The molecular karyotype of *Leishmania major* and mapping of α and β tubulin gene families to multiple unlinked chromosomal loci

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ABSTRACT

The arrangement of tubulin genes in the genome of the protozoan parasite *Leishmania major* was studied by genomic Southern blot analysis and mapping of genes to chromosomes fractionated by pulsed field gradient gel (PFG) electrophoresis. α -tubulin genes exist as a tandem array of 2.4 kb PstI fragments. β -tubulin genes are found as a tandem array of 3.9 kb AvaI or PvuI fragments, but additional genes are also found on other genomic DNA fragments. Chromosome-sized DNA molecules released from promastigotes of *L.major* were fractionated into at least 17 chromosome bands of approximate size 400-4000 kb by PFG gel electrophoresis. Some bands may be present in non-equimolar amounts suggesting that there may be more than 17 chromosomes. All α -tubulin genes were localized to a single band (chromosome 7). β -tubulin genes were localized to four bands (chromosomes 6, 10, 16 and 17). This shows that the α - and β -tubulin gene families are unlinked in *L.major*. There is a single chromosomal locus for the α -tubulin tandem array whereas β -tubulin genes exist both as a tandem array and as dispersed genes at four chromosomal loci.

INTRODUCTION

The arrangement of tubulin genes in the protozoan parasite *Leishmania* is novel among eukaryotes. With most organisms, α - and β -tubulin genes exist as multiple copies which are dispersed in the genome (1-4). In *Trypanosoma brucei*, these genes exist as a tandem array of alternating α - and β -tubulin gene pairs (5). In contrast, *Leishmania* possess separate tandem repeat arrays of α - and β -tubulin genes (6-8). This unique arrangement in *Leishmania* may reflect a gene organization which has evolved to allow the efficient control of gene expression in an organism which exhibits rapid changes in the levels of tubulins expressed during its life cycle (9).

An interesting feature of the tubulin system in *Leishmania* is the flexibility that exists in the type of control of tubulin gene expression exhibited by different species during differentiation from the flagellated promastigote form to the non-flagellated amastigote form. In *L.enriettii*, the expression of the single size class of α and β tubulin mRNA is controlled at the level of mRNA accumulation (10). In *L.mexicana amazonensis*, the expression of the

single size class of α tubulin mRNA is translationally controlled: in contrast, three size classes of β tubulin mRNA exist and, although the total amount of β tubulin mRNA is constant during differentiation, the level of accumulation of each of these three size classes is differentially regulated (11,12). These results suggest that multiple β tubulins exist in L.m.amazonensis and show that the control of tubulin expression in species of Leishmania is both flexible and complex. Our understanding of the molecular basis of this flexibility in control will require a comparative analysis of the chromosomal arrangement of α and β tubulin genes in different Leishmania. This is necessary in order to answer several important questions concerning tubulin gene arrangement such as the number of non-allelic tubulin genes, whether the α and β tubulin gene families are linked, whether dispersed genes also exist and whether these dispersed genes are located on the same chromosome as the tandem repeat arrays.

Recently, the technique of pulsed field gradient (PFG) gel electrophoresis has permitted the fractionation of chromosome-sized DNA molecules from yeast, trypanosomes and other kinetoplastid protozoa including L.tropica (13-16). The localization of tubulin genes in L.tropica to two distinct chromosomal size classes was shown by Southern blotting and probing with an $\alpha\beta$ -tubulin probe from T.brucei (16). However, this experiment could not map individual α - or β -tubulin genes to specific chromosomes, show the linkage of the two gene families or reveal the number of loci for α - and β -tubulin.

Here, we report our initial studies on the chromosomal arrangement of tubulin genes in L.major. Using genomic Southern blot analysis, we show that α and β tubulin genes exist as separate tandem repeat arrays and that β -tubulin genes also exist as dispersed gene copies. By PFG gel electrophoresis, we find that a cloned line of L.major contains at least 17 chromosomes and have mapped the chromosomal location of the multiple α - and β -tubulin genes. The results show that the α - and β -tubulin gene families are unlinked, that there is a single locus for α -tubulin but multiple dispersed loci for the β -tubulin family.

