

# Chapter 2

## Microtubules

Pavel Dráber and Eduarda Dráberová

**Abstract** Microtubules, assembled from heterodimers of  $\alpha$ - and  $\beta$ -tubulin, are hollow tubes of about 25 nm in diameter, participating in essential cellular functions such as maintenance of cell shape, cell division, cell motility, and ordered intracellular transport. Tubulin dimers form protofilaments running lengthwise along the microtubule wall with the  $\beta$ -tubulin facing the microtubule plus end conferring a structural polarity.  $\alpha$ - and  $\beta$ -Tubulins are highly conserved and consist of isotypes encoded by different genes. Numerous posttranslational modifications of tubulin subunits diversify the surfaces of microtubules and provide a mechanism for their functional specialization. A third member of the tubulin family,  $\gamma$ -tubulin, plays a role in microtubule nucleation. Microtubules display dynamic instability characterized by alternating phases of growth and shrinkage separated by catastrophe and rescue events. The dynamic nature of microtubules is dependent on many microtubule-regulatory proteins.

### 2.1 Introduction

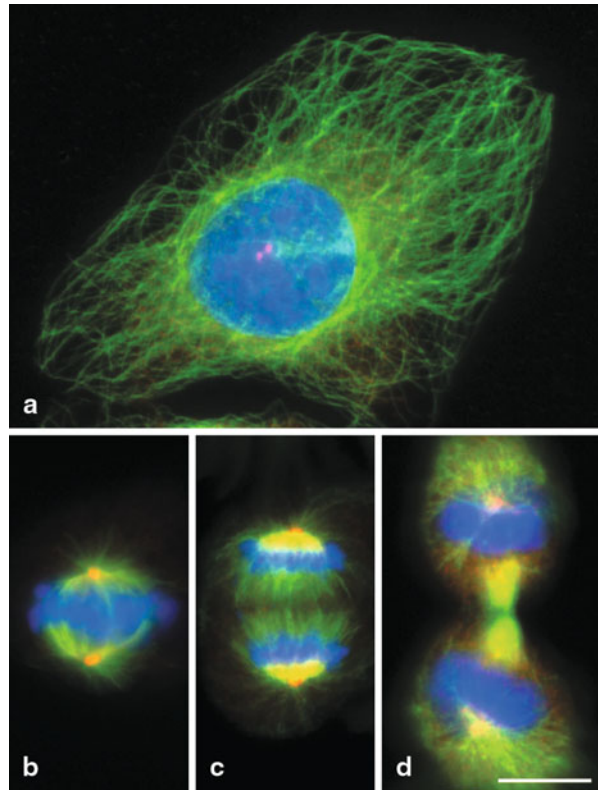
Microtubules are cylindrical cytoskeletal polymers indispensable for many vital cellular activities such as maintenance of cell shape, division, migration, and ordered vesicle transport powered by motor proteins. They are also essential in organizing the spatial distribution of organelles in interphase cells. Microtubules can be organized into microtubule-based organelles with a specialized function, including the radial cytoplasmic network, axonemes, centrioles, midbodies during cytokinesis, and the mitotic/meiotic spindles. Singlet microtubules are the most ubiquitous form of the polymer, however, microtubules can also form doublets (in cilia) or triplets (in centrioles and basal bodies; [1, 2]). Microtubule structures appearing during the cell cycle are shown in Fig. 2.1. The basic building blocks of microtubules are heterodimers of globular  $\alpha$ - and  $\beta$ -tubulin subunits. Tubulins are arranged in a head-to-tail fashion to form 13 protofilaments that constitute microtubules with outer diameter around 25 nm. Microtubules are thus inherently polar and contain two structurally distinct

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**Fig. 2.1** Changes in microtubules during the cell cycle. Microtubule structures (green) undergo marked morphological changes during the cell cycle. Human osteosarcoma cell U2OS in **a** interphase, **b** metaphase, **c** anaphase, and **d** telophase was stained for  $\alpha\beta$ -tubulin dimer with polyclonal antibody (green) and for  $\gamma$ -tubulin with monoclonal anti- $\gamma$ -tubulin antibody TU-30 ([55]; red). DNA is stained blue. Scale bar, 10  $\mu$ m



