

Distinct Localization and Cell Cycle Dependence of COOH Terminally Tyrosinolated α -Tubulin in the Microtubules of *Trypanosoma brucei brucei*

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Abstract. α -Tubulin can be posttranslationally modified in that its COOH-terminal amino acid residue, tyrosine, can be selectively removed and replaced again. This reaction cycle involves two enzymes, tubulin carboxypeptidase and tubulin tyrosine ligase. The functional significance of this unusual modification is unclear. The present study demonstrates that posttranslational tyrosinolation of α -tubulin does occur in the parasitic hemoflagellate *Trypanosoma brucei brucei* and that posttranslational tyrosinolation can be detected in both α -tubulin isoforms found in this organism. Trypanosomes contain a number of microtubular

structures: the flagellar axoneme; the subpellicular layer of singlet microtubules which are closely associated with the cell membrane; the basal bodies; and a cytoplasmic pool of soluble tubulin. Tyrosinolated α -tubulin is present in all these populations. However, immunofluorescence studies demonstrate a distinct localization of tyrosinolated α -tubulin within individual microtubules and organelles. This localization is subject to a temporal modulation that correlates strongly with progress of a cell through the cell cycle. Our results indicate that the presence of tyrosinolated α -tubulin is a marker for newly formed microtubules.

MICROTUBULES (MTs)¹ can exert a number of different functions, assume various configurations in different cellular compartments, and exhibit vastly different stabilities even within individual cells. A number of biochemical events are thought to contribute to the amazing structural and functional flexibility of these rigidly conserved polymers. These include the variation in the activity and localization of microtubule-organizing centers (Tucker, 1979), changing properties and local concentrations of microtubule-associated proteins, and the presence within different MTs of tubulins with differing amino acid sequences (Ponstingl et al., 1982; Cleveland and Sullivan, 1985). A further means of generating MT heterogeneity is via posttranslational modification of the tubulin proteins themselves (Eipper, 1974; Sandoval and Cuatrecasas, 1976; Feit and She-lansky, 1975; L'Hernault and Rosenbaum, 1983).

An unusual type of modification has been observed by Barra et al. (1973), who reported the posttranslational addition of tyrosine to a brain protein which was subsequently identified as α -tubulin. Further studies have demonstrated that the tyrosine is added to the carboxy terminus of α -tubulin. In most organisms, α -tubulin is initially synthesized with a tyrosine as its COOH-terminal amino acid (Tyr-tubulin) (Valenzuela et al., 1981; Cleveland and Sullivan, 1985). This tyrosine can be posttranslationally removed in vivo by a specific carboxy-peptidase (Argarana et al., 1980;

Martensen, 1982), exposing the penultimate amino acid residue, which is glutamic acid (Glu-tubulin). Most likely, the substrate for this enzyme activity is the tubulin of the MTs (Thompson et al., 1979; Kumar and Flavin, 1981). Such de-tyrosinolated α -tubulin then becomes the substrate for a cytoplasmic tubulin tyrosine ligase which restores a tyrosine residue at the COOH terminus of α -tubulin (Tyr-tubulin) (Raybin and Flavin, 1975; Raybin and Flavin, 1977; Thompson, 1982; Flavin and Murofushi, 1984). This enzyme has recently been purified to homogeneity (Schröder et al., 1985).

Tubulin tyrosine ligase activity has been demonstrated in cell lysates from vertebrates (Preston et al., 1979) as well as from a number of invertebrates (Kobayashi and Flavin, 1981; Gabius et al., 1983), but no such activity has yet been detected in unicellular organisms. Nevertheless, the in vivo occurrence of posttranslational tyrosinolation of α -tubulin has recently been demonstrated for the hemoflagellate *Trypanosoma brucei* (Stieger et al., 1984). Remarkably, in this organism both the α - and the β -tubulins carry a tyrosine as their COOH-terminal amino acid (Kimmel et al., 1985), but only the α -tubulin can serve as a substrate for the posttranslational tyrosinolation reaction.

Despite much effort, very little is understood about the functional significance of the de-tyrosinolation/tyrosinolation reaction cycle. Changes in COOH-terminal tyrosine content or in tubulin tyrosine ligase activity have been correlated with changes in cell shape (Deanin et al., 1981), cell differen-

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

tiation (Nath and Flavin, 1979; Cumming et al., 1984), redox status of the cell (Nath and Gallin, 1984), progression through the cell cycle (Forrest and Klevecz, 1978), and ageing (Gabijs et al., 1983). Also, a role for tyrosinolated α -tubulin in the functioning of voltage-gated sodium channels has been proposed (Matsumoto et al., 1984a, b). Immunocytochemical studies using monoclonal antibodies specific for the COOH-terminal tyrosine (Kilmartin et al., 1982) or polyclonal antibodies raised against synthetic peptides representing the tyrosinolated as well as the detyrosinolated COOH terminus of α -tubulin (Gundersen et al., 1984) have led to a general picture in which most microtubules are seen to contain both tyrosinolated and detyrosinolated α -tubulin, though in varying ratios (Wehland et al., 1983; Gundersen et al., 1984). In some instances, individual microtubules have been detected which seem to contain exclusively one or the other of the two forms of α -tubulin (Cumming et al., 1984; Gundersen et al., 1984).

Trypanosomes are attractive for investigating a possible correlation between the tyrosinolation status of α -tubulin and microtubular localization and/or function. Interphase cells of these organisms contain three distinct populations of MTs; namely (a) the cytoplasmic tubulin pool, (b) the subpellicular array of membrane-associated singlet MTs, and (c) the MTs of the flagellar axoneme. These microtubular structures can be visualized by electron microscopy (Angelopoulos, 1970) and can be biochemically separated with relative ease (Russell et al., 1984; Russell and Gull, 1984; Schneider et al., 1987). The present report demonstrates that tyrosinolated α -tubulin is present in all three cellular compartments outlined above, and that both α -tubulin isoforms present in trypanosomes (Schneider et al., 1987) can be posttranslationally tyrosinolated. Immunofluorescence studies show that tyrosinolated α -tubulin is nonrandomly distributed in the MTs. Our observations suggest a temporal control of tubulin tyrosinolation through the cell cycle.