MATERIALS AND METHODS

Parasites

The cloned line of L.major, LRC-L137/7/V121 (termed V121), was isolated and grown in NNN medium as described (17).

Isolation and analysis of genomic DNA

Genomic DNA was isolated from promastigotes of V121 as described (18).

DNA (1 µg) was digested for 16h at 37°C with 10 units of restriction enzyme under the conditions recommended by the supplier. Digested DNA was fractionated by electrophoresis in a 0.7% agarose gel in TBE buffer (19). The gel was processed for Southern blotting, the DNA was transferred to a nitrocellulose filter and prepared for hybridization as described (19). Filters were hybridized at 42°C for 20-24h in hybridization medium (19) containing $1-5 \times 10^6$ cpm/ml of nick-translated (20) probes, washed in 2 x SSC, 0.1% SDS at 65°C for 4h, dried and exposed to X-ray film for 2-4 days.

PFG gel electrophoresis of chromosomes

Promastigotes of strain V121 were harvested, washed and suspended in phosphate buffered saline (PBS) at a cell density of 3×10^9 /ml. Following addition of an equal volume of molten 2% low melting agarose (Seakem) in PBS at 37°C, the molten mixture was embedded in the wells of a slot holder (1.6 x 5 x 5 mm slots) and allowed to set. The agarose inserts were placed in lysing buffer (0.5 M EDTA, 0.01 M Tris, 1% Sarkosyl, pH 9.5 at 42°C) containing 2 mg/ml Proteinase K (Merck) and incubated at 42°C for 48h. The inserts were stored at 4°C in lysing buffer. For fractionation, an agarose insert was sliced into four pieces and incubated for 1h in 0.9 x TBE buffer. Each piece was placed in the well of a 1.5% agarose (Seakem ME) gel in 0.9 x TBE, the well was filled with 1% low melting agarose in 0.9 x TBE and subjected to PFG gel electrophoresis (13). The samples were subjected to 200V (10V/cm) for 2h in the north-south direction (top-bottom) and then fractionated for 21-24h with 40-80 sec pulses, 250V (12.5V/cm) in the north-south direction and 100V (5V/cm) in the east-west (left-right) direction at 18°C-23°C. Following fractionation, the gel was stained with ethidium bromide (1 µg/ml) for 16h and photographed. Chromosomal DNA was blotted from the gel onto nitrocellulose filters as described above. The approximate molecular weights of the chromosomes of L.major were estimated relative to the reported approximate sizes of the yeast chromosomes which were prepared from S.cerevisiae strain J69b1 as described (13). Due to the uncertainty in the sizes of the yeast chromosomes and the nature of the fractionation observed, the precise size of the L.major chromosomes is uncertain.

RESULTS

Genomic structure of tubulin genes

Genomic DNA was isolated from promastigotes of the cloned line V121 of L.major LRC-L137, digested with various restriction endonucleases, fractionated by agarose gel electrophoresis and blotted onto nitrocellulose filters.

Figure 1. Genomic Southern blot analysis of tubulin genes. Genomic DNA from promastigotes of *L.major* LRC-L137/7/V121 was digested with PstI (lanes 1,4), AvaI (lane 2) or PvuI (lane 3), fractionated, blotted and hybridized to probes for α -tubulin (lane 1) or β -tubulin (lanes 2-4). The probes were plasmid DNA isolated from clones pP94/1 and pP121/4 which contain cloned cDNA sequences for α - and β -tubulin respectively. These clones were isolated from cDNA libraries synthesized from poly A⁺ mRNA isolated from *L.major* V121 as will be described elsewhere using cloned α - and β -tubulin cDNA sequences from chicken (1). The sizes of the genomic DNA fragments homologous to the probes is shown beside the lanes in kb. In lane 3, the hybridizing fragments are of size 3.9, 6.8, 7.9, 9.2 and >25 kb.

