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Trypanosome hybrids generated in tsetse flies by nuclear fusion

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Genetic exchange may occur between two particular *Trypanosoma brucei* clones simultaneously transmitted by the same tsetse fly. We report here that this exchange takes place in the fly, through nuclear fusion. The resulting hybrids appear to be sub-tetraploid, some particular DNA sequences from one of the parental stocks being lost before enough cloned hybrid trypanosomes could be harvested for DNA analysis. A further reduction of the DNA content of these hybrids occurs gradually upon growth and yields near diploid value in a major part of the population. This mode of hybrid generation is different from the fusion of haploid gametes, which is thought to occur normally upon inoculation of metacyclic trypanosomes in their mammalian host. In this respect, the sub-tetraploid hybrids appear to undergo meiosis in the fly, generating sub-diploid metacyclic forms, then fusion in the mammalian blood.

Key words: Hybrid trypanosomes/karyogamy/parasexual recombination/parasitic cycle

Introduction

Different lines of evidence suggest that trypanosomes are diploid and may undergo genetic exchange (for a review, see Tait 1983). First, the nuclear DNA content, as measured by fluorescence cytophotometry, appears to be twice the genome complexity measured by DNA renaturation kinetics (Borst *et al.*, 1982). Secondly, analysis of isoenzyme electrophoretic patterns (Tait 1980, 1983; Letch, 1984) and restriction endonuclease site polymorphism (Gibson *et al.*, 1985; P.Paindavoine, unpublished results) reveals allelic variants with possible heterozygous combinations. However, direct evidence for the existence of a sexual process in African trypanosomes has been found only very recently by the demonstration that the metacyclic trypanosomes from tsetse saliva have a nuclear DNA content half that of the bloodstream forms, indicating that trypanosomes undergo meiosis during their development in the fly (Zampetti-Bosseler *et al.*, 1986).

A direct demonstration of genetic exchange between trypanosomes was provided following simultaneous cyclical development of two distinct trypanosome clones in the same tsetse flies (Jenni *et al.*, 1986). Indeed, trypanosomes cloned following such mixed infections appeared to be hybrid with regard to a variety of genetic markers. However, these results were not fully interpretable in Mendelian terms and, in particular, a comparison of Southern blots from hybrid and parental genomic digests revealed unexplained differences in the labelling intensity of the same DNA fragments. In order to obtain an insight into the mechanism involved in the generation of these hybrids, we have analyzed the DNA content of parental and hybrid trypanosome clones, following three approaches: (i) cytofluorometry of individual Feulgen-pararosaniline stained cells; (ii) quantitation on Southern blots of defined DNA fragments; and (iii) chromosome characterization by pulse-field gradient (PFG) electrophoresis.

The results show unambiguously that these hybrids are sub-tetraploid synkaryons, most probably resulting from the fusion of diploid nuclei during development in the fly, prior to their differentiation into metacyclic forms. This process appears to be distinct from trypanosome gametogamy, which is thought to occur in the mammalian host (Zampetti-Bosseler *et al.*, 1986).

Results

Hybrids are generated in double infected flies

The production of the hybrid trypanosomes in glossina simultaneously infected with two different *T. brucei* ssp. clones has been reported previously (Jenni *et al.*, 1986). Further analysis of the same and other clones has been carried out. Figure 1 provides a scheme of the mixed infection experiments as well as the pedigree of all the *T. brucei* clones mentioned in the text.