Materials and Methods

The procedures used in this study are described in the preceding paper (Schneider et al., 1987). Conditions for inhibiting protein synthesis, for in vivo labeling of trypanosomes with [3 H]tyrosine, and for Taxol-induced polymerization of tubulin were exactly as described earlier (Stieger et al., 1984).

The tyrosinolation-specific anti- α -tubulin antibody YL 1/2 was produced by Kilmartin et al. (1982). All other antibodies used are described in the preceding paper.

Immunofluorescence

Cells were harvested by centrifugation, washed twice in phosphate-buffered saline (PBS), and were allowed to settle onto poly-L-lysine-coated slides for 1 h at 25°C in a moist atmosphere. The slides were washed in PBS and fixed in cold acetone for 10 min, then rehydrated in PBS. Slides were incubated in the first antibody for 1 h at 25°C, then washed three times in PBS for 10 min each wash. The slides were then incubated in fluorescein-conjugated second antibody and washed in PBS as before. Excess PBS was drained off, and 25 μ l of 1 μ g/ml 4,6-diamidino-2-phenylindole (DAPI; Williamson and Fennell, 1975) were applied. The cells were mounted under coverslips using Mowiol containing 1 mg/ml *p*-phenylene diamine as an antifade.

Results

Both α -Tubulin Isoforms Can Be Posttranslationally Tyrosinolated In Vivo

Posttranslational tyrosinolation of the carboxy terminus of

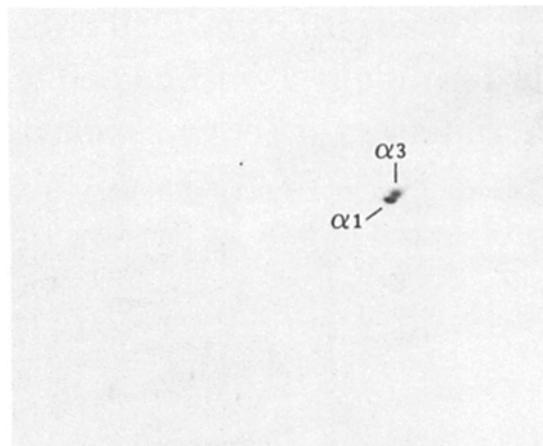


Figure 1. Fluorogram of posttranslationally tyrosinolated α -tubulin isoforms. Cells were labeled with [3 H]tyrosine for 2 h in the presence of protein synthesis inhibitors. A whole cell lysate was fractionated by two-dimensional gel electrophoresis. The fluorogram represents the entire gel, demonstrating the absence of labeled proteins other than α -tubulin.

α -tubulin has been demonstrated for *Trypanosoma brucei* (Stieger et al., 1984), and optimal conditions for in vivo labeling have been established. These were used for studying the posttranslational tyrosinolation of the two α -tubulin isoforms now detected in this organism (Schneider et al., 1987). Preliminary experiments have established that in the presence of protein synthesis inhibitors, incorporation of [3 H]tyrosine into tubulin reaches a plateau after 2 h. A lysate from cells labeled for 2 h was analyzed by two-dimensional gel electrophoresis, and the resulting autoradiograph is presented in Fig. 1. The figure clearly demonstrates that under the conditions used, radiolabeled tyrosine is incorporated exclusively into α -tubulin. It is notable that both α -tubulin isoforms, $\alpha 1$ and $\alpha 3$ (Schneider et al., 1987), are similarly labeled. Taking into account the large excess of $\alpha 3$ -tubulin over $\alpha 1$ -tubulin which has been demonstrated in the accompanying paper (Schneider et al., 1987; also compare Fig. 2 below), these results indicate that the $\alpha 1$ -isoform is much more heavily labeled than the $\alpha 3$ -isoform.

Inspection of numerous autoradiograms from such in vivo labeling experiments demonstrated that posttranslational tyrosinolation occurs exclusively in the α -tubulins, and not in β -tubulin. This specificity was not a priori to be expected since, in *Trypanosoma brucei*, β -tubulin also contains a tyrosine as its carboxy-terminal amino acid (Kimmel et al., 1985). Furthermore, an antibody previously believed to be specific for the tyrosinolated carboxy terminus of α -tubulin has recently been shown to cross-react with some other proteins which carry a carboxy-terminal tyrosine or phenylalanine residue (Wehland et al., 1984).

Subcellular fractions prepared from trypanosomes labeled with [3 H]tyrosine in the presence of protein synthesis inhibitors were analyzed by two-dimensional gel electrophoresis. In all these experiments, whole subcellular fractions were used, rather than purified tubulin, in order to avoid selective losses due to the purification procedures. Fig. 2 *a* represents an autoradiogram of a total cellular lysate, where again only the $\alpha 1$ -tubulin and $\alpha 3$ -tubulin isoforms are labeled with [3 H]tyrosine. A very different pattern is seen when the soluble cytoplasmic pool is analyzed (Fig. 2 *b*). The Coomassie-