The filters were hybridized to nick-translated probes derived from plasmid cDNA clones pP94/1 and pP121/4 which contain cDNA inserts corresponding to genes for α - and β -tubulin of *L.major*, respectively. These cDNA sequences were isolated from an *L.major* cDNA library using cloned probes for the α - and β -tubulin genes of chicken (1) (legend, Fig. 1). The 1.5 kb insert in the α -tubulin plasmid pP94/1 lacks any site for PstI. When hybridized to PstI-digested genomic DNA, this probe detects two homologous fragments; a minor fragment of 2.7 kb and a dominant fragment of 2.4 kb (Fig. 1, lane 1). Two lines of evidence suggest that the dominant 2.4 kb PstI fragment represents

the tandemly repeated α -tubulin gene of L.major: with blots of partial PstI digests of genomic DNA a ladder of homologous fragments separated by about 2.4 kb is observed and other enzymes also release a dominant 2.4 kb homologous fragment (data not shown). This is analogous to the arrangement of α -tubulin genes in L.enriettii on tandem 2 kb PstI fragments (6).

The 0.85 kb insert in the β -tubulin plasmid pP121/4 lacks sites for AvaI or PvuI and contains a single site for PstI about 20bp from one end. In blots of genomic DNA digested with these three enzymes, the pP121/4 probe detects one dominant homologous fragment which appears to be reiterated in the genome and 2 (PstI), 3 (AvaI) or 4 (PvuI) additional minor fragments (Fig. 1, lanes 2-4). With both AvaI and PvuI digests, the repeated fragment is of size 3.9 kb and the same fragment is observed with other enzymes (data not shown). The fact that several enzymes generate repeated fragments of identical size strongly suggests that the 3.9 kb fragment represents the unit length size of the β -tubulin genes located in a direct tandem repeat array which is similar in size to the repeat observed in L.enriettii and L.tropica (6-8). With PstI, the dominant fragment is of size 1.5 kb and presumably represents a genomic DNA fragment released from within the 3.9 kb unit length repeat (c.f. 6-8). The additional DNA fragments homologous to the β -tubulin probe (or the single DNA fragment homologous to the α -tubulin probe) could represent genes located on DNA fragments flanking the repeat array or additional dispersed tubulin genes. In addition, the presence of introns within tubulin genes or heterogeneity in restriction sites within the genes could result in the appearance of additional homologous fragments in Southern blots. The question of whether these additional fragments represent dispersed tubulin gene copies is addressed below.

Molecular karyotype of L.major

Chromosome-sized DNA molecules from L.major were fractionated by PFG gel electrophoresis (13). Promastigotes of L.major were embedded in inserts of agarose, lysed in situ and slices of the inserts were placed in the wells of a 1.5% agarose gel and subjected to PFG gel electrophoresis. As shown in Fig. 2A, with a pulse time of 80 secs, chromosomal DNAs were fractionated into about 17 dominant chromosome-sized DNA bands although not all 17 bands are resolved in this gel (see below). The peculiar bending of the DNA in the lanes is due to the gradient electrical field which also effects the resolution obtained with the identical DNA samples loaded in the different lanes (c.f. 13-16). By comparison of chromosomes of L.major with the estimated size of yeast chromosomal DNAs (13), the DNA molecules from L.major are of approximate

Figure 2. Fractionation of chromosomes of *L. major* V121 by pulsed field gradient gel electrophoresis. Panel A. Fractionation of chromosomes from *L. major* V121 and yeast with a pulse time of 80 secs. Lanes 1, 2, 4, 5, 7, 8, *L. major*; lanes 3, 6, *S. cerevisiae*. The low molecular weight DNA smear(S) may represent fragments of larger molecules since in separate experiments this DNA shows weak homology to each of several probes. The positions of chromosome bands 1-17 are indicated by white dots where the smallest chromosome is designated chromosome 1. Not all 17

