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Abstract:

Microtubules are core components of the eukaryotic cytoskeleton with essential roles in cell division, shaping, intracellular transport, and motility. Despite their functional heterogeneity, microtubules have a highly conserved structure made from almost identical molecular building blocks; the tubulin proteins. Alternative tubulin isotypes and a variety of posttranslational modifications control the properties and functions of the microtubule cytoskeleton, a concept known as the 'tubulin code'. This concept first emerged with the discovery that α- and β-tubulin are each encoded by multiple genes, but it took decades before its functional importance begun to emerge. Here we review the current understanding of the molecular components of the tubulin code, and how they impact microtubule properties and functions. We discuss how tubulin isotypes and posttranslational modifications control microtubule behaviour at the molecular level, and how this translates into physiological functions at the cellular and organism levels. We further show how the fine-tuning of microtubule functions by some tubulin modifications affects homeostasis, and how its perturbation can lead to a large variety of dysfunctions, many of them linked to human disorders.

Introduction

Microtubules are cytoskeletal filaments with an outer diameter of 25 nm. Their hollow shape endows them with a unique mechanical rigidity[1] that allows for the assembly of large intracellular structures. Microtubules are intrinsically dynamic; they constantly alternate between phases of polymerization and spontaneous depolymerization, a process known as dynamic instability[2]. How to tame such a fluctuating system into highly ordered and controlled structures such as mitotic and meiotic spindles ensuring the correct division of cells[3-5], axonemes that are the central molecular machines of cilia and flagella[6-8], or the cytoskeleton of neurons that controls neuronal connectivity and function over an entire lifetime[9-11] is a fascinating problem that has caught the attention of a large scientific community for over half a century[12].

Since the early ultrastructural analyses of microtubules by electron microscopy[13,14], huge advances have been made in understanding the molecular structure of the microtubule lattice and the arrangement of the α/β-tubulin heterodimers within[15-19]. The discovery of many microtubule-associated proteins (MAPs) as factors that influence microtubule assembly and dynamics revealed that microtubule assemblies could attain specific characteristics, and thus functions, by associating with selected subsets of MAPs[20]. Specific combinations of active molecular motors and structural MAPs can thus explain the mechanisms of self-organizing assemblies such as mitotic and meiotic spindles[21,22].

The understanding of how microtubules form characteristic assemblies together with a plethora of MAPs and motors, and how these assemblies fulfil specific functions has strongly advanced in all areas of cell biology. Some of these MAPs are specific end-binding proteins that control microtubule dynamics and attachment to other cellular structures[23]. Other MAPs bind the entire microtubule lattice, and are thus considered to regulate microtubule dynamics and stability, but might also have more specific roles that remain to be explored[20]. By contrast, how microtubules themselves are functionally modulated by incorporation of specific tubulin gene products, called isotypes, or by tubulin posttranslational modifications (PTMs), has remained unclear until the beginning of the 21st century. Why these molecular processes, commonly conceptualised under the term 'tubulin code'[24] (Fig. 1), have for a long time resisted a thorough functional characterization became only recently apparent. Emerging molecular and functional studies reveal that in many cases, the tubulin code acts as a fine-regulator, and not as a binary switch of microtubule functions. In this review we will

summarize the current understanding of the tubulin code, its elements and their regulation, and discuss the functional implications of this code on the cell and organism levels.

The tubulin code elements

Microtubules exist in every eukaryotic cell. The striking sequence conservation of tubulins throughout evolution is reflected in an almost identical fold of tubulin in virtually every species investigated so far[25,26], with the consequence that tubulin of a variety of eukaryotic organisms assembles into highly similar microtubules: hollow tubes mostly, but not exclusively, built of 13 protofilaments in cells. As tempting as it appears to talk of evolutionary conservation in this case, in reality microtubules can be different between species, and even within single species functionally specialized microtubules have been observed.

1) Tubulin isotypes

Tubulin isotypes arise from the expression of alternative tubulin genes, and their numbers vary largely between species and phyla. In yeast, for instance, there are two genes for α-[27] and only one for β-tubulin[28], whereas the human genome contains nine genes for each, α- and β-tubulin[29]. There is no clear evolutionary trajectory of these tubulin genes, which is why orthologs can only be identified in evolutionary close species. This is reflected in the rather confusing nomenclature of the tubulin genes[30]. 'Generic' α- and β-tubulins are highly conserved between evolutionarily distant species, while more unique isotypes appear to have evolved when novel microtubule functions arose. A striking example is the co-evolution of blood platelets and β1-tubulin (TubB1)[31]. Platelets are small cell fragments essential for blood coagulation that exist only in mammals. Platelets assemble a specialized microtubule array, the marginal band, which requires β1-tubulin[32], a highly divergent isotype in the vertebrate phylum. Another example is β3-tubulin (βTub60D) in *Drosophila melanogaster*, an isotype that is only expressed in subsets of cells during development. Genetic experiment demonstrated that this isotype cannot replace the generic β2-tubulin (βTub85D) in key microtubule functions such as axoneme assembly or spindle formation[33], suggesting that it had evolved for a specific developmental processes in fly. While these particular cases clearly

underpin the notion that tubulin isotypes can be essential to form functionally specialized microtubules, it still remains an open question why so many other tubulin isotypes (Fig. 1) are almost identical in many species, including mammals. We will try to provide some answers to this question in this review.

2) Tubulin posttranslational modifications

Tubulin is subjected to a large number of PTMs (Fig. 1; Table 1). Some of them are found on a broad range of proteins such as phosphorylation[34-50], acetylation[51], methylation[52], palmitoylation[53], ubiquitination[54,55], or polyamination[56], while others were initially discovered on tubulin. Examples for such 'tubulin-specific' PTMs are the enzymatic, ribosome-independent incorporation of tyrosine (tyrosination)[57,58], glutamate ([poly]glutamylation)[59-61], or glycine ([poly]glycylation)[62], or the enzymatic removal of single amino acids from the C-terminus of tubulin, such as detyrosination[63,64], or the generation of Δ2-[65,66] or Δ3-tubulin[67] (Fig. 1; Table 1; Box 1).

Most PTMs label distinct microtubule subpopulations in cells, and are expected to 'encode' those microtubules for specific functions. Enzymes catalysing detyrosination[68], acetylation[69] and polyglutamylation[70] were shown to preferentially modify microtubules vs. the soluble tubulin dimers, underpinning that a targeted modification of selected microtubules in cells is mechanistically feasible.

In the past decade, great advances in the understanding of the biological roles of tubulin acetylation, [de]tyrosination, [poly]glutamylation and [poly]glycylation have been made, which is why we will focus on those PTMs in this review. Most research has focussed on the role of those PTMs on tubulin, which appears to be the main substrate for glutamylation and glycylation, however other, non-tubulin substrates have also been described (Box S1).

Regulation of microtubule properties

The concept that the incorporation of different tubulin variants can affect intrinsic properties of microtubules, such as flexibility, or assembly/disassembly dynamics, is as old as the discovery of tubulin isotypes[71]. However, mechanistic insights into how tubulin isotypes and PTMs control microtubule properties have mostly been obtained in the recent years.

1) Control of mechanic properties

1.1) The tubulin code can determine structural features of microtubules

Recent advances in cryo-electron microscopy provided high-resolution structures of entire microtubules[16,17,25,72,73] that now directly visualise which amino acid residues of α- and β-tubulins are critically involved in the formation of the microtubule lattice. The availability of these structures makes it now possible to model how different tubulin isotypes, which often differ in only a few amino acids, could alter the properties of microtubules, for instance due to their involvement in lattice contacts. Indeed, evolutionary distant mammalian and yeast tubulins both assemble into highly similar 13-protofilament microtubules, but show differences in microtubule structure and mechanics[25,74]. Novel approaches to generate recombinant mammalian tubulin[75-77] allowed to directly demonstrate a strong impact of mammalian β-tubulin isotypes on structural features of the microtubules: while α1B/β2B-tubulin (TubA1B/TubB2B) assembled preferentially into 14-protofilament microtubules *in vitro*, α1B/β3- (TubA1B/TubB3) microtubules mostly formed 13-protofilament microtubules[77].

Caenorhabditis elegans, a worm built of only 959 somatic cells, shows a large structural divergence between microtubules of different cell types. Most somatic cells contain 11-protofilament microtubules, however some neurons assemble hyper-stable 15-protofilament tubes, and cilia form their axonemal microtubule doublets with A-tubules of 13 protofilaments[78] (Fig. 2a). This diversity of microtubule structures is mirrored by a relatively large sequence variability of *C. elegans* tubulin isotypes. Indeed, specific α- and β-tubulin isotypes are required for the assembly of 15-protofilament[79,80], or ciliary microtubules[81,82] in this organism. The concept that tubulin isotypes are determinants of protofilament numbers was further corroborated by a cross-species study. The formation of 16-protofilament accessory microtubules, normally found in the sperm tails of the moth

Heliothis virescens, but not in the fly *Drosophila melanogaster*, could be induced by expressing the testis-specific *TUBB2* gene from *Heliothis virescens*[83] in *Drosophila*.

Direct evidence for the intrinsic capacity of tubulin isotypes to determine microtubule structure was recently provided by assembling microtubules from purified *C. elegans* and bovine brain tubulin *in vitro*. Similar to previous observations in cells, *C. elegans* tubulin preferentially assembled into 11-protofilament microtubules, while bovine brain tubulin formed 13- and 14-protofilament microtubules[26]. While it cannot be excluded that PTMs present on these purified tubulins influence protofilament numbers, these *in vitro* experiments provide strong evidence for the concept that isotypes do directly determine the structure of microtubules. In cells, however, microtubules assemble in the presence of interacting proteins such as doublecortin[84], or the yeast orthologue for EB1 - Bim1p[74], which can further influence protofilament number.

Finally, emerging evidence suggests that tubulin PTMs can also influence the structure of microtubules. The assembly of the characteristic 15-protofilament microtubules in *C. elegans* touch receptor neurons, for instance, is dependent on Mec-17 (aTAT1)-mediated tubulin acetylation, and absence of this enzyme leads to irregularities in protofilament numbers[85]. Mice lacking the polyglutamylase TTLL9 show defects in the characteristic structure of ciliary axonemes, where some microtubule doublet are missing[86].

1.2) Tubulin isotypes determine mechanical features of microtubules

Mechanical bending of microtubules requires sliding of adjacent protofilaments, which is controlled by non-covalent inter-protofilament interactions. Tubulin isotypes might affect those interactions, however so far, no direct evidence for the involvement of isotypes in microtubule flexibility has been reported. Indirect support comes from studies of blood platelets. Platelets attain their specific round shape and defined diameter by the assembly of a microtubule coil of precisely 12 turns; the marginal band[87]. The extreme bending of platelet microtubules depends on a specific β-tubulin isotype, TUBB1[31], as mutation or absence of this gene lead to severe defects in the architecture of the marginal band[32,88] (Fig. 2b). TUBB1 is the most divergent tubulin isotype in mammals and does not have close homologs in other phyla that do not have platelets. It thus appears that this particular β-tubulin isotype has specifically evolved to sustain the high degree of microtubule bending required for correct

platelet functions[89,90], however, direct biophysical evidence for an increased flexibility of β1-tubulin-containing microtubules is still missing.