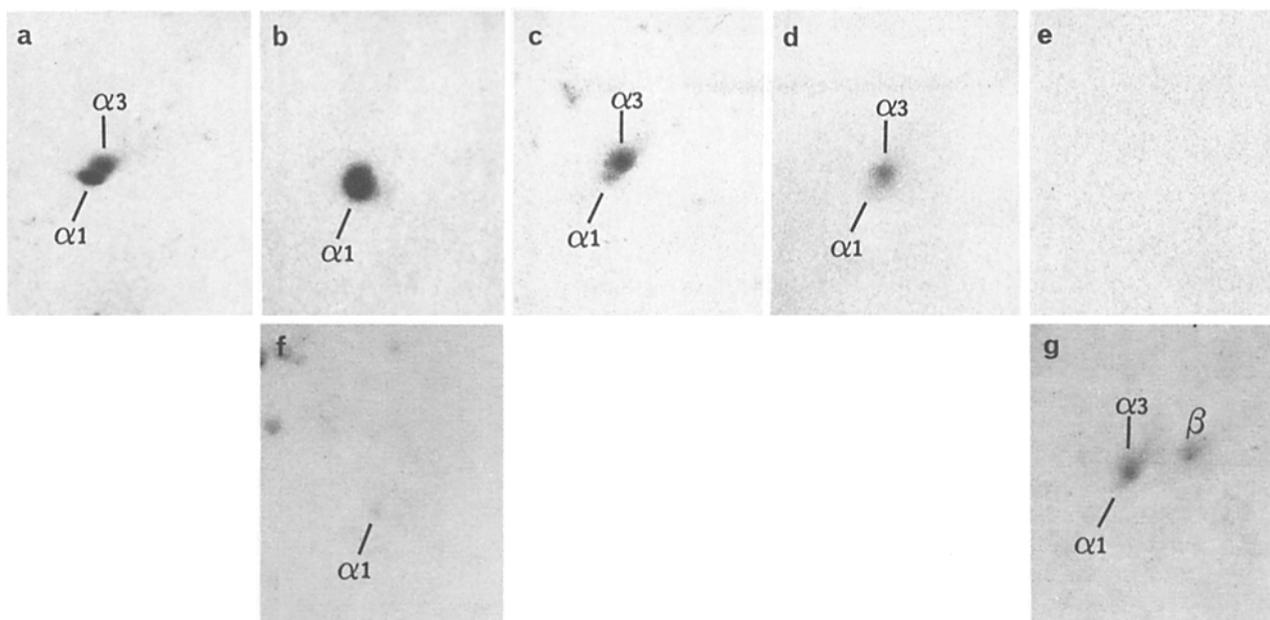


Figure 2. Differential incorporation of posttranslationally tyrosinolated tubulin into MTs of different subcellular compartments. Cells were labeled in vivo with [^3H]tyrosine as described in the text, and subcellular fractions were prepared and analyzed on two-dimensional gels. The resulting autoradiographs represent the following: (a) whole cell extract; (b) pool of soluble tubulin; (c) cytoskeleton; (d) subpellicular MTs; and (e) flagella. The Coomassie-stained gels containing the pool of soluble tubulin and containing flagella are shown in order to demonstrate the scarcity of tubulin in the soluble pool (f) and the abundance of tubulin in the flagellar preparation (g).

stained gel shows that very little tubulin is present in this fraction, all of which is the $\alpha 1$ isoform (Fig. 2 f). The corresponding autoradiogram reveals that this $\alpha 1$ isoform is very heavily labeled (Fig. 2 b). Again a different labeling pattern is again observed both with total cytoskeletons (Fig. 2 c) and with subpellicular MTs (Fig. 2 d), the $\alpha 3$ -isoform being the dominant labeled species. In contrast to these results, no incorporation of labeled tyrosine was found in the flagella (Fig. 2 e), though Coomassie-staining clearly demonstrated the presence of α -tubulin in the gel (Fig. 2 g).

This latter observation was followed up by a more quantitative estimation of this difference. Subpellicular and flagellar MTs were prepared from trypanosomes labeled for 2 h with [^3H]tyrosine in the absence of protein synthesis and were then analyzed by gel electrophoresis. A typical set of results is presented in Fig. 3. Coomassie-staining was used to ascertain that gels to be compared with each other indeed contained similar amounts of tubulin (Fig. 3 A). Nevertheless, autoradiography revealed that very different amounts of radioactivity (i.e., of posttranslationally added tyrosine) are present in tubulin from subpellicular MTs and flagellar tubulin, respectively. Titration experiments have shown that under these conditions 10–50 times more [^3H]tyrosine is incorporated into subpellicular MTs than into flagellar MTs. No significant change in this ratio was observed when the labeling time was extended up to 24 h (results not shown).

All Major Types of Trypanosomal MTs Contain Tyrosinolated α -Tubulin (Tyr-tubulin)

The in vivo labeling experiments reported above have demonstrated that both α -tubulin isoforms can be posttranslationally tyrosinolated, but that these modified α -tubulins are incorporated into subpellicular and into flagellar MTs with vastly different kinetics. However, an analysis of the post-translational tyrosinolation does not necessarily detect total

Tyr-tubulin. Rather, an important part of the Tyr-tubulin population may consist of primary translation products, which have not undergone a detyrosinolation/retyrosinolation cycle. To assess the steady-state tyrosinolation status of α -tubulin in the different subcellular compartments, immunoblotting was performed with two different α -tubulin-specific antibodies. The monoclonal antibody DM1A recognizes α -tubulin irrespective of its tyrosinolation status (Blöse et al., 1984), and was used to estimate the total amounts of α -tubulin. The second antibody used, YL 1/2 (Kilmartin et al., 1982), is specific for tyrosinolated α -tubulin only (Wehland et al., 1983; Wehland et al., 1984). Lysates of whole trypanosome cells, cytoskeletons and flagella were subjected to two-dimensional gel electrophoresis, and the resulting blots were stained with the two antibodies. A representative set of results is given in Fig. 4. Clearly, both antibodies produce very similar staining patterns with all samples tested, and these are also similar to the ones obtained with Coomassie stain-

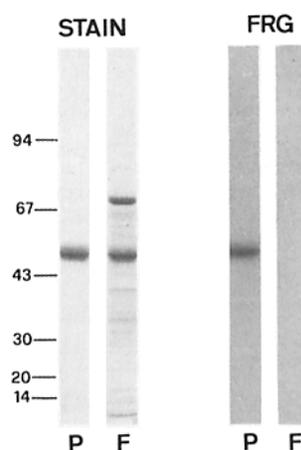


Figure 3. α -Tubulin which is posttranslationally tyrosinolated in the absence of protein synthesis is not incorporated into the flagellar axoneme. Subpellicular MTs and flagella isolated from cells after a 2-h labeling with [^3H]tyrosine in the absence of protein synthesis were analyzed by SDS gel electrophoresis. *STAIN*, Coomassie stain; *FRG*, fluorogram of the same gel. *P*, Subpellicular MTs; *F*, flagella. No radioactivity is detectable in the flagellar MTs.

