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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 Cs_2SO_4 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 and 1.708 g/cm^3 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, 3.7×10^7 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].

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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 Cs_2SO_4, 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, R_f, of 0.14 was used. The ligand and Cs_2SO_4 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/cm^3 and a major one at about 1.708 g/cm^3. The CsCl profiles of the fractions confirm

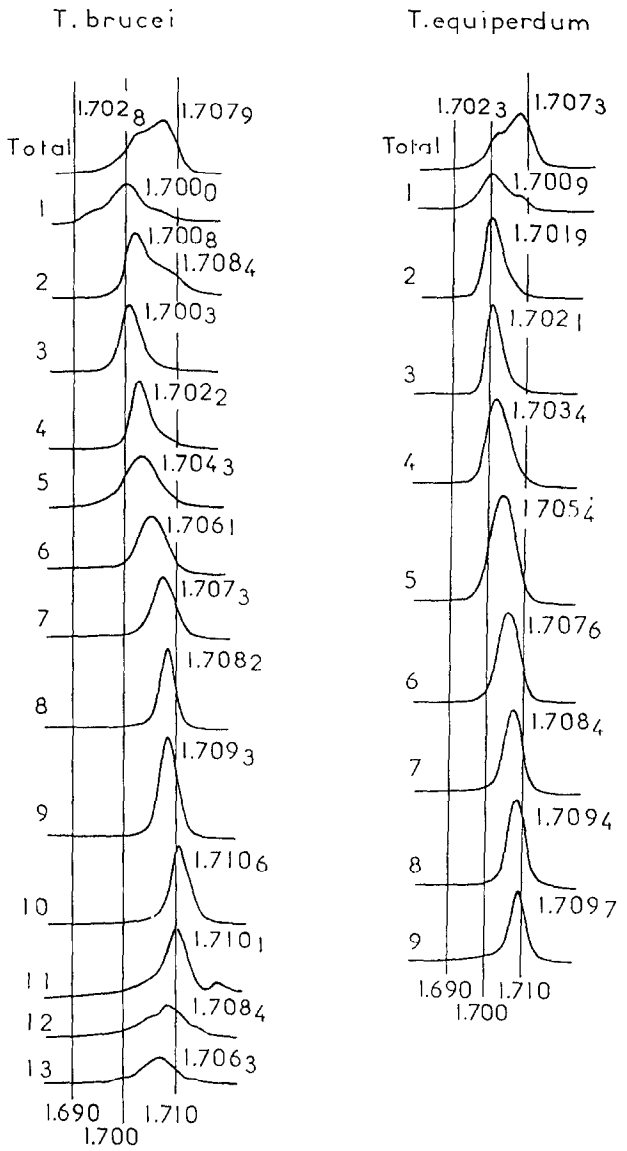

Fig. 1. Analytical CsCl profiles of unfractionated nuclear DNAs from *T. brucei* and *T. equiperdum* and of the compositional fractions obtained from them by preparative centrifugation in a Cs_2SO_4/BAMD gradient.

this bimodality and show, in addition, the presence in fraction 1 of relatively large amounts of DNA banding at 1.701 g/cm^3 which is part, like that banding at 1.703 g/cm^3 of fraction 4, of the major component centered at 1.702 g/cm^3 (fractions 2 and 3).

A second, major component appears to be centered around 1.708 g/cm^3. This component represents about 64% of total DNA whereas the 1.702 g/cm^3 component only corresponds to about 36%, if an intermediate component centered at 1.705 g/cm^3 is assigned to the second major component. The minute amount of a 1.708g/cm^3 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/cm^3 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 unfractionated DNA, with two major components banding at about 1.703 g/cm^3 and about 1.708 g/cm^3, respectively. They also represent about 36% and 64%, respectively of the genome, if the intermediate component (1.704 g/cm^3) 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 Cs_2SO_4/BAMD gradient. These comprise a 1.692 g/cm^3 component, present in fraction 1, possibly corresponding to the satellite DNA described by Sloof et al. [23], a 1.708 g/cm^3 component (fractions 1 and 2) similar to that seen in *T. equiperdum*, a 1.718 g/cm^3 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. equiper-*

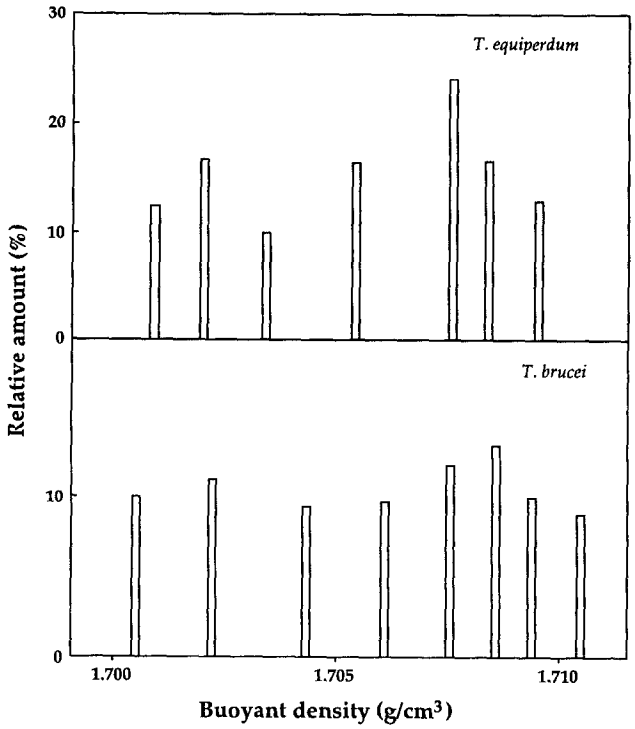

Fig. 2. Histograms displaying the relative amounts of the major DNA components of *T. brucei* and *T. equiperdum* , as estimated from the data of Fig. 1. In *T. equiperdum*, fractions 2 and 3 and fractions 8 and 9, respectively, were pooled because of their very close modal buoyant densities. Likewise, in *T. brucei*, fractions 1, 2 (except for the 1.708 g/cm^3 component) and 3, and fractions 10 and 11, respectively, were pooled.

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/cm^3 and representing 2/3 of the genome and a less abundant major component centered at 1.702–1.703 g/cm^3 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/cm^3, with a shoulder at 1.702 g/cm^3. A number of minor components, corresponding to satellite DNAs and, in the case of the 1.718 g/cm^3 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* [26,28]. In conclusion, compositional compartmentalization appears to be a phylogenetically very widespread situation, at least in eukaryotes, as already predicted some years ago [3].

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