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Subcellular Sequestration of an Antigenically Unique β-Tubulin

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Tubulin from *Trypanosoma brucei* was characterized by Western blotting using well defined monoclonal antibodies reacting with α- or β-tubulin and a new monoclonal antibody, 1B41, raised against a microtubule-enriched fraction of *T. brucei*, which specifically reacts with the β-subunit of tubulin from either *T. brucei* or rat brain. This antibody has been used to examine the subcellular distribution of the corresponding antigen in *T. brucei* by indirect immunofluorescence. The epitope recognized by 1B41 is restricted to a thin line extending from the basal body region to the anterior end of the cell body. To determine the relationship between the immunoreactive zone and the flagellum, double-label immunofluorescence was performed in both interphase and mitotic cells with 1B41 and a flagellar marker, the monoclonal antibody 5E9, specific for the paraflagellar rod polypeptides of trypanosomes. These experiments revealed that the immunoreactive tubulin was contained in a part of the subpellicular cytoskeleton that remained in a constant spatial correspondence with the flagellum throughout the cell division cycle. The β-tubulin recognized by 1B41 may be segregated into the microtubular structures associated with a cisterna of the endoplasmic reticulum forming the *subflagellar microtubule quartet* (SFMQ). These results suggest that the presence of an antigenically unique β-tubulin defines a subpopulation of microtubules possessing specific dynamic properties that may be involved in the morphogenesis of daughter cells during the division of *T. brucei*.

Key words: cytoskeleton, microtubules, monoclonal antibodies, cell morphogenesis, tubulin, *Trypanosoma brucei*, subflagellar microtubule quartet

INTRODUCTION

The involvement of microtubules in a wide range of cellular functions such as ciliary and flagellar motility, chromosome movement, maintenance of cell shape, and secretion implies that, in many cases, they are organized into morphologically different arrays. The diversity of microtubular structures and functions may result from the nature of the different α- and β-tubulin subunits and/or from the binding to different associated proteins. In many organisms, tubulin is encoded by multigene families that might produce a complex pattern of α- and β-tubulin isotype expression [Bond et al., 1984; Havercroft and Cleveland, 1984; Raff, 1984]. Nevertheless, multigene families are not the only pathway used by a cell to generate distinct tubulin forms; studies of tubulin polypeptides showed that this protein can be posttranslationally modified by acetylation of the ε-amino group of a lysine residue on the α-subunit [L'Hernault and Rosenbaum, 1985], by a detyrosination-tyrosination cycle of α-tubulin [Raybin and Flavin, 1977; Kumar and Flavin, 1981], and by phosphorylation of β-tubulin [Eipper, 1972]. In the eukaryotic cells so far examined, posttranslationally modified α-tubulin has been shown to belong to subpopulations of microtubules. For instance, the acetylated α-tubulin is restricted to drug-resistant microtubules [Le Dizet and Piperno, 1986; Piperno et al., 1987] and tyrosinated α-tubulin to newly assembled microtu-

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bules [Gundersen and Bulinski, 1986b; Sherwin et al., 1987]. Although β-tubulin isotypes display a tissue-specific distribution [see Sullivan and Cleveland, 1986], there is no evidence for a possible sequestration of a β-tubulin form into a subpopulation of microtubules within a single cell [Lewis et al., 1987].

Trypanosoma brucei, the causative agent of sleeping sickness, is well suited to investigate the heterogeneity of tubulin subunits. Ultrastructural studies reveal several microtubular structures: flagellar axoneme, subpellicular microtubules, and mitotic spindle [Vickerman and Preston, 1976]. In the genome of *T. brucei*, the tubulin genes are arranged into a single cluster of alternating α- and β-tubulin genes [Seebeck et al., 1983; Thomashow et al., 1983]. The α- and β-tubulin mRNAs differ in size, 1.95 kb and 2.3 kb, respectively; in that each one migrates as a single band [Imboden et al., 1986], there is no evidence for the production of multiple tubulin isotypes. However, it has been shown that at least two kinds of posttranslational processing occur to generate modified α-tubulin. Schneider et al. [1987] detected two α-tubulin species, termed α1 and α3, by two-dimensional polyacrylamide gel electrophoresis and showed that the α1-tubulin was the direct translation product of the α-tubulin mRNA, whereas the α3-tubulin resulted from the acetylation of α1-tubulin. Another demonstrated posttranslational modification is the detyrosination-tyrosination cycle of α-tubulin [Sherwin et al., 1987].

In this paper, we use a mouse monoclonal antibody, 1B41, elicited against *T. brucei* microtubule proteins and specifically reacting with β-tubulin, to show that the corresponding epitope is restricted to only a part of the subpellicular cytoskeleton. Furthermore, the assembly of the tubulin subset reacting with 1B41 in the daughter cells appears to take place early in the cell division of trypanosomes and could be one of the key events in the morphogenesis of growing cells.

MATERIALS AND METHODS

Growth and Harvesting of Cells

Bloodstream forms of *T. brucei brucei*, clone AnTat 1.1 (a generous gift from Dr. Le Ray, Institute of Tropical Medicine, Antwerp, Belgium), were grown in Swiss mice and harvested from blood by using the cell chromatography method of Lanham [1968]. Procyclic trypanosomes were cultured in glucose-lactalbumin-serum-hemoglobin (GLSH) medium [Le Ray, 1975]. Cells were washed twice at 1,500g for 5 min at 4°C and resuspended in phosphate-saline-glucose (PSG; 57 mM Na_2HPO_4, 3 mM NaH_2PO_4, 40 mM NaCl, 60 mM glucose, pH 8.2).