1.3) Can tubulin PTMs affect microtubule mechanics?

Acetylation of lysine 40 of α-tubulin[51,91] was for many years the most enigmatic PTM of tubulin, as it occurs in the lumen of microtubules (Fig. 1), thus causing a number of controversial discussions on its potential functions (reviewed in ref.[92]). The famous ambiguity was whether acetylation actually stabilises microtubules, or just labels stable microtubules. Recent work has now provided evidence that K40-acetylation protects microtubules from mechanical aging, a process in which microtubules lose their flexural rigidity following repetitive bending[93]. Consequently, acetylation avoids microtubule breakage inside cells, thus making them longer-lived[94] (Fig. 2c). A structural study showed that the modification of K40, located in an unstructured loop of α-tubulin, reduces inter-protofilament interactions[95] (Fig. 2c), and might thus facilitate protofilament sliding and increase microtubule flexibility. Therefore, K40-acetylation of α-tubulin is a tubulin PTM that directly regulates microtubule mechanics. Intriguingly, the loop containing K40 is one of the hotspots of sequence variation between tubulin isotypes, and might thus adapt different conformations as already shown for tubulin from budding yeast[74] and *C. elegans*[26]. This suggests that acetylation and expression of different α-tubulin isotypes could cooperate to adjust mechanical features of microtubules in cells.

Little is so far known on how other tubulin PTMs affect microtubule mechanics. A potential role of detyrosination could be deduced by studying the role of a specific α-tubulin isotype, α4A-tubulin (TUBA4A). Loss of this isotype in blood platelets affects the architecture of the microtubule marginal band[96] (Fig. 2b), indicating that α4A-tubulin plays an essential role in the assembly of this coiled microtubule structure. However, as α4A-tubulin is a rather conserved, 'generic' α-tubulin, it is rather unlikely that it contains unique structural features that change microtubule mechanics. By contrast, a distinct feature of α4A-tubulin is the lack of the gene-encoded C-terminal tyrosine residue, which mimics detyrosination. Though it has not yet been tested whether detyrosination directly affects microtubule bending in platelets, the essential role of this PTM in microtubule flexing during heart and skeletal muscle contraction[97,98] suggests so. It remains to be determined if detyrosination directly renders

microtubules more flexible, or rather attracts proteins to the microtubules that then change their mechanical behaviour.

2. Control of microtubule dynamics

2.1) Tubulin isotypes can control polymerization dynamics of microtubules

Structural work demonstrates that the contacts between tubulin molecules within the microtubule lattice determine microtubule dynamics[19]. Microtubule dynamics is in fact a summary term for several of their properties: growth speed and persistence, as well as the propensity to spontaneously depolymerize, a.k.a catastrophe[2]. First experiments using β-isotype-specific monoclonal antibodies to fractionate brain tubulin[99] showed that different β-tubulin isotypes do indeed affect the dynamic properties of microtubules[100-103]. The use of recombinant tubulin with defined isotype composition confirmed these early experiments by demonstrating that microtubules assembled from pure α1B/β2B-tubulin dimers were more resistant to spontaneous, or catalysed depolymerization as compared to α1B/β3-microtubules[77,104] (Fig. 2d). Considering that β3-tubulin is predominantly expressed in neuronal cells[105], this suggests that neuronal microtubules are more dynamic, a concept that was suggested earlier based on the observation that brain tubulin which was biochemically depleted of β3-tubulin shows an increased assembly speed[100].

An even more striking impact of tubulin isotypes on microtubule dynamics was found with the more divergent *C. elegans* tubulin, which *in vitro* assembled more than three times faster as compared to mammalian brain tubulin[26]. Together, these observations have provided solid and direct evidence that isotypes control the dynamic instability of microtubules.

2.2) Regulation of microtubule dynamics by tubulin PTMs

So far there are a few examples of PTMs that can directly modulate microtubule dynamics. Phosphorylation of S172 of β-tubulin by the cyclin-dependent kinase Cdk1[48], or by the dual-specificity tyrosine-regulated kinase (DYRK)[50], as well as acetylation of K252 of β-tubulin by San acetyl transferase[106] preclude the tubulin dimer from incorporation into microtubules (Fig. 2d). On the other hand, polyamination of tubulin stabilises microtubules and prevents their depolymerisation[56] (Fig. 2d).

Other tubulin PTMs can control microtubule dynamics indirectly, by regulating the MAPs that affect microtubule stability. Detyrosination, for instance, can control binding of CLIP170 or p150^{glued}, which in turn affects microtubule growth speed and persistence[107-109] (Fig. 3a). At the same time, detyrosination also regulates the active disassembly of microtubules by the depolymerizing motors of the kinesin-13 family[110] (Fig. 3a). Polyglutamylation controls enzymatic severing of microtubules by spastin and katanin[111-113] (Fig. 3b), and could thus modulate the microtubule mass and dynamics in cells[114]. Additionally, polyglutamylation might control the binding of a variety of microtubule-associated proteins (MAPs)[115,116], which could eventually stabilize microtubules (Fig. 3b)[20].

Control of microtubule-MAP interactions

Microtubules are interaction platforms for a myriad of proteins, commonly referred to as MAPs[20]. While the term MAP is often associated with non-motile proteins that bind with high affinity to microtubules, in a larger sense, all proteins that interact with microtubules, including molecular motors, plus- and minus-end tracking proteins, and even microtubule depolymerizing proteins could be considered as MAPs. One of the central concepts of the tubulin code is that it could regulate interactions between MAPs and microtubules in a selective manner, thus introducing specificity and selectivity. Intuitively, the PTMs are perfectly situated as dynamic, rapidly adjustable regulators of such interactions, as they can take place on tubulin dimers within existing microtubules. Tubulin isotypes can also control MAP-microtubule interactions, though this type of regulation might be less dynamic, as newly synthetized isotypes need to be incorporated into microtubules via de-novo polymerization.

1) Tubulin isotypes and MAPs

In the past ten years many novel structures of MAPs bound to the microtubule lattice have been solved (for example ref.[73,117-124]). As these structures reveal the precise interaction sites, i.e. amino acid residues, between a MAP and tubulin, it can now be deduced how sequence differences between tubulin isotypes could affect these interactions.

A domain of the tubulin molecule that is involved in many, but not all microtubule-MAP interactions is the unfolded C-terminal tubulin tail. Notwithstanding the rather subtle differences in the primary sequences of tubulin tails in mammals, first direct experimental

evidence with chimeric yeast tubulins has demonstrated that single amino-acid differences, such as the presence of a lysine residue in the tail of β3-tubulin, is sufficient to substantially reduce the run length of kinesin-1 on microtubules. Strikingly, this effect could be counteracted by adding additional glutamate residues in the form of a side chain similar to polyglutamylation on the β3-tubulin tail, or by simply removing the lysine residue[125]. This example illustrates the potential cross-talk between isotypes and PTMs. Similarly, α4A-tubulin (TUBA4A), an isotype lacking the genetically encoded C-terminal tyrosine, thus mimicking tubulin detyrosination, can be enzymatically tyrosinated[126,127]. Finally, the distribution of glutamate residues within the C-terminal tails (i.e. the modification sites for glutamylation and glycylation) might affect the patterns of these two PTMs, as the modifying enzymes have some, yet not fully explored preferences for those sites (Box 1).

2) Tubulin PTMs and MAPs

2.1) The detyrosination/retyrosination cycle

The idea that tubulin PTMs could dynamically regulate the interaction landscape of microtubules emerged together with the discovery of these modifications. Experiments in the 1980ies already suggested differences in the interactions of MAPs with microtubules depending on their tyrosination state[128], but were surprisingly not followed up. More recently, it was demonstrated that the C-terminal tyrosine of α-tubulin plays an essential role for the localisation of CAP-Gly domain-containing proteins to the +TIP complex[129,130]. The underlying molecular mechanism was revealed by structural work showing that CAP-Gly domains specifically recognise C-terminal -EEY/F sequences, which are characteristic for the tyrosinated form of α-tubulin[131].

Another molecular mechanism that depends on the presence of tyrosinated tubulin in the microtubule lattice is the kinesin-13-mediated microtubule disassembly. Complete detyrosination can thus protect microtubules from active depolymerization with motor proteins of this family, such as mitotic centromere-associated kinesin (MCAK) and Kif2A[110] (Fig. 3a). This discovery provided a mechanistic rationale for the established notion that detyrosinated microtubules are more stable, which was mostly derived from observations in cells[132-134], where depolymerizing kinesins might selectively spare detyrosinated microtubules, and consequently making them longer-lived.

Other microtubule interactors have greater affinity to detyrosinated microtubules. Studies using chimeric yeast tubulin revealed that kinesin-2, but not kinesin-1, has an increased motility and processivity on detyrosinated microtubules[125] (Fig. 3a). Similarly, CENP-E, a kinetochore-associated kinesin-7 motor, shows stronger interactions, and is thus more processive, on detyrosinated as compared to fully tyrosinated microtubules purified from Hela cells[135,136] (Fig. 3a).

The minus-end directed motor dynein, in contrast, was not affected by the tyrosination status of microtubules[125], whereas a complex of dynein, dynactin and the adaptor protein BicD2 required tyrosination for its initial loading onto microtubules (Fig. 3a). This dependency on tyrosination is mediated by the $p150^{glued}$ subunit of dynactin – a CAP-Gly protein. Strikingly, once the complex is loaded on microtubules, it can walk through patches of detyrosinated microtubules without changes in motility[137].

2.2) The concept of fine-tuning microtubule-MAP interactions

Two tubulin PTMs, polyglutamylation and polyglycylation, generate a variety of lateral glutamate or glycine peptide chains at different glutamate residues within the C-terminal tails of α- and β-tubulins (Box 1). Using chimeras of yeast tubulin bodies with mammalian C-terminal tails, on which controlled patterns of polyglutamylation were generated by chemically adding glutamate chains of defined length allowed for the first time to show a differential sensitivity of kinesin motors to glutamylation patterns: kinesin-2 motility was already induced by glutamylation with chains of 3 glutamate residues, whereas for activating kinesin-1, glutamate chains of 10 residues length were required[125] (Fig. 3b). In contrast, neither the motility of dynein, nor the depolymerizing activity of kinesin-13 were affected by the presence of either of these glutamate chains[125]. These observations have far-reaching functional implications in the light of polyglutamylation levels found in cells. In brain, α-tubulins with 10 glutamate residues have not been detected, and the majority of α-tubulin carries about 3 glutamate residues[59]. This implies that only kinesin-2, but not kinesin-1, might be directly regulated by tubulin polyglutamylation in neurons.

