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Short Communication

Tubulin is not Posttranslationally Modified (Acetylated or Polyglutamylated) in *Tetrahymena* Macronucleus

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Summary. In the interphase of the cell cycle of *Tetrahymena pyriformis* GL tubulin was not demonstrated in the macronucleus by confocal microscopy using antibodies to α-tubulin, acetylated α-tubulin and polyglutamylated tubulin antibodies or Flutax-1. During cell division, from the formation of the new oral apparatus to the beginning of cytokinesis, tubulin was diffusely present in the nucleus, however (i) it did not form visible microtubules in confocal microscope- and (ii) it was not acetylated or polyglutamylated. As acetylation stabilizes microtubules, the results point to the dynamism of nuclear tubulin which could have a role in the transport of the numerous macronuclear chromosomes.

INTRODUCTION

Ciliates are relatively large and highly polarized cells, whose locomotion, feeding, cortical architecture and development are heavily dependent on diverse microtubules (Frankel 1999). These diverse microtubules have distinct morphologies and stabilities. *Tetrahymena* expresses only α- and two β-tubulin genes (and also γ-tubulin gen), thus it is unlikely that the diversity of microtubules are derived from diverse tubulin genes (Gaertig *et al*. 1993). Considering this diversity of microtubules it may derive mainly from (a) association with different microtubule-associated proteins (MAPs), with structural MAPs or molecular motors; or from (b) different posttranslational modifications (PTMs), as acetylation (α-tubulin), detyrosination (α-tubulin), glutamylation (α-and β-tubulins), glycylation (α-and β-tubulins), or phosphorylation (α-and β-tubulins); the different properties of microtubule species and their stability emerge from the regulation of post-translational modifications (Gaertig 2000).

α-Tubulin acetylation appears to be the most widespread PTM as it was detected in ciliary, cortical and intramicronuclear microtubules of *Tetrahymena* (MacRae 1997). This PTM affects the stability of microtubules, and this is due to the binding of specific MAPs to the walls of acetylated microtubules (Piperno and Fuller 1985). It was found that microtubule bundles formed in MAP_{2c}- or tau-transfected cells were stabilized against microtubule depolymerizing reagents and were enriched in acetylated alpha tubulin (Marute *et al*. 1986). Thus,

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probably acetylation (and possibly also the detyrosination or polyglutamylation) may play a role in the maintenance of stable populations of microtubules. It was found that stable microtubules defined as those that had not undergone polymerization within 1 h after injection of biotin-tubulin and they were posttranslationally modified; furthermore dynamic microtubules were all unmodified. Posttranslational modification, however, is not a prerequisite for microtubule stability and vice versa. *Potorous tridactylis* kidney cells have no detectable acetylated microtubules but do have a sizable subset of stable ones, and chick embryo fibroblast cells are extensively modified however, hardly have stable microtubules (Schulze *et al*. 1987).

Tubulin have been reported within the dividing macronucleus of ciliated protozoa (Williams and Williams 1976), but little is known about their precise role in the division process itself, or about their posttranslational modification(s).

In our previous experiments connection of microtubular and signaling systems was observed in *Tetrahymena* (Kovács *et al*. 2000, Kovács and Pintér 2001), and data were obtained on the effect of drugs affecting microtubular assembly (Kovács and Csaba 2005). The structure of microtubular system (longitudinal and transverse microtubule bands) in cells exposed to 0.1 mM indomethacin became frequently irregular, a phenomenon which was not seen in control cells. In a considerable amount of cells stomatogenesis were seen, whereas in these cells the macronucleus did not elongate. Considering these facts we concluded that indomethacin impaired the signaling system which was able to connect the events of divisional morphogenesis; it ceased the junction between the cytoskeletal and nuclear processes (Kovács and Pállinger 2003). The aim of the present study is to obtain additional data on the posttranslational modification (eg. acetylation) of tubular stuctures in the nuclei of *Tetrahymena*, and on the possible role of microtubular stability/dynamics in the mitotic events of this unicellular organism.

