УДК 665.939.14:54-116

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GAMMA-TUBULINS AND THEIR FUNCTIONS

γ-Tubulin is a ubiquitous phylogenetically conserved member of tubulin superfamily. In comparison with αβ-tubulin dimers, it is a low abundance protein present within the cells in both various types of microtubule-organizing centers and cytoplasmic protein complexes. γ-Tubulin small complexes are subunits of the γ-tubulin ring complex, which is involved in microtubule nucleation and capping of the minus ends of microtubules. In the past years important findings have advanced the understanding of the structure and function of γ-tubulin ring complexes. Recent evidences suggest that the functions of γ-tubulin extend beyond microtubule nucleation.

Abbreviations: a.a., amino acid; GCP, γ-tubulin complex protein; γTuRC, γ-tubulin ring complex; γTuSC, γ-tubulin small complex; MAPs, microtubule-associated proteins; MTOCs, microtubule-organizing centers; SPB, spindle pole body.

Introduction. Microtubules are highly dynamic cytoskeletal components that are essential for many cellular functions in eukaryotes such as intracellular organization, ordered vesicle transport and cell division. The basic building blocks of microtubules are heterodimers of globular α- and β-tubulin subunits. They are arranged in a head-to-tail fashion to form 13 protofilaments that constitute cylindrical microtubules with outer diameter around 25 nm [1]. Microtubules are thus inherently polar, with α-tubulin at one end of the polymer, the (–) end, and β-tubulin at the other, the (+) end. Protofilaments are slightly offset from one another and form a «3-start» helix. This means that the helix spans three subunits of a protofilament before it completes one turn. There is a «seam» in the microtubule wall, where each helix makes a complete turn. While the protofilaments interact laterally with each other mainly through α-α and β-β contacts, at the seam the α-subunit interacts with a β-subunit [2]. Microtubule ends also differ in their assembly and disassembly characteristics. Tubulin subunits are added to the fast growing (+) ends of microtubules and are preferentially lost from the (–) ends. Microtubules can self-assemble in vitro from high concentrations of tubulin heterodimers in the presence of GTP and Mg^{2+}, but such microtubules are randomly organized. However the concentration of αβ-tubulin heterodimers in cells is below the level necessary for spontaneous nucleation in vitro and microtubules are precisely organized there. This is because microtubules in cells are anchored in microtubule-organizing centers (MTOCs) and these organelles also assist in microtubule nucleation. Besides tubulin heterodimers the microtubules are also built up with microtubule-associated proteins (MAPs) that participate in the regulation of microtubule assembly and are responsible for interactions of microtubules with other cellular components.

Different types of MTOCs. In various cell types, MTOCs have different structural arrangements. In spite of it they have similar functions, they initiate and maintain the polar assembly of cellular microtubules [3]. Centrosomes and basal bodies of cilia and flagella are the most frequently studied MTOCs in animal cells. The centrosomes are formed by an electrodense fibrogranular pericentriolar matrix that surrounds two perpendicular short microtubule structures — the centrioles. Centrosomes are usually located in perinuclear regions, and microtubules emanate from centrosomal peripheral regions [4]. Microtubule polymerization is thus oriented, i. e. the (–) ends of microtubules are in MTOC and the growing (+)