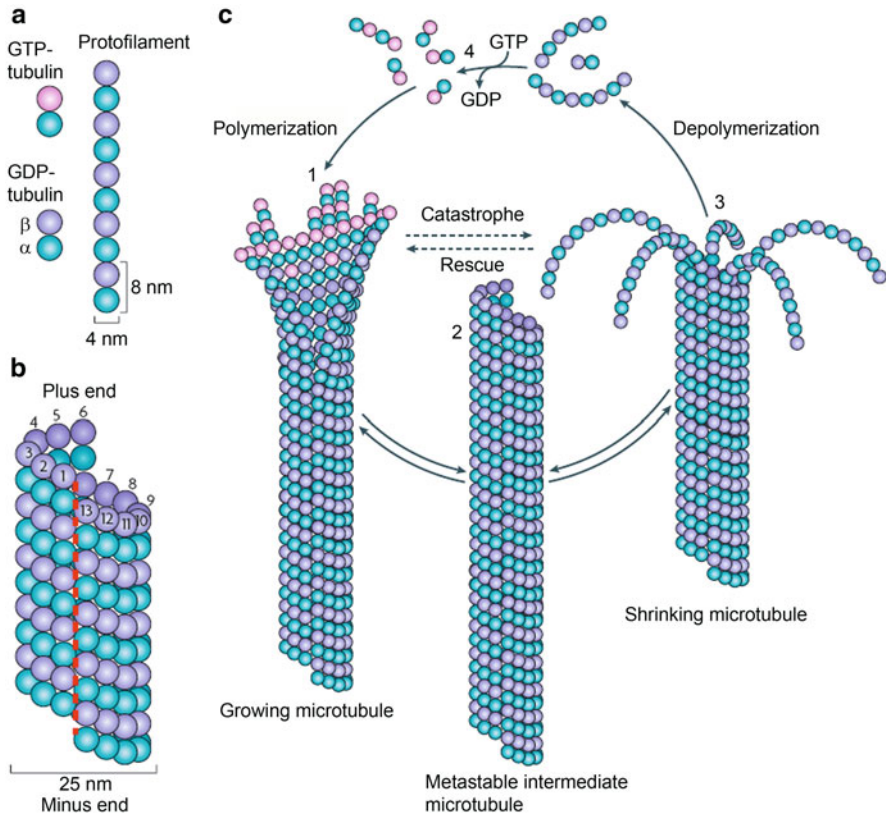
ends: a slow-growing minus end, exposing  $\alpha$ -tubulin subunits, and a fast-growing plus end, exposing  $\beta$ -tubulin subunits [3, 4]. Typically in mammalian cells, the microtubule minus ends are stably anchored in microtubule-organizing centers (MTOC), whereas the plus ends are highly dynamic and switch between phases of growth and shrinkage. There are, however, exceptions to this organization. In dendrites of nerve cells, some microtubules are oriented with minus ends away from the cell body. Microtubules vary considerably in their stability. While microtubules that form the axonemes in cilia and flagella are stable, cytoplasmic microtubules turn over rapidly. Microtubule dynamics help remodel the microtubular network during the cell cycle. Although the structure of microtubules is conserved among various cell types, it can be adapted to highly divergent tasks by mechanisms that are not yet fully understood. Incorporation of alternative tubulin isotypes and posttranslational modification of tubulin subunits can regulate microtubule properties. Intracellular microtubule organization is further controlled by the distribution of nucleation sites and by the activity of microtubule-regulatory proteins. This chapter will focus on microtubule fundamentals, briefly reviewing tubulin and microtubule structure, microtubule dynamics, tubulin isotypes, microtubule nucleation, tubulin posttranslational modifications, and proteins that stabilize or destabilize microtubules.

