comp. Neurol. 374 (1), 52–69.
Barisic, M., Silva e Sousa, R., Tripathy, S.K., Magiera, M.M., Zaytsev, A.V., Pereira, A.L., Janke, C., Grishchuk, E.L., Maiato, H., 2015. Mitosis. Microtubule detyrosination guides chromosomes during mitosis. Science 348 (6236), 799–803.
Braun, A., Breuss, M., Salzer, M.C., Flint, J., Cowan, N.J., Keays, D.A., 2010. "Tuba8 is expressed at low levels in the developing mouse and human brain.". Am. J. Hum. Genet. 86 (5), 819–822 author reply 822-813.
Breuss, M., Heng, J.I., Poirier, K., Tian, G., Jaglin, X.H., Qu, Z., Braun, A., Gstrein, T., Ngo, L., Haas, M., Bahi-Buisson, N., Moutard, M.L., Passemard, S., Verloes, A., Gressens, P., Xie, Y., Robson, K.J., Rani, D.S., Thangaraj, K., Clausen, T., Chelly, J., Cowan, N.J., Keays, D.A., 2012. Mutations in the beta-tubulin gene TUBB5 cause microcephaly with structural brain abnormalities. Cell Rep. 2 (6), 1554–1562.
Breuss, M., Keays, D.A., 2014. Microtubules and neurodevelopmental disease: the movers and the makers. Adv. Exp. Med. Biol. 800, 75–96.
Breuss, M., Morandell, J., Nimpf, S., Gstrein, T., Lauwers, M., Hochstoeger, T., Braun, A., Chan, K., Sanchez Guajardo, E.R., Zhang, L., Suplata, M., Heinze, K.G., Elsayad, K., Keays, D.A., 2015. The expression of Tubb2b undergoes a developmental transition in murine cortical neurons. J. Comp. Neurol.
Burgoyne, R.D., Cambray-Deakin, M.A., Lewis, S.A., Sarkar, S., Cowan, N.J., 1988. Differential distribution of beta-tubulin isotypes in cerebellum. EMBO J. 7 (8), 2311–2319.
Carpenter, J.L., Ploense, S.E., Snustad, D.P., Silflow, C.D., 1992. Preferential expression of an alpha-tubulin gene of Arabidopsis in pollen. Plant Cell 4 (5), 557–571.
Cearns, M.D., Escuin, S., Alexandre, P., Greene, N.D., Copp, A.J., 2016. Microtubules, polarity and vertebrate neural tube morphogenesis. J. Anat. 229 (1), 63–74.
Cederquist, G.Y., Luchniak, A., Tischfield, M.A., Peeva, M., Song, Y., Menezes, M.P., Chan, W., Andrews, C., Chew, S., Jamieson, R.V., Gomes, L., Flaherty, M., Grant, P.E., Gupta, M.L., Engle, E.C., 2012. An inherited TUBB2B mutation alters a kinesin-binding site and causes polymicrogyria, CFEOM and axon dysinnervation. Hum. Mol. Genet. 21 (26), 5484–5499.
Chakraborti, S., Natarajan, K., Curiel, J., Janke, C., Liu, J., 2016. The emerging role of the tubulin code: from the tubulin molecule to neuronal function and disease. Cytoskeleton 73 (10), 521–550.
Chalfie, M., Thomson, J.N., 1982. Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. J. Cell Biol. 93 (1), 15–23.
Chretien, D., Metoz, F., Verde, F., Karsenti, E., Wade, R.H., 1992. Lattice defects in microtubules: protofilament numbers vary within individual microtubules. J. Cell Biol. 117 (5), 1031–1040.
Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J., Kirschner, M.W., 1980. Number and evolutionary conservation of alpha- and beta-tubulin and cytoplasmic beta- and gamma-actin genes using specific cloned cDNA probes. Cell 20 (1), 95–105.
Cochella, L., Hobert, O., 2012. Diverse functions of microRNAs in nervous system development. Curr. Top. Dev. Biol. 99, 115–143.
