FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 44, No. 2, 2006
pp. 133-138

Microtubules with different diameter, protofilament number and protofilament spacing in *Ornithogalum umbellatum* ovary epidermis cells

Maria Kwiatkowska[1], Katarzyna Popłonska[1], Dariusz Stepinski[1] and Zygmunt Hejnowicz[2]

[1]Department of Cytophysiology, University of Łódz;
[2]Department of Biophysics and Biology of Cell, Silesian University, Katowice, Poland

Abstract: Microtubules present in the epidermis of *Ornithogalum umbellatum* ovary in the area of lipotubuloids (*i.e.* aggregates of lipid bodies surrounded by microtubules) are 25-51 nm in diameter. They consist mainly of 10 and 11, sometimes 9 and 12 protofilaments. An average diameter of microtubule consisting of 9 subunits is about 32 nm, of 10 - 35 nm, of 11 - 38 nm and of 12 - 43 nm, however, individual microtubules in each category significantly vary in size. These differences result from varying distance between protofilaments in microtubule walls and diameters of protofilaments: in thin microtubules they are densely packed and smaller while in thicker ones they are loosely arranged and bigger. A hypothesis has been put forward that changes in microtubule diameter depend on structural changes associated with their functional status and are executed by modifications of protofilament arrangement density and their diameters in microtubule wall. The above hypothesis seems to be in agreement with the opinion formed on the basis of *in vitro* image of microtubules, that lateral contact between tubulin subunits in neighboring protofilaments indicates some flexibility and changeability during microtubule function. (www.cm-uj.krakow.pl/FHC)

Key words: Microtubules - Protofilaments - Lipotubuloid - Rotary motion - *Ornithogalum umbellatum*

Introduction

Microtubule walls consist of two types of tubulin: α and β joined into dimers and arranged into vertical protofilaments. Adjacent dimmers at the same time form a spiral.

Plant cell microtubules described by Ledbetter and Porter [20] who used a negative staining technique consisted of 13 subunits visible in a cross-section. Tilney *et al.* [31] fixing the material with glutaraldehyde supplemented with tannic acid obtained very convincing pictures which seemed to indicate that different types of animal microtubules also consisted of 13 subunits. The same number of subunits was revealed in plant microtubules fixed *in vivo* [9] and in those polymerized *in vitro* [10]. 13 subunits is the number usually accepted as characteristic of microtubules [2], however, some variations were observed. Burton *et al.* [3] noted that wider than average microtubules (30 nm in diameter) in crayfish sperm contained 15 subunits and only a few 14 or 16. However, in crayfish nerve cord 12 subunits were found. Also in cockroach *Blatella germanica*, a dominating population of thick microtubules (40 nm in diameter) consisted of 15 protofilaments, while a few smaller ones (18 nm in diameter) of 13 protofilaments [24]. Variations in the microtubule protofilament number were also observed in *Caenorhabditis elegans* depending on microtubule localization. In the nervous tissue of this nematode there were usually microtubules 24 nm in diameter consisting of 11, sometimes 12 protofilaments, while in the receptors of sensory neurons microtubules 30 nm in diameter consisted of 15 protofilaments [4]. Microtubules polymerized from *C. elegans* extract contained 9, 10 or 11 protofilaments while in the ox the majority of microtubules obtained by the same method consisted of 13 protofilaments [1]. Microtubules polymerized in vitro from *Xenopus* egg extract mainly contained 14 protofilaments [5], moreover, the authors found out that the same microtubule may consist of a various number of subunits in different places.