DNA blots of both parental and progeny clones have been hybridized with specific probes. Figure 2 shows the hybridiza-

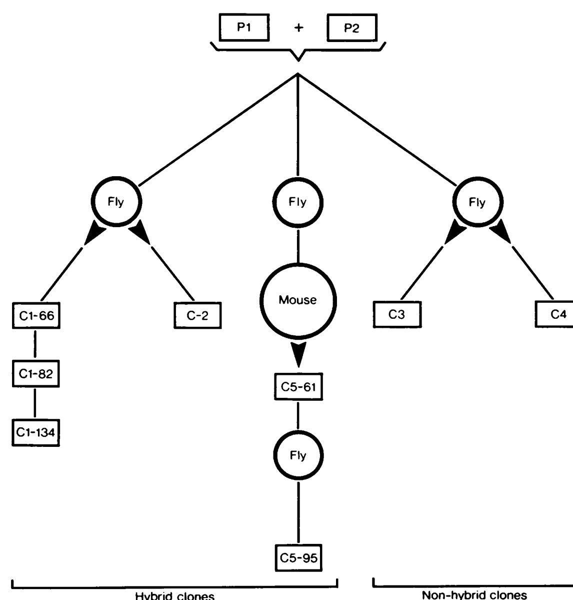

Fig. 1. Abridged pedigree of the *T. brucei* clones obtained from three individual mixed infections with the parental clones P1 (STIB 386 AA) and P2 (STIB 247 L). Clones C1 (STIB 723 VIL), C2 (STIB 723 VIM) and C5 (STIB 723 CA) are hybrids, whereas clones C3 (STIB 723 G) and C4 (STIB 723 H) are not. The numbers following C1 and C5 indicate the time (in days) between infection of the fly with the parental trypanosomes and collection of the hybrids for DNA analyses. The arrowheads indicate cloning, either from metacyclic (C1, C2, C3 and C4) or from bloodstream (C1-66) forms.

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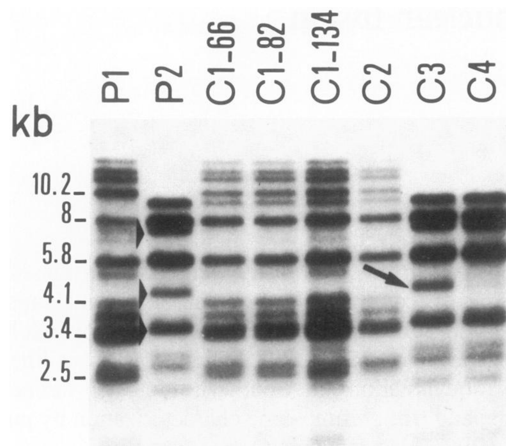

Fig. 2. Genetic exchange between two distinct trypanosome clones infecting the same tsetse fly. The total DNAs from the bloodstream forms of the trypanosome clones in parental (P1, P2) and progeny trypanosome clones (C1, C2, C3, C4) were digested by *Pst*I, then blotted onto nitrocellulose according to Southern (1975). Hybridization was carried out with the AnTat 1.8 cDNA probe (Pays *et al.*, 1980). C1 has been grown for different lengths of time beginning at fly infection: 66, 82 and 134 days, for C1−66, C1−82, C1−134 respectively. Arrowheads refer to fragments not found in hybrids. The arrow points to a fragment referred to in the first part of the discussion.

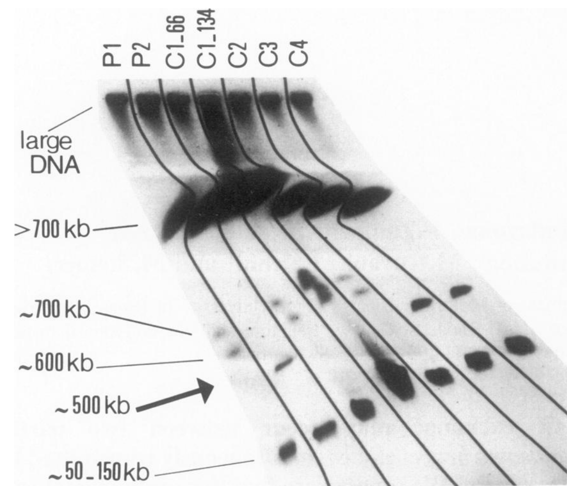

Fig. 3. Electrophoretic separation of chromosome-sized DNAs from parental and progeny clones. Agarose blocks containing DNA from parental (P1 and P2), hybrid (C1 and C2) or non-hybrid clones (C3 and C4) were electrophoresed in a 1% agarose gel, according to Schwartz and Cantor (1984), with 63-s pulses for 20 h. The gel was then stained with ethidium bromide and its photograph presented here as a negative. A 500 kb molecule (arrowed) is not transmitted to hybrid clones.

