Compositional compartmentalization of the nuclear genomes of *Trypanosoma brucei* and *Trypanosoma equiperdum*

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High molecular weight DNA preparations from *Trypanosoma brucei* and *Trypanosoma equiperdum* were fractionated by preparative centrifugation in a Cs₂SO₄ density gradient in the presence of BAMD, bis(acetatomercurimethyl)dioxane, a sequence-specific DNA ligand. Analytical centrifugation in CsCl of the DNA fractions so obtained showed that both DNAs had a bimodal distribution with two major peaks banding at 1.702-1.703 g/cm³ and 1.708 g/cm³ and representing 1/3 and 2/3 of total DNA, respectively. Several minor components were also detected. These results indicate that a compositional compartmentalization is not only found in the genome of vertebrates and plants, as already described, but also in those of protozoa such as Trypanosomes.

*Isochore; Trypanosome; Genome compartmentalization*

1. INTRODUCTION

The genomes of the vast majority of bacteria are characterized by narrow, unimodal compositional distributions of the large fragments in which they are broken down during DNA preparation [1,2]. Other genomes show, however, a compositional compartmentalization. For instance, the human genome is a mosaic of isochores (see [3-6] for reviews), very long (> 300 kb, on the average) DNA segments that are compositionally homogeneous above a size of 3 kb (see [7,8]) and that belong to a small number of families which cover a very extended (30–60%) GC range. Random physical and enzymatic degradation occurring during DNA extraction breaks down isochores into the large fragments, 50–100 kb in size, which form routine high molecular weight DNA preparations. The compositional distribution of such DNA fragments reflects that of the isochores from which they derive. This brief description of compositional features of the human genome basically applies to the genomes of all warm-blooded vertebrates. Cold-blooded vertebrates show a much lower degree of compositional heterogeneity in their isochores [9-11]. An isochore organization has also been detected and characterized in plants, *Gramineae* showing a large degree of heterogeneity and all other plants investigated so far a small degree [12-14].

Under these circumstances, an interesting question concerns the phylogenetic spread of such a compartmentalized genome organization. Here we have investigated this problem in two Trypanosomes: *T. brucei* and *T. equiperdum*. Trypanosomes (Class Zoomastigophora, Order *Kinetoplastida*) are very ancient [15-17] flagellated protozoa, whose extant species are only known as parasites. In the case of *T. brucei*, the (haploid) genome has a small size, 3.7 x 10⁷ bp [18], and comprises about 50 mini-chromosomes of 50–150 kb and at least 9 large chromosomes, the largest of which measures about 5.7 Mb (see [19] for detailed references). Mini-chromosomes represent about 10% of the nuclear genome [19].

2. MATERIALS AND METHODS

Nuclear DNA from *T. equiperdum* was a preparation described elsewhere [20], whereas nuclear DNA from *T. brucei* was provided by Prof. Piet Borst. Fractionation of high molecular weight (50–100 kb) nuclear DNA from the two species was carried out by equilibrium centrifugation in a preparative density gradient of Cs₂SO₄ in the presence of a sequence-specific DNA ligand, BAMD, as described [21] BAMD is 3,6-bis(acetatomercurimethyl)dioxane. It binds to AT-rich sequences making 'heavy' the DNA molecules that are rich in those sequences. A ligand/nucleotide molar ratio, R₀, of 0.14 was used. The ligand and Cs₂SO₄ were removed by dialysis against 10 mM Tris, 10 mM EDTA, pH 7.5, at room temperature overnight and against 10 mM Tris, 1 mM EDTA, pH 7.5, at 4°C for 4 days. The fractions so obtained were centrifuged in a CsCl density gradient using an analytical ultracentrifuge, as described elsewhere [22].

3. RESULTS

The analytical CsCl profiles of the compositional DNA fractions from the two Trypanosomes are shown in Fig. 1, the relative amounts of the DNA components in Fig. 2.

