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Genetics

Evidence for haploidy in metacyclic forms of *Trypanosoma brucei*

(parasitology/Feulgen staining/sexual cycle/mating/meiosis)

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ABSTRACT **The parasitic flagellate *Trypanosoma brucei* undergoes a series of morphologic and metabolic changes during its passage in the digestive organs of its insect vector, a *Glossina* or tsetse fly. This morphogenesis ends by the differentiation, in the salivary gland of the fly, of the metacyclic form, which will be transmitted in the bloodstream of the mammalian host. On the basis of DNA microfluorometric measurements, we propose that these metacyclic trypanosomes have a haploid amount of DNA, compared to that of bloodstream forms and also of the proventricular forms, which initiate the invasion of the salivary glands. It can be inferred that trypanosomes undergo meiosis during their developmental cycle in the tsetse fly's salivary glands and syngamy shortly after cyclic transmission.**

During its life cycle, *Trypanosoma brucei* differentiates into a series of cytologically and metabolically distinct forms. The principal forms identified in the blood of the mammalian host are the slender form, which rapidly divides and can undergo antigenic variation, and the stumpy, nondividing form. After the tsetse fly has fed on blood from an infected mammalian host, the ingested trypanosomes differentiate into the procyclic and proventricular forms in the digestive tract and subsequently into the epimastigote and metacyclic forms in the salivary glands of the fly. Although many aspects of this parasitic cycle are now well documented (see ref. 1 for a recent review), the existence of a sexual cycle in trypanosomes has been and still remains an open question (2). The identification of mitosis, meiosis, and the ploidy of these parasites is not possible by direct cytogenetic analysis because their genome does not condense into defined chromosomes at any stage of the cell cycle. However, evidence based on isoenzyme studies (2, 3) and on the recent demonstration of hybrid formation between two clones of *T. brucei* (4) shows that genetic exchange does take place in trypanosomes. Analysis of restriction site polymorphisms (5) and direct measurements of DNA content (6) and genome complexity (7) indicate that *T. brucei* is diploid. Borst *et al.* (6) measured the nuclear DNA content of *T. brucei* using quantitative absorption and fluorescence cytophotometry of individual Feulgen reaction–pararosaniline-stained cells. They performed their analyses on bloodstream and procyclic culture forms and concluded that both forms have the same DNA content. However, the amount of DNA has never been measured in trypanosomes during their developmental cycle in the tsetse fly vector. Therefore, we estimated by direct microfluorometry the nuclear DNA content, after Feulgen reaction–pararosaniline staining of proventricular and metacyclic trypanosomes. These forms are found in the saliva of flies just before and after development of the parasite in the salivary glands, respectively. The quantitative cytochemical method we used is based on the observation of a linear relationship between the amount of fluorescence and the DNA content of Feulgen-stained nuclei under appropriate fixation, hydrolysis, and staining-procedure conditions (8–10).

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MATERIAL AND METHODS

Bloodstream forms of *T. brucei* clone STIB 247-LF were grown in mice, purified from blood components by anion-exchange chromatography (11), and smeared on glass slides. Proventricular and metacyclic forms were obtained directly from *T. brucei* (clone STIB 247-LF or clone STIB-247 IAB)-infected *Glossina morsitans centralis*. The flies were fed either through a membrane on bloodstream trypanosomes or on infected mice and, at various times afterwards, were allowed to probe individually onto warm glass slides. The salivary probes were then checked for the presence of proventricular or mature metacyclic forms. Trypanosome preparations were fixed in methanol/35% (wt/vol) formaldehyde/glacial acetic acid, 85:10:5 (vol/vol), for 60 min and stained with Feulgen-reaction pararosaniline after acid hydrolysis (60 min, at 22°C in 5 M HCl) as described in detail by others (9, 10). The intensity of the Feulgen reaction was estimated fluorometrically, with a Leitz MPV cytophotometer equipped with Ploem's incident light illuminator as described (12).

RESULTS AND DISCUSSION

Fig. 1 shows the distribution of Feulgen-reaction fluorescence intensity of individual nuclei in bloodstream, proventricular, and metacyclic forms of *T. brucei*. In the histogram of bloodstream trypanosomes, different subpopulations can be recognized on the basis of their nuclear DNA content (Fig. 1*A*). The main peak of cells with a fluorescence emission value close to 12 represents the cells in G_1 having a 2 C DNA content, assuming that the genome of *T. brucei* is diploid. Some cells show higher amounts of DNA and are probably in S and G_2 phase. The DNA histogram of proventricular trypanosomes (Fig. 1*B*) shows a peak with a main emission value comparable to that of the bloodstream forms in G_1 phase. Increased scattering of individual readings may be due to some inherent technical difficulties in relation to the highly elongated shape of the nuclei and the frequent proximity of the kinetoplast to the nucleus in this population, which includes some proventricular transition forms (data not shown; ref. 13). In metacyclic trypanosomes, the majority of nuclei have a fluorescence value of 6, which is half that observed for G_1 cells in both bloodstream and proventricular forms (Fig. 1*C*). Moreover, the histogram of metacyclic forms shows a single peak with no evidence of a replicative phase. This observation is in agreement with previous cytological observations (1, 14) that mature metacyclic trypanosomes do not divide.