MATERIALS AND METHODS

Chemicals. Mouse monoclonal anti-acetylated tubulin, anti-polyglutamylated-tubulin and anti-α-tubulin antibodies, FITC-labeled anti-mouse goat IgG, Flutax-1 and tryptone were obtained from Sigma (St Louis, MO, USA). Yeast extract was purchased from Oxoid (Unipath, Basingstoke, Hampshire, UK). All other chemicals used were of analytical grade available from commercial sources.

Cultures. In the experiments, *Tetrahymena pyriformis* GL strain was tested in the logarithmic phase of growth. The cells were cultivated at 28°C in 0.1 % yeast extract containing 1 % tryptone medium. Before the experiments the cells were washed with fresh culture medium and were resuspended at a concentration of 5×10^4 cells ml^{-1}.

Confocal scanning laser microscopy. To localize tubulin-containing structures, cells were fixed in 4% paraformaldehyde dissolved in PBS, pH 7.2. After washing with wash buffer [WB] (0.1% BSA in 20 mM Tris-HCl; 0.9% NaCl; 0.05% Tween 20, pH 8.2) the cells were incubated with monoclonal anti-acetylated tubulin antibody or monoclonal anti-a-tubulin antibody diluted 1:500 with antibody [AB] buffer (1% BSA in 20 mM Tris-HCl; 0.9% NaCl; 0.05% Tween 20, pH 8.2) for 45 min at room temperature. After three washings with WB the anti-tubulin antibody treated cells were incubated with FITC-labelled anti-mouse goat IgG (diluted to 1:500 with AB buffer) for 45 min at room temperature.

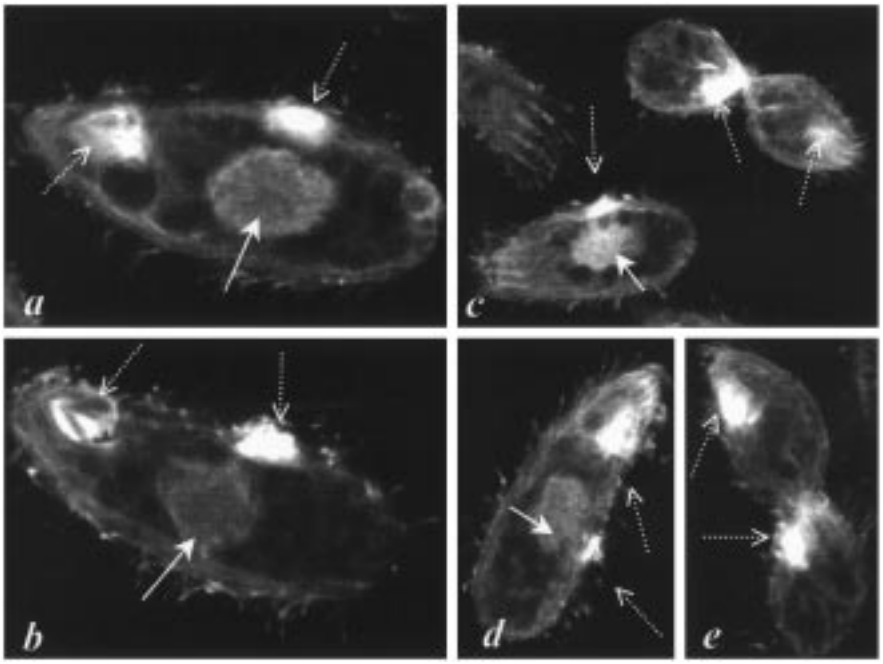
Beyond that some cells were labeled with fluorescent taxoid derivative 7-O-[N-(4'-fluoresceincarbonyl)-L-alanyl]taxol (Flutax-1). As Flutax-1 reversibly interacts with the taxol binding sites of microtubules with high affinity, it serves to image the microtubule cytoskeleton (Arregui *et al*. 2002). The binding of Flutax-1 was analysed on fixed cells (in 4% paraformaldehyde dissolved in PBS, pH 7.2). The stock solution of Flutax-1 was 10^{-4} M dissolved in DMSO, and diluted with AB buffer to 10^{-6}M. The cell suspensions were incubated with 10^{-6}M Flutax-1 (v/v) for 20 min. After these incubations the cells were washed four times with WB, and were mounted onto microscopic slides.