chromosomal DNA bands are resolved at this pulse time. The positions of chromosomes 11 and 17 are shown. Panel B. Fractionation of chromosomes from L.major and yeast with a pulse time of 50 secs. Lanes 2-5, L.major; lanes 1, 6, S.cerevisiae. The position of chromosome bands 1-6 are indicated by white dots and the position of chromosome 6 is shown. Panel C. Fractionation of chromosomes from L.major with a pulse time of 40 secs. The positions of chromosome bands 1-11 are indicated by white dots and the position of chromosomes 1,6,7 and 10 is shown. Note the variation in apparent stoichiometry in bands 6 and 9.

size 400-4000 kb. Each lane in Fig. 2A also contains material in the slot which may represent very large linear DNA or kinetoplast DNA networks which do not enter the gel. In addition, each lane also contains a DNA smear (S) that is about 50-200 kb in size which may represent fragments of larger molecules.

The banding pattern obtained varies with the pulse time since shorter pulse times give improved resolution of the small chromosomal DNAs (13,16). As shown in Figs. 2B and 2C, with a pulse time of 40-50 secs, the smallest DNA bands seen in Fig. 2A are fractionated into at least 11 bands. By comparison of the banding patterns observed at different pulse times in different gels, the total number of chromosomal DNA bands observed in L.major V121 is at least 17.

The observation of DNA molecules which separate into discrete bands with a size range comparable to that observed with chromosomes from yeast (13) and T.brucei (14-16) suggests that these DNA molecules represent the chromosomes of L.major. The molecular karyotype of L.major is shown in Fig. 3 which depicts the 17 bands that can be separated under our present electrophoresis conditions. The karyotype shows that L.major V121 contains several large chromosomes (>2000 kb) and many small chromosomes. Chromosomes 15 and 16 have been observed on several occasions although in many gels these molecules are not clearly resolved (Fig. 2A). The 17 DNA bands detected probably represent an underestimate of the total number of discrete chromosomes since some bands are present in non-stoichiometric amounts. For example, bands 6, 9 and 11 appear to be present in greater amounts as judged by the intensity of their fluorescence in the presence of ethidium bromide (Fig. 2A-C). This estimate of chromosome number for L.major is comparable to that deduced for L.tropica (16). By summation of these approximate chromosome sizes, we estimate a genome size of at least 30,000 kb, compared with previous estimates for Leishmania of about 10-50,000 kb (21,22).

Leishmania major

V121

Figure 3. The molecular karyotype of L. major V121. Each box represents a chromosome band identified in various gels fractionating chromosomes of V121 at different pulse times. The figure is a composite of several gels. Spacing between the bands (and their width) is representative of the relative mobilities and width of each band. Shaded bands (chromosomes 6, 9, 11) represent chromosomal DNAs which appear to be present in non-equimolar amounts as judged by their relative fluorescence in the presence of ethidium bromide. The 17 bands depicted may therefore represent more than 17 different chromosomes and is a minimal estimate of the total chromosome number.

Chromosomal location of tubulin genes

The resolution of individual chromosomes of L. major into discrete DNA bands provided an approach to establishing three points concerning α - and β -tubulin gene arrangement: are the tandem repeat arrays of the α - and β -tubulin gene families linked on a single chromosome? Do multiple dispersed genes for α - and β -tubulin exist and if so, are these genes linked within each family? In order to study these questions, the gel shown in Fig. 2A was divided between lanes 4 and 5, the chromosomal DNA blotted onto a nitrocellulose filter, and hybridised to probes for α - and β -tubulin (Fig. 4). The pP94/1 α -tubulin probe hybridized to chromosome 7 although some weak hybridization to DNA in the slot and the DNA smear was observed (Fig. 4A, lanes 1-4). This result suggests that in L. major there is a single chromosomal locus for the array of α -tubulin tandem repeats on chromosome 7. The pP121/4 β -tubulin probe hybridized to four distinct chromosomes (chromosomes 6, 10, 16, 17), to DNA in the slot as well as showing weak hybridization to the DNA smear (Fig. 4A, lanes 5-8). The strongest hybridization was observed to chromosomes 16 and 17. This result shows conclusively that the arrangement of β -tubulin genes in L. major is quite complex. There are clearly four chromosomal loci

