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Differential expression of mRNAs for α- and β-tubulin during differentiation of the parasitic protozoan *Leishmania mexicana*

(trypanosomatid flagellate/microtubule/RNA blot/cell differentiation)

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ABSTRACT **The parasitic protozoan *Leishmania mexicana amazonensis* has two developmental stages: a motile flagellated promastigote stage and a sessile intracellular amastigote stage. In our previous work, cells of the promastigote stage were found to synthesize more tubulin protein than those of the amastigote stage. Here, tubulin mRNAs in these leishmanias were analyzed. Based on dot blot hybridization between total leishmanial RNA and tubulin-specific cDNA probes derived from chicken brain, amastigotes and promastigotes were found to have approximately equal amounts of α- and β-tubulin mRNAs. RNA blotting of leishmanial RNA, using chicken tubulin cDNA probes, showed that amastigotes and promastigotes both gave a single mRNA species of 2100 nucleotides for α-tubulin in roughly similar quantities. However, such analysis for β-tubulin revealed mainly a single mRNA species of 3600 nucleotides for amastigotes and three species of 2800, 3600, and 4400 nucleotides for promastigotes, the smallest mRNA being the most predominant. Thus, regulation of gene expression appears to be different only for β-tubulin between the two developmental stages of this protozoan.**

Species of the parasitic protozoa in the genus *Leishmania* or *Trypanosoma* are etiologic agents of a variety of diseases in humans and animals (1). A characteristic feature of these protozoa is the presence of abundant and functionally distinct microtubules. Flagellar microtubules are involved in locomotion, subpellicular microtubules are involved in the maintenance of cell shape, and nuclear spindle microtubules are involved in cell division (2). It has been reported that taxol, a microtubule stabilizing drug, blocks cell division of *Trypanosoma cruzi* but has no effect on its cellular motility (3). This finding not only indicates the functional importance of microtubules but also suggests their type or structural heterogeneity.

The major structural unit of microtubules is the protein tubulin, which is a dimer composed of α and β subunits (4). Tubulins of trypanosomatid protozoa have been analyzed at both the protein and nucleic acid levels. The identities of tubulin and microtubules have been verified in *Leishmania donovani* (5), *Leishmania tropica* (6), and *Leishmania mexicana* (7). Anti-tubulin antibodies have been detected in the sera of dogs with naturally occurring visceral leishmaniasis (8). With respect to the different types of microtubules, monoclonal antibodies to bovine brain microtubules and yeast tubulin can distinguish flagellar and subpellicular microtubules of *Trypanosoma brucei* (9) and *Trypanosoma rhodesiense*, respectively (10). Using materials derived from flagellar and subpellicular microtubules and from the cytoplasmic tubulin pool of *Crithidia fasciculata*, *Leishmania tarentolae*, and *T. brucei*, three separate assembly-competent tubulin fractions have been purified and found by two-dimensional gel electrophoresis and peptide mapping to differ in molecular structure (11, 12). Differences in tubulin proteins suggest the existence of different tubulin genes. Indeed, most eukaryotes, including the trypanosomatid protozoa, have multiple genes encoding for tubulin (13, 14). The α- and β-tubulin genes of *T. brucei* are tandemly duplicated (15, 16), whereas those of *Leishmania enriettii* are in separate α and β clusters (17). Whether the multiple tubulin genes of trypanosomatid protozoa give rise to different tubulin proteins remains to be investigated.

We have studied leishmanias as a model for eukaryotic cell differentiation (7, 18–20), because they can be induced *in vitro* to change back and forth between a flagellated motile promastigote stage and an intracellular sessile amastigote stage. Our previous work on protein biosynthesis during leishmanial differentiation showed a good correlation between the biosynthetic activity of tubulin and the length of microtubules in leishmanial flagella and cytoskeleton during cell differentiation (7). We further isolated the polyadenylylated RNAs from both stages of leishmanias and carried out *in vitro* translation using the rabbit reticulocyte cell-free system. This led to the unexpected finding that amastigotes and promastigotes have similar amounts of translatable tubulin RNA, although the former synthesize much less tubulin protein than the latter *in vivo* (18). Thus, our previous finding indicated post-transcriptional control for tubulin biosynthesis during leishmanial differentiation.