Cochrane, D.R., Spoelstra, N.S., Howe, E.N., Nordeen, S.K., Richer, J.K., 2009. MicroRNA-200c mitigates invasiveness and restores sensitivity to microtubule-targeting chemotherapeutic agents. Mol. Cancer Ther. 8 (5), 1055–1066.
Cowan, N.J., Dudley, L., 1983. Tubulin isotypes and the multigene tubulin families. Int. Rev. Cytol. 85, 147–173.
Cowan, N.J., Wilde, C.D., Chow, L.T., Wefald, F.C., 1981. Structural variation among human beta-tubulin genes. Proc. Natl. Acad. Sci. U. S. A. 78 (8), 4877–4881.
Creppe, C., Malinouskaya, L., Volvert, M.L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S., Belachew, S., Malgrange, B., Chapelle, J.P., Siebenlist, U., Moonen, G., Chariot, A., Nguyen, L., 2009. Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. Cell 136 (3), 551–564.
Cueva, J.G., Hsin, J., Huang, K.C., Goodman, M.B., 2012. Posttranslational acetylation of alpha-tubulin constrains protofilament number in native microtubules. Curr. Biol. 22 (12), 1066–1074.
Cushion, T.D., Dobyns, W.B., Mullins, J.G., Stoodley, N., Chung, S.K., Fry, A.E., Hehr, U., Gunny, R., Aylsworth, A.S., Prabhakar, P., Uyanik, G., Rankin, J., Rees, M.I., Pilz, D.T., 2013. Overlapping cortical malformations and mutations in TUBB2B and TUBA1A. Brain 136 (Pt 2), 536–548.
Dickson, B.J., 2002. Molecular mechanisms of axon guidance. Science 298 (5600), 1959–1964.
Fanarraga, M.L., Avila, J., Zabala, J.C., 1999. Expression of unphosphorylated class III beta-tubulin isotype in neuroepithelial cells demonstrates neuroblast commitment and differentiation. Eur. J. Neurosci. 11 (2), 517–527.
Feng, R., Sang, Q., Kuang, Y., Sun, X., Yan, Z., Zhang, S., Shi, J., Tian, G., Luchniak, A., Fukuda, Y., Li, B., Yu, M., Chen, J., Xu, Y., Guo, L., Qu, R., Wang, X., Sun, Z., Liu, M., Shi, H., Wang, H., Feng, Y., Shao, R., Chai, R., Li, Q., Xing, Q., Zhang, R., Nogales, E., Jin, L., He, L., Gupta Jr., M.L., Cowan, N.J., Wang, L., 2016. Mutations in TUBB8 and human oocyte meiotic arrest. N. Engl. J. Med. 374 (3), 223–232.
Florio, M., Huttner, W.B., 2014. Neural progenitors, neurogenesis and the evolution of the neocortex. Development 141 (11), 2182–2194.
Fukushige, T., Siddiqui, Z.K., Chou, M., Culotti, J.G., Gogonea, C.B., Siddiqui, S.S., Hamelin, M., 1999. MEC-12, an alpha-tubulin required for touch sensitivity in *C. elegans*. J. Cell Sci. 112 (Pt 3), 395–403.
Gebauer, F., Hentze, M.W., 2004. Molecular mechanisms of translational control. Nat. Rev. Mol. Cell Biol. 5 (10), 827–835.
Gibney, E.R., Nolan, C.M., 2010. Epigenetics and gene expression. Heredity 105 (1), 4–13.
Glisovic, T., Bachorik, J.L., Yong, J., Dreyfuss, G., 2008. RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. 582 (14), 1977–1986.
Gloster, A., Wu, W., Speelman, A., Weiss, S., Causing, C., Pozniak, C., Reynolds, B., Chang, E., Toma, J.G., Miller, F.D., 1994. The T alpha 1 alpha-tubulin promoter specifies gene expression as a function of neuronal growth and regeneration in transgenic mice. J. Neurosci. 14 (12), 7319–7330.
