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CELL SCIENCE AT A GLANCE

# The tubulin code at a glance

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## ABSTRACT

Microtubules are key cytoskeletal elements of all eukaryotic cells and are assembled of evolutionarily conserved α-tubulin–β-tubulin heterodimers. Despite their uniform structure, microtubules fulfill a large diversity of functions. A regulatory mechanism to control the specialization of the microtubule cytoskeleton is the 'tubulin code', which is generated by (i) expression of different α- and β-tubulin isotypes, and by (ii) post-translational modifications of tubulin. In this Cell Science at a Glance article and the accompanying poster, we provide a comprehensive overview of the molecular components of the tubulin code, and discuss the mechanisms by which these components contribute to the generation of functionally specialized microtubules.

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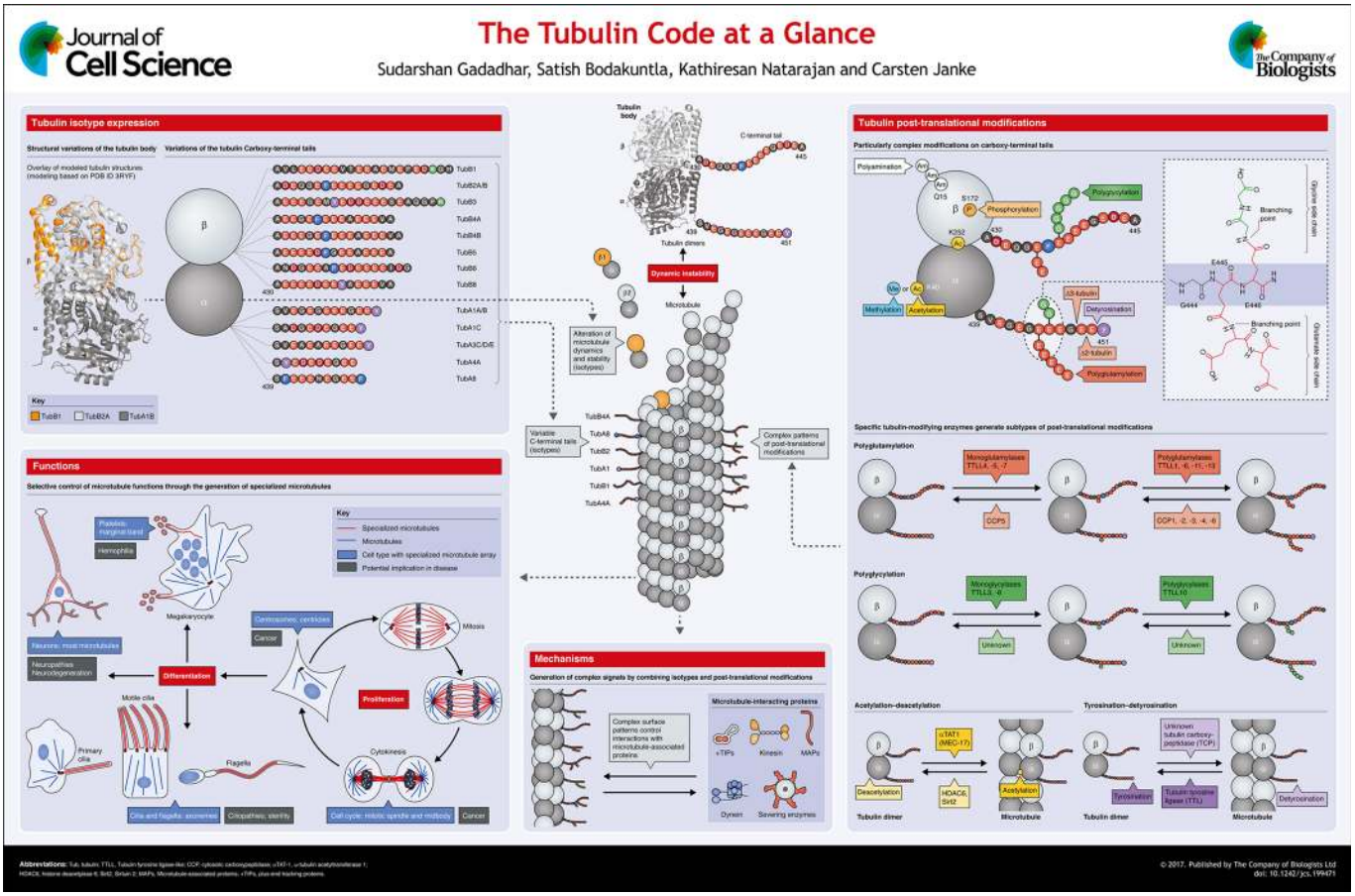
## Introduction

