

REVIEW

SUBJECT COLLECTION: CYTOSKELETON

Tubulin isotypes – functional insights from model organisms

Emmanuel T. Nsamba and Mohan L. Gupta, Jr*

ABSTRACT

The microtubule cytoskeleton is assembled from the α - and β -tubulin subunits of the canonical tubulin heterodimer, which polymerizes into microtubules, and a small number of other family members, such as γ -tubulin, with specialized functions. Overall, microtubule function involves the collective action of multiple α - and β -tubulin isotypes. However, despite 40 years of awareness that most eukaryotes harbor multiple tubulin isotypes, their role in the microtubule cytoskeleton has remained relatively unclear. Various model organisms offer specific advantages for gaining insight into the role of tubulin isotypes. Whereas simple unicellular organisms such as yeast provide experimental tractability that can facilitate deeper access to mechanistic details, more complex organisms, such as the fruit fly, nematode and mouse, can be used to discern potential specialized functions of tissue- and structure-specific isotypes. Here, we review the role of α - and β -tubulin isotypes in microtubule function and in associated tubulinopathies with an emphasis on the advances gained using model organisms. Overall, we argue that studying tubulin isotypes in a range of organisms can reveal the fundamental mechanisms by which they mediate microtubule function. It will also provide valuable perspectives on how these mechanisms underlie the functional and biological diversity of the cytoskeleton.

KEY WORDS: Isotype, Microtubule, Tubulin

Introduction

Microtubules (MTs) are essential cytoskeletal filaments that underlie diverse processes in eukaryotes including mitosis, intracellular transport and axon formation. Despite their involvement in a wide range of contexts, their basic structure and intrinsically dynamic behavior are widely conserved. They are long, hollow cylinders assembled from the protein tubulin, a heterodimer of α - and β -subunits (Fig. 1). Tubulin polymerizes in a head-to-tail manner, bestowing MTs with a polarity that facilitates the organization of MT structures, directed transport, force generation and other scenarios that require spatial fidelity (Goodson and Jonasson, 2018). Another conserved property, termed dynamic instability, is the propensity of MTs, driven by the hydrolysis of GTP bound to β -tubulin, to stochastically switch between periods of polymerization and depolymerization (Mitchison and Kirschner, 1984). A key reason MTs can function in diverse roles is that their organization and dynamics can be spatially and temporally controlled by regulatory factors and MT-associated proteins (MAPs) (Bodakuntla et al., 2019). Apart from the α -tubulin- β -tubulin heterodimer ($\alpha\beta$ -tubulin), the tubulin family includes γ -tubulin, which is also ubiquitous and key for MT nucleation (Oakley et al., 2015). In addition, the specialized δ -, ϵ - and ζ -tubulins contribute to the structure and/or

function of centrioles and basal bodies in a subset of eukaryotes (Chang and Stearns, 2000; Turk et al., 2015; Vaughan et al., 2000).

Model organisms have been indispensable for understanding how MTs achieve diverse biological processes. Here, we review the role of α - and β -tubulin isotypes in MT function with an emphasis on the advances gained using model organisms.

Microtubule diversity and the tubulin code

Despite the high conservation in MT structure and dynamic behavior, diversity is introduced at three levels. First, the tubulin family has undergone evolutionary expansion to produce multiple variants, or isotypes, of γ -tubulin in some species (Findeisen et al., 2014), and α - and β -tubulin in most species (Cleveland et al., 1980; Roll-Mecak, 2019). Second, post-translational modifications (PTMs) can alter tubulin molecules and influence MT function, including their flexibility (Xu et al., 2017) and stability (Janke and Bulinski, 2011). Variation can also arise from altered expression of tubulin-modifying enzymes or the absence of PTM-target sites (modifiable amino acids such as glutamate, lysine and tyrosine) in specific isotypes (Fig. 1). Third, MT-associated activities such as polymerization, organization and directed transport can be controlled by MAPs and regulatory proteins (Bodakuntla et al., 2019). Altogether these observations form the basis of the multi-tubulin (Cleveland, 1987; Fulton and Simpson, 1976) or tubulin code (Janke, 2014; Verhey and Gaertig, 2007; Yu et al., 2015) hypotheses, which postulate specific contributions to MT function from isotype composition and/or PTMs. Work from many researchers has yielded impressive insights into tubulin structure, MT dynamics, PTMs, MAPs and regulatory proteins. However, a detailed understanding of how tubulin isotypes contribute to complex MT properties has lagged behind these other advances.

Tubulin isotypes and the tubulin code

For 40 years, the role of tubulin isotypes in the functional diversity of the MT cytoskeleton has remained relatively unclear. One obstacle has been the difficulty in obtaining single-isotype tubulins for biochemical study. The formation of functional tubulin heterodimers requires a complex chaperone-mediated folding pathway (Lewis et al., 1997), and historically, successful exogenous expression in prokaryotic cells or overexpression in eukaryotic systems has not been reported. Until recently, single-isotype preparations were essentially limited to yeast tubulins (Bode et al., 2003) and heterogeneous mammalian brain tubulins immunodepleted of specific isotypes (Banerjee et al., 1988; Panda et al., 1994). A second obstacle has been the entanglement of isotype-specific effects and secondary phenotypes in cell-based experiments. α/β -stoichiometry is one factor, as surplus β -tubulin can be toxic (Burke et al., 1989; Weinstein and Solomon, 1990). Another factor is assigning isotype contribution, as removal of one isotype can also change the relative ratios of the remaining subunits. Thus, interpretation of phenotypes following knockdown or overexpression of tubulin isotypes can be complicated.

Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA.

*Author for correspondence (mgupta@iastate.edu)

DOI: E.T.N., 0000-0002-1626-1868; M.L.G., 0000-0002-4996-5458

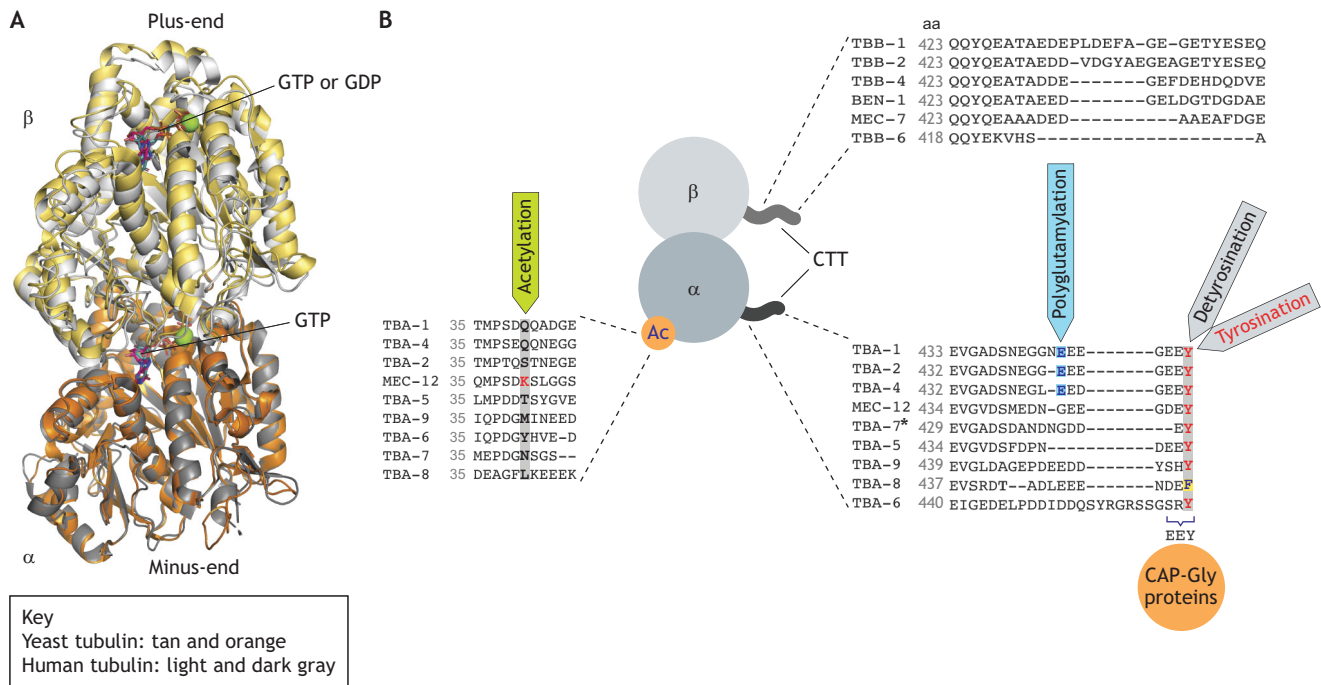


Fig. 1. The tubulin structure is highly conserved but notable sequence differences exist between isoforms. (A) Overlay of human (light and dark gray) and yeast (tan and orange) tubulin heterodimers modeled from their cryo-electron microscopy structures (PDB human: 6E7B, yeast: 5W3F). The root-mean-square deviation (RMSD) is 1.18 Å (1 Å=0.1 nm), indicative of strong structural conservation between these divergent eukaryotes. (B) Sites of post-translation modification vary among isoforms (*C. elegans* proteins are shown as examples). Left, lysine 40, the target of an α -tubulin acetylation (Ac) that influences MT flexibility and kinesin binding, is present only in MEC-12. Right, variation among isoforms is typically concentrated in the final ~20 amino acids, or C-terminal tail (CTT). Several α -tubulin isoforms lack the preferred site of polyglutamylation (glutamate; blue squares). TUBA-8 lacks the terminal tyrosine residue that undergoes detyrosination/tyrosination. Additionally, TBA-6 and TBA-9 lack any conservation with the EEY binding motif for interaction with CAP-Gly domain-containing proteins that bind to MT plus-ends. aa, amino acid.

Several advances are helping to unravel this longstanding mystery. First, improvements in gene editing and isotype replacements have begun to reveal the importance of individual isoforms in MT dynamics and spindle positioning (Honda et al., 2017; Nsamba et al., 2021; Parker et al., 2018), cilia and flagella assembly (Fackenthal et al., 1995; Hoyle and Raff, 1990; Hurd et al., 2010), and neurogenesis (Bittermann et al., 2019; Latremoliere et al., 2018; Lockhead et al., 2016; Zheng et al., 2017). Second, the successful purification of functional recombinant tubulin is beginning to yield insights into the biochemical properties of various isoforms (Ayukawa et al., 2021; Minoura et al., 2013; Pamula et al., 2016; Ti et al., 2018; Vemu et al., 2017). Combining these advances with insights from pathological tubulin variants will help to elucidate individual isotype function in specific cellular contexts (Fourel and Boscheron, 2020; Minoura et al., 2016; Pham and Morrisette, 2019; Ti et al., 2016).

Most eukaryotes have multiple isoforms

Overall, the number of tubulin isoforms generally increases with organismal and developmental complexity (Table 1), which supports the idea that they facilitate specialized or diverse interactions with MT-interacting proteins. In general, isoforms readily co-polymerize (Lewis et al., 1987; Lu and Luduena, 1994; Panda et al., 1994). Strikingly, even a chicken–yeast chimeric β -tubulin is incorporated into MTs in mouse cells (Bond et al., 1986). This co-polymerization likely reflects evolutionary constraints on isoforms to preserve fundamental MT structure and dynamics. Indeed, most isotype-specific changes are enriched within the C-terminal tail (CTT), a short region not required for basic polymerization (Bhattacharyya et al., 1985; Serrano et al., 1984).

The CTT is also where most known PTMs occur (Janke, 2014). It remains unknown whether specific α - and β -tubulin isoforms have preferred and/or incompatible heterodimerization partners.

Although most eukaryotes express multiple tubulin isoforms, the question of why has remained largely open. There are at least four, non-mutually exclusive hypotheses: (1) numerous genes ensure a stable and ample supply of tubulin heterodimers; (2) distinct loci allow cell type-, cell state- or developmental stage-specific regulation of tubulin levels (Raff, 1984); (3) isoforms possess unique biochemical properties that support particular MT-based functions (Fulton and Simpson, 1976; Luduena, 1993), and (4) isoforms have enhanced, or diminished, association with specific MAPs, thus altering MT behavior and/or function (Denarier et al., 2021; Nsamba et al., 2021). Additional isotype-specific complexity results from PTMs that can be limited to a subset of isoforms, such as in *C. elegans* touch neurons where only MEC-12 (an α -tubulin isotype) harbors the acetylatable lysine at position 40 (Fukushige et al., 1999). Work in model organisms is thus crucial in understanding how tubulin isoforms underlie fundamental, MT-based processes (Fig. 2).

