Protoplasma (2006) 227: 65–76
DOI 10.1007/s00709-005-0152-0

PROTOPLASMA
Printed in Austria

Multiple tubulin forms in ciliated protozoan *Tetrahymena* and *Paramecium* species

Review article

L. Libusová[1,2] and **P. Dráber**[2,*]

[1] Department of Animal Physiology and Developmental Biology, Faculty of Sciences, Charles University, Prague
[2] Department of Biology of the Cytoskeleton, Institute of Molecular Genetics, Czech Academy of Sciences, Prague

Received May 15, 2005; accepted August 26, 2005; published online May 30, 2006

Summary. *Tetrahymena* and *Paramecium* species are widely used representatives of the phylum Ciliata. Ciliates are particularly suitable model organisms for studying the functional heterogeneity of tubulins, since they provide a wide range of different microtubular structures in a single cell. Sequencing projects of the genomes of members of these two genera are in progress. Nearly all members of the tubulin superfamily (α-, β-, γ-, δ-, ε-, η-, θ-, ι-, and κ-tubulins) have been identified in *Paramecium tetraurelia*. In *Tetrahymena* spp., the functional consequences of different posttranslational tubulin modifications (acetylation, tyrosination and detyrosination, phosphorylation, glutamylation, and glycylation) have been studied by different approaches. These model organisms provide the opportunity to determine the function of tubulins found in ciliates, as well as in humans, but absent in some other model organisms. They also give us an opportunity to explore the mechanisms underlying microtubule diversity. Here we review current knowledge concerning the diversity of microtubular structures, tubulin genes, and posttranslational modifications in *Tetrahymena* and *Paramecium* species.

Keywords: Microtubule; Tubulin; Posttranslational modification; Ciliate; *Paramecium tetraurelia*; *Tetrahymena* spp.

Introduction

Microtubules represent one of the major cytoskeletal systems of all eukaryotic cells. They play a key role in organizing the spatial arrangement of the organelles, in intracellular transport, during nuclear and cell division, and in ciliar or flagellar motility. Microtubules are hollow tubes built of αβ-tubulin heterodimers that assemble in a head-to-tail arrangement. Both α- and β-tubulin bind GTP prior to becoming part of the microtubule wall, but only β-tubulin cleaves it to GDP afterwards. The head-to-tail assembly defines the polarity of microtubules (Dustin 1984). In cells, the minus end of the microtubule is usually anchored in a microtubule-organizing centre, represented by the centrosome, basal body, or spindle pole body depending on the organism. The plus ends explore cellular space, switching rapidly between phases of growth and shrinkage, a behaviour called dynamic instability (Mitchison and Kirschner 1984). Besides these dynamic microtubules, more stable microtubules also exist in cells. The finding that the microtubular structures present in a single cell can have different properties is connected with the fact that most eukaryotic cells possess a multigene tubulin family in their genomes and thus express several isotypes of α- and β-tubulin. This tubulin diversity is further elaborated by a wide range of posttranslational modifications, creating different tubulin isoforms (Westermann and Weber 2003). In addition to the main microtubular building blocks, eukaryotic cells encode other members of the growing tubulin family.

Tetrahymena and *Paramecium* species contain a wide range of different microtubular structures in a single cell, and many new members of the tubulin family have been described in these organisms, as well as different forms of posttranslationally modified tubulin. They are, therefore, particularly suitable models for studies of tubulin heterogeneity. Moreover, these organisms have provided many insights into cilia (including the discovery of the first microtubular motor dynein) and the cellular principles of

* Correspondence and reprints: Institute of Molecular Genetics, Czech Academy of Sciences, Vídeňská 1083, 14220 Prague, Czech Republic. E-mail: paveldra@biomed.cas.cz

self-templated cortical patterning. Therefore, this review focuses on the diversity of microtubular structures, tubulin genes, and posttranslational modifications in *Tetrahymena* and *Paramecium* species.

Ciliates as model organisms

Ciliates are single-cell organisms grouped together with dinoflagellates and apicomplexans as alveolates. This is one of the biggest groups in the kingdom Protozoa (Cavalier-Smith 1993). Members of the genera *Tetrahymena* and *Paramecium* are model organisms that are often used in investigations of the microtubular cytoskeleton. The main reason is that their cells possess a wide range of morphologically and functionally distinct microtubular structures. Both *Tetrahymena* and *Paramecium* species are free-living, fresh-water organisms. Although their complex structures and physiology are comparable to those of other eukaryotes, they are as easy to maintain as prokaryotic organisms. They can be cultured in simple axenic media, they double every 2.5–3 h at 30 °C and can reach densities as high as 10^6 cells per ml of medium. On the other hand, their cells are big enough to be easily micromanipulated. A huge number of established clonal strains are available and cell lines with genotypically different macronuclei and micronuclei (called heterokaryons) can be readily constructed. They are useful for the maintenance of lethal mutations, chromosome losses, and knockouts of essential genes because all these unfavourable changes in the genome can be kept in the transcriptionally inactive micronucleus. Precise homologous recombination gives complete control over the placement of the inserted gene and can be used for gene disruption (knockout) or replacement.

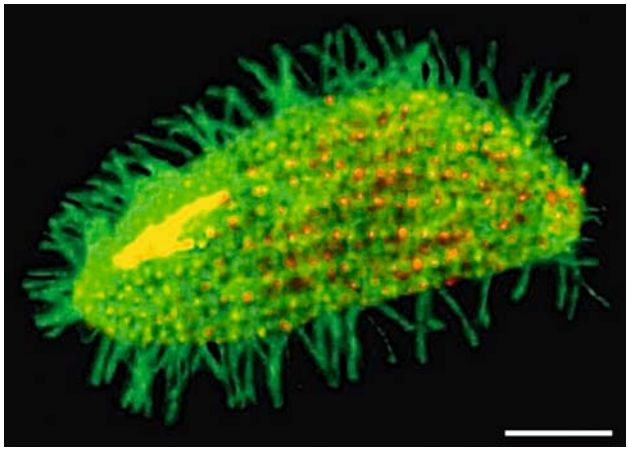

Fig. 1. Immunofluorescence microscopy of *T. thermophila* cell. αβ-Tubulin dimers are decorated by polyclonal antibody (green), γ-tubulin by monoclonal antibody (red). Bar: 10 μm

The sequencing of several ciliates is in progress. Most effort is focused on the genus *Tetrahymena*, particularly on the species *T. thermophila* (http://www.lifesci.ucsb.edu/~genome/Tetrahymena/ and http://www.ciliate.org/) and *T. pyriformis* (http://www.tigr.org/tdb/e2k1/ttg/index.shtml). Another sequencing project involves *Paramecium tetraurelia* (http://www.genoscope.cns.fr/externe/English/Projets/Projet_FN/organisme_FN.html). It is assumed that the whole *P. tetraurelia* genome codes for approximately 30,000 genes, similar to the human genome (Zagulski et al. 2004). A typical representative of this protozoan group is *T. thermophila*, which is depicted in Fig. 1.

Diversity of microtubular structures

Up to 17 distinct microtubular structures have been described so far in *T. thermophila*: central pair and outer doublets of ciliary microtubules, basal bodies, longitudinal bundle of basal microtubules, transverse fibres, postciliary ribbons (Allen 1967), oral connectives (Nilsson and Williams 1966), postoral deep fibre (Williams and Bakowska 1982), cytoproct (Allen and Wolf 1974), contractile vacuole pore (Elliott and Bak 1964), micronuclear and macronuclear spindles, pronuclear transfer basket (Orias et al. 1983), intracytoplasmic microtubular network, and different structures connected with micronuclear meiosis or conjugation (Gaertig and Fleury 1992).