## 2.2 Microtubule Structure

In mammals, tubulin heterodimers represent 3–4% of the total protein content in cells and reach up to 20% in brain. The secondary and tertiary structures of the  $\alpha$ - and  $\beta$ -monomers are essentially identical, as expected from their identity of >40% over the entire sequence of above 445 amino acids of the sequence [5].  $\alpha$ - and  $\beta$ -Tubulins are globular proteins with a molecular weight approximately 55 kDa and isoelectric points between 5.2 and 5.8 [6]. Each monomer is formed by three sequential and functionally distinctive domains: the nucleotide-binding N-terminal domain, intermediate domain, and C-terminal domain whose C-terminal tail (CTT) part is exposed on the surface of microtubules [7]. Dimers of  $\alpha$ - and  $\beta$ -tubulin are stable and rarely dissociate at the 10–20  $\mu$ M concentration of tubulin found in cells. Each tubulin monomer binds one molecule of GTP, nonexchangeably in  $\alpha$ -subunit (N-site) and exchangeably in  $\beta$ -subunit (E-site). Tubulin dimers also bind divalent cations. Within the microtubule, each tubulin heterodimer forms extensive noncovalent bonds with its neighbors. These bonds form longitudinally as well as laterally between dimers in a protofilament, linking adjacent protofilaments. The N-site is buried at the monomer-monomer interface within the dimer, explaining the nonexchangeability at that site. On the other hand, the nucleotide at the E-site is partially exposed on the surface of the dimer, allowing its exchange with the solution. The cylindrical and left-handed helical microtubule wall typically comprises 13 parallel protofilaments *in vivo* (Fig. 2.2a). Microtubules assembled from purified tubulin *in vitro* have a broad distribution of protofilament numbers centered on 14 [8]. A major advance step in understanding the microtubule functions is marked by the solution of its structure, based on docking the high-resolution structure of brain tubulin, studied by electron crystallography [5, 9], into lower-resolution microtubule maps imaged by electron cryomicroscopy [10, 11]. These studies have confirmed that tubulin dimers form a B lattice, where the main lateral contacts across protofilaments are between subunits of the same type (i.e.,  $\alpha$ - $\alpha$ ,  $\beta$ - $\beta$ ). Most of the studied microtubules appear to have a seam along their length in which lateral contacts are reversed (i.e.,  $\alpha$ - $\beta$ ,  $\beta$ - $\alpha$ ; [12]). This is due to 12-nm helical pitch in combination with the 8-nm longitudinal repeat between  $\alpha\beta$ -tubulin dimers (Fig. 2.2b). There are fenestrations of about  $1.5 \times 2$  nm between the contact regions of protofilaments, thus giving direct access to the microtubule lumen [13]. The microtubular surface displays a surprisingly large number of binding sites, with numerous proteins binding to the outside surface and a multitude of small ligands binding to the inside of microtubules [14]. Some structural interactions with other molecules including nucleotides, drugs, microtubule-associated proteins (MAPs), and motor proteins have been predicted [3].

## 2.3 Microtubule Dynamics

Assembly (polymerization) and disassembly (depolymerization) of microtubules is driven by the binding, hydrolysis, and exchange of GTP on the  $\beta$ -tubulin monomer. GTP hydrolysis is not required for microtubule assembly *per se* but is necessary



**Fig. 2.2** Microtubule structure and dynamics instability. **a** Microtubules are composed of stable  $\alpha\beta$ -tubulin heterodimers that are aligned in a polar head-to-tail fashion to form protofilaments. **b** The cylindrical and helical microtubule wall typically comprises 13 protofilaments in vivo. A discontinuity in the structure of the microtubule wall (lattice seam) is marked by red dashed line. **c** Dynamic instability of microtubules. Polymerization of microtubules is initiated from a pool of GTP-loaded tubulin subunits. GTP hydrolysis changes the conformation of a protofilament from a slightly curved tubulin-GTP to a more intensely curved tubulin-GDP structure. The curved tubulin-GDP is forced to remain straight when it is part of the microtubule wall. Growing microtubule sheets presumably maintain the “cap” of tubulin-GTP subunits to stabilize the straight tubulin conformation within the microtubule lattice 1. Closure of the terminal sheet structure generates a metastable, blunt-ended microtubule intermediate 2, which might pause, undergo further growth or switch to the depolymerization phase. A shrinking microtubule is characterized by fountain-like arrays of ring and spiral protofilament structures 3. The polymerization–depolymerization cycle is completed by exchanging GDP of the disassembly products with GTP 4. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews in Molecular Biology (Akhmanova and Steinmetz 2008), copyright (2008)