Tubulin isoforms across diverse species

Overall, α - and β -tubulin are highly conserved with any sequence variation between isoforms largely concentrated within the CTT. Owing to the CTT being on the exterior of the MT, where it is optimally positioned to interact with MAPs, motors, regulatory proteins and other cellular targets (Nogales, 2000), this isotype-specific variation could easily lead to context-specific changes in MT behavior. It should also be noted, however, that single amino acid changes outside the CTT can have striking effects on MT

Table 1. Tubulin isotypes and their known functions in diverse model organisms

Species	Division/ Phylum	Tubulin isotypes		Known functional groups	Isotypes involved*		References	
		α	β		α	β		
<i>Mus musculus</i> (mouse)	Chordate	<i>TUBA1A</i>	<i>TUBB1/</i>	Neuronal specific; brain development	<i>TUBA1A</i>	<i>TUBB2A,</i> <i>TUBB2B,</i> <i>TUBB3,</i> <i>TUBB5</i>	For isotype numbers, see Khodiyar et al., 2007; Hausrat et al., 2021; for function, see Aiken et al., 2019; Belvindrah et al., 2017; Bittermann et al., 2019; Breuss et al., 2012; Keays et al., 2007; Latremoliere et al., 2018; Stottmann et al., 2013; Tischfield et al., 2010	
		<i>TUBA1B</i>	<i>TUBB2A</i>					
		<i>TUBA1C</i>	<i>TUBB2B</i>					
		<i>TUBA4A</i>	<i>TUBB3</i>					
		<i>TUBA3A</i>	<i>TUBB4A</i>	Platelet biogenesis	<i>TUBA4A</i>	<i>TUBB1</i>	Kunishima et al., 2009; Schwer et al., 2001; Strassel et al., 2019	
<i>TUBA3B</i>	<i>TUBB4B</i>							
<i>TUBA8</i>	<i>TUBB5</i>							
<i>TUBAL3</i>	<i>TUBB6</i>							
				Spermatogenesis	<i>TUBA8</i>		Diggle et al., 2017	
<i>Danio rerio</i> (zebrafish)	Chordate	<i>TUBA1A</i>	<i>TUBB1</i>	Retina and brain development	<i>TUBA1A</i>		For isotype numbers, see https://zfinfo.org/ ; for function, see Veldman et al., 2010	
		<i>TUBA1B</i>	<i>TUBB6</i>					
		<i>TUBA1C</i>	<i>TUBB2</i>					
		<i>TUBA2</i>	<i>TUBB4B</i>					
		<i>TUBA4L</i>	<i>TUBB5</i>					
		<i>TUBA5</i>	<i>ZDB-</i>					
		<i>TUBA7L</i>	<i>GENE-</i>					
		<i>TUBA8L2</i>	<i>030131-</i>					
		<i>TUBA8L3</i>	<i>7741</i>					
		<i>TUBA8L4</i>						
		<i>TUBA8L5</i>						
		<i>ZDB-</i>						
		<i>GENE-</i>						
		<i>051127-7</i>						
		<i>ZDB-</i>						
		<i>GENE-</i>						
		<i>120214-26</i>						
<i>Xenopus laevis</i> (frog)	Chordate	<i>TUBA1A</i>	<i>TUBB</i>				For isotype numbers, see Brown et al., 2007; Hirst et al., 2020	
		<i>TUBA1B</i>	<i>(TUBB5)</i>					
		<i>TUBA1C</i>	<i>TUBB2B</i>					
		<i>TUBA4B</i>	<i>TUBB3</i>					
		<i>TUBA5</i>	<i>TUBB4A</i>					
		<i>TUBA8</i>	<i>TUBB4B</i>					
		<i>TUBB6</i>						
<i>Caenorhabditis elegans</i> (nematode)	Nematode	<i>TBA-1</i>	<i>TBB-1,</i>	Mitotic divisions; neuronal	<i>TBA-1,</i>	<i>TBB-1,</i>	For isotype numbers, Chalfie and Thomson, 1982; Fukushige et al., 1999; Hurd, 2018; Siddiqui et al., 1989; for function, see Baran et al., 2010; Driscoll et al., 1989; Honda et al., 2017; Phillips et al., 2004	
		<i>TBA-2</i>	<i>TBB-2,</i>					
		<i>MEC-12</i>	<i>MEC-7</i>					
		<i>TBA-4,</i>	<i>TBB-4</i>					
		<i>TBA-5,</i>	<i>BEN-1</i>	Sensory neurons	<i>TBA-5,</i>	<i>TBB-4</i>		
		<i>TBA-6,</i>	<i>TBB-6</i>					
		<i>TBA-7,</i>						
		<i>TBA-8</i>						
		<i>TBA-9</i>						
				Mechanosensory	<i>TBA-6,</i>	<i>MEC-7</i>		
<i>Drosophila melanogaster</i> (fruit fly)	Arthropod	<i>TUB67C</i>	<i>TUB85D</i>	Germ cells; spermatogenesis; oogenesis; early embryo	<i>TUB67C,</i>	<i>TUB85D</i>	For isotype numbers, see Fackenthal et al., 1995; Hoyle and Raff, 1990; Hutchens et al., 1997; Kempfues et al., 1982; for function, Matthews et al., 1993; Raff et al., 2000; Tao et al., 2021; Theurkauf, 1992	
		<i>TUB84B</i>	<i>TUB65B</i>					
		<i>TUB84D</i>	<i>TUB97EF</i>					
		<i>TUB85E</i>	<i>TUB60D</i>					
		<i>TUB90E</i>	<i>TUB56D</i>	Developmentally regulated	<i>TUB85E</i>	<i>TUB65B</i>		
				Thermosensitive		<i>TUB97EF</i>		

Continued

Table 1. Continued

Species	Division/ Phylum	Tubulin isotypes		Known functional groups	Isotypes involved*		References
		α	β		α	β	
<i>Tetrahymena thermophila</i>	Ciliophora	<i>ATU1</i> <i>ALT1</i> <i>ALT2</i> <i>ALT3</i>	<i>BTU1</i> <i>BTU2</i> <i>BLT1</i> <i>BLT2</i> <i>BLT3</i> <i>BLT4</i> <i>BLT5</i> <i>BLT6</i>	Mitotic divisions Meiotic divisions Somatic cilia; basal bodies		<i>BLT1</i> , <i>BLT4</i> <i>BLT1</i> <i>BTU2</i>	For isotype numbers, Eisen et al., 2006; Gaertig et al., 1993; McGrath et al., 1994; for function, Pucciarelli et al., 2012 Pucciarelli et al., 2012 Pucciarelli et al., 2012
<i>Aspergillus nidulans</i>	Fungi	<i>TUBA</i> <i>TUBB</i>	<i>BENA</i> <i>TUBC</i>	Vegetative mitosis; nuclear movement Asexual sporulation Sexual reproduction	<i>TUBA</i> <i>TUBB</i>	<i>BENA</i> <i>TUBC</i>	For isotype numbers, Doshi et al., 1991; May et al., 1985; Morris et al., 1979; for function, Oakley and Morris, 1980; Oakley and Morris, 1981a May and Morris, 1988; May et al., 1985; Weatherbee et al., 1985 Doshi et al., 1991; Kirk and Morris, 1991; Morris et al., 1979; Oakley and Morris, 1981b
<i>Fusarium graminearum</i>	Fungi	<i>FGTUB4</i> <i>FGTUB5</i>	<i>FGTUB1</i> <i>FGTUB2</i>	Vegetative growth Sexual reproduction		<i>FGTUB2</i> <i>FGTUB1</i>	For isotype numbers, Chen et al., 2009; Zhao et al., 2014; for function, Liu et al., 2013; Zhao et al., 2014 Zhao et al., 2014
<i>Saccharomyces cerevisiae</i> (budding yeast)	Fungi	<i>TUB1</i> <i>TUB3</i>	<i>TUB2</i>	Early spindle positioning Late spindle positioning	<i>TUB3</i> <i>TUB1</i>		For isotype numbers, Neff et al., 1983; Schatz et al., 1986a; for function, Nsamba et al., 2021 Nsamba et al., 2021
<i>Schizosaccharomyces pombe</i> (fission yeast)	Fungi	<i>NDA2</i> <i>TBA2</i>	<i>NDA3</i>				Adachi et al., 1986; Hiraoka et al., 1984; Toda et al., 1984

*Zebrafish genome annotation is ongoing and currently reports a relatively high number of tubulin variants. It is unclear how many may be duplicative or nonfunctional, as several appear to lack important regions.

dynamics (Driver et al., 2017; Gupta et al., 2002; Huang and Huffaker, 2006), motor protein interactions (Cederquist et al., 2012; Minoura et al., 2016; Tischfield et al., 2010) and MAP binding (Denarier et al., 2019, 2021). Perhaps the strongest argument that tubulin isotypes make unique and meaningful contributions to MT function is that they are evolutionarily conserved within biological classes, for example, in mammals (Khodiyar et al., 2007). Isotypes also display common cell- or tissue-specific expression profiles. For instance, the β -tubulin isotype Tubb3 is conserved across mammals, both in sequence and neuronal-specific expression (Jiang and Oblinger, 1992; Katsetos et al., 2003). This conserved expression pattern further suggests that Tubb3 possesses properties important for MT function specifically in the neuronal setting.

Despite being highly conserved among clades, isotypes are less so across diverse organisms. For example, there is no obvious homolog of the mammalian *TUBB3* in zebrafish. In some cases, isotypes simply do not have direct homologs, for instance in the case of *Saccharomyces cerevisiae*, in which a second α -tubulin isotype likely arose via whole-genome duplication (Kellis et al., 2004). However, although zebrafish and mammalian isotypes do not strongly cluster phylogenetically, as isotypes do when comparing mammals only, zebrafish also possess neuronal-specific tubulin isotypes (Gulati-Leekha and Goldman, 2006; Oehlmann et al., 2004; Veldman et al., 2010). Similarly, testis- or oocyte-specific isotypes exist in the fly (Kemphues et al., 1982), nematode (Nishida et al., 2021), frog (Wu and Morgan, 1994), chicken (Pratt et al.,

1987) and mouse (Distel et al., 1984; Feng et al., 2016). Although grouping isotypes from distant clades into functionally similar groups based on sequence alone is not trivial, these observations suggest that certain isotypes fulfill neuronal- and gamete-specific roles in diverse organisms.

Although some studies show stark differences in the ability of isotypes to promote specific MT structures, such as cilia (Hoyle and Raff, 1990), others have found substantial redundancy in basic MT functions (Adachi et al., 1986; Honda et al., 2017; Phillips et al., 2004; Weatherbee et al., 1985). To co-polymerize, tubulin isotypes must remain sufficiently conserved to retain MT structure and dynamic properties. This places unifying constraints on the isotypes and, thus, can result in significant overlap in their performance of basic MT functions. With just one isotype, the entire complement of MT-dependent activities would be limited to a single tubulin or MT-binding interface. Divergence of this interface to accommodate new functions would be sharply restricted by the cost of disrupting existing processes. In contrast, if an organism harbored two isotypes that co-polymerized, the original binding interface on one isotype could ensure the effectiveness of existing activities, while the 'extra' binding and/or regulatory elements of the second isotype could co-evolve with a subset of current or new functions. The structures of α - and β -tubulin are highly similar to the bacterial tubulin homologue FtsZ, which is monomeric (Silber et al., 2020). Sequence conservation is even higher with the apparently monomeric homologues in the archaea Thaumarchaeota (Yutin and

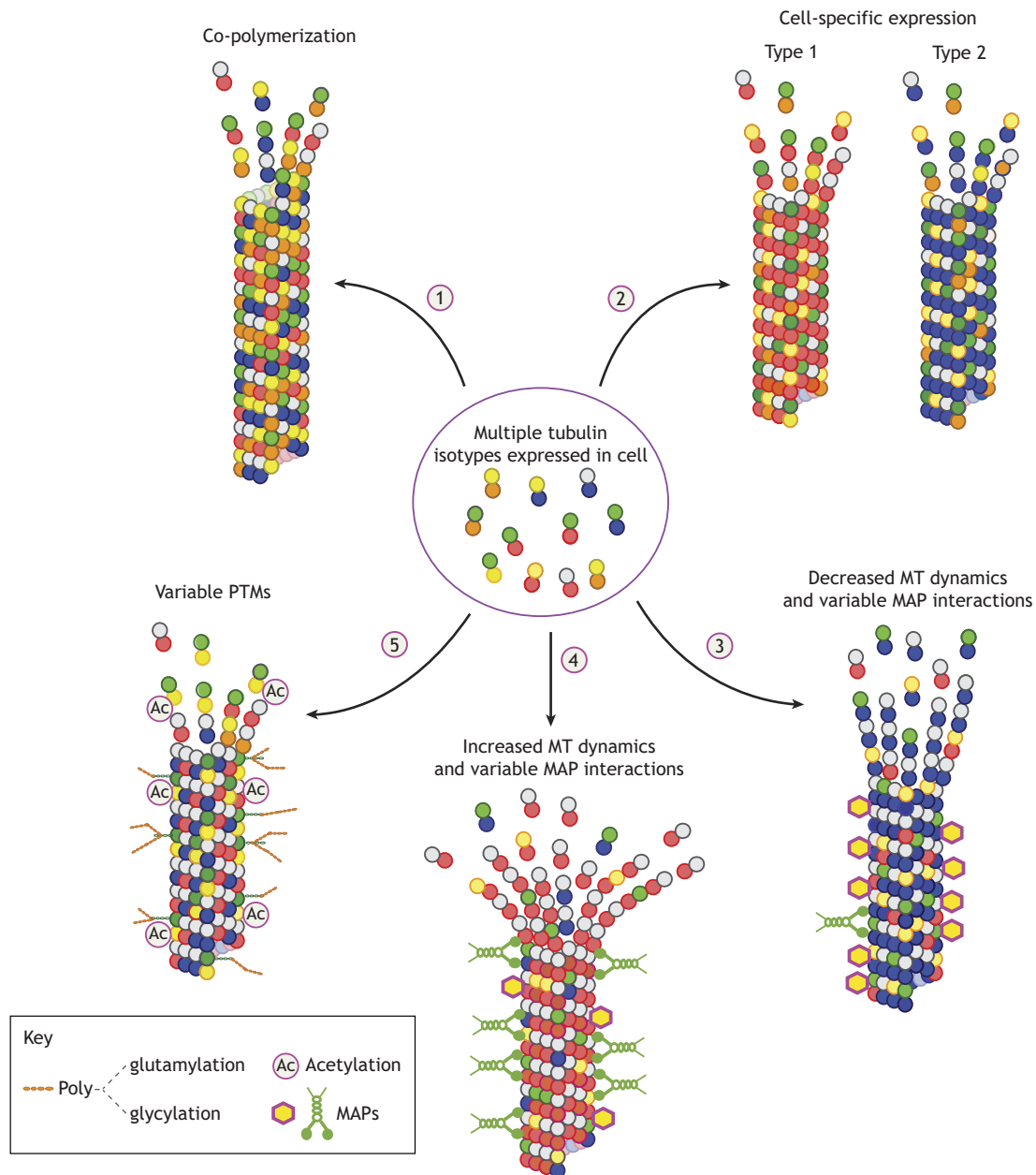


Fig. 2. Impact of tubulin isotypes on the tubulin code. Tubulin isotypes (different colors) exhibit diversity in several aspects of microtubule (MT) function. (1) Tubulin isotypes can co-polymerize, resulting in MTs with distinct composition and thus function. (2) Tubulin isotypes can display cell-, tissue- or developmental-specific expression profiles, for instance expression of *TUBA1A* and *TUBB3* in neurons versus that of *TUBA4A* and *TUBB1* in blood platelets. (3 and 4) Tubulin isotypes can affect MT dynamics, as well as either (3) decrease or (4) increase MT-associated protein (MAP) localization and activity on MTs. (5) Subsets of tubulin isotypes can be diminished in or enriched for distinct post-translational modifications (PTMs), which can subsequently influence the properties and function of MTs. See also Fig. 1B for specific examples.