The main aim of the present studies was to investigate whether various diameters of microtubules described in

Correspondence: M. Kwiatkowska, Dept. Cytophysiology, University of Łódz, Pilarskiego 14, 90-231 Łódz, Poland; e-mail kwiat@biol.uni.lodz.pl

Ornithogalum umbellatum ovary epidermis [17] were correlated with different numbers of protofilaments or depended on the structural changes of microtubules. In order to reveal the microtubules, epidermal cells were fixed in glutaraldehyde + OsO_4 with addition of tannic acid. These microtubules are built of typical tubulin binding to anti-βtubulin antibodies (Kwiatkowska, unpubl.). They are more stable than most microtubules, as they can be fixed in buffered OsO_4 [16, 17], similarly as nerve cell microtubules [30]. Fixation in OsO_4 or in the mixture of OsO_4 and glutaraldehyde due to quick penetration of the fixative allows "freezing" of cell structures in the state as it is in a living cell [27].

Materials and methods

Ovary epidermis of fully developed *O. umbellatum* flowers was used as the material. The sections of epidermis were untreated or treated for 30 min with 8% tannic acid in cacodylate buffer (pH 7.4), fixed in a freshly prepared 1:1 mixture of 1% OsO_4 and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h and postfixed in 1% OsO_4 in the same buffer at 4°C. The material was dehydrated in an alcohol series and embedded in Spurr-Kit and Epon-Kit (Polysciences) mixture. Ultrathin sections were contrasted by the method of Reynolds [28].

Microtubule diameters and protofilament numbers in cross-sections were measured on micrographs at × 110 000 magnification with the aid of magnifying glass and microscopic micrometer (exact to 0.1 mm = 1.7 nm). Protofilament diameters and distances between them were measured on micrographs at × 450 000 magnification also with a micrometer.

Results

O. umbellatum microtubules, observed in this study, are part of specific structures - lipotubuloids (in the past referred to as elaioplasts) - cytoplasm domains rich in lipid bodies and microtubules [17, 19]. Lipotubuloids also contain abundance of ribosomes and endoplasmic reticulum and single dictyosomes, mitochondria, microbodies and autolytic vacuoles [see also 16 , 17, 18]. They do not have their own membrane. On the large area they are surrounded by a tonoplast as they invaginate into vacuoles. The lipotubuloids move as one body because microtubules surrounding the adjacent lipid bodies stabilize their position running in different directions (Fig. 1). Lipotubuloids move forwards in a complex progressive and rotary way with varying speed and direction of rotations.

The lipid bodies and microtubules are very difficult to fix simultaneously. All techniques recommended for visualization of microtubule subunits destroy lipotubuloids. Moreover, the arrangement of microtubules in a lipotubuloid is not perfectly ordered as they are not completely straight and parallel to each other (Fig. 1) and because of that if some microtubule subunits are clearly seen, the others are not discernible. In spite of these difficulties it was possible to define an approximate number of protofilaments and diameter of microtubules.

The analysis of microtubule cross-sections showed that in *O. umbellatum* they were mainly composed of 10 or 11 protofilaments (56% and 34.4% respectively). Only 9% consisted of 12 and 1.6% of 9 subunits.

The average diameter of microtubules varied with the number of subunits. Microtubules containing 9 subunits were about 32 nm in diameter, 10 - 35 nm, 11 - 38 nm, 12 - 43 nm. However, diameters of individual microtubules varied considerably in every category of microtubules (Figs. 2 and 3). These data suggest that microtubules consisting of 10 and 11 protofilaments fall into two categories: thinner and more numerous - diameter 25-39 nm and thicker and less numerous - diameter 41-51 nm.

Pictures of microtubules consisting on average of 12 subunits and with extremely different diameters (Fig. 2a-c) show varying density of protofilament arrangement. Since due to material characteristics not the whole microtubule periphery is well seen in pictures, 4 best discernible subunits forming 1/3 of the periphery were chosen and measured while the remaining subunits were supplemented according to the model (Fig. 2 a'-c'). The analysis of the cross-sections and drawings indicates that together with changes in the distance between the particular protofilaments in a microtubule wall, the diameter of these subunits also varies. They are thicker (mean 5.65±0.57 nm) in microtubules with the biggest diameter than in those with smaller diameter (mean 4.32±0.68 nm) and the smallest diameter (mean 3.56±0.82 nm) as the same number of protofilaments is loosely or densely arranged, respectively (Fig. 2a-c). The distance between the particular protofilaments is 5.15±1.3 nm in microtubules with the biggest diameters, 2.42±0.8 nm in middle-sized microtubules and 1.9±0.8 nm in the smallest ones. Differences in diameters of protofilaments and distances between them in the particular types of microtubules are statistically significant except interprotofilament distance between middle-sized and smallest microtubules (Student's t-test; $p=0.05$).