The cytochemical results presented here suggest that the nuclei of metacyclic *T. brucei* contain an amount of DNA that is half that found in nuclei of both bloodstream and proventricular forms. Alternatively, this observation could

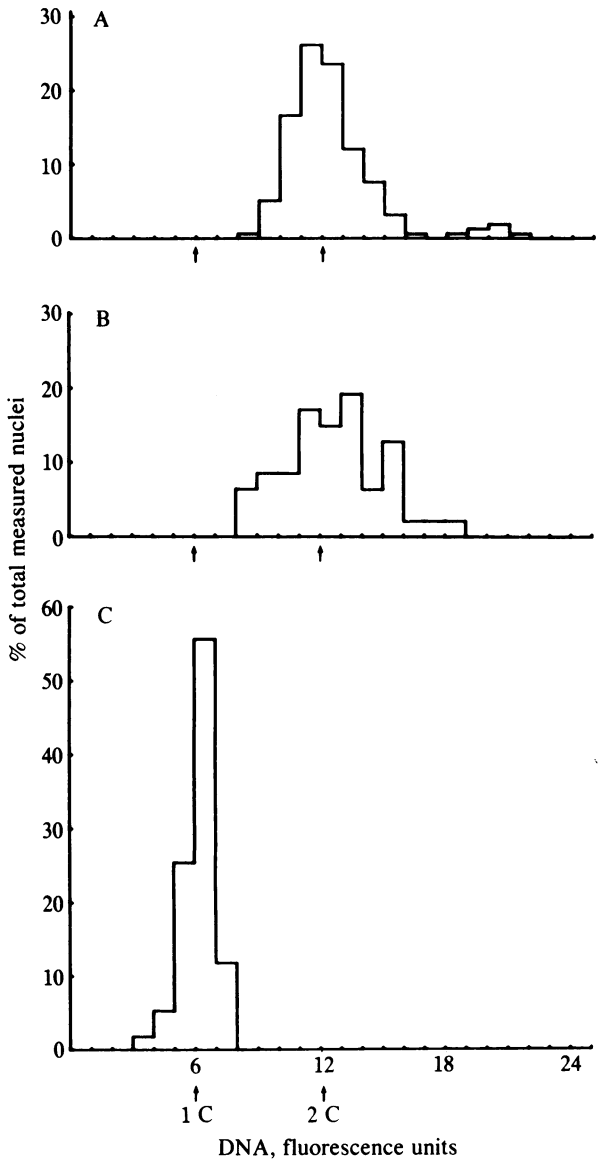

FIG. 1. Frequency distribution of DNA contents obtained by microfluorometry, expressed in arbitrary fluorescence emission units of randomly chosen nuclei of bloodstream (*A*), proventricular (*B*), and metacyclic (*C*) forms of *T. brucei*. A minimum of 500 nuclei have been measured from each trypanosome form. The results for bloodstream and metacyclic trypanosomes were pooled from 3 and 11 distinct transmissions, respectively. The proventricular forms were collected from a single fly. Emission intensity was measured exactly 7 sec after the onset of excitation, and the background fluorescence for each individual measurement, which was measured immediately after, was subtracted.

result from some difference in DNA packaging or chromatin structure between metacyclics and the two other forms—i.e., bloodstream and proventricular trypanosomes. Although this possibility cannot be completely ruled out, we think it is unlikely because it is not supported by any available evidence. In particular, no known factor in the DNA structure, conformation, or compactness can account for a 2-fold difference in the Feulgen-reaction fluorescence intensity (8, 15–17). In addition, the microscopic examination of the chromatin in metacyclic trypanosomes shows no trace of pycnosis or of any other feature that could account for this difference in fluorescence. Therefore, the simplest and most likely interpretation of our data is that metacyclic forms have a haploid amount of DNA, assuming that bloodstream and proventricular forms are diploid (5–7). In this case, the trypanosomes would undergo meiosis in the salivary glands of tsetse flies, and nondividing, mature metacyclic forms should be considered as gametes. Moreover, on the basis of this explanation of our data, we predict that conjugation of these trypanosome gametes occurs in the "chancre," a local skin and subcutaneous reaction that develops following a bite by an infected tsetse fly (18–20) or syringe injection of metacyclic trypanosomes (21). In conclusion, our cytochemical investigation, together with previous biochemical studies (2–4), provides strong evidence for the existence of trypanosome meiosis and gametogenesis in the salivary glands of the vector and for mating occurring in the mammalian host. Since cloned *T. brucei* are capable of completing a normal parasitic cycle, they should be monoecious (homothallic).

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