Microtubules (MTs) are the largest filamentous components of the eukaryotic cytoskeleton and are essential for every cell as they control cell shape, division, motility and differentiation. MTs fulfill many of their functions by forming specific assemblies, such as the mitotic spindle to separate the chromosomes during cell division, and the axoneme to form cilia and flagella. MTs are dynamically assembled from evolutionarily highly conserved heterodimers of α- and β-tubulin. Considering the extraordinary conservation of α- and β-tubulins, one of the key challenges is to understand how these filaments can adapt to a huge variety of functions. MTs can functionally specialize by interacting with a variety of MT-associated proteins (MAPs). These proteins can regulate MT dynamics by either stabilizing or destabilizing them, and can generate forces (motor proteins) or connect MTs to other cellular structures, such as membranes or other cytoskeletal components. Furthermore, MTs can themselves be programmed by the 'tubulin code' – a combination of



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the differential expression of α- and β-tubulin genes (tubulin isotypes) and a plethora of post-translational modifications (PTMs) – to exert specific functions as presented in the accompanying poster. Here, we review the components and mechanisms of the tubulin code, and briefly discuss their potential role in controlling MT functions.

## Tubulin isotypes

MTs are highly conserved protein assemblies that show little structural variation throughout eukaryotic evolution. As their assembly is essentially driven by the spontaneous longitudinal and lateral association of α-tubulin–β-tubulin dimers, it is obvious that there is a restricted freedom for the evolution of the atomic structure of the tubulin dimers (Ludueña, 2013). Nevertheless, α- and β-tubulins are encoded by multiple genes in most organisms, giving rise to highly conserved but still different gene products that can form MTs (reviewed in Ludueña and Banerjee, 2008). In mammals, nine α-tubulin and nine β-tubulin genes have been identified, which have, regrettably, been given a confusing variety of names in the literature (for nomenclature see: www.genenames.org/cgi-bin/genefamilies/set/778). The expression and incorporation of different tubulin isotypes into the MT lattice can have two principal functions: (i) either the structure of the highly conserved core of the α- or β-tubulin protein is slightly altered, thus affecting assembly, dynamics and mechanical properties of MTs, or (ii) flexible regions, especially the C-terminal tails that decorate the surface of MTs, vary and thus may affect the interactions of MTs with MAPs and/or the PTMs that are generated on these tails.

Initially, tubulin isotypes were thought to form individual MTs with specialized functions in cells and tissues (Lewis et al., 1985), but this expectation has not been confirmed as different isotypes can freely intermingle into mosaic MTs (Lewis et al., 1987). Nevertheless, specialized MTs, such as axonemes (Raff et al., 2008), neuronal MTs (Joshi and Cleveland, 1989) and the MTs of the marginal band of platelets (Lecine et al., 2000; Schwer et al., 2001), are selectively enriched in specific β-tubulin isotypes, which might in part be responsible for the specific functions of these MTs. Less is known about the roles of α-tubulin isotypes, which in mammals show higher sequence conservation than the β-tubulins. One striking feature of the α-tubulin isotype TubA4A is that it does not contain the characteristic C-terminal tyrosine residue, and thus its expression could influence the tyrosination–detyrosination cycle in cells.

## Molecular mechanisms of isotypes

So far, little is known regarding the impact of specific tubulin isotypes on MT properties and functions. Tubulin isotypes could affect the primary structure of tubulin dimers and thus the assembly dynamics, stability and also physical properties of MTs. To demonstrate this visually, we have modeled the structures of TubA1B with TubB2A, and TubA1B with TubB1, using the crystal structure of brain tubulin (PDB ID 3RYF) as a template. This overlay shows that some of the structural elements are differently positioned in TubB1, which is the most divergent of all mammalian tubulin isotypes. This suggests that incorporation of this isotype could indeed affect the properties of the MT lattice.