In this paper, we report our results based on a direct analysis of tubulin mRNAs from amastigotes and promastigotes of *L. mexicana*. Evidence presented indicates that the two leishmanial stages have, indeed, similar amounts of α- and β-tubulin mRNA, but they may have different species of the latter.

MATERIALS AND METHODS

Parasites. *L. mexicana amazonensis* amastigotes and promastigotes were grown *in vitro* and harvested as described (7, 21). For some experiments, the Josefa strain of *L. mexicana amazonensis* (UISS 150492, LV81, WR364R) was also used. Josefa strain amastigotes were maintained *in vivo* as skin lesions on the tail base or foot pad of BALB/c mice. Lesions were excised and homogenized in Hanks' balanced salt solution. After a low-speed centrifugation to remove debris, amastigotes were pelleted, washed, and counted. Viability of amastigotes was determined by the erythrosin B dye exclusion test (22). For each batch of RNA extraction, at least 2.5×10^9 amastigotes were used. Josefa strain amastigotes were also maintained *in vitro* in J774.1n− macrophages

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(21). Infected cells were collected from each 150-cm^2 tissue culture flask, pelleted, and suspended in 1 ml of hypotonic solution (42.5 mM KCl/5 mM $MgCl_2$) for 4 min (23). This was followed by several rapid needle passages and suspension in 35 ml of phosphate-buffered saline with 2 mM EDTA. Amastigotes were pelleted, washed, counted, and their viability was determined as described. Josefa strain promastigotes were grown in Schneider's *Drosophila* medium supplemented with 15% heat-inactivated fetal bovine serum (24). For RNA extraction, 10^{10} logarithmic phase promastigotes were used.

Purification of Leishmanial RNA. Total leishmanial cellular RNA was isolated by two different methods. In Method 1 (18, 25), cell pellets were dissolved in 5 ml of lysis buffer (0.1 M Tris·HCl, pH 9/0.1 mM EDTA/1% $NaDodSO_4$/0.4 M NaCl/100 μg of heparin per ml) and extracted once with an equal volume of saturated phenol containing 0.1% 8-hydroxyquinoline. This was mixed with a Vortex for 1 min and centrifuged at 20,000 × *g* for 10 min at 4°C. The aqueous phase was collected and the phenol phase was reextracted with 4 ml of lysis buffer. The combined aqueous phase was extracted once with phenol and once with chloroform/isoamyl alcohol, 24:1 (vol/vol). The RNA was precipitated by the addition of 2.5 vol of ethanol at −20°C overnight. Contaminating DNA was removed by spooling with a glass rod or by LiCl solubilization, and RNA was collected by centrifugation at 20,000 × *g* for 30 min at 2°C. Finally, the leishmanial RNA was dissolved in 0.5 ml of 10 mM Hepes (pH 7.5) and stored at −70°C. In Method 2 (26), 8 ml of buffer A (0.1 M sodium acetate, pH 6/0.001 M EDTA/2% $NaDodSO_4$/10 mg of bentonite per ml) was mixed with 5 ml of phenol (90% phenol/10% *m*-cresol/1% 8-hydroxyquinoline) at 4°C for the extraction of leishmanial RNA. The remainder of the procedure was as described for Method 1. From total cellular RNA prepared by Methods 1 or 2, polyadenylylated RNA was isolated using an oligo(dT)-cellulose column as described (18, 26, 27). Quantitation of RNA was determined spectrophotometrically at 260 nm or, for small amounts, by ethidium bromide dye binding with known standards (28).