Monoclonal Antibodies (mAb)

The hydridoma cell line secreting mAb 1B41 of the IgM type was selected after the fusion of the myeloma X-63 Ag8-653 with spleen cells from high responder mice [Biozzi et al., 1979] (generously provided by Dr. G. Biozzi, Curie Institute, Paris) immunized with a microtubule-enriched preparation from *T. b. brucei* [Gallo and Schrével, 1985]. Positive hybridomas were identified following an indirect immunofluorescence test (see below) and were cloned three times by limiting dilution. Cell culture supernatants or ascites fluids were used as the source of antibody. The selection of the monoclonal mouse IgG 5E9 against the paraflagellar rod polypeptides of trypanosomes has already been described [Gallo and Schrével, 1985]. A set of monoclonal antitubulin antibodies was also used: 16D3, a general antitubulin antibody [Gallo and Anderton, 1983]; the rat antibody YL 1/2 [Kilmartin et al., 1982] (Serotec, Oxon, U.K.) against the tyrosinated form of α-tubulin; 6.11B.1 against the acetylated form of α-tubulin [Piperno and Fuller, 1985] (kindly donated by Dr. G. Piperno, the Rockefeller University, New York, NY); and an antibody specific for β-tubulin (Amersham, Little Chalfont, U.K.), referred to as anti-β in this paper. Controls were performed with the mAb 23D9 of unknown specificity [Gallo and Schrével, 1985] and with two mouse IgMs against human lymphocyte surface antigens, DR [Bodger et al., 1983] and CD6 [Prentice et al., 1984].

Electrophoresis and Immunoblotting

One-dimensional electrophoresis was carried out as described by Laemmli [1970] in 7.5% (w/v) polyacrylamide gels containing 6 M urea. Microtubule-enriched preparations from *T. b. brucei* and rat brain microtubules purified by two assembly-disassembly cycles [Shelanski et al., 1973] were dissolved in Laemmli's sample buffer containing 6 M urea. Following electrophoresis, polypeptides were transferred to nitrocellulose sheets (BA 85; Schleicher & Schuell, Dassel, Federal Republic of Germany) according to the method of Towbin et al. [1979]. In some experiments, blots were incubated with carboxypeptidase A or alkaline phosphatase after the blocking step. Carboxypeptidase A (C 9268; Sigma, St. Louis, MO) was used at 10 μg/ml in Tris-buffered saline (TBS; 140 mM NaCl, 10 mM Tris- HCl, pH 7.4) for 30 min at 37°C. Alkaline phosphatase (P 5521; Sigma) was used at 30 U/ml in 100 mM Tris- HCl, pH 8.0, for 2.5 hr at 37°C. Treated and untreated blots were then processed with antibodies. Ascites fluids were diluted in 3% (w/v) hemoglobin in TBS as follows: 1B41, 1:250; 16D3, 1:500; YL 1/2, 1:600; anti-β, 1:800. Hybridoma culture supernatant of mAb 6.11B1 was diluted 1:20. Antibody binding was visualized by autoradiography; the appropriate second antibodies were radioiodinated by the chloramine T method and used at 5.10^5 cpm/ml. Autoradiograms were exposed for 2–5 days at −80°C on Hyperfilm MP (Amersham, U.K.) with intensifying screens.

Immunofluorescence Microscopy

Air-dried parasites were fixed for 5 min with acetone. Cells were incubated for 30 min with dilutions of ascites fluids (1B41, 1:750; anti-β, 1:100) and washed in TBS for 30 min. A second layer of antibodies consisting of fluorescein isothiocyanate (FITC)-conjugated goat antimouse Ig (GAM/FITC, Nordic, Copenhagen, Denmark) diluted 1:40 was then applied. The slides were counterstained with 0.02% (w/v) Evans blue in TBS and mounted in Citifluor AF1 (Citifluor Ltd, London, U.K.) to reduce fading. For double immunofluorescence, 5E9 was conjugated with tetramethylrhodamine isothiocyanate (TRITC) by using the following protocol: the antibody was purified by anion-exchange chromatography on Mono Q column using a fast protein liquid chromatography device (Pharmacia Fine Chemicals, Uppsala, Sweden); washed in 250 mM $NaHCO_3$, 250 mM Na_2CO_3, pH 9.0, 100 mM NaCl; and concentrated to 20 mg/ml with Centricon 30 filters (Amicon, Danvers, MA). The coupling was carried out over 12 hr at 4°C with 0.05 mg of TRITC (Sigma) by milligram of antibody. The conjugated antibody was separated from the free TRITC by filtration through a Sephadex G 25 column (Pharmacia) and washed twice in Dulbecco's phosphate-buffered saline with Centricon 30 filters. The cells were first incubated with 1B41 (ascites fluid diluted 1:750), then with fluoresceinylated goat antimouse IgM antibodies (GAM/IgM/FITC, Nordic) diluted 1:40 and eventually with 5E9-TRITC (11 μg/ml). Afterwards the cells were incubated with the A-T pair-specific fluorochrome 2′-[4-hydroxyphenyl] -5- [4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole (Hoescht 33258). Slides were mounted in 50% (w/v) glycerol in TBS and observed with an Optiphot microscope (Nikon, Tokyo, Japan) fitted with filter cubes B2 (excitation 495 nm, emission 520 nm) for FITC, G (excitation 546 nm, emission 580 nm) for TRITC, and UV (excitation 365 nm, emission 420 nm) for Hoescht 33258.