Koonin, 2012) and Odinarchaeota (Zaremba-Niedzwiedzka et al., 2017). It is therefore tempting to speculate that the emergence of α - and β -subunits represents the initial ‘isotype’, such that duplication of these early tubulins would double the unique binding interface available on the polymer. The presence of many isotypes would allow MTs in modern eukaryotes to accommodate an even greater range of specific interactions. In situations where an evolving interaction significantly disrupts other functions, cell or developmental expression of isotypes could become a solution.

Unicellular organisms provide tractability that can facilitate learning the mechanistic details of tubulin isotypes. More complex models can help discern tissue- and structure-specific isotype

functions. Model organisms are also essential for elucidating the molecular etiologies of tubulin-related disorders, known as tubulinopathies. Below, we summarize our understanding of tubulin isotype function in major model organisms.

S. cerevisiae* and *Schizosaccharomyces pombe

Budding and fission yeasts have served as useful models for elucidating the mechanisms controlling MT function. Despite being evolutionarily distant, both contain a single β -tubulin and two α -tubulin isotypes (Table 1). Only one fission yeast α -tubulin isotype, *NDA2*, is essential for survival (Adachi et al., 1986). However, in its absence, increased expression of the other, *ATB2*, restores viability

(Toda et al., 1984). Curiously, the same is seen in budding yeast. Only *TUB1* is essential, but in its absence, increased *TUB3* rescues viability (Schatz et al., 1986b). These data suggested that *TUB1* and *TUB3* are functionally interchangeable, with the main difference being that *TUB1* is expressed at higher levels than *TUB3* (Schatz et al., 1986b). This idea is supported by studies that found purified yeast tubulin contained more Tub1 than Tub3 (Barnes et al., 1992; Bode et al., 2003) and western blots of cell lysates showing ~70% Tub1 and ~30% Tub3 (Aiken et al., 2019).

However, two recent studies challenge the idea that Tub1 and Tub3 differ only in expression and provide evidence based on western blot analysis and either quantitative cell imaging (Denarier et al., 2021) or reverse transcription real-time quantitative PCR (RT-qPCR) (Nsamba et al., 2021) that they are comparably expressed in cells. This suggests that in *tub3Δ* cells, the ~50% remaining Tub1 supports one or more essential functions that similar levels of Tub3 cannot achieve in *tub1Δ* cells. Consistent with this, the dynein regulator She1 displays a preference for Tub3- over Tub1-MTs *in vitro* (Denarier et al., 2021). She1 localization to mitotic spindles is also significantly increased in *tub1Δ* cells overexpressing *TUB3*, although they also have more spindle MTs (Denarier et al., 2021). An open reading frame (ORF)-replacement strategy created cells that express only either *TUB1* or *TUB3*, but at levels comparable to overall α -tubulin in wild-type cells (Nsamba et al., 2021). This revealed that Tub1 and Tub3 differentially support the recruitment of key components and the functions of the Kar9- and dynein-dependent pathways, the two major spindle-positioning mechanisms in mitosis. Furthermore, the isotypes display negative synthetic interactions with different subsets of genes, suggesting the absence of either one alters MT function in a specific manner (Nsamba et al., 2021). However, it remains unclear why more Tub1 was found in tubulin purified from wild-type cells (Barnes et al., 1992; Bode et al., 2003). It is possible that Tub1–Tub2 heterodimers are extracted more efficiently under non-denaturing conditions, or they better survive purification conditions. It's also unknown whether Tub1 levels increase in later-stage cultures that are aimed to maximize biomass (Bode et al., 2003) or for industrial scale production (Barnes et al., 1992). Differences in Tub1:Tub3 ratios measured by western blot analysis could similarly result from potential culture stage- and/or cell cycle-related changes in expression (Aiken et al., 2019; Denarier et al., 2021; Hanson et al., 2016; Nsamba et al., 2021). If such changes existed, it would imply additional function specialization of the isotypes. Taken together, data from yeast support the notion that tubulin isotypes share redundancy for basic MT properties, but their molecular differences optimally support the biochemical mechanisms employed by diverse MAPs and regulatory factors.

Aspergillus nidulans* and *Fusarium graminearum

The filamentous fungi *Aspergillus nidulans* and *Fusarium graminearum* are among the few ascomycetes that contain multiple β -tubulins (Zhao et al., 2014). *A. nidulans* contains two α -tubulins, *TUBA* and *TUBB*, and two β -tubulin isotypes, *BENA* and *TUBC*, with either α - or β -isotype able to support viability in the absence of the other (Table 1). Although *BENA* is needed for efficient mitosis and nuclear movement, and disruption of *BENA* expression is lethal, placing *TUBC* behind the *BENA* promoter rescues viability (Oakley and Morris, 1980, 1981a). Therefore, at sufficient levels, either β -tubulin isotype can support essential functions. However, they are not absolutely interchangeable, and at least subtle differences exist (May, 1989). Conversely, *TUBC* disruption, although non-lethal, causes defects during asexual

sporulation (conidiation), while it remains untested whether *BENA* upregulation rescues these defects (May and Morris, 1988; May et al., 1985; Weatherbee et al., 1985). There are also distinct phenotypes upon disruption of either α -tubulin gene. Whereas *TUBA* disruption causes a block in vegetative mitosis (Doshi et al., 1991; Oakley and Morris, 1981b), disrupting *TUBB*, whose transcript increases during germination (Doshi et al., 1991), perturbs sexual development leading to the first meiotic division (Kirk and Morris, 1991) and results in atypical cell and nuclear morphology (Doshi et al., 1991; Morris et al., 1979; Oakley and Morris, 1981b). The defects associated with the loss of either isotype were subsequently found to be largely complemented by overexpression of the other remaining isotype. (Kirk and Morris, 1993). Why either α -tubulin isotype, at lower-than-normal levels, differentially impacts MT-dependent processes remains unclear.

F. graminearum is a pathogen that causes destructive disease of cereal crops (Audenaert et al., 2013). It harbors two β -tubulins, *FGTUB1* and *FGTUB2*, and two α -tubulin isotypes, *FGTUB4* and *FGTUB5* (Zhao et al., 2014) (Table 1). Initial studies found that mutations in *FGTUB1* or *FGTUB2* differed in their ability to confer resistance to MT-destabilizing benzimidazole fungicides (Chen et al., 2009). Subsequent work revealed differences in function during hyphal growth (Liu et al., 2013). Loss of either differentially affects growth rates, ascosporeogenesis and the perithecia (Zhao et al., 2014). Thus, the β -tubulin isotypes differ in their ability to support cellular functions, with *FGTUB1* and *FGTUB2* being more important during sexual reproduction and vegetative growth, respectively.

Immunolocalization showed that both proteins localize to the same MT structures in sexually (ascus) and asexually (conidia) reproducing cells, indicating that subcellular localization does not underlie their functional diversification (Wang et al., 2019). Disruption of *FGKIN1*, which encodes a kinase that might influence MT stability by mediating phosphorylation of MAPs, causes FgTub1, but not FgTub2, to hyper-localize to the nucleolus (Luo et al., 2014; Zhao et al., 2014). Thus, tubulin isotypes in *F. graminearum* might potentially regulate MTs by controlling their behavior and/or interactions with specific regulatory factors.

Tetrahymena thermophila

In addition to the canonical α -tubulin *ATU1* and two identical β -tubulin isotypes *BTU1* and *BTU2*, *Tetrahymena thermophila* contains an additional three α - and six β -tubulin genes that represent relatively divergent tubulins, named ALTs and BLTs, respectively (Eisen et al., 2006; Gaertig et al., 1993; McGrath et al., 1994) (Table 1). Loss of *ATU1* is lethal (Hai et al., 1999), as is the simultaneous loss of *BTU1* and *BTU2* (Xia et al., 2000). Evidence for individualized function came from expression of GFP-tagged versions of the β -tubulin isotypes, which demonstrated that they segregate into distinct intracellular MT structures, likely due to regulated expression and isotype-specific nucleoporin-mediated transport (Pucciarelli et al., 2012). While GFP-labeled *BTU2* is prevalent in basal bodies and somatic cilia, *BLT1* and *BLT4* are undetectable. Conversely, both these isotypes are incorporated into the MT arrays of the macronucleus and the mitotic spindle of the micronucleus, where *BTU2* is absent. Furthermore, only *BLT1* is included in the meiotic spindle during conjugation (Pucciarelli et al., 2012).

Tubulin in *Tetrahymena* axonemes contains multiple PTMs, making this a useful model for investigating their roles. Indeed, work in *Tetrahymena* has demonstrated the importance of the CTT, as well as PTMs contained within it, for critical MT functions in

vivo (Duan and Gorovsky, 2002; Redeker et al., 2005), and revealed the role of α -tubulin acetylation, although it likely occurs indirectly (Walter et al., 2012), in promoting kinesin binding and motility (Reed et al., 2006). Subsequently, polyglutamylation of the B-tubule of outer doublet MTs was shown to be required to regulate the force production by ciliary dynein (Suryavanshi et al., 2010). Overall, *Tetrahymena* has proven valuable in revealing mechanisms that segregate tubulins into distinct MT populations, as well as the role of PTMs.

Caenorhabditis elegans

The *C. elegans* genome has a large collection of tubulin genes including six β - and nine α -tubulin isotypes (Hurd, 2018). Notably, they harbor more sequence diversity than typically seen in other organisms (Gogonea et al., 1999). Although the canonical 13-protofilament structure is present in axonemes, *C. elegans* MTs are largely composed of 11-protofilaments, whereas touch receptor neuron (TRN) cells possess 15-protofilament MTs (Chalfie and Thomson, 1982). *C. elegans* MTs are also characterized by fast growth rates (Chaaban et al., 2018), up to ~55-fold faster than the relatively slow *S. cerevisiae* tubulin growth seen *in vivo* (Gupta et al., 2002; Honda et al., 2017; Srayko et al., 2005) and ~10-fold the level seen *in vitro* (Bode et al., 2003; Chaaban et al., 2018). *C. elegans* has the advantage of allowing developmental processes to be studied through gene manipulation by RNAi and more recently CRISPR technologies (Lockhead et al., 2016; Zheng et al., 2017). Genetic and cytological studies have classified *C. elegans* isotypes into four functional groups, including one with unknown function(s) (Table 1). Here, we provide a brief summary and refer the reader to a comprehensive review (Hurd, 2018) and a recent study evaluating isotype expression and localization by endogenous GFP tagging (Nishida et al., 2021).

Genetic screens in *C. elegans* provided early evidence for the role of tubulin isotypes in MT structure and/or function and mechanosensation (Chalfie and Sulston, 1981; Chalfie and Thomson, 1982). These and other studies revealed that the α -tubulin *MEC-12* and β -tubulin *MEC-7* are important for generating the 15-protofilament MTs found in TRNs. Mutations in either cause the loss of some 15-protofilament MTs, with 11-protofilament MTs appearing instead, often accompanied by perturbation of mechanosensation signaling components and loss of touch sensitivity (Bounoutas et al., 2009, 2011; Fukushige et al., 1999; Hsu et al., 2014; Huang et al., 1995; Kirszenblat et al., 2013; O'Hagan et al., 2005; Savage et al., 1989, 1994). Other tubulin isotypes are expressed in specific, yet overlapping, subsets of sensory neurons, where they largely localize to axonemes (Hurd et al., 2010), with at least some being delivered via intraflagellar transport (Hao et al., 2011). Perturbations in the α -tubulin isotypes *TBA-6*, *TBA-9* or β -tubulin *TBB-4* are associated with defective cilia (Hurd et al., 2010; Silva et al., 2017). Although cilia still form in null mutants of these isotypes, they display a range of structural abnormalities and functional defects, including reduced intraflagellar transport and impaired signaling mechanisms, which compromise behaviors involved in locomotion, exploration and response to contact with food, and nose retraction upon touch, as well as male mating.

Several *C. elegans* isotypes are important for mitotic and meiotic spindle function. Despite broad expression and likely function in neurons (Baran et al., 2010; Fukushige et al., 1993; Nishida et al., 2021), the α -tubulins *Tba-1* and *Tba-2* are mainly observed in dividing cells, where they have partially redundant roles. Knockdown of either, which share 98.5% identity, is tolerated,

whereas double knockdown sufficiently disrupts spindle function to be embryonic lethal (Honda et al., 2017; Lu and Mains, 2005; Phillips et al., 2004). Two closely related β -tubulins, *TBB-1* and *TBB-2*, with 97.6% identity, are also broadly expressed and partially redundant in motor neurons and spindle function (Honda et al., 2017; Lockhead et al., 2016; Nishida et al., 2021). Loss of *TBB-2* causes significant embryonic lethality that is rescued by *TBB-1* overexpression (Honda et al., 2017). Notably, loss of *TBB-2* itself causes upregulation of *TBB-1* in early embryos, which may underlie their natural redundancy (Ellis et al., 2003). Mutants in the β -tubulin *BEN-1* confer resistance to benomyl, a MT destabilizer that causes slow growth, reduced process outgrowth from motor neurons and uncoordinated movement (Driscoll et al., 1989). *BEN-1* is expressed broadly in the nervous system, thus implicating it in motor neuron development and function.