***In Vitro* Labeling of Plasmid DNA.** Whole plasmids containing tubulin inserts were labeled by nick-translation using [α-^{32}P]dCTP, and the nick-translated plasmid DNA (about 10^8 cpm/μg) was separated from unincorporated dCTP by Sephadex G-50 column chromatography (29, 30). The plasmids used were as follows: α-253 and β-37 corresponding to α- and β-tubulin cDNAs of *Chlamydomonas* (ref. 31; courtesy of Carolyn Silflow, University of Minnesota), and pT1 and pT2 corresponding to chicken brain α- and β-tubulin cDNAs (ref. 32; courtesy of Don Cleveland, Johns Hopkins University).

Analysis of Leishmanial RNA by Dot Blot and RNA Blot Hybridization. Relative tubulin mRNA levels of amastigotes and promastigotes were compared by dot hybridization (33), with the exception that the total RNA (3 μg) was used instead of a cytoplasmic fraction. For RNA blot hybridization, total RNA (up to 10 μg) or poly(A)$^+$ RNA (1 μg) samples were electrophoresed on horizontal 1.5% agarose gels containing 6% formaldehyde (34). After electrophoresis, the gels were soaked in 0.5 M ammonium acetate containing 1 μg of ethidium bromide per ml for 15 min, and they were then treated with 50 mM NaOH for 15 min. The RNA samples were transferred from the agarose gels to nitrocellulose filters (35). RNA blot hybridization was carried out according to Wallach *et al.* (36), except that both the 6-hr prehybridization [in a solution containing 50% formamide/2× Denhardt's solution/50 mM Tris·HCl, pH 7.5/0.1% sodium pyrophosphate/0.1% $NaDodSO_4$/10% dextran sulfate/0.9 M NaCl/100 μg of calf thymus DNA per ml (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)] and the 16-hr hybridization (with nick-translated DNA probe added at 10 ng/ml) were done at 42°C. Blot washings and autoradiography were carried out according to Thomas (35), and the blots were stained afterward with methylene blue to check the amount and size of transferred rRNA (30).

RESULTS

Analysis of Tubulin mRNAs in Leishmanial Promastigotes. Total cellular RNA and poly(A)$^+$ RNA were extracted (Method 2) and electrophoresed under denaturing conditions on agarose gels. The RNA was transferred to nitrocellulose and hybridized with nick-translated *Chlamydomonas* tubulin cDNA probes (Fig. 1). These heterologous probes hybridized well with leishmanial RNA under stringent conditions. With the α-253 probe, one band was detected in promastigote poly(A)$^+$ RNA (lane 4). With the β-37 probe, three bands were seen (lane 6). Based on rRNA as size markers, the estimated sizes are 2100 nucleotides for leishmanial α-tubulin, and 2800, 3600, and 4400 nucleotides for leishmanial β-tubulin. The smallest species (2800 nucleotides) is the predominant β-tubulin mRNA in promastigotes. However, in addition to the single α-tubulin band and the three β-tubulin bands, other faint, discrete bands of smaller size were always detectable in the samples using total RNA (lanes 3 and 5) but not in those using poly(A)$^+$ RNA. These discrete bands of smaller size were also detected in poly(A)$^-$ samples, which contained little, if any, of the predominant tubulin RNA bands (data not shown). As they were hybridizable to tubulin cDNA probes, these bands may indicate specific degradation of the tubulin message in leishmanias. However, the exact nature of these additional bands is unclear.

Comparison of the Relative Amounts of Tubulin RNA in Amastigotes and Promastigotes. Initially, we found that it was relatively difficult to isolate enough amastigotes and to extract undegraded RNA from these cells. A rapid amastigote

