Characterization of tubulin genes in *Trypanosoma rangeli*

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Tubulin genes in *Trypanosoma rangeli*, the only trypanosome besides *T. cruzi* to infect humans in America, are organized in homogeneous, alternate α and β gene tandem repeats of 3.8 kb. The basic repeat was cloned, mapped and partially sequenced. In contrast to most other eukaryotes, where tubulin genes are scattered throughout the genome, trypanosomatids so far studied are characterized by tandem arrangements of these genes with the genus *Trypanosoma* displaying an alternating α- and β-tubulin tandem repeat.

Key words: *Trypanosoma rangeli*; Tubulin; Gene organization; Tandem repeat; DNA sequence; Sequence homology

Introduction

Alpha and beta tubulins are the main constituents of microtubules. These proteins are very conserved through evolution, although heterogeneous subtypes have been detected [1]. In trypanosomes, the existence of different classes of microtubules brings up the possibility of a diversity of tubulin proteins, that could be accounted for by either a transcriptional or a post-translational event. Also, the existence of different developmental forms in trypanosomatids, some with and some without flagella, makes the participation of the expression of tubulin genes in the cycle necessarily important, and a good model system for the study of gene regulation in these organisms.

Tubulin genes are found in multiple copies in all organisms so far studied [1]. Trypanosomatids were the first eukaryotes in which these genes were found in tandem repeats, either of separate or of alternating α- and β-tubulin gene blocks. In two different species of *Leishmania*, the majority of tubulin genes were found in separate α and β repeats [2,3]. In two subspecies of *T. brucei*, alternating α- and β-tubulin genes were found mainly in repeats of 3.7 and 3.6 kb [4-6]. A similar arrangement was found in *T. equiperdum* (personal communication of U. Hibner to T. Seebeck, see ref. 5). In *T. cruzi*, a more complex structure was found, although at least part of the genes is in a α and β tandem repeat of approximately 4 kb (ref. 7, and our unpublished observations).

*Trypanosoma rangeli* [8] is the only trypanosome to infect humans in the Americas besides *T. cruzi*, the causative agent of Chagas' disease. Although this organism apparently does not cause a specific pathology in man, it is important from an epidemiological point of view. Its geographical distribution overlaps that of *T. cruzi* [9,10], both flagellates are transmitted by triatomine bugs [10,11] and infect the same mammal hosts [10-12]. Morphologically they are similar, and serological cross-reactions have been described, although there are some conflicting results [13-15]. Not much has been done to characterize *T. rangeli* at the genomic level. Frasch has demonstrated a high level of K-DNA similarity comparing schizo-
demes of *T. rangeli* and *T. cruzi* [16].

In the present study we demonstrate that *T. rangeli* has tubulin genes organized in a similar way to the African trypanosomes.

**Materials and Methods**

**Growth of *T. rangeli*.** *T. rangeli* Venezuela was isolated by Dr. M.P. Deane from a mixed infection with *T. cruzi* from Venezuela. *T. rangeli* José is an isolate from a human mixed infection with *T. cruzi* from the Amazon region (I.K.F. Miranda et al., in preparation). Cells were grown in PHI-Lit [17] supplemented with 20% fetal calf serum at 28°C with weekly passages and initial concentrations of 10⁶ cells ml⁻¹.

**DNA preparation and digestion.** *T. rangeli* and plasmid DNAs were prepared as described [18] and digested with restriction enzymes according to instructions of manufacturer (New England BioLabs, Beverly, MA, and FIOCruz, Rio de Janeiro).

**Southern transfer and hybridization.** Unidirectional or bidirectional Southern transfers were done as described [20, 21]. Overnight hybridization to nick-translated probes [19] was done in 6 × SSC (1 × SSC is 150 mM NaCl, 15 mM Na citrate), 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% ficoll, 50 mM Tris-HCl, pH 7.5, 0.1% Na pyrophosphate, 1% sodium dodecyl sulphate (SDS), 100 μg/ml sonicated and denatured herring sperm DNA and approximately 10⁶ cpm of probe ml⁻¹, at 65°C. Filters were washed 4 times at 65°C with 1 × SSC and 1% SDS.

**Probes.** The chimeric plasmid pLT1 contains a 2-kb *PstI* insert of *L. enrietti* genomic DNA containing the entire α-tubulin gene plus some flanking sequences, cloned into pBR322 [2]. Clone pLEβ3 has a 4-kb fragment of *L. enrietti* genomic DNA that contains one complete β-tubulin gene and spacer sequences cloned in pBR322 [22]. Both were gifts from Dr. Dyann Wirth.

**Construction and partial sequencing of clones containing a repeat of tubulin genes of *T. rangeli*.** *T. rangeli* Venezuela DNA (50 μg) was digested with *BamHI* and run in a 0.7% agarose gel. A slice of gel containing the 3.8-kb DNA region was excised, the DNA electroeluted by a modification of the method of McDonnel et al. (23) and cloned into pBR322, using HB101 for transformation. Colonies were transferred to nitrocellulose and the filters treated as described [24]. Filters were probed with pLT1. Nucleotide sequencing was done according to standard protocols of the Sanger [25] technique.

**Results**

**Restriction analysis of genomic DNA.** Total DNA of *T. rangeli* Venezuela and José was digested with *BamHI*, *HindIII*, *PstI* and *SalI* and run in a 0.8% agarose gel. The gel was transferred bidirectionally and the filters hybridized to either pLT1 or pLEβ3. Fig. 1 shows that the same bands lit up upon hybridization with both probes. The two strains evidenced the same bands with all but one enzyme. *HindIII* did not release the 3.8-kb band repeat in Venezuela. *BamHI*, *HindIII* and

![Fig. 1. Southern blot analysis of genomic DNA (5 μg/lane) of *T. rangeli* Venezuela (V) and José (J). DNA was digested with (1), *BamHI*; (2), *HindIII*; (3), *PstI*; (4), *SalI*, and run in a 0.8% agarose gel. After transfer, the filter was hybridized to nick-translated pLT1. The same result was obtained when hybridization was to pLEβ3.](image-url)
SalI gave rise to a band of 3.8 kb, and PstI to a rather broad band of approximately 1.9 kb that could contain half the repeat. These results were an indication of the existence of tandem repeats of alternating α and β-tubulin genes in *T. rangeli*.