How a single biological process can be fine-tuned by different polyglutamylation levels has been first demonstrated for microtubule severing. Comparing virtually non-glutamylated and differentially glutamylated microtubules showed that spastin is activated by polyglutamylation of its substrate, the microtubule[111]. Using the polyglutamylase TTLL7 to

generate microtubules with controlled polyglutamylation patterns further revealed an even more exciting aspect of spastin regulation: while the initial increase of tubulin polyglutamylation gradually induced the severing activity of spastin, further accumulation of the PTM reversed this effect[112] (Fig. 3b). This demonstrated that polyglutamylation can act as a rheostat, an exciting concept implying that the length of the glutamate chains, or/and the accumulation of glutamylation on different sites within a single tubulin molecule, could fine-tune the functional readout of this PTM. A similar concept had been proposed earlier for several other MAPs that showed binding differences to differentially glutamylated tubulin in blot-overlay assays[115,116,138,139], however more direct evidence will be required to confirm those conclusions. Ultimately, the discovery that different polyglutamylases can specifically determine the length and distribution (α- vs. β-tubulin) of glutamate chains[140] shows that the concept that many microtubule interactors are coordinated by different degrees and patterns of polyglutamylation is a realistic scenario in cells. Expressed in a cell- and tissue-specific manner, the large variety of modifying and demodifying enzymes could cooperate to generate defined glutamylation patterns (Box 1) to control intracellular distribution of microtubule-interacting proteins and organelles.

2.3) Regulatory mechanisms of tubulin PTMs in cells

The well-characterised roles of tubulin detyrosination in controlling CAP-Gly protein-microtubule interactions[129,130] and kinesin-13-mediated microtubule depolymerisation[110] are almost binary switches between two different functional states of microtubules. However, PTMs can also have more subtle effects on the interactions between MAPs and microtubules, and are consequently much harder to measure. Many observations were first made in cells, and were not always confirmed by *in-vitro* reconstitution assays with purified components.

In neurons, excessive detyrosination of tubulin abolished the preference of kinesin-1 motors to move into axons, suggesting that differential detyrosination between axons and dendrites could guide kinesin-1 into axons[141]. A preference of kinesin-1 to detyrosinated microtubules in cells has also been reported in non-differentiated cells[142]. Lysosomes accumulate on detyrosinated stretches of microtubules in a kinesin-1-dependent manner, and as a result their fusion with autophagosomes preferentially takes place at those microtubule sections[143]. These experiments suggested a preference of kinesin-1 motors for detyrosinated microtubule tracks, however *in vitro* experiments did not confirm this notion[125].

Changes in acetylation also induced alterations of cargo transport in cultured cells[144], particularly in neurons[145-149]. While the evidence for transport regulation in most of these studies is compelling, it is still an open question if acetylation alone leads to this effect. Indeed, neither mice lacking the tubulin deacetylase HDAC6[150], nor the acetyl-transferase aTAT1[151,152], show obvious defects in neuronal functions, which would be expected when neuronal transport is perturbed. Moreover, *in-vitro* assays with purified components have shown that the motility of kinesin-1 is not affected by the acetylation status of the tubulin tracks[153,154], which makes it difficult to directly link the effects observed in cells with the molecular functions of this tubulin PTM.

Discrepancies between cell-based and *in-vitro* experiments might be explained by other factors that influence transport processes in cells, for instance a combined effect of multiple PTMs on microtubule tracks[155]. Indeed, a recent *in-vitro* study comparing microtubules assembled from brain (many PTMs) and Hela (no PTMs) tubulin found a clear difference in the motility of the kinesin-3 KIF1A. So far it is not clear if this is caused by a single PTM or isotype, or the combination of them[156]. Moreover, motor proteins are not alone on transported vesicles and organelles[157-159], and the additional adapter or helper proteins[160] could, in combination with the motor proteins, sense the PTM status of the microtubules. Another possibility is that MAPs that bind the microtubule tracks affect the use of these tracks by specific motors[161], and that the preferential binding of certain MAPs is regulated by tubulin PTMs. It thus appears that while some of the published data are contradictory and confusing, they in fact open a large window of novel options of how the interplay between the tubulin code and a hypothetical MAP code[162] could control microtubule-based functions, which is an exciting field to be explored in the near future.

Cellular and physiological roles

Microtubules adapt to an amazing variety of structures and behaviours in different cell types of multicellular organisms, and even within single cells. Tubulin isotypes and PTMs contribute to the assembly of those microtubule structures by modulating their intrinsic properties, as well as their interactions with a multitude of interacting proteins. On the organism scale, the tubulin code can help microtubules adapt to changing physiological requirements in long-lived cells, ensuring homeostasis. Indeed, a growing number of studies shows that perturbations of the tubulin isotypes and PTMs can have devastating consequences at the organism level.

1) The tubulin code in cilia and flagella

Eukaryotic cilia and flagella are based on an evolutionarily conserved microtubule structure, the axoneme, which consists of nine circularly arranged doublet microtubules, plus two central singlet microtubules for motile cilia and flagella[163]. In motile cilia, the microtubule doublets are interconnected with ciliary dynein motors, thus forming the machinery to generate the characteristic ciliary beating. Motile cilia and flagella are important for cell movement, for example for spermatozoids or ciliated microorganisms such as *Tetrahymena* or *Paramecium*[164], or for the generation of liquid flow, as the multiciliated ependymal cells in the brain ventricles, or in the trachea of the respiratory system[165]. Many PTMs of tubulin are strongly enriched on axonemal microtubules, and even appear to be evolutionarily linked to this organelle (Box 2).

1.1) Tubulin PTMs play key roles in cilia and flagella

Whenever glutamylation is perturbed in different cellular or organism models, motile cilia and flagella are among the most obvious structures showing functional aberrations. Deletion of polyglutamylating enzymes directly affected ciliary beating in the unicellular organisms *Chlamydomonas reinhardtii*[166] and *Tetrahymena thermophila*[167], or in the multiciliated ependymal cells in mice[168]. On the ultrastructural levels, glutamylation is predominantly found on the B-tubules[169,170], which are the interaction sites of the axonemal dynein heads that generate the ciliary beating. It was thus intuitive to assume that polyglutamylation levels of axonemal microtubules directly control dynein activity, and thus the beating of the cilia, which indeed is the case[166,167] (Fig. 4a).

In mice, many of the enzymes involved in glutamylation appear to be important for sperm development and function, as a recurrent phenotype of knockout models is male infertility. The morphological defects range from impaired flagellar motility to erroneous axoneme assembly, which could be related to dysfunctions of either centrioles serving as basal bodies for axoneme assembly, or the axonemes themselves[86,171-173]. Even early steps of spermatogenesis can be perturbed. In mice lacking the deglutamylase CCP5, the sperm manchette, a transient microtubule structure essential for the formation of sperm heads, is dysfunctional. Spermatozoids fail to evacuate their cytoplasm, show supernumerary basal bodies, and are unable to assemble functional flagella[174]. Perturbed polyglutamylation in mice

also induces defects in other motile cilia such as airway cilia[86,175], which could lead to respiratory disorders as pathogens cannot be efficiently cleaned out of the trachea.

Tubulin glycylation was considered a PTM highly specific to axonemes of motile cilia and flagella[176] until the recent demonstration of its presence in some primary cilia[177]. Depletion of glycylation led to loss of motile cilia from ependymal cells in mice[168], and to a significant shortening of primary cilia in cultured cells[177]. Photoreceptors of the mammalian retina contain the highly specialised connecting cilia, which progressively shortened in the absence of glycylation. The late-onset retina degeneration observed in mice lacking the glycylase TTLL3[178] is likely related to a suboptimal cargo transport through the connecting cilium, a process that is highly solicitated in photoreceptors[179] (Fig. 4a).

Intriguingly, loss of glycylation in murine photoreceptor cells is accompanied by an increase of glutamylation[178], indicating that, as shown earlier in *Tetrahymena thermophila*[180,181], both PTMs compete for the same modification sites on tubulin, and are therefore functionally interconnected. Indeed, patients with mutations in the deglutamylase CCP5 also develop retina degeneration[182-185]. Loss of CCP5 is likely to lead to an accumulation of polyglutamylation, similar what has been demonstrated for mice lacking the deglutamylase CCP1[178,186]. The concept emerging from these observations is that mutations in a range of different tubulin-modifying enzymes can not only functionally, but biochemically lead to similar defects, and thus, be linked to similar diseases. Along these lines, mutations in the glutamylase TTLL5 have also lead to retina degeneration in humans[187,188], however it appears that in this case it is not the perturbation of tubulin glutamylation, but of another substrate (Box S1), which causes the loss of photoreceptors in the corresponding mouse model[189].

Other tubulin PTMs such as detyrosination, Δ2-tubulin[190] and acetylation[176] are also enriched on axonemes, but little is so far known on their functional roles. Mice lacking aTAT1 are subfertile[152], suggesting that this PTM is needed for proper axoneme function, perhaps due to its capacity of rendering microtubules more resistant to mechanical fatigue[93,94].

Finally, primary cilia are also modified with a range of tubulin PTMs. Those non-specialised types of cilia are present on many cells in the vertebrate organism, and serve as sensory organelles and signalling hubs. Defective primary cilia can lead to a variety of diseases commonly referred to as ciliopathies[191]. Tubulin PTMs might play similar roles in primary cilia as in their motile counterparts, however much less is so far known about direct regulation of their functions by tubulin PTMs. First examples show that acetylation[69],

glutamylation[192,193] and glycylation[177] are required for correct assembly and function of primary cilia (Fig. 4a).

1.2) Cilia-specific roles of tubulin isotypes

Early studies demonstrated the presence of distinct tubulin isotypes in cilia of different species[194], however it was at the time not clear if this heterogeneity was related to tubulin isotypes or PTMs. The development of antibodies specific to mammalian isotypes[99,101] revealed β4-tubulin (TUBB4) as a major β-tubulin isotype in two functionally different types of cilia: the connecting cilia of photoreceptor cells, as well as in the motile airway cilia in trachea[195]. It is therefore likely that β4-tubulin possesses properties that are essential for the formation of the axoneme.

The idea that specific tubulin isotypes convey unique properties to axonemal microtubules was recently experimentally supported by the observation that purified axonemal tubulin from *Chlamydomonas* displayed a distinct assembly/disassembly behaviour as compared to mammalian brain tubulin[170]. Strikingly, *Chlamydomonas* β-tubulin shares specific sequence motifs with mammalian TUBB4A, which are absent in other mammalian tubulin isotypes. This strongly suggests that the primary peptide sequence of ciliary β-tubulin isotypes determines some of the characteristic features of axonemal microtubules, such as particularly low growth and shrinkage rates[170]. Work in *Drosophila* further demonstrated that a specific amino acid residue encoded in all axonemal β-tubulins, glycine 56, is essential for the attachment of the outer dynein arms, and thus, for the motility of the axonemes[196] (Fig. 4a).