for switching between alternating phases of growth and shrinkage separated by catastrophe (transition from growth to shrinkage) and rescue (transition from shortening to growth) events. Polymerization is typically initiated from a pool of GTP-loaded tubulin subunits (Fig. 2.2c 1; [15]). Growing microtubule ends fluctuate between slightly bent and straight protofilament sheets. GTP hydrolysis

and release of inorganic phosphate occur shortly after incorporation, and is promoted by burial and locking of the partially exposed nucleotide as a result of the head-to-tail assembly of dimers. It has been postulated that GTP hydrolysis changes the conformation of protofilament from a slightly curved tubulin-GTP to a more profoundly curved tubulin-GDP structure [16]. This nucleotide-dependent conformational model predicts that the curved tubulin-GDP is forced to remain straight when it is part of the microtubule wall. Growing microtubule sheets maintain a “cap” of tubulin-GTP subunits to stabilize the straight tubulin conformation within the microtubule lattice [17]. A loss of this cap results in rapid depolymerization. A closure of the terminal sheet structure generates a metastable, blunt-ended microtubule intermediate (Fig. 2.2c 2), which may pause, undergo further growth or switch to the depolymerization phase. A shrinking microtubule is characterized by fountain-like arrays of ring and spiral protofilament structures (Fig. 2.2c 3). This conformational change, presumably directed by tubulin-GDP, may destabilize lateral contacts between adjacent protofilaments. The polymerization–depolymerization cycle is completed by exchanging GDP of the disassembly products with GTP (Fig. 2.2c 4). These characteristics result in dynamic instability [18], an essential feature of microtubules that allows them to search through the cell for targets, such as the chromosomal kinetochores, the cell cortex, and actin cytoskeleton [19, 20].

In some cells, microtubules that are not anchored to the centrosome undergo a special form of turnover called treadmilling. If the concentration of tubulin dimer exceeds a critical concentration ( $C_c$ ), the dimers polymerize into microtubules, whereas microtubules depolymerize at concentrations below the  $C_c$ . With differing concentrations at the opposite microtubule ends, and when the microtubules are at, or near, the steady state, higher  $C_c$  at the minus end results in shortening at this end, whereas lower  $C_c$  at the plus end of microtubule results in net growth [21]. When the overall concentration of soluble tubulin is maintained in between the two different  $C_c$  at opposite ends, continuous flow of subunits through microtubules will occur. One of the major sites of microtubule treadmilling is the mitotic spindle. In this situation the ends of microtubules, although tethered to kinetochores and spindle poles, remain free for subunit exchange and rapid flow of tubulin from plus to minus ends. This flow in spindle microtubules has been termed flux [22].

The stability and dynamics of microtubules are actively regulated by a number of cellular factors [19] as well as a variety of ligands, some of them with important anticancer properties. A host of well-known drugs (e.g., vinblastine, colchicine, and paclitaxel) potentially suppress the dynamic instability and treadmilling dynamics of microtubules, and can thereby perturb cellular processes dependent on these dynamics [23, 24].

## 2.4 Tubulin Isotypes

In mammals both  $\alpha$ - and  $\beta$ -tubulin consist of isotypes encoded by different genes and differing in amino acid sequences. Alignment of amino acid sequences of the  $\alpha$ - and  $\beta$ -tubulin isotypes revealed that most of the divergence is contained in the last