Similar to what is seen in the microorganisms described above, although the *C. elegans* tubulin isotypes show specific contributions to MT functions, there is also sufficient redundancy such that loss of one rarely produces extreme phenotypes (Fukushige et al., 1999; Lockhead et al., 2016; Nishida et al., 2021; Zheng et al., 2017). Nevertheless, RNAi depletion of specific isotypes with substitute expression by another shows that the isotype composition has distinct effects on MT dynamics and MT-mediated processes, such as spindle movement (Honda et al., 2017). Moreover, the combinatorial loss of two isotypes can result in severe phenotypes, such as the embryonic lethality upon loss of *TBA-1* and *TBB-2*, suggesting only they are redundant for spindle function and/or support processes that are synthetically required for viability (Honda et al., 2017). Finally, *Tba-1* and *Tba-2*, and *Tbb-1* and *Tbb-2* display differential interactions with and sensitivities to the MT-severing protein Katanin (encoded by *MEI-1* and *MEI-2*) (Lu and Mains, 2005; Lu et al., 2004; Wright and Hunter, 2003). This is reminiscent of the budding yeast isotypes that optimize the function of specific motors and MAPs during spindle positioning (Nsamba et al., 2021).

Drosophila melanogaster

The *Drosophila* genome contains five isotypes of both α - and β -tubulin (Table 1). Three α -tubulin isotypes, *TUB67C*, *TUB84B* and *TUB84D*, are known to function during oogenesis and early embryogenesis. Of these, *TUB67C*, whose expression is strictly maternal, is more divergent and required for nuclear division in these cells (Matthews et al., 1993; Theurkauf, 1992). Conversely, excessive *Tub67c* with reduced *Tub84b* and *Tub84d* does not disrupt meiosis but instead compromises spindle function in mitosis (Matthews et al., 1993).

In *Drosophila*, the α -tubulin *TUB84B* and β -tubulin *TUB85D* are required for axoneme structure and function during spermatogenesis (Fackenthal et al., 1995; Hoyle and Raff, 1990; Hutchens et al., 1997; Kaltschmidt et al., 1991; Kempfues et al., 1980; Raff et al., 2000). *TUB84B* is indispensable for the formation of the axonemal central pair and for accessory MTs with luminal filaments (Hutchens et al., 1997), whereas *TUB85D* supports the assembly of doublet MTs (Hoyle and Raff, 1990). In most studies, alternative isoforms could not complement *TUB84B* or *TUB85D*, further demonstrating their specificity in spermatogenesis. Strikingly, even though *Tub85e* is 98% similar to *Tub84b*, if it comprises more than 50% of cellular tubulin, it dominantly disrupts the spermatogenesis-specific role of *Tub84b* (Hutchens et al., 1997). A similar relationship exists between the developmental *Tub65b* and the spermatogenesis-specific *Tub85d* (Fackenthal et al., 1995; Hoyle and Raff, 1990). Recently, α -tubulin *TUB67C* was found to be

critical for germline stem cell maintenance in testis (Tao et al., 2021).

The *Drosophila* model has the potential to uncover isotype-specific function in various contexts, including under lower temperatures, which typically destabilizes MTs. Indeed, genome-wide expression profiling revealed that the β -tubulin *TUB97EF* is upregulated at lower temperatures, where it functions to increase MT stability during development (Myachina et al., 2017).

Xenopus laevis

The *Xenopus* genome was only annotated recently and new genes may still be discovered, but it contains at least six α - and six β -tubulin isotypes (www.xenbase.org; Table 1). Although little investigation into *Xenopus* tubulin isotypes has been done to date, the power of this model was recently demonstrated with the discovery that they control spindle size. *Xenopus* egg extracts have been invaluable for the study of spindle assembly. Extracts from *X. laevis* generate spindles that are longer than those assembled from extracts of the smaller species, *X. tropicalis* (Brown et al., 2007). Additionally, *Xenopus* spindles scale with cell size *in vivo* (Wühr et al., 2008), suggesting the existence of size-regulating mechanisms. Subsequent studies have shown that cytosolic volume (Good et al., 2013; Hazel et al., 2013), as well as the concentration and activity of factors such as Katanin (Loughlin et al., 2011), XMAP215 (Milunović-Jevtić et al., 2018; Reber et al., 2013) and TPX2 (Helmke and Heald, 2014), contribute to spindle size. A recent study has also shown that despite 96–99.8% and 98.6–100% sequence identity between the *X. laevis* and *X. tropicalis* α - and β -tubulin isotypes respectively, they display different dynamic properties, which contributes to the observed differences in spindle size (Hirst et al., 2020). Therefore, the relatively small differences between the *X. laevis* and *X. tropicalis* isotype sequences might have a significant impact on MT dynamics and potentially control interactions with various spindle factors. Although the expression of some *Xenopus* tubulin isotypes are restricted to germ cells (Bieker and Yazdani-Buicky, 1992) or neurons (Moody et al., 1996), the specific contribution of individual isotypes to MT function remains largely unexplored.

Danio rerio

Annotation of the zebrafish (*Danio rerio*) genome is ongoing and thus far, a relatively high number of α -tubulin variants have been reported. However, it is unclear how many of the 13 α - and six β -tubulin sequences found may represent the same gene, or possibly be pseudogenes (https://zfinfo.org/; Table 1). Although limited findings have been reported, there is evidence for tissue specificity, developmental regulation and functional variability. For instance, *in situ* hybridization shows that the β -tubulin *TUBB5* (ZDB-GENE-031110-4) is expressed within the developing peripheral and central nervous system, and eventually shifts to a subset of the adult brain (Oehlmann et al., 2004). Additionally, α -tubulin *TUBA1A* (ZDB-GENE-090507-4) is needed for proper central nervous system development (Veldman et al., 2010). Moreover, induced *TUBA1A* expression is required for axonal regeneration in retinal ganglion cells following optic injury (Veldman et al., 2010). Similar mechanisms have been observed with *TUBA1A* in mice, as discussed below.

Mus musculus

The eight α - and eight β -tubulin isotypes in mouse (*Mus musculus*) are nearly identical to their counterparts in humans (Findeisen et al., 2014; Khodiyar et al., 2007) (Table 1). Their cell, tissue and

Box 1. Tubulin isotypes and tubulinopathies

Mutations in human isotypes cause disorders collectively called tubulinopathies [reviewed in Chakraborti et al. (2016) with additional citations below]. Mutations in *TUBA1A*, *TUBB2A*, *TUBB2B* or *TUBB5* often produce malformation of cortical development (MCD), while those in *TUBB3* frequently cause axon guidance disorders, even in the absence of MCD, hallmarked by congenital fibrosis of the extraocular muscles (CFEOM). However, mutations in *TUBA1A* (Jurgens et al., 2021) or *TUBB2B* can also produce CFEOM, and some in *TUBB3* generate MCD, with or without CFEOM. Depending on the specific residue involved, *TUBB4A* mutations give rise to congenital neurological disorders and/or neurodegenerative phenotypes (Chakraborti et al., 2016). Some *TUBB3* syndromes also include neurodegenerative aspects (Tischfield et al., 2010), whereas *TUBA4A* mutations are associated with amyotrophic lateral sclerosis, a late-onset motor neurodegenerative disorder (Smith et al., 2014). Outside the nervous system, mutations in *TUBA4A* or *TUBB1* cause macrothrombocytopenia, with impaired blood platelet formation and function (Kunishima et al., 2009; Strassel et al., 2019). In oocytes, *TUBB8* is essential for meiotic spindle function and mutations in this gene cause female infertility (Chen et al., 2019; Feng et al., 2016). In less frequent incidences, two mutations in *TUBB4B* are associated with Usher syndrome, a disorder affecting both hearing and vision (Luscan et al., 2017). Two *TUBA3D* mutations have been linked to the degenerative corneal disorder keratoconus (Hao et al., 2017). A single *TUBA3E* mutation is associated with MCD (Alazami et al., 2015). Another mutation in *TUBB6* is implicated in congenital facial palsy, bilateral ptosis and velopharyngeal dysfunction, likely due to impaired cranial innervation (Fazeli et al., 2017).

As described for *TUBB4A* above, the manifestation of specific phenotypes often depends on the mutated residue. With *TUBB3* for example, R262C results in isolated CFEOM, whereas E410K causes CFEOM with accompanying facial weakness, developmental delay, commissural axon dysgenesis and late-onset polyneuropathy (Tischfield et al., 2010). In contrast, E205K produces MCD with axonal abnormalities and developmental delays in the absence of CFEOM (Poirier et al., 2010). In the case of *TUBB5*, certain mutations produce microcephaly with severe developmental delays (Breuss et al., 2012), whereas others cause facial abnormalities and excessive circumferential skin folding known as 'Michelin baby syndrome' (Isrie et al., 2015). Thus, isotypes have distinct roles in development and health, and specific residues within isotypes mutated in disease likely mediate MT interactions with key cellular factors, giving rise to the respective disease phenotypes.

developmental expression pattern is also generally similar to that in humans (Bittermann et al., 2019; Braun et al., 2010; Keays et al., 2007; Tischfield et al., 2010). Moreover, the phenotypes resulting from perturbations of specific isotypes are comparable to those observed in humans (Aiken et al., 2019; Cederquist et al., 2012; Feng et al., 2016; Keays et al., 2007; Stottmann et al., 2013; Tischfield et al., 2010).

Perhaps the most productive aspect of studying tubulin isotypes in mouse has been the elucidation of mechanisms underlying tubulin-related diseases, termed tubulinopathies (Box 1). Pioneering studies have shown that the β -tubulin isotype *TUBB1* is required to form the marginal band, a ring-like assembly of highly curved MTs that supports the structure and function of blood platelets (Cuenca-Zamora et al., 2019). Although the alternative β -tubulins *TUBB5* and *TUBB2A* are upregulated in these cells, they could not compensate for the loss of *TUBB1* (Schwer et al., 2001). Recently, the α -tubulin *TUBA4A* was also found to be required for marginal band formation and platelet biogenesis (Strassel et al., 2019). Mutations in *TUBB1* or *TUBA4A* are implicated in macrothrombocytopenia, a group of disorders characterized by

defective platelet formation and function (Kunishima et al., 2009; Strassel et al., 2019). In addition to blood disorders, mutagenic screens uncovered the relationship between *TUBA1A* perturbation and lissencephaly (Keays et al., 2007). Subsequent mutagenesis verified the role of *TUBB2B* in cortical development (Stottmann et al., 2013). Furthermore, modeling human mutations in the corresponding mouse genes confirmed the causative links between *TUBA1A*, *TUBB2B* or *TUBB3* mutations and a range of neurological disorders (Aiken et al., 2019; Belvindrah et al., 2017; Cederquist et al., 2012; Keays et al., 2007; Minoura et al., 2016; Tischfield et al., 2010).

The investigation of mouse tubulin isotypes has led to the recent generation of animals lacking *TUBA1A*, *TUBB2A* and/or *TUBB2B* (Bittermann et al., 2019), *TUBA8* (Diggle et al., 2017; Bittermann et al., 2019) or *TUBB3* (Latremoliere et al., 2018). These studies show that, although loss of *TUBB2A* or *TUBB2B* does not reduce viability, the absence of both results in mild cortical malformations (Bittermann et al., 2019). However, loss of *TUBA1A* causes substantial forebrain dysmorphology and perinatal lethality (Bittermann et al., 2019). The function of *TUBA1A* in neuronal migration cannot be compensated for by *TUBA8* (Belvindrah et al., 2017; Kawauchi, 2017). Although not lethal, partial loss of *TUBA1A* results in disrupted axon pathfinding across commissural structures, reduced MT-dependent trafficking and impaired synaptic function (Buscaglia et al., 2020). Loss of *TUBA8* does not significantly affect brain development (Diggle et al., 2017; Bittermann et al., 2019), but likely functions in spermatogenesis (Diggle et al., 2017). Moreover, *Tuba8* cannot compensate for the straightness and polymerization speed of MTs in neurons that is normally achieved by *Tuba1a*, indicating they are not functionally equivalent (Belvindrah et al., 2017). Strikingly, the canonical neuronal tubulin *TUBB3* (Tuj1) is not required for general nervous system development, but needed for the timely regeneration of peripheral nervous system axons (Latremoliere et al., 2018). Indeed, this role of *TUBB3* cannot be substituted for by other β -tubulin isotypes (Latremoliere et al., 2018).

Model organisms and tubulinopathies

The tubulinopathies have highlighted the need to better understand the role of tubulin isotypes, particularly in humans (Box 1). This presents several challenges. Although recombinant or mutated tubulin for biochemical study has been available from yeast for some time (Davis et al., 1994), it was only obtained from higher eukaryotes more recently (Minoura et al., 2013, 2016; Pamula et al., 2016; Ti et al., 2016; Vemu et al., 2017). Examining isotype function in cells has also been challenging, as dampening expression of one can result in upregulation of others and/or change the ratios of the remaining subunits along with any specific properties (Latremoliere et al., 2018). Thus, using knockdown or deletion to assess the activity of specific isotypes *in vivo* can be confounded by the effect that changes in the remaining isotypes have on MT function and cell health.

