Subpellicular and Flagellar Microtubules of
Trypanosoma brucei brucei Contain the Same α-Tubulin Isoforms

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Abstract. The cytoskeleton of the parasitic hemoflagellate *Trypanosoma brucei brucei* essentially consists of two microtubule-based structures: a subpellicular layer of singlet microtubules, which are in close contact with the cell membrane, and the flagellar axoneme. In addition, the cells contain a small pool of soluble tubulin. Two-dimensional gel electrophoretic analysis of the tubulins present in these subcellular compartments revealed two distinct electrophoretic isoforms of α-tubulin, termed α1 and α3. α1-Tubulin most likely represents the primary translation product, while α3-tubulin is a posttranslationally acetylated derivative of α1-tubulin. In the pool of soluble cytoplasmic tubulin, α1 is the predominant species, while the very stable flagellar microtubules contain almost exclusively the α3-tubulin isoform. The subpellicular microtubules contain both isoforms. Neither of the two α-tubulin isoforms is organelle specific, but the α3 isoform is predominantly located in stable microtubules.

MICROTUBULES (MTs)[1] are involved in a wide variety of cellular functions, such as maintenance of cellular architecture, cell motility, and intracellular and axonal transport (Roberts and Hyams, 1979; Dustin, 1984; Borisy et al., 1984). Despite their functional versatility, the overall structure of MTs is invariably that of a defined polymer formed by the reversible polymerization of α/β-tubulin heterodimers (Margolis and Wilson, 1981; Mitchison and Kirschner, 1984; Purich and Kristofferson, 1984). Each of the two tubulins is coded for by one (Neff et al., 1983; Callahan et al., 1984) or several genes (Cleveland et al., 1980; Wilde et al., 1982; for a review see Cleveland and Sullivan, 1985). In the latter case, such multiple gene families may code for identical (Youngblom et al., 1984) or nonidentical (Ponstingl et al., 1982; Hall et al., 1983) tubulin proteins, and different tubulin genes may be active in different organs (Kalfayan and Wensink, 1981; Murphy and Wallis, 1983; Cowan et al., 1983) or during different stages of development (Kemphues et al., 1979; Bond and Farmer, 1983; Lewis et al., 1985).

Analysis of the tubulin proteins has revealed extensive microheterogeneity of both α- and β-tubulin. This microheterogeneity is detected both in complex organs (Field et al., 1984) as well as within individual cells (Gozes and Sweadner, 1981). While some of the electrophoretic isotypes are due to differences in amino acid sequence, hence representing products of nonidentical tubulin genes, many may be generated by posttranslational modifications. In general, little is known about the function of these modifications. A significant exception is the flagellar α-tubulin isotype of *Chlamydomonas* and *Polytomella* (Brunke et al., 1982a; McKeithan et al., 1983; L'Hernault and Rosenbaum, 1983). The genome of *Chlamydomonas* contains two α- and two β-tubulin genes, which are coordinately regulated (Brunke et al., 1982b). The two α-tubulin genes code for two slightly different proteins, one of which is found as the predominant species in vivo (Silflow et al., 1985). The β-tubulin genes of *Chlamydomonas*, though containing introns of different sequence and being transcribed into two mRNAs of different length, code for identical proteins (Youngblom et al., 1984). The tubulin isoform pattern of *Chlamydomonas* cells displays just two isoforms of α-tubulin and a single isoform of β-tubulin (McKeithan et al., 1983). One of the two α-tubulin isoforms, α1, represents the primary translation product, while the other, α3, which is enriched in the flagellum, is its acetylated derivative (L'Hernault and Rosenbaum, 1983; L'Hernault and Rosenbaum, 1985a). The acetylation of α1-tubulin to α3-tubulin is coupled to the growth of the flagellum (McKeithan et al., 1983) and is reversible upon flagellar resorption (L'Hernault and Rosenbaum, 1985b).

A very similar tubulin isotype pattern was also found in an unrelated organism, the flagellate *Crithidia fasciculata* (Russell et al., 1984). Also in analogy to *Chlamydomonas*, the generation of one α-tubulin isotype was seen to depend on flagellar growth, and the particular isotype was found to be the sole α-tubulin isotype in the flagellum (Russell et al., 1984; Russell and Gull, 1984).

1. *Abbreviation used in this paper*: MT, microtubule.

The present study analyzes the tubulin isotype pattern of the parasitic hemoflagellate *Trypanosoma brucei*. This organism is the causative agent of human sleeping sickness in Africa, as well as of economically disastrous cattle diseases (Ormerod, 1979). It undergoes a complex life cycle, which includes both a mammalian and a fly host, and during which extensive morphological and metabolic transformations of the trypanosomes take place (Hoare, 1972). Interphase trypanosome cells contain three major microtubular structures; namely, the flagellar axoneme, the subpellicular layer of membrane-bound singlet microtubules, and the cytoplasmic pool of soluble tubulin (Vickerman and Preston, 1976). Tubulin from *Trypanosoma brucei* has been isolated and characterized earlier (Stieger et al., 1984). The trypanosomal genome contains ~10 α- and 10 β-tubulin genes (Imboden et al., 1986) in a tightly clustered array of alternating α and β genes (Thomashow et al., 1983; Seebeck et al., 1983). Only a single size class each of α- and of β-tubulin mRNA is present in these cells (Sather and Agabian, 1985; Imboden et al., 1986).