**Table 2.1** Overview of human tubulin isotypes

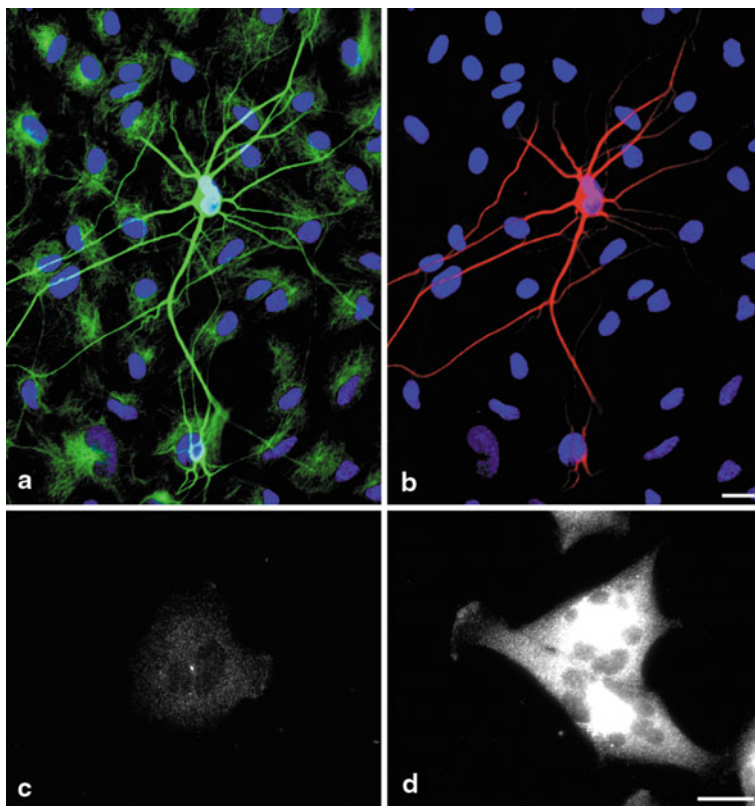
Isotype <sup>a</sup>	Gene name	Protein ID (NCBI)	Length (amino acids)	C-terminal sequence (beyond residue 430)
α1A	<i>TUBA1A</i>	NP_006000	451	DYEEVGVDSVEGEGEEEGEEY
α1B	<i>TUBA1B</i>	NP_006073	451	DYEEVGVDSVEGEGEEEGEEY
α1C	<i>TUBA1C</i>	NP_116093	449	DYEEVGADSADGEDEGEY
α4A	<i>TUBA4A</i>	NP_005991	448	DYEEVGIDSYEDEDEGEE
α3C/D	<i>TUBA3C</i>	NP_005992	450	DYEEVGVDSVEAAEEEGEEY
α3C/D	<i>TUBA3D</i>	NP_525125	450	DYEEVGVDSVEAAEEEGEEY
α3E	<i>TUBA3E</i>	NP_997195	450	DCEEVGVDSVEAAEEEGEEY
α8	<i>TUBA8</i>	NP_061816	449	DYEEVGTDSEEEENEGEEF
α-like 3	<i>TUBAL3</i>	NP_079079	446	DLAALERDYEEVAQSF
βI	<i>TUBB</i>	NP_821133	444	EEEEDFGEEAEEEA
βII	<i>TUBB2A</i>	NP_001060	445	DEQGEFEEEEGEDEA
βII	<i>TUBB2B</i>	NP_821080	445	DEQGEFEEEEGEDEA
βIII	<i>TUBB3</i>	NP_006077	450	EEEGEMYEDDEESEAQGPK
βIVa	<i>TUBB4</i>	NP_006078	444	EEGEFEEEAEEVA
βIVb	<i>TUBB2C</i>	NP_006079	445	EEGEFEEEAEEVA
βV	<i>TUBB6</i>	NP_115914	446	NDGEEAFDEEEIDG
βVI	<i>TUBB1</i>	NP_110400	451	VLEEDEEVTEEAEMEPEDKGH

<sup>a</sup>The nomenclature of α-tubulins follows the recent revision [27], and that of β-tubulins is based on recent reviews [28, 172]

20 amino acids [25], a region of the protein that lies on the exterior of the microtubule and is the putative binding site for MAPs [26]. Differences among isotypes are often highly conserved in evolution, suggesting that they have functional significance. In humans, eight α-tubulin and seven β-tubulin isotypes, were identified ([25, 27, 28]; see Table 2.1). In addition, other very different forms of tubulin have been discovered, designated as γ, δ, ε, ζ, η, θ, ι, and κ [29]. Interestingly, all have been found either in the centrosome or a very similar basal body. Some of these tubulins play a significant role in the assembly of these two organelles [30]. Together with α- and β-subunits, these tubulins constitute the tubulin superfamily. Several of them are widespread among eukaryotes (α, β, γ, δ), while other are more restricted [29, 30, 31, 32].