Cilia and cortical structures

Longitudinal ciliary rows cover the vast majority of the ciliate cell surface. In *Tetrahymena* spp., 18–21 of these rows represent the locomotive organ (Lee 2000). In *Paramecium* spp., the beat frequency is dependent on the cyclic AMP (cAMP) level in the cytoplasm, and K^+ channels seem to be directly coupled to adenylate cyclase. Opening of these channels leads to an increased intracellular concentration of cAMP and to fast forward swimming of the cell. On the other hand, an increased concentration of Ca^{2+} causes reversal of the beat direction (Satir et al. 1993). Cilia are not just used for oriented movement but also make these organisms capable of coping with the phylogenetically oldest stimulus, gravity. Cilia enable the cells to overcome sedimentation by negative gravitaxis (Machemer 1996). The distal ends of ciliary microtubules are attached to the membrane by microtubule-capping structures. These structures are part of the regulatory system that directs ciliary microtubule assembly and maintains their stability (Suprenant and Dentler 1988, Miller et al. 1990). The cilia axoneme has the conventional "9 + 2" structure, consisting of a central

pair of microtubules surrounded by nine microtubular doublets comprising one complete (A) and one partial microtubule (B) fused together (Sale and Satir 1976). The basal bodies underlying cilia are composed of nine sets of triplet microtubules, each triplet containing one complete (A) fused to two incomplete microtubules (B and C). The basal bodies are surrounded by a set of fibrous rootlets, constituting together a structural unit called a kinetid (Lynn 1981). The canonical set of these rootlets comprises the postciliary microtubular ribbons, transverse microtubular fibres, and the nonmicrotubular striated rootlet (known as the kinetodesmal fibre) directed to the anterior pole of the cell (Allen 1967). The longitudinal bundle of basal microtubules also lies in close vicinity of the basal bodies. The kinetids can possess either one (monokinetids) or two basal bodies (dikinetids). In dikinetids, the basic pattern of rootlets is slightly modified (Iftode and Fleury-Aubusson 2003). In *T. thermophila*, the cell is covered with monokinetids except for the anterior ends of ciliary rows, where dikinetids form an asymmetrical apical crown (Jerka-Dziadosz 1981). In contrast, monokinetids and dikinetids alternate in the antero-posterior rows in *P. tetraurelia* (Iftode and Fleury-Aubusson 2003).

In all ciliates, the basal bodies and associated rootlets are anchored in the submembranous skeletal layer, called the epiplasm. In *Tetrahymena* spp., it is a continuous, amorphous, dense layer, whereas in *Paramecium* spp., the architecture is more complex. The epiplasm is divided into separate curved scales forming a regular pattern under the plasma membrane. It contains microtubular structures and the alveolar system, as well as dense core secretory granules called mucocysts for *Tetrahymena* spp. and trichocysts for *Paramecium* spp. The rapid and synchronous exocytosis of mucocyst or trichocyst contents through their opening is in fact an important part of cellular self-defence response (Galvani and Sperling 2000). The alveolar system consists of membrane-delimited interconnected sacs that serve as a vast Ca^{2+} storage compartment involved in control of trichocyst exocytosis, ciliary beating, and cytoskeletal dynamics during division (Stelly et al. 1991, Plattner et al. 1999). A schematic view of the cortical ultrastructure of *Tetrahymena* species is depicted in Fig. 2.

Oral apparatus

The oral apparatus architecture is exceedingly complex; it includes microtubular structures, such as modified cilia, oral connectives, and the postoral deep fibre, as well as membranelles, undulating membranes and nonmicrotubular cytoskeletal systems (Nilsson and Williams 1966).

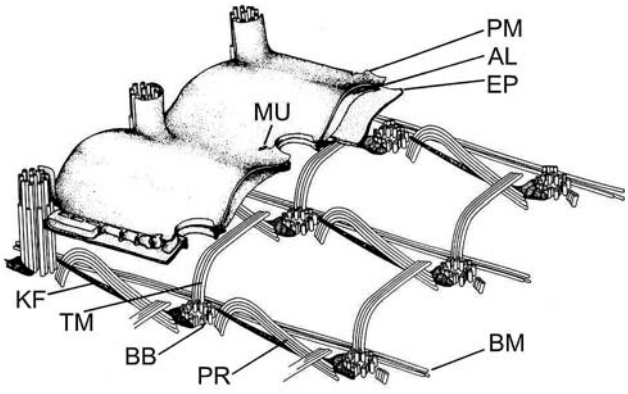

Fig. 2. Cortical ultrastructure of *Tetrahymena* sp. *AL* Alveoli, *BB* basal body, *BM* basal microtubules, *EP* epiplasm, *KF* kinetodesmal fibre, *MU* mucocyst opening, *PM* plasma membrane, *PR* postciliary microtubular ribbon, *TM* transverse microtubular fibre. Modified from Lynn and Small (1988) and Lee et al. (2000). With the permission of Allen Press

Contractile vacuole pore and intracytoplasmic microtubules

In contractile vacuole systems, the circular and radial network of microtubules is involved in enhancing membrane tubulation activity and governs the exocytotic cycle (Tominaga et al. 1999). The intracytoplasmic network of microtubules was first described in conjugating cells (Gaertig and Fleury 1992), although it is also present in vegetative cells (Stargell et al. 1992). Its role is still poorly understood.

Microtubules in nuclear division

Several microtubular structures are connected with nuclear division in ciliated protozoans, because each cell contains two distinct nuclei – the transcriptionally silent micronucleus and the transcriptionally active macronucleus. Although microtubules are required for proper division of both nuclei, macronuclear chromosomes lack centromeres and the role of microtubules in the macronucleus has not yet been fully elucidated. Both nuclei divide without nuclear-envelope breakdown. As the dividing macronucleus starts to elongate, an elliptical marginal band of intranuclear microtubules assembles. Most of these are oriented parallel to the longitudinal axis of the nucleus throughout elongation. The intranuclear microtubular band breaks down shortly after macronuclear elongation is completed (Tucker et al. 1980). The peripheral sheath of microtubules is also present during macronuclear division but there are no kinetochore microtubules (Davidson and LaFountain 1975).

The micronuclear mitotic spindle contains several distinctive elements. Approximately 150 continuous micro-

tubules form a peripheral sheath inside the nuclear membrane, while the kinetochore bundles traverse the centre of the nucleoplasm. A new set of microtubules, called the separation spindle, appears during micronuclear elongation in late anaphase (Davidson and LaFountain 1975). The peripheral sheath, as well as the midportion of the separation spindle, consists of microtubules with an atypical diameter of 27–32 nm. Moreover, microtubules in the midportions of well-elongated spindles are constructed from 14–16 protofilaments. Only a few 24 nm diameter microtubules with the canonical 13 protofilaments are present. Polymerization of microtubules with atypical diameters continues as spindle elongation progresses (Tucker et al. 1985).

Microtubules in the process of conjugation

Another set of microtubular structures is connected with sexual mating in ciliates, termed conjugation. Conjugation involves a succession of nuclear processes – reduction of the amount of genetic material by micronuclear meiosis, postmeiotic division, and formation of gametic micronuclei (known as pronuclei), reciprocal pronuclear exchange between the partners, fertilization, and development of somatic macronuclei and micronuclei together with destruction of the old macronuclei (Frankel 2000). In meiotic micronuclear division, microtubular structures analogous to mitotic structures (peripheral sheath, kinetochore microtubules) play a role (Davidson and LaFountain 1975). After meiotic division, all four haploid daughter micronuclei are connected by the microtubular network to the paroral region and are moved closer to this region. Under physiological conditions, only one of the four germ nuclei is moved into the paroral cone and is covered with microtubules. This nucleus survives, while the other three degenerate. This surviving germ nucleus divides once more and produces a migratory and a stationary pronucleus. Prior to the reciprocal exchange of the migratory nuclei, microtubules assemble around the migratory pronuclei again, forming the pronuclear transfer basket (Orias et al. 1983). The migratory pronucleus then moves into the partner cell and fuses with the resident stationary pronucleus. This postmeiotic migration is also mediated by microtubular structures (Nakajima et al. 2002).

Tubulins and their genes

The sequencing of *T. thermophila*, *P. tetraurelia* and also partly of *T. pyriformis* is in progress. Although the *T. thermophila* macronuclear genome sequencing project is nearly finished, none has yet been fully completed. An overview of the tubulin genes from these organisms recorded in the NCBI database is summarized in Table 1. Most of tubulin genes listed in Table 1 are well annotated, their properties have been studied and localization or functional analysis of the corresponding proteins published.