tion patterns specific for the AnTat 1.8 antigen gene (Pays *et al.*, 1980) in the two parental clones P1 and P2, as well as for some clones resulting from the mixed infection experiments, C1, C2, C3 and C4.

Clones C1 and C2 exhibit hybrid patterns with respect to P1 and P2, whereas C3 and C4 do not appear to be hybrid. Both C3 and C4 show the DNA pattern of clone P2, except for the arrowed DNA fragment which will be referred to in the Discussion. Since each of these C1−C4 clones are derived from single metacyclic trypanosomes isolated from the fly's saliva, it would seem that the hybrid patterns observed in C1 and C2 result from a genetic transfer taking place between parental cells in the insect vector.

Particular DNA sequences seem to be excluded from the hybrids

As shown in Figure 2, the hybrid DNA pattern results in a combination of both parental DNA patterns, since at least one P2 specific band (9.5 kb) is found in C1. However, other DNA bands from P2 (arrowheads) are clearly not transmitted. The same pattern has been reproducibly observed in several independent clones from two different mixed-infection experiments with the same parental clones (data not shown).

Similarly, limited electrophoretic analysis of chromosome-sized DNA from parental and progeny clones shows that at least one chromosome, a 500-kb chromosome specific to P2, is not transmitted to the hybrids (Figure 3). The chromosome pattern of the latter appears to be identical to that of P1, within the limits of resolution of this method. In contrast, non-hybrid clones (C3 and C4) appear to be identical to the other parental clone, P2.

The hybrids are sub-tetraploid

Figure 4 shows the distribution of Feulgen-fluorescence intensity of individual nuclei in bloodstream trypanosomes from parental and progeny clones. In the parental populations (A and B), the first main peak of cells in G1 phase has a fluorescence emission value which most probably represents the diploid amount of DNA (Zampetti-Bosseler *et al.*, 1986). The mean DNA content of the

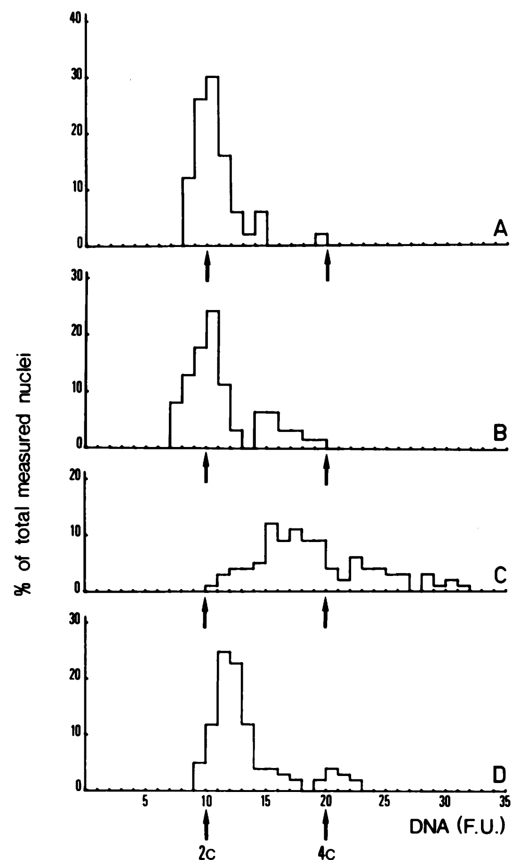

Fig. 4. Nuclear DNA contents in parental and hybrid bloodstream trypanosomes. The DNA content of P1 and P2 (**A** and **B**, respectively) and C1−66 and C1−134 (**C** and **D**, respectively) was measured by microfluorometry following Feulgen reaction. Fluorescence is expressed in arbitrary units (F.U.) and the background fluorescence for each individual measurement was substracted. At least 200 nuclei have been analyzed from each trypanosome clone.