Figure 4. Localization of tubulin genes on chromosomes of *L. major*. Panel A. The gel shown in Fig. 2A was divided between lanes 4 and 5, processed and the chromosomal DNAs blotted onto nitrocellulose filters. The filters were hybridized to probes representing α tubulin (pP94/1, lanes 1-4) or β -tubulin (pP121/4, lanes 5-8) and washed in 5 x SSC, 0.1% SDS at 65°C. The α -tubulin probe hybridizes to chromosome 7 (see also Fig. 5). The β -tubulin probe hybridizes to chromosomes 6, 10, 16, 17 (see Fig. 5). Panel B. The filters shown in Figure 4A were each re-hybridized to probes for β -tubulin (lanes 1-4) or α -tubulin (lanes 5-8), washed and exposed as above. In both A and B, the filters were re-assembled before autoradiographing to reflect the original orientation of the lanes shown in Figure 2A. Note that in both Panels A and B the hybridization of the β tubulin probe to chromosomes 16 and 17 is quantitatively greater than that observed to chromosomes 6 and 10.

Figure 5. Localization of tubulin genes on small chromosomes of *L. major*. A Southern blot of the chromosomal DNAs fractionated with a pulse time of 40 secs (Fig. 2C) was hybridized to probes for β -tubulin (pP121/4) (Panel A) and subsequently to an α -tubulin probe (pP94/1) (Panel B). Filters were washed in 2 x SSC, 0.1% SDS at 65°C. The position of chromosomes 6, 7, 10, 16 and 17 which show homology with the two probes is indicated.

for β -tubulin which are distinct from the single α -tubulin locus.

The localization of α and β -tubulin genes to the separate chromosomes was confirmed in two ways. Firstly, the filters shown in Figure 4A were re-hybridized to α - and β -tubulin probes and in each case the predicted hybridization to the different chromosomes was observed (Fig. 4B). Secondly, the tubulin probes were hybridized to a Southern blot of chromosomal DNAs fractionated at a pulse time of 40 secs. (Fig. 2C). As shown in Figure 5A, the β -tubulin probe hybridized to chromosomes 6 and 10 that are well-resolved in this gel as well as hybridizing more strongly to the unresolved chromosomes 16 and 17. When the α -tubulin probe was hybridized to the same blot (Fig. 5B), homology was detected with chromosome 7 that is clearly resolved in this gel from chromosomes 6 and 10 bearing the β -tubulin genes.

DISCUSSION

The genomic arrangement and chromosomal location of genes for α - and β -tubulin in *L. major* has been studied by Southern blot analysis of isolated genomic DNA and of DNA from intact chromosomes fractionated by PFG gel electrophoresis. Conventional genomic Southern analysis revealed that α -tubulin

genes occur as tandemly repeated genes on 2.4 kb PstI fragments which is similar to the arrangement in L.enriettii (6). β -tubulin genes also occur as a tandem repeat of a 3.9 kb unit length fragment as observed in L.enriettii and L.tropica (6-8). In addition, genomic DNA analysis revealed the presence of additional DNA fragments homologous to each of the tubulin probes. The nature of these additional DNA fragments is considered below.

The technique of PFG gel electrophoresis has permitted the determination of the molecular karyotype of L.major. Promastigotes of the cloned line of L.major V121 appear to contain at least 17 chromosomes which fall into two main size classes: about 12 small chromosomes of approximate size 400-1500 kb and at least 5 large chromosomes about 2000-4000 kb in size. The smallest chromosome 1 migrates with a mobility comparable to the smallest yeast chromosome (~300 kb) and slower than a pentamer of λ Amp3 phage DNA (225 kb) (data not shown). Our estimates of the chromosome sizes are based on a comparison with the estimated value for yeast chromosomes (13).