α- and β-tubulins

The haploid genome of *T. thermophila* contains only one α-tubulin gene (*ATU*), encoding a single protein (Callahan et al. 1984). The expression of this gene is not stable. α-Tubulin mRNA levels oscillate in a cell-cycle-dependent fashion due to changes in both transcription and mRNA decay rates. α-Tubulin mRNA levels increased in growing cells and decreased in starving cells or during conjugation (McGrath et al. 1994). On the other hand, the translational efficiency of α-tubulin mRNA was largely unaffected when the rate of tubulin synthesis was raised more than 100-fold. Thus, differential regulation of α-tubulin mRNA translation initiation does not contribute to the induction of tubulin synthesis observed during cilia regeneration and in growing cells (Calzone et al. 1988). The key role of the α-tubulin gene was verified by Hai and Gorovsky (1997). One of the two copies of the *ATU* gene in the diploid germ-line micronucleus was disrupted by biolistic bombardment transformation. The heterozygous germline transformants were made homozygous in the micronucleus, although they still had wild-type α-tubulin genes in the macronucleus. When these cells were mated, the exconjugant progeny cells failed to grow because the new somatic macronuclei had no functional α-tubulin genes. However, the conjugants were rescued by transformation with a functional marked α-tubulin gene.

The two β-tubulin genes of *T. thermophila* (*BTU1* and *BTU2*) encode the same 443-amino-acid peptide, which is 99.7% identical to the β-tubulin proteins of *T. pyriformis* and 95% identical to human beta-1 tubulin (Gaertig et al. 1993). A mutation in BTU1 that confers hypersensitivity to the microtubule-stabilizing drug paclitaxel leads to nuclear-division defects in *T. thermophila*, producing cells with a widely variable DNA content, including a complete lack of macronuclei. Immunofluorescence studies revealed elongated microtubular structures surrounding macronuclei that failed to migrate to the cleavage furrows. In contrast, other cytoplasmic microtubule-dependent processes, such as cytokinesis, cortical patterning, and oral apparatus assembly, appeared to be unaffected in the mutant. Normal macronuclear division was restored when the *btu1-1* mutant allele was inactivated by targeted disruption or expressed as a truncated protein (Smith et al. 2004). The expression of

Table 1. Tubulin genes in *Tetrahymena* and *Paramecium* species

Organism	Gene	DNA sequence name, NCBI nucleotide database accession nr.	Reference(s) or source	Protein length, NCBI protein database accession nr.
T. thermophila	α-tubulin	ATU, M86723	McGrath et al. 1994	449 aa, AAA21350
	β-tubulin	BTU1, L01415	Gaertig et al. 1993	443 aa, AAA30110
		BTU2, L01416	Gaertig et al. 1993	443 aa, AAA30111
	γ-tubulin	GTU, U96076	Shang et al. 2002	449 aa, AAB65830
T. pyriformis	α-tubulin	αTT, X12767	Barahona et al. 1988	449 aa, CAA31256
	β tubulin	βTT1, X12768	Barahona et al. 1988	443 aa, CAA31257
		βTT2, X12769	Barahona et al. 1988	443 aa, CAA31258
	γ-tubulin	AF293353	E. Joachimiak et al., Warsaw, unpubl. results	441 aa, AAG44954
P. tetraurelia	α-tubulin	αPT1, X99489	Dupuis-Williams et al. 1996	449 aa, CAA67847
		αPT2, X99490	Dupuis-Williams et al. 1996	449 aa, CAA67848
	β-tubulin	βPT1, X67237	Dupuis 1992	442 aa, CAA47663
		βPT2, AJ608918	Ruiz et al. 2004	442 aa, CAE75645
		βPT3, AJ608919	Ruiz et al. 2004	442 aa, CAE75646
	γ-tubulin	γPT1, AJ012329	F. Ruiz et al., Gif-sur-Yvette, unpubl. results	455 aa, CAA09991
		γPT2, AJ012330	F. Ruiz et al., Gif-sur-Yvette, unpubl. results	455 aa, CAA09992
	δ-tubulin	δPT1, AJ401299	Ruiz et al. 2000, Garreau de Loubresse et al. 2001	399 aa, CAC10567
	ε-tubulin	εPT1, AJ427411	Dupuis-Williams et al. 2002	469 aa, CAD20554
	η-tubulin	sm19, AJ272425	Ruiz et al. 2000	476 aa, CAB99490
	θ-tubulin	theta-tubulin, AJ427480	P. Dupuis-Williams, Paris, unpubl. results	437 aa, CAD20607
	ι-tubulin	iota-tubulin, AJ427481	P. Dupuis-Williams, Paris, unpubl. results	408 aa, CAD20608
	κ-tubulin	κPT1, AJ576069	F. Ruiz, Gif-sur-Yvette, unpubl. results	448 aa, CAE11219

the β-tubulin gene *BTU1* is preferentially induced by antimicrotubule drug treatment in *T. thermophila*. In contrast, deciliation induces expression of both β-tubulin genes. This implies the existence of at least two distinct pathways regulating β-tubulin gene transcription. While one pathway must sense the loss of cilia per se, the other must operate through the cytoplasmic microtubule system. It also shows that the cytoplasmic microtubule cytoskeleton itself participates in a signal transduction pathway that regulates specific tubulin gene transcription (Gu et al. 1995).

The detailed study of α- and β-tubulin C-terminal tails in *T. thermophila* has emphasized the essential function of these parts of the molecules, which could not be visualized by electron crystallography. Mutations, deletions, C-terminal tail switches, and duplications of the last 13 amino acid residues of α-tubulin and the last 17 of β-tubulin have been created by mutagenesis and homologous gene replacement. Although the presence of the very end part is indispensable, the α- and β-tubulin tails are interchangeable and cells grow normally with either an α- or a β-tubulin tail on both tubulins. However, an α-tubulin gene containing a duplicated α-tubulin C terminus cannot rescue deletion of the β-tubulin tail (Duan and Gorovsky 2002).

Tetrahymena pyriformis, like *T. thermophila*, carries one α- (*αTT*) and two β-tubulin (*βTT1* and *βTT2*) genes. The β-tubulin proteins differ only in two amino acid residues (Barahona et al. 1988). Although different tubulin isotypes perform specific functions in many organisms, it is hard to imagine that such a minor change as in this case would alter the functional properties of the molecule.

For *P. tetraurelia*, analysis of the tubulin gene family has revealed the existence of two α- and three β-tubulin genes. Sequencing of the two α-tubulin genes, *αPT1* and *αPT2*, has shown that they are intronless and code for very similar polypeptides, differing only in their C-terminal amino acids, glycine and alanine, respectively. Distribution of these two proteins throughout the microtubular network is uniform. The striking similarity between all α-tubulin genes suggests a recent common origin by gene duplication

or homogenization by gene conversion. The C-terminal amino acid tyrosine is absent from α-tubulin molecules in *P. tetraurelia*, although it is present in *Tetrahymena* tubulin (Dupuis-Williams et al. 1996).

The coding sequence of the first β-tubulin gene (*βPT1*) is interrupted by two short noncoding sequences of 27 bp each (Dupuis 1992). The amino acid sequences of the other two β-tubulin genes (*βPT2* and *βPT3*) show 100% identity with *βPT1* (Ruiz et al. 2004).

All β-tubulin isotypes of *Tetrahymena* spp. and *P. tetraurelia* carry the amino acid sequence EGEFEEE very close to the C terminus. It has been proposed that the EGEFXXX sequence in this region of β-tubulin allows the molecule to be incorporated into axonemal microtubules (Raff et al. 1997).

Apart from defined microtubular structures, the αβ-tubulin dimers are also found in the soluble pool of protein molecules in the cytoplasm (Seyfert and Sawatzki 1986). Tubulin has also been identified as a membrane component in ciliates, although the experimental data vary considerably. Whereas Adoutte et al. (1980) found only trace amounts in *T. pyriformis* and *P. tetraurelia* cilia, Dentler (1980) detected tubulin as a major protein in *T. pyriformis* ciliary membranes. These discrepancies may be caused by different membrane removal techniques, proteolytic cleavage of tubulin, or other methodological reasons.