Different α- and β-tubulin isotypes often differ in their cellular and tissue distribution. Besides, purified isotypes display different properties including microtubule assembly, conformation, GTPase, dynamics, and ability to interact with antitumor drugs [24, 28]. Vertebrate β-tubulin isotypes have fairly distinct tissue distributions [25, 33]. βI-Tubulin expression is essentially ubiquitous. The distribution of βIVb-tubulin is also widespread among tissues and cell types but is especially prominent in axonemes (cilia and flagella; [34]). βII-tubulin particularly abounds in brain, peripheral nerves, and muscles, but is also expressed to a lesser degree in other tissues. It has also been described in tumor cell nuclei of various cancer types [35, 36, 37]. βIII-Tubulin occurs largely in neurons and testicular Sertoli cells, and in low amounts in a very small number of other tissues. Specific expression of βIII-tubulin in neuronal cell is depicted in Fig. 2.3a, b. βIVa-Tubulin is expressed only in brain, while βVI-tubulin is restricted to hematopoietic-specific cell types (megakaryocytes and





**Fig. 2.3** Differential expression of  $\beta$ III-tubulin and  $\gamma$ -tubulin. **a, b** Primary culture of rat neurons and glial cells stained for tubulin with polyclonal antibody (**a** green) and for neuron-specific  $\beta$ III-tubulin with monoclonal antibody TU-20 [173] (**b** red). Nuclei are shown in blue. **c, d** Comparison of  $\gamma$ -tubulin expression in primary culture of human astrocytes (**c**) and human glioblastoma cells T98G (**d**) using monoclonal antibody TU-30 [55]. Note that  $\gamma$ -tubulin in astrocyte is concentrated in centrosome, while in glioblastoma cells a high amount of  $\gamma$ -tubulin is also present in cytoplasm. Fluorescence images were captured and processed in exactly the same manner. Scale bars, 20  $\mu$ m

platelets). The distribution of  $\beta$ V is still largely unknown. On the other hand, tissue distribution of  $\alpha$ -tubulin isotypes seems to be much less complex when compared to  $\beta$ -tubulin.

In many cases, the isotype distribution among tissues and even among different cell types within the same tissue is complex [38]. In addition, the pattern changes during development [39]. Interestingly, expression of tubulin isotypes is altered in drug-resistant and tumorous cells [40–42]. There is some evidence suggesting that cells alter the synthesis of certain tubulin isotypes in order to overcome drug exposure. Tubulin isotype expression profiling has been assessed by RT-PCR of mRNA [43, 44], specific antibodies [43] or by mass spectrometry [45]. While antibodies directed against C-terminal, isotype-defining sequences of most  $\beta$ -tubulin isotypes are readily available, antibodies to  $\alpha$ -tubulin isotypes are scarce.

### 2.4.1 $\beta$ III-Tubulin

Compared to other  $\beta$ -tubulin isotypes,  $\beta$ III-tubulin possesses certain distinctive properties, which may account for its unique function(s) [28]. Unlike the  $\beta$ I,  $\beta$ II, and  $\beta$ IV isotypes,  $\beta$ III-tubulin lacks in this regard the widely conserved and oxidation-sensitive residue cys239, which is replaced by ser239 [46]. It has been hypothesized that the absence of cys239 may permit  $\alpha\beta$ III-tubulin dimers to assemble in the presence of free radicals [28]. In addition, the  $\beta$ III-tubulin contains an uncommon cys124 residue, in contrast to other  $\beta$ -tubulin isotypes which share the ser/ala124 residue [46]. The remarkable phylogenetic conservation of  $\beta$ III-tubulin across various vertebrate species indicates that cys124 and ser239 may have some functional roles. Unlike the  $\beta$ II- and  $\beta$ IV-tubulin isotypes,  $\beta$ III-tubulin is phosphorylated at a serine in the C-terminus [47]. Also, in contrast to other  $\beta$ -tubulin isotypes, the presence of thr429 in  $\beta$ III-tubulin strongly favors microtubule assembly [48]. When tubulin is reduced and carboxymethylated,  $\beta$ III-tubulin shows a unique electrophoretic mobility on polyacrylamide gels [49]. Finally, despite its restricted and highly selective (predominantly neuronal) cell type distribution in normal organs and tissues, the  $\beta$ III isotype is widely, albeit differentially, expressed in a broad range of human tumors of neuronal and nonneuronal origin. Abnormal  $\beta$ III-tubulin expression is probably associated with more aggressive and drug-resistant cancers [33, 50, 51].