The data presented here will document the tubulin isotype pattern of *Trypanosoma brucei*, both of the procyclic (insect gut) and the bloodstream form. One of the two α-tubulin subtypes detected represents the primary translation product of the α-tubulin mRNA, and the other is a posttranslational isoform thereof. This isoform is highly enriched not only in the flagellum, but also in the subpellicular microtubules. No differences in isoform pattern are detected between bloodstream trypanosomes and culture-grown procyclic (insect gut) forms. The accompanying paper (Sherwin et al., 1987) will present an analysis of another posttranslational modification of the trypanosomal α-tubulin (tyrosinolation) and the temporal control of its intracellular distribution.

Materials and Methods

Trypanosomes

Trypanosoma brucei brucei trypomastigotes maintained by passage through mice were harvested from rats 3–4 d after intraperitoneal inoculation. Trypomastigotes were separated from blood cells by DEAE–cellulose chromatography (Lanham and Godfrey, 1970).

Procyclic *Trypanosoma brucei brucei*, stock STIB 366, were grown in tissue culture flasks in SDM-79 medium (Brun and Schönenberger, 1979). Cells were harvested during exponential growth; i.e. at ~5 × 10^6 cells/ml.

Cell Fractionation

Soluble Cytosplasmic Pool. Two procedures for cell lysis were used with similar results. (*a*) Bloodstream trypanosomes were chilled and then sonicated five times for 5 s with an MSE bench sonicator with a 5-mm probe operating at a 9-μm amplitude. The lysis buffer contained 0.1 M Pipes, pH 6.9, 2 mM EGTA, 1 mM $MgSO_4$, 0.1 mM EDTA, and 10 μg/ml leupeptin (PEME buffer). The 100,000 g supernatant from this lysate was retained and designated the cytoplasmic fraction. (*b*) Procyclic trypanosomes were washed once in PBS (50 mM Na phosphate, pH 7.2, 150 mM NaCl). The pellet was suspended in 1/100 of the original culture volume MME buffer (10 mM morpholino propane sulfonic acid [MOPS], pH 6.9, 1 mM EGTA, 1 mM $MgSO_4$), frozen in liquid nitrogen, and then thawed again. The 100,000 g supernatant from this lysate was retained and designated the cytoplasmic fraction.

Cytoskeletons. Cells were incubated in lysis buffer (MME or PEME) containing 0.2% Triton X-100 and left in ice for 1–10 min. Cytoskeletons were collected by centrifugation at 3,000 g and the extraction step was repeated once more.

Subpellicular Fraction. Trypanosomes were suspended in PMN buffer (10 mM Na phosphate, pH 7.2, 150 mM NaCl, 1 mM $MgCl_2$) containing

0.1% Triton X-100. The resulting cytoskeletons were washed twice in the same buffer and were then suspended in the same buffer containing 1 mM $CaCl_2$. This suspension was incubated in ice for 45 min and subsequently centrifuged at 100,000 g for 1 h. The supernatant is highly enriched in subpellicular tubulin. This method represents an adaptation of the procedure for the selective depolymerization of pellicular MTs described by Dolan et al. (1986), where complete disassembly of the pellicular MTs was found to occur at 100 μM Ca^{2+}, with complete retention of the flagellar MTs. Increasing the Ca^{2+} concentration to 500 μM incurred no further release of tubulin, the maximum release being ~53% of the total cytoskeletal tubulin. We also observe complete depolymerization of the subpellicular MTs and complete retention of the axoneme, with the pellicle accounting for approximately half of the tubulin in the cytoskeleton.

Flagella. Cells were extracted with a buffer consisting of 0.1 M Pipes, pH 6.9, 2 mM EGTA, 1 mM $MgSO_4$, 0.1 mM EDTA, 1% NP-40. Under these conditions, the flagellum remains attached to the cell body only at its base. Flagella were detached from these cytoskeletons by agitation in a Polytron at setting 3 for 10 s. The suspension was then layered onto 0.1 M sucrose and centrifuged for 5 min at 500 g. Flagella remained at the sample/sucrose interphase and were collected for two-dimensional gel analysis. Alternatively, cytoskeletons (see above) were resuspended in MME buffer containing 0.1% Triton X-100 and 1 M NaCl. After leaving for 10 min in ice, flagella were collected by centrifugation at 16,000 g for 10 min.

Usually 5 × 10^7 cells were used for soluble cytoplasmic pool preparations, while 2 × 10^7 cells were used for the preparation of cytoskeletons, flagella, and the subpellicular fraction. Total proteins of the resulting fractions were solubilized for two-dimensional gel analysis to a final volume of 200 μl, of which 20–50 μl was loaded per gel.

In Vivo Acetylation of α-Tubulin

Conditions for inhibition of protein synthesis and in vivo labeling of trypanosomes with [³H]acetate (New England Nuclear, Boston, MA; 3.4 Ci/mmol) were similar to those described by Stieger et al. (1984) for labeling cells with [³H]tyrosine. Exponentially growing trypanosomes were collected, washed twice, and finally resuspended in ME-83 medium (Seebeck and Kurath, 1985). Cells were preincubated for 30 min in the presence of 50 μg/ml cycloheximide, 25 μg/ml each of puromycin and chloramphenicol. The cells were then labeled with 200 μCi/ml [³H]acetate for 2 h and then processed for two-dimensional gel electrophoresis and fluorography.