Tubulin dimers are target molecules for many different drugs with stabilizing (e.g., taxol) or destabilizing (e.g., colchicine) effects on microtubules (Amos 2004). Although tubulin in ciliates is more resistant to these drugs and higher concentrations must be used, the microtubular structures are also affected. When *T. pyriformis* cells were treated with 50 μM taxol for 90 min, the intracellular microtubular arrays were replaced by bundles of microtubules located around the macronucleus. In cells treated with 10 mM colchicine for 90 min, the cortical microtubules disappeared. An analysis of total protein extracts showed that the level of tubulin did not change significantly during taxol treatment. After colchicine treatment, the amount of tubulin in total protein extracts decreased (Casalou et al. 2001). On the other hand, taxol treatment induced a slight increase in the synthesis of tubulin in *T. thermophila* (Stargell et al. 1992). The drugs influence many important processes in cells, including nuclear division and conjugation. Another microtubular drug, vinblastine, inhibited the migration of the four daughter nuclei into the paroral region in *P. caudatum*. All nuclei degenerated shortly afterwards and nuclear migration failed (Yanagi and Hiwatashi 1985).

γ-Tubulin

The presence of γ-tubulin and its permanent association with basal bodies was observed for the first time in *T. thermophila* and *P. tetraurelia* with polyclonal antibodies raised against phylogenetically conserved parts of the protein (Liang et al. 1996). *Tetrahymena thermophila* carries a single-copy γ-tubulin gene (*GTU*) which is essential (Shang et al. 2002). γ-Tubulin function has been studied by placing the *GTU* gene under the control of an inducible promoter. The tagged version of γ-tubulin localized to four distinct microtubule-organizing centres: basal bodies, macronuclear envelopes, micronuclear envelopes, and contractile vacuole pores. Overexpression of γ-tubulin had no detectable effect on cell growth or morphology. *GTU* knockouts showed apparent changes in cell morphology and in microtubule bundling. Among all the microtubule-organizing centres, basal bodies seemed to be the most sensitive to γ-tubulin depletion. γ-Tubulin was found to be required not only for the formation of new basal bodies but also for the maintenance of mature ones. After depletion of γ-tubulin, basal bodies also gradually lost other markers of microtubule-organizing centres, such as centrin and glutamylated tubulin. When GTU expression was reinduced in cells, basal bodies re-formed rapidly, and the normal, highly organized structure of the *T. thermophila* cell cortex was reestablished, indicating that the precise patterning of the cortex can be formed de novo (Shang et al. 2002). One γ-tubulin gene has also been detected in *T. pyriformis* (Joachimiak et al., NCBI database). As expected, the protein shows high similarity to *T. thermophila* γ-tubulin, although there are some differences, particularly in the C-terminal region.

In contrast to *Tetrahymena* spp., *P. tetraurelia* cells carry two γ-tubulin genes, *γPT1* and *γPT2*. Inactivation of the *P. tetraurelia* γ-tubulin genes leads to the inhibition of basal body duplication (Ruiz et al. 1999). γ-Tubulin has been found in a soluble form in the cytosol. It is also permanently localized to four types of sites: basal bodies, the micronuclear compartment, the pores of the contractile vacuoles, and the cytoproct. In addition, γ-tubulin associates transiently with the region of nuclear exchange during conjugation (Klotz et al. 2003).

Other members of the tubulin family

Apart from the conventional α-, β-, and γ-tubulin genes, a number of new members of the tubulin family have been described in *P. tetraurelia*. The first of these is the single

gene *δPT1* coding for δ-tubulin (Ruiz et al. 2000). Inactivation of this gene led to a loss of the C-tubule in basal bodies but had no effect on ciliogenesis. This deficiency did not directly affect basal body duplication but perturbed the architecture of the cortical cytoskeleton, progressively leading to mislocalization and loss of basal bodies and to altered cell size and shape. Furthermore, a loss of B- and even A-tubules at one or more triplet sites was detectable in some cases. The remaining doublets were positioned according to the canonical ninefold symmetry, regardless of the missing microtubules. This study confirmed a role for δ-tubulin in C-tubule assembly and also demonstrated the existence of a prepattern for the ninefold symmetry of the basal body (Garreau de Loubresse et al. 2001).

Another component localizing to the basal bodies is ε-tubulin. The ε-tubulin gene (*εPT1*) is essential for the assembly and anchorage of the basal bodies. Gene silencing led to a reduction in basal body number and a disorganization of the basal body pattern in the cortex and oral apparatus. The changes were not immediately apparent; they gradually evolved over several cell cycles. This contrasts with the drastic arrest of basal body duplication induced by γ-tubulin silencing (Ruiz et al. 1999), suggesting that ε-tubulin could be involved at a later stage of basal body biogenesis (Dupuis-Williams et al. 2002).

The gene originally named *SM19* was later found to encode η-tubulin. Mutations of the *SM19* gene led to a progressive reduction in the number of basal bodies, accompanied by reduced cell length and modified cell shape (Ruiz et al. 1987). Mutant cells also showed a delocalization of γ-tubulin. This delocalization suggests that η-tubulin might tether γ-tubulin complexes to the nucleation site in basal bodies (Ruiz et al. 2000). Further studies have revealed an interaction between η- and β-tubulin molecules. The results indicate that η-tubulin can cap the microtubule minus end via a direct or indirect interaction with the β-tubulin subunit and thus either stabilize nascent microtubules in developing basal bodies or transduce a signal for basal body duplication (Ruiz et al. 2004).

The gene databases are also complemented with the sequences of θ-, ι-, and κ-tubulins from *Paramecium* spp. Phylogenetic analysis of their sequences has shown that κ-tubulin is grouped with α-tubulin, whereas θ-tubulin belongs to the β-tubulin branch (Dutcher 2003). However, the role of these minor tubulins in cell architecture and organization of the microtubular network remains unknown.

Newly predicted tubulins

The NCBI database obviously does not contain all tubulin sequences occurring in *Tetrahymena* and *Paramecium* species. The Tetrahymena Genome Database (TGD; http://www.ciliate.org/), *T. thermophila* Gene Index (TIGR; http://www.tigr.org/tdb/tgi/protist.shtml), and *P. tetraurelia* sequencing project on Genoscope (http://www.genoscope.cns.fr) harbour further molecules, which apparently belong to the tubulin family. However, these sequences are only very roughly annotated and no experimental data on them are available. We have performed blastp searches in the above databases using the *T. thermophila* β-tubulins and revealed the presence of as yet undisclosed tubulin isotypes in the *T. thermophila* and *P. tetraurelia* genomes. Upon careful inspection, some of these genes turned out to contain errors in the underlying genomic sequence (due to incorrect assembly of sequencing reads) or exon-intron structures incorrectly determined during the automated annotation. Although the level of similarity varies among the corresponding proteins, the most conserved areas are the GTP-binding domain in the N-terminal region and a part of the C-terminal region. It is known that the C-terminal part of the tubulin molecule is involved in interactions with microtubular motors and several microtubule-associated proteins (Sackett 1995, Kikkawa et al. 2000, Niederstrasser et al. 2002).

Phylogenetic analysis of the corrected tubulin sequences revealed novel paralogues to already known tubulins in both *T. thermophila* and *P. tetraurelia*. *Tetrahymena thermophila* contains δ- (ID 34.m00277 in TGD) and ε-tubulin (ID 8.m00446 in TGD), whereas three novel α-tubulin sequences (ID GSPATP00002927001, GSPATP00021912001 and two parts of one protein GSPATP00030885001 and GSPATP00030884001 in the Genoscope database) were found in *P. tetraurelia*. Moreover, another ι-tubulin (ID GSPATP00032612001 in the Genoscope database) was detected, although there is already one ι-tubulin sequence in the NCBI database. The same could hold true for an additional κ-tubulin (ID GSPATP00034751001 in the Genoscope Paramecium database), but the sequence similarity is weaker in this case. However, the localization, function, and possible posttranslational modifications of all these anticipated tubulins are completely unknown. In this context, it is worth mentioning that no obvious orthologues of η-, θ-, ι-, and κ-tubulin genes have been sequenced in *Tetrahymena* spp. so far. One cannot exclude that further thorough analysis of tubulin-like sequences in the completely sequenced genomes would identify still more tubulins in *Tetrahymena* and *Paramecium* species.