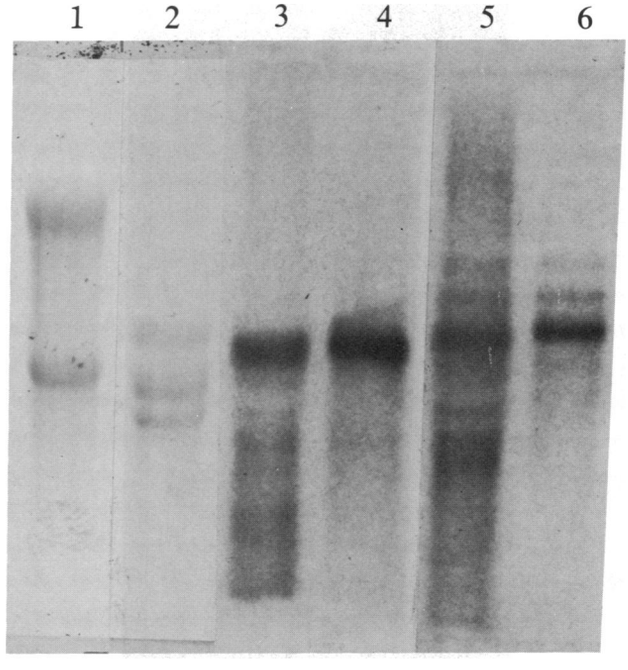

FIG. 1. RNA blot hybridization of *L. mexicana* promastigote RNA. Lanes: 1 and 2, hamster poly(A)$^-$ and leishmanial total RNA, methylene blue-stained bands of rRNA as size markers; 3 and 4, total and poly(A)$^+$ leishmanial RNA hybridized to *Chlamydomonas* α-tubulin probe; 5 and 6, total and poly(A)$^+$ RNA hybridized to *Chlamydomonas* β-tubulin probe. [The α-tubulin band of the total RNA lane does not migrate exactly as that of the poly(A)$^+$ RNA lane because of the presence of rRNA in the tubulin banding region.]

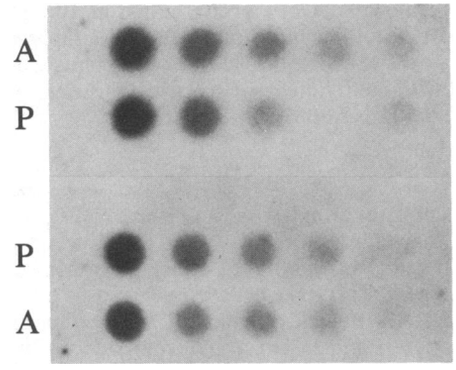

FIG. 2. Dot blot hybridization of total RNA from *L. mexicana* promastigotes and amastigotes. Top two rows, equal amount (3 μg) of amastigote (A) and promastigote (P) RNA in serial 1:2 dilutions, hybridized to chicken brain β-tubulin probe (the fourth promastigote dot was not filtered properly); bottom two rows, promastigote and amastigote RNA hybridized to chicken brain α-tubulin probe.

isolation method was subsequently used, and total RNA was obtained from leishmanial amastigotes derived from mouse foot pad lesions (Method 1). Equal amounts of total RNA from amastigotes and promastigotes, in serial dilutions, were loaded onto nitrocellulose for dot hybridization, and the RNA was hybridized to the chicken brain tubulin cDNA probes pT1 and pT2 (Fig. 2). As with the *Chlamydomonas* probes, the chicken probes also hybridized to leishmanial RNA under stringent conditions. As determined by densitometric scanning, the corresponding dots of amastigote and promastigote RNAs showed similar levels of hybridization with both probes, with an average amastigote/promastigote ratio of 1:1.35 for α-tubulin and 1:0.91 for β-tubulin. Thus, the quantity of tubulin RNAs is quite comparable between the two developmental stages.

Comparison of Tubulin RNA Species Between Amastigotes and Promastigotes. Equal amounts of total RNA from amastigotes and promastigotes were electrophoresed under denaturing conditions, transferred to nitrocellulose, and hybridized with the pT1 probe. (The amount of RNA transferred was consistent, as determined by ethidium bromide staining of the three leishmanial rRNA bands.) Both amastigote and promastigote total RNA contain a single band of comparable intensity that was hybridizable to pT1 (chicken α-tubulin probe; Fig. 3). This result confirms that both amastigotes and promastigotes of *L. mexicana* have similar amounts of α-tubulin RNAs (per unit of total cellular RNA) and that they are the same size (2100 nucleotides; see Fig. 1).