In the case of *T. equiperdum*, the CsCl profile of unfractionated DNA is bimodal with a minor component at about 1.702 g/cm³ and a major one at about 1.708 g/cm³. The CsCl profiles of the fractions confirm...
this bimodality and show, in addition, the presence in fraction 1 of relatively large amounts of DNA banding at 1.701 g/cm³ which is part, like that banding at 1.703 g/cm³ of fraction 4, of the major component centered at 1.702 g/cm³ (fractions 2 and 3).

A second, major component appears to be centered around 1.708 g/cm³. This component represents about 64% of total DNA whereas the 1.702 g/cm³ component only corresponds to about 36%, if an intermediate component centered at 1.705 g/cm³ is assigned to the second major component. The minute amount of a 1.708 g/cm³ component present in fraction 1 is likely to represent a satellite DNA in view of its anomalous behavior in the preparative gradient. Indeed, because of its high buoyant density in CsCl, this 1.708 g/cm³ component should normally be present in one of the last fractions of the gradient.

The genome of *T. brucei* is similar to that of *T. equiperdum*, as shown by a basically identical profile of unfractonated DNA, with two major components banding at about 1.703 g/cm³ and about 1.708 g/cm³, respectively. They also represent about 36% and 64%, respectively of the genome, if the intermediate component (1.704 g/cm³) of fraction 5 is partitioned between them. The *T. brucei* DNA shows, however, a larger number of minor components, most of which should correspond to satellite DNAs, in view of their anomalous behavior in the preparative Cs₂SO₄/BAMD gradient. These comprise a 1.692 g/cm³ component, present in fraction 1, possibly corresponding to the satellite DNA described by Sloof et al. [23], a 1.708 g/cm³ component (fractions 1 and 2) similar to that seen in *T. equiperdum*, a 1.718 g/cm³ component (in fraction 11; not seen in *T. equiperdum*), and possibly corresponding to ribosomal DNA, and several very minor components present in the last two fractions.

### 4. DISCUSSION

The results just presented indicate a striking bimodality in the nuclear genomes of *T. brucei* and *T. equiperdum*.
dum. These two genomes are very similar in the compositional distribution of their DNA fragments. Both of them show a predominant major component centered at 1.708 g/cm³ and representing 2/3 of the genome and a less abundant major component centered at 1.702–1.703 g/cm³ representing the remaining 1/3. These features correct an old report based on preparative CsCl banding [18], according to which the main band of nuclear DNA of T. brucei is centered at 1.703 g/cm³, with a shoulder at 1.702 g/cm³. A number of minor components, corresponding to satellite DNAs and, in the case of the 1.718 g/cm³ component of fraction 11 of T. brucei, possibly to ribosomal DNA, were also detected.

The overall GC range (35–55%) covered by large DNA fragments from these two genomes is extremely wide. The bimodality of the CsCl profile is very striking in that both components, at least very largely, correspond to protein-encoding DNA. This is indicated by several lines of evidence. (i) Hybridization experiments have located ESAG (expression site associated) genes in the low GC compartment and housekeeping genes in the high GC compartment of T. brucei (paper in preparation). (ii) The compositional distribution of third codon positions of T. brucei is bimodal, and the ESAG genes have a lower GC content than housekeeping genes ([24]; H. Musto, H. Rodriguez-Maseda and G. Bernardi, submitted for publication). (iii) The scarcity of satellite and middle repetitive DNA in the T. brucei genome.

It appears, therefore, that a compositional compartmentalization also exists in primitive, unicellular organisms, like Trypanosomes. This is not a unique case, however, since compositional compartmentalization has also been demonstrated in the nuclear DNA of Plasmodium cynomologi, which consists of isochores likely to average 100 kb [25], and in chromosome III of Saccharomyces cerevisiae [26]. In conclusion, compositional compartmentalization appears to be a phylogenetically very widespread situation, at least in eukaryotes, as already predicted some years ago [3].

REFERENCES