The mounted cells were analyzed in a Bio-Rad MRC 1024 confocal scanning laser microscope (CSLM) equipped a krypton/argon mixed gas laser as a light source. Excitation carried out with the 480 nm line from the laser.

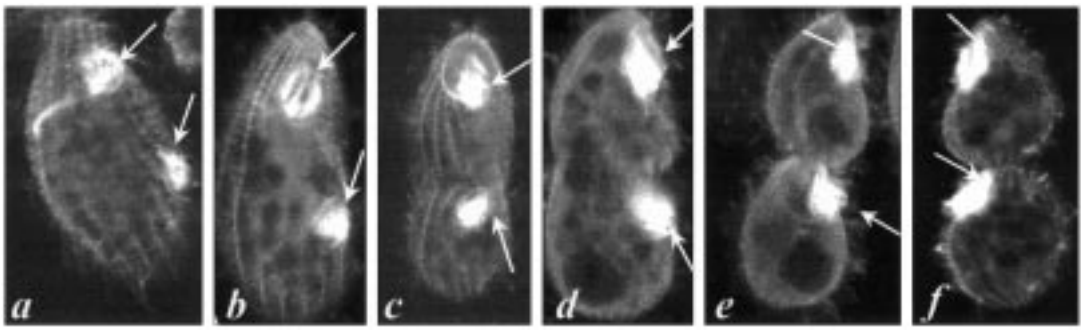
RESULTS AND DISCUSSION

According to Fujiu and Numata (1999) the macronuclear division of *Tetrahymena* is classified into six stages, considering the morphology of the macronucleus. They found that tubulin is substantially excluded from interphase nuclei, but it is present in dividing (amitotic?) ones - from the appearance of „anarchic field", the formation of new oral apparatus (about stage 2) to the beginning of cytokinesis, when the division furrow appears (about stage 5). In their observations tubulin forms microtubules throughout the macronuclear division however, these microtubules are biochemically different from the cytoskeletal (cytoplasmic) ones. Investigating electron-microspically, Williams and Williams (1976) demonstrated the presence of these nuclear microtubules mainly in connection with the nuclear envelope.

In our present experiments in the interphase nucleus tubulin was not demonstrable (Figs 4a-c), it was labeled