RESULTS

mAb 1B41 Selectively Binds to β-Tubulin

The polypeptides carrying the epitope defined by mAb 1B41 were identified by Western blotting using a gel system resolving tubulin into α- and β-subunits; this was achieved by including 6 M urea in both sample and separating gel buffers. The two major polypeptides stained by Coomassie blue in the electrophoretogram of proteins from a microtubule-enriched preparation from *T. b. brucei*, presented in Figure 1a (lane 1), are α- and β-tubulins since they comigrate with the corresponding subunits of rat brain microtubules (Fig. 1a, lane 2) and react with the general antitubulin antibody 16D3 (Fig. 1b). Two well characterized anti-α-tubulin antibodies, 6.11B.1 and YL 1/2 against the acetylated [Piperno and Fuller, 1985] and tyrosinated [Wehland et al., 1983] forms of tubulin, respectively, were used to position tubulin subunits in the electrophoretic system used. The specificity of 6.11B.1 for acetylated α-tubulin was assessed by its lack of reactivity with purified rat brain tubulin (Fig. 1c). Both antibodies bound to the upper band of either *T. b. brucei* or rat brain tubulin (Fig. 1c,d), so the slowest migrating subunit is α-tubulin and the fast migrating subunit is β-tubulin, as confirmed by its reaction with anti-β (Fig. 1e). The immunoblot incubated with 1B41 demonstrated that this antibody specifically bound to β-tubulin in either *T. b. brucei* extracts or rat brain microtubules (Fig. 1f). Antibody staining of proteins trans-

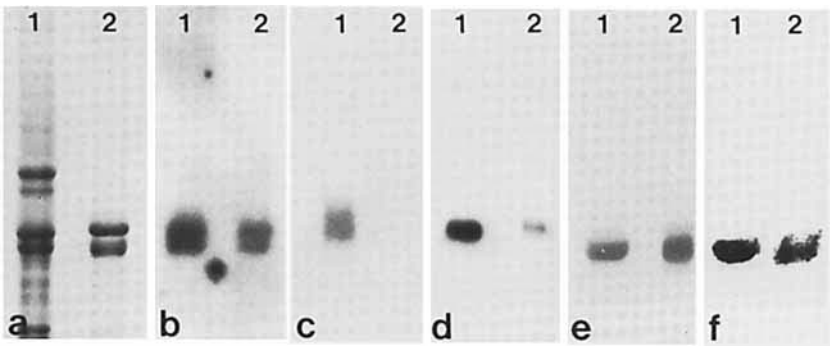

Fig. 1. Binding of mAb to polypeptides from *T. b. brucei* microtubule-enriched fraction (lanes 1) and rat brain microtubules (lanes 2) separated in a sodium dodecyl sulfate-urea polyacrylamide gel. **a:** Coomassie blue-stained gel. **b–f:** Autoradiograms of nitrocellulose transfers of corresponding gels probed with several antitubulin monoclonal antibodies. b, 16D3; c, 6.11B.1; d, YL 1/2; e, anti-β; f, 1B41. Autoradiogram in f was exposed for a longer time than in b–e to enhance the visualization of the band reacting with 1B41.

ferred from a conventional Laemmli gel lacking urea revealed no cross-reactivity of 1B41 with polypeptides other than tubulin in whole cell extract from *T. b. brucei* (data not shown).

A quantitative evaluation of the binding of 1B41 to tubulin was obtained by counting the radioactivity of the β-tubulin bands cut out from blots incubated with similar concentrations of 1B41 or 16D3 (which is also an IgM). The proportion of the total β-tubulin reacting with 1B41 was roughly estimated at 12% for trypanosome or brain tubulin. The pattern of 1B41 reactivity remained unchanged after treatment of blots with carboxypeptidase A or alkaline phosphatase prior to antibody incubation.

mAb 1B41 Displays an Antigenically Unique β-Tubulin Sequestration in *T. b. brucei*

To establish the subcellular distribution of β-tubulin epitopes in *T. b. brucei*, indirect immunofluorescence was performed with 1B41 and another mAb against β-tubulin; anti-β. The flagella and the whole cell body reacted with anti-β (Fig. 2a,b); the staining was especially localized near the cell membrane and reflected the

