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REVIEW

SUBJECT COLLECTION: CYTOSKELETON

Tubulin isotypes – functional insights from model organisms

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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 (α/β-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 immuno-depleted 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.

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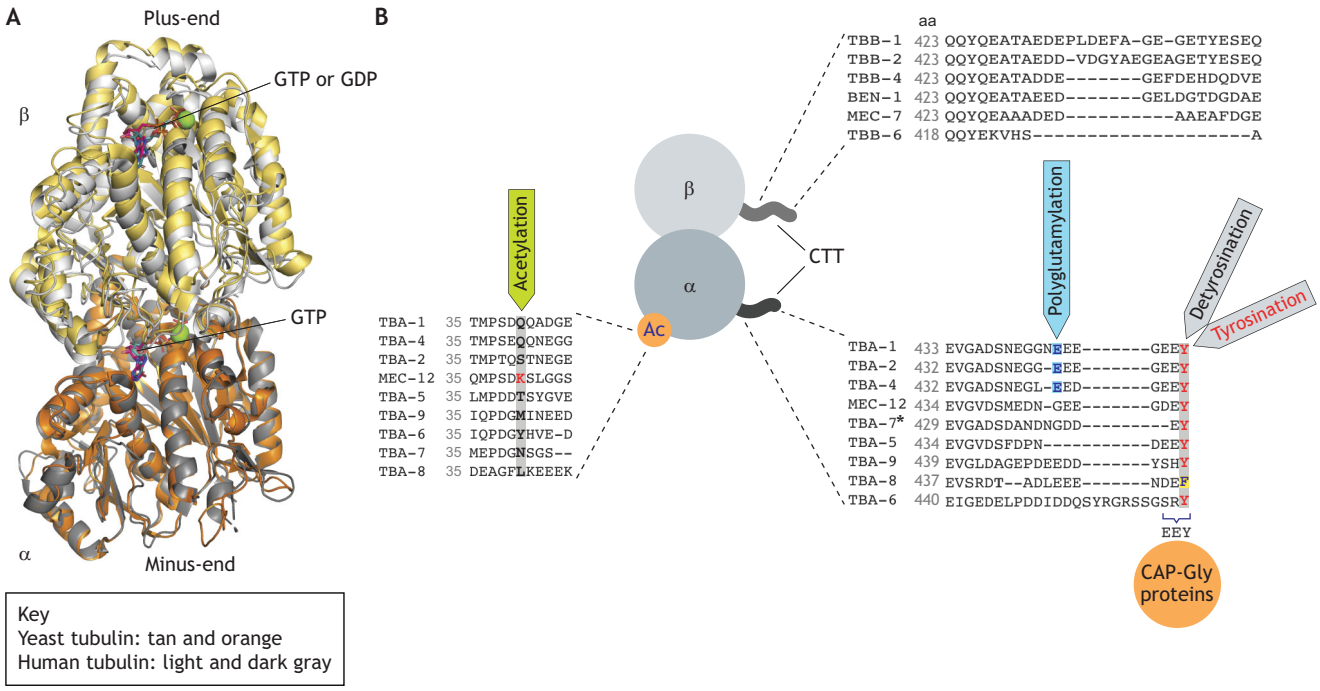

Fig. 1. The tubulin structure is highly conserved but notable sequence differences exist between isotypes. (A) Overlay of human (light and dark gray) and yeast (tan and orange) tubulin heterodimers modeled from their cryo-electron microscopy structures (PDB human: 6E7B, yeast: 5W3F). The root-mean-square 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 isotypes (*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 isotypes is typically concentrated in the final ∼20 amino acids, or C-terminal tail (CTT). Several α-tubulin isotypes lack the preferred site of polyglutamylation (glutamate; blue squares). *TUBA-8* lacks the terminal tyrosine residue that undergoes detyrosination/tyronsination. 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 isotypes 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 isotypes (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 Morrissette, 2019; Ti et al., 2016).

Most eukaryotes have multiple isotypes

Overall, the number of tubulin isotypes 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, isotypes 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 isotypes 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 isotypes have preferred and/or incompatible heterodimerization partners.