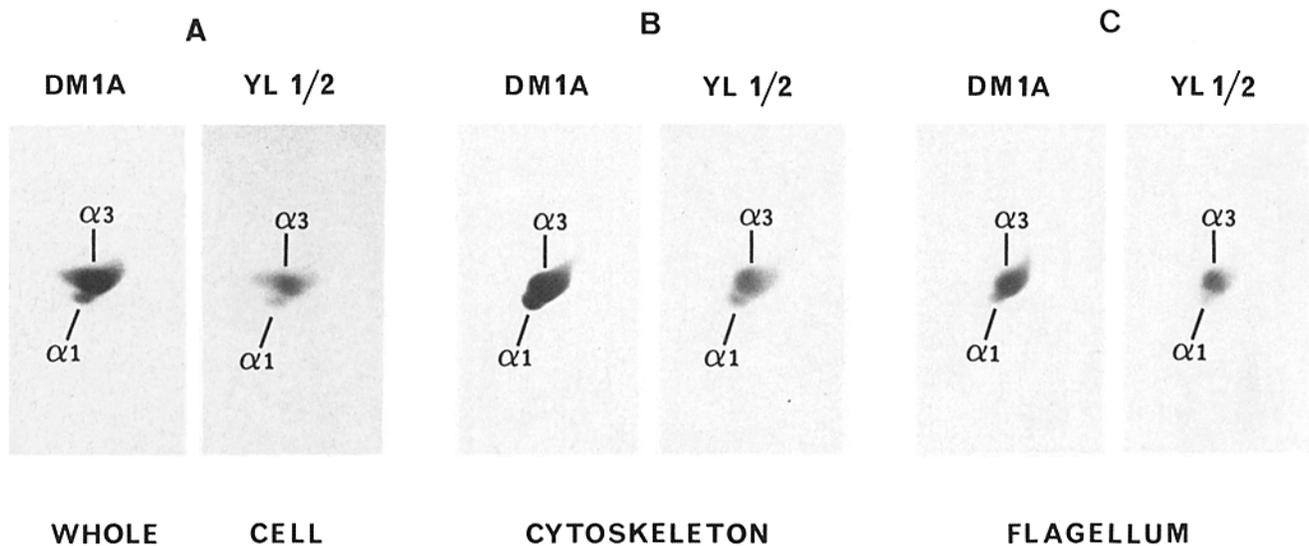


Figure 4. All three populations of MTs contain tyrosinolated α -tubulin. Subcellular fractions were analyzed by two-dimensional electrophoresis, and α -tubulin isotypes were stained with two different antibodies: DM1A (α -tubulin specific) and YL 1/2 (tyrosinolation specific). (A) Whole cell lysate; (B) cytoskeleton; (C) flagellum.

ing. Most notably, antibody YL 1/2 detects the $\alpha 1$ as well as the $\alpha 3$ tubulin isotype. This observation is in agreement with the above findings with *in vivo* labeling, and it establishes that both tubulin isotypes can be tyrosinolated. Furthermore, similar quantitative ratios between $\alpha 1$ and $\alpha 3$ isotypes are observed by staining with either of the two antibodies. Fig. 4 C demonstrates that the tyrosine-specific antibody YL 1/2 also reacts with flagellar tubulin. Thus, the markedly different *in vivo* labeling behavior of subpellicular and flagellar α -tubulins (see Figs. 2 and 3) may reflect a kinetic, rather than a steady-state difference between these two tubulin pools.

Distinct Localization of Tyr-tubulin in the Trypanosomal Cell

The experiments outlined above demonstrate that both α -tubulin isotypes can be tyrosinolated, and that Tyr-tubulin is found in comparable proportions in whole cells, cytoskeletons, and flagella. Immunofluorescence microscopy was now used to investigate the localization of Tyr-tubulin within the trypanosomal cell. Staining blood stream trypanosomes with an anti- α -tubulin monoclonal antibody whose epitope is not subject to posttranslational modification (DM1A) or an anti- β -tubulin monoclonal antibody (KMX) reveals the pattern seen in Fig. 5 A. The cell body is intensely fluorescent due to the massive numbers and homogenous distribution of the subpellicular microtubules. The flagellum is seen as a fluorescent wavy line along the side of the cell body. However, immunofluorescent staining with the YL 1/2 antibody (tyrosinolation specific) reveals a completely different pattern (Fig. 5 B). Fluorescence is localized in the posterior third of the cell body, in the basal bodies and in the newly formed daughter flagellum. Identical results were obtained with several different fixation methods. Studies of many cells revealed that these particular patterns of YL 1/2 immunofluorescence staining are subject to change as the cells progress through their division cycle. In Fig. 6, we have arranged a series of immunofluorescent micrographs representing this temporal