Glotzer, M., 2009. The 3Ms of central spindle assembly: microtubules, motors and MAPs. Nat. Rev. Mol. Cell Biol. 10 (1), 9–20.
Gotz, M., Huttner, W.B., 2005. The cell biology of neurogenesis. Nat. Rev. Mol. Cell Biol. 6 (10), 777–788.
Gu, J., Firestein, B.L., Zheng, J.Q., 2008. Microtubules in dendritic spine development. J. Neurosci. 28 (46), 12120–12124.

Gulledge, A.T., Kampa, B.M., Stuart, G.J., 2005. Synaptic integration in dendritic trees. J. Neurobiol. 64 (1), 75–90.
He, M., Zhang, Z.H., Guan, C.B., Xia, D., Yuan, X.B., 2010. Leading tip drives soma translocation via forward F-actin flow during neuronal migration. J. Neurosci. 30 (32), 10885–10898.
Heng, J.I., Chariot, A., Nguyen, L., 2009. Molecular layers underlying cytoskeletal remodelling during cortical development. Trends Neurosci.
Hutchens, J.A., Hoyle, H.D., Turner, F.R., Raff, E.C., 1997. Structurally similar *Drosophila* alpha-tubulins are functionally distinct in vivo. Mol. Biol. Cell 8 (3), 481–500.
Isrie, M., Breuss, M., Tian, G., Hansen, A.H., Cristofoli, F., Morandell, J., Kupchinsky, Z.A., Sifrim, A., Rodriguez-Rodriguez, C.M., Dapena, E.P., Doonanco, K., Leonard, N., Tinsa, F., Moortgat, S., Ulucan, H., Koparir, E., Karaca, E., Katsanis, N., Marton, V., Vermeesch, J.R., Davis, E.E., Cowan, N.J., Keays, D.A., Van Esch, H., 2015. Mutations in either TUBB or MAPRE2 cause circumferential skin creases Kunze type. Am. J. Hum. Genet. 97 (6), 790–800.
Jaglin, X.H., Poirier, K., Saillour, Y., Buhler, E., Tian, G., Bahi-Buisson, N., Fallet-Bianco, C., Phan-Dinh-Tuy, F., Kong, X.P., Bomont, P., Castelnau-Ptakhine, L., Odent, S., Loget, P., Kossorotoff, M., Snoeck, I., Plessis, G., Parent, P., Beldjord, C., Cardoso, C., Represa, A., Flint, J., Keays, D.A., Cowan, N.J., Chelly, J., 2009. Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. Nat. Genet. 41 (6), 746–752.
Janke, C., Bulinski, J.C., 2011. Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. Nat. Rev. Mol. Cell Biol. 12 (12), 773–786.
Jansen, A.C., Oostra, A., Desprechins, B., De Vlaeminck, Y., Vlaeminck, Y., Verhelst, H., Regal, L., Verloo, P., Bockaert, N., Keymolen, K., Seneca, S., De Meirleir, L., Meirleir, L., Lissens, W., 2011. TUBA1A mutations: from isolated lissencephaly to familial polymicrogyria. Neurology 76 (11), 988–992.
Keays, D.A., Tian, G., Poirier, K., Huang, G.J., Siebold, C., Cleak, J., Oliver, P.L., Fray, M., Harvey, R.J., Molnar, Z., Pinon, M.C., Dear, N., Valdar, W., Brown, S.D., Davies, K.E., Rawlins, J.N., Cowan, N.J., Nolan, P., Chelly, J., Flint, J., 2007. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell 128 (1), 45–57.
Kemphues, K.J., Kaufman, T.C., Raff, R.A., Raff, E.C., 1982. The testis-specific beta-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. Cell 31 (3 Pt 2), 655–670.
Kevenaar, J.T., Hoogenraad, C.C., 2015. The axonal cytoskeleton: from organization to function. Front. Mol. Neurosci. 8, 44.