Although most eukaryotes express multiple tubulin isotypes, 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) isotypes possess unique biochemical properties that support particular MT-based functions (Fulton and Simpson, 1976; Luduena, 1993), and (4) isotypes 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 isotypes, 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 isotypes underlie fundamental, MT-based processes (Fig. 2).

Tubulin isotypes across diverse species

Overall, α- and β-tubulin are highly conserved with any sequence variation between isotypes largely concentrated within the CTT. Owing to the CTT being on the exterior of the MT, where its 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

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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	
Mus musculus (mouse)	Chordate	*TUBA1A* *TUBA1B* *TUBA1C* *TUBA4A* *TUBA3A* *TUBA3B* *TUBA8* *TUBAL3*	*TUBB1/* *TUBB2A* *TUBB2B* *TUBB3* *TUBB4A* *TUBB4B* *TUBB5* *TUBB6*	Neuronal specific; brain development	*TUBA1A*	*TUBB2A,* *TUBB2B,* *TUBB3,* *TUBB5*	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
				Platelet biogenesis	*TUBA4A*	*TUBB1*	Kunishima et al., 2009; Schwer et al., 2001; Strassel et al., 2019
				Spermatogenesis	*TUBA8*		Diggle et al., 2017
Danio rerio (zebrafish)	Chordate	*TUBA1A* *TUBA1B* *TUBA1C* *TUBA2* *TUBA4L* *TUBA5* *TUBA7L* *TUBA8L2* *TUBA8L3* *TUBA8L4* *TUBA8L5* *ZDB-GENE-051127-7* *ZDB-GENE-120214-26*	*TUBB1* *TUBB6* *TUBB2* *TUBB4B* *TUBB5* *ZDB-GENE-030131-7741*	Retina and brain development	*TUBA1A*		For isotype numbers, see https://zfin.org/; for function, see Veldman et al., 2010
Xenopus laevis (frog)	Chordate	*TUBA1A* *TUBA1B* *TUBA1C* *TUBA4B* *TUBA5* *TUBA8*	*TUBB* *(TUBB5)* *TUBB2B* *TUBB3* *TUBB4A* *TUBB4B* *TUBB6*				For isotype numbers, see Brown et al., 2007; Hirst et al., 2020
Caenorhabditis elegans (nematode)	Nematode	*TBA-1* *TBA-2* *MEC-12* *TBA-4*, *TBA-5*, *TBA-6*, *TBA-7*, *TBA-8*	*TBB-1*, *TBB-2*, *MEC-7* *TBB-4* *BEN-1* *TBB-6*	Mitotic divisions; neuronal	*TBA-1*, *TBA-2*	*TBB-1*, *TBB-2*, *BEN-1*	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
		TBA-9		Sensory neurons	*TBA-5*, *TBA-6*, *TBA-9*	*TBB-4*	Hao et al., 2011; Hurd et al., 2010; Silva et al., 2017
				Mechanosensory	*MEC-12*	*MEC-7*	Bounoutas et al., 2011; Chalfie and Thomson, 1982; Fukushige et al., 1999; Savage et al., 1989
Drosophila melanogaster (fruit fly)	Arthropod	*TUB67C* *TUB84B* *TUB84D* *TUB85E* *TUB90E*	*TUB85D* *TUB65B* *TUB97EF* *TUB60D* *TUB56D*	Germ cells; spermatogenesis; oogenesis; early embryo	*TUB67C,* *TUB84B,* *TUB84D*	*TUB85D*	For isotype numbers, see Fackenthal et al., 1995; Hoyle and Raff, 1990; Hutchens et al., 1997; Kemphues et al., 1982; for function, Matthews et al., 1993; Raff et al., 2000; Tao et al., 2021; Theurkauf, 1992
				Developmentally regulated	*TUB85E*	*TUB65B*	Fackenthal et al., 1995; Hoyle and Raff, 1990; Hutchens et al., 1997
				Thermosensitive		*TUB97EF*	Myachina et al., 2017