In *Caenorhabditis elegans*, an organism without motile cilia, cells with primary cilia express characteristic tubulin genes[81]. Deleting one of them, the α-tubulin gene TBA-6, led to a loss of the microtubule doublet structure in the sensory cilia, which instead contained 18 singlet microtubules, and displayed defects in intra-flagellar transport and vesicle-sorting[82]. A unique feature of TBA-6 is its C-terminal tail, which, in contrast to all other α-tubulin isotypes, is longer, contains positively charged amino acid residues, and, most strikingly, no glutamate residues that could serve as sites for posttranslational glutamylation. In the light of *in vitro* reconstitution experiments demonstrating that the C-terminal tails of brain tubulin hinder the formation of B-tubules[197], it is appealing to hypothesise that the particular tail of TBA-6 permits doublet formation due to its different biophysical features, and perhaps because it cannot be polyglutamylated.

Thus, while still little is known about the underlying mechanisms, solid evidence for essential roles of particular tubulin isotypes in axonemal structure and function exist. So far, these data stem mostly from two model organisms in which the primary sequences of tubulin isotypes are more divergent than in mammals. While this makes it difficult to draw direct parallels to other organisms, these examples show that single amino-acid substitutions in the highly structured tubulin body, as well as variations in the peptide sequence of the C-terminal tails can be essential to build and maintain axonemes. Most excitingly, these sequence variations can influence the posttranslational modification of a given isotype, thus directly linking the two core elements of the tubulin code in one single biological function.

2) The tubulin code in neurons

2.1) A differential distribution of tubulin PTMs in neurons?

In contrast to most other cell types of multicellular organisms, neurons are particular as their entire microtubule cytoskeleton is highly posttranslationally modified. Neuronal α-tubulin is acetylated at K40[198,199], detyrosinated[199,200], and further converted into Δ2-tubulin[66]. Moreover, neuronal microtubules are abundantly polyglutamylated on α-[59] and β-tubulin[60,61]. All these PTMs accumulate as neurons differentiate and mature[199,201,202], underpinning the concept of tubulin PTMs as neuronal differentiation markers. Biochemical analyses of purified brain tubulin so far provided approximate measures of the levels of individual PTMs[59,65,93,203-205].

A first careful mapping of tubulin tyrosination and acetylation by immunofluorescence and immune-electron microscopy revealed that acetylation is present all-along the axon, but much less so at the growing end of the axon, where reversely, tyrosinated (i.e., non-detyrosinated) tubulin is predominant[206]. This fits the expectation of axonal microtubules being long-lived, and thus more acetylated and detyrosinated, while the growing end of the axon, including the growth cone, contains freshly assembled, non-modified microtubules. An elegant approach used fraying of microtubules of cultured neurons to show that single, continuous microtubules change their PTM status towards distal end of the axon[207], which might have important implications for their functions in growth cones (Fig. 4b).

It took two decades and the advent of superresolution microscopy until another study described the presence of two different microtubule populations, one acetylated and barely tyrosinated (i.e. detyrosinated or Δ2-tubulin) in the centre of neuronal dendrites, and the other

tyrosinated and barely acetylated at the dendrite periphery[208]. Amazingly, these two different microtubule species show opposite polarity, thus supporting two different types of transport: retrograde, kinesin-1 driven transport on the central, highly modified microtubules, and anterograde transport by kinesin-3 on the peripheral, less modified microtubules (Fig. 4b). At this point it is not clear if the different PTMs are merely markers of different microtubule subtypes, or if they directly control the motors that walk on them. In axons, this polarity and PTM segregation of microtubules does not exist, and consequently all kinesin motors walk towards the axon distal ends.

2.2) Functions of tubulin PTMs in neurons

Different PTMs in neurons might play district roles in neuronal development and homeostasis. The balance between detyrosination and tyrosination appears to be important in early neuronal development, as massive accumulation of detyrosination in TTL-knockout mice leads to perinatal death due to neurodevelopmental defects[209]. Cultured hippocampal neurons from these mice lack tyrosinated microtubules in axonal growth cones and show massive abnormalities in neuronal pathfinding[210]. Perturbations of the tubulin detyrosination cycle can also lead to human disease. While mutations of TTL might be rare to find in adult patients due to the expected massive developmental defects induced by the nearly-complete absence of tyrosinated tubulin[209], they are more likely to be found in genes encoding enzymes of the detyrosinase family[211,212]. Indeed, mutations in SVBP were recently linked to microcephaly and intellectual disability[213,214], thus confirming the importance of this PTM in neurodevelopment.

Phosphorylation of β-tubulin S172 by the DYRK kinase controls microtubule dynamics in differentiating neurons in *Drosophila melanogaster*[50]. Alterations in this PTM lead to defects in dendrite branching and excitability of these neurons, which results in neurological defects similar to defects found in Down syndrome and autism spectrum disorders.

Acetylation is a prominent tubulin PTM in neurons, however its absence in aTAT-knockout mice induced surprisingly mild neurological defects[151], the most remarkable being the loss of touch sensation[215]. This mirrors touch-sensation defects in acetylation-defective *Drosophila*[216], as well as in *C. elegans*, where aTAT1 (Mec-17)-mediated acetylation[217] is important for the formation of the characteristic 15-protofilament microtubules[85] that are essential for touch sensation[218]. Mutation of K40 in the major neuronal α-tubulin isotype in

560 *Drosophila* (αTub84B) further highlighted the importance of acetylation in dendritic
561 refinement of sensory neurons[219]. A number of reports have linked tubulin acetylation to
562 neurodegeneration, mostly via the deacetylase HDAC6[149,220-224]. The interpretation of these
563 experiments is however not straight-forward, as HDAC6 deacetylates not only α-tubulin
564 K40[225], but also the mitochondria transport adaptor protein Miro1[226] and the actin regulator
565 cortactin[227] (Box S1).

566 Polyglutamylation, on the other hand, has been demonstrated to directly and cell-
567 autonomously cause neurodegeneration using genetic approaches in mice. The well-
568 established mouse model for Purkinje cell degeneration, the *pcd* mouse[173], carries a mutation
569 in the gene Nna1[228], later shown to be the deglutamylase CCP1 (also known as
570 Nna1/AGTPBP1)[229]. CCP1 deficiency causes accumulation of hyperglutamylated tubulin in
571 the cerebellum, the main brain region undergoing degeneration in *pcd* mice[229]. The rapid
572 degeneration of Purkinje cells can be avoided for the entire lifetime if TTLL1, the major
573 α-tubulin polyglutamylase in neurons[230], is deleted selectively in Purkinje cells of *pcd* mice.
574 This demonstrates the causality of TTLL1-catalysed hyperglutamylation for the degeneration
575 of these neurons[231] (Fig. 4b).

576 But why then do not all brain regions in *pcd* mice degenerate sooner or later? Another
577 member of the CCP family[232,233], CCP6, was found to be expressed specifically in brain
578 regions that do not degenerate in *pcd* mice[229]. Indeed, deletion of CCP6 additional to CCP1
579 induced a massive hyperglutamylation in the entire mouse brain, resulting in the degeneration
580 of neurons that were unaffected in *pcd* mice[231].

581 The discovery of a novel infant-onset human condition linked to inactivating mutations in
582 CCP1 with remarkable similarity to the *pcd* mouse model[234-236] established deregulated
583 polyglutamylation as a novel cause of human neurodegeneration. It is conceivable that more
584 subtle alterations of this PTM could be linked, or even causative, for other, late-onset human
585 pathologies.

586 Exploring the molecular mechanisms by which abnormal polyglutamylation leads to
587 neurodegeneration provide a handle to decipher the physiological role of this PTM in the
588 nervous system. So far, defects in axonal transport have been reported in different types of
589 neurons[231,237], while a causative role of the microtubule-severing enzyme spastin was
590 excluded[231]. However, it is likely that other microtubule-based processes, such as the binding
591 and distribution of neuronal MAPs, could also be affected if polyglutamylation is perturbed.

2.3) Tubulin isotypes in the nervous system

Two β-tubulin isotypes, β2- (TUBB2) and β3- (TUBB3) tubulin are strongly enriched in neuronal microtubules[238]. While β2-tubulin is also expressed in other cell types, β3-tubulin is almost exclusively found in neurons[239,240]. In the light of a recent study showing that β3-tubulin-containing microtubules depolymerise faster[77], previous observations of differential expression of this TUBB3 in different types of neurons[241], or its upregulation during regeneration of sensory nerves[242] now suggest that TUBB3 expression directly regulates microtubule dynamics in a cell-type and function-dependent context. This concept was confirmed in a TUBB3-knockout mouse, which displays defects in axonal regeneration[243] (Fig. 4b). Those defects are reminiscent of phenotypes found in aTAT1-knockout mice[215], indicating once again that different elements of the tubulin code concur in optimising microtubule functions. Indeed, an increase of tubulin acetylation and polyglutamylation was detected in TUBB3-knockout mice[243], suggesting that neurons attempted to compensate for the loss of β3-tubulin by adjusting microtubule dynamics, or interactions with MAPs and molecular motors.

3) Microtubule functions in muscles

The observation that enzymatic activity of TTL in muscles is about two times higher as compared to the brain[244], and reaches a temporal maximum during myofiber development in skeletal muscles[245] indicated very early that the detyrosination/tyrosination cycle could play a particularly important role for muscle microtubules. The first functional insight, however, came only recently from the observation that mechanotransduction in skeletal and heart muscle is affected by the detyrosination status of muscle microtubules[97] (Fig. 4c). High-speed imaging revealed that microtubules in the heart muscle buckle with every beat – an impressive example of the mechanical resistance of microtubules. The buckling of microtubule provides a viscous resistance to the actin-myosin force, thus controlling the viscoelasticity of the muscle.

The viscoelasticity of muscles is directly dependent on tubulin detyrosination, which controls the anchorage of microtubules to the desmin structures of muscle fibres. Absence of

623 detyrosination leads to disruption of microtubule-desmin contacts, and consequently perturbs
624 cardiac muscle function[98] (Fig. 4c). Abnormally high detyrosination levels of microtubules
625 lead to overly stiff cardiac muscles, and related to human heart failure[246]. Strikingly,
626 myocardiocytes from heart-failure patients recovered elasticity when treated with the drug
627 parthenolide to reduce tubulin detyrosination[247], or with the microtubule-destabilizing drug
628 colchicine[246]. Genetically, increased detyrosination levels could originate from two
629 mechanisms, upregulation of the detyrosinating enzymes Vash1 or Vash2, or overexpression
630 of α4A-tubulin (TUBA4A), which lacks C-terminal tyrosine and mimics detyrosination.
631 Indeed, TUBA4A was found to be overexpressed in failing hearts[246].

632 The discovery of the role of microtubules and their posttranslational detyrosination in
633 controlling muscle functions provides a striking example for an unexpected role of
634 microtubules and the tubulin code. Other tubulin PTMs and isotypes[248,249], yet to be explored,
635 might also play important roles in the regulation of muscle functions.