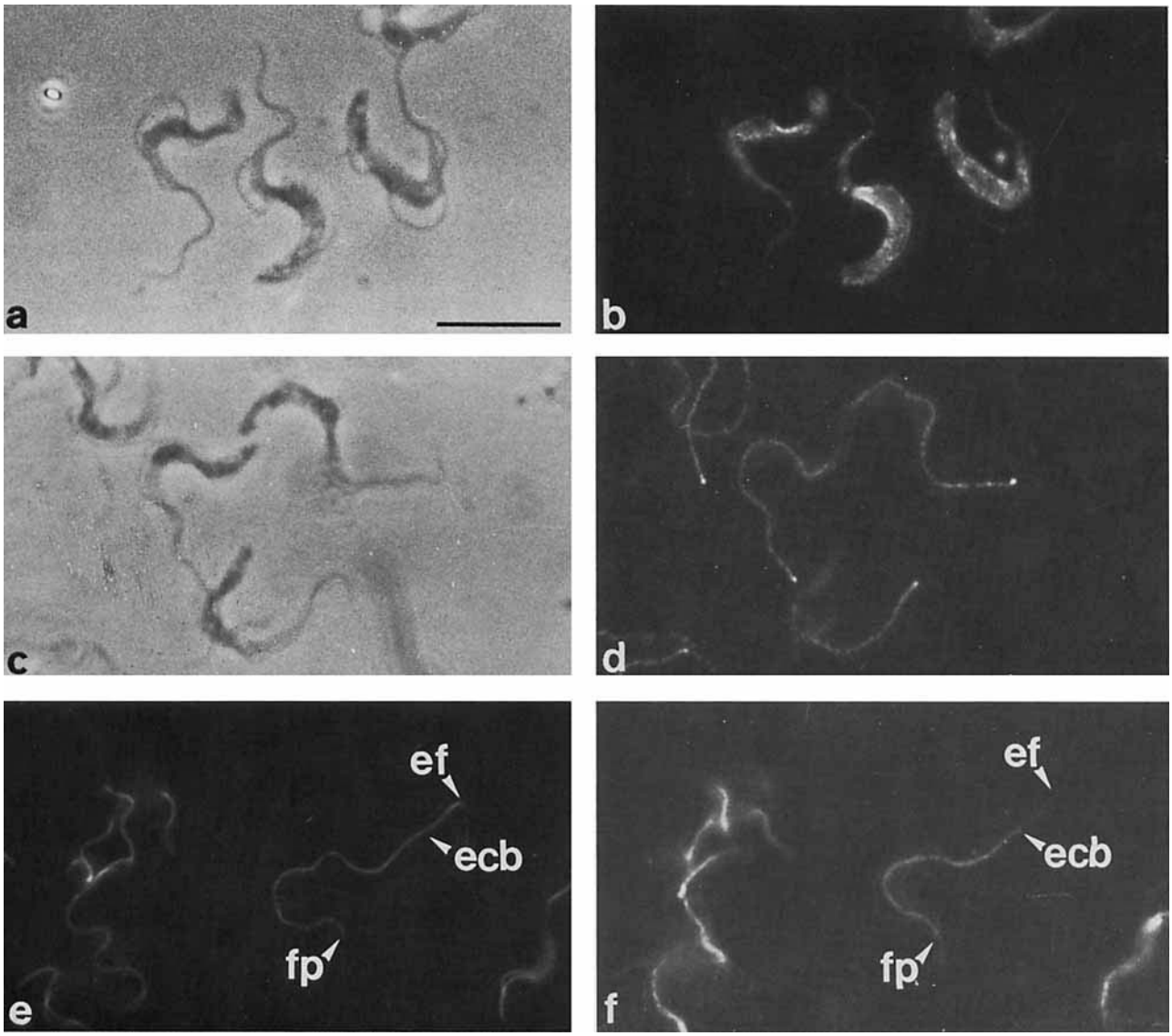

Fig. 2. Localization of β-tubulin epitopes in the bloodstream form of *T. b. brucei*. Interphase cells were labeled in indirect immunofluorescence with mAb. Phase-contrast and corresponding fluorescent images of cells stained with anti-β **(a, b)** and 1B41 **(c, d)**. Double-label immunofluorescence of *T. brucei* with 5E9 specific for paraflagellar rod polypeptides **(e)** and 1B41 against β-tubulin. **(f)** fp, flagellar pocket; ef, end of the flagellum; ecb, end of the cell body. Bar = 10 μm.

distribution of subpellicular microtubules. By contrast, the 1B41 antibody gave only a slightly punctate staining along a line located in the flagellar zone (Fig. 2c,d). This pattern displayed in slender forms was also obtained in all mammalian forms (anteronuclear stumpy, postnuclear stumpy, and intermediate forms) and in tse-tse midgut form (procyclic forms) [for the characteristics of the different forms, see Vickerman, 1985].

To localize the zone reacting with 1B41 relatively to the flagellum, cells were processed for double immunofluorescence labeling. mAb 5E9 recognizes the paraflagellar rod proteins of trypanosomes [Gallo and Schrével, 1985]; consequently, conjugated to TRITC, it can be used as a good marker of the flagellum in trypanosomes. 5E9 stained the flagellum from the flagellar pocket to its distal end (Fig. 2e). The immunofluorescence images obtained with 1B41 clearly indicated that the specific labeling was limited to the part of the cell body where the flagellar membrane adhered to the plasma membrane (Fig. 2f). The zone reacting with 1B41 is shorter than the flagellum and, more precisely, begins in the flagellar pocket and extends up to the anterior end of the cell body. A slight bleed-through of TRITC fluorescence could be observed into the FITC channel, although it was bleached for more than 10 min. No staining was observed in the controls performed with mAb 23D9 of unknown specificity and mouse IgMs CD6 and DR against human lymphocyte surface antigens.

Evolution of the 1B41 Reactive Zone During the Cell Division Cycle of *T. b. brucei*

To pinpoint the development of the 1B41 reactive zone during the division of *T. b. brucei*, cells were stained at different stages of the cell cycle by double immunofluorescence, similar to what had been done for interphase cells. The position of a given cell in the cell cycle was estimated by 1) the presence and the length of a daughter flagellum and 2) the division and the position of nucleus and kinetoplast. The flagellum was stained by 5E9-TRITC, and DNA was visualized with the intercalating dye Hoescht 33258. Figure 3 displays a series of fluorescence images of cells arranged according to their position in the division cycle.

1) *At the start of the bipartition cycle*, the flagellum was seen as an undulating line attached to the cell body, and the kinetoplast was detected as a bright point at the posterior part of the cell; the 1B41 reactive zone displayed the pattern observed in interphase cells (Fig. 3a–c).

2) *When the daughter flagellum began to lengthen*, and kinetoplast and nucleus began to divide, it was very difficult to distinguish any modification of the staining obtained with 1B41 (Fig. 3d–f).

3) *When the newly forming flagellum reached about the middle of the cell*, the two kinetoplasts were separated, the nucleus underwent mitosis and the presence of a new 1B41 reactive zone was then obvious (Fig. 3g–i).

4) *At a late stage of the cycle*, the daughter flagellum had grown nearly to its full length, and nuclear division was completed; two 1B41 reactive zones were clearly visible lying along the maternal and daughter flagella (Fig. 3 j–l).