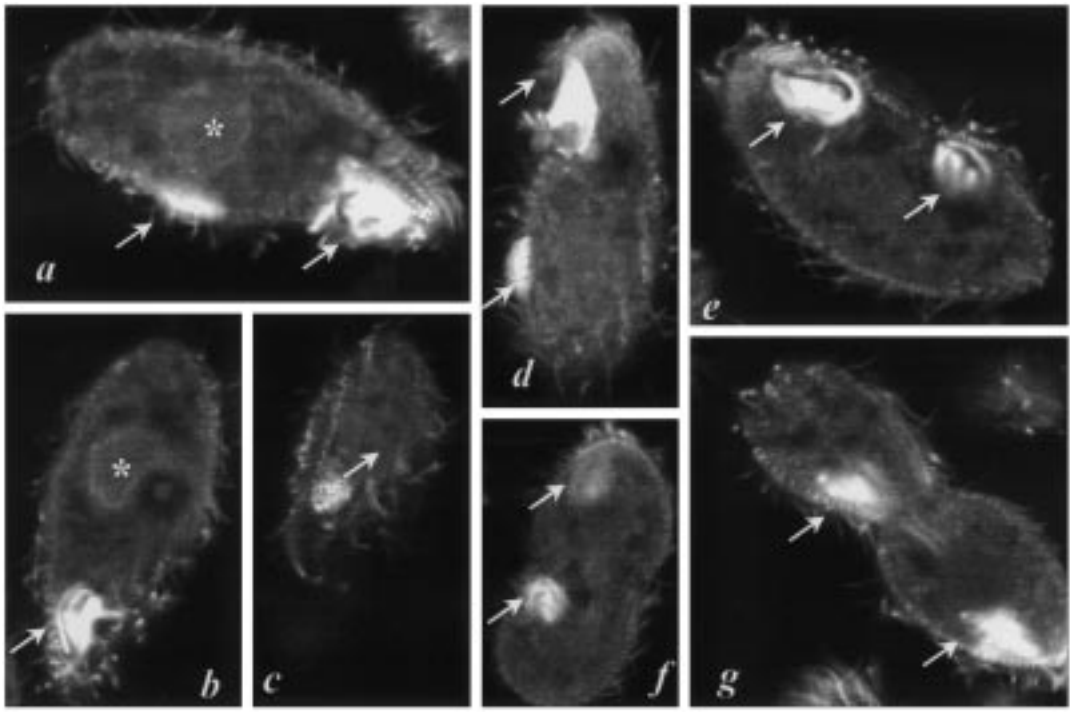
Figs 1a-e. Binding of FITC-labeled anti α-tubulin antibody to the tubulin containing systems of *Tetrahymena*. The nuceli are labeled from the formation of new oral apparatus to the beginning of appearance of division furrow. Arrows - nuclei; dotted arrows - oral apparatus. Confocal scanning laser microscopic pictures. Magnification: 1400× (1a, b); 900× (1c-e).



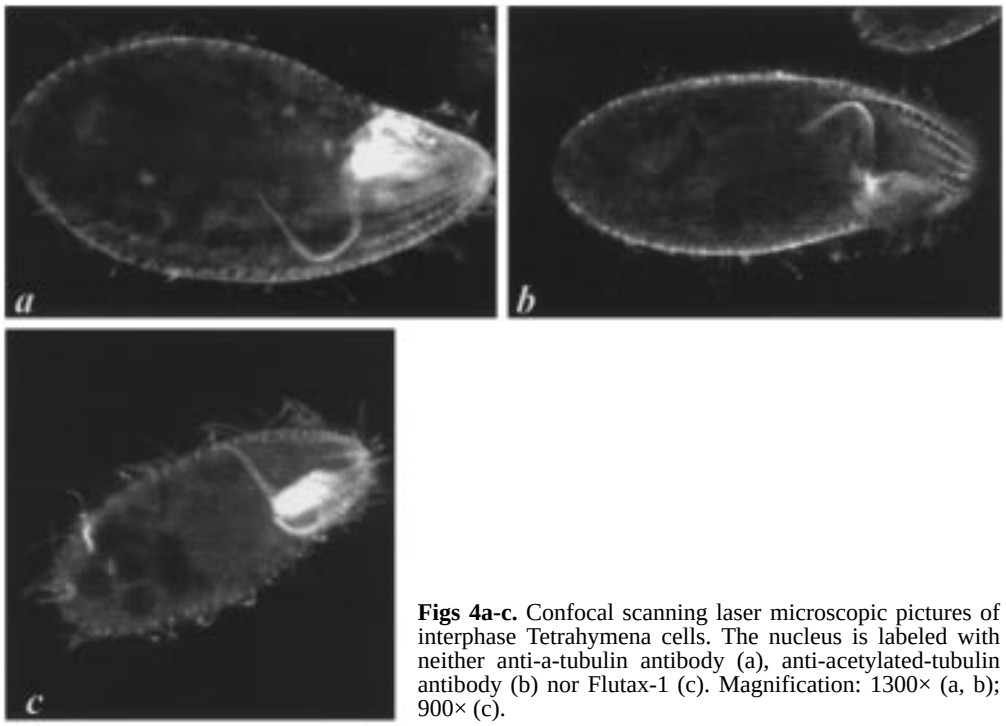
Figs 2a-f. Binding of FITC-labeled anti acetylated-tubulin antibody to the microtubular system of *Tetrahymena*. Confocal scanning laser microscopic pictures. Arrows - oral apparatus. The labeling is absent in all stages of cell division. Magnification 1100×.