636

637 4) The tubulin code in cell division

638 *4.1) Regulation of the mitotic and meiotic spindles*

639 The division of eukaryotic cells essentially depends on microtubules as components of mitotic
640 and meiotic spindles. Spindles are amazingly complex[250], and yet highly dynamic assemblies
641 of microtubules[251] that ensure the correct separation of the genetic material into two daughter
642 cells. A huge amount of work has so far gone into explaining how the self-assembly of
643 different molecular compounds can give rise to such complex, highly controlled microtubule
644 structure[252,253], but the potential impact of the tubulin code has so far rarely been explored.

645 A first direct evidence for a role of tubulin isotypes in controlling spindle behaviour was
646 found in *C. elegans*, where two α- and two β-tubulins are expressed in the embryo. Despite
647 the high similarity of those two α- and two β-tubulin isotypes, each isotype confers distinct
648 dynamic properties to mitotic spindle microtubules, and thus each of them was essential for
649 proper spindle function[254].

650 Several tubulin PTMs have been found on spindle microtubules. In mammalian cells,
651 detyrosination is enriched on inner spindle microtubules, but virtually absent from astral
652 microtubules[255]. A similar distribution has been shown for polyglutamylation[111], which

653 together with an increased polyglutamylase activity in mitosis[256] suggested a role of this PTM
654 in cell division (Fig. 4d).

655 A recent study has now uncovered a mechanistic role for detyrosination in cell division. In
656 mitosis, unaligned chromosomes are transported to the metaphase plate by the kinetochore-
657 associated kinesin-7 motor CENP-E[257]. This mechanism was perturbed by a complete
658 inhibition of detyrosination in dividing cells, suggesting that CENP-E can 'read' the
659 detyrosination of spindle microtubules. Indeed, *in vitro* reconstitution experiments revealed a
660 preference of CENP-E for detyrosinated microtubules[135] (Fig. 4d).

661 Finally, phosphorylation of serine 172 of β-tubulin by the cyclin-dependent kinase Cdk1
662 prevents the incorporation of the tubulin dimer into the microtubule lattice, which might
663 control microtubule dynamics in mitosis[48]. Indeed, mimicking S172 phosphorylation in
664 budding yeast perturbed cell division, thus confirming the importance of this PTM for correct
665 spindle function[258].

666

667 *4.2) Generating asymmetries in dividing cells*

668 In mammals, TTL is the sole enzyme to catalyse re-tyrosination of tubulin, thus its absence
669 leads to a massive accumulation of detyrosinated tubulin, and TTL-knockout mice die
670 perinatally[209]. Among the dysfunctions observed in these mice, one striking phenotype was a
671 severe disorganisation of the brain. This defect could be explained by the failure of spindle
672 alignment in TTL-knockout cells[129]: Spindle position depends on the interactions of astral
673 microtubules with the cell cortex[259], which are mediated by CAP-Gly proteins[260]. The +TIP
674 localisation of CAP-Gly proteins depends on tyrosination[130], which is normally enriched on
675 astral microtubules[255]. Therefore, the nearly-complete absence of tyrosinated tubulin in TTL-
676 knockout cells leads to dysfunctional +TIP complexes, and consequently to impaired spindle
677 orientation, which in turn determines the faith of daughter cells after neuronal progenitor
678 division[261,262].

679 During meiosis in mouse oocytes, detyrosination is asymmetrically distributed between the
680 two meiotic half-spindles, and thus involved in non-Mendelian segregation of chromosomes,
681 known as meiotic drive[263]. Strikingly, the half-spindle that migrates toward the oocyte cortex
682 progressively accumulates tyrosination, which implies an active role of TTL rather than of a
683 detyrosinating enzyme in the generation of this asymmetry[264].

684

4.3) Controlling centrosome functions

Centrosomes are microtubule organising centres, which in many different cell types serve as facilitators of mitotic spindle bipolarity, and converted into basal bodies become the organising centres of cilia and flagella[265]. Polyglutamylation is particularly enriched on the centrioles[266]; complex microtubule structures at the core of the centrosome[267] (Fig. 4d). Different patterns of polyglutamylation have recently been mapped to distinct domains of centrioles, suggesting that the modification could serve as a guidance signal for centriole-associated proteins that localise to highly defined positions within these complex structures[268,269].

So far, no experiments selectively abolishing polyglutamylation of centrioles were reported. Injection of anti-glutamylation antibodies into dividing cells[270] led to centriole disassembly and cell-cycle defects[266,271], thus providing a first glimpse onto a potential importance of polyglutamylation in centriole maintenance and functions. In the light of the central role centrosomes play in cell division[5], this could indicate that polyglutamylation, by tightly controlling centriole assembly, and perhaps also centriole maturation and duplication, could control cell cycle timing and fidelity. Indeed, spermatozoids of *CCP5*-knockout mice show supernumerary centrioles, suggestive of a centriole duplication defect due to increased polyglutamylation[174]. Considering the large number of implications of centrosome duplication defects in human diseases[272], it is possible that aberrant centriole polyglutamylation could be one of the causes of such disorders.

4.4) Controlling cell proliferation via the primary cilium

As mentioned above, many tubulin PTMs are enriched in ciliary axonemes and basal bodies, and might thus affect cell proliferation by controlling the functions of primary cilia. Primary cilia are signalling centres that control cell division and proliferation, and their dysfunction might lead to cancer[273,274]. The first direct link between a tubulin PTM, cell proliferation and cancer was found for the glycylase TTLL3. Mice lacking *TTLL3* show a reduced number of primary cilia in the colon epithelium, which hyper-proliferates and shows faster tumour growth[275]. To which extent perturbed primary cilia are the unique reason for this phenotype remains to be established. A remarkable observation in this study was that hyperproliferation of the colon tissue led to no visible morphological changes in non-cancerous colon tissue, and the defect only became apparent after tumour induction. This illustrates how subtle effects of

717 defective tubulin PTMs can be overlooked despite their role in a key physiological process
718 and tumorigenesis.

719

720 **Conclusions and perspectives**

721 Here we have reviewed current advances in the functional understanding of the tubulin code.
722 So far, exciting new links between the tubulin code and a range of cellular functions have
723 been discovered, however many questions still remain open. Considering that the elements of
724 the tubulin code, i.e. tubulin PTMs and multiple tubulin genes, were discovered in the
725 1970ies, it is surprising that so little advance had been made. Why is this so?

726 In 1976, Fulton and Simpson formulated the first 'multi-tubulin hypothesis': *"The surfaces of*
727 *a tubulin molecule must interact with many other tubulin surfaces … as well as with*
728 *associated molecules …. Many of these structural interactions appear to have been conserved*
729 *throughout evolution, and this probably imposes severe restraints on variations in the amino*
730 *acid sequence. … On the other hand, subtle changes may have occurred that do not alter the*
731 *basic topology of tubulin but do provide specialized associative properties or binding sites for*
732 *particular functions."*[71].

733 The discovery of tubulin isotypes that are often highly similar, or tubulin PTMs that label
734 specific microtubule species in cells without being 'essential' in the classical cell-biological
735 sense has beautifully confirmed this early hypothesis, but also somewhat dampened the
736 interest in the tubulin code. At the end of the 1980ies, it became clear that most tubulin
737 isotypes are interchangeable without obvious functional consequences in cells[276], which led to
738 the questioning of their functional importance (vs. evolutionary redundancy)[277,278]. At the
739 same time, research on tubulin PTMs was impeded by the absence of appropriate means of
740 manipulation, which was overcome mostly in the 21st century by the discovery of a number of
741 modifying enzymes, and some were discovered only recently[211,212]. Surprisingly however,
742 many of the tubulin-modifying enzymes showed only mild phenotypic defects when deleted,
743 even though in same cases the levels of tubulin PTMs changed significantly when only one
744 modifying enzyme was mutated. In most cases, only some specific cell types or organs show
745 signs of dysfunction, or degeneration, and only in rare cases, such as TTL[209], deletion of a
746 single enzyme has severe consequences for development and survival.

747 It thus appears that tubulin isotypes and PTMs might have in many cases rather subtle effects
748 on gross microtubule functions, but could be important to control complex, long-lasting

cellular functions, in some cases by regulating only selected microtubule populations in a cell. While this confirms the initial predictions of the multi-tubulin hypothesis[71], it made and makes the functional analyses of the tubulin code challenging: Detecting subtle alterations of microtubule functions requires more sensitive methods to measure microtubule behaviour in cells or in purified systems, or long-term observations of organism, including detailed histological and behavioural analyses. However, it also bears a great opportunity for a conceptual leap in cell biology. Evolution has shown that both, tubulin isotypes and PTMs would be eradicated if they were not needed for cell survival (Box 2), which strongly suggests that tubulin isotypes and PTMs are bound to be functionally important in organisms that have retained them.

The fact that so far both elements of the tubulin code have almost systematically slipped through the meshes of various analytical approaches indicates the urgent need of more adapted methodology, and, more importantly, the need to broaden our concept of biological functions in space and time. Indeed, cellular processes can last over a lifetime, and cells such as neurons span lengths of over 1 m in our body. Regulatory processes that can easily be neglected in the cell culture dish might have a key role in controlling such complex systems over a longer time. The role of the tubulin code in these processes has recently been proven by the discovery that deregulation of tubulin PTMs can lead to the degeneration of neurons[231,234] or photoreceptors[178], and there is a whole spectrum of neurological disorders linked to mutations of tubulin isotypes (Box S2). Exploring the role of the tubulin code is a great challenge for the coming years, and will certainly contribute to uncover novel, so far unexplored principles of the regulation of cellular functions.

Display items

Box 1: Complex PTMs on the C-terminal tubulin tails

The complexity of tubulin PTMs is particularly high on the C-terminal tails of these proteins, where detyrosination, polyglutamylation and polyglycylation take place (Fig. 1). The complexity that arises from these PTMs, and their interplay with tubulin isotypes, will be briefly discussed in this box.

The majority of α-tubulin genes in most organisms encode a C-terminal tyrosine or phenylalanine, which can be enzymatically removed[64] and re-added without the requirement of ribosomes[58]. These discoveries were surprising in two ways: first, the initial PTM is actually the removal, and not the addition of a functional group, and second, it was the first time an enzymatic incorporation of an amino acid into a peptide chain without mRNA and ribosome was observed. Thus, while the tubulin PTM became known as tubulin tyrosination, it is more appropriate to consider the detyrosination as the actual modification – with one exception: in cells expressing tubulin isotypes missing the C-terminal tyrosine, such as the mammalian α-tubulin TubA4A[279].

The enzymatic removal of C-terminal tyrosine can be followed by further amino acid cleavages, which on mammalian α-tubulin give rise to Δ2- and Δ3-tubulins (lacking the first and second glutamates before the C-terminal tyrosine; Fig. 1)[66,67]. It thus appears that tubulin C-terminal tails might be subjected to extensive amino acid editing, most likely beyond what is currently known. A first glimpse of this possibility was found with the discovery that an antibody specific to Δ3-tubulin also labelled β-tubulin, which implied that four C-terminal amino acids of β-tubulin have been removed to generate the specific epitope for this antibody[67]. Structural data of the enzyme adding tyrosine to tubulin, the tubulin-tyrosine ligase (TTL) show that the mode of binding between enzyme and the tubulin is so specific that even Δ2-tubulin cannot be retyrosinated[122].