Posttranslational modifications of tubulin dimers

The heterogeneity of tubulins observed in most eukaryotes, including ciliates, can be attributed to the expression of distinct tubulin genes, to posttranslational modifications of tubulin molecules, or to combinations of both. In *T. pyriformis*, two-dimensional electrophoretic analysis of the ciliary tubulins showed the presence of eight α-tubulins and four β-tubulin isoforms (Barahona et al. 1988). Another study identified between five and seven α-tubulin isoforms in cilia and four or five in the cytoskeletal fraction of *T. pyriformis* cells (Penque et al. 1991). Similarly, Nakamura et al. (1992) detected seven α- and four β-tubulin isoforms among *Tetrahymena* ciliary proteins. In *P. tetraurelia*, analysis of the soluble tubulin pool has led to the identification of at least six α- and five β-tubulin isoforms (Dupuis-Williams et al. 1996). The number of detected α- and β-tubulin isoforms could exceed the number of tubulin genes present in ciliates. Thus, posttranslational modifications are one of the mechanisms by which distinct tubulin isoforms can arise. The known posttranslational modifications in *Tetrahymena* and *Paramecium* species are summarized in Table 2.

Acetylation

In *T. thermophila*, like in most eukaryotes, α-tubulin is acetylated at the ε-amino group of lysine 40. This posttranslational modification is detectable in cilia and numerous other microtubular structures, with the exception of the most labile intracytoplasmic microtubules. Replacement of the wild-type α-tubulin gene in the macronucleus with a version encoding arginine instead of lysine 40, and therefore preventing acetylation at this position, produced no detectable phenotypic changes. Thus, acetylation of α-tubulin at lysine 40 is nonessential in *T. thermophila* (Gaertig et al. 1995). This result was unexpected because of the high phylogenic conservation of this acetylation site in α-tubulin, and also because α-tubulin is fully acetylated in some other protozoan species (Schneider et al. 1997, 1998; Weber et al. 1997). On the other hand, α-tubulin acetylation seems to be redundant in *Chlamydomonas reinhardtii* and maybe even in mammalian cells (Kozminski et al. 1993, Zhang et al. 2003). The tubulin acetyltransferase and the enzyme involved in α-tubulin deacetylation remain unidentified in ciliates. In *P. tetraurelia*, acetylated α-tubulin is localized in ciliary axonemes and a set of stable microtubular arrays associated with the cortex (Adoutte et al. 1991).

Tyrosination and detyrosination

Another posttranslational modification occurring in ciliates is the tyrosination–detyrosination cycle of α-tubulin. The C-terminal tyrosine of α-tubulin can be removed by tubulin tyrosine carboxypeptidase to uncover the penultimate amino acid, which is usually glutamate. The modified tubulin is then designated Glu-tubulin. The C-terminal tyrosine can be added back through the enzymatic activity of tubulin tyrosine ligase (Westermann and Weber 2003). The majority of α-tubulin is detyrosinated in *T. thermophila* (Redeker et al. 2005). A novel type of tyrosine carboxypeptidase is probably involved in the removal of the tyrosine residue, because the penultimate amino acid is glycine instead of the usual glutamate in these cells. In *P. tetraurelia*, the α-tubulin genes have divergent C-terminal sequences and entirely lack the terminal tyrosine (Dupuis-Williams et al. 1996). The C-terminal tyrosine apparently has no intrinsic functional role in ciliates. On the other hand, the tubulin tyrosination–detyrosination cycle is a well-established posttranslational modification in a great variety of species, ranging from humans through plants to trypanosomes. However, the situation in ciliates suggests that this cycle has no universal significance

Table 2. Posttranslational modifications of tubulins in *Tetrahymena* and *Paramecium* species[a]

Modification	Tubulin	*Tetrahymena* spp.	*P. tetraurelia*
Acetylation	α	+ (Lys40)	+ (Lys40)
Tyrosination/detyrosination	α	+ (Tyr449)	−
Phosphorylation	β	+	ND
Glutamylation	α	+	+
	β	+	+
Glycylation	α	+ (Glu445, Glu446, Glu 447)	+ (Glu445)
	β	+ (Glu437, Glu438, Glu439, Glu440, Glu 442)	+ (Glu437, Glu438, Glu439, Glu441)

[a] +, modification is present; −, modification is not present; ND, not determined. The known positions of modified amino acids are indicated in brackets. References are in the text

for the morphogenesis of highly sophisticated microtubular structures in these organisms.

Phosphorylation

Phosphorylation is a very important modification for microtubule-associated protein regulation but seems to play only a minor role for tubulin dimers. In *Tetrahymena* ciliary axonemes, β-tubulin has been found to be exclusively phosphorylated by endogenous Ca^{2+}/calmodulin-dependent protein kinase(s) (Hirano-Ohnishi and Watanabe 1989). The functional relevance of this modification in ciliates is still unknown.

(Poly)glutamylation

Glutamylation involves the addition of a variable number of glutamate residues to the side γ-carboxyl group of specific glutamic acids near the C termini of α- and β-tubulin (Edde et al. 1990). Both axonemal and cytoplasmic tubulin is glutamylated in *Paramecium* and *Tetrahymena* species. The glutamylation site is located in the C-terminal region that can be removed by subtilisin treatment. Unlike tubulin acetylation, glutamylation is not restricted to superstable microtubules (Bré et al. 1994). Axonemal tubulin is glutamylated at a much higher level in *T. thermophila* than in *P. tetraurelia*, and α-tubulin seems to be more glutamylated than β-tubulin (Redeker et al. 2005). A gradient of glutamylated tubulin labelling has been observed in axonemal microtubules of *P. tetraurelia* (Pechart et al. 1999). Actually, although the basal bodies are polyglutamylated, arising axonemes are bi- or monoglutamylated just at their base (Kann et al. 2003). In mammalian cells, microinjection of antibody against glutamylated tubulin led to the complete disassembly of centrioles, which are basal body analogues (Bobinnec et al. 1998). Polyglutamylation probably also influences the interaction between microtubules and microtubule-associated proteins (Larcher et al. 1996, Bonnet et al. 2001).

Recently, enzymes associated with polyglutamylase activity have been detected in *T. thermophila*. These proteins possess a tubulin tyrosine ligase-like domain. Ttll11p has a polyglutamylase activity directed towards α-tubulin, whereas Ttll6Ap is a β-tubulin-preferring enzyme. Overexpression of Ttll6Ap led to a strong increase in polyglutamylation of cilia and cortical microtubules. The excessive polyglutamylation of β-tubulin (and also partly α-tubulin) negatively affected cell proliferation and cilia motility, which became paralyzed several hours after overexpression induction (Janke et al. 2005).