change in YL 1/2 staining during the cell cycle. Decisions on the position of a particular cell in the cycle can be made by considering the size of the cell, the presence or absence of a daughter flagellum and the length of any such flagellum, the position of the basal bodies, and the position (and division) of nucleus and kinetoplast as visualized by the DNA intercalating dye DAPI. After staining with YL 1/2, cells at the start of the cycle show bright fluorescence at the posterior third of the cell. The flagellum is not stained (Fig. 6, a and b). As the cell cycle progresses, a short daughter flagellum forms on the new basal body. This new flagellum stains very brightly with YL 1/2 (Fig. 6, c-e). As this flagellum lengthens over the next period of the cycle, its bright fluorescence is maintained, as is the generally bright fluorescence of the posterior third of the cell (Fig. 6, f-h). The pattern of fluorescence then starts to change, and the time of this change correlates with the separation of the kinetoplast DNA complexes, as judged by DAPI staining, and the separation of the closely associated basal bodies, as judged by YL 1/2 staining (Fig. 6, i and j). At this time, the intensity of fluorescence of the posterior portion of the cell is reduced, and the very bright staining of the daughter flagellum is lost almost completely. The kinetoplast DNA complexes separate before division of the nucleus, and in cells at this stage, there is only weak fluorescence of the posterior cell region and none in the flagellum, and yet there is bright fluorescence of the two basal bodies. The nucleus then goes through mitosis and at this stage, DAPI fluorescence shows single cells with two nuclei and two kinetoplasts (Fig. 6, l-n). Although there is some low level of general fluorescence in the cell body at this stage, the most brightly staining objects are the basal bodies. The axonemes of both the maternal and daughter flagella are not stained by YL 1/2 at this late stage of the cell cycle, just before actual cell cleavage.

Thus, the monoclonal antibody YL 1/2 reveals a temporal regulation of tyrosinolation during the trypanosome cell cycle. In the early part of the cycle, the posterior portion of the cell stains brightly, while the flagellum does not stain. How-

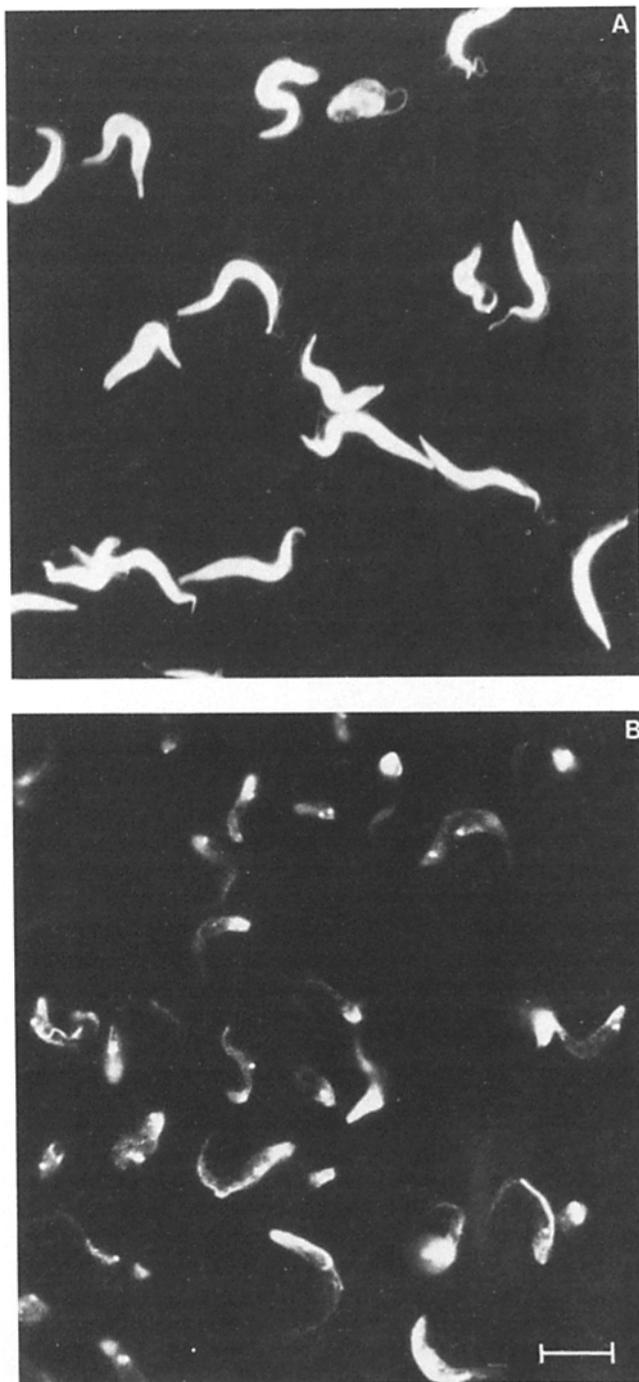


Figure 5. Immunofluorescence of bloodstream trypanosomes. Cells were stained with KMX (A) or YL 1/2 (B), followed by the appropriate FITC-labeled second antibody. Bar, 10 μ m.