Khan, I.A., Luduena, R.F., 1996. Phosphorylation of beta III-tubulin. Biochemistry 35 (12), 3704–3711.
Kimble, M., Incardona, J.P., Raff, E.C., 1989. A variant beta-tubulin isoform of *Drosophila melanogaster* (beta 3) is expressed primarily in tissues of mesodermal origin in embryos and pupae, and is utilized in populations of transient microtubules. Dev. Biol. 131 (2), 415–429.
Kosik, K.S., 2006. The neuronal microRNA system. Nat. Rev. Neurosci. 7 (12), 911–920.
Kosodo, Y., Suetsugu, T., Suda, M., Mimori-Kiyosue, Y., Toida, K., Baba, S.A., Kimura, A., Matsuzaki, F., 2011. Regulation of interkinetic nuclear migration by cell cycle-coupled active and passive mechanisms in the developing brain. EMBO J. 30 (9), 1690–1704.
Kriegstein, A.R., Gotz, M., 2003. Radial glia diversity: a matter of cell fate. Glia 43 (1), 37–43.
Kriegstein, A.R., Noctor, S.C., 2004. Patterns of neuronal migration in the embryonic cortex. Trends Neurosci. 27 (7), 392–399.
Kuijpers, M., Hoogenraad, C.C., 2011. Centrosomes, microtubules and neuronal development. Mol. Cell. Neurosci. 48 (4), 349–358.
Kumar, R.A., Pilz, D.T., Babatz, T.D., Cushion, T.D., Harvey, K., Topf, M., Yates, L., Robb, S., Uyanik, G., Mancini, G.M.S., Rees, M.I., Harvey, R.J., Dobyns, W.B., 2010. TUBA1A mutations cause wide spectrum lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. Hum. Mol. Genet. 19 (14), 2817–2827.
Kunishima, S., Kobayashi, R., Itoh, T.J., Hamaguchi, M., Saito, H., 2009. Mutation of the beta1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. Blood 113 (2), 458–461.
Kwiatkowska, M., Poplonska, K., Stepinski, D., Hejnowicz, Z., 2006. Microtubules with different diameter, protofilament number and protofilament spacing in *Ornithogalum umbellatum* ovary epidermis cells. Folia Histochem. Cytobiol. 44 (2), 133–138.
Leandro-Garcia, L.J., Leskela, S., Landa, I., Montero-Conde, C., Lopez-Jimenez, E., Leton, R., Cascon, A., Robledo, M., Rodriguez-Antona, C., 2010. Tumoral and tissue-specific expression of the major human beta-tubulin isotypes. Cytoskeleton 67 (4), 214–223.
Lee, H.O., Norden, C., 2013. Mechanisms controlling arrangements and movements of nuclei in pseudostratified epithelia. Trends Cell Biol. 23 (3), 141–150.
Leskela, S., Leandro-Garcia, L.J., Mendiola, M., Barriuso, J., Inglada-Perez, L., Munoz, I., Martinez-Delgado, B., Redondo, A., de Santiago, J., Robledo, M., Hardisson, D., Rodriguez-Antona, C., 2011. The miR-200 family controls beta-tubulin III expression and is associated with paclitaxel-based treatment response and progression-free survival in ovarian cancer patients. Endocr. Relat. Cancer 18 (1), 85–95.
Lewis, S.A., Lee, M.G., Cowan, N.J., 1985. Five mouse tubulin isotypes and their regulated expression during development. J. Cell Biol. 101 (3), 852–861.
Lewis, S.A., Tian, G., Cowan, N.J., 1997. The alpha- and beta-tubulin folding pathways. Trends Cell Biol. 7 (12), 479–484.
Lewis, S.A., Tian, G., Vainberg, I.E., Cowan, N.J., 1996. Chaperonin-mediated folding of actin and tubulin. J. Cell Biol. 132 (1–2), 1–4.