The first recognized tubulinopathy, *TUBA1A* mutations that cause lissencephaly, was identified by genetic screens for developmental and behavioral disorders in mice (Keays et al., 2007). In addition, work in yeast revealed that mutations in *TUBB3* that cause congenital fibrosis of the extraocular muscles (CFEOM), stabilize MTs and perturb their interactions with kinesins (Tischfield et al., 2010). These findings guided analyses that confirmed similar consequences in the more complex mouse model (Tischfield et al., 2010). A similar approach reinforced this paradigm by showing that different CFEOM-associated mutations,

in *TUBB2B*, similarly disrupt MT interaction with the kinesin Kip3 when modeled in yeast cells (Cederquist et al., 2012). Moreover, work in mouse revealed that a compensatory mutation in the kinesin *KIF5B*, which restores motility on CFEOM-mutant MTs, rescues axon growth defects in the *TUBB3* mutant (Minoura et al., 2016). Some *TUBA1A* mutants that cause lissencephaly support normal kinesin function but disrupt dynein activity when modeled in yeast cells (Aiken et al., 2019), and dominantly perturb neuronal migration in mice (Aiken et al., 2019; Belvindrah et al., 2017). The impaired motor activity of kinesin and dynein on the mutant MTs in axonal- and migration-related disorders, respectively, is consistent with their known key roles in each process. Additional work in yeast and mouse helped elucidate the defects in mutant *TUBB8* that block meiotic spindle function, leading to female infertility (Feng et al., 2016). Furthermore, using yeast, it has been shown that the F265L mutation of *TUBB2B*, which causes malformation of cortical development (MCD) (Jaglin et al., 2009), perturbs MT interaction with the plus-end-binding protein Bim1 (yeast Eb1) and disrupts its role in mitotic spindle positioning (Denarier et al., 2019). The perturbation of specific motors or MAPs by various mutations can therefore explain the segregation of certain tubulinopathy phenotypes with the respective isotype and/or the individual residues mutated therein.

Although simple models like yeast can provide mechanistic insights into the effects of tubulin isotype mutations, their utility can nevertheless be limited with regard to processes observed in higher eukaryotes, such as development, and more complex models are needed. For example, CRISPR technology was recently used in *C. elegans* to examine the effects of tubulin mutations on MT stability and neurite growth pattern, which appears to correlate with the tubulin region mutated (Zheng et al., 2017). Thus, model systems will remain valuable in defining the molecular basis of tubulinopathies, which may also reveal further insights into how specific isotypes support diverse MT functions (Fourel and Boscheron, 2020).

Conclusions

Elucidating how tubulin isotypes support MT functions has been challenging, but from work in model organisms an overall picture is emerging. At first glance, many isotypes appear interchangeable, and this may stem from their common need to co-polymerize, support dynamic instability and not disrupt core MT activities. Essentially, an isotype must remain, at heart, a tubulin. Outside of these constraints, isotypes could evolve new behaviors and/or co-evolve with specific MT regulators or interacting factors, provided any changes do not significantly perturb other essential MT-dependent functions. Cell- or developmental context-specific expression, however, could alleviate such incompatibilities. Indeed, beyond their fundamental redundancy, the tubulin isotypes that have been examined generally display distinct differences in their support of various MT activities. Notably, isotypes can enhance specialized functions at sub-stoichiometric levels in MTs. This can explain how mutations in one allele of a single isotype can alter MTs to produce tubulinopathies (Chakraborti et al., 2016).

A central role of tubulin isotypes appears to be to allow MTs to more effectively execute diverse functions. In budding yeast, *TUB1* and *TUB3* optimize different mitotic spindle-positioning mechanisms (Nsamba et al., 2021). In *Drosophila*, *TUB84B* and *TUB85D* fulfill distinct functions during spermatogenesis (Hoyle and Raff, 1990; Hutchens et al., 1997). The emergence of a new isotype increases the evolutionary space in which MT functions can diversify. It is, therefore, unsurprising that complex organisms

generally harbor more isotypes (Table 1). Since tubulin isotype specification would be guided by the MT functions required in specific organisms, it takes different routes in distinct evolutionary branches. Hence, isotype number and functional specialization is not comparable across distantly-related species, for example, yeast, nematode and vertebrates. Examples are also seen in more closely related species, for instance, *TUBB3* is not found in zebrafish, *TUBB1* appears unique to mammals, as are blood platelets in which *TUBB1* is expressed, and *TUBB8* exists only in primates. The challenges in addressing isotype function and the expanding spectrum of tubulinopathies, together with advances in genome editing and cytological techniques present exciting opportunities to further leverage model organisms. Findings from these systems might reveal fundamental mechanisms and valuable paradigms to better understand how tubulin isotypes support diverse MT processes, and how they function in healthy development and human disease.

Acknowledgements

We apologize to the many researchers whose work in various model systems was omitted due to space limitations. We thank Ambuj Kumar for assistance with Fig. 1.

Competing interests

The authors declare no competing or financial interests.

Funding

Our work in this area is supported by the National Science Foundation grant number MCB-1846262 to M.L.G.

References

- Adachi, Y., Toda, T., Niwa, O. and Yanagida, M. (1986). Differential expressions of essential and nonessential alpha-tubulin genes in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **6**, 2168–2178.
- Aiken, J., Moore, J. K. and Bates, E. A. (2019). TUBA1A mutations identified in lissencephaly patients dominantly disrupt neuronal migration and impair dynein activity. *Hum. Mol. Genet.* **28**, 1227–1243. doi:10.1093/hmg/ddy416
- Alazami, A. M., Patel, N., Shamseldin, H. E., Anazi, S., Al-Dosari, M. S., Alzahrani, F., Hijazi, H., Alshammari, M., Aldahmesh, M. A., Salih, M. A. et al. (2015). Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. *Cell Rep.* **10**, 148–161. doi:10.1016/j.celrep.2014.12.015
- Audenaert, K., Vanheule, A., Höfte, M. and Haesaert, G. (2013). Deoxynivalenol: a major player in the multifaceted response of fusarium to its environment. *Toxins* **6**, 1–19. doi:10.3390/toxins6010001
- Ayukawa, R., Iwata, S., Imai, H., Kamimura, S., Hayashi, M., Ngo, K. X., Minoura, I., Uchimura, S., Makino, T., Shirouzu, M. et al. (2021). GTP-dependent formation of straight tubulin oligomers leads to microtubule nucleation. *J. Cell Biol.* **220**, e202007033. doi:10.1083/jcb.202007033
- Banerjee, A., Roach, M. C., Wall, K. A., Lopata, M. A., Cleveland, D. W. and Luduena, R. F. (1988). A monoclonal antibody against the type II isotype of beta-tubulin. Preparation of isotypically altered tubulin. *J. Biol. Chem.* **263**, 3029–3034.
- Baran, R., Castelblanco, L., Tang, G., Shapiro, I., Goncharov, A. and Jin, Y. (2010). Motor neuron synapse and axon defects in a *C. elegans* alpha-tubulin mutant. *Plos One* **5**, e9655.
- Barnes, G., Louie, K. A. and Botstein, D. (1992). Yeast proteins associated with microtubules in vitro and in vivo. *Mol. Biol. Cell* **3**, 29–47. doi:10.1091/mbc.3.1.29
- Belvindrah, R., Natarajan, K., Shabajee, P., Bruel-Jungerman, E., Bernard, J., Goutierre, M., Moutkine, I., Jaglin, X. H., Savariradjane, M., Irinopoulou, T. et al. (2017). Mutation of the α -tubulin Tuba1a leads to straighter microtubules and perturbs neuronal migration. *J. Cell Biol.* **216**, 2443–2461. doi:10.1083/jcb.201607074
- Bhattacharyya, B., Sackett, D. L. and Wolff, J. (1985). Tubulin, hybrid dimers, and tubulin S. Stepwise charge reduction and polymerization. *J. Biol. Chem.* **260**, 10208–10216.
- Bieker, J. J. and Yazdani-Buick, M. (1992). The multiple β -tubulin genes of *Xenopus*: Isolation and developmental expression of a germ-cell isotype β -tubulin gene. *Differentiation* **50**, 15–23. doi:10.1111/j.1432-0436.1992.tb00481.x
- Bittermann, E., Abdelhamed, Z., Liegel, R. P., Menke, C., Timms, A., Beier, D. R. and Stottmann, R. W. (2019). Differential requirements of tubulin genes in mammalian forebrain development. *PLoS Genet.* **15**, e1008243. doi:10.1371/journal.pgen.1008243
- Bodakuntla, S., Jijumon, A. S., Villablanca, C., Gonzalez-Billault, C. and Janke, C. (2019). Microtubule-Associated Proteins: Structuring the Cytoskeleton. *Trends Cell Biol.* **29**, 804–819. doi:10.1016/j.tcb.2019.07.004
- Bode, C. J., Gupta, M. L., Suprenant, K. A. and Himes, R. H. (2003). The two α -tubulin isotypes in budding yeast have opposing effects on microtubule dynamics in vitro. *EMBO Rep.* **4**, 94–99. doi:10.1038/sj.embor.embor716
- Bond, J. F., Fridovich-Keil, J. L., Pillus, L., Mulligan, R. C. and Solomon, F. (1986). A chicken-yeast chimeric β -tubulin protein is incorporated into mouse microtubules in vivo. *Cell* **44**, 461–468. doi:10.1016/0092-8674(86)90467-8
- Bounoutas, A., O'Hagan, R. and Chalfie, M. (2009). The multipurpose 15-protofilament microtubules in *C. elegans* have specific roles in mechanosensation. *Curr. Biol.* **19**, 1362–1367.
- Bounoutas, A., Kratz, J., Emtage, L., Ma, C., Nguyen, K. C. and Chalfie, M. (2011). Microtubule depolymerization in *Caenorhabditis elegans* touch receptor neurons reduces gene expression through a p38 MAPK pathway. *Proc. Natl. Acad. Sci. USA* **108**, 3982–3987. doi:10.1073/pnas.1101360108
- Braun, A., Breuss, M., Salzer, M. C., Flint, J., Cowan, N. J. and Keays, D. A. (2010). Tuba8 is expressed at low levels in the developing mouse and human brain. *Am. J. Hum. Genet.* **86**, 819–822; author reply 822–3.
- Breuss, M., Heng, J. I.-T., Poirier, K., Tian, G., Jaglin, X. H., Qu, Z., Braun, A., Gstrein, T., Ngo, L., Haas, M. et al. (2012). Mutations in the β -tubulin gene TUBB5 cause microcephaly with structural brain abnormalities. *Cell Rep.* **2**, 1554–1562. doi:10.1016/j.celrep.2012.11.017
- Brown, K. S., Blower, M. D., Maresca, T. J., Grammer, T. C., Harland, R. M. and Heald, R. (2007). *Xenopus tropicalis* egg extracts provide insight into scaling of the mitotic spindle. *J. Cell Biol.* **176**, 765–770. doi:10.1083/jcb.200610043
- Burke, D., Gasdaska, P. and Hartwell, L. (1989). Dominant effects of tubulin overexpression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **9**, 1049–1059.
- Buscaglia, G., Northington, K. R., Moore, J. K. and Bates, E. A. (2020). Reduced TUBA1A tubulin causes defects in trafficking and impaired adult motor behavior. *Eneuro* **7**, ENEURO.0045-20.2020.
- Cederquist, G. Y., Luchniak, A., Tischfield, M. A., Peeva, M., Song, Y., Menezes, M. P., Chan, W.-M., Andrews, C., Chew, S., Jamieson, R. V. et al. (2012). An inherited TUBB2B mutation alters a kinesin-binding site and causes polymicrogyria, CFEOM and axon dysinnervation. *Hum. Mol. Genet.* **21**, 5484–5499.
- Chaaban, S., Jariwala, S., Hsu, C.-T., Redemann, S., Kollman, J. M., Müller-Reichert, T., Sept, D., Bui, K. H. and Brouhard, G. J. (2018). The structure and dynamics of *C. elegans* tubulin reveals the mechanistic basis of microtubule growth. *Dev. Cell* **47**, 191–204.e8.
- Chakraborti, S., Natarajan, K., Curriel, J., Janke, C. and Liu, J. (2016). The emerging role of the tubulin code: From the tubulin molecule to neuronal function and disease. *Cytoskeleton (Hoboken, NJ)* **73**, 521–550. doi:10.1002/cm.21290
- Chalfie, M. and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* **82**, 358–370. doi:10.1016/0012-1606(81)90459-0
- Chalfie, M. and Thomson, J. N. (1982). Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. *J. Cell Biol.* **93**, 15–23. doi:10.1083/jcb.93.1.15
- Chang, P. and Stearns, T. (2000). δ -Tubulin and ϵ -tubulin: two new human centrosomal tubulins reveal new aspects of centrosome structure and function. *Nat. Cell Biol.* **2**, 30–35. doi:10.1038/71350
- Chen, C.-J., Yu, J.-J., Bi, C.-W., Zhang, Y.-N., Xu, J.-Q., Wang, J.-X. and Zhou, M.-G. (2009). Mutations in a β -tubulin confer resistance of *Gibberella zeae* to benzimidazole fungicides. *Phytopathology* **99**, 1403–1411. doi:10.1094/PHYTO-99-12-1403
- Chen, B., Wang, W., Peng, X., Jiang, H., Zhang, S., Li, D., Li, B., Fu, J., Kuang, Y., Sun, X. et al. (2019). The comprehensive mutational and phenotypic spectrum of TUBB8 in female infertility. *Eur. J. Hum. Genet.* **27**, 300–307.
- Cleveland, D. W. (1987). The multitubulin hypothesis revisited: what have we learned? *J. Cell Biol.* **104**, 381–383. doi:10.1083/jcb.104.3.381
- Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. and Kirschner, M. W. (1980). Number and evolutionary conservation of α - and β -tubulin and cytoplasmic β - and γ -actin genes using specific cloned cDNA probes. *Cell* **20**, 95–105. doi:10.1016/0092-8674(80)90238-X
- Cuenca-Zamora, E. J., Ferrer-Marín, F., Riverá, J. and Teruel-Montoya, R. (2019). Tubulin in Platelets: When the Shape Matters. *Int. J. Mol. Sci.* **20**, 3484. doi:10.3390/ijms20143484
- Davis, A., Sage, C., Dougherty, C. and Farrell, K. (1994). Microtubule dynamics modulated by guanosine triphosphate hydrolysis activity of beta-tubulin. *Science* **264**, 839–842.
- Denarier, E., Brousse, C., Sissoko, A., Andrieux, A. and Boscheron, C. (2019). A neurodevelopmental TUBB2B β -tubulin mutation impairs Bim1 (yeast EB1)-dependent spindle positioning. *Biol. Open* **8**, bio.038620.
- Denarier, E., Ecklund, K. H., Berthier, G., Favier, A., O'Toole, E. T., Gory-Fauré, S., Macedo, L. D., Delphin, C., Andrieux, A., Markus, S. M. et al. (2021). Modeling a disease-correlated tubulin mutation in budding yeast reveals insight into MAP-mediated dynein function. *Mol. Biol. Cell* **32**, ar10. doi:10.1091/mbc.E21-05-0237