DISCUSSION

mAb 1B41 Recognizes an Epitope Located on β-Tubulin

The mAb 1B41 has been shown to react specifically with the β-subunit of tubulin from *T. b. brucei* and rat brain. The specificity of the antibody for β-tubulin in the former organism was demonstrated in microtubule-enriched fractions that contained all the tubulin species of this cell [Schneider et al., 1987]. The antigenic site recognized by 1B41 may be related either to a peptide sequence or to the covalent addition of chemical groups. Since every β-tubulin gene of this cell codes for the same polypeptide [Imboden et al., 1986; Schneider et al., 1987], a putative peptidic 1B41 binding site would exist on all tubulin molecules present in *T. b. brucei*.

Schneider et al. [1987] did not observe any electrophoretic shift in the two-dimensional gel coordinates of β-tubulin either from in vitro translation products of mRNAs or from cell lysates, so there is no evidence yet for a posttranslational modification of β-tubulin in *T. b. brucei*. Since 1B41 binding to β-tubulin was not modified by treatment with carboxypeptidase A or alkaline phosphatase, the antigenic site recognized by this antibody is probably not linked to the phosphorylation or to the C-terminus of β-tubulin of *T. b. brucei*, which displays the unusual feature of having a C-terminal tyrosine residue coded for by the corresponding gene [Kimmel et al., 1985]. Although the actual nature of the epitope defined by 1B41 remains to be investigated, the specificity of this mAb allows its use as a probe for a molecular domain of β-tubulin in *T. b. brucei*.

1B41 Immunoreactive β-Tubulin Is Sequestrated Into a Subset of Tubulin in *T. brucei*

Immunofluorescence experiments showed that the epitope defined by 1B41 was exposed to the antibody only in a specialized assembly of tubulin confined to the flagellar adhesion zone. Double-label immunofluorescence with 1B41 and the mAb 5E9 against the paraflagellar rod polypeptides demonstrated the association of the 1B41 reactive tubulin with the flagellum. However, the 1B41 epitope is not located inside the flagellum, since

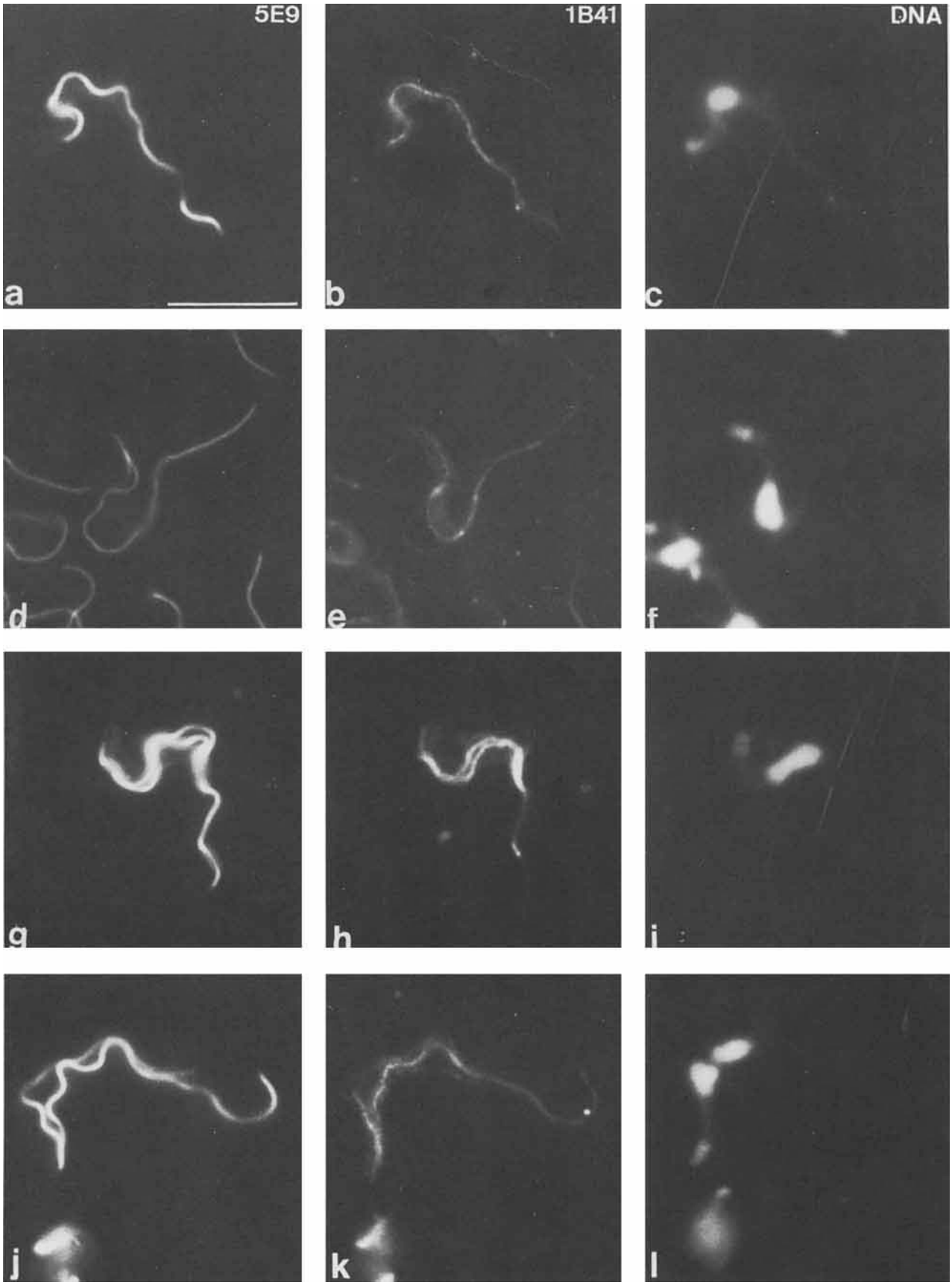

Fig. 3. Changes in the pattern of 1B41 staining during cell division of *T. b. brucei*. Double-label immunofluorescence with 5E9 **(a, d, g, j)** and 1B41 **(b, e, h, k)** together with DNA staining (Hoescht 33258; **c, f, i, l**). Photographs are arranged in the order of increasing length of the daughter flagellum. Bar = 10 μm.