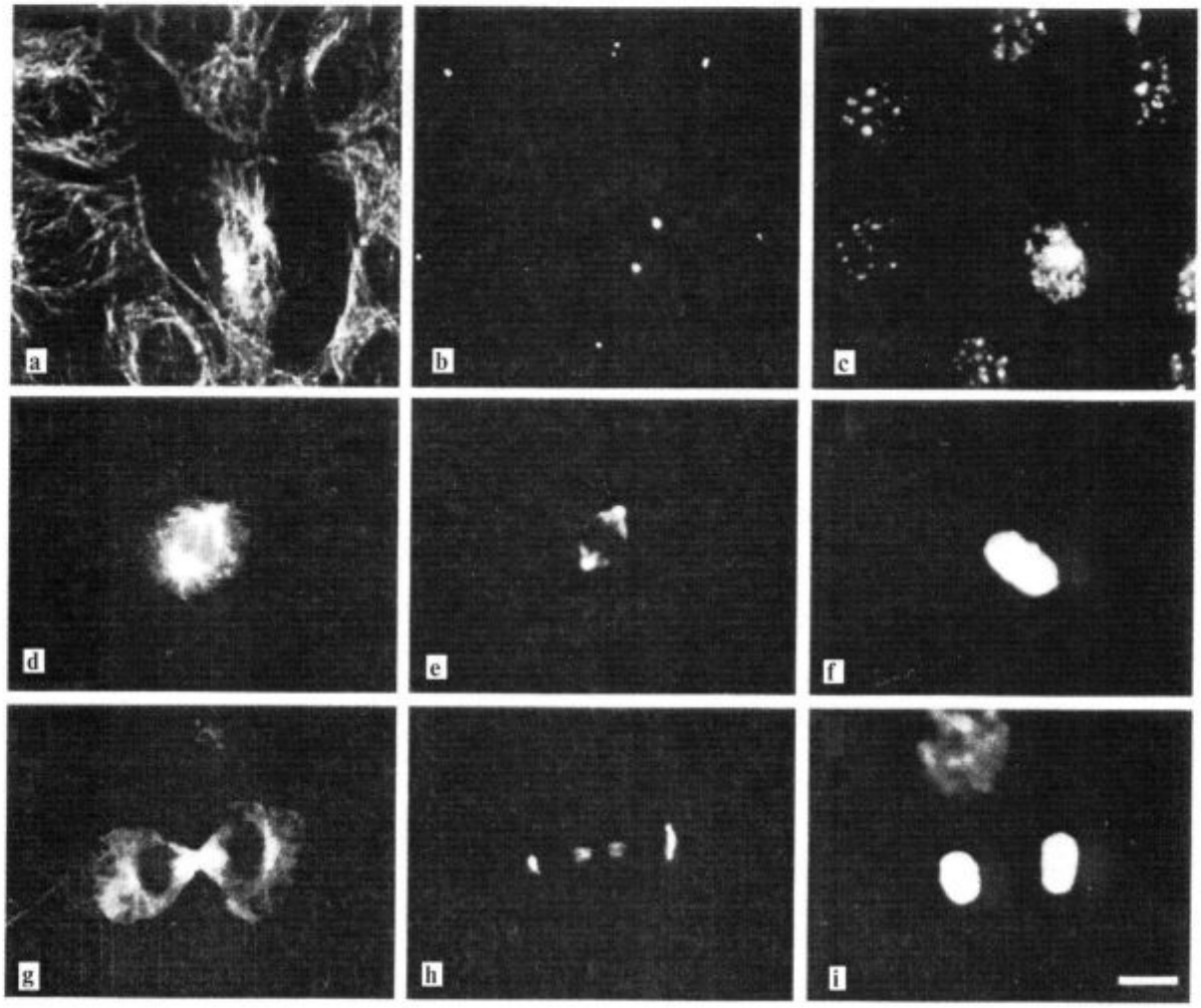
ends are directed to the cell periphery [5]. Microtubules can also arise from centrosomes without centrioles. The structure of basal bodies resembles that of centrioles but, in contrast to centrosomes, microtubules grow directly from basal bodies [6]. In yeast MTOC is located in nuclear membrane and is called the spindle pole body (SPB). This three-layer electrodense structure participates in the organization of cytoplasmic as well as nuclear microtubules [7]. In plant cells there is not a simple organizing center; instead of this, the nuclear envelope and cell cortex serve as MTOCs [8]. One of the key questions in microtubule organization is how such morphologically divergent MTOCs can perform similar functions. It was suggested that they might contain the same structural proteins, which take part in microtubule organization. One possible candidate of such protein is the γ-tubulin, which was found in MTOCs in cells of many different organisms.

Multiple γ-tubulins. γ-Tubulin was first discovered in filamentous fungus *Aspergillus nidulans* as a product of the *mipA* gene during genetic screening for proteins that interact with β-tubulin [9]. Experimental depletion of γ-tubulin led to a depletion of microtubules and to growth arrest. Immunolocalization of γ-tubulin revealed enrichment in MTOCs at the mitotic poles. On the basis of these observations it was proposed that γ-tubulin might play a role in microtubule nucleation [10]. Using γ-tubulin cDNA sequence from *A. nidullans* for screening of cDNA from the fruit fly *Drosophila melanogaster*, a corresponding γ-tubulin sequence was cloned, and the obtained sequence was subsequently used for cloning of human γ-tubulin [11]. Up to now γ-tubulin cDNAs and genes have been cloned and sequenced from a huge variety of organisms, and it seems certain that γ-tubulin is present in all eukaryotes.