**Southern blots of partially digested DNA.** The accuracy of the proposed structure was verified by partial digestion of the DNA and hybridization to the α-tubulin probe. *T. rangeli* Venezuela DNA was digested for different lengths of time with BamHI, run in a 0.7% agarose gel and transferred to nitrocellulose. Upon hybridization (Fig. 2) up to 5 multiples of the repeat unit are visible. The higher molecular weight fragments are not resolved and form a cluster of strong hybridization on the top of the gel.

**Quantification of tubulin genes.** DNA of *T. rangeli* Venezuela (3 μg) was digested with *SalI* and run in a 0.8% agarose gel. Increasing amounts (0.1, 0.25, 0.5 and 1 ng) of linearized pLT1 were run in the same gel. The Southern blot of the gel was hybridized to pLT1 (Fig. 3). Estimating a genome size of $5 \times 10^7$ for *T. rangeli* from results obtained with other trypanosomatids [26,27], we calculate an approximate number of 8 repeats of tubulin genes per haploid genome. This should be considered only a rough approximation, as the exact genome size for *T. rangeli* has not yet been determined.

**Cloning of the unit repeat of tubulin genes.** Two BamHI clones hybridizing against pLT1 were selected for further characterization, pRM2 and pRM4. They differed in that pRM4 contained a site for HindIII and pRM2 did not (Fig. 4), and in that they were cloned in opposite directions. These clones were cut with various restriction enzymes and the gels were transferred and hybridized to pLT1 and pLEβ3 inserts. From the patterns obtained we constructed a map that is shown in Fig. 5.

**Partial sequencing of clone pRM2.** The sequencing strategy is shown in Fig. 5. The BamHI insert of pRM2 was cut with PstI and the three fragments obtained were cloned in M13mp18 and M13mp19 in both directions. The sequences obtained were compared to published *T. brucei* tub-
Fig. 4. Mapping of clones pRM2 and pRM4. 1 μg of DNA from clones pRM2 (B and D) and pRM4 (A and C) was cut with BamHI (B), HindIII (H), PstI (P) and SalI (S), run in a 0.7% agarose gel and transferred bidirectionally. The filters were hybridized to labelled pLT1 insert (A and B) and pLEβ3 insert (C and D). The arrows indicate the bands that hybridize only with β tubulin.

Fig. 5. Map of clones pRM2 and pRM4. B, BamHI; P, PstI; H, HindIII; S, SalI. Arrows, sequencing strategy; bold lines, position of genes deduced from sequencing and known size of T. brucei tubulin genes.

Discussion

In T. rangeli, as in other trypanosomes so far studied, tubulin genes are arranged in an alternating repeat of α and β-tubulin sequences. Only one major 3.8-kb band is seen in genomic Southern blots, even upon very long exposures, with the appearance of a faint band of 5.2 kb that should represent one tail end of the repeat. It would be very difficult, with this kind of monotonous structure, to have heterogeneity at the structural level of tubulin genes. If trypanosomes really have different kinds of tubulin involved in the construction of the distinct classes of microtubules, as was shown previously in Crithidia [28], this heterogeneity should most probably be given by a post-translational event. Evidence for a post-translational modification was provided by the treatment of T. rhodesiense with an antibody specific for tyrosinated tubulin, which stained only the flagellar microtubules [5].

The estimated number of 8 copies of the tubulin gene repeat per haploid genome falls within what has been found for other flagellates studied [2-4].

When we compared genomic Southern blots of two different isolates of T. rangeli, namely Ven-
ezuela andJosé, we noticed that the only difference between the two was the absence of a HindIII site in the Venezuela isolate, where we could not visualize the 3.8-kb band upon hybridization with the α and β probes. One of the clones obtained from *T. rangeli* Venezuela did contain the HindIII site. This class of repeat must be in the minority in the genome of this strain, since it is not visible in the genomic Southern blot. Its existence, though, is evidence that an ancestral cryptic sequence remains in the genome of this organism, and for the differential amplification of these two classes of repeats in the two strains studied.

Although *T. rangeli* is transmitted by the bite of the infected triatomine vector, it has been positioned in the subgenus *Herpetosoma* of the section Stercoraria [29]. More recently, this systematic position has been questioned by Añez [30] who created a subgenus, Tejerai, within the section Salvaria, to position this species. This reconsideration is given support in a WHO publication [31]. This new classification positions *T. rangeli* closer to the African trypanosomes than to *T. cruzi*. It would be necessary to study the structure of tubulin genes in more subgenera of *Trypanosoma* to generalize any finding, but so far it seems that the very simple organization of alternating α and β-tubulin genes is a characteristic of the trypanosomes from the section Salvaria. The recently published results on *T. cruzi* show a much more complex organization of these genes [7]. Our preliminary results with a few species of the section Stercoraria follow this complex pattern. In higher eukaryotes, tubulin genes are dispersed. If, as stated by Hoare [32], trypanosomes transmitted by inoculation are of more recent origin than those with a contaminative method of transmission, the more dispersed structure of these genes in the Stercoraria would make sense from an evolutionary point of view. We understand this may be an oversimplification of the available data, considering the apparent very complex genetic make-up of trypanosomes as a whole [33,34].

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