neither with antibodies against a-tubulin and acetylated tubulin nor Flutax-1. During cell division of *Tetrahymena* the nuclear tubulin appears, however, it did not form confocal microscopically demonstrable microtubules. It was at all times labeled with anti-a-tubulin antibodies (Figs 1a-d) and Flutax-1 (Figs 3a,b), however this tubulin is neither acetylated (Figs 2a-f) nor polyglutamylated (data not shown). In late cytokinesis some microtubular structures were visible between the isolated two macronuclei - largely in the Flutax-labeled cells (Fig. 5c).

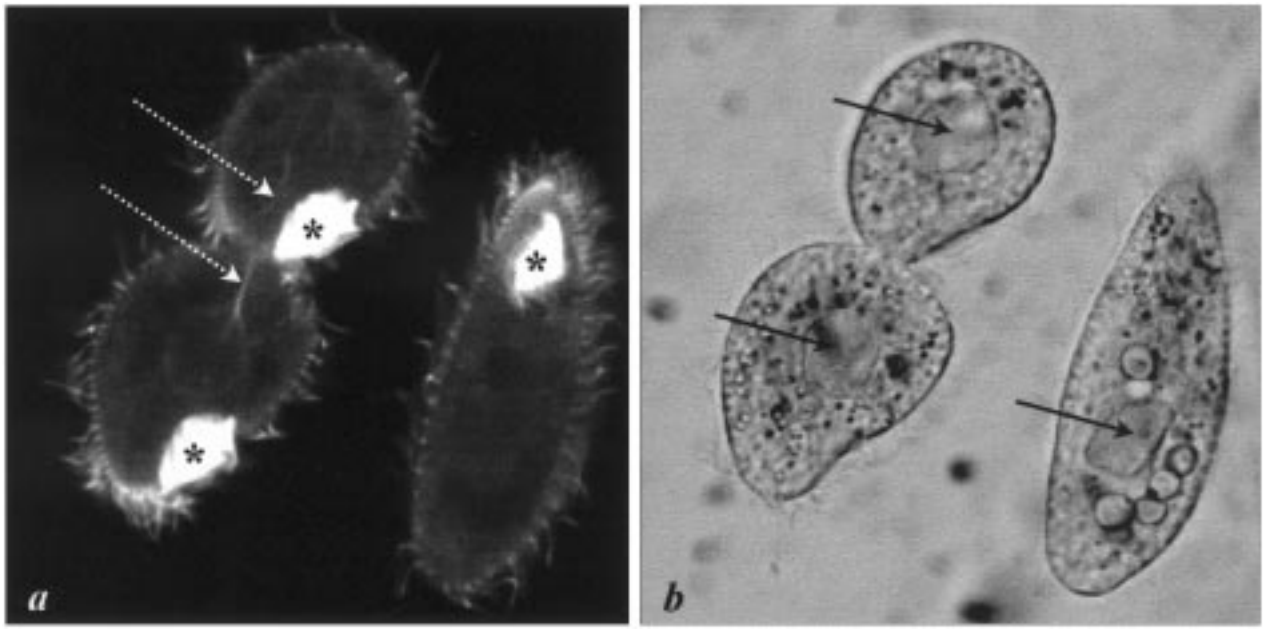
In tissue-culture of mammalian cells tubulin has been implicated as one of the nuclear proteins. It was found distributed throughout the nucleus and particularly in association with the chromatin. Tubulin comprised about 6.5% of the nonhistone chromosomal proteins. Nuclear tubulin appears in a nonmicrotubular form (as in our present experiments). Fluorescence microscopical examinations on metaphase chromosomes revealed that tubulin was present on the chromosomes. These data suggest a structural role for chromatin-associated tubulin