While the α- and β-tubulins are encoded by multiple families [12], only a limited number of γ-tubulin genes were found in the majority of organisms studied up to now. For example, in *Arabidopsis thaliana* [13], *D. melanogaster* [14] and *Homo sapiens* [15] there are only two functional genes. Human γ-tubulin genes encode proteins with greater than 98 % amino acid (a.a.) identity. The two *D. melanogaster* γ-tubulins are relatively divergent, sharing approximately 82 % a.a. identity, and have different patterns of expression during gametogenesis and development [14]. In the myxomycete *Physarum polycephalum*, there was only a single γ-tubulin gene described that encoded two γ-tubulins differing in relative molecular weight, sedimentation properties and strength of their binding to the MTOCs. It is not known however, whether these two forms arise from posttranslational modifications or from alternative splicing of mRNA [16]. What might be the selective advantage of retaining two genes that code for very similar γ-tubulins is still unclear. One possibility is that their expression could be differentially regulated, and consequently the level of γ-tubulin expression would be increased in particular tissues or cells. Alternatively, γ-tubulin may be so important that there is a selective advantage in functional redundancy. If one γ-tubulin is mutated so as to render it non-functional, the other γ-tubulin gene might substitute the production of functional γ-tubulin [15].

γ-Tubulins are highly conserved proteins. When sequences from extremely divergent organisms are compared, they share at least 66 % a.a. identity. A remarkably high a.a. identity, reaching 98 %, exists between γ-tubulins of *H. sapiens* and *Xenopus laevis*. γ-Tubulins are similar to aβ-tubulin heterodimers and they share approximately 28–35 % a.a. identity with α- and β-tubulins. When conserved substitutions are taken into consideration they share more than 60 % similarity [17]. Compared to tubulin dimers, γ-tubulin is a minor protein. Quantification of γ-tubulin mRNA in *A. nidulans* revealed that it represented only 5 % of β-tubulin mRNA [9]. In the cell line XTC from *X. laevis*, γ-tubulin represents only 0.005 % of total proteins, while the aβ-tubulin dimers account for 2.5 % of total proteins [18].

γ-Tubulin structure. A comparison of γ-tubulin sequences with those of α- and β-tubulins revealed a similar secondary and tertiary structure, and implied that highly conserved regions among tubulins might be involved in the binding of GTP. In contrast to α- and β-tubulin subunits, those have on their C-terminal regions acidic a.a. residues binding the MAPs, γ-tubulin has no substantially acidic C-terminal end [19]. Little is known about the self-assembly of γ-tubulin and/or interaction of γ-tubulin with αβ-tubulin dimers and microtubules. As the high-resolution structural knowledge on αβ-dimer and tubulin-tubulin interactions in the microtubule is extensive, the sequence/structure analysis resulted in a structural model of possible interactions of γ-tubulin. γ-Tubulin is likely to contact the (−) end of α-tubulin and may associate laterally with α- or β-tubulin. γ-Tubulin should also be capable of self-assembling to form



Distribution of γ-tubulin at different stages of the cell cycle in cultured cell line 3T3. Triple-label staining of cells with antibody against α-tubulin (a, d, g), antibody against γ-tubulin (b, e, h) and DNA-binding dye (c, f, i). Interphase (a–c), metaphase (d–f), telophase (g–i). Bar, 10 μm

either a dimer or a protofilament-like oligomer [20]. Lateral association of γ-tubulin with aβ-dimer has been suggested from a systematic search for αβ-tubulin binding sites on the γ-tubulin using the SPOT peptide technique [21].

In several model systems multiple γ-tubulin charge variants were identified [22-24]. These findings indicate that γ-tubulin, like the α- and β-tubulin counterparts, could be subject to posttranslational modifications. Recently phosphorylation of the γ-tubulin residue Tyr455, that is invariant in all γ-tubulins, has been reported in budding yeast [25].

γ-Tubulin localization. The intracellular location of γ-tubulin was first determined in *A. nidulans*, where it was located in SPB. Immunofluorescence experiments on various cell lines of different tissue origin and derived from different species revealed that γ-tubulin in metazoan cells was located in centrosomes of interphase cells and concentrated in poles of mitotic spindles [11, 18, 26]. γ-Tubulin was found along microtubules of mitotic spindles, yet was lacking on astral microtubules, and was also detectable in late telophase in midbodies [27–29]. γ-Tubulin was further located in the core of centrioles [22, 30]. The distribution of γ-tubulin in different stages of the cell cycle is shown in Figure. γ-Tubulin is in basal bodies in multi-ciliated epithelial cells [31], green alga *Chlamydomonas reinhardtii* [32] as well as in ciliate [33] and flagellate protozoa [34, 35].

The distribution of γ-tubulin in MTOC is independent of the presence of centrioles, as can be inferred from its detection in acentriolar centrosomes in fertil-

ized oocytes [36] and in centrosomes of acentriolar cell line of *D. melanogaster* [37]. A unique situation occurs in acentriolar cells of higher plants, where punctuate γ-tubulin staining was found in association with all microtubule arrays [38] and was also detected in the kinetochore/centromeric region [39, 40]. Collectively these findings document that γ-tubulin is a universal component of active MTOCs irrespective of their structural diversity.