The above data indicate that microtubule diameter varies not only because they consist of a different number of protofilaments but also due to diverse diameters of protofilaments and density of their arrangement in microtubule wall.

Discussion

Cross-section measurements of diameters of microtubules show that in *O. umbellatum* ovary epidermis two microtubule populations - thinner and more numerous and thicker and less numerous - are present. This is in agreement with the earlier measurements of widths of longitudinally oriented microtubules from the same ma-

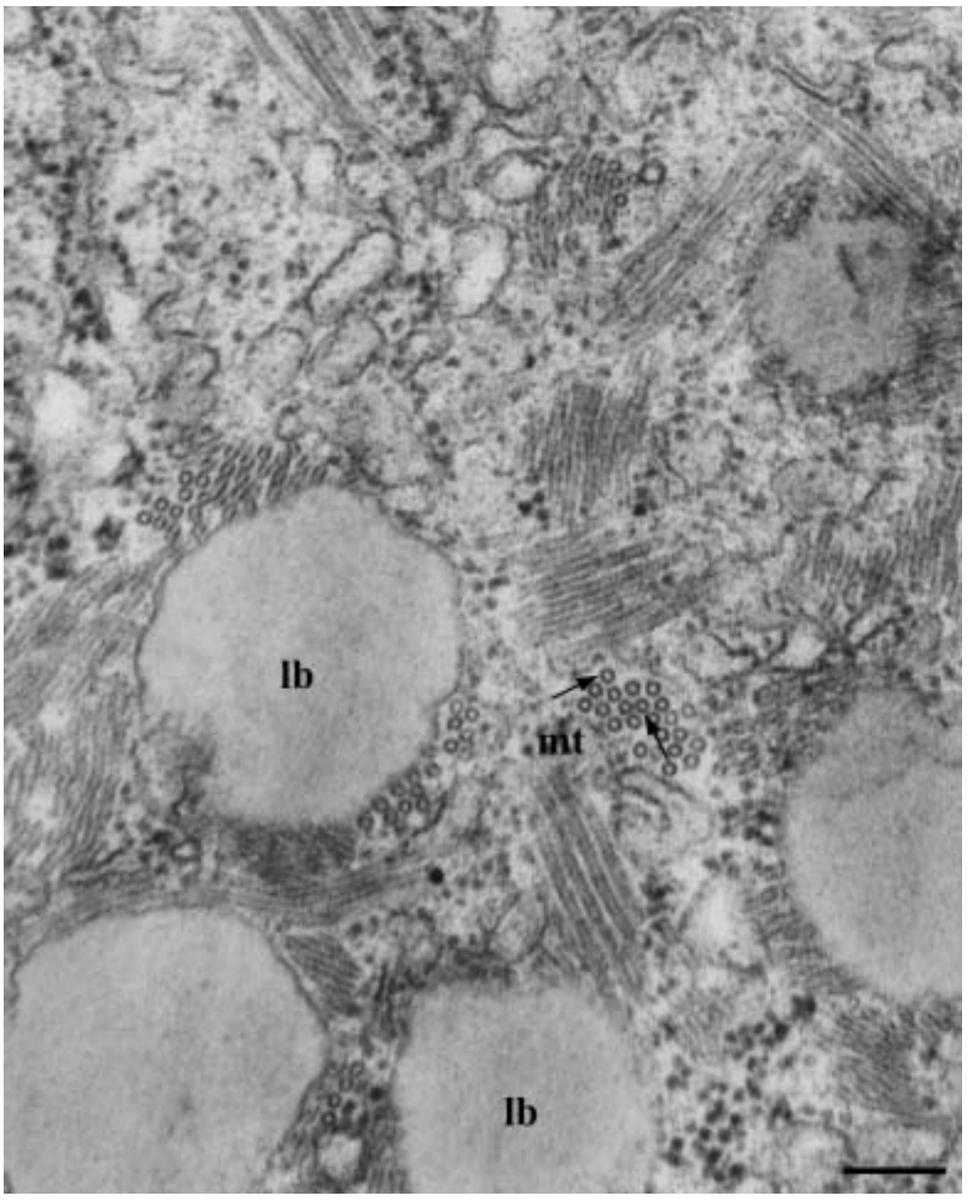
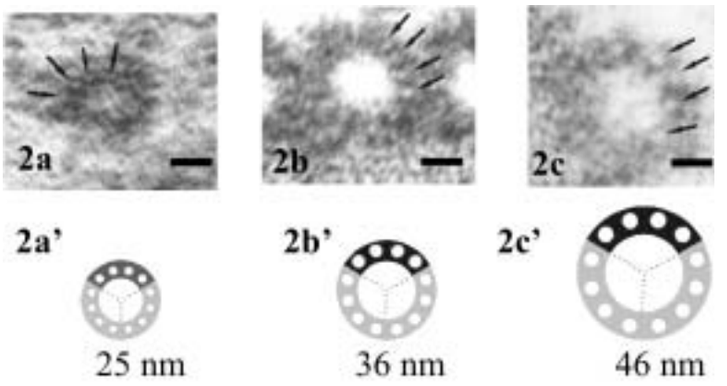