RNA Isolation and Translation

Total RNA was isolated from bloodstream trypanosomes by the phenol/acetate procedure described previously (Russell et al., 1984) and from procyclic trypanosomes by guanidinium rhodanide extraction (Imboden et al., 1986). Tubulin-specific mRNA was isolated by hybridization to filters carrying DNA of plasmid pTBtu9A, which contains an α-tubulin and a β-tubulin gene of *T. brucei* (Seebeck et al., 1983), or appropriate subclones which are specific for either of the two genes (pTBtu 9A-IV and pTBtu 9A-VII). Total polyA⁺ RNA was purified by oligo-dT cellulose chromatography. In vitro translation was performed in commercial rabbit reticulocyte lysates (Amersham Corp., Arlington Heights, IL or Promega Biotec, Madison, WI). Total translation mixtures were directly used for two-dimensional PAGE analysis.

Two-dimensional Gel Electrophoresis and Immunoblotting

Two-dimensional gel electrophoresis was performed essentially according to the procedure of O'Farrell (1975), with the modifications described by Burland et al. (1983). Transfer of proteins to nitrocellulose and immunostaining with monoclonal antibodies was done as previously described (Birkett et al., 1985). The following antibodies were used: YOL/34 (Kilmartin et al., 1982; α-tubulin specific), DM1A (Blose et al., 1984; α-tubulin specific), and KMX-1 (Birkett et al., 1985; β-tubulin specific).

Results

Trypanosomes Contain Two α-Tubulin Isoforms

Total proteins from procyclic trypanosomes were analyzed by two-dimensional gel electrophoresis. Coomassie Blue staining visualized three distinct spots in the gel region
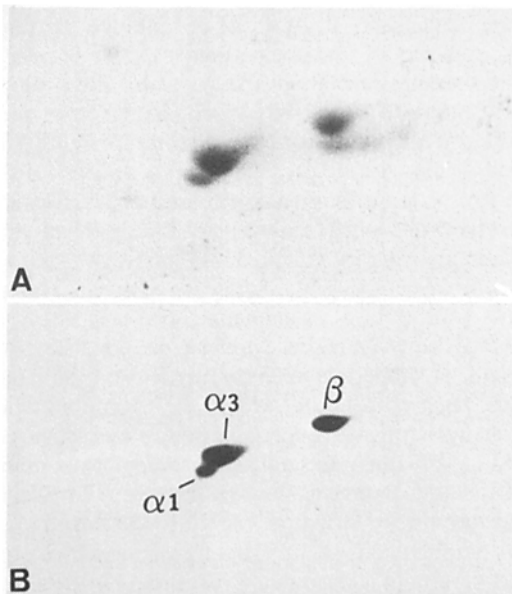
Figure 1. A whole cell lysate was analyzed by two-dimensional gel electrophoresis. Proteins were visualized by Coomassie staining (*A*) and the tubulins were detected by immunostaining with a cocktail of DM1A antibody (α-tubulin specific) and KMX1 (β-tubulin specific). Only the tubulin region of the gel is presented in the figure.

Figure 2. Analysis of tubulin translated in vitro from hybrid-selected α-tubulin mRNA. The whole translation mix was supplemented with unlabeled tubulin as an internal reference and was analyzed by two-dimensional gel electrophoresis. An autoradiogram of the entire gel is presented. The only detectable signal is at the position of α1-tubulin. The position of α3-tubulin is indicated by an open circle.

where the tubulins were expected. Immunostaining with α-tubulin– and β-tubulin–specific antibodies revealed that the two more basic spots, with smaller apparent molecular weights, are α-tubulin, while the third spot is β-tubulin. In accordance with the nomenclature applied previously to the tubulins of *Chlamydomonas*, we have termed the more basic of the two α-tubulin isoforms α1, while the apparently higher molecular weight, less basic variety has been termed α3. The relative coordinates of α1 and α3 tubulin isoforms on a two-dimensional gel have been established with the related flagellate *Crithidia fasciculata* (Russell and Gull, 1984). Mixing experiments performed with different subcellular fractions from this trypanosome have shown that these coordinates are constant, and that α1 and α3 are distinct tubulin isoforms. Fig. 1 presents a comparison between a Coomassie-stained gel (Fig. 1 *A*) and a gel stained with a combination of an α-tubulin–specific (DM1A) and a β-tubulin–specific (KMX1) antibody. A large quantitative difference between the two α-tubulin isoforms is observed both in Coomassie-stained (Fig. 1 *A*) and in immunostained (Fig. 1 *B*) gels, the α3-tubulin being the most abundant isoform.