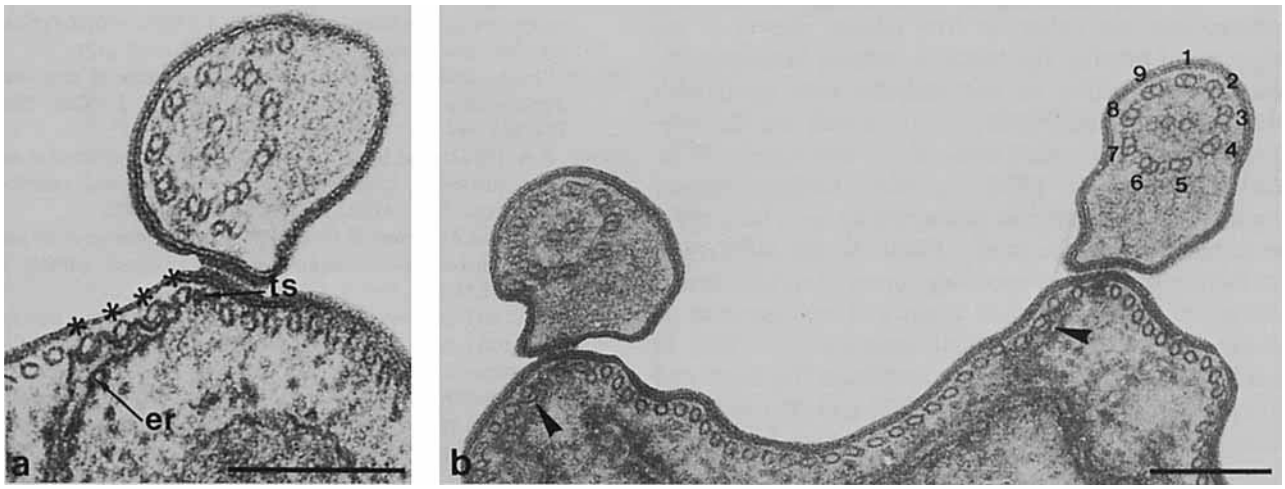

Fig. 4. Electron micrographs of cross sections through the cortical region of *T. b. brucei* cells. **a:** High magnification showing the four microtubules (asterisks) forming the SFMQ associated with a cisterna of the endoplasmic reticulum (er) and the tubular structure (ts) between the SFMQ and the junctional complex. **b:** Lower magnification of a dividing cell showing the SFMQs associated with each of the daughter flagella (arrowheads). Axonal doublets are numbered 1–9. Bar = 200 nm.

the structure labeled by 1B41 is clearly distinct from the one labeled by 5E9. Therefore, the 1B41 antibody is likely to bind to a part of the subpellicular cytoskeleton. A particular structure running along the flagellum, called the "subpellicular organelle" by Taylor and Godfrey [1969], consists of a group of four microtubules immediately adjacent to the junctional complex between the flagellum and the cell body and closely associated to a cisterna of the endoplasmic reticulum; it is always located on the same side as doublets 6–9 of the axoneme (Fig. 4); this structure will be referred to here as the *subflagellar microtubule quartet* or SFMQ. In addition, Vickerman [1969] described a tubular structure, 9 nm in diameter, in the gap between the SFMQ and the junctional complex (Fig. 4a, ts). There are several lines of evidence indicating that the SFMQ is a good candidate for the segregation of the unique β-tubulin recognized by 1B41. First, similarly to the SFMQ, the immunoreactive zone extends from the basal body region to the anterior end of the cell body; second, comparison of the immunofluorescence patterns obtained with 1B41 and 5E9 in dividing cells shows that the 1B41 reactive zone remains associated with the flagellum throughout the cell division cycle as does the SFMQ [Taylor and Godfrey, 1969] (Fig. 4b). mAb have already been reported to be able to detect sequestration of tubulin antigens in a part of the tubulin pool of several cell types including *T. brucei* [Gallo and Anderton, 1983; Sherwin et al., 1987]. This is especially well documented for acetylated α-tubulin, which is located in stable microtubules of mammalian cells [Piperno et al., 1987] and of several unicellular organisms: *Chlamydomonas* [Le Dizet and Piperno, 1986] and *Physarum* [Diggins and Dove, 1987; Sasse et al., 1987].

Clear evidence of the association of particular β-tubulin species with specialized microtubular structures arose from the study of the marginal band of avian erythrocytes [Murphy et al., 1986]. Nevertheless, there is no segregation of genetically encoded β-tubulin isotypes in HeLa cells or mouse hematopoietic cells; furthermore, all these isotypes appear to be functionally interchangeable [Lewis et al., 1987]. The only report of a somewhat uneven distribution of a β-tubulin form inside a single cell deals with the phosphorylation of β-tubulin accompanying neurite outgrowth during neuroblastoma cell differentiation [Gard and Kirschner, 1985]. The results presented in this paper are the first report of a restricted distribution of a β-tubulin epitope within a single cell.