Fig. 1. Lipotubuloid fragment from *O. umbellatum* ovary epidermis not treated with tannic acid. lb - lipid bodies , mt - microtubules; arrows - mt of different diameter. Bar = 0.2 μm.



Figs 2. *O. umbellatum* lipotubuloid microtubules treated with 8% tannic acid before fixation, varying in diameter and consisting of 12 protofilaments. Bar = 14.3 nm. **2 a',b',c'** – drawings of microtubules from a,b,c micrographs based on measurements of 4 adjacent subunits and distances between them (black area).

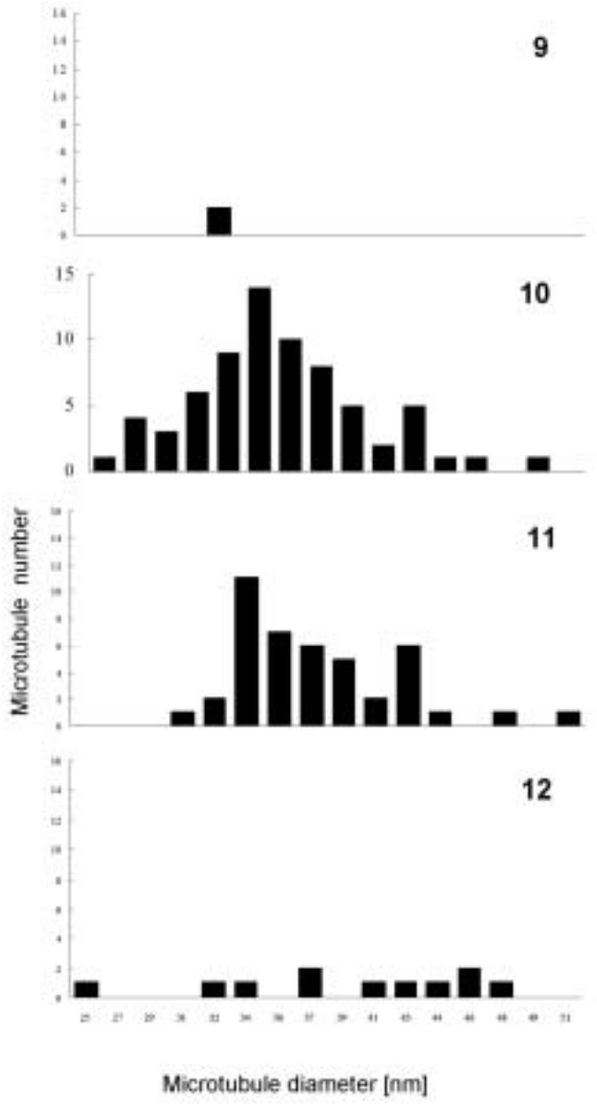


Fig. 3. Populations of microtubules with different diameters composed of 9, 10, 11 and 12 protofilaments, based on measurements of 126 microtubules.

terial fixed only in buffered OsO_4 (cf. Fig. 12a in [17]). However, the diameters of the microtubules presented in this study are larger which might be due to a different fixation method. In cells fixed only in OsO_4, microtubules are usually smaller in diameter than those fixed in glutaraldehyde and OsO_4 [16, 17]. The increase in microtubule diameters is also caused by tannic acid [8].

Experiments with the use of 2,4-dinitrophenol (DNP) inhibiting ATP synthesis combined with recording cell movement in *O. umbellatum* stipule epidermis suggest that a rotary movement of lipotubuloids is generated by a motor drive present in these structures, as the peripheral speed of the rotating lipotubuloids is 6.2 times faster than the maximum speed of the cytoplasmic motion and DNP stops cyclosis earlier than the rotary motion of the lipotubuloid [17].

Combination of movement recording and electron microscopy revealed that the more dynamic was lipotubuloid movement (*e.g.* in stipule epidermis), the larger was the population of thicker microtubules and the less dynamic movement (*e.g.* in ovary epidermis) the smaller was the population of such microtubules (cf. Fig. 12a,b in [17]). DNP-induced inhibition of a dynamic movement of lipotubuloids in stipule epidermis resulted in appearance of one population of microtubules intermediate in size (cf. Fig. 12c in [17]). Removal of DNP restored dynamic movement of lipotubuloids and diversification of microtubule size into two populations (cf. Fig. 12d in [17]).