Soluble forms of γ-tubulins. Although MTOCs or microtubule arrays represent the most obvious location of γ-tubulin, many cells contain a large amount of γ-tubulin in soluble form. Soluble γ-tubulin complexes were first detected in embryonal extracts of *D. melanogaster*. Besides γ-tubulin they contained proteins DMAP190 and DMAP60 [41]. Cytosolic forms of γ-tubulin were later detected in oocytes of *X. laevis* as the 25S complexes, γ-somes [42], and subsequently in many other cell types, including the postmitotic nucleated erythrocytes [24] and plant cells [43]. Cytosolic γ-tubulin appears in two main complexes: the large γ-tubulin ring complexes (γTuRC; around 2.2 MDa) and the γ-tubulin small complex (γ-TuSC; around 280 kDa). At a higher salt concentration the large complexes dissociate into γ-TuSC. The human γ-TuSC comprises two molecules of γ-tubulin and one molecule each of GCP2 and GCP3 (γ-tubulin complex proteins) [44, 45], which are homologues of the *Saccharomyces cerevisiae* proteins Spc97p and Spc98p associated with SPB [46]. The Spc97p and Spc98p proteins are related to each other and are both capable of binding to γ-tubulin [47]. Homologous proteins in *Drosophila* are designated Dgrip84 and Dgrip91 (Dgrip: Drosophila gamma ring protein) [45] and Xgrip109 and Xgrip 110 in *Xenopus* (Xgrip: Xenopus gamma ring protein) [48]. The γTuRC derives from the 5–7 γTuSC by condensation and association with other proteins. These proteins have been denoted as GCP4, GCP5 and GCP6, using the standardized nomenclature for proteins of γ-tubulin complexes. Comparison of the five human GCP protein sequences demonstrated that they shared 31–44 % sequence similarity in five conserved regions [49].

Electron microscopy revealed in γTuRC open-ring structure approximately 25 nm in diameter, and defined a subunit structure similar to microtubule cross section. The individual subunits visible within the ring walls have been interpreted as representing the γTuSC [45, 50]. Electron microscopic tomography indicates that associated proteins, not involved in γTuSC, form the cap of the ring structure [51]. Hundreds of γTuRC-like rings were found in pericentriolar material of *Drosophila* [52] centrosomes. The existence of these rings correlated with the ability of centrosomes to nucleate microtubules [53].

Apart from γTuRC and γTuSC, a part of γ-tubulin is probably associated with the TCP-1 chaperonin complex, which is responsible for folding of α-, β- and γ-tubulins [54]. It was also found that γ-tubulin in HeLa cells under natural conditions existed as a homodimer or a heterodimer [55]. Interestingly, like the αβ-dimers, γ-tubulin in porcine microtubule proteins has the ability to form oligomers under the conditions of native electrophoresis (Sulimenko et al., submitted).

γ-Tubulin and microtubule nucleation. γ-Tubulin is the key functional component of MTOCs. Disruption of the γ-tubulin gene in *A. nidulans* caused a loss of mitotic spindles [10], and microinjection of anti-γ-tubulin antibody into mammalian cells prevented the nucleation of new microtubules by the centrosome, and disrupted the morphogenesis of mitotic spindle [26]. Functional conservation of γ-tubulin was demonstrated in experiments in which human γ-tubulin was expressed in *Schizosaccharomyces pombe* with defective endogenous γ-tubulin. Although the homology between *S. pombe* and *H. sapiens* attains only about 71 %, the human γ-tubulin did replace the functions of yeast tubulin and was localized in SPB [56]. In addition, *Xenopus* egg extract depleted of the γ-tubulin-containing protein complex lost much of its ability to support the microtubule asters originating from the centrosome [57]. On the other hand, an overexpression of γ-tubulin in mammalian cells caused γ-tubulin accumulation in cytoplasm where it formed ectopic microtubule nucleation sites [58]. These experiments provided convincing evidence that γ-tubulin is the key functional component of the MTOCs.

Two models were proposed to explain how the γTuRC nucleates microtubules, the so-called «template» and «protofilament» models. It is presumed by the «template model» that γTuRC contains laterally associated γ-tubulin subunits and nucleates by making longitudinal contacts with the terminal α-tubulin [50]. In the «protofilament» model, a ring of longitudinally stacked γ-tubulin subunits forms a short protofilament that nucleates microtubules via lateral contacts with αβ-dimers to seed a bidimensional microtubule lattice [59]. Although electron tomogra-

phy and immunoelectron microscopy studies seem to confirm the template model [51, 60], biochemical evidences [61] rather support a modified protofilament model [62].