Figs 3a-g. a - binding of Flutax-1 to the microtubular system of *Tetrahymena*; **b, c** - same cell; optical sections at middle of the cell (b) and the surface of cell (c). Nuclar-labeling is visible at the formation of new oral apparatus, later this labeling disappears. Arrows - oral apparatus; asterisk - nuclei. Confocal scanning laser microscopic pictures Magnification: 1200× (a, e, g); 900× (b-d); 800× (f).



Figs 4a-c. Confocal scanning laser microscopic pictures of interphase Tetrahymena cells. The nucleus is labeled with neither anti-a-tubulin antibody (a), anti-acetylated-tubulin antibody (b) nor Flutax-1 (c). Magnification: 1300× (a, b); 900× (c).



Figs 5a, b. Interphase and dividing *Tetrahymena* cells. **a** - labeled with Flutax; **b** - same cells in brightfield. Arrows - nuclei; dotted arrows - microtubule bundles between the nuclei of daughter cells. Asterisk - oral apparatus. Confocal scanning laser microscopy Magnification 1200×.

(Menko and Tan 1980). The ciliate macronucleus (the transcriptionally active „somatic" nucleus) typically does not show recognizable chromosomes, and conventional mitotic spindles are not seen. Cell division (amitosis?) in this case involves an elongation of the nucleus within the persisting nuclear envelope, and an eventual pulling apart into the two daughter nuclei (Williams and Williams 1976). It was shown that colchicine applied to *Tetrahymena* late in the cell cycle had no blocking effect on either cell or macronuclear division. However, nuclear cleavage was unequal; many daughter cells were produced with nuclei which were either smaller or larger than normal, and some had no nuclei at all (Tamura *et al.* 1969). It has been shown that significant elongation of the macronucleus can occur in the absence of tubulin, and that microtubules are not required to maintain an elongation and separation once it has occured normally. However, autonomous nuclear constriction and separation do not occur without nuclear tubulin; the macronucleus is cut in two by the advancing cytoplasmic fission furrow.

In ciliated protoza the macronucleus contains the somatic genome, which is fragmented, polyploid and actively expressed. In the *Tetrahymena thermophila* the macronucleus consists of 200-300 identifiable autonomously replicating pieces („MAC chromosomes"), derived from the five pairs of germline-derived chromosomes (Conover and Brunk 1986). Macronuclear chromosomes lack centromeres and kinetochores (Davidson and LaFountain Jr. 1975), sister chromatids segregate randomly and spindle pole bodies do not form (Doerder 1979); accordingly the macronuclear microtubules operate in no way like the „real" kinetochor-microtubules. The huge number of MAC chromosomes to which tubulin can be sticked could explain the diffuse appearance of anti-tubulin fluorescence during cell division. This tubulin which does not form microtubules could have a role in the transport of „MAC chromosomes". The nuclear envelope associated microtubules which were not demonstrated immunocytochemically, but were shown in electron microscopic pictures have a role in the terminal stages of nuclear elongation and separation (Williams and Williams 1976).

It is likely that the macronuclear microtubules are very dynamic structures: the enhanced stability of microtubules alters normal microtubule dynamics, leading to the nuclear aberrant phenotypes. In a b-tubulin mutant *Tetrahymena thermophila* (btu1-1), where microtubules are strongly stabilized polymers, and increasing amount of amacronucleate cell formation occurs. Also

the microtubule-stabilizing drugs (taxol and DMSO) promote amacronucleate cell formation (Smith *et al*. 2004). This helps to understand why acetylated (stabilized) tubulin in the macronucleus of dividing *Tetrahymena* was not found and can explain the biochemical difference between nuclear and cytoplasmic tubulin, mentioned by Fujiu and Numata.

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