Equal amounts of total RNA from amastigotes and promastigotes were also hybridized to pT2 (chicken β-tubulin probe) under stringent conditions (Fig. 4). Whereas the total RNA of promastigotes gave three bands (2800, 3600, and 4400 nucleotides), with the 2800-nucleotide RNA species predominant (compare to Fig. 1), the total RNA of amastigotes gave a different pattern. The predominant band for β-tubulin RNA of amastigotes was the 3600-nucleotide species (corresponding to the middle band in promastigote RNA species), although there may be a faint lower band at 2800 nucleotides. Moreover, the relative intensity of the single β-tubulin band of amastigotes appears to equal the combined band-

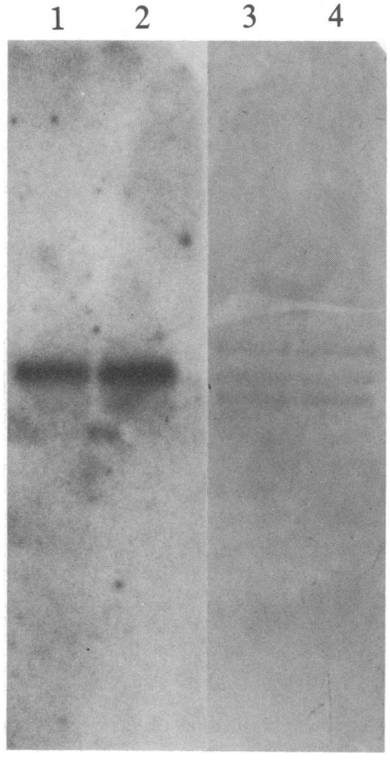

FIG. 3. RNA blot hybridization of leishmanial RNA. Equal amounts of total RNA from amastigotes (lane 1) and promastigotes (2) were hybridized to chicken brain α-tubulin probe. Lanes 3 and 4 are the corresponding methylene blue-stained bands for rRNA of the same nitrocellulose filter.

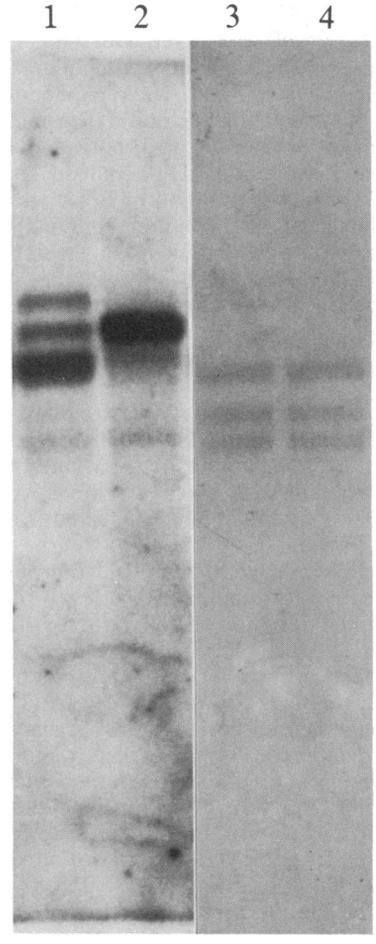

FIG. 4. RNA blot hybridization of leishmanial RNA. Equal amounts (6.5 μg) of total RNA from promastigotes (lane 1) and amastigotes (lane 2) were hybridized to chicken brain β-tubulin probe. Lanes 3 and 4 are the corresponding methylene blue-stained bands for rRNA of the same nitrocellulose filter.