α1-Tubulin Is the Primary Translation Product

The genome of *Trypanosoma brucei* contains numerous tubulin genes, which are all very similar, though not necessarily identical (Seebeck et al., 1983; Thomashow et al., 1983). Thus, the two α-tubulin isoforms could either represent two different products of two different genes or, alternatively, be derived one from the other through posttranslational modification. To resolve this question, α-tubulin mRNA was selected by preparative hybridization and was then translated in vitro. Analysis of the in vitro translation products revealed only a single electrophoretic component,
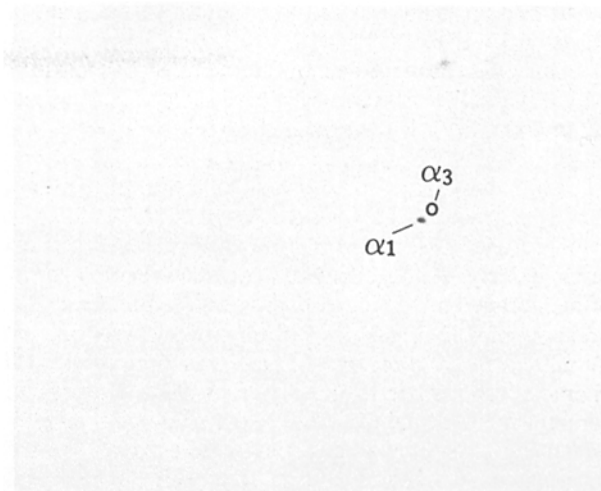
which co-migrates with α1-tubulin (Fig. 2). Thus, α1-tubulin, which is the minor species in total cell extracts, presumably represents the primary translation product, while α3-tubulin is most likely a modified derivative of α1-tubulin. This is in agreement with previous results from pulse-chase radiolabeling experiments performed with *Crithidia fasciculata* (Russell and Gull, 1984), where [35S]methionine label was shown to move from the α1-tubulin isoform to α3 in cells which were regenerating flagella in the absence of protein synthesis.

α3 Is an Acetylated Form of Tubulin

The above results showing that the α3-tubulin isoform is generated via a posttranslational modification is directly analogous to the situation in *Chlamydomonas reinhardtii*. In this latter organism, the modification has been shown, by a variety of criteria, to be an acetylation. We have radiolabeled trypanosomes with [3H]acetate under conditions of stringent protein synthesis inhibition. Subsequent analysis of cellular proteins by two-dimensional gel electrophoresis and fluorography has revealed that under these conditions only the α3-tubulin becomes radiolabeled (Fig. 3), strongly indicating that the modification that generates the *Trypanosoma brucei* α3-tubulin isoform is also an acetylation. Confirmation of this conclusion comes from recent work using a monoclonal antibody which is selective for acetylated tubulin (Piperno and Fuller, 1985) (Sasse, R., unpublished data).

Distribution of Tubulin Isoforms in Different Cellular Compartments

Cell fractionation experiments were undertaken to investigate the distribution of the two α-tubulin isoforms in the different subcellular fractions of the trypanosome cell. In a previous study with *Crithidia fasciculata* (Russell et al., 1984), we have purified the tubulins from the different sub-
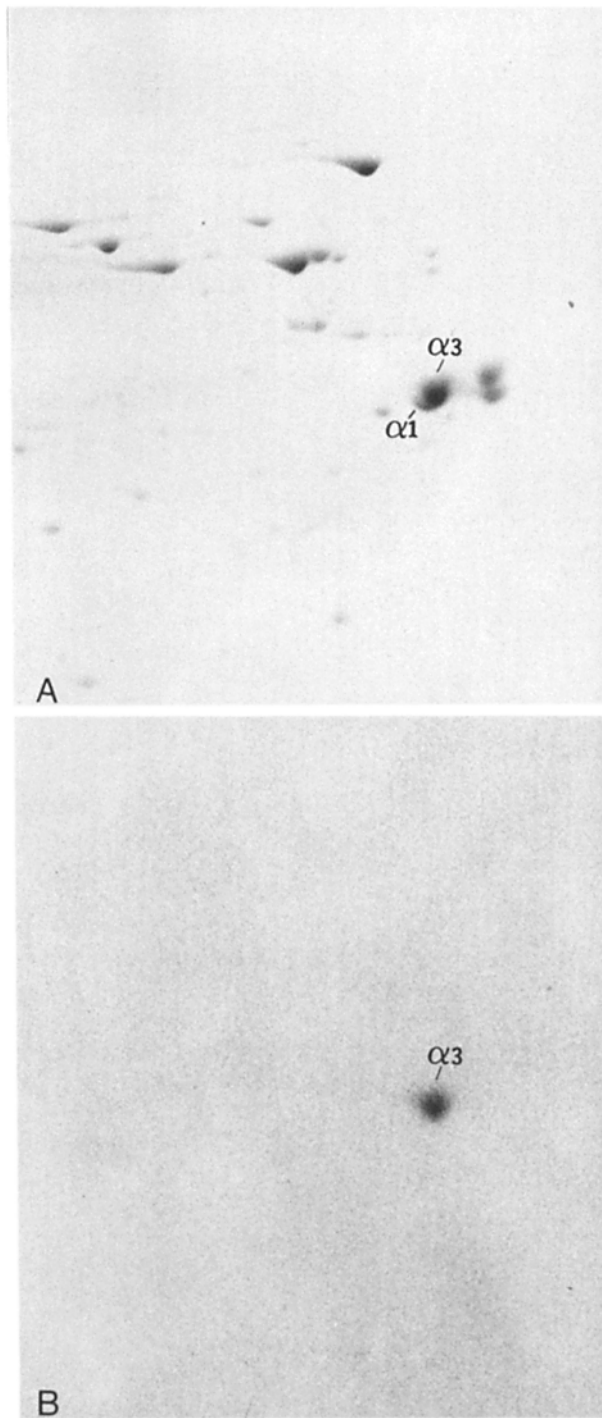
Figure 3. Posttranslational acetylation of the α3-tubulin isoform. Cells were incubated with [³H]acetate under conditions of stringent protein synthesis inhibition and were then processed for two-dimensional analysis. (*A*) Tubulin region of a gel stained with Coomassie Blue. (*B*) Fluorograph of the same region. Label is detected only in the α3-tubulin isoform.

the subpellicular and flagellar MTs, and the subsequent selective removal of the subpellicular MTs, leaving the flagellar axonemes. Isolation and purity of these preparations were carefully monitored by light and electron microscopy. Electron micrographs of the different subcellular fractions used for analysis are presented in Fig. 4. Fig. 4 *A* shows a transverse section through a trypanosome cell, illustrating the three fractions to be analyzed: (*a*) the pool of soluble cytoplasmic protein (not visible on the micrograph), (*b*) the subpellicular layer of singlet, membrane-associated microtubules, and (*c*) the flagellar axoneme.