(Poly)glycylation

The basic mechanism of polyglycylation is the same as that of polyglutamylation. A polyglycine side chain of variable length is covalently attached to the γ-carboxyl group of glutamate residues that are in the C termini of α- and β-tubulin (Westermann and Weber 2003). This modification was detected for the first time in the C-terminal region of axonemal tubulin from *P. tetraurelia*. Both α- and β-tubulin subunits are modified by polyglycylation, containing up to 34 glycyl units covalently bound to glutamyl residues. The side polyglycine chains are linked to Glu445 in α-tubulin and Glu437 in β-tubulin (Redeker et al. 1994). Additional glycylation sites have been discovered in the C-terminal part of β-tubulin. Altogether, β-tubulin from *P. tetraurelia* can be glycylated on each of the last four C-terminal glutamate residues Glu437, Glu438, Glu439 and Glu441. In both cytoplasmic and axonemal microtubules, the most abundant β-tubulin isoform contains six posttranslationally added glycine residues: two on both Glu437 and Glu438 and one on both Glu439 and Glu441 (Vinh et al. 1999). The polyglycylation results in bulky C-terminal domains which may affect interactions between microtubules and microtubule-associated proteins. In fact, the entire axonemal α/β-tubulin of *P. tetraurelia* is glycylated (Redeker et al. 2005). The level of glycylation has been studied with antibodies with different affinities towards mono- and polyglycylated tubulins. This approach revealed that cytoplasmic tubulin is polyglycylated at a much lower level than is the highly modified axonemal tubulin (Bré et al. 1998). Polyglycylation has been shown to take place early in the course of morphogenesis; and the lengthening of the polyglycine chains occurs after a great delay following addition of the first glycine residue, and following microtubule assembly in *P. tetrautelia* (Iftode et al. 2000). Deglycylase activity has been detected in cytoplasmic extracts of *P. tetraurelia* (Bré et al. 1998).

Glycylated tubulin has also been found in cilia and cortical microtubules of *Tetrahymena* spp., though nonmodified ciliary α-tubulin was readily detected in *T. thermophila* (Redeker et al. 2005). In *T. thermophila*, three polyglycylation sites have been identified near the C terminus of α-tubulin (Glu445, Glu446, Glu447) and five in β-tubulin (Glu437, Glu438, Glu439, Glu440, Glu442). Whereas the polyglycylation sites on α-tubulin are dispensable, elimination of five to three of the glutamic acids required as polyglycylation sites on β-tubulin was either lethal or caused slow growth, low motility, and defects in *T. thermophila* cell division (Xia et al. 2000). Viable mutants with three

polyglycylation sites on β-tubulin produced immotile 9 + 0 axonemes; in addition, cytokinesis was impaired. The glycylation domain of β-tubulin is also required for maintenance of the length of growing and already assembled cilia. In contrast to the aberrant cilia, several types of cortical systems showed an abnormally high number of microtubules in the same mutant cell. Deficiencies in tubulin glycylation are structure-specific and lead to either insufficient assembly of cilia axonemes or excessive assembly and stability of cortical microtubules (Thazhath et al. 2004). In *Tetrahymena* spp., a substantial portion of tubulin isoforms, especially of α-tubulin, is both glutamylated and glycylated on the same molecule, and each level of glutamylation corresponds with one or more levels of glycylation. Mutations of β-tubulin glycylation sites dramatically affect both polyglycylation and polyglutamylation in α-tubulin. This strong effect of mutations on the nonmutated subunit suggests the existence of cross-talk between the α- and β-subunits of the tubulin dimers. This cross-talk could take place either within the same dimer or between two subunits of adjacent tubulin dimers within a microtubule, given that both tubulin and microtubules could be substrates of polymodification enzymes. Specifically, the tubulin glutamylase could have its binding site on β-tubulin and glutamylate the α-subunit. Mutation of the tail domain of β-tubulin could affect the enzyme binding either directly or through a conformational change in β-tubulin. Similarly, a certain level of glycylation on the β-tubulin tail might be required for modification of β-tubulin itself. It has been proposed that polyglycylation and/or polyglutamylation stabilizes the B-microtubule of the axonemal outer doublets and regulates intraflagellar transport (Redeker et al. 2005).

Concluding remarks

The research on microtubular structures in ciliates has shed more light on new members of the growing tubulin superfamily and on the mechanisms underlying microtubule diversity. The role of δ-, ε-, and η-tubulin is apparently connected with the triplet microtubules of basal bodies underlying ciliary axonemes. Localization and function of θ-, ι-, and κ-tubulin are yet to be uncovered. Future full analysis of sequenced genomes could bring further surprises in the tubulin field, including identification of new tubulin family members or new isotypes of already established tubulins. In addition to this, posttranslational modifications are considerable generators of tubulin diversity in *Tetrahymena* and *Paramecium* species. Investigation of these modifications has revealed that polyglycylation and polyglutamylation play pivotal roles in the generation of basal bodies and axonemes. On the other hand, tubulin acetylation and tyrosination and detyrosination do not seem to have essential functions in ciliates, although they are assumed to be important in many other cells. The functional consequence of posttranslational tubulin modifications can be tested in *Tetrahymena* and *Paramecium* species by knockout and gene dominance or replacement approaches. Since these modifications may affect the assembly properties of tubulins, it would be interesting and instructive to know more about their localizations in distinct structures and about their interactions with other proteins. Much work also remains to be done to ascertain the mechanisms and regulation of posttranslational modifications in ciliates. Most of the enzymes involved in *Tetrahymena* and *Paramecium* species have not yet been isolated and properly characterized. This would substantially enhance our understanding of tubulin modifications.

Since respiratory, reproductive, and neural tissues comprise cells carrying cilia, an insight into the role of tubulin in cilia assembly is also important for understanding human diseases. We already know that abnormal ciliary activity affects egg movement through the oviduct, mucus clearance from the respiratory tract, or the role of ependymal cells in the nervous system. Ciliates are also excellent model organisms for investigations into the structure of microtubule-organizing centres. Research on microtubule-organizing centre duplications and on their functions in cells is crucial for a better understanding of mitotic and meiotic spindles. Defects in these processes lead to unequal chromosome segregation and, moreover, a variety of human cancers exhibits centrosome amplification.

Acknowledgments

This work was supported by grant LC545 from the Ministry of Education of the Czech Republic and by the Institutional Research Support AVOZ 50520514. We thank Marek Eliáš (Faculty of Science, Charles University, Prague) for phylogenetic analysis of tubulin sequences.

References

Adoutte A, Ramanathan R, Lewis RM, Dute RR, Ling KY, Kung C, Nelson DL (1980) Biochemical studies of the excitable membrane of *Paramecium tetraurelia*. III. Proteins of cilia and ciliary membranes. J Cell Biol 84: 717–738

Adoutte A, Delgado P, Fleury A, Levilliers N, Laine MC, Marty MC, Boisvieux-Ulrich E, Sandoz D (1991) Microtubule diversity in ciliated cells: evidence for its generation by post-translational modification in the axonemes of *Paramecium* and quail oviduct cells. Biol Cell 71: 227–245

Allen RD (1967) Fine structure, reconstruction and possible functions of components of the cortex of *Tetrahymena pyriformis*. J Protozool 14: 553–565

Allen RD, Wolf RW (1974) The cytoproct of *Paramecium caudatum*: structure and function, microtubules, and fate of food vacuole membranes. J Cell Sci 14: 611–631

Amos LA (2004) Microtubule structure and its stabilisation. Org Biomol Chem 2: 2153–2160

Barahona I, Soares H, Cyrne L, Penque D, Denoulet P, Rodrigues-Pousada C (1988) Sequence of one alpha- and two beta-tubulin genes of *Tetrahymena pyriformis*. Structural and functional relationships with other eukaryotic tubulin genes. J Mol Biol 202: 365–382

Bobinnec Y, Khodjakov A, Mir LM, Rieder CL, Edde B, Bornens M (1998) Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. J Cell Biol 143: 1575–1589

Bonnet C, Boucher D, Lazereg S, Pedrotti B, Islam K, Denoulet P, Larcher JC (2001) Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J Biol Chem 276: 12839–12848

Bré MH, de Nechaud B, Wolff A, Fleury A (1994) Glutamylated tubulin probed in ciliates with the monoclonal antibody GT335. Cell Motil Cytoskeleton 27: 337–349

Bré MH, Redeker V, Vinh J, Rossier J, Levilliers N (1998) Tubulin polyglycylation: differential posttranslational modification of dynamic cytoplasmic and stable axonemal microtubules in paramecium. Mol Biol Cell 9: 2655–2665

Callahan RC, Shalke G, Gorovsky MA (1984) Developmental rearrangements associated with a single type of expressed alpha-tubulin gene in *Tetrahymena*. Cell 36: 441–445