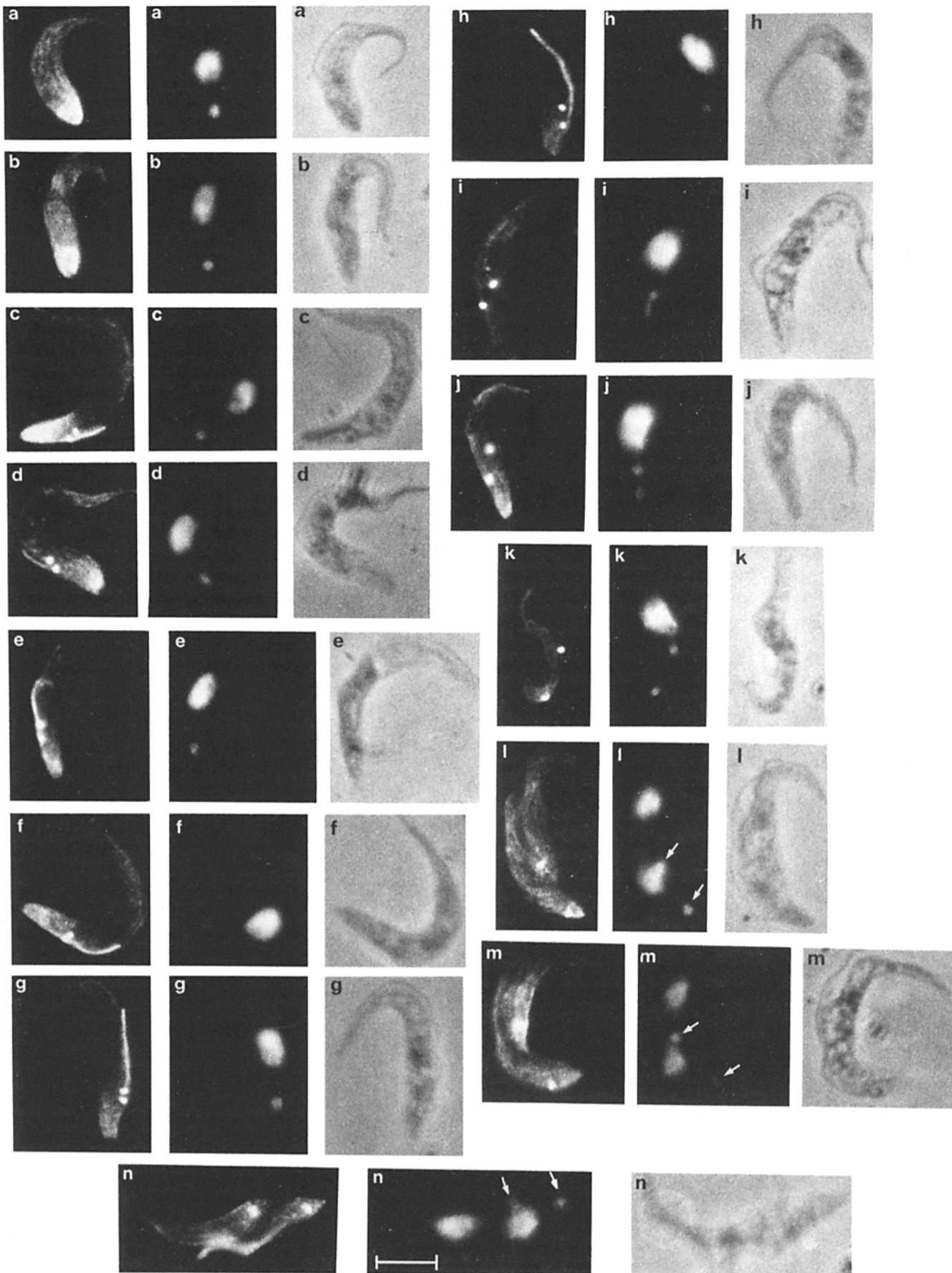
ever, the newly forming daughter flagellum stains intensely. At a particular stage of the cell cycle, this daughter flagellum loses its ability to stain with YL 1/2. This point is reached when the flagellum has grown to its full length, which is before the separation of the two basal bodies. It is interesting that both the old and the new basal body stain intensely with YL 1/2 throughout the cell cycle, indicating that the α -tubulin in these structures remains highly tyrosinolated throughout the cell cycle.

Discussion

The data presented above demonstrate that in *Trypanosoma brucei* the α -tubulin can be posttranslationally tyrosinolated. Notably, only α -tubulin can undergo this reaction though, in trypanosomes, β -tubulin also has a tyrosine as its carboxy-terminal amino acid (Kimmel et al., 1985). These observations, along with the earlier ones of Stieger et al. (1984) are the first evidence for the occurrence of this reaction in protozoa. Both in vivo labeling and immunoblotting with a tyrosination-specific antibody have served to demonstrate that both of the two trypanosomal α -tubulin isoforms (Schneider et al., 1987) are tyrosinolated. A marked difference in the kinetics of incorporation of posttranslationally tyrosinolated tubulin into subpellicular and into flagellar MTs has been observed. On the other hand, immunoblotting experiments have demonstrated similar steady-state levels of tyrosination in both types of MTs. Since the in vivo labeling experiments had to be conducted in the presence of protein synthesis inhibitors, this may well have affected the transport or the incorporation of labeled α -tubulin into the flagellar axoneme. A more unlikely conclusion to be drawn from these observations is the possibility of different α -tubulin pools within cells. Following this line, newly synthesized α -tubulin could be ferried directly into the flagellum, whereas recycled (i.e., detyrosinolated) and posttranslationally retyrosinolated α -tubulin would be directed to the subpellicular MTs.

Immunofluorescence studies on the localization of Tyr-tubulin within the trypanosomal cytoskeleton revealed a very distinct localization of this isoform in some, but not other, cytoskeletal domains. The subpellicular MTs of the posterior third of the cell body, the nascent daughter flagellum of dividing cells, and the basal bodies all react strongly with the tyrosination-specific antibody YL 1/2. A very weak or no reaction is detected in the subpellicular MTs of the anterior two-thirds of the cell body and in the maternal flagellum. The distribution of Tyr-tubulin within the trypanosomal cytoskeleton observed in this study is in total contrast to the one reported in an earlier study (Cumming and Williamson, 1984). These authors concluded that Tyr-tubulin is specifically and exclusively located in the flagella of trypanosomes. The observations presented above clearly disprove their conclusions. Our analysis of the intracellular distribution of Tyr-tubulin through the cell cycle has revealed a strong correlation between the pattern of tyrosinolated MTs and the progression through the cell cycle.