An Antigenically Unique β-Tubulin May Define the Exclusive Dynamics of a Class of Microtubules Related to Morphogenesis

The restricted availability to 1B41 of the corresponding antigenic site of tubulin may be linked to chemical alterations of tubulin or may be induced by the binding of an accessory protein to tubulin; the dot staining observed along the reactive zone is similar to the reaction of an mAb to the microtubule-associated protein MAP1 on brain microtubules [Asai et al., 1985]. The modulation of the exposition of the epitope by chemical modifications of tubulin could be related to differential dynamic properties of microtubule classes in *T. brucei*.

Indeed, the enzymes acting on tubulin identified thus far preferentially act either on free tubulin dimers or on polymerized tubulin. For instance, tubulin carboxypeptidase is more active on microtubules than on tubulin dimers [Kumar and Flavin, 1981], which are the substrate for tubulin-tyrosine ligase [Nath and Flavin, 1979; Wehland and Weber, 1987]. Similarly, *Chlamydomonas* α-tubulin acetyltransferase preferentially acetylates polymeric tubulin [Maruta et al., 1986], so the differential distribution of the corresponding forms of tubulin inside a same cell reflects different kinetics of incorporation of tubulin dimers into individual microtubules. This is underlined by the association of acetylated [Le Dizet and Piperno, 1986; Piperno et al., 1987] and detyrosinated [Gundersen and Bulinski, 1986a,b] tubulin with microtubules having long half-lives. Furthermore, the existence of microtubules with different turnover rates within a single cell has been demonstrated directly by Schulze and Kirschner [1987].

Recently, Kirschner and Mitchison [1986] explained the morphogenesis of microtubular organelles in terms of microtubule dynamics. The most tempting hypothesis suggested by the results presented in this paper is that the special form of β-tubulin contained in the SFMQ leads it to play a role in the establishment of the polarity of daughter cells during the division of *T. brucei* through specific dynamic properties.

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REFERENCES

Asai, D.J., Thompson, W.C., Wilson, L., Dresden C.F., Schulman, H., and Purich, D.L. (1985): Microtubule-associated protein (MAPs): A monoclonal antibody to MAP1 decorates microtubules in vitro but stains stress fibers and not microtubules in vivo. Proc. Natl. Acad. Sci. USA 82:1434–1438.

Biozzi, G., Mouton, D., Sant'Anna, O.A., Passos, H.C., Gennari, M., Reis, M.H., Ferreira, V.C.A., Heumann, A.M., Bouthillier, Y., Ibanez, O.M., Stiffel, C., and Siqueira, M. (1979): Genetics of immunoresponsiveness to natural antigens in the mouse. Curr. Top. Microbiol. Immunol. 85:31–98.

Bodger, M.P., Izaguirre, C.A., Blacklock, H.A., and Hoffbrand, A.V. (1983): Surface antigenic determinants on human pluripotent and unipotent hematopoietic progenitor cells. Blood 61:1006–1014.

Bond, J.F., Robinson, G.S., and Farmer, S.R. (1984): Differential expression of two neural cell-specific β-tubulin mRNAs during rat brain development. Mol. Cell. Biol. 4:1313–1319.

Diggins, M.A., and Dove, W.F. (1987): Distribution of acetylated alpha-tubulin in *Physarum polycephalum*. J. Cell Biol. 104:303–309.

Eipper, B.A. (1972): Rat brain microtubule protein: Purification and determination of covalently bound phosphate and carbohydrate. Proc. Natl. Acad. Sci. USA 69:2283–2287.

Gallo, J.-M., and Anderton, B.H. (1983): A subpopulation of trypanosome microtubules recognized by a monoclonal antibody to tubulin. EMBO J. 2:479–483.

Gallo, J.-M., and Schrével, J. (1985): Homologies between paraflagellar rod proteins from trypanosomes and euglenoids revealed by a monoclonal antibody. Eur. J. Cell Biol. 36:163–168.

Gard, D.L., and Kirschner, M.W. (1985): A polymer-dependent increase in phosphorylation of β-tubulin accompanies differentiation of a mouse neuroblastoma cell line. J. Cell Biol. 100:764–774.

Gundersen, G.G., and Bulinski, J.C. (1986a): Distribution of tyrosinated and nontyrosinated α-tubulin during mitosis. J. Cell Biol. 102:1118–1126.

Gundersen, G.G., and Bulinski, J.C. (1986b): Microtubule arrays in differentiated cells contain elevated levels of a post-translationally modified form of tubulin. Eur. J. Cell. Biol. 42:288–294.

Havercroft, J.C., and Cleveland, D.W. (1984): Programmed expression of β-tubulin genes during development and differentiation of the chicken. J. Cell Biol. 99:1927–1935.

Imboden, M., Blum, B., De Lange, T., Braun, R., and Seebeck, T. (1986): Tubulin mRNAs of *Trypanosoma brucei*. J. Mol. Biol. 188:393–402.

Kilmartin, J.V., Wright B., and Milstein, C. (1982): Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. J. Cell Biol. 93:576–582.

Kimmel, B.E., Samson, S., Wu, J., Hirschberg, R., and Yarbrough, L.R. (1985): Tubulin genes of the african trypanosome *Trypanosoma brucei rhodesiense*: Sequence of a 3.7 Kb fragment containing genes for α- and β- tubulins. Gene 35:237–248.

Kirschner, M., and Mitchison, T. (1986): Beyond self-assembly: From microtubules to morphogenesis. Cell 45:329–342.

Kumar, N., and Flavin, M. (1981): Preferential action of a brain detyrosinolating carboxypeptidase on polymerized tubulin. J. Biol. Chem. 256:7678–7686.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lanham, S.M. (1968): Separation of trypanosomes from the blood of infected rats and mice by anion exchangers. Nature 218:1273–1274.