- Diggie, C. P., Martinez-Garay, I., Molnár, Z., Brinkworth, M. H., White, E., Fowler, E., Hughes, R., Hayward, B. E., Carr, I. M., Watson, C. M. et al. (2017). A tubulin alpha 8 mouse knockout model indicates a likely role in spermatogenesis but not in brain development. *PLoS One* **12**, e0174264. doi:10.1371/journal.pone.0174264
- Distel, R. J., Kleene, K. C. and Hecht, N. B. (1984). Haploid expression of a mouse testis α -tubulin gene. *Science* **224**, 68-70. doi:10.1126/science.6701535
- Doshi, P., Bossie, C. A., Doonan, J. H., Mays, G. S. and Morris, N. R. (1991). Two α -tubulin genes of *Aspergillus nidulans* encode divergent proteins. *Mol. Gen. Genet. Mgg.* **225**, 129-141. doi:10.1007/BF00282651
- Driscoll, M., Dean, E., Reilly, E., Bergholz, E. and Chalfie, M. (1989). Genetic and molecular analysis of a *Caenorhabditis elegans* beta-tubulin that conveys benzimidazole sensitivity. *J. Cell Biol.* **109**, 2993-3003. doi:10.1083/jcb.109.6.2993
- Driver, J. W., Geyer, E. A., Bailey, M. E., Rice, L. M. and Asbury, C. L. (2017). Direct measurement of conformational strain energy in protofilaments curling outward from disassembling microtubule tips. *eLife* **6**, e28433. doi:10.7554/eLife.28433
- Duan, J. and Gorovsky, M. A. (2002). Both carboxy-terminal tails of α - and β -tubulin are essential, but either one will suffice. *Curr. Biol.* **12**, 313-316. doi:10.1016/S0960-9822(02)00651-6
- Eisen, J. A., Coyne, R. S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J. R., Badger, J. H., Ren, Q., Amedeo, P., Jones, K. M. et al. (2006). Macronuclear genome sequence of the ciliate *tetrahymena thermophila*, a model eukaryote. *PLoS Biol.* **4**, e286. doi:10.1371/journal.pbio.0040286
- Ellis, G. C., Phillips, J. B., O'Rourke, S., Lyczak, R. and Bowerman, B. (2003). Maternally expressed and partially redundant β -tubulins in *Caenorhabditis elegans* are autoregulated. *J. Cell Sci.* **117**, 457-464. doi:10.1242/jcs.00869
- Fackenthal, J. D., Hutchens, J. A., Turner, F. R. and Raff, E. C. (1995). Structural analysis of mutations in the *Drosophila* beta 2-tubulin isoform reveals regions in the beta-tubulin molecular required for general and for tissue-specific microtubule functions. *Genetics* **139**, 267-286. doi:10.1093/genetics/139.1.267
- Fazeli, W., Herkenrath, P., Stiller, B., Neugebauer, A., Fricke, J., Lang-Roth, R., Nürnberg, G., Thoenes, M., Becker, J., Altmüller, J. et al. (2017). A TUBB6 mutation is associated with autosomal dominant non-progressive congenital facial palsy, bilateral ptosis and velopharyngeal dysfunction. *Hum. Mol. Genet.* **26**, 4055-4066. doi:10.1093/hmg/ddx296
- Feng, R., Sang, Q., Kuang, Y., Sun, X., Yan, Z., Zhang, S., Shi, J., Tian, G., Lechniak, A., Fukuda, Y. et al. (2016). Mutations in TUBB8 and human oocyte meiotic arrest. *N. Engl. J. Med.* **374**, 223-232. doi:10.1056/NEJMoa1510791
- Findeisen, P., Mühlhausen, S., Dempewolf, S., Hertzog, J., Zietlow, A., Carlomagno, T. and Kollmar, M. (2014). Six subgroups and extensive recent duplications characterize the evolution of the eukaryotic tubulin protein family. *Genome. . Evol.* **6**, 2274-2288. doi:10.1093/gbe/evu187
- Fouriel, G. and Boscheron, C. (2020). Tubulin mutations in neurodevelopmental disorders as a tool to decipher microtubule function. *FEBS Lett.* **594**, 3409-3438. doi:10.1002/1873-3468.13958
- Fukushige, T., Yasuda, H. and Siddiqui, S. S. (1993). Molecular Cloning and Developmental Expression of the Alpha-2 Tubulin Gene of *Caenorhabditis elegans*. *J. Mol. Biol.* **234**, 1290-1300. doi:10.1006/jmbi.1993.1685
- Fukushige, T., Siddiqui, Z. K., Chou, M., Culotti, J. G., Gogonea, C. B., Siddiqui, S. S. and Hamelin, M. (1999). MEC-12, an alpha-tubulin required for touch sensitivity in *C. elegans*. *J. Cell Sci.* **112**, 395-403. doi:10.1242/jcs.112.3.395
- Fulton, C. and Simpson, P. A. (1976). Selective synthesis and utilization of flagellar tubulin. The multi-tubulin hypothesis. *Cell Motil. Cytoskeleton* **3**, 987-1005.
- Gaertig, J., Thatcher, T. H., McGrath, K. E., Callahan, R. C. and Gorovsky, M. A. (1993). Perspectives on tubulin isotype function and evolution based on the observation that *Tetrahymena thermophila* microtubules contain a single α - and β -tubulin. *Cell Motil. Cytoskel* **25**, 243-253. doi:10.1002/cm.970250305
- Gogonea, C. B., Gogonea, V., Ali, Y. M., Merz, K. M. and Siddiqui, S. S. (1999). Computational prediction of the three-dimensional structures for the *Caenorhabditis elegans* tubulin family11Color Plates for this article are on pages 126-130. *J. Mol. Graph. Model.* **17**, 90-100. doi:10.1016/S1093-3263(99)00025-X
- Good, M. C., Vahey, M. D., Skandarajah, A., Fletcher, D. A. and Heald, R. (2013). Cytoplasmic volume modulates spindle size during embryogenesis. *Science* **342**, 856-860. doi:10.1126/science.1243147
- Goodson, H. V. and Jonasson, E. M. (2018). Microtubules and microtubule-associated proteins. *Csh Perspect Biol* **10**, a022608.
- Gulati-Leekha, A. and Goldman, D. (2006). A reporter-assisted mutagenesis screen using α 1-tubulin-GFP transgenic zebrafish uncovers missteps during neuronal development and axonogenesis. *Dev. Biol.* **296**, 29-47. doi:10.1016/j.ydbio.2006.03.024
- Gupta, M. L. J., Bode, C. J., Thrower, D. A., Pearson, C. G., Suprenant, K. A., Bloom, K. S. and Himes, R. H. (2002). beta-Tubulin C354 mutations that severely decrease microtubule dynamics do not prevent nuclear migration in yeast. *Mol. Biol. Cell* **13**, 2919-2932. doi:10.1091/mbc.e02-01-0003
- Hai, B., Gaertig, J. and Gorovsky, M. A. (1999). Chapter 28 knockout heterokaryons enable facile mutagenic analysis of essential genes in tetrahymena. *Methods Cell Biol.* **62**, 513-531. doi:10.1016/S0091-679X(08)61554-X
- Hanson, M. G., Aiken, J., Sietsema, D. V., Sept, D., Bates, E. A., Niswander, L. and Moore, J. K. (2016). Novel α -tubulin mutation disrupts neural development and tubulin proteostasis. *Dev. Biol.* **409**, 406-419. doi:10.1016/j.ydbio.2015.11.022
- Hao, L., Thein, M., Brust-Mascher, I., Civelekoglu-Scholey, G., Lu, Y., Acar, S., Prevo, B., Shaham, S. and Scholey, J. M. (2011). Intraflagellar transport delivers tubulin isotypes to sensory cilium middle and distal segments. *Nat. Cell Biol.* **13**, 790-798.
- Hao, X., Chen, P., Zhang, Y., Li, S., Shi, W. and Gao, H. (2017). De novo mutations of TUBA3D are associated with keratoconus. *Sci. Rep.* **7**, 13570. doi:10.1038/s41598-017-13162-0
- Hausrat, T. J., Radwitz, J., Lombino, F. L., Breiden, P. and Kneussel, M. (2021). Alpha- and beta-tubulin isotypes are differentially expressed during brain development. *Dev. Neurobiol.* **81**, 333-350. doi:10.1002/dneu.22745
- Hazel, J., Krutkramelis, K., Mooney, P., Tomschik, M., Gerow, K., Oakey, J. and Gatlin, J. C. (2013). Changes in cytoplasmic volume are sufficient to drive spindle scaling. *Science* **342**, 853-856. doi:10.1126/science.1243110
- Helmke, K. J. and Heald, R. (2014). TPX2 levels modulate meiotic spindle size and architecture in *Xenopus* egg extracts. *J. Cell Biol.* **206**, 385-393. doi:10.1083/jcb.201401014
- Hiraoka, Y., Toda, T. and Yanagida, M. (1984). The NDA3 gene of fission yeast encodes beta-tubulin: a cold-sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**, 349-358. doi:10.1016/0092-8674(84)90013-8
- Hirst, W. G., Biswas, A., Mahalingan, K. K. and Reber, S. (2020). Differences in intrinsic tubulin dynamic properties contribute to spindle length control in *Xenopus* species. *Curr. Biol.* **30**, 2184-2190.e5. doi:10.1016/j.cub.2020.03.067
- Honda, Y., Tsuchiya, K., Sumiyoshi, E., Haruta, N. and Sugimoto, A. (2017). Tubulin isotype substitution revealed that isotype combination modulates microtubule dynamics in *C. elegans* embryos. *J. Cell Sci.* **130**, 1652-1661.
- Hoyle, H. D. and Raff, E. C. (1990). Two *Drosophila* beta tubulin isoforms are not functionally equivalent. *J. Cell Biol.* **111**, 1009-1026. doi:10.1083/jcb.111.3.1009
- Hsu, J.-M., Chen, C.-H., Chen, Y.-C., McDonald, K. L., Gurling, M., Lee, A., Garriga, G. and Pan, C.-L. (2014). Genetic analysis of a novel tubulin mutation that redirects synaptic vesicle targeting and causes neurite degeneration in *C. elegans*. *PLoS Genet.* **10**, e1004715. doi:10.1371/journal.pgen.1004715
- Huang, B. and Huffaker, T. C. (2006). Dynamic microtubules are essential for efficient chromosome capture and biorientation in *S. cerevisiae*. *J. Cell Biol.* **175**, 17-23. doi:10.1083/jcb.200606021
- Huang, M., Gu, G., Ferguson, E. L. and Chalfie, M. (1995). A stomatin-like protein necessary for mechanosensation in *C. elegans*. *Nature* **378**, 292-295. doi:10.1038/378292a0
- Hurd, D. D. (2018). Tubulins. In *C. elegans*. (August 4, 2018), WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook.1.182.1, <http://www.wormbook.org>.
- Hurd, D. D., Miller, R. M., Núñez, L. and Portman, D. S. (2010). Specific alpha- and beta-tubulin isotypes optimize the functions of sensory cilia in *Caenorhabditis elegans*. *Genetics* **185**, 883-896. doi:10.1534/genetics.110.116996
- Hutchens, J. A., Hoyle, H. D., Turner, F. R. and Raff, E. C. (1997). Structurally similar *Drosophila* alpha-tubulins are functionally distinct in vivo. *Mol. Biol. Cell* **8**, 481-500. doi:10.1091/mbc.8.3.481
- 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. et al. (2015). Mutations in either TUBB or MAPRE2 cause circumferential skin creases kunze type. *Am. J. Hum. Genet.* **97**, 790-800. doi:10.1016/j.ajhg.2015.10.014
- 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. et al. (2009). Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. *Nat. Genet.* **41**, 746-752. doi:10.1038/ng.380
- Janke, C. (2014). The tubulin code: molecular components, readout mechanisms, and functions. *J. Cell Biol.* **206**, 461-472. doi:10.1083/jcb.201406055
- 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. doi:10.1038/nrm3227
- Jiang, Y. Q. and Oblinger, M. M. (1992). Differential regulation of beta III and other tubulin genes during peripheral and central neuron development. *J. Cell Sci.* **103**, 643-651. doi:10.1242/jcs.103.3.643
- Jurgens, J. A., Barry, B. J., Lemire, G., Chan, W.-M., Whitman, M. C., Shaaban, S., Robson, C. D., MacKinnon, S., England, E. M., McMillan, H. J. et al. (2021). Novel variants in TUBA1A cause congenital fibrosis of the extraocular muscles with or without malformations of cortical brain development. *Eur. J. Hum. Genet.* **29**, 816-826. doi:10.1038/s41431-020-00804-7
- Kaltschmidt, B., Glätzer, K. H., Michiels, F., Leiss, D. and Renkawitz-Pohl, R. (1991). During *Drosophila* spermatogenesis beta 1, beta 2 and beta 3 tubulin isotypes are cell-type specifically expressed but have the potential to coassemble into the axoneme of transgenic flies. *Eur. J. Cell Biol.* **54**, 110-120.