Fig. 4 *B* presents a purified cytoskeleton, consisting of the intact "cage" of subpellicular microtubules and the flagellar structures. Such preparations are devoid of soluble tubulin. Fig. 4 *C* shows a transverse section through such a cytoskeleton, and Fig. 4 *D* illustrates a flagellar preparation, containing the flagellar axoneme as the sole microtubular structure. The other prominent structure visible in the flagellum, the paraxial complex, consists of a set of proteins which are not related to tubulin (Vickerman and Preston, 1976; Russell et al., 1983; Gallo and Schrevel, 1985).

The proteins of the subcellular fractions were analyzed by two-dimensional electrophoresis and the tubulin regions of resulting gels are depicted in Fig. 5. Fig. 5 *A* represents the pool of soluble cytoplasmic protein. In striking contrast to the pattern observed with total cellular protein (Fig. 1), only the α1-tubulin isoform (i.e., the presumed primary translation product, but not α3-tubulin) is present in this fraction. A small amount of α3-tubulin can occasionally be detected with immunoblotting, which may represent a slight contamination by protein from other subcellular fractions. Alternatively, and more interestingly, it may represent the small steady-state pool of newly posttranslationally modified α-tubulin.

In contrast to the pattern seen with the soluble tubulin pool, both α-tubulin isoforms are present when cytoskeletons are analyzed (Fig. 5 *B*). In this structure, the α3-tubulin isoform is the major species. However, when the two different types of stable MTs which constitute the cytoskeleton, the subpellicular MTs, and the flagellar axonemal MTs are analyzed separately, further differences become evident. The subpellicular MTs (Fig. 5 *C*) contain both isoforms, with α3-tubulin being the major and α1-tubulin the minor species, with an overall ratio which is quite similar to that detected with whole cell extracts (compare Fig. 1 *C*). Quite a different ratio between α1- and α3-tubulin is detected in the flagellar MTs, which contain almost exclusively α3-tubulin, with no more than a trace of α1-tubulin (Fig. 5 *D*). In all subcellular fractions, as well as in whole cell extracts, the same, single species of β-tubulin was always observed.

The observed differential distribution of α-tubulin isoforms in the various subcellular fractions is compatible with the idea that the α1-tubulin isoform is the primary translation product in vivo, which is subsequently incorporated into the stable MTs of the cytoskeleton. Upon incorporation, α1-tubulin is modified into α3-tubulin, the modification occurring to different extents in the subpellicular layer and in the flagellum. Alternatively, the observed isoform patterns may represent differences in the rates of modification and/or microtubule assembly between the two microtubular organelles.

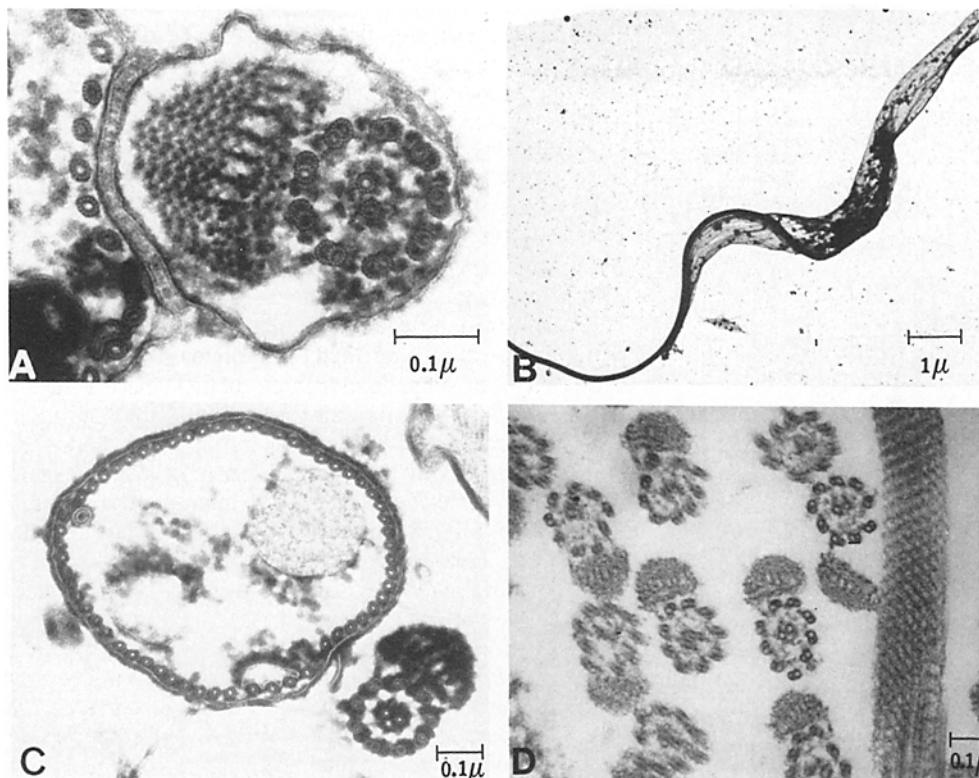A closer inspection of many two-dimensional gels revealed that in the cytoplasmic pool, α-tubulin is often in ex-

cellular compartments. However, in the present paper, the subcellular fractions were analyzed in their totality in order to avoid the possible selection of tubulin isoforms during the purification steps. The experimental procedure involved the isolation of a detergent-insoluble cytoskeleton containing