## 2.5 Microtubule Nucleation

One of the key components required for microtubule nucleation and stabilization is  $\gamma$ -tubulin [52], a highly conserved, albeit minor, member of the tubulin superfamily concentrated in interphase cells mainly in MTOCs [53]. There is about 30% identity between  $\gamma$ -tubulin, with molecular weight approximately 48 kDa, and tubulin dimers. In mitotic cells the  $\gamma$ -tubulin appears on spindle poles and it is also distributed along spindle fibers [54, 55]. During cytokinesis it is found in midbodies [56]. Localization of  $\gamma$ -tubulin in different phases of the cell cycle is shown in Fig. 2.1. The  $\gamma$ -tubulin is associated in complexes with other proteins. The human  $\gamma$ -tubulin small complex ( $\gamma$ TuSC; around 280 kDa) comprises two molecules of  $\gamma$ -tubulin and one molecule each of GCP( $\gamma$ -tubulin complex protein) 2 and 3 [57]. It has a form of Y-shaped flexible structure with  $\gamma$ -tubulins located on the two arms [58, 59]. The large  $\gamma$ -tubulin-ring complex ( $\gamma$ TuRC) derives from 5 to 7  $\gamma$ -TuSCs by condensation and association with proteins GCP4, GCP5, GCP6 [60], and GCP-WD/NEDD1 [61]. Electron microscopic tomography indicates that the associated proteins absent in  $\gamma$ TuSC form the cap of the ring structure [62]. Hundreds of  $\gamma$ TuRC-like rings were found in pericentriolar material of centrosomes, and the presence of these rings correlated with the ability of centrosomes to nucleate microtubules [63]. Apart from the nucleation from MTOC,  $\gamma$ TuRCs are also involved in the regulation of microtubule minus-end dynamics [64]. There is some evidence that  $\gamma$ -tubulin may associate with



the microtubule wall [65, 66] and with cellular membranes [67–69] where it can participate in noncentrosomal microtubule nucleation [67, 70, 71].  $\gamma$ -Tubulin complexes apparently also play a role in the regulation of microtubule plus-end dynamics and in the spindle assembly checkpoint signaling [72, 73].

Whereas multiple gene families encode  $\alpha$ - and  $\beta$ -tubulin, only two functional genes exist in mammalian cells (*TUBG1*, *TUBG2*) that code very similar  $\gamma$ -tubulins [74]. The  $\gamma$ -tubulin is posttranslationally modified [66, 75, 76], and phosphorylation [77–79] as well as monoubiquitination [80] of  $\gamma$ -tubulin have been described. Complexes of  $\gamma$ -tubulin with protein tyrosine kinases of Src family [78, 81, 82], polo-like kinase [83], microtubule affinity-regulating kinase 4 (MARK 4; [84]) or phosphoinositide 3-kinase [71, 85, 86] have been documented. Collectively taken, the data strongly suggest that kinases might be involved in the regulation of  $\gamma$ -tubulin interactions.

Increased  $\gamma$ -tubulin expression has been reported in cells of breast carcinoma [87, 88] and gliomas [89–91]. The  $\gamma$ -tubulin abnormalities in cancer cells can result in dysfunction of centrosomes due to the presence of supernumerary centrosomes, and/or in aberrant nucleation due to ectopic  $\gamma$ -tubulin localization [92]. Differential subcellular distribution of  $\gamma$ -tubulin in human astrocytes and glioblastoma cells is shown in Fig. 2.3c,d. Numerous proteins and protein complexes, including ninein, augmin, Cep192/SPD2, AKAP450/CG-NAP, pericentrin/kendrin, and CDK5RAP2/centrosomin contribute to the anchoring of  $\gamma$ TuRC to MTOCs. Localization of these factors can be specific for a particular cell type or cell cycle stage [72, 93].