- Katsetos, C. D., Legido, A., Perentes, E. and Mörk, S. J.** (2003). Class III β -tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. *J. Child Neurol.* **18**, 851–866. doi:10.1177/088307380301801205
- Kawauchi, T.** (2017). Tubulin isotype specificity in neuronal migration: Tuba8 can't fill in for Tuba1a. *J. Cell Biol.* **216**, 2247–2249. doi:10.1083/jcb.201705172
- Keays, D. A., Tian, G., Poirier, K., Huang, G.-J., Siebold, C., Cleak, J., Oliver, P. L., Fray, M., Harvey, R. J., Molnár, Z. et al.** (2007). Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell* **128**, 45–57. doi:10.1016/j.cell.2006.12.017
- Kellis, M., Birren, B. W. and Lander, E. S.** (2004). Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* **428**, 617–624. doi:10.1038/nature02424
- Kemphues, K. J., Raff, E. C., Raff, R. A. and Kaufman, T. C.** (1980). Mutation in a testis-specific β -tubulin in *Drosophila*: analysis of its effects on meiosis and map location of the gene. *Cell* **21**, 445–451. doi:10.1016/0092-8674(80)90481-X
- Kemphues, K. J., Kaufman, T. C., Raff, R. A. and Raff, E. C.** (1982). The testis-specific β -tubulin subunit in *drosophila melanogaster* has multiple functions in spermatogenesis. *Cell* **31**, 655–670. doi:10.1016/0092-8674(82)90321-X
- Khodiyar, V. K., Maltais, L. J., Sneddon, K. M. B., Smith, J. R., Shimoyama, M., Cabral, F., Dumontet, C., Dutcher, S. K., Harvey, R. J., Lafanechère, L. et al.** (2007). A revised nomenclature for the human and rodent α -tubulin gene family. *Genomics* **90**, 285–289. doi:10.1016/j.ygeno.2007.04.008
- Kirk, K. E. and Morris, N. R.** (1991). The tubB alpha-tubulin gene is essential for sexual development in *Aspergillus nidulans*. *Gene Dev.* **5**, 2014–2023. doi:10.1101/gad.5.11.2014
- Kirk, K. E. and Morris, N. R.** (1993). Either alpha-tubulin isogene product is sufficient for microtubule function during all stages of growth and differentiation in *Aspergillus nidulans*. *Mol. Cell Biol.* **13**, 4465–4476.
- Kirszenblat, L., Neumann, B., Coakley, S. and Hilliard, M. A.** (2013). A dominant mutation in *mec-7*/ β -tubulin affects axon development and regeneration in *Caenorhabditis elegans* neurons. *Mol. Biol. Cell* **24**, 285–296.
- Kunishima, S., Kobayashi, R., Itoh, T. J., Hamaguchi, M. and Saito, H.** (2009). Mutation of the β 1-tubulin gene associated with congenital macrothrombocytopenia affecting microtubule assembly. *Blood* **113**, 458–461. doi:10.1182/blood-2008-06-162610
- Latremoliere, A., Cheng, L., DeLisle, M., Wu, C., Chew, S., Hutchinson, E. B., Sheridan, A., Alexandre, C., Latremoliere, F., Sheu, S.-H. et al.** (2018). Neuronal-specific TUBB3 is not required for normal neuronal function but is essential for timely axon regeneration. *Cell Rep.* **24**, 1865–1879.e9.
- Lewis, S. A., Gu, W. and Cowan, N. J.** (1987). Free intermingling of mammalian β -tubulin isotypes among functionally distinct microtubules. *Cell* **49**, 539–548.
- Lewis, S. A., Tian, G. and Cowan, N. J.** (1997). The alpha- and beta-tubulin folding pathways. *Trends Cell Biol.* **7**, 479–484.
- Liu, S., Duan, Y., Ge, C., Chen, C. and Zhou, M.** (2013). Functional analysis of the β 2-tubulin gene of *Fusarium graminearum* and the β -tubulin gene of *Botrytis cinerea* by homologous replacement. *Pest Manag. Sci.* **69**, 582–588. doi:10.1002/ps.3474
- Lockhead, D., Schwarz, E. M., O'Hagan, R., Bellotti, S., Krieg, M., Barr, M. M., Dunn, A. R., Sternberg, P. W. and Goodman, M. B.** (2016). The tubulin repertoire of *C. elegans* sensory neurons and its context-dependent role in process outgrowth. *Mol. Biol. Cell* **27**, 3717–3728.
- Loughlin, R., Wilbur, J. D., McNally, F. J., Nédélec, F. J. and Heald, R.** (2011). Katanin contributes to interspecies spindle length scaling in *Xenopus*. *Cell* **147**, 1397–1407. doi:10.1016/j.cell.2011.11.014
- Lu, Q. and Luduena, R. F.** (1994). In vitro analysis of microtubule assembly of isotopically 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, C. and Mains, P. E.** (2005). Mutations of a redundant α -tubulin gene affect *Caenorhabditis elegans* early embryonic cleavage via MEI-1/katanin-dependent and -independent pathways. *Genetics* **170**, 115–126. doi:10.1534/genetics.104.030106
- Lu, C., Srayko, M. and Mains, P. E.** (2004). The *Caenorhabditis elegans* microtubule-severing complex MEI-1/MEI-2 katanin interacts differently with two superficially redundant β -tubulin isotypes. *Mol. Biol. Cell* **15**, 142–150. doi:10.1091/mbc.e03-06-0418
- Luduena, R. F.** (1993). Are tubulin isotypes functionally significant. *Mol. Biol. Cell* **4**, 445–457. doi:10.1091/mbc.4.5.445
- Luo, Y., Zhang, H., Qi, L., Zhang, S., Zhou, X., Zhang, Y. and Xu, J.** (2014). FgKin1 kinase localizes to the septal pore and plays a role in hyphal growth, ascospore germination, pathogenesis, and localization of Tub1 beta-tubulins in *Fusarium graminearum*. *New Phytol.* **204**, 943–954. doi:10.1111/nph.12953
- Luscan, R., Mechaussier, S., Paul, A., Tian, G., Gérard, X., Defoort-Dellhemmes, S., Loundon, N., Audo, I., Bonnin, S., LeGargasson, J.-F. et al.** (2017). Mutations in TUBB4B Cause a Distinctive Sensorineural Disease. *Am. J. Hum. Genet.* **101**, 1006–1012. doi:10.1016/j.ajhg.2017.10.010
- Matthews, K. A., Rees, D. and Kaufman, T. C.** (1993). A functionally specialized alpha-tubulin is required for oocyte meiosis and cleavage mitoses in *Drosophila*. *Dev. Camb. Engl.* **117**, 977–991.
- May, G. S.** (1989). The highly divergent beta-tubulins of *Aspergillus nidulans* are functionally interchangeable. *J. Cell Biol.* **109**, 2267–2274. doi:10.1083/jcb.109.5.2267
- May, G. S. and Morris, N. R.** (1988). Developmental regulation of a conidiation specific β -tubulin in *Aspergillus nidulans*. *Dev. Biol.* **128**, 406–414. doi:10.1016/0012-1606(88)90302-8
- May, G. S., Gambino, J., Weatherbee, J. A. and Morris, N. R.** (1985). Identification and functional analysis of beta-tubulin genes by site specific integrative transformation in *Aspergillus nidulans*. *J. Cell Biol.* **101**, 712–719. doi:10.1083/jcb.101.3.712
- McGrath, K. E., Yu, S. M., Heruth, D. P., Kelly, A. A. and Gorovsky, M. A.** (1994). Regulation and evolution of the single alpha-tubulin gene of the ciliate *Tetrahymena thermophila*. *Cell Motil Cytoskel* **27**, 272–283. doi:10.1002/cm.970270308
- Milunovic-Jevtic', A., Jevtic', P., Levy, D. L. and Gatlin, J. C.** (2018). In vivo mitotic spindle scaling can be modulated by changing the levels of a single protein: the microtubule polymerase XMAP215. *Mol. Biol. Cell* **29**, 1311–1317. doi:10.1091/mbc.E18-01-0011
- Minoura, I., Hachikubo, Y., Yamakita, Y., Takazaki, H., Ayukawa, R., Uchimura, S. and Muto, E.** (2013). Overexpression, purification, and functional analysis of recombinant human tubulin dimer. *FEBS Lett.* **587**, 3450–3455. doi:10.1016/j.febslet.2013.08.032
- Minoura, I., Takazaki, H., Ayukawa, R., Saruta, C., Hachikubo, Y., Uchimura, S., Hida, T., Kamiguchi, H., Shimogori, T. and Muto, E.** (2016). Reversal of axonal growth defects in an extraocular fibrosis model by engineering the kinesin-microtubule interface. *Nat. Commun.* **7**, 10058. doi:10.1038/ncomms10058
- Mitchison, T. J. and Kirschner, M.** (1984). Dynamic instability of microtubule growth. *Nature* **312**, 237–242. doi:10.1038/312237a0
- Moody, S. A., Miller, V., Spanos, A. and Frankfurter, A.** (1996). Developmental expression of a neuron-specific β -tubulin in frog (*Xenopus laevis*): a marker for growing axons during the embryonic period. *J. Comp. Neurol.* **364**, 219–230. doi:10.1002/(SICI)1096-9861(19960108)364:2<219::AID-CNE3>3.0.CO;2-8
- Morris, N. R., Lai, M. H. and Oakley, C. E.** (1979). Identification of a gene for α -tubulin in *Aspergillus nidulans*. *Cell* **16**, 437–442. doi:10.1016/0092-8674(79)90019-9
- Myachina, F., Bosshardt, F., Bischof, J., Kirschmann, M. and Lehner, C. F.** (2017). *Drosophila* β -Tubulin 97EF is upregulated at low temperature and stabilizes microtubules. *Development* **144**, 4573–4587.
- Neff, N. F., Thomas, J. H., Grisafi, P. and Botstein, D.** (1983). Isolation of the beta-tubulin gene from yeast and demonstration of its essential function in vivo. *Cell* **33**, 211–219.
- Nishida, K., Tsuchiya, K., Obinata, H., Onodera, S., Honda, Y., Lai, Y.-C., Haruta, N. and Sugimoto, A.** (2021). Expression patterns and levels of all tubulin isotypes analyzed in GFP Knock-In *C. elegans* strains. *Cell Struct. Funct.* **46**, 51–64. doi:10.1247/csf.21022
- Nogales, E.** (2000). Structural insights into microtubule function. *Annu. Rev. Biochem.* **69**, 277–302. doi:10.1146/annurev.biochem.69.1.277
- Nsamba, E. T., Bera, A., Costanzo, M., Boone, C. and Gupta, M. L.** (2021). Tubulin isotypes optimize distinct spindle positioning mechanisms during yeast mitosis. *J. Cell Biol.* **220**, e202010155. doi:10.1083/jcb.202010155
- Oakley, B. R. and Morris, N. R.** (1980). Nuclear movement is β -tubulin-dependent in *Aspergillus nidulans*. *Cell* **19**, 255–262. doi:10.1016/0092-8674(80)90407-9
- Oakley, B. R. and Morris, N. R.** (1981a). A β -tubulin mutation in *Aspergillus nidulans* that blocks microtubule function without blocking assembly. *Cell* **24**, 837–845. doi:10.1016/0092-8674(81)90109-4
- Oakley, E. C. and Morris, N. R.** (1981b). Phenotype and mapping of tubA1. *Aspergillus Newsletter* **15**, 39.
- Oakley, B. R., Paolillo, V. and Zheng, Y.** (2015). γ -Tubulin complexes in microtubule nucleation and beyond. *Mol. Biol. Cell* **26**, 2957–2962. doi:10.1091/mbc.E14-11-1514
- Oehlmann, V. D., Berger, S., Sterner, C. and Korsching, S. I.** (2004). Zebrafish beta tubulin 1 expression is limited to the nervous system throughout development, and in the adult brain is restricted to a subset of proliferative regions. *Gene Expr. Patterns* **4**, 191–198. doi:10.1016/j.modgep.2003.09.001
- O'Hagan, R., Chalfie, M. and Goodman, M. B.** (2005). The MEC-4 DEG/ENAC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. *Nat. Neurosci.* **8**, 43–50. doi:10.1038/nn1362
- Pamula, M. C., Ti, S.-C. and Kapoor, T. M.** (2016). The structured core of human β tubulin confers isotype-specific polymerization properties. *J. Cell Biol.* **213**, 425–433. doi:10.1083/jcb.201603050
- Panda, D., Miller, H. P., Banerjee, A., Luduena, R. F. and Wilson, L.** (1994). Microtubule dynamics in vitro are regulated by the tubulin isotype composition. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11358–11362. doi:10.1073/pnas.91.24.11358
- Parker, A. L., Teo, W. S., Pandzic, E., Vicente, J. J., McCarroll, J. A., Wordeman, L. and Kavallaris, M.** (2018). β -Tubulin carboxy-terminal tails exhibit isotype-specific effects on microtubule dynamics in human gene-edited cells. *Life Sci. Alliance* **1**, e201800059-e16. doi:10.26508/lsa.201800059