Liu, N., Xiong, Y., Ren, Y., Zhang, L., He, X., Wang, X., Liu, M., Li, D., Shui, W., Zhou, J., 2015. Proteomic profiling and functional characterization of multiple post-translational modifications of tubulin. J. Proteome Res. 14 (8), 3292–3304.
Lui, J.H., Hansen, D.V., Kriegstein, A.R., 2011. Development and evolution of the human neocortex. Cell 146 (1), 18–36.
Marcos, S., Moreau, J., Backer, S., Job, D., Andrieux, A., Bloch-Gallego, E., 2009. Tubulin tyrosination is required for the proper organization and pathfinding of the growth cone. PLoS One 4 (4), e5405.
Marin, O., Rubenstein, J.L., 2003. Cell migration in the forebrain. Annu. Rev. Neurosci. 26, 441–483.
McKean, P.G., Vaughan, S., Gull, K., 2001. The extended tubulin superfamily. J. Cell Sci. 114 (Pt 15), 2723–2733.
Messier, P.E., 1978. Microtubules, interkinetic nuclear migration and neurulation. Experientia 34 (3), 289–296.
Mishima, Y., Tomari, Y., 2016. Codon usage and 3′ UTR length determine maternal mRNA stability in zebrafish. Mol. Cell 61 (6), 874–885.
Mitchison, T., Kirschner, M., 1984. Dynamic instability of microtubule growth. Nature 312 (5991), 237–242.
Morris-Rosendahl, D., Najm, J., Lachmeijer, A., Sztriha, L., Martins, M., Kuechler, A., Haug, V., Zeschnigk, C., Martin, P., Santos, M., Vasconcelos, C., Omran, H., Kraus, U., Van der Knaap, M., Knaap, M., Schuierer, G., Kutsche, K., Uyanik, G., 2008. Refining the phenotype of α-1a tubulin (TUBA1A) mutation in patients with classical lissencephaly. Clin. Genet. 74 (5), 425–433.
Nadarajah, B., Brunstrom, J.E., Grutzendler, J., Wong, R.O., Pearlman, A.L., 2001. Two modes of radial migration in early development of the cerebral cortex. Nat. Neurosci. 4 (2), 143–150.
Namba, T., Funahashi, Y., Nakamuta, S., Xu, C., Takano, T., Kaibuchi, K., 2015. Extracellular and intracellular signaling for neuronal polarity. Physiol. Rev. 95 (3), 995–1024.
Nirschl, J.J., Magiera, M.M., Lazarus, J.E., Janke, C., Holzbaur, E.L., 2016. Alpha-tubulin tyrosination and CLIP-170 phosphorylation regulate the initiation of dynein-driven transport in neurons. Cell Rep. 14 (11), 2637–2652.
Orphanides, G., Reinberg, D., 2002. A unified theory of gene expression. Cell 108 (4), 439–451.
Park, I.Y., Powell, R.T., Tripathi, D.N., Dere, R., Ho, T.H., Blasius, T.L., Chiang, Y.C., Davis, I.J., Fahey, C.C., Hacker, K.E., Verhey, K.J., Bedford, M.T., Jonasch, E., Rathmell, W.K., Walker, C.L., 2016. Dual chromatin and cytoskeletal remodeling by SETD2. Cell 166 (4), 950–962.
Poirier, K., Keays, D.A., Francis, F., Saillour, Y., Bahi, N., Manouvrier, S., Fallet-Bianco, C., Pasquier, L., Toutain, A., Tuy, F.P., Bienvenu, T., Joriot, S., Odent, S., Ville, D., Desguerre, I., Goldenberg, A., Moutard, M.L., Fryns, J.P., van Esch, H., Harvey, R.J., Siebold, C., Flint, J., Beldjord, C., Chelly, J., 2007. Large spectrum of lissencephaly and pachygyria phenotypes resulting from de novo missense mutations in tubulin alpha 1A (TUBA1A). Hum. Mutat. 28 (11), 1055–1064.