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Table 1. Continued

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

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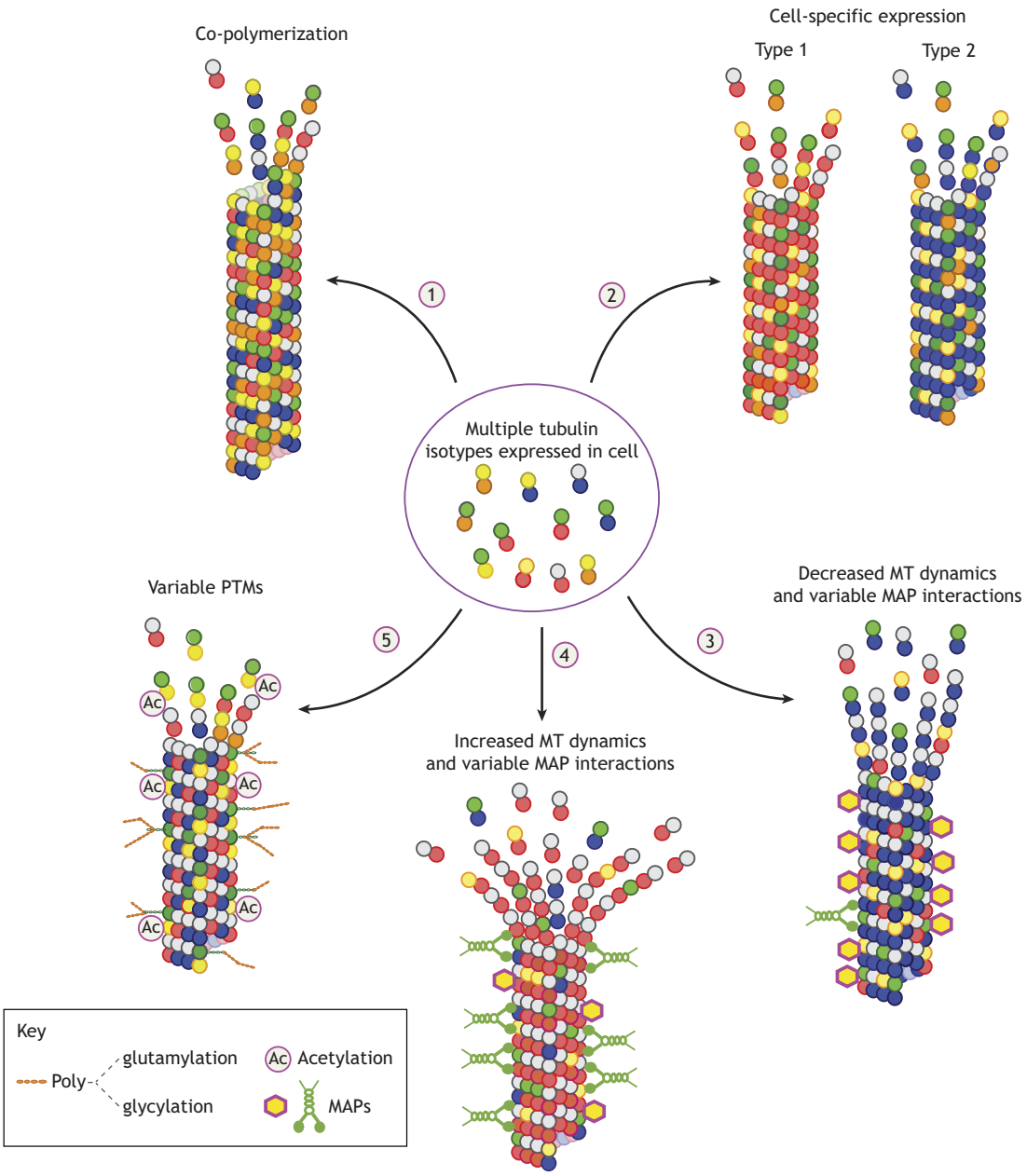

Fig. 2. Impact of tubulin isotypes on the tubulin code. Tubulin isotypes (different colors) exhibit diversity in several aspects of microtubule (MT) function. (1) Tubulin isotypes can co-polymerize, resulting in MTs with distinct composition and thus function. (2) Tubulin isotypes can display cell-, 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, ascosporogenesis and the perithecium (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; Kemphues 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

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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 psuedogenes (https://zfin.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 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

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 phonotypes (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.

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.

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