This striking correlation suggests that the tyrosinolated state of α -tubulin is a marker of newly formed MTs. According to this view, Tyr-tubulin is the species which enters the polymerization reaction. This pool of Tyr-tubulin consists of the primary translation products containing the carboxy-terminal tyrosine coded for by the mRNA, as well as of "recycled" Glu-tubulin to which a terminal tyrosine has been added by the activity of the tubulin tyrosine ligase. Once incorporated into the polymer, Tyr-tubulin is gradually detyrosinolated by the action of the MT-associated tubulin carboxypeptidase. This is consistent with the known substrate specificity and subcellular localization of tubulin tyrosine ligase (Schröder et al., 1985) and of tubulin carboxypeptidase (Thompson et al., 1979; Kumar and Flavin, 1981). Furthermore, it is strongly supported by recent observations (Gun-



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dersen et al., 1985) that in mammalian cells recovering from Nocodazole-induced loss of MTs, the α -tubulin of all newly forming MTs is heavily tyrosinolated.

The inference from this concept for the trypanosomal cytoskeleton is twofold. First, the brilliant staining of the basal body MTs throughout the cell cycle would imply a continuous turnover of their tubulins. However, the possible absence of decarboxylase activity from these structures cannot be ruled out at present. Second, the strong immunofluorescence regularly observed in the posterior moiety of the cells during the early stages of the cell cycle would suggest the abundant presence of growing poles of subpellicular MTs in this region.

The results presented here provide considerable evidence against some of the functions that have been proposed for Tyr-tubulin, such as being a marker of organelle-specific or membrane-associated MTs. Rather, they demonstrate a correlation between the steady-state tyrosination status of α -tubulin and the progression through the cell cycle, an idea put forward earlier by Forrest and Klevecz (1978) for mammalian cells in culture. In turn, this correlation suggests that the presence of tyrosinated α -tubulin is a marker for newly generated MTs. In this context, it is interesting to remember that extensive posttranslational tyrosination has been detected upon chemotactic stimulation of macrophages (Nath et al., 1981) or under oxygen stress (Nath and Gallin, 1984), conditions which are accompanied by extensive remodeling of the cellular architecture and hence a significant turnover of MTs.

While the detailed function of the detyrosination/detyrosination reaction pair still remains poorly understood, the simply structured cytoskeleton of *Trypanosoma brucei* clearly represents a highly suitable model system for a further investigation of this unusual type of protein modification.

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References

- Angelopoulos, E. 1970. Pellicular microtubules in the family trypanosomatidae. *J. Protozool.* 17:39-51.
- Argarana, C. E., H. S. Barra, and R. Caputto. 1980. Tubulin-tyrosine carboxypeptidase from chicken brain: properties and partial purification. *J. Neurochem.* 34:114-118.
- Barra, H. S., J. A. Rodriguez, C. A. Arce, and R. Caputto. 1973. A soluble preparation from rat brain that incorporates into its own proteins ^{14}C -arginine by a ribonuclease-sensitive system and ^{14}C -tyrosine by a ribonuclease-insensitive system. *J. Neurochem.* 20:97-108.
- Blose, S. H., D. I. Meltzer, and J. R. Ferramisco. 1984. 10-nm filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. *J. Cell Biol.* 98:847-858.
- Cleveland, D. W., and K. F. Sullivan. 1985. Molecular biology and genetics of tubulin. *Annu. Rev. Biochem.* 54:331-365.
- Cumming, R., R. D. Burgoyne, and N. A. Lytton. 1984. Immunocytochemical demonstration of α -tubulin modification during axonal maturation in cerebellar cortex. *J. Cell Biol.* 98:347-351.
- Cumming, R., and J. Williamson. 1984. Differential labelling of trypanosome microtubules using tubulin subunit monoclonal antibodies. *Cell Biol. Int. Rep.* 8:2.
- Deanin, G. G., S. F. Preston, and M. W. Gordon. 1981. Carboxyl terminal tyrosine metabolism of α -tubulin and changes in cell shape: Chinese hamster ovary cells. *Biochem. Biophys. Res. Commun.* 100:1642-1650.
- Eipper, B. A. 1974. Rat brain tubulin and protein kinase activity. *J. Biol. Chem.* 249:1398-1406.
- Feit, H., and M. L. Shelanski. 1975. Is tubulin a glycoprotein? *Biochem. Biophys. Res. Commun.* 66:920-927.
- Flavin, M., and H. Murofushi. 1984. Tyrosine incorporation in tubulin. *Methods Enzymol.* 106:223-237.
- Forrest, G. L., and R. R. Klevecz. 1978. Tyrosyltubulin ligase and colchicine binding activity in synchronized Chinese hamster cells. *J. Cell Biol.* 78:441-450.
- Gabius, H. J., G. Graupner, and F. Cramer. 1983. Activity patterns of aminoacyl-tRNA synthetases, tRNA methylases, arginyl-transferase and tubulin: tyrosine ligase during development and aging of *Caenorhabditis elegans*. *Eur. J. Biochem.* 131:231-234.
- Gundersen, G. G., S. Khawaja, and J. C. Bulinski. 1985. Microtubules containing nontyrosinated alpha tubulin arise from post-polymerization modification of tyrosinated tubulin in vivo. *J. Cell Biol.* 101(5, Pt. 2):257a. (Abstr.)
- Gundersen, G. G., M. H. Kalnoski, and J. C. Bulinski. 1984. Distinct populations of microtubules tyrosinated and nontyrosinated α -tubulin are distributed differently in vivo. *Cell.* 38:779-789.
- Kilmartin, J. V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell Biol.* 93:576-582.
- Kimmel, B. E., S. Samson, J. Wu, R. Hirschberg, and L. R. Yarbrough. 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.
- Kobayashi, T., and M. Flavin. 1981. Tubulin tyrosylation in invertebrates. *Comp. Biochem. Physiol.* 69B:387-392.
- Kumar, N., and M. Flavin. 1981. Preferential action of a brain detyrosinating carboxypeptidase on polymerized tubulin. *J. Biol. Chem.* 256:7678-7686.
- L'Hernault, S. W., and J. L. Rosenbaum. 1983. *Chlamydomonas* α -tubulin is posttranslationally modified in the flagella during flagellar assembly. *J. Cell Biol.* 97:258-263.
- Martensen, T. M. 1982. Preparation of brain Tyrosinotubulin Carboxypeptidase. In *Methods in Cell Biology*. Vol. 24. D. W. Prescott, editor. Academic Press, New York. 265-269.
- Matsumoto, G., M. Ichikawa, A. Tasaki, H. Murofushi, and H. Sakai. 1984a. Axonal microtubules necessary for generation of sodium current in squid giant axons. I. Pharmacological study on sodium current and restoration of sodium current by microtubule proteins and 260 k protein. *J. Membr. Biol.* 77:77-91.
- Matsumoto, G., M. Ichikawa, and A. Tasaki. 1984b. Axonal microtubules necessary for generation of sodium current in squid giant axons. II. Effect of colchicine upon asymmetrical displacement current. *J. Membr. Biol.* 77:93-99.
- Nath, J., and M. Flavin. 1979. Tubulin tyrosylation in vivo and changes accompanying differentiation of cultured neuroblastoma-glioma hybrid cells. *J. Biol. Chem.* 254:11505-11510.
- Nath, J., M. Flavin, and E. Schiffmann. 1981. Stimulation of tubulin tyrosination in rabbit leukocytes evoked by the chemoattractant, formyl-methionyl-leucyl-phenylalanine. *J. Cell Biol.* 91:232-239.
- Nath, J., and J. I. Gallin. 1984. Modulation of tubulin tyrosination in human polymorphonuclear leukocytes (PMN). In *White Cell Mechanics*. H. J. Meiselman, editor. Alan R. Liss Inc., New York. 95-110.
- Ponstingl, H., E. Krauhs, M. Little, T. Kempf, R. Hofer-Warbinek, and W. Ade. 1982. Amino acid sequences of α - and β -tubulins from pig brain: heterogeneity and regional similarity to muscle proteins. *Cold Spring Harbor Symp. Quant. Biol.* 46:191-197.
- Preston, S. F., G. G. Deanin, R. K. Hanson, and M. W. Gordon. 1979. The phylogenetic distribution of tubulin tyrosine ligase. *J. Mol. Biol.* 13:233-244.
- Raybin, D., and M. Flavin. 1975. An enzyme tyrosylating α -tubulin and its role in microtubule assembly. *Biochem. Biophys. Res. Commun.* 65:1088-1095.