Poirier, K., Saillour, Y., Bahi-Buisson, N., Jaglin, X.H., Fallet-Bianco, C., Nabbout, R., Castelnau-Ptakhine, L., Roubertie, A., Attie-Bitach, T., Desguerre, I., Genevieve, D., Barnerias, C., Keren, B., Lebrun, N., Boddaert, N., Encha-Razavi, F., Chelly, J., 2010. Mutations in the neuronal beta-tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. Hum. Mol. Genet. 19 (22), 4462–4473.
Poirier, K., Saillour, Y., Fourniol, F., Francis, F., Souville, I., Valence, S., Desguerre, I., Lepage, J.M., Boddaert, N., Jacquemont, M.L., Beldjord, C., Chelly, J., Bahi-Buisson, N., 2012. Expanding the spectrum of TUBA1A-related cortical dysgenesis to Polymicrogyria. Eur. J. Hum. Genet.
Preitner, N., Quan, J., Nowakowski, D.W., Hancock, M.L., Shi, J., Tcherkezian, J., Young-Pearse, T.L., Flanagan, J.G., 2014. APC is an RNA-binding protein, and its interactome provides a link to neural development and microtubule assembly. Cell 158 (2), 368–382.
Presnyak, V., Alhusaini, N., Chen, Y.H., Martin, S., Morris, N., Kline, N., Olson, S., Weinberg, D., Baker, K.E., Graveley, B.R., Coller, J., 2015. Codon optimality is a major determinant of mRNA stability. Cell 160 (6), 1111–1124.
Raff, E.C., Fackenthal, J.D., Hutchens, J.A., Hoyle, H.D., Turner, F.R., 1997. Microtubule architecture specified by a beta-tubulin isoform. Science (New York, N.Y.) 275 (5296), 70–73.
Rakic, P., 1972. Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. 145 (1), 61–83.
Reed, N.A., Cai, D., Blasius, T.L., Jih, G.T., Meyhofer, E., Gaertig, J., Verhey, K.J., 2006. Microtubule acetylation promotes kinesin-1 binding and transport. Curr. Biol. 16 (21), 2166–2172.
Schoenberg, D.R., Maquat, L.E., 2012. Regulation of cytoplasmic mRNA decay. Nat. Rev. Genet. 13 (4), 246–259.
Sgro, F., Bianchi, F.T., Falcone, M., Pallavicini, G., Gai, M., Chiotto, A.M., Berto, G.E., Turco, E., Chang, Y.J., Huttner, W.B., Di Cunto, F., 2016. Tissue-specific control of midbody microtubule stability by Citron kinase through modulation of TUBB3 phosphorylation. Cell Death Differ. 23, 801–813.
Smith, B.N., Ticozzi, N., Fallini, C., Gkazi, A.S., Topp, S., Kenna, K.P., Scotter, E.L., Kost, J., Keagle, P., Miller, J.W., Calini, D., Vance, C., Danielson, E.W., Troakes, C., Tiloca, C., Al-Sarraj, S., Lewis, E.A., King, A., Colombrita, C., Pensato, V., Castellotti, B., de Belleroche, J., Baas, F., ten Asbroek, A.L., Sapp, P.C., McKenna-Yasek, D., McLaughlin, R.L., Polak, M., Asress, S., Esteban-Perez, J., Munoz-Blanco, J.L., Simpson, M., Consortium, S., van Rheenen, W., Diekstra, F.P., Lauria, G., Duga, S., Corti, S., Cereda, C., Corrado, L., Soraru, G., Morrison, K.E., Williams, K.L., Nicholson, G.A., Blair, I.P., Dion, P.A., Leblond, C.S., Rouleau, G.A., Hardiman, O., Veldink, J.H., van den Berg, L.H., Al-Chalabi, A., Pall, H., Shaw, P.J., Turner, M.R., Talbot, K., Taroni, F., Garcia-Redondo, A., Wu, Z., Glass, J.D., Gellera, C., Ratti, A., Brown Jr., R.H., Silani, V., Shaw, C.E., Landers, J.E., 2014. Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. Neuron 84 (2), 324–331.