Polyglutamylation and polyglycylation were initially discovered on tubulin. Both PTMs consist of the generation of secondary peptide chains as branches from the main chain, using the (γ) carboxy-group of a glutamate as modification site (Fig. 1). As C-terminal tails of tubulin are rich in glutamates, there are many potential sites on which these two PTMs could be added. Theoretically, this could give rise to a large variety of combinatory signals on both, α- and β-tubulin, however so far, only little insight has been gained in the complexity of these PTMs in living cells. Both PTMs were discovered by mass spectrometry approaches that were

804 particularly designed to analyse the C-terminal tubulin tails, as otherwise these highly acidic
805 tails are mostly lost in proteomic analyses. Analysing purified brain tubulin – the gold
806 standard in tubulin biochemistry – the main modification sites found were E445 on α1-tubulin
807 (TubA1)[59], E435 on β2-tubulin (TubB2)[61], and E438 on β3-tubulin (TubB3)[60]. Using a
808 similar approach, polyglycylation was discovered on ciliary tubulin isolated from
809 *Paramecium tetraurelia*, and accumulations of up to 34 glycine residues per tubulin molecule
810 were observed[62].

811 The enzymes catalysing the glutamylation and glycylation reactions, both members of the
812 tubulin tyrosine ligase like (TTLL) family (Table 1) show enzymatic preferences for either α-
813 or β-tubulin, or for the generation of short vs. long glutamate or glycine chains[140,280]. To
814 which extent these enzymes also modify specific positions out of the many possible
815 modification sites within the tubulin tails has so far remained an open question. Nevertheless,
816 the existing selectivity of the modifying enzymes already provides an indication that these
817 two PTMs generate highly controlled patterns on cellular microtubules. To do so, TTLL
818 enzymes need to be selectively activated, or localized. Little is so far known about regulatory
819 circuits involved in such control mechanisms, however first insights indicate that such control
820 mechanisms exist: the protein CSAP was shown to directly activate TTLL enzymes[281], and
821 some other proteins were shown to interact with TTLLs thus localising them to specific
822 organelles such as cilia[193] or centrosomes[282].

823

824 **Box 2: An evolutionary link between tubulin PTMs and cilia and flagella**

825 Tubulin PTMs are strongly enriched on axonemal microtubules, and most of them have
826 essential ciliary functions[176] (Fig. 4a). Strikingly, most of the known tubulin PTMs appear to
827 be evolutionarily linked to cilia and flagella. TTLL enzymes, which catalyse tubulin
828 glutamylation[140] and glycylation[181,280], for instance, can be easily identified in different
829 organisms based on their highly conserved TTL domain[140,181,230,280]. Homologs of *TTLL* genes
830 are absent from eukaryotes without cilia, such as the yeasts *Saccharomyces cerevisiae* or
831 *Schizosaccharomyces pombe*, as well as many plants. However, whenever an organism has
832 ciliated cells, *TTLL* genes can be identified in its genome, given that a well-annotated genome
833 sequence is available. For example, *Batrachochytrium dendrobatidis* is a fungus that can
834 grow cilia, and consequently assembles basal bodies and axonemes[283]. Performing a BLAST
835 search with murine TTLL1, a polyglutamylase, reveals the presence of highly homologous

proteins. While so far, no systematic evolutionary study has been published, the presence of TTLL homologs could be considered a strong indication for the presence of glutamylation and/or glycylation, and could be used as a starting point for a subsequent functional characterisation.

Figure 1. The elements of the tubulin code.

Microtubules dynamically assemble from dimers of α- and β-tubulins. Tubulins are highly structured, forming the 'tubulin bodies', while their C-terminal amino acids form unstructured tails that protrude from the microtubule surface. The tubulin code stands for the concept that different tubulin gene products together with a variety of posttranslational modifications (PTMs) modulate the composition of individual microtubules. Tubulin isotypes (depicted in different colours: dark grey and brown for α-tubulins, light grey and pink for β-tubulins) are encoded by different tubulin genes, and can intermingle during microtubule polymerisation. Tubulin PTMs are catalysed by a range of enzymes (Table 1), and are located either at the globular, highly structured tubulin bodies (e.g. acetylation, phosphorylation), or at the unstructured C-terminal tails of tubulin (e.g. detyrosination, Δ2- and Δ3-tubulin, (poly)glutamylation, (poly)glycylation). Tubulin PTMs can generate binary switches (on/off signals) by adding/removing single functional residues (acetylation, phosphorylation, detyrosination, Δ2- and Δ3-tubulin), or can gradually modulate the strength of their signals by adding different numbers of residues (polyamination, (poly)glutamylation, (poly)glycylation).

Figure 2. The impact of the tubulin code on microtubule properties.

a. Tubulin isotypes can determine protofilament numbers. In *C. elegans*, two isotypes specific to touch-receptor neurons (mec-7 and mec-12) determine the 15-protofilament microtubule architecture in these cells [78-80]. **b.** Tubulin isotypes can be essential for the formation of a geometrically defined microtubule array, the marginal band. This band assembles from microtubules along the outer rim of blood platelets, and is essential for the shape and correct function of the platelets. Two tubulin isotypes, α4A- (TubA4A)[96] and β1-tubulin (TubB1)[32] are essential for the correct assembly of the marginal band, and lack of either of these isotypes leads to dysfunctions of blood platelets. **c.** Tubulin PTMs can change mechanical properties of microtubules. Acetylation of α-tubulin at K40 structures the loop of α-tubulin in a way that weakens the interactions between neighbouring protofilaments (red arrowheads)[95]. At the same time, acetylation reduces the flexural rigidity of microtubules, making them more

resistant to mechanical bending, thus avoiding microtubule breakage and disassembly[93,94]. (upper panels are adapted from ref.[95]) **d.** Tubulin isotypes can control microtubule dynamics. Microtubules containing β3-tubulin are more dynamic than the ones assembled from β2B-tubulin[77,104]. Phosphorylation of β-tubulin S172 by Cdk1[48] or DYRK[50], or acetylation of K252 by San[106] impede the incorporation of tubulin dimers into microtubules. Tubulin polyamination, in contrast, renders microtubules particularly resistant to depolymerisation[56].

Figure 3. The impact of the tubulin code on MAP-microtubule interactions.

a. Both, tyrosinated and detyrosinated microtubules can attract specific subsets of MAPs. MCAK[110], CLIP-170[129,130] and dynein in complex with BicD2 [137] are attracted to tyrosinated microtubules, while the kinesin motors CENP-E[135] and kinesin-2[125] preferentially associate with detyrosinated microtubules. **b.** Different levels of tubulin polyglutamylation can fine-tune functions of microtubule-interacting proteins. The activity of the microtubule-severing enzyme spastin is upregulated by initial polyglutamylation of this substrate microtubules[111,112], however, further accumulation of this PTM inhibit spastin activity[112]. Molecular motors, such as kinesin-1 and kinesin-2[125], or flagellar dynein[166] can be also differentially regulated by varying degrees of polyglutamylation. While kinesin-2 is induced by moderate levels of polyglutamylation, kinesin-1 requires higher levels of this PTM to stimulate its performance[125].

Figure 4. Cellular and physiological role of the tubulin code.

Functions for specific tubulin PTMs and isotypes are summarised. Note that only the known functions are highlighted, which does not exclude that other PTMs or isotypes are present, and/or have additional functions on those microtubules. Overview representations of cells show all known tubulin PTMs using colour coding, while in zoom representations only specific PTMs and isotypes are shown for clarity.

a. Cilia and flagella. Axonemal microtubules are highly modified with a range of tubulin PTMs. Glycylation has so far only been found on axonemes. Both, polyglutamylation and glycylation accumulate toward the proximal part of the cilia, while acetylation appears to be equally distributed all-along axonemes[168,177,284]. Basal bodies are highly polyglutamylated. In axonemes polyglutamylation specifically decorates the B-tubules of the microtubule doublets[169,170] and controls dynein activity and ciliary beating[166,167]. In *D. melanogaster*, the

β2-tubulin isotype is essential for the binding of outer dynein arms[196]. In all types of cilia, glycylation controls cilia length and stability[168,177,178], and its absence is linked to photoreceptor degeneration[178], or cell-cycle defects due to loss of primary cilia[275]. **b. Neurons.** Acetylation and detyrosination decorate distinct microtubules of opposite polarities in dendrites. These two microtubule subpopulations control transport directionality in dendrites, but it is not known if the PTMs directly control the motor proteins involved[208] (note that polyglutamylation is also present, but not shown). Polyamination stabilises yet unidentified microtubule populations in neurons[56], while the presence of β3-tubulin (TubB3) in neurons enhances microtubule dynamics[77], which is essential for axon regeneration[243]. Polyglutamylation regulates bidirectional axonal transport driven by kinesins and dynein[231], and abnormal accumulation of this PTM leads to neurodegeneration[231,234]. Most neuronal microtubules are highly posttranslational modified, except for the highly dynamic ones in the growth cone[207]. Tyrosinated microtubules are essential for growth cone guidance[210]. **c. Muscles.** Detyrosinated α-tubulin isotype TubA4A and posttranslational detyrosination of microtubules in muscle cells are essential for their buckling, which in turn defines their capacity to bear load and influences the viscoelastic behaviour of muscle cells during contraction[98,285]. Aberrant detyrosination is linked to heart failure[246]. **d. Cell cycle and centrosome.** Tubulin acetylation, polyglutamylation[111] and detyrosination[255] are enriched in central mitotic spindles and on midbody microtubules. Tyrosinated microtubules are essential for spindle orientation[261,262] due to the requirement of this PTM for dynein loading onto astral microtubules[137]. The enrichment of detyrosinated microtubules on central spindle microtubules[255] guides the kinetochore-associated CENP-E motor towards the metaphase plate, thus assuring correct chromosome congression and separation[135]. Centriolar microtubules are highly polyglutamylated[266], with a specific localisation of this PTM at the C-tubules[268]. The high levels of polyglutamylation on centrioles is essential for centrosome integrity throughout mitosis[266,271].