- Pham, C. L. and Morrisette, N. S. (2019). The tubulin mutation database: A resource for the cytoskeleton community. *Cytoskeleton* **76**, 186–191. doi:10.1002/cm.21514
- Phillips, J. B., Lyczak, R., Ellis, G. C. and Bowerman, B. (2004). Roles for two partially redundant α -tubulins during mitosis in early *Caenorhabditis elegans* embryos. *Cell Motil. Cytoskel* **58**, 112–126. doi:10.1002/cm.20003
- Poirier, K., Saillour, Y., Bahi-Buisson, N., Jaglin, X. H., Fallet-Bianco, C., Nabbout, R., Castelnau-Ptakine, L., Roubertie, A., Attie-Bitach, T., Desguerre, I. et al. (2010). Mutations in the neuronal β -tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. *Hum. Mol. Genet.* **19**, 4462–4473. doi:10.1093/hmg/ddq377
- Pratt, L. F., Okamura, S. and Cleveland, D. W. (1987). A divergent testis-specific alpha-tubulin isotype that does not contain a coded C-terminal tyrosine. *Mol. Cell. Biol.* **7**, 552–555.
- Pucciarelli, S., Ballarini, P., Sparvoli, D., Barchetta, S., Yu, T., Detrich, H. W. and Miceli, C. (2012). Distinct functional roles of β -tubulin isotypes in microtubule arrays of *Tetrahymena thermophila*, a model single-celled organism. *PLoS One* **7**, e39694. doi:10.1371/journal.pone.0039694
- Raff, E. C. (1984). Genetics of microtubule systems. *J. Cell Biol.* **99**, 1–10. doi:10.1083/jcb.99.1.1
- Raff, E. C., Hutchens, J. A., Hoyle, H. D., Nielsen, M. G. and Turner, F. R. (2000). Conserved axoneme symmetry altered by a component β -tubulin. *Curr. Biol.* **10**, 1391–1394. doi:10.1016/S0960-9822(00)00784-3
- Reber, S. B., Baumgart, J., Widlund, P. O., Pozniakovsky, A., Howard, J., Hyman, A. A. and Jülicher, F. (2013). XMAP215 activity sets spindle length by controlling the total mass of spindle microtubules. *Nat. Cell Biol.* **15**, 1116–1122. doi:10.1038/ncb2834
- Redeker, V., Levilliers, N., Vinolo, E., Rossier, J., Jaillard, D., Burnette, D., Gaertig, J. and Bré, M.-H. (2005). Mutations of tubulin glycosylation sites reveal cross-talk between the C termini of α - and β -tubulin and affect the ciliary matrix in *tetrahymena*. *J. Biol. Chem.* **280**, 596–606. doi:10.1074/jbc.M408324200
- Reed, N. A., Cai, D., Blasius, T. L., Jih, G. T., Meyhofer, E., Gaertig, J. and Verhey, K. J. (2006). Microtubule acetylation promotes kinesin-1 binding and transport. *Curr. Biol.* **16**, 2166–2172. doi:10.1016/j.cub.2006.09.014
- Roll-Mecak, A. (2019). How cells exploit tubulin diversity to build functional cellular microtubule mosaics. *Curr. Opin. Cell Biol.* **56**, 102–108. doi:10.1016/j.cob.2018.10.009
- Savage, C., Hamelin, M., Culotti, J. G., Coulson, A., Albertson, D. G. and Chalfie, M. (1989). *mec-7* is a beta-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Gene Dev.* **3**, 870–881. doi:10.1101/gad.3.6.870
- Savage, C., Xue, Y., Mitani, S., Hall, D., Zakhary, R. and Chalfie, M. (1994). Mutations in the *Caenorhabditis elegans* beta-tubulin gene *mec-7*: effects on microtubule assembly and stability and on tubulin autoregulation. *J. Cell Sci.* **107**, 2165–2175. doi:10.1242/jcs.107.8.2165
- Schatz, P. J., Pillus, L., Grisafi, P., Solomon, F. and Botstein, D. (1986a). Two functional alpha-tubulin genes of the yeast *Saccharomyces cerevisiae* encode divergent proteins. *Mol. Cell. Biol.* **6**, 3711–3721.
- Schatz, P. J., Solomon, F. and Botstein, D. (1986b). Genetically essential and nonessential alpha-tubulin genes specify functionally interchangeable proteins. *Mol. Cell. Biol.* **6**, 3722–3733.
- Schwer, H. D., Lecine, P., Tiwari, S., Italiano, J. E., Hartwig, J. H. and Shivdasani, R. A. (2001). A lineage-restricted and divergent β -tubulin isoform is essential for the biogenesis, structure and function of blood platelets. *Curr. Biol.* **11**, 579–586. doi:10.1016/S0960-9822(01)00153-1
- Serrano, L., de la Torre, J., Maccioni, R. B. and Avila, J. (1984). Involvement of the carboxyl-terminal domain of tubulin in the regulation of its assembly. *Proc. Natl. Acad. Sci. USA* **81**, 5989–5993. doi:10.1073/pnas.81.19.5989
- Siddiqui, S., Aamodt, E., Rastinejad, F. and Culotti, J. (1989). Anti-tubulin monoclonal antibodies that bind to specific neurons in *Caenorhabditis elegans*. *J. Neurosci.* **9**, 2963–2972. doi:10.1523/jneurosci.09-08-02963.1989
- Silber, N., de Opitz, C. L. M., Mayer, C. and Sass, P. (2020). Cell division protein FtsZ: from structure and mechanism to antibiotic target. *Future Microbiol.* **15**, 801–831. doi:10.2217/fmb-2019-0348
- Silva, M., Morsci, N., Nguyen, K. C. Q., Rizvi, A., Rongo, C., Hall, D. H. and Barr, M. M. (2017). Cell-specific α -tubulin isotype regulates ciliary microtubule ultrastructure, intraflagellar transport, and extracellular vesicle biology. *Curr. Biol.* **27**, 968–980. doi:10.1016/j.cub.2017.02.039
- 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. et al. (2014). Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. *Neuron* **84**, 324–331. doi:10.1016/j.neuron.2014.09.027
- Srayko, M., Kaya, A., Stamford, J. and Hyman, A. A. (2005). Identification and characterization of factors required for microtubule growth and nucleation in the early *C. elegans* embryo. *Dev. Cell* **9**, 223–236. doi:10.1016/j.devcel.2005.07.003
- Stottmann, R. W., Donlin, M., Hafner, A., Bernard, A., Sinclair, D. A. and Beier, D. R. (2013). A mutation in *Tubb2b*, a human polymicrogyria gene, leads to lethality and abnormal cortical development in the mouse. *Hum. Mol. Genet.* **22**, 4053–4063.
- Strassel, C., Magiera, M. M., Dupuis, A., Batzenschlager, M., Hovasse, A., Pleines, I., Guéguen, P., Eckly, A., Moog, S., Mallo, L. et al. (2019). An essential role for α 4A-tubulin in platelet biogenesis. *Life Sci. Alliance* **2**, e201900309. doi:10.26508/lsa.201900309
- Suryavanshi, S., Eddé, B., Fox, L. A., Guerrero, S., Hard, R., Hennessey, T., Kabi, A., Mallison, D., Pennock, D., Sale, W. S. et al. (2010). Tubulin glutamylation regulates ciliary motility by altering inner dynein arm activity. *Curr. Biol.* **20**, 435–440. doi:10.1016/j.cub.2009.12.062
- Tao, X., Dou, Y., Huang, G., Sun, M., Lu, S. and Chen, D. (2021). α -Tubulin regulates the fate of germline stem cells in *drosophila* testis. *Sci. Rep.* **11**, 10644. doi:10.1038/s41598-021-90116-7
- Theurkauf, W. E. (1992). Behavior of structurally divergent α -tubulin isotypes during *Drosophila* embryogenesis: evidence for post-translational regulation of isotype abundance. *Dev. Biol.* **154**, 205–217. doi:10.1016/0012-1606(92)90060-T
- Ti, S.-C., Pamula, M. C., Howes, S. C., Duellberg, C., Cade, N. I., Kleiner, R. E., Forth, S., Surrey, T., Nogales, E. and Kapoor, T. M. (2016). Mutations in human tubulin proximal to the kinesin-binding site alter dynamic instability at microtubule plus- and minus-ends. *Dev. Cell* **37**, 72–84. doi:10.1016/j.devcel.2016.03.003
- Ti, S.-C., Alushin, G. M. and Kapoor, T. M. (2018). Human β -tubulin isotypes can regulate microtubule protofilament number and stability. *Dev. Cell* **47**, 175–190.e5. doi:10.1016/j.devcel.2018.08.014
- Tischfield, M. A., Baris, H. N., Wu, C., Rudolph, G., Maldergem, L. V., He, W., Chan, W.-M., Andrews, C., Demer, J. L., Robertson, R. L. et al. (2010). Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell* **140**, 74–87.
- Toda, T., Adachi, Y., Hiraoka, Y. and Yanagida, M. (1984). Identification of the pleiotropic cell division cycle gene *NDA2* as one of two different α -tubulin genes in *schizosaccharomyces pombe*. *Cell* **37**, 233–241. doi:10.1016/0092-8674(84)90319-2
- Turk, E., Willis, A. A., Kwon, T., Sedzinski, J., Wallingford, J. B. and Stearns, T. (2015). Zeta-tubulin is a member of a conserved tubulin module and is a component of the centriolar basal foot in multiciliated cells. *Curr. Biol.* **25**, 2177–2183. doi:10.1016/j.cub.2015.06.063
- Vaughan, S., Attwood, T., Navarro, M., Scott, V., McKean, P. and Gull, K. (2000). New tubulins in protozoal parasites. *Curr. Biol.* **10**, R258–R259.
- Veldman, M. B., Bembien, M. A. and Goldman, D. (2010). *Tuba1a* gene expression is regulated by *KLF6/7* and is necessary for CNS development and regeneration in zebrafish. *Mol. Cell. Neurosci.* **43**, 370–383. doi:10.1016/j.mcn.2010.01.004
- Vemu, A., Atherton, J., Spector, J. O., Moores, C. A. and Roll-Mecak, A. (2017). Tubulin isoform composition tunes microtubule dynamics. *Mol. Biol. Cell* **28**, 3564–3572. doi:10.1091/mbc.e17-02-0124
- Verhey, K. J. and Gaertig, J. (2007). The tubulin code. *Cell Cycle (Georgetown, Tex)* **6**, 2152–2160. doi:10.4161/cc.6.17.4633
- Walter, W. J., Beránek, V., Fischermeier, E. and Diez, S. (2012). Tubulin acetylation alone does not affect kinesin-1 velocity and run length in vitro. *PLoS One* **7**, e42218. doi:10.1371/journal.pone.0042218
- Wang, H., Chen, D., Li, C., Tian, N., Zhang, J., Xu, J.-R. and Wang, C. (2019). Stage-specific functional relationships between *Tub1* and *Tub2* beta-tubulins in the wheat scab fungus *Fusarium graminearum*. *Fungal Genet. Biol.* **132**, 103251. doi:10.1016/j.fgb.2019.103251
- Weatherbee, J. A., May, G. S., Gambino, J. and Morris, N. R. (1985). Involvement of a particular species of beta-tubulin (beta 3) in conidial development in *Aspergillus nidulans*. *J. Cell Biol.* **101**, 706–711. doi:10.1083/jcb.101.3.706
- Weinstein, B. and Solomon, F. (1990). Phenotypic consequences of tubulin overproduction in *Saccharomyces cerevisiae*: differences between alpha-tubulin and beta-tubulin. *Mol. Cell. Biol.* **10**, 5295–5304.
- Wright, A. J. and Hunter, C. P. (2003). Mutations in a β -tubulin disrupt spindle orientation and microtubule dynamics in the early *caenorhabditis elegans* embryo. *Mol. Biol. Cell* **14**, 4512–4525.
- Wu, W. and Morgan, G. T. (1994). Ovary-specific expression of a gene encoding a divergent α -tubulin isotype in *Xenopus*. *Differentiation* **58**, 9–18. doi:10.1046/j.1432-0436.1994.5810009.x
- Wühr, M., Chen, Y., Dumont, S., Groen, A. C., Needleman, D. J., Salic, A. and Mitchison, T. J. (2008). Evidence for an upper limit to mitotic spindle length. *Curr. Biol.* **18**, 1256–1261. doi:10.1016/j.cub.2008.07.092
- Xia, L., Hai, B., Gao, Y., Burnette, D., Thazhath, R., Duan, J., Bré, M.-H., Levilliers, N., Gorovsky, M. A. and Gaertig, J. (2000). Polyglycylation of tubulin is essential and affects cell motility and division in *tetrahymena thermophila*. *J. Cell Biol.* **149**, 1097–1106. doi:10.1083/jcb.149.5.1097
- Xu, Z., Schaedel, L., Portran, D., Aguilar, A., Gaillard, J., Marinkovich, M. P., Théry, M. and Nachury, M. V. (2017). Microtubules acquire resistance from mechanical breakage through intraluminal acetylation. *Science* **356**, 328–332. doi:10.1126/science.aai8764
- Yu, I., Garnham, C. P. and Roll-Mecak, A. (2015). Writing and reading the tubulin code*. *J. Biol. Chem.* **290**, 17163–17172. doi:10.1074/jbc.R115.637447

- Yutin, N. and Koonin, E. V. (2012). Archaeal origin of tubulin. *Biol. Direct* **7**, 10-10. doi:10.1186/1745-6150-7-10
- Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., Seitz, K. W., Anantharaman, K., Starnawski, P., Kjeldsen, K. U. et al. (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* **541**, 353-358. doi:10.1038/nature21031
- Zhao, Z., Liu, H., Luo, Y., Zhou, S., An, L., Wang, C., Jin, Q., Zhou, M. and Xu, J.-R. (2014). Molecular evolution and functional divergence of tubulin superfamily in the fungal tree of life. *Sci. Rep.* **4**, 6746. doi:10.1038/srep06746
- Zheng, C., Diaz-Cuadros, M., Nguyen, K. C. Q., Hall, D. H. and Chalfie, M. (2017). Distinct effects of tubulin isotype mutations on neurite growth in *Caenorhabditis elegans*. *Mol. Biol. Cell* **28**, 2786-2801. doi:10.1091/mbc.e17-06-0424