The above data indicate that microtubule diameters change significantly according to the functional state of the lipotubuloid which supports a hypothesis that their structural transformation may generate lipotubuloid rotary movements.

In the literature there is usually a direct relation between microtubule diameter and the number of protofilaments: in thicker microtubules the numbers of protofilaments forming their walls are higher than in thinner microtubules. Moor [23] suggested that microtubule diameter depended on the number of subunits per one coil of a spiral observed in a microtubule and could be 21, 22.4 and 25 nm. He assumed that one type of microtubules could change into another one by reorganization of subunits so that thicker and shorter or thinner and longer microtubules could be formed from the same number of subunits. Other authors believed that formation of thick microtubules, 35 nm in diameter, resulted from disappearance of typical ones (25 nm) caused by an increased osmotic pressure [13].

It seems very unlikely that DNP-induced changes in microtubule diameter observed in *O. umbellatum* were caused by changes in the number of protofilaments forming microtubule walls. We believe that density of their arrangement and diameter of protofilaments in the walls of microtubules is a more probable reason for different microtubule diameters in different functional states of lipotubuloids. Microtubules with extremely large and small diameter were also observed close to each other in lipotubuloids of cells (Fig. 1, arrows and Figs 9 and 11 in [17]). The above hypothesis formed on the basis of the pictures of *in vivo* fixed cells seems to be in agreement with the opinion of Chrétien *et al.* [6] that microtubules assembled *in vitro* indicate some flexibility of the inter-protofilament bonds in cryo-electron microscopic image: they are capable of changes in the range from -0.05 nm to +0.09 nm of the lateral interactions between tubulin subunits in adjacent protofilamants. This corresponds with observations using high resolution techniques *in vitro* revealing that lateral con-