It is clear that L.major probably contains more than 17 individual chromosomes since certain DNA bands appear to be present in non-stoichiometric amounts (e.g. chromosomes 6 and 9) and an accurate estimate of the total number will only be possible if conditions are devised for improving the resolution in these regions. However, it is not clear at present whether bands 6, 9 and 11 contain several different chromosomes of similar size or multiple copies of the same chromosome, i.e. that L.major is an aneuploid organism which is trisomic (or tetrasomic, etc.) for particular chromosomes. It is uncertain whether Leishmania promastigotes are haploid or diploid (22).

The utility of PFG gel electrophoresis in establishing the linkage relationship between complex gene loci has been demonstrated for the α - and β -tubulin genes in L.major. The chromosomal Southern blot analysis using cloned cDNAs corresponding to α - and β -tubulin of L.major revealed that all α -tubulin genes are located on a single band (chromosome 7) whereas the multiple β -tubulin genes are dispersed and located on four bands (chromosomes 6, 10, 16, 17). This shows conclusively that the α - and β -tubulin gene families are unlinked in the genome of L.major. This arrangement raises the question of the mode of control of tubulin gene expression during differentiation in L.major. For α tubulin, precedent suggests that control could be at the level of mRNA accumulation (L.enriettii) (10) or translation (L.m.amazonensis) (11,12). For β tubulin the situation is more complex as control could be at the level of accumulation of one or more classes of mRNA (10,12) or at the translational level. An intriguing possibility is that the chromosomal location of tubulin genes may determine the mode of control of their expression.

This can be tested by comparing the arrangement of tubulin genes in different species of *Leishmania*. If control is exerted at the transcriptional level as observed in many systems (4) the unlinked arrangement of tubulin genes observed in *L.major* would imply the co-ordinated control of their expression at separate chromosomal locations.

The presence of a single locus for the α -tubulin array on chromosome 7 is consistent with genomic Southern analysis reported here and in *L.enriettii* (6). This suggests that the additional 2.7 kb PstI genomic DNA fragment homologous to α -tubulin probably represents a fragment flanking the tandem repeat array although it is possible that an extra α -tubulin gene exists on chromosome 7 which is separate from the repeat array. With respect to β -tubulin, the identification of multiple β -tubulin loci in *L.major* is consistent with the genomic Southern analysis described here for *L.major* and similar results have been obtained with *L.enriettii* and *L.tropica* (6-8). However, the precise number of non-allelic β -tubulin loci present in *L.major* is uncertain. Genomic Southern analysis with AvaI suggests that in *L.major* there should be a locus for a tandemly repeated β -tubulin array as well as three additional loci (Fig. 1) if the three extra homologous fragments represent dispersed gene copies unlinked to the tandem array. This would be consistent with the identification of four chromosomal loci in *L.major* if each locus was unlinked. Another more likely possibility is that one of the extra genomic DNA fragments represents a DNA fragment flanking the tandem repeat array: enzymes such as AvaI and PvuI which cut the repeat unit once should release a flanking fragment which is homologous to the probe whereas PstI which cuts at least twice within the repeat should not. Thus the three extra AvaI fragments observed probably represent a flanking fragment plus two dispersed gene copies in which case we would expect to observe a maximum of only three chromosomal loci for the unlinked dispersed β tubulin genes. The three bands observed with PstI are also consistent with a model of a repeat array and two dispersed gene copies. It is therefore possible that *L.major* contains only three non-allelic β -tubulin loci (a tandem β -tubulin array and its flanking fragment, plus two single dispersed genes) located on four chromosomes. This would strongly suggest that one of the four chromosomes carrying the β -tubulin loci contains a duplicated copy of one of the β -tubulin genes. This could arise by gene duplication and transposition to a new chromosome or by duplication of a complete chromosome followed by chromosomal size divergence. One interesting possibility is that chromosomes 16 and 17 each contain copies of the tandemly repeated β tubulin array since the intensity of hybridization of the β tubulin probe to chromosomes 16 and 17 is several fold greater than