Le Dizet, M., and Piperno, G. (1986): Cytoplasmic microtubules containing acetylated α-tubulin in *Chlamydomonas reinhardtii*: Spatial arrangement and properties. J. Cell Biol. 103:13–22.

Le Ray, D. (1975): Structures antigéniques de *Trypanosoma brucei* (Protozoa, Kinetoplastida). Analyse immunoélectrophorétique et études comparatives. Ann. Soc. Belge Méd. Trop. 55:129–311.

Lewis, S.A., Gu, W., and Cowan, N.J. (1987): Free intermingling of mammalian β-tubulin isotypes among functionally distinct microtubules. Cell 49:539–548.

L'Hernault, S.W., and Rosenbaum, J.L. (1985): *Chlamydomonas* α-tubulin is posttranslationally modified by acetylation on the ε-amino group of a lysine. Biochemistry 24:473–478.

Maruta, H., Greer, K., and Rosenbaum, J.L. (1986): The acetylation of alpha-tubulin and its relationship to the assembly and disas-

sembly of microtubules. J. Cell Biol. 103:571–579.

Murphy, D.B., Grasser, W.A., and Wallis, K.T. (1986): Immunofluorescence examination of beta-tubulin expression and marginal band formation in developing chicken erythroblasts. J. Cell Biol. 103:628–635.

Nath, J., and Flavin, M. (1979): Tubulin tyrosylation in vivo and changes accompanying differentiation of cultured neuroblastoma-glioma hybrid cells. J. Biol. Chem. 254:11505–11510.

Piperno, G., and Fuller, M.T. (1985): Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. 101:2085–2094.

Piperno, G., Le Dizet, M., and Chang, X.J. (1987): Microtubules containing acetylated α-tubulin in mammalian cells in culture. J. Cell Biol. 104:289–302.

Prentice, H.G., Blacklock, H.A., Janossy, G., Gilmore, M.J.M.L., Price-Jones, L., Tidman N., Trejdosiewicz, L.K., Skeggs, D.B.L., Panjwani, D., Ball, S., Graphakos, S., Patterson, J., Ivory, K., and Hoffbrand, A.V. (1984): Depletion of T lymphocytes in donor marrow prevents significant graft-versus-host disease in matched allogenic leukaemic marrow transplant recipients. Lancet 1:472–475.

Raff, E.C. (1984): Genetics of microtubule systems. J. Cell Biol. 99:1–10.

Raybin, D., and Flavin, M. (1977): Modification of tubulin by tyrosylation in cells and extracts and its effect on assembly in vitro. J. Cell Biol. 73:492–504.

Sasse, R., Glyn, M.C.P., Birkett, C.R., and Gull, K. (1987): Acetylated α-tubulin in *Physarum:* Immunological characterization of the isotype and its usage in particular microtubular organelles. J. Cell Biol. 104:41–49.

Schneider, A., Sherwin, T., Sasse, R., Russell, D.G., Gull K., and Seebeck, T. (1987): Subpellicular and flagellar microtubules of *Trypanosoma brucei brucei* contain the same α-tubulin isoforms. J. Cell Biol. 104:431–439.

Schulze, E., and Kirschner, M. (1987): Dynamic and stable populations of microtubules in cells. J. Cell Biol. 104:277–288.

Seebeck, T., Whittaker, P.A., Imboden, M.A., Hardman, N., and Braun, R. (1983): Tubulin genes of *Trypanosoma brucei*: A tightly clustered family of alternating genes. Proc. Natl. Acad. Sci. USA 80:4634–4638.

Shelanski, M.L., Gaskin, F. and Cantor, C.R. (1973): Microtubule assembly in the absence of added nucleotides. Proc. Natl. Acad. Sci. USA 70:765–768.

Sherwin, T., Schneider, A., Sasse, R., Seebeck, T., and Gull, K. (1987): Distinct localization and cell cycle dependence of COOH terminally tyrosinated α-tubulin in the microtubules of *Trypanosoma brucei brucei*. J. Cell Biol. 104:439–446.

Sullivan, K.F., and Cleveland, D.W. (1986): Identification of conserved isotype-defining variable region sequences for four vertebrate β-tubulin polypeptide classes. Proc. Natl. Acad. Sci. USA 83:4327–4331.

Taylor, A.E.R., and Godfrey, D.G. (1969): A new organelle in bloodstream salivarian trypanosomes. J. Protozool. 16:466–470.

Thomashow, L.S., Milhausen, M., Rutter, W.J., and Agabian, N. (1983): Tubulin genes are tandemly linked and clustered in the genome of *Trypanosoma brucei*. Cell 32:35–43.

Towbin, H., Staehelin, T., and Gordon, J. (1979): Electrophoretic transfers of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350–4354.

Vickerman, K. (1969): On the surface coat and flagellar adhesion in trypanosomes. J. Cell Sci. 5:163–193.

Vickerman, K. (1985): Developmental cycles and biology of pathogenic trypanosomes. Br. Med. Bull. 41:105–114.

Vickerman, K., and Preston, T.M. (1976): Comparative cell biology of Kinetoplastida flagellates. In Lumsden, W.H.R., and Evans, D.A. (eds.): "Biology of the Kinetoplastida," London: Academic Press, pp. 35–130.

Wehland, J., and Weber, K. (1987): Tubulin-tyrosine ligase has a binding site on β-tubulin: A two domain structure of the enzyme. J. Cell Biol. 104:1059–1068.

Wehland, J., Willingham, M.C., and Sandoval, I.V. (1983): A rat monoclonal antibody reacting specifically with the tyrosinated form of α-tubulin. I. Biochemical characterization, effects on microtubule polymerization in vitro, and microtubule polymerization and organization in vivo. J. Cell Biol. 97:1467–1475.