Figure 4. Electron micrographs of subcellular fractions. (*A*) Cross section through an intact trypanosome cell; (*B*) cytoskeleton (negative stain); (*C*) cross section through a cytoskeleton; (*D*) cross section through a flagellar preparation.

cess over β-tubulin, whereas in the subpellicular MTs or in the flagellar axoneme, the two tubulin species are always present in stoichiometric amounts. The excess of α-tubulin in the cytoplasmic pool is observed irrespective of the procedure used to visualize the tubulin (Coomassie staining, silver staining, or immunostaining), indicating that it is not a trivial staining artefact.

The Tubulin Isoform Pattern Does Not Change during the Life Cycle of Trypanosomes

Trypanosoma brucei goes through a number of morphologically and metabolically distinct states during its life cycle, which includes as host both a fly vector and a mammal (Hoare, 1972). This high degree of biological variability led us to question whether the basic biochemistry of the MTs is different in these different life cycle stages. The studies presented so far have been performed with culture-grown procyclic (insect gut form) trypanosomes. For a comparison, bloodstream trypanosomes were isolated from infected rats and were analyzed for their tubulin isoforms as described above. A synopsis of these results is given in Fig. 6. Total cellular tubulin displays again two α-tubulin isoforms, which co-migrate with the α1- and α3-tubulins previously identified in procyclic trypanosomes. Also, only a single β-tubulin species can be detected (Fig. 6 *A*). For cell fractionation, a procedure was used which was different from that used above for procyclic cells (see Materials and Methods). Nevertheless, very similar results were obtained. The pool of soluble tubulin essentially consists of α1- and β-tubulin, with no, or very little, discernible α3-tubulin isoform present (Fig. 6 *B*). The cytoskeleton contains both α1- and α3-tubulin isoforms, with α3-tubulin being the major species (Fig. 6 *C*), while

in the flagellum, only the α3-tubulin isoform can be detected (Fig. 6 *D*).

Primary translation products from bloodstream form mRNA were analyzed by in vitro translation of total mRNA and subsequent analysis of the products by two-dimension electrophoresis. Since tubulin mRNA is very abundant in bloodstream trypanosomes, the tubulins synthesized in vitro can be directly identified among the translation products of total mRNA. In agreement with the results found in procyclic trypanosomes, only one α-tubulin isoform, α1, is synthesized (Fig. 6 *E*). The satellite spot near α-tubulin in Fig. 6 *E* (X) is neither α1- nor α3-tubulin, nor is there a corresponding cellular polypeptide with these coordinates. Spots similar to this have been observed previously in in vitro translation products by McKeithan et al. (1983) for *Chlamydomonas* and by Green and Dove (1984) for *Physarum*.

The comparison of the tubulin isoform patterns from procyclic and from bloodstream stage trypanosomes shows that they are very similar indeed. This observation indicates that the basic biochemistry and dynamics of the microtubular structures remain unchanged throughout the different stages of the life cycle of these organisms.

Discussion

Trypanosomes contain a number of different microtubular structures, which together form the dominant component of their cellular architecture (Vickerman and Preston, 1979). Despite their variability in structure and function, they are all composed of tubulins which display a surprisingly simple isoform pattern. Besides the primary translation product, termed α1-tubulin, only one other isoform of α-tubulin, α3,
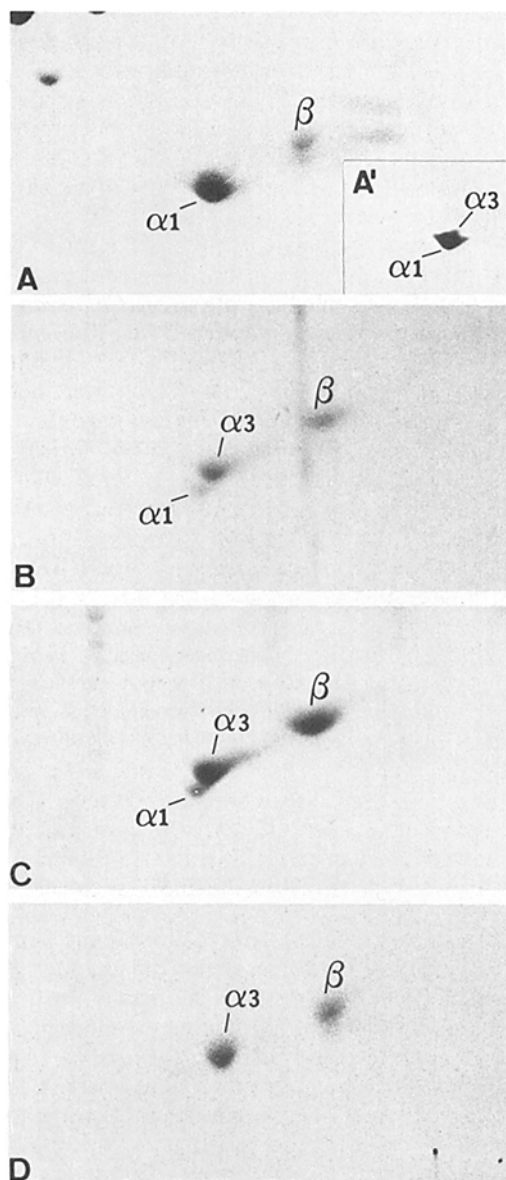
Figure 5. Distribution of tubulin isotypes in different subcellular fractions. (*A*) Silver staining of the pool of soluble protein; (*A'*) Immunostaining with DM1A (α-tubulin specific); (*B*) cytoskeleton (Coomassie staining); (*C*) subpellicular MTs (Coomassie staining); (*D*) flagella (Coomassie staining). Only the tubulin region of the gel is presented.