Calzone FJ, Callahan R, Gorovsky MA (1988) Direct measurement of tubulin and bulk message distributions on polysomes of growing, starved and deciliated *Tetrahymena* using RNA gel blots of sucrose gradients containing acrylamide. Nucleic Acids Res 16: 9597–9609

Casalou C, Cyrne L, Rosa MR, Soares H (2001) Microtubule cytoskeleton perturbation induced by taxol and colchicine affects chaperonin containing TCP-1 (CCT) subunit gene expression in *Tetrahymena* cells. Biochim Biophys Acta 1522: 9–21

Cavalier-Smith T (1993) Kingdom Protozoa and its 18 phyla. Microbiol Rev 57: 953–994

Davidson L, LaFountain JR Jr (1975) Mitosis and early meiosis in *Tetrahymena pyriformis* and the evolution of mitosis in the phylum Ciliophora. Biosystems 7: 326–336

Dentler WL (1980) Microtubule-membrane interactions in cilia. I. Isolation and characterization of ciliary membranes from *Tetrahymena pyriformis*. J Cell Biol 84: 364–380

Duan J, Gorovsky MA (2002) Both carboxy-terminal tails of alpha- and beta-tubulin are essential, but either one will suffice. Curr Biol 12: 313–316

Dupuis P (1992) The beta-tubulin genes of *Paramecium* are interrupted by two 27 bp introns. EMBO J 11: 3713–3719

Dupuis-Williams P, Klotz C, Mazarguil H, Beisson J (1996) The tubulin gene family of *Paramecium*: characterization and expression of the alpha PT1 and alpha PT2 genes which code for alpha-tubulins with unusual C-terminal amino acids, GLY and ALA. Biol Cell 87: 83–93

Dupuis-Williams P, Fleury-Aubusson A, de Loubresse NG, Geoffroy H, Vayssie L, Galvani A, Espigat A, Rossier J (2002) Functional role of epsilon-tubulin in the assembly of the centriolar microtubule scaffold. J Cell Biol 158: 1183–1193

Dustin P (1984) Microtubules, 2nd edn. Springer, Berlin

Dutcher SK (2003) Long-lost relatives reappear: identification of new members of the tubulin superfamily. Curr Opin Microbiol 6: 634–640

Edde B, Rossier J, Le Caer JP, Desbruyeres E, Gros F, Denoulet P (1990) Posttranslational glutamylation of alpha-tubulin. Science 247: 83–85

Elliott AM, Bak IJ (1964) The contractile vacuole and related structures in *Tetrahymena pyriformis*. J Protozool 11: 250–261

Frankel J (2000) Cell biology of *Tetrahymena thermophila*. In: Asai D, Forney J, Wilson L, Matsudaira P (eds) *Tetrahymena thermophila*. Methods in cell biology, vol 62. Academic Press, London, pp 27–125

Gaertig J, Fleury A (1992) Spatiotemporal reorganization of intracytoplasmic microtubules is associated with nuclear selection and differentiation during developmental process in the ciliate *Tetrahymena thermophila*. Protoplasma 167: 74–87

Gaertig J, Thatcher TH, McGrath KE, Callahan RC, Gorovsky MA (1993) Perspectives on tubulin isotype function and evolution based on the observation that *Tetrahymena thermophila* microtubules contain a single alpha- and beta-tubulin. Cell Motil Cytoskeleton 25: 243–253

Gaertig J, Cruz MA, Bowen J, Gu L, Pennock DG, Gorovsky MA (1995) Acetylation of lysine 40 in alpha-tubulin is not essential in *Tetrahymena thermophila*. J Cell Biol 129: 1301–1310

Galvani A, Sperling L (2000) Regulation of secretory protein gene expression in *Paramecium*. Role of the cortical exocytotic sites. Eur J Biochem 267: 3226–3234

Garreau de Loubresse N, Ruiz F, Beisson J, Klotz C (2001) Role of delta-tubulin and the C-tubule in assembly of *Paramecium* basal bodies. BMC Cell Biol 2: 4–10

Gu L, Gaertig J, Stargell LA, Gorovsky MA (1995) Gene-specific signal transduction between microtubules and tubulin genes in *Tetrahymena thermophila*. Mol Cell Biol 15: 5173–5179

Hai B, Gorovsky MA (1997) Germ-line knockout heterokaryons of an essential alpha-tubulin gene enable high-frequency gene replacement and a test of gene transfer from somatic to germ-line nuclei in *Tetrahymena thermophila*. Proc Natl Acad Sci USA 94: 1310–1315

Hirano-Ohnishi J, Watanabe Y (1989) Ca^{2+}/calmodulin-dependent phosphorylation of ciliary beta-tubulin in *Tetrahymena*. J Biochem (Tokyo) 105: 858–860

Iftode F, Fleury-Aubusson A (2003) Structural inheritance in *Paramecium*: ultrastructural evidence for basal body and associated rootlets polarity transmission through binary fission. Biol Cell 95: 39–51

Iftode F, Clerot JC, Levilliers N, Bré MH (2000) Tubulin polyglycylation: a morphogenetic marker in ciliates. Biol Cell 92: 615–628

Janke C, Rogowski K, Wloga D, Regnard C, Kajava AV, Strub JM, Temurak N, van Dijk J, Boucher D, Van Dorsselaer A, Suryavanshi S, Gaertig J, Edde B (2005) Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science 308: 1758–1762

Jerka-Dziadosz M (1981) Cytoskeleton-related structures in *Tetrahymena thermophila*: microfilaments at the apical and division-furrow rings. Cell Sci 51: 241–253

Kann ML, Soues S, Levilliers N, Fouquet JP (2003) Glutamylated tubulin: diversity of expression and distribution of isoforms. Cell Motil Cytoskeleton 55: 14–25

Kikkawa M, Okada Y, Hirokawa N (2000) 15 Å resolution model of the monomeric kinesin motor, KIF1A. Cell 100: 241–252

Klotz C, Ruiz F, Garreau de Loubresse N, Wright M, Dupuis-Williams P, Beisson J (2003) Gamma-tubulin and MTOCs in *Paramecium*. Protist 154: 193–209

Kozminski KG, Diener DR, Rosenbaum JL (1993) High level expression of nonacetylatable alpha-tubulin in *Chlamydomonas reinhardtii*. Cell Motil Cytoskeleton 25: 158–170

Larcher JC, Boucher D, Lazereg S, Gros F, Denoulet P (1996) Interaction of kinesin motor domains with alpha- and beta-tubulin subunits at a tau-independent binding site. Regulation by polyglutamylation. J Biol Chem 271: 22117–22124

Lee JJ, Leedale GF, Bradbury P (eds) (2000) An illustrated guide to the Protozoa, 2nd edn. Society of Protozoologists, Lawrence, Kan

Liang A, Ruiz F, Heckmann K, Klotz C, Tollon Y, Beisson J, Wright M (1996) Gamma-tubulin is permanently associated with basal bodies in ciliates. Eur J Cell Biol 70: 331–338

Lynn DH (1981) The organisation and evolution of microtubular organelles in ciliated Protozoa. Biol Rev 56: 243–249

Lynn DH, Small EB (1988) An update on the systematics of the phylum Ciliophora Doflein, 1901: the implications of kinetid diversity. Biosystems 21: 317–322

Machemer H (1996) A theory of gravikinesis in *Paramecium*. Adv Space Res 17: 11–20

McGrath KE, Yu SM, Heruth DP, Kelly AA, Gorovsky MA (1994) Regulation and evolution of the single alpha-tubulin gene of the ciliate *Tetrahymena thermophila*. Cell Motil Cytoskeleton 27: 272–283

Miller JM, Wang W, Balczon R, Dentler WL (1990) Ciliary microtubule capping structures contain a mammalian kinetochore antigen. J Cell Biol 110: 703–714

Mitchison T, Kirschner M (1984) Dynamic instability of microtubule growth. Nature 312: 237–242

Nakajima Y, Ishida M, Mikami K (2002) Microtubules mediate germ-nuclear behavior after meiosis in conjugation of *Paramecium caudatum*. J Eukaryot Microbiol 49: 74–81