929 **Table 1:** Known tubulin posttranslational modifications (PTMs) and enzymes

Tubulin PTM	Chemistry	Modification sites	Forward enzymes	Reverse enzymes
Acetylation	Enzymatic addition of acetyl-moiety to lysine residue	α-tubulin K40[91]	α-tubulin acetyl-transferase 1 (aTAT1)[69,217]	histone deacetylase 6 (HDAC6)[225]; sirtuin 2 (Sirt2)[286]
		β-tubulin K252[106]	San acetyl transferase[106]	*Not known*
Methylation	Enzymatic addition of methyl-moiety to lysine residue	α-tubulin K40[52]	SET-domain-containing 2 methyltransferase (SETD2)[52]	*Not known*
Detyrosination; retyrosination	Enzymatic removal of C-terminal tyrosine residue from α-tubulin[64,287]; ribosome-independent incorporation of tyrosine[58,288]	α-tubulin C-terminal Y	Detyrosinases are vasohibin proteins[211,212] in complex with the small vasohibin-binding protein (SVBP)[289-293]	Tubulin tyrosine ligase (TTL)[294]
Generation of Δ2-tubulin; Δ3-tubulin	Enzymatic removal of C-terminal glutamates from α-tubulin after detyrosination[66,67]	α-tubulin penultimate C-terminal E	cytosolic carboxypeptidases (CCP)[229,295,296]	*No reverse reaction known to date, tyrosination of Δ2-tubulin with TTL is not possible[66,122]*
[poly]glutamylation	Enzymatic addition of glutamate to γ-carboxy-group of glutamate side chains, followed by elongation of the nascent chain with further glutamates	α- and β-tubulin tubulin C-terminal tails (multiple residues are modified)[59-61]	tubulin tyrosine ligase like (TTLL) protein, multiple members in most organisms (9 glutamylases in mammals)[140,230,297]	cytosolic carboxypeptidases (CCP), multiple members in most organisms (6 deglutamylases in mammals)[229,295,296]
[poly]glycylation	Enzymatic addition of glycine to γ-carboxy-group of glutamate side chains, followed by elongation of the nascent chain with further glycines	α- and β-tubulin tubulin C-terminal tails (multiple residues are modified)[62,284]	tubulin tyrosine ligase like (TTLL) protein, multiple members in most organisms (3 glycylases in mammals)[181,280,298]	*No reverse reaction or enzymes known*
Polyamination	Enzymatic addition of polyamines to the γ-carboxamide group of a glutamine residue side chains	α- and β-tubulin, major modification: α-tubulin Q15[56]	Transglutaminases (TG)[56]	*No reverse reaction or enzymes known*
Phosphorylation	Enzymatic addition of phosphate group to serine/threonine/tyrosine residue	β-tubulin S172[48]	Cyclin-dependent kinase 1[48]	*Not known*
		β-tubulin S172[50]	dual-specificity tyrosine-regulated kinase (DYRK)[50]	*Not known*
		β3-tubulin S444[42]	*Not known*	*Not known*
		α-tubulin Y432 (determined on C-terminal α-tubulin peptide, unsensitive to carboxypeptidase A	Spleen tyrosine kinase (Syk)[46]	*Not known*

		treatment, which excludes Y451)[46]		
		α- and β-tubulin Y residues (not identified)[37,44]	Src kinase[37,44]	*Not known*
Ubiquitinylation	Enzymatic addition of the small protein ubiquitin to lysine residues of tubulin[54,299]	α-tubulin, major modification: site K304[55]	*Not known*	*No reverse reaction or enzymes known*
Sumoylation	Enzymatic addition of the small protein sumo to lysine residues of tubulin[300]	α-tubulin (modification site unknown)[300]	*Not known*	*No reverse reaction or enzymes known*
Palmitoylation	Enzymatic addition of long-chain fatty acid palmitate to tubulin[53,301]	α-tubulin, major modification site: K376[53]	*Not known*	*No reverse reaction or enzymes known*

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Author contributions

Both authors researched data for the article, contributed to discussion of the content, wrote the article and reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Glossary

Axonemes

A structure built from microtubules and associated proteins at the core of all eukaryotic cilia and flagella. In motile cilia, axonemes consist of nine microtubule doublets arranged around a central microtubule pair, accessory proteins and flagellar dynein motors that assure the beating of cilia. Primary cilia lack the motor protein and central-pair microtubules.

A-tubule, B-tubule

Components of the microtubule doublets of axonemes. The A-tubules are generic microtubules made of 13 protofilaments, while B-tubules are partial microtubules made of 10 protofilaments that partially share the wall of the A-tubules (Fig. 4a).

CAP-Gly domain-containing proteins

Cytoskeleton-Associated-Proteins (CAP) containing a glycine (Gly)-rich domain. CAP-Gly proteins contain a well-conserved GKNDG sequence motive that specifically recognizes -EEY/F sequences[131], which targets them to the plus ends of tyrosinated microtubules.

+TIP complex

A group of microtubule-interacting proteins localized to the plus ends of microtubules[302]. For most of these proteins, plus-end localisation is mediated by a group of the end-cinding (EB) protein, such as mammalian EB1, EB2 and EB3, or yeast Bim1p.

Meiotic drive

The preferential, non-Mendelian transmission of a particular allele or locus during meiosis.

Ependymal cells

Glial cells lining the ventricles of the mammalian brain, as well as the central canal of the spinal cord. Ependymal cells have multiple motile cilia, whose coordinated beating

980 determines the direction of flow of cerebrospinal fluid[165]. They are also called
981 ependymocytes.
982
983 Basal body
984 A microtubule-based multiprotein structure at the base of cilia and flagella[303]. The core
985 microtubule structure, the centriole (Fig. 4d), is the same that constitutes the centrosomes of
986 dividing cells[304].
987
988 Primary cilia
989 A solitary microtubule-based organelle emanating from the cell surface of most mammalian
990 cells. Primary cilia are thought to be environmental sensors and signalling hubs of the cell[305],
991 and their dysfunction was linked to a variety of ciliopathies and cancers[274]. Primary cilia
992 contain axonemes without dynein motors and are thus non-motile.
993
994 Connecting cilia
995 A highly modified primary cilium connecting the cell body to the outer segment of
996 photoreceptor cells in the retina[306].
997
998 Growth cone
999 Dynamic structure at the tip of a growing neurites, able to sense the environment and guide
1000 neurite outgrowth and connection[307]. Growth cones are temporal structure in developing
1001 neurons.
1002
1003 Purkinje cell
1004 GABAergic neurons located in the cerebellar cortex. Purkinje cell are among the largest
1005 neurons in the brain with highly ramified dendritic tree.
1006
1007 Microcephaly

A medical condition in which the brain and head of patients is smaller than expected[308].

Viscoelasticity

The property of materials that exhibit both viscous and elastic characteristics when undergoing deformation.

Desmin

Muscle-specific intermediate filament assembly essential for the structural integrity and function of muscle fibres[309].

Kinetochore

A multiprotein structure associated with the centromeres of duplicated chromosomes in eukaryotic cells. Kinetochores are the docking sites for spindle microtubules to pull sister chromatids apart[310]. Kinetochores further control correct sister chromatid attachment via checkpoints[311].

Astral microtubules

A microtubule population that exists only during mitosis. Astral microtubules connect the centrosome to the cell cortex and serve to orient the mitotic spindle in the cell[312].