## 2.6 Tubulin Posttranslational Modifications

High-resolution isoelectric focusing that separates polypeptides differing in their net charge has revealed that tubulin subunits can be resolved into more than 20 isoforms, far more than expected from the number of isotypes that are actually expressed [94, 95]. This fact reflects extensive posttranslational modifications (PTMs) of both tubulin subunits. Most PTMs of tubulin subunits take place after polymerization into microtubules and modified tubulins are nonuniformly distributed along microtubules. Strongly modified stable microtubules are concentrated in specialized organelles, such as centrioles and cilia or in axons of neurons. Currently known PTMs are summarized in Table 2.2. Well-characterized PTMs include acetylation, detyrosination, polyglutamylation, and polyglycylation.

### 2.6.1 Tubulin Acetylation

Acetylation of the  $\epsilon$ -amino group of lys40 of  $\alpha$ -tubulin [96, 97] is the sole modification that occurs on the amino acid moiety that extends to microtubule lumen [5]. The acetyl-lys40 of  $\alpha$ -tubulin is therefore obscured in unfixed cells [98]. Several

**Table 2.2** Overview of tubulin posttranslational modifications

Modification <sup>a</sup>	Subunit	Residue(s)	Forward enzyme	Reverse enzyme
Acetylation	α	K40	MEC-17	HDAC6, SIRT2
Detyrosination	α	C-terminal Y	CCP1	TTL
Δ2	α	Penultimate E		
Glutamylation	α, β	Multiple E in CTT	TTLL1, 4, 5, 6, 7, 9, 11, 13	CCP5
Glycylation	α, β	Multiple G in CTT	TTLL3, 8, 10	
Phosphorylation	α, β	S172 β-tubulin	Cdk1 PSK, Syk, Fes	
Glycosylation	α, β			
Arginylation	α, β			
Methylation on K	α			
Methylation on E	α, β	E434 α-tubulin		
Palmitoylation	α, β	C376 α-tubulin		
Sumoylation	α			
Ubiquitylation	α	Multiple K	Parkin	
Nitration on Y	α			

<sup>a</sup>See text for references  
CTT C-terminal tail

additional acetylation sites on α-tubulin and one on β-tubulin were found by mass spectrometry, and some of them are exposed on the outer surface of microtubules [99]. However, lys40 of α-tubulin seems to be the main acetylation site [100]. The acetyl-lys40 mark appears with some delay after microtubule assembly and is an indicator of the microtubule age [101, 102]. MEC-17 protein related to GCN5 histone acetyltransferases was identified as the acetyltransferase that exclusively acetylates lys40 of α-tubulin [103]. Deacetylation of acetyl-lys40 on α-tubulin is catalyzed by HDAC6 [104, 105] and SIRT2 [106] deacetylases. Acetylation is a characteristic feature of stable microtubules.

**2.6.2 Tubulin Detyrosination and Generation of Δ2-Tubulin**

The reversible detyrosination/tyrosination of α-tubulin is the best characterized tubulin modification [107]. Detyrosination removes the gene-encoded C-terminal tyrosine residue on α-tubulin after incorporation of tubulin dimer into microtubule [108, 109]. Detyrosination is likely to be generated by the cytosolic carboxypeptidase 1 (CCP1), since mutation of this enzyme in mice substantially decreases the level of microtubule detyrosination [110]. It remains to be determined whether or not CCP1 has α-tubulin detyrosination activity in vitro. Retyrosination of tubulin heterodimers released from microtubules is generated by tubulin tyrosine ligase (TTL) [111]. Detyrosinated α-tubulin can be further converted into Δ2-tubulin by irreversible removal of penultimate glutamate residue [112]. This PTM can limit the amount of α-tubulin that undergoes recycling because Δ2-tubulin released from microtubules cannot revert to an unmodified state. Detyrosinated α-tubulin is characteristic of stable microtubules and Δ2-tubulin is found on very stable microtubules.



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