that observed to chromosomes 6 and 10. This would be consistent with the detection of five large PvuI fragments (Fig. 1): the 3.9 kb repeat array, two dispersed gene copies and two polymorphic flanking PvuI fragments containing the same flanking AvaI fragment. This conclusion is also supported by AvaI/PvuI double digests which release a 2.4 kb repeated fragment and the same three extra AvaI fragments seen in Fig. 1 (data not shown). Experiments are in progress to confirm this possibility. It is of interest that two chromosomes of *Plasmodium falciparum* appear to be genetically-related and represent duplications of large segments of chromosomal DNA (23).

The homogeneity of the α tubulin family contrasts with the structural heterogeneity shown for the β -tubulin family and raises the possibility that the four β tubulin loci represent functionally distinct genes encoding specialized β -tubulin proteins. Since *Leishmania* possess at least three functionally distinct microtubules, in the flagellum, subpellicular membranes and mitotic spindle apparatus (24), it is possible that the β -tubulin genes on separate chromosomes encode unique β -tubulin proteins for different microtubules. The presence of multiple β -tubulin proteins or mRNAs have been demonstrated in *L.m.amazonensis* (12), pigs (25), humans (26,27), chickens (28), rats (29), *Drosophila* (30,31), and *Physarum* (32). However, it has also been shown that functional heterogeneity of tubulins does not necessarily imply a genetic heterogeneity since a single tubulin gene can determine a single gene product active in several functional classes of microtubules (30,33,34). Direct sequence analysis of tubulin genes cloned from the four separate chromosomal loci will be needed to test these possibilities and to eliminate the options that some loci simply represent copies of the same alleles or unexpressed pseudogenes (4,26).

The work described here extends recent observations analysing the molecular karyotype and genetic content of several kinetoplastida including *L.tropica* (16). It was reported that *L.tropica* contains more than 20 chromosomes all of which are larger than 700 kb and that α - and β -tubulin genes are located in two chromosomal locations: large DNA (>2000 kb) and small DNA (<2000 kb). Our findings that *L.major* contains at least 17 chromosomes confirms that two species of *Leishmania* carry many small chromosomes rather than a few large ones. This is also true for other species of *Leishmania* (unpublished observations). Chromosome 1 in *L.major*, however, would appear to be smaller than 700 kb. Although chromosome size estimates using PFG gel electrophoresis are still tentative, we have found one very small chromosome (~200 kb) in a strain of *L.mexicana* sp. (unpublished observations) which shows that some *Leishmania* do indeed possess chromosomes smaller than 700 kb. The assignment of α - and

β -tubulin genes to individual chromosomes in L.major has extended the finding in L.tropica. It is clear that α -tubulin is located only on small DNA (chromosome 7) whereas β -tubulin is located on both small (chromosomes 6 and 10) and large (chromosomes 16 and 17) DNA. In preliminary experiments comparing the chromosomal arrangement of tubulin genes in various strains and species of Leishmania, we have found that tubulin gene location appears to be conserved in certain strains of L.major but shows variability among different species. It will be of interest to map more precisely the location of individual tubulin genes in L.tropica.

It is clear that PFG gel electrophoresis is a very useful tool for comparing molecular karyotypes of closely related organisms and has already been used to distinguish strains of T.brucei (14,15) and P.falciparum (23) and species of Trypanosoma (16). This technique may also prove valuable in classifying strains, subspecies and species of Leishmania. From our preliminary studies comparing the karyotypes of different Leishmania, it appears that different species and strains of one species share many chromosomal DNA bands but also exhibit unique bands suggesting that some chromosome bands of Leishmania can vary both in size and apparent copy number whereas other bands appear to be conserved between isolates. When combined with Southern blot analysis of individual genes, it will be possible to develop a genetic map for Leishmania which is not possible to date using conventional genetic techniques.

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