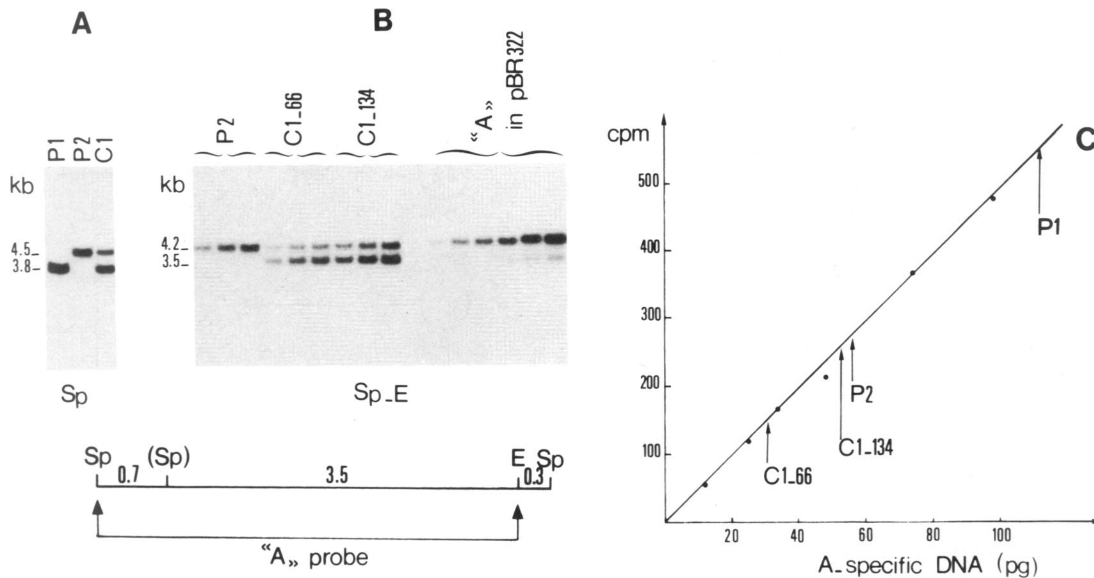

Fig. 5. DNA content in parental and hybrid trypanosomes as estimated by Southern blot analysis. **(A)** Restriction fragment length polymorphisms in parental (P1 and P2) and hybrid (C1) trypanosomes. *Sph*I (Sp) digests of genomic DNAs from P1, P2 and C1 procyclic forms were hybridized with a ^{32}P-labelled 4.2-kb *Sph*I−*Eco*RI fragment ('A' probe), showing one single band of 3.8 kb and 4.5 kb in clones P1 and P2, respectively, and both bands in the C1 clone. A 0.7-kb *Sph*I fragment which hybridizes only weakly to the probe is not visible here. Interpretation of the data is given in the map below with the polymorphic *Sph*I site between brackets. **(B)** 'A' sequence quantitation in clones P2, C1−66 and C1−134. *Sph*I−*Eco*RI (Sp-E) digests of pBR322 DNA carrying the 4.2-kb 'A' sequence and of genomic DNAs from P2, C1−66 and C1−134 bloodstream trypanosomes were hybridized with the ^{32}P-labelled 'A' probe. In each case an *Sph*I−*Eco*RI fragment of 4.2 kb is revealed. The labelling intensity of this fragment was quantitated in 0.5, 0.75 and 1 μg (from left to right) of each one of the three genomic DNAs, using 12, 25, 33, 49, 74, 98 pg (from left to right) of the 'A' sequence in pBR322 as calibration references. **(C)** Plotting of the data from panel B. The labelling intensity of sequence 'A' from genomic and plasmid DNAs was estimated by liquid scintillation counting of the relevant nitrocellulose area. The calibration curve is drawn from the data obtained with known amounts of the 'A' sequence in pBR322 (see Figure 5B), after substraction of a 68 c.p.m. background activity. The mean radioactivities found for 1 μg of C1−66, C1−134 and P2 DNAs, 150.2, 252.1 and 260.5 c.p.m. (arrows), correspond respectively to 31.5, 53.0 and 55.6 pg of A-specific sequences. According to Borst *et al.* (1982), the DNA content of one nucleus for *T. brucei* is around 0.1 pg, with a complexity of about 74 000 kb. If haploid, the 'A' sequence (4.2-kb) would then represent $0.1 \times 4.2 \times 10^7/74\ 000$ pg of DNA, or 56.7 pg in 10^7 nuclei (1 μg). Accordingly, the values obtained here give an estimate of one 'A' sequence per 75 500, 134 500 or 79 600 kb, for P2, C1−66 and C1−134 clones, respectively. The reliability of these measurements can be assessed by the following considerations: (i) The size of the fragment to titrate is the same in genomic DNA as in the calibration samples. As all DNAs are aligned in the same gel, possible differences due to variations in the blotting efficiency are minimized. Moreover, the results presented here have been reproduced in three independent experiments. (ii) The titration has been determined by comparing values in the linear portion of the calibration curve. As an internal control of the measurement accuracy, three different amounts of each genomic DNA have been loaded on the gel, and quantitation of the A sequence in these samples proved to be strictly proportional to the amount of DNA loaded.