is detected. However, the quantitative ratio between these two isoforms differs strongly in different subcellular compartments. The cytoplasmic pool of soluble tubulin almost exclusively contains the α1-tubulin isoform, considered to represent the unmodified protein. In contrast, the membrane-associated subpellicular MTs contain both isoforms, α3-tubulin being the major species by far, while the flagellar axonemal MTs are almost completely devoid of the α1-tubulin isoform. In all subcellular fractions, the same single β-tubulin variant is present, which is likely to represent the primary unmodified translation product.

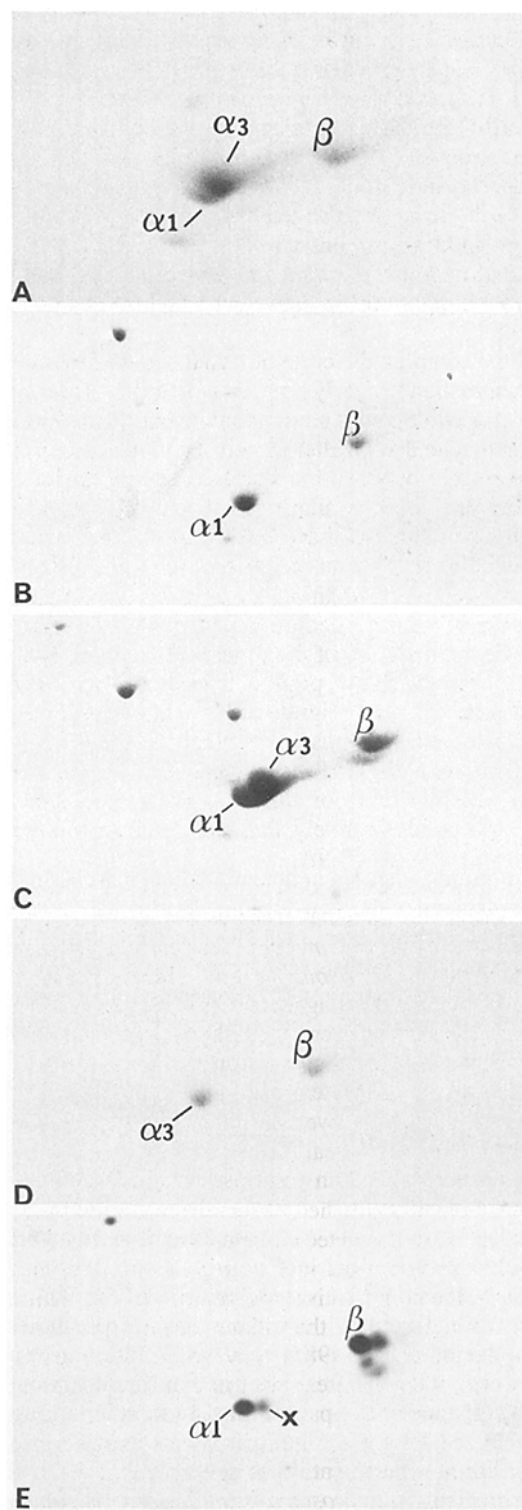Based on its coordinates of migration in a two-dimensional gel system, the α3-tubulin isoform of trypanosomes is very

Figure 6. Tubulin isotypes of bloodstream trypanosomes. (*A*) Whole cell lysate; (*B*) pool of soluble cytoplasmic proteins; (*C*) cytoskeleton; (*D*) flagella. *A–D* are Coomassie stains. (*E*) Autoradiograph of a gel displaying the tubulin translated in vitro from total mRNA. The spot nearby the α1-tubulin position (*X*) is neither α1 nor α3 and represents an artefact of the translation system (see text for details). In all panels, only the tubulin region of the gels is presented.

similar to the acetylated α3-tubulin isoform detected in *Chlamydomonas* (L'Hernault and Rosenbaum, 1985). In vivo labeling experiments with [³H]acetate provide strong evidence that the trypanosomal α3 isoform is indeed an acetylated derivative of α1. This conclusion is further supported by the observation that an acetylation-specific monoclonal antibody (Piperno and Fuller, 1985) strongly reacts with the α3, but not with the α1 tubulin isoform. Furthermore, our results are in good agreement with previous experiments with the related flagellate *Crithidia fasciculata*, in which a precursor/product relationship between α1 and α3 had been demonstrated.