Stanchi, F., Corso, V., Scannapieco, P., Ievolella, C., Negrisolo, E., Tiso, N., Lanfranchi, G., Valle, G., 2000. TUBA8: a new tissue-specific isoform of alpha-tubulin that is highly conserved in human and mouse. Biochem. Biophys. Res. Commun. 270 (3), 1111–1118.
Tabata, H., Nakajima, K., 2003. Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. J. Neurosci. 23 (31), 9996–10001.

Tian, G., Lewis, S.A., Feierbach, B., Stearns, T., Rommelaere, H., Ampe, C., Cowan, N.J., 1997. Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. J. Cell Biol. 138 (4), 821–832.

Tischfield, M.A., Baris, H.N., Wu, C., Rudolph, G., Van Maldergem, L., He, W., Chan, W.M., Andrews, C., Demer, J.L., Robertson, R.L., Mackey, D.A., Ruddle, J.B., Bird, T.D., Gottlob, I., Pieh, C., Traboulsi, E.I., Pomeroy, S.L., Hunter, D.G., Soul, J.S., Newlin, A., Sabol, L.J., Doherty, E.J., de Uzcategui, C.E., de Uzcategui, N., Collins, M.L., Sener, E.C., Wabbels, B., Hellebrand, H., Meitinger, T., de Berardinis, T., Magli, A., Schiavi, C., Pastore-Trossello, M., Koc, F., Wong, A.M., Levin, A.V., Geraghty, M.T., Descartes, M., Flaherty, M., Jamieson, R.V., Moller, H.U., Meuthen, I., Callen, D.F., Kerwin, J., Lindsay, S., Meindl, A., Gupta Jr., M.L., Pellman, D., Engle, E.C., 2010. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell 140 (1), 74–87.

Tsai, J.W., Lian, W.N., Kemal, S., Kriegstein, A.R., Vallee, R.B., 2010. Kinesin 3 and cytoplasmic dynein mediate interkinetic nuclear migration in neural stem cells. Nat. Neurosci. 13 (12), 1463–1471.

Tsai, L.H., Gleeson, J.G., 2005. Nucleokinesis in neuronal migration. Neuron 46 (3), 383–388.

Villasante, A., Wang, D., Dobner, P., Dolph, P., Lewis, S.A., Cowan, N.J., 1986. Six mouse alpha-tubulin mRNAs encode five distinct isotypes: testis-specific expression of two sister genes. Mol. Cell. Biol. 6 (7), 2409–2419.

Wade, R.H., Chretien, D., 1993. Cryoelectron microscopy of microtubules. J. Struct. Biol. 110 (1), 1–27.

Wang, D., Villasante, A., Lewis, S.A., Cowan, N.J., 1986. The mammalian beta-tubulin repertoire: hematopoietic expression of a novel, heterologous beta-tubulin isotype. J. Cell Biol. 103 (5), 1903–1910.

Webster, D.R., Gundersen, G.G., Bulinski, J.C., Borisy, G.G., 1987. Differential turnover of tyrosinated and detyrosinated microtubules. Proc. Natl. Acad. Sci. U. S. A. 84 (24), 9040–9044.

Westermann, S., Weber, K., 2003. Post-translational modifications regulate microtubule function. Nat. Rev. Mol. Cell Biol. 4 (12), 938–947.

Wilde, C.D., Crowther, C.E., Cowan, N.J., 1982. Isolation of a multigene family containing human alpha-tubulin sequences. J. Mol. Biol. 155 (4), 533–538.

Yu, I., Garnham, C.P., Roll-Mecak, A., 2015. Writing and reading the tubulin code. J. Biol. Chem. 290 (28), 17163–17172.