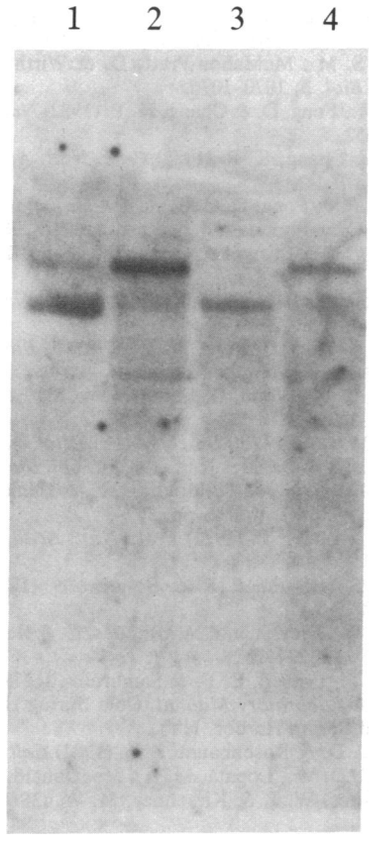

FIG. 5. RNA blot hybridization of leishmanial RNA. Total RNA from promastigotes (lane 1) and amastigotes (lane 2) of the Josefa strain, and from promastigotes (lane 3) and amastigotes (lane 4) of *L. mexicana amazonensis* were hybridized to chicken brain β-tubulin probe. Amastigotes of lane 2 were derived from animal skin lesions, and those of lane 4 were from infected macrophages in culture.

ing intensities of the three β-tubulin bands of promastigotes. The same results were obtained in the hybridization experiment of total RNAs with the chicken β-tubulin probe, irrespective of the sources of amastigotes from mouse lesions or cultured macrophages (Fig. 5). Thus, the β-tubulin RNA species of *L. mexicana* are different between amastigotes and promastigotes.

DISCUSSION

In the present study, we have examined the α- and β-tubulin mRNAs in the two developmental stages of *L. mexicana amazonensis*. As assayed by dot blot hybridization, both amastigotes and promastigotes were found to have similar amounts of tubulin RNA. This result confirms our previous finding on the production of the same amount of tubulin protein by *in vitro* translation with the polyadenylylated RNAs from the two leishmanial stages (18). Since the amastigotes synthesize much less tubulin protein than the promastigotes *in vivo* (7), there must be post-transcriptional regulation of tubulin biosynthesis during leishmanial differentiation. The tubulin mRNA may be localized within ribonucleoproteins, masked and unavailable for translation in amastigotes, but becomes readily available for translation during the early phase of amastigote-to-promastigote differentiation (7). In the insect trypanosome *Crithidia*, ≈45% of translatable polyadenylylated RNA has been found to be localized in a nonpolysomal compartment (37).

By RNA blot hybridization, we have found in the present study that both amastigotes and promastigotes possess a 2100-nucleotide α-tubulin mRNA in roughly equal amounts. However, for β-tubulin mRNA, amastigotes have mainly a single species of 3600 nucleotides, while promastigotes have three—namely, a 2800-, a 3600-, and a 4400-nucleotide species (with the smallest being the most predominant). The quantity of the single species of amastigote β-tubulin mRNA appears to be equal to the sum of the three promastigote tubulin mRNAs. Since we have previously shown that the two leishmanial stages have similar amounts of translatable tubulin mRNAs by *in vitro* translation (18), these mRNA species are most likely translatable. Similar high molecular weight tubulin mRNAs have been noted before. There are three high molecular weight β-tubulin mRNAs (between 3500 and 4000 nucleotides) in the chicken. These mRNAs have also been found to be translatable, and they are differentially expressed in a variety of chicken cell lines and tissues (38).

The estimated sizes for *L. mexicana* tubulin mRNAs are in accord with published data on tubulin genes of the guinea pig leishmania *L. enriettii* (17). *L. enriettii* has α-tubulin genes of 2000 nucleotides and β-tubulin genes of 4000 nucleotides (≈15 copies each in two separate tandem repeats). Our results with *L. mexicana* RNA are also similar to those of previous investigators, who showed that tubulin genes of *L. enriettii* are hybridizable to probes for β-tubulin derived from *Chlamydomonas* and for α-tubulin derived from *Drosophila* (17, 39). Three tubulin RNA bands, similar to those seen by us in *L. mexicana* promastigotes, were reported for *L. enriettii* promastigotes (40). Molecular species of *L. enriettii* amastigote tubulin RNAs have not been reported. In contrast to our finding of equal amounts of tubulin mRNAs in both amastigotes and promastigotes of *L. mexicana*, *L. enriettii* was found to have 5- to 10-fold more tubulin mRNA in the promastigotes than in the amastigotes (41). This discrepancy can be explained by species differences between human and guinea pig leishmanias or by a difference in experimental procedures. The amastigotes of *L. mexicana* used in our work were derived from animal lesions and from cultured macrophages, while those of *L. enriettii* used by others were obtained by temperature-induced transformation of promastigotes (40, 42).