Nakamura K, Okuya Y, Katahira M, Yoshida S, Wada S, Okuno M (1992) Analysis of tubulin isoforms by two-dimensional gel electrophoresis using SDS-polyacrylamide gel electrophoresis in the first dimension. J Biochem Biophys Methods 24: 195–203

Niederstrasser H, Salehi-Had H, Gan EC, Walczak C, Nogales E (2002) XKCM1 acts on a single protofilament and requires the C terminus of tubulin. J Mol Biol 316: 817–828

Nilsson JR, Williams NE (1966) An electron microscope study of the oral apparatus of *Tetrahymena pyriformis*. C R Trav Lab Carlsberg 35: 119–141

Orias JD, Hamilton EP, Orias E (1983) A microtubule meshwork associated with gametic pronucleus transfer across a cell-cell junction. Science 222: 181–184

Pechart I, Kann ML, Levilliers N, Bré MH, Fouquet JP (1999) Composition and organization of tubulin isoforms reveals a variety of axonemal models. Biol Cell 91: 685–697

Penque D, Galego L, Rodrigues-Pousada C (1991) Multiple alpha-tubulin isoforms in cilia and cytoskeleton of *Tetrahymena pyriformis* generated by post-translational modifications. Studies during reciliation. Eur J Biochem 195: 487–494

Plattner H, Flotenmeyer M, Kissmehl R, Pavlovic N, Hauser K, Momayezi M, Braun N, Tack J, Bachmann L (1999) Microdomain arrangement of the SERCA-type Ca^{2+} pump (Ca^{2+}-ATPase) in subplasmalemmal calcium stores of paramecium cells. J Histochem Cytochem 47: 841–854

Raff EC, Fackenthal JD, Hutchens JA, Hoyle HD, Turner FR (1997) Microtubule architecture specified by a beta-tubulin isoform. Science 275: 70–73

Redeker V, Levilliers N, Schmitter JM, Le Caer JP, Rossier J, Adoutte A, Bré MH (1994) Polyglycylation of tubulin: a posttranslational modification in axonemal microtubules. Science 266: 1688–1691

Redeker V, Levilliers N, Vinolo E, Rossier J, Jaillard D, Burnette D, Gaertig J, Bré MH (2005) Mutations of tubulin glycylation sites reveal cross-talk between the C termini of alpha- and beta-tubulin and affect the ciliary matrix in *Tetrahymena*. J Biol Chem 280: 596–606

Ruiz F, Garreau de Loubresse N, Beisson J (1987) A mutation affecting basal body duplication and cell shape in *Paramecium*. J Cell Biol 104: 417–430

Ruiz F, Beisson J, Rossier J, Dupuis-Williams P (1999) Basal body duplication in *Paramecium* requires gamma-tubulin. Curr Biol 9: 43–46

Ruiz F, Krzywicka A, Klotz C, Keller A, Cohen J, Koll F, Balavoine G, Beisson J (2000) The SM19 gene, required for duplication of basal bodies in *Paramecium*, encodes a novel tubulin, eta-tubulin. Curr Biol 10: 1451–1454

Ruiz F, Dupuis-Williams P, Klotz C, Forquignon F, Bergdoll M, Beisson J, Koll F (2004) Genetic evidence for interaction between eta- and beta-tubulins. Eukaryot Cell 3: 212–220

Sackett DL (1995) Structure and function in the tubulin dimer and the role of the acidic carboxyl terminus. Subcell Biochem 24: 255–302

Sale WS, Satir P (1976) Splayed *Tetrahymena* cilia. A system for analyzing sliding and axonemal spoke arrangements. J Cell Biol 71: 589–605

Satir P, Barkalow K, Hamasaki T (1993) The control of ciliary beat frequency. Trends Cell Biol 3: 409–412

Schneider A, Plessmann U, Weber K (1997) Subpellicular and flagellar microtubules of *Trypanosoma brucei* are extensively glutamylated. J Cell Sci 110: 431–437

Schneider A, Plessmann U, Felleisen R, Weber K (1998) Posttranslational modifications of trichomonad tubulins; identification of multiple glutamylation sites. FEBS Lett 429: 399–402

Seyfert HM, Sawatzki G (1986) An estimation of the soluble tubulin content in *Tetrahymena* cells of normal and of size-altered phenotype. Exp Cell Res 162: 86–96

Shang Y, Li B, Gorovsky MA (2002) *Tetrahymena thermophila* contains a conventional gamma-tubulin that is differentially required for the maintenance of different microtubule-organizing centers. J Cell Biol 158: 1195–1206

Smith JJ, Yakisich JS, Kapler GM, Cole ES, Romero DP (2004) A beta-tubulin mutation selectively uncouples nuclear division and cytokinesis in *Tetrahymena thermophila*. Eukaryot Cell 3: 1217–1226

Stargell LA, Heruth DP, Gaertig J, Gorovsky MA (1992) Drugs affecting microtubule dynamics increase alpha-tubulin mRNA accumulation via transcription in *Tetrahymena thermophila*. Mol Cell Biol 12: 1443–1450

Stelly N, Mauger JP, Claret M, Adoutte A (1991) Cortical alveoli of *Paramecium*: a vast submembranous calcium storage compartment. J Cell Biol 113: 103–112

Suprenant KA, Dentler WL (1988) Release of intact microtubule-capping structures from *Tetrahymena* cilia. J Cell Biol 107: 2259–2269

Thazhath R, Jerka-Dziadosz M, Duan J, Wloga D, Gorovsky MA, Frankel J, Gaertig J (2004) Cell context-specific effects of the beta-tubulin glycylation domain on assembly and size of microtubular organelles. Mol Biol Cell 15: 4136–4147

Tominaga T, Naitoh Y, Allen RD (1999) A key function of non-planar membranes and their associated microtubular ribbons in contractile vacuole membrane dynamics is revealed by electrophysiologically controlled fixation of *Paramecium*. J Cell Sci 112: 3733–3745

Tucker JB, Beisson J, Roche DL, Cohen J (1980) Microtubules and control of macronuclear "amitosis" in *Paramecium*. J Cell Sci 44: 135–151

Tucker JB, Mathews SA, Hendry KA, Mackie JB, Roche DL (1985) Spindle microtubule differentiation and deployment during micronuclear mitosis in *Paramecium*. J Cell Biol 101: 1966–1976

Vinh J, Langridge JI, Bré MH, Levilliers N, Redeker V, Loyaux D, Rossier J (1999) Structural characterization by tandem mass spectrometry of the posttranslational polyglycylation of tubulin. Biochemistry 38: 3133–3139

Weber K, Schneider A, Westermann S, Muller N, Plessmann U (1997) Posttranslational modifications of alpha- and beta-tubulin in *Giardia lamblia*, an ancient eukaryote. FEBS Lett 419: 87–91

Westermann S, Weber K (2003) Post-translational modifications regulate microtubule function. Nat Rev Mol Cell Biol 4: 938–947

Williams NE, Bakowska J (1982) Scanning electron microscopy of cytoskeletal elements in the oral apparatus of *Tetrahymena*. J Protozool 29: 382–389

Xia L, Hai B, Gao Y, Burnette D, Thazhath R, Duan J, Bré MH, Levilliers N, Gorovsky MA, Gaertig J (2000) Polyglycylation of tubulin is essential and affects cell motility and division in *Tetrahymena thermophila*. J Cell Biol 149: 1097–1106

Yanagi A, Hiwatashi K (1985) Intracellular positional control of survival or degeneration of nuclei during conjugation in *Paramecium caudatum*. J Cell Sci 79: 237–246

Zagulski M, Nowak JK, Le Mouel A, Nowacki M, Migdalski A, Gromadka R, Noel B, Blanc I, Dessen P, Wincker P, Keller AM, Cohen J, Meyer E, Sperling L (2004) High coding density on the largest *Paramecium tetraurelia* somatic chromosome. Curr Biol 14: 1397–1404

Zhang Y, Li N, Caron C, Matthias G, Hess D, Khochbin S, Matthias P (2003) HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo. EMBO J 22: 1168–1179