Figure 6. The pattern of YL 1/2 staining changes through the cell cycle. Cells are arranged (a-n) according to their position in the cell cycle (see text for details). Each cell is represented threefold: stained with the YL 1/2 antibody (leftmost photograph of each panel); stained with DAPI to visualize nuclei and kinetoplasts (middle photographs); and in phase-contrast (rightmost photographs). Arrows in l, m, and n point to the separated kinetoplasts. Bar, 5 μm . All panels are shown at the same magnification.

- Raybin, D., and M. Flavin. 1977. Enzyme which specifically adds tyrosine to the α -chain of tubulin. *Biochemistry*. 16:2189-2194.
- Russell, D. G., D. Miller, and K. Gull. 1984. Tubulin heterogeneity in the trypanosome *Crithidia fasciculata*. *Mol. Cell. Biol.* 4:779-790.
- Russell, D. G., and K. Gull. 1984. Flagellar regeneration in the trypanosome *Crithidia fasciculata* involves the posttranslational modification of cytoplasmic α -tubulin. *Mol. Cell. Biol.* 4:1182-1185.
- Sandoval, I. V., and P. Cuatrecasas. 1976. Protein kinase associated with tubulin: affinity chromatography and properties. *Biochemistry*. 15:3424-3432.
- Schneider, A., T. Sherwin, R. Sasse, D. Russell, K. Gull, and T. Seebeck. 1987. Subpellicular and flagellar microtubules of *Trypanosoma brucei brucei* contain the same α -tubulin isotypes. *J. Cell Biol.* 104:431-438.
- Schröder, H. C., J. Wehland, and K. Weber. 1985. Purification of brain tubulin-tyrosine ligase by biochemical and immunological methods. *J. Cell Biol.* 100:276-281.
- Stieger, J., T. Wyler, and T. Seebeck. 1984. Partial purification and characterization of microtubular protein from *Trypanosoma brucei*. *J. Biol. Chem.* 259:4596-4602.
- Thompson, W. C. 1982. The cyclic tyrosination/detyrosination of α -tubulin. *In Methods in Cell Biology*. Vol. 24. D. W. Prescott, editor. Academic Press, Inc., New York. 235-255.
- Thompson, W. C., G. G. Deanin, and M. W. Gordon. 1979. Intact microtubules are required for rapid turnover of carboxyl-terminal tyrosine of α -tubulin in cell culture. *Proc. Natl. Acad. Sci. USA*. 76:1318-1322.
- Tucker, J. B. 1979. Spatial organization of microtubules. *In Microtubules*. K. Roberts, and J. S. Hyams, editors. Academic Press, Inc. New York. 315-358.
- Valenzuela, P., M. Quiroga, J. Zaldivar, W. J. Rutter, M. Kirschner, and D. W. Cleveland. 1981. Nucleotide and corresponding amino acid sequences encoded by α - and β -tubulin mRNAs. *Nature (Lond.)*. 289:650-655.
- Wehland, J., M. C. Willingham, and I. V. Sandoval. 1983. A rat monoclonal antibody reacting specifically with the tyrosylated 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.
- Wehland, J., H. C. Schröder, and K. Weber. 1984. Amino acid sequence requirements in the epitope recognized by the α -tubulin-specific rat monoclonal antibody YL 112. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1295-1300.
- Williamson, D. H., and D. J. Fennell. 1975. The use of fluorescent DNA-binding agent for detecting and separating yeast mitochondrial DNA. *In Methods in Cell Biology*, Vol. 12. D. M. Prescott, editor. Academic Press, Inc., New York. 335-351.