The discovery of disease-related mutations in tubulin isotypes (see Box 1) has provided the first insights into molecular mechanisms that could be regulated by these isotypes. The phenotypes generated by these mutations could be explained in different ways. One possibility is that single amino-acid replacements slightly alter the properties of tubulin, which can be tolerated in most MT functions but result in aberrations in some particularly challenging functions, such as long-range neuronal migration (discussed in Chakraborti et al., 2016). Indeed, single mutations in different isotypes can affect GTP hydrolysis and catastrophe rate of MTs (Geyer et al., 2015), as well as MT dynamic instability (Ti et al., 2016). Another proposed mechanism is that mutations in tubulin isotypes change MT dynamics in cells by inducing defects in chaperone-mediated folding and in the heterodimerization pathway of α- and β-tubulin (Keays et al., 2007; Tian et al., 2010, 2008), thus unbalancing the tubulin pool. Overall, the discovery of tubulin mutations has revealed that subtle alterations of the primary sequence of tubulins can have a strong impact on MT functions.

### Box 1. Tubulin mutations

The discovery of mutations in specific tubulin isotypes in the past years has been instrumental in the efforts to better understand the functions of these isotypes in organisms and cells. For instance, mutations in TubB1, an isotype expressed specifically in platelets, invariably lead to bleeding disorders owing to dysfunctional or low numbers of platelets (Fiore et al., 2016; Kunishima et al., 2009, 2014). Similarly, mutations in the neuron-specific isotype TubB3 lead to neuronal disorders (Tischfield et al., 2010), and TubB8 mutations lead to impaired oocyte maturation, suggesting a specific role of this isotype in the germline (Feng et al., 2016).

A large number of mutations have been identified in broadly expressed tubulin isotypes, but strikingly, these mutations cause tissue- and cell-type-specific pathologies. For now, most of these mutations have been identified in neurodevelopmental or neurodegenerative disorders (reviewed in Tischfield et al., 2011). The reasons for these cell-type-specific effects remain unclear. One possibility is that these mutations introduce very subtle changes into the tubulin structure, which only marginally affect MT functions. These defects would only become apparent in cellular processes (such as neuronal migration) that essentially depend on fine-tuned MT dynamics (discussed in detail in Chakraborti et al., 2016). Even more intriguingly, mutations of different amino acid residues in the same tubulin isotype can have distinct pathological outcomes. Mutations of TubB4A, for instance, can affect either neurons or oligodendrocytes depending on the amino acid residue mutated (discussed in detail in Chakraborti et al., 2016).

## Tubulin PTMs

The second component of the tubulin code is tubulin PTMs. Some of these PTMs, such as detyrosination–tyrosination, (poly) glutamylation and (poly)glycylation were initially discovered on tubulin and are therefore known as tubulin PTMs (Arce et al., 1975; Eddé et al., 1990; Hallak et al., 1977; Redeker et al., 1994). Although this holds true for tyrosination, which has been shown to be specific to α-tubulin owing to a unique structural fit between the enzyme tubulin tyrosine ligase (TTL) and tubulin (Prota et al., 2013), glutamylation and glycylation also occur on other substrates; however, so far, only a few of these have been identified (for example, see van Dijk et al., 2008). Other tubulin PTMs, such as acetylation, methylation and phosphorylation, are well-known protein PTMs that occur on a variety of substrates. Most, if not all, tubulin PTMs are generated on the MT polymer, thus allowing the generation of locally restricted and controlled marks on tubulin. Here, we present only the best-studied tubulin PTMs.

## Detyrosination and tyrosination

Tyrosination is an ATP-dependent and tRNA-independent addition of tyrosine to tubulin (Arce et al., 1975) that is reversible (Hallak et al., 1977). Most α-tubulin genes are expressed with a gene-encoded C-terminal tyrosine residue, which is removed by

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detyrosination and re-added by tyrosination. Thus, it is the detyrosination step that initiates the detyrosination–tyrosination cycle. Detyrosinated tubulin was initially called 'Glu-tubulin' for its C-terminal glutamate residue; however, currently the term 'detyr-tubulin' is used to avoid confusion with glutamylated tubulin.

Tyrosination is catalyzed by TTL, the first tubulin-modifying enzyme to be purified (Murofushi, 1980; Schröder et al., 1985) and identified (Ersfeld et al., 1993). Surprisingly, the enzyme catalyzing detyrosination has not yet been discovered. An initial suggestion that detyrosination is mediated by cytosolic carboxypeptidase 1 (CCP1; also known as AGTPBP1) (Kalinina et al., 2007; Rodriguez de la Vega et al., 2007) has not been confirmed (Rogowski et al., 2010).