tacts through M loop and H3 helix seem to cause flexibility in the interprotofilament contacts that must be capable of accommodating different angles between adjacent protofilaments [25, 26, 33]. Other authors also believe that there are strong arguments in favor of microtubules being inhomogeneous and anisotropic, as well as composed of subunits that are more strongly bound in the longitudinal direction (within protofilaments) than laterally (between protofilaments) [12, 29, 15, 32]. Meurer-Grob *et al.* [22] conclude that lateral contact between tubulin subunits in neighboring protofilaments has a decisive role for microtubule stability, rigidity and architecture. However, experiments using high resolution technique revealed the structural changes in microtubules during their functioning. *In vitro* interaction between kinesin and tubulin shows that microtubule structure changes during this process: α tubulin subunits in protofilaments come closer to each other [14]. Besides, non-claret disjunctional (ncd), a kinesin-like motor tail fragment induces formation of large protofilament sheets, suggesting a tail-induced modification of lateral protofilament contacts [34].

Recent studies indicate that microtubules contribute to the generation of different kinds of movements, not only employing dynein and kinesin but also cooperating with actin-myosin system [7, 11, 21]. That is why it seems important that *O. umbellatum* lipotubuloid microtubules are connected with microfilaments lying parallel to their surface. These microfilaments probably are actin filaments, as the rotary motion of lipotubuloids is sensitive to cytochalasin B [19].

Lipotubuloids are the site of a very intense incorporation of ^{3}H-palmitic acid and after 15 h postincubation with isotope-free medium a migration of the labeled substance from lipotubuloids to the whole cells occurs [18]. It seems that progressive-rotary motion of lipotubuloids greatly facilitates both the entry of components necessary for lipid synthesis and the release of substances which are later distributed inside the cell.

References

[1] Aamodt EJ, Culotti JG (1986) Microtubules and microtubule-associated proteins from the nematode *Caenorhabditis elegans*: periodic cross-links connect microtubules *in vitro*. J Cell Biol 103: 23-31

[2] Alberts B, Bray D, Johnson A, Lewis J, Raff M, Roberts K, Walter P (1998) Essential Cell Biology. Garland Publishing Inc, New York-London

[3] Burton PR, Hinkley RE, Pierson GB (1975) Tannic acid-stained microtubules with 12, 13 and 15 protofilaments. J Cell Biol 65: 227-233

[4] Chalfie M, Thomson JN (1982) Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. J Cell Biol 93: 15-23

[5] Chrétien D, Metoz F, Verde F, Karsenti E, Wade RH (1992) Lattice defects in microtubules: protofilament numbers vary within individual microtubules. J Cell Biol 117: 1031-1040

[6] Chrétien D, Flyvbjerg H, Fuller SD (1998) Limited flexibility of the inter-protofilament bonds in microtubules assemled from pure tubulin. Eur Biophys J 27: 490-500

[7] Goode BL, Drubin DG, Barnes G (2000) Functional cooperation between the microtubule and actin cytoskeletons. Cell Biol 12: 63-71

[8] Hayat MA (2000) Principles and techniques of electron microscopy. Biological applications. Cambridge University Press, Cambridge

[9] Hilaire E, Paulsen AQ, Brown ChS, Guikema JA (1995) Cortical microtubules in sweet clover columella cells developed in microgravity. Plant Cell Physiol 36: 1387-1392

[10] Hugdahl JD, Bokros CL, Hanesworth VR, Aalund GR, Morejohn LC (1993) Unique functional characteristics of the polymerization and MAP binding regulatory domains of plant tubulin. Plant Cell 5: 1063-1080

[11] Hwang E, Kusch J, Barral Y, Huffaker TC (2003) Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. J Cell Biol 161: 483-488

[12] Kis A, Kasas S, Babic B, Kulik AJ, Benoit W, Briggs GAD, Schönenberger C, Catsicas S, Forró L (2002) Nanomechanics of microtubules. Phys Rev Lett 89: 1-4