The multitubulin hypothesis proposes that different microtubular structures are assembled from different tubulins, and that tubulin heterogeneity has functional significance (43). Conceivably, the three β-tubulin mRNAs detected in the promastigotes of *L. mexicana* may reflect a heightened level of differential expression for tubulins needed in their varied metabolic activities involving flagellar, mitotic, and cytoskeletal microtubules. However, while there are ample cases for β-tubulins encoded by different genes (such as the two human β-tubulins with different carboxyl-terminal residues; see ref. 14), there are also cases for a single β-tubulin with multiple functions (such as the genetic evidence for a *Drosophila* testis-specific β-tubulin that is involved in meiosis, nuclear shaping, and axoneme assembly; see ref. 44). Thus, whether tubulin heterogeneity is a reflection of strict functional specialization must await careful analysis in each individual system.

Our work suggests that there are different regulatory mechanisms for α- and β-tubulin biosynthesis during leishmanial differentiation. This is demonstrated in our previous study of tubulin biosynthesis *in vivo*—i.e., a more rapid change in the synthesis for β-tubulin than for α-tubulin during leishmanial transformation (7). Additional evidence is our finding of the same α-tubulin mRNA for both amastigotes and promastigotes and different species of β-tubulin mRNA for amastigotes and promastigotes in the present study. Whereas the α-tubulin may be under strict post-transcriptional control, the β-tubulin regulation may be a complex phenomenon. For β-tubulin, the relationship between the single amastigote mRNA and the three promastigote mRNAs is unclear, although the amastigote mRNA may cor-

respond to one of the three based on size similarity. The occurrence of three promastigote β-tubulin mRNAs suggests either differential processing of a common mRNA precursor synthesized from a single gene, or different levels of expression of tubulin mRNAs from several genes. Since both amastigote and promastigote mRNAs direct the *in vitro* synthesis of a similar amount of tubulin protein (18), it is more likely that the three mRNAs are different species rather than the products of a common precursor. Whether the three β-tubulin mRNAs of promastigotes are derived from the same or different genes can be determined by cDNA cloning and nucleotide sequencing of the β-tubulin mRNAs.