### Δ2- and Δ3-tubulin

Following detyrosination, additional amino acid residues are removed from the C-terminal tail of α-tubulin to generate Δ2-tubulin (Paturle-Lafanechere et al., 1991) and Δ3-tubulin (Aillaud et al., 2016; Berezniuk et al., 2012). This process is catalyzed by enzymes of the CCP family (Kalinina et al., 2007; Kimura et al., 2010; Rodriguez de la Vega et al., 2007; Rogowski et al., 2010; Tort et al., 2014). Δ2-tubulin cannot undergo tyrosination (Paturle-Lafanechere et al., 1991; Rüdiger et al., 1994) and it has remained unclear whether the glutamate residue can be re-added to generate detyr-tubulin (Aillaud et al., 2016; Berezniuk et al., 2012).

### Tubulin acetylation

The best-characterized acetylation site on tubulin is residue lysine 40 (K40) of α-tubulin (L'Hernault and Rosenbaum, 1985). K40 acetylation is catalyzed by the tubulin acetyl transferase αTAT1 (encoded by *ATAT1* in mammals; MEC-17 in *Caenorhabditis elegans*) (Akella et al., 2010; Shida et al., 2010) and removed by histone deacetylase 6 (HDAC6) (Hubbert et al., 2002) or sirtuin 2 (SIRT2) (North et al., 2003). The acetylation of K40 is different from most other tubulin PTMs as it occurs in the lumen of MTs. Therefore, the modifying enzymes need to access the lumen to generate this modification, and the mechanism of luminal entry has recently been described for αTAT1 (Coombes et al., 2016; Ly et al., 2016).

Recent advances in proteomics have suggested the existence of additional acetylation sites on α- and β-tubulin (Choudhary et al., 2009; Liu et al., 2015); however, their distribution and functional roles remain to be studied. One confirmed acetylation event was found on lysine 252 (K252) of β-tubulin; it is catalyzed by the San acetyl transferase (encoded by *NAA50*) and expected to regulate MT polymerization (Chu et al., 2011).

### Glutamylation and glycylation

(Poly)glutamylation and (poly)glycylation are PTMs that are generated by the enzymatic addition of one or more glutamate or glycine residues as branched peptide chains to the C-terminal tails of α- and/or β-tubulin (Eddé et al., 1990; Redeker et al., 1994). These PTMs are initiated by the addition of a glutamate or glycine to the γ-carboxyl group of one of the gene-encoded glutamate residues of the C-terminal tubulin tails, thus creating a branched peptide structure. Subsequent elongation of these branch chains then generates polyglutamylation or polyglycylation. Owing to this intrinsic complexity and the presence of multiple potential modification sites on α- and β-tubulins, polymodifications generate non-binary complex signals.

Glutamylases and glycylases are members of the TTL-like (TTLL) family. Each enzyme has a reaction and substrate preference and, therefore, initiating and elongating enzymes with preferences for either α- or β-tubulin exist (Janke et al., 2005; Rogowski et al., 2009; van Dijk et al., 2007; Wloga et al., 2009). Some enzymes appear to be highly specific for tubulin, whereas others have a range of substrates (Rogowski et al., 2009; van Dijk et al., 2008). Enzymes catalyzing the reverse reaction have so far only been found for glutamylation. Deglutamylases are members of the CCP family, which, similar to TTLLs, have preferential activities either to shorten long glutamate chains or remove glutamylation at the branching point (Kimura et al., 2010; Rogowski et al., 2010).

### Other tubulin PTMs

Apart from the above-described well-characterized tubulin PTMs, there is a plethora of other PTMs – including phosphorylation, polyamination, palmitoylation, arginylation, ubiquitylation, glycosylation, sumoylation (reviewed in Janke and Bulinski, 2011) and methylation (Park et al., 2016) – that have been found on tubulin. So far, only few of them have been characterized in more detail. For instance, phosphorylation on serine 172 of β-tubulins, which is catalyzed by the cyclin-dependent kinase Cdk1, has been shown to affect MT assembly (Fourest-Lieuvin et al., 2006). The recently discovered methylation of K40 (Park et al., 2016) is highly intriguing as it provides a PTM that competes with the well-known acetylation of this site. Polyamination is also a newly identified polymodification that adds positively charged branch chains to glutamine residues of tubulin and is involved in the stabilization of MTs (Song et al., 2013).