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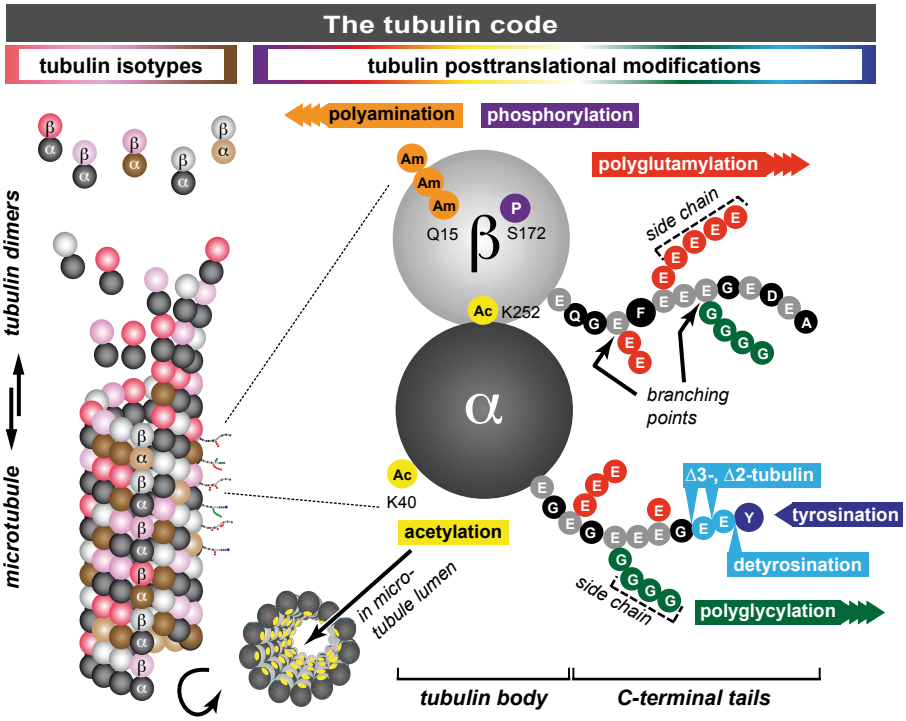
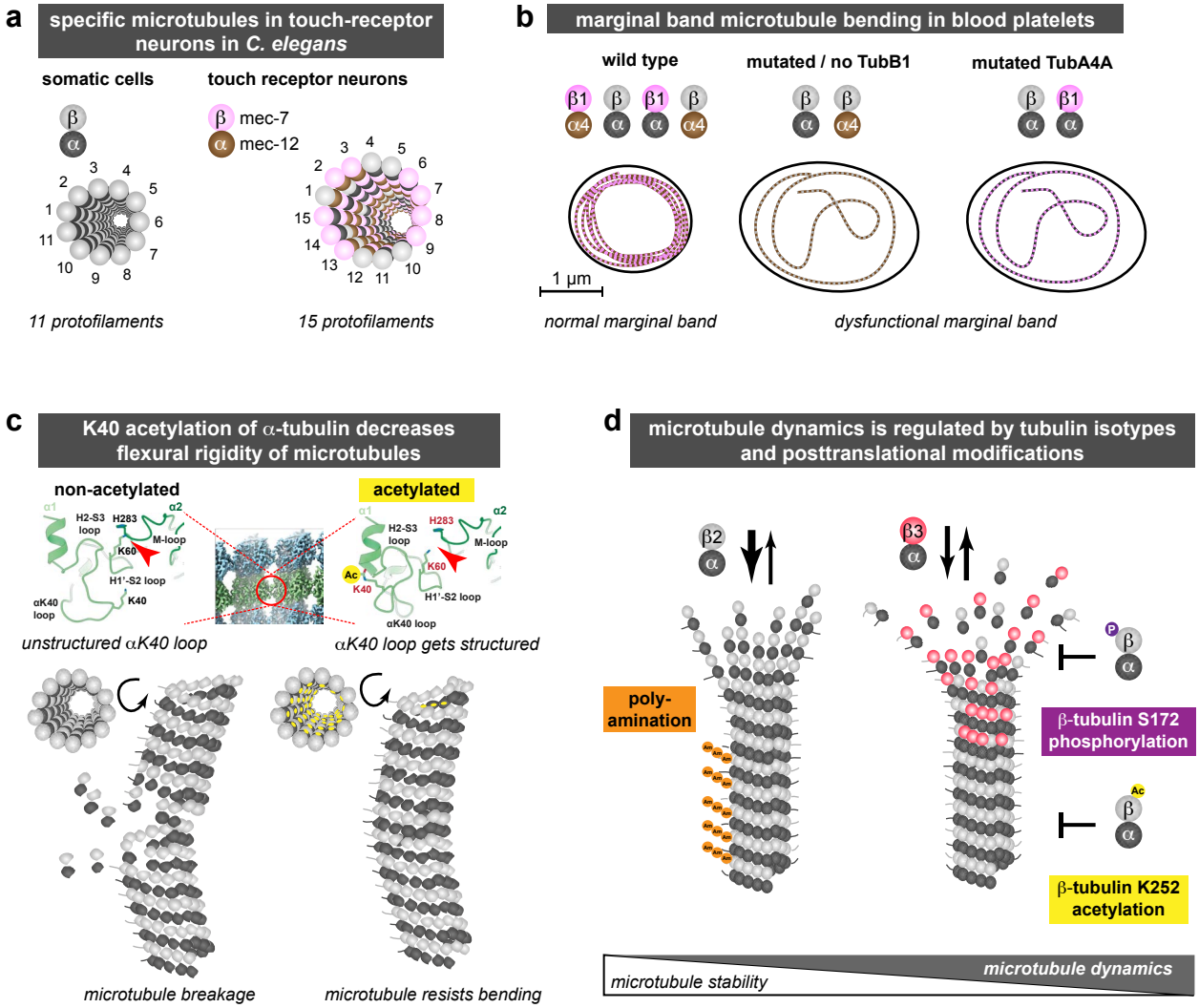
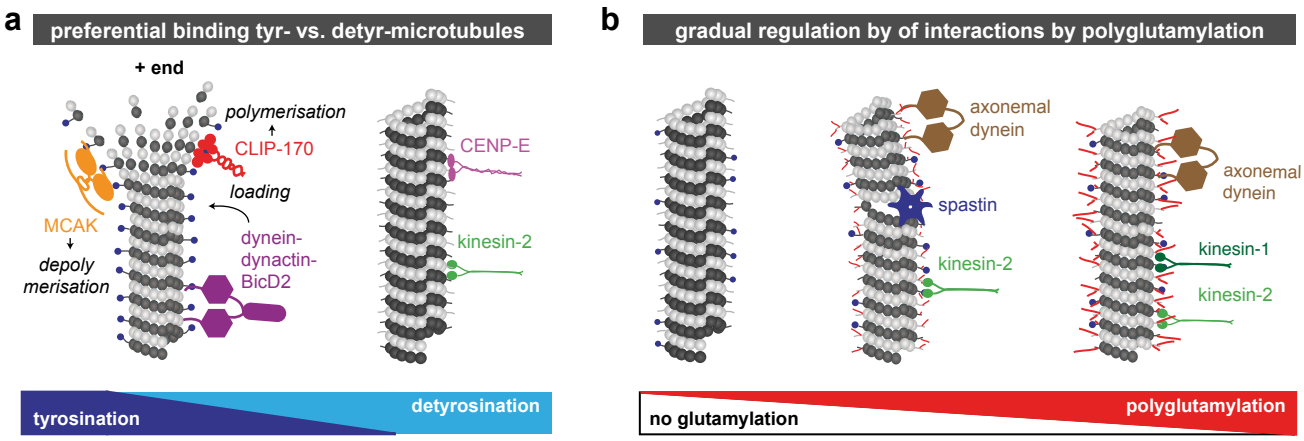
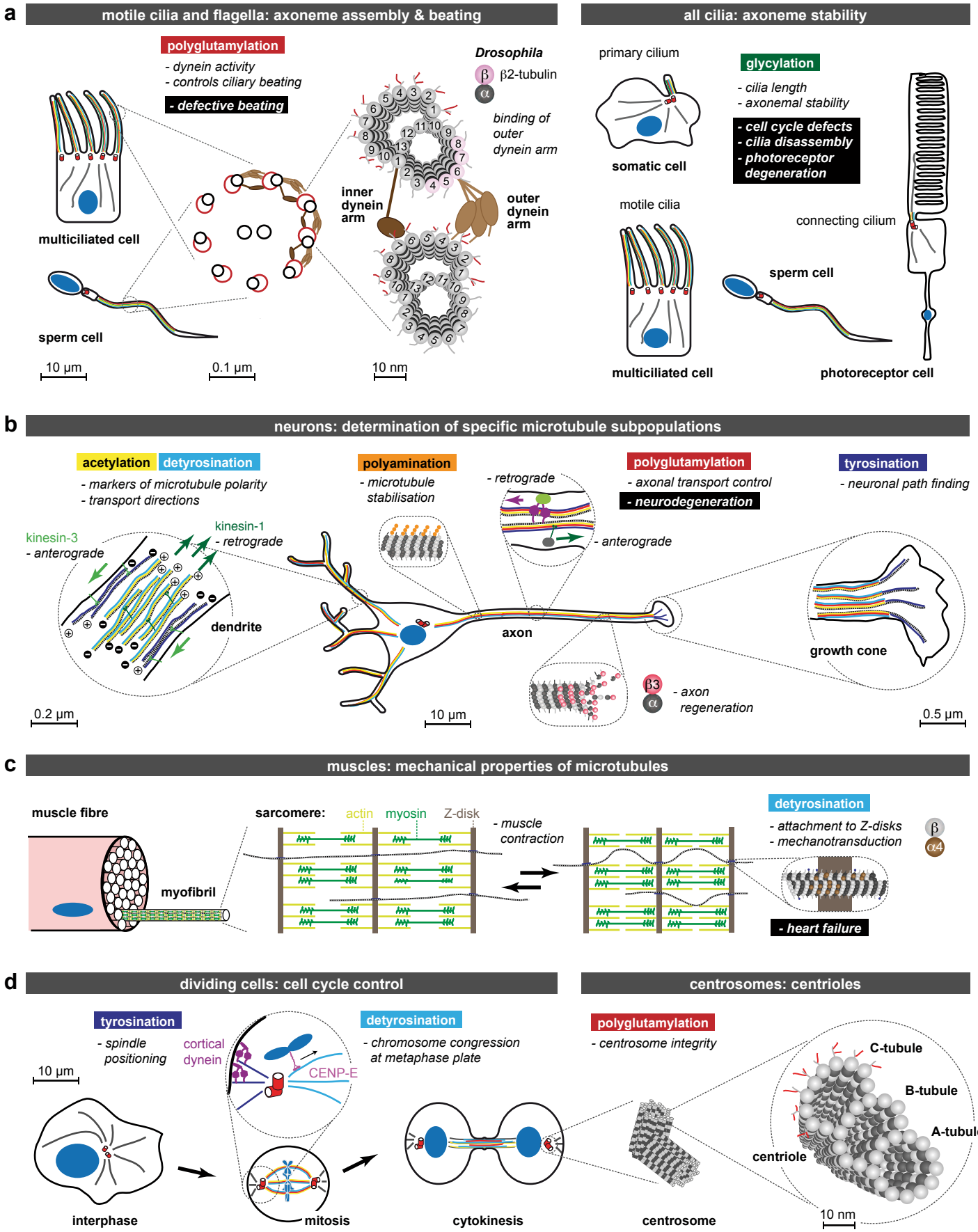

The tubulin code
tubulin isotypes
tubulin posttranslational modifications
polyamination
phosphorylation
polyglutamylation
tubulin dimers
microtubule
Am
Am
Am
Q15
P
S172
Ac
K252
side chain
branching points
Ac
K40
acetylation
in micro-tubule lumen
Δ3-, Δ2-tubulin
tyrosination
detyrosination
side chain
polyglycylation
tubulin body
C-terminal tails


a
specific microtubules in touch-receptor neurons in C. elegans
somatic cells
touch receptor neurons
β
α
mec-7
mec-12
11 protofilaments
15 protofilaments
b
marginal band microtubule bending in blood platelets
wild type
mutated / no TubB1
mutated TubA4A
β1
α4
1 µm
normal marginal band
dysfunctional marginal band
c
K40 acetylation of α-tubulin decreases flexural rigidity of microtubules
non-acetylated
acetylated
H2-S3 loop
H283
M-loop
K60
H1'-S2 loop
αK40 loop
K40
Ac
unstructured αK40 loop
αK40 loop gets structured
microtubule breakage
microtubule resists bending
d
microtubule dynamics is regulated by tubulin isotypes and posttranslational modifications
β2
β3
poly-amination
β-tubulin S172 phosphorylation
β-tubulin K252 acetylation
microtubule stability
microtubule dynamics


a
preferential binding tyr- vs. detyr-microtubules
+ end
polymerisation
CLIP-170
loading
MCAK
depoly
merisation
dynein-
dynactin-
BicD2
CENP-E
kinesin-2
tyrosination
detyrosination
b
gradual regulation by of interactions by polyglutamylation
axonemal
dynein
spastin
kinesin-2
axonemal
dynein
kinesin-1
kinesin-2
no glutamylation
polyglutamylation


a
motile cilia and flagella: axoneme assembly & beating
polyglutamylation
- dynein activity
- controls ciliary beating
- defective beating
multiciliated cell
sperm cell
10 µm
0.1 µm
Drosophila
β β2-tubulin
α
binding of outer dynein arm
inner dynein arm
outer dynein arm
10 nm
all cilia: axoneme stability
primary cilium
somatic cell
glycylation
- cilia length
- axonemal stability
- cell cycle defects
- cilia disassembly
- photoreceptor degeneration
motile cilia
multiciliated cell
connecting cilium
sperm cell
photoreceptor cell
b
neurons: determination of specific microtubule subpopulations
acetylation
detyrosination
- markers of microtubule polarity
- transport directions
kinesin-3
- anterograde
kinesin-1
- retrograde
dendrite
0.2 µm
polyamination
- microtubule stabilisation
- retrograde
- anterograde
axon
10 µm
polyglutamylation
- axonal transport control
- neurodegeneration
β3
α
- axon regeneration
tyrosination
- neuronal path finding
growth cone
0.5 µm
c
muscles: mechanical properties of microtubules
muscle fibre
myofibril
sarcomere:
actin
myosin
Z-disk
- muscle contraction
detyrosination
- attachment to Z-disks
- mechanotransduction
β
α4
- heart failure
d
dividing cells: cell cycle control
tyrosination
- spindle positioning
cortical dynein
CENP-E
10 µm
detyrosination
- chromosome congression at metaphase plate
interphase
mitosis
cytokinesis
centrosomes: centrioles
polyglutamylation
- centrosome integrity
centrosome
C-tubule
B-tubule
A-tubule
centriole
10 nm
color code used for microtubules (all panels)
non-modified (or PTMs not known)
tyrosinated
detyrosinated
acetylated (K40)
glutamylated
glycylated