[13] Komis G, Apostolakos P, Galatis B (2002) Hyperosmotic stress induces formation of tubulin macrotubules in root-tip cells of *Triticum turgidum*: Their probable involvement in protoplast volume control. Plant Cell Physiol 43: 911-922

[14] Krebs A, Goldie KN, Hoenger A (2004) Complex formation with kinesin motor domains affects the structure of microtubules. J Mol Biol 335: 139-153

[15] Krebs A, Goldie KN, Hoenger A (2005) Structural rearrangements in tubulin following microtubule formation. EMBO Rep 6: 227-232

[16] Kwiatkowska M (1971) Fine structure of the lipotubuloids (elaioplasts) in *Ornithogalum umbellatum* L. Acta Soc Bot Pol 40: 451-465

[17] Kwiatkowska M (1972) Changes in the diameter of microtubules connected with the autonomous rotary motion of the lipotubuloids (elaioplast). Protoplasma 75: 345-357

[18] Kwiatkowska M (2004) The incorporation of ^{3}H-palmitic acid into *Ornithogalum umbellatum* lipotubuloids, which are a cytoplasmic domain rich in lipid bodies and microtubules. Light and EM autoradiography. Acta Soc Bot Pol 73: 181-186

[19] Kwiatkowska M, Popłonska K, Stepinski D (2005) Actin filaments connected with the microtubules of lipotubuloids - cytoplasmic domains rich in lipid bodies and microtubules. Protoplasma 226: 163-167

[20] Ledbetter MC, Porter KR (1964) Morphology of microtubules of plant cells. Science 144: 872-874

[21] Manneville J-B, Etiene-Manneville S, Skehel P, Carter T, Ogden D, Ferenczi M (2003) Interaction of the actin cytoskeleton with microtubules regulates secretory organelle movement near the plasma membrane in human endothelial cells. J Cell Sci 116: 3927-3938

[22] Meurer-Grob P, Kasparian J, Wade RH (2001) Microtubule structure at improved resolution. Biochemistry 40: 8000-8008

[23] Moor H (1967) Der Feinbau der Microtubuli in Hefe nach Gefrierätzung. Protoplasma 64: 89-103

[24] Nagano T, Suzuki F (1975) Microtubules with 15 subunits in cockroach epidermal cells. J Cell Biol 64: 242-245

[25] Nogales E (1999) A structural view of microtubule dynamics. Cell Mol Life Sci 56: 133-142

[26] Nogales E, Whittaker M, Milligan RA, Downing KH (1999) High-resolution model of the microtubule. Cell 96: 79-88

[27] Omoto ChK, Kung Ch (1980) Rotation and twist of central-pair microtubules in the cilia of *Paramecium*. J Cell Biol 87: 33-46

[28] Reynolds SS (1963) The use of lead citrate of high pH as an electron-opaque stain microscopy. J Cell Biol 17: 208-212

[29] Sept D, Baker NA, Mccammon JA (2003) The physical basis of microtubule structure and stability. Protein Sci 12: 2257-2261
[30] Tani E, Ametani T (1970) Substructure of microtubules in brain nerve cells as revealed by ruthenium red. J Cell Biol 46: 159-165
[31] Tilney L, Bryan J, Bush D, Fujiwara K, Mooseker MS, Murphy DB, Snyder DH (1973) Microtubules: evidence for 13 protofilaments. J Cell Biol 59: 312-326
[32] Tuszynski JA, Luchko T, Porter S, Dixon JM (2005) Anisotropic elastic properties of microtubules. Eur Phys J E Soft Matter 17: 29-35
[33] Wang H-W, Nogales E (2005) Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly. Nature 435: 911-915.
[34] Wendt T, Karabay A, Krebs A, Gross H, Walker R, Hoenger A (2003) A structural analysis of the interaction between ncd tail and tubulin protofilaments. J Mol Biol 333: 541-552

Received: August 16, 2005
Accepted after revision: January 5, 2006