### Molecular mechanisms controlled by tubulin PTMs

Most of the better-studied tubulin PTMs are found within the C-terminal tails of α- and/or β-tubulin. These tails are exposed to the outer surface of assembled MTs and are key interaction sites for MAPs. It is thus obvious that these PTMs can influence, potentially in a selective manner, the interactions between MTs and MAPs. Moreover, the primary sequence of C-terminal tails varies between different tubulin isotypes, providing another layer of complexity to this regulatory mechanism.

Detyrosination has been demonstrated to regulate the molecular motors kinesin-1 (Dunn et al., 2008; Kaul et al., 2014; Konishi and Setou, 2009; Kreitzer et al., 1999; Liao and Gundersen, 1998), kinesin-2 (Sirajuddin et al., 2014) and CENP-E (Barisic et al., 2015). Moreover, detyrosination prevents the kinesin-13 motors MCAK (also known as Kif2C) and Kif2A from disassembling MTs (Peris et al., 2009), thus providing a mechanism by which detyrosination could regulate MT stability. Tyrosination also has a strong effect on the processivity of dynein in complex with dynactin and BicD2 (McKenney et al., 2016). Moreover, the tyrosination status of MTs controls the interaction with some plus-end tracking proteins, such as the cytoplasmic linker protein 170 (CLIP170; encoded by *CLIP1*) (Bieling et al., 2008; Nirschl et al., 2016; Peris et al., 2006).

Equally, polyglutamylation has been demonstrated to regulate the interactions of MTs with a range of MAPs. Initial blot-overlay experiments have suggested that polyglutamylation selectively regulates some neuronal MAPs, such as tau (encoded by *MAPT*), MAP2, MAP1A and MAP1B (Bonnet et al., 2001; Boucher et al., 1994), whereas other MAPs, such as MAP6, were unaffected (Bonnet et al., 2002). More recent work using chimeric tubulins that mimic polyglutamylation suggests that kinesin-1 is sensitive to polyglutamylation (Sirajuddin et al., 2014). This confirmed the earlier observation that polyglutamylation regulates kinesin-1-dependent transport of postsynaptic cargos in neurons (Maas et al., 2009). Polyglutamylation also regulates flagellar dynein motors, as

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modulation of this PTM *in vivo* leads to altered ciliary beating (Kubo et al., 2010; Suryavanshi et al., 2010). Moreover, polyglutamylation influences MT dynamics by regulating the activity of the MT-severing enzyme spastin (Lacroix et al., 2010; Valenstein and Roll-Mecak, 2016). So far, no molecular mechanism through which polyglycylation controls MT functions has been identified.

The mechanisms by which acetylation of residue K40 controls MT functions have so far remained ambiguous. As K40 is positioned at the luminal face of MTs, it could potentially regulate the interaction with MT inner proteins (MIPs) (Linck et al., 2014) or MT lattice interactions, and thus MT dynamics. Indeed, in *C. elegans* touch receptor neurons, the organization of the particular 15-protofilament MTs depends on K40 acetylation (Cueva et al., 2012; Topalidou et al., 2012). In contrast, the ultrastructure of mammalian 13-protofilament MTs is unaffected by K40 acetylation (Howes et al., 2014).

Strikingly, alteration of tubulin acetylation in cells affects intracellular transport driven by kinesin-1 or dynein (Dompierre et al., 2007; Reed et al., 2006). However, a direct regulation of kinesin-1 motility by K40 acetylation of tubulin has not been confirmed *in vitro* (Kaul et al., 2014; Walter et al., 2012), suggesting that other mechanisms contribute to the transport phenotype observed in cells.

## Functions of the tubulin code in health and disease

The tubulin code is expected to adapt MTs to specialized cellular functions. So far, the incorporation of specific tubulin isotypes has been found in a few specialized MT structures and cells. In mammals, for instance, TubB1 is expressed exclusively in platelets and megakaryocytes (Wang et al., 1986), and is essential for their function (see Box 1). Apart from TubB1, TubB3 and TubB4 have also been found in specific tissues and structures – TubB3 is most prominent in neurons (Denoulet et al., 1986; Joshi and Cleveland, 1989; Lewis et al., 1985), whereas TubB4 is particularly enriched in the axonemes of cilia and flagella (Renthal et al., 1993). A striking example of isotype specialization is found in the nematode *C. elegans*. In this organism, MTs usually comprise 11 protofilaments, whereas in touch receptor neurons, the tubulin isotypes MEC-12 (α-tubulin) and MEC-7 (β-tubulin) assemble into 15-protofilament MTs that are essential for the function of these neurons (Fukushige et al., 1999; Lockhead et al., 2016; Savage et al., 1989).