The γTuSC also nucleates microtubules, but with a much lower efficiency than the γTuRC, suggesting that the assembly into a larger complex enhances the nucleating activity [45]. Even monomeric γ-tubulin could nucleate microtubules. Partially purified γ-tubulin, prepared *in vitro* in a reticulocyte lysate, induced microtubule nucleation at low tubulin concentration, decreasing the size of the nucleus to three tubulin heterodimers. One γ-tubulin per microtubule was sufficient to block further growth from the (−) end. These data suggest that a single γ-tubulin forms a tight lateral bond with β-tubulin in an oligomer of three tubulin dimers [61].

The γTuRCs not only nucleate microtubules, but they also have separate capping activities. γTuRCs are located at the (−) ends of microtubules when they are nucleated in their presence. γTuRC thus may be important for modulating the (−) end dynamics [63].

Other functions of γ-tubulin. While a substantial progress has been made in understanding of the role of γ-tubulin in microtubule nucleation, other data suggest that γ-tubulin might have some additional functions. A γ-tubulin mutation in *S. pombe* was isolated that permitted microtubule nucleation but at a restrictive temperature, altered interphase microtubule arrays and caused an arrest of mitosis in anaphase A [64]. γ-Tubulin could thus have a role in the functioning of assembled microtubules. It was shown that γ-tubulin and kinesin-like protein KLPA have overlapping roles in the establishment of spindle bipolarity [65]. An involvement of γ-tubulin in the coordination of postmetaphase events, anaphase and cytokinesis is implicated by experiments based on systematic alanine-scanning mutagenesis of human γ-tubulin and analysis of phenotypes of each mutant allele in *S. pombe* [66].

Accumulating data support the idea that γ-tubulin could participate in microtubule stabilization. γ-Tubulin has been found on stable kinetochore microtubules that are resistant to anti-microtubule drugs in plants [39, 67] and in the cold-stable fraction of microtubules in animal cells [23]. γ-Tubulin could play a role in the duplication of centrioles or stabilization of centriolar microtubules.

An association of polo-like kinase with α-, β- and γ-tubulins in a stable complex was found in mouse mammary carcinoma cells [68] whereas in basophilic leukemia cells a direct or indirect association of γ-tubulin with protein tyrosine kinase p53/p56^{lyn} was detected [69]. It appears possible that phosphorylations could modify the interaction of γ-tubulin with αβ-dimers or other proteins. It has been reported that γ-tubulin interacts with hyperphosphorylated BRCA1 protein (a suppressor of tumorigenesis in breast and ovary) and this could regulate the mitosis [70]. Attenuated interaction between BRCA1 and γ-tubulin may induce an increase in the proportion of aneuploid cell population and contribute to tumorigenesis [71].

This work was supported in part by grants from the Grant Agency of the Czech Republic 304/00/0553 and from Ministry of Education of the Czech Republic (LN00A026).

РЕЗЮМЕ. γ-Тубулин — широко распространенный филогенетически консервативный представитель надсемейства тубулинов. По сравнению с димерами αβ-тубулина это белок с низким относительным содержанием, который присутствует в клетках как в различных типах центров организации микротрубочек, так и в комплексах цитоплазматических белков. Малый комплекс γ-тубулина является субъединицей кольцевого комплекса γ-тубулина, который участвует в инициации микротрубочек и кеппировании их минус-концов. Полученные в прошедшие годы важные результаты расширяют представления о структуре и функциях кольцевого комплекса γ-тубулина. Последние данные свидетельствуют о том, что функции γ-тубулина заключаются не только в инициации микротрубочек.

РЕЗЮМЕ. γ-Тубулин — широко розповсюджений філогенетично консервативний представник надродини тубулінів. У порівнянні з димерами αβ-тубуліна це білок з низьким відносним вмістом, присутній у клітинах як у різних типах центрів організації мікротрубочок, так і у комплексах цитоплазматичних білків. Малий комплекс γ-тубуліна є субодиницею кільцевого комплексу γ-тубуліна, що бере участь в ініціації мікротрубочок і кепуванні їх мінус-кінців. Отримані в останні роки важливі результати розширюють уявлення про структуру і функції кільцевого комплексу γ-тубуліна. Останні дані свідчать про те, що функції γ-тубуліна полягають не лише в ініціації мікротрубочок.

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Received 12.03.02