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1. Molyneux, D. H. & Ashford, R. W. (1983) *The Biology of Trypanosoma and Leishmania, Parasites of Man and Domestic Animals* (Taylor & Francis, London).
2. Vickerman, K. & Preston, T. M. (1976) in *Biology of the Kinétoplastida*, eds. Lumsden, W. H. R. & Evans, D. A. (Academic, London), Vol. 1, pp. 25–130.
3. Baum, S. G., Wittner, M., Nadler, J. P., Horwitz, S. B., Dennis, J. E., Schiff, P. B. & Tanowitz, H. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4571–4575.
4. Gunning, B. E. S. & Hardham, A. R. (1982) *Annu. Rev. Plant Physiol.* **33**, 651–698.
5. Dwyer, D. M. (1981) in *The Biochemistry of Parasites*, ed. Slutzky, G. M. (Pergamon, Oxford), pp. 10–28.
6. Bordier, C., Garavito, R. M. & Armbruster, B. (1982) *J. Protozool.* **29**, 560–565.
7. Fong, D. & Chang, K. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7624–7628.
8. Pateraki, E., Portocala, R., Labrousse, H. & Guesdon, J. L. (1983) *Infect. Immun.* **42**, 496–500.
9. Gallo, J. M. & Anderton, B. H. (1983) *EMBO J.* **2**, 479–483.
10. Cumming, R. & Williamson, J. (1984) *Cell Biol. Int. Rep.* **8**, 2.
11. Russell, D. G. (1983) *J. Protozool.* **30**, 41A (abstr.).
12. Russell, D. G., Miller, D. & Gull, K. (1984) *Mol. Cell. Biol.* **4**, 779–790.
13. Cleveland, D. W. (1983) *Cell* **34**, 330–332.
14. Cowan, N. J. & Dudley, L. (1983) *Int. Rev. Cytol.* **85**, 147–173.
15. Thomashow, L. S., Milhausen, M., Rutter, W. J. & Agabian, N. (1983) *Cell* **32**, 35–43.
16. Seebeck, T., Whittaker, P. A., Imboden, M. A., Hardman, N. & Braun, R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4634–4638.
17. Landfear, S. M., McMahon-Pratt, D. & Wirth, D. F. (1983) *Mol. Cell. Biol.* **3**, 1070–1076.
18. Wallach, M., Fong, D. & Chang, K. P. (1982) *Nature (London)* **299**, 650–652.
19. Fong, D. & Chang, K. P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7366–7370.
20. Chang, K. P. & Fong, D. (1983) in *Cytopathology of Parasitic Diseases*, Ciba Foundation Symposium, eds. Evered, D. & Collins, G. M. (Pitman, London), Vol. 99, pp. 113–137.
21. Chang, K. P. (1980) *Science* **209**, 1240–1242.
22. Hodgkinson, V. H., Herman, R. & Semprevivo, L. (1980) *Exp. Parasitol.* **50**, 397–408.
23. Harrison, E. H. & Bowers, W. E. (1983) *J. Biol. Chem.* **258**, 7134–7140.
24. Hendrick, L. D., Wood, D. E. & Hajduk, M. E. (1978) *Parasitology* **76**, 309–316.
25. Wallach, M. (1982) *Mol. Biochem. Parasitol.* **6**, 335–342.
26. Melera, P. W. & Davide, J. P. (1982) in *Cell Biology of Physarum and Didymium*, eds. Aldrich, H. C. & Daniel, J. W. (Academic, New York), Vol. 2, pp. 307–317.
27. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
28. Luttke, A., Lurquin, P. F. & Bonotto, S. (1980) *Anal. Biochem.* **108**, 1–5.
29. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
30. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
31. Silflow, C. D. & Rosenbaum, J. L. (1981) *Cell* **24**, 81–88.
32. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20**, 95–105.
33. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572.
34. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **77**, 4743–4751.
35. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
36. Wallach, M., Cully, D. F., Haas, L., Trager, W. & Cross, G. A. M. (1984) *Mol. Biochem. Parasitol.* **12**, 85–94.
37. Rondinelli, E., Soares, M. C. M., de Castro, J. F. & de Castro, F. T. (1981) *Arch. Biochem. Biophys.* **209**, 349–355.
38. Lopata, M. A., Havercroft, J. C., Chow, L. T. & Cleveland, D. W. (1983) *Cell* **32**, 713–724.
39. Wirth, D. F. & Slater, C. (1983) *Mol. Biochem. Parasitol.* **9**, 83–92.
40. Wyman, C. & Landfear, S. (1983) *Biol. Bull.* **165**, 539 (abstr.).
41. Landfear, S. (1983) *Biol. Bull.* **165**, 493–494 (abstr.).
42. Hunter, K. W., Cook, C. L. & Hansen, S. A. (1982) *Acta Trop.* **39**, 143–150.
43. Fulton, C. & Simpson, P. A. (1976) in *Cell Motility*, eds. Goldman, R., Pollard, T. & Rosenbaum, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Book A, pp. 987–1005.
44. Kemphues, K. J., Kaufman, T. C., Raff, R. A. & Raff, E. C. (1982) *Cell* **31**, 655–670.