Considering the specialized functions of certain tubulin isotypes, changes in their expression levels could influence the properties of the MTs and thus alter MT functions in cells. For instance, differential isotype expression has been observed in various cancers (reviewed in Parker et al., 2014) and could be involved in rendering these cancers more resistant to therapeutic drugs (Kamath et al., 2005; Leandro-Garcia et al., 2012; Yang et al., 2016).

Similar to changes in isotype expression, point mutations in tubulin isotypes could alter the properties and, thus, the functions of MTs. Indeed, tubulin mutations are linked to a wide spectrum of human pathologies (see Box 1).

Tubulin PTMs are differentially distributed on functionally distinct MTs and are mostly enriched on stable long-lived MTs, such as neuronal, axonemal and centriolar MTs. Furthermore, detyrosination preferentially occurs on a subset of MTs in the mitotic spindle (Geuens et al., 1986; Gundersen and Bulinski, 1986), as well as on neuronal MTs (Brown et al., 1993; Cambray-Deakin and Burgoyne, 1987; Robson and Burgoyne, 1989). Deregulation of the detyrosination–tyrosination cycle has been shown to influence tumorigenesis (Kato et al., 2004; Lafanechere et al., 1998; Mialhe et al., 2001; Souček et al., 2006), affect neuronal differentiation (Erck et al., 2005; Marcos et al., 2009) and impede proper chromosome segregation during mitosis (Barisic et al., 2015). Furthermore, detyrosination is an important regulator of cardiac muscle function (Kerr et al., 2015; Robison et al., 2016).

The specific role of K40 acetylation is not yet fully understood. Acetylation has been associated with stable MTs and thus used as a marker for MT stability. So far, it has been shown to have a role in the maturation of megakaryocytes and platelet formation (Iancu-Rubin et al., 2012; Sadoul et al., 2012) and to be essential for touch-sensing in mice (Morley et al., 2016) and *C. elegans* (Topalidou et al., 2012).

Glutamylation occurs on neuronal MTs during neuronal differentiation (Audebert et al., 1993, 1994). Balanced levels of polyglutamylation in neurons play an essential role in neuronal survival (Rogowski et al., 2010). Glutamylation is further enriched on mitotic spindles and midbodies (Bobinnec et al., 1998b; Lacroix et al., 2010), where it could have a role in the control of the cell cycle. Centrioles and basal bodies are hotspots of polyglutamylation (Bobinnec et al., 1998b; Geimer et al., 1997), and blocking this PTM with anti-glutamylation antibodies results in the disassembly of centrioles (Bobinnec et al., 1998a). Moreover, polyglutamylation is prominent on ciliary and flagellar axonemes (Bré et al., 1994; Fouquet et al., 1994; O'Hagan et al., 2011), where it regulates the beating behavior and integrity of these organelles (reviewed in Konno et al., 2012). In contrast to glutamylation, glycylation has so far been exclusively observed on axonemal MTs (Bré et al., 1996; Redeker et al., 1994; Rüdiger et al., 1995; Weber et al., 1996; Xia et al., 2000) and has been implicated in the mechanical stabilization of the axoneme (Pathak et al., 2011; Rogowski et al., 2009; Wloga et al., 2009).

## Conclusions and perspectives

After decades of research, the mechanisms and functions of the 'tubulin code' have only just begun to be unraveled. Some insights at the organism level have already been gained as knockout mice for tubulin-modifying enzymes show a variety of phenotypes, and tubulin mutations (see Box 1) are found in a range of human pathologies. To determine the roles of the tubulin code at the cellular level, it will be important to establish cell biology approaches that are sensitive enough to reveal the impact of subtle alterations in MT behavior. Another great challenge in the field is the development of methods to produce tubulin 'à la carte' – i.e. recombinant tubulin with controlled PTMs – in order to study the mechanisms of the tubulin code *in vitro*. The first exciting advances in this direction have recently been made (Barisic et al., 2015; Minoura et al., 2013; Pamula et al., 2016; Sirajuddin et al., 2014; Vemu et al., 2016).

Taken together, it appears that the tubulin code controls physiological processes through a plethora of mechanisms. Disruption of these processes can lead to diseases such as ciliopathies, cancer and neurodegeneration. Thus, understanding the molecular mechanisms of the tubulin code and their impact on physiology is the key challenge for the coming years.

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#### Competing interests

The authors declare no competing or financial interests.

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### Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.199471.supplemental

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