In view of the complex life cycle of trypanosomes, including both a mammalian and a fly host, in which they undergo a number of morphological and metabolic transformations, it is important to note that no alteration in the tubulin isoform pattern is detectable between bloodstream and insect form trypanosomes. This close similarity is all the more significant as different procedures have been used for preparing analogous subcellular fractions from procyclic and bloodstream trypanosomes. In addition, it provides an internal control for the absence of procedure-dependent fractionation artefacts. In vitro translation of the tubulin mRNAs demonstrated that a single translation product of each α-tubulin and β-tubulin is made. These co-migrate with the in vivo–synthesized α1- and β-tubulin isoforms, respectively. This result indicates that all of the 10 α- and β-tubulin genes in the genome of *Trypanosoma brucei* code for identical or extremely similar gene products or, alternatively, that only a subset of them is being transcribed into mRNA.

The simple isoform pattern described here for *Trypanosoma brucei* is very similar to patterns observed in other protozoa such as the algae *Chlamydomonas reinhardtii* (McKeithan et al., 1983) and *Polytomella agilis* (McKeithan and Rosenbaum, 1981) and the flagellate *Crithidia fasciculata* (Russell et al., 1984). However, in all these organisms, including trypanosomes, the tubulin isoform pattern is operationally defined by the gel system used for the analysis, but it most likely represents an oversimplified picture. Higher resolving gel systems may reveal further isoforms within the ones defined above (McKeithan and Rosenbaum, 1981; Russell et al., 1984; our unpublished observations), and monoclonal antibodies have also detected heterogeneity within individual tubulin isotype spots in *Physarum* (Birkett et al., 1985). Similarly, the comparatively large number of tubulin isotypes recently detected in the ciliate *Tetrahymena thermophila* (Suprenant et al., 1985) may reflect the greater resolving power of the gel system used in their study, rather than a basic difference to the pattern in the aforementioned organisms.

Cell fractionation experiments have demonstrated that the α3-tubulin isoform in *Trypanosoma brucei* is not restricted to a particular subcellular compartment. Three lines of evidence serve to rule out that this conclusion might be influenced by crosscontamination between the different subcellular fractions. (a) Two different cell fractionation procedures have both led to very similar results. (b) In total cell lysates, the α3-tubulin isoform is by far the major α-tubulin species. Since the flagellar tubulin cannot account for more than half of the total polymerized cellular tubulin, the α3-tubulin isoform has to be present in the subpellicular MTs, which account for the bulk of total cell tubulin. (c) In the accompany-

ing paper, Sherwin et al. (1987) demonstrate that in vivo tyrosinolation of α3-tubulin is easily detected in subpellicular microtubules even after short-term labeling periods with [³H]tyrosine, while little is detected in the flagellum under similar experimental conditions. In such experiments, crosscontamination of subpellicular with flagellar MTs can be excluded, since the flagellar MTs are not radiolabeled under the conditions used. These biochemical data are in agreement with recent reports (Piperno and Fuller, 1985; LeDizet and Piperno, 1986) where the authors have demonstrated immunologically that the α3-tubulin isoform in *Chlamydomonas* is present in different compartments of the cell. Similarly, Russell et al. (1984) have also detected the α3-tubulin isoform in the subpellicular MTs of *Crithidia*, but were not able to rule out the possibility of flagellar contamination.

The role of α-tubulin acetylation is not clear at present. The reaction is reversible in that tubulin is modified while it is being deposited in the flagellar axoneme (L'Hernault and Rosenbaum, 1983; Brunke et al., 1982a; Russell and Gull, 1984) and is deacetylated upon flagellar resorption (L'Hernault and Rosenbaum, 1985b). In *Chlamydomonas*, an α-tubulin–specific acetyl transferase has been detected in the flagellum as well as in the cytoplasm (Greer et al., 1985). These recent results, as well as those of Piperno and Fuller (1985) and LeDizet and Piperno (1986) fully support the contention of the present study that acetylation may be involved in the regulation of microtubule stability. Such a notion is suggested by the very different ratios of α1- to α3-tubulin in the various trypanosomal microtubules. The cytoplasmic pool of soluble tubulin contains very little or no α3-tubulin isoform, while the very stable MTs of the flagellar axoneme contain almost exclusively α3-tubulin. The MTs of the membrane-associated subpellicular array, which are of intermediate stability, are also intermediate in their content of α3-tubulin isoform.

Any interpretation of the function of the α3-tubulin isoform, and, hence, of the putative tubulin acetylation, should be made with great care and is possibly inadequate, since additional modifications, which are not detected in the gel system used, can be expected. One such modification, the posttranslational reversible tyrosinolation of the trypanosomal α-tubulin (Stieger et al., 1984) has been detected to occur on both α-tubulin isoforms (see the accompanying paper by Sherwin et al. [1987]). To understand the function of tubulin modifications, much further work and suitable model organisms are needed. The simply structured cytoskeleton of *Trypanosoma brucei*, in conjunction with the fact that structure and function of its tubulin genes have been worked out in great detail (Thomashow et al., 1983; Seebeck et al., 1983; Kimmel et al., 1985; Sather and Agabian, 1985; Imboden et al., 1986), makes this organism highly accessible for studying the complex functions of such simple modifications.

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