hybrid trypanosomes prepared at the earliest possible time after the mixed infection of the fly (C1−66, 58 days of development in the fly and 8 days of growth in mice) appear to be about 1.7 times higher. These hybrids could thus be considered as sub-tetraploids. In contrast, the DNA content of non-hybrid clones (C3 and C4) has been found to be diploid (data not shown).

These observations were confirmed by quantitative analysis of probe hybridization to DNA fragments in Southern blots. As observed in Figure 2, showing hybridization to genomic blots containing calibrated amounts of DNA, the labelling intensity of DNA fragments specific for only one of the parental clones is reduced in hybrid DNA, as compared to the parents (see for instance the four upper bands in this figure). This may be accounted for by the expansion of the genome size in the hybrids resulting from nuclear fusion. A detailed analysis, presented in Figure 5, demonstrates the extent of this expansion. The probe used here, sequence A, whose function is unknown, is a 4.2-kb *Sph*I−*Eco*RI DNA fragment cloned from large chromosomal DNA of stock EATRO 1125 (AnTaR 1). It reveals only a single band in genomic DNA digests, with a variety of restriction endonucleases, of all wild-type *T. brucei* examined (51 stocks, results not shown). However, according to the stock, this band is either 3.8 or 4.5 kb, due to an *Sph*I site polymorphism (see map in Figure 5). In addition, variations in band density suggest that the sequence may be present in either one or two copies. The relative amount of sequence A was titrated in parental (P1 and P2) and hybrid (C1) clones, using a cloned fragment as reference (Figure 5B). The results are plotted on Figure 5C. The copy number of sequence A was estimated to be one in 35 300 kb in P1 and 65 500 kb in P2. Since the diploid nuclear DNA content is estimated to be around 74 000 kb (Borst *et al.*, 1982), it seems that a single copy of the 4.2-kb *Sph*I−*Eco*RI sequence is present in P2, and two copies of its allelic variant (3.5-kb *Sph*I−*Eco*RI) in P1. In C1−66, collected 66 days after infection of the fly, the copy number of the 4.2-kb *Sph*I−*Eco*RI fragment appears to be only one in 134 500 kb by the same criteria, an estimate which could be accounted for by diltuion due to an increase of 78% in total DNA content of C1−66 as compared to P2. This observation supports the view that C1−66 is sub-tetraploid, in accordance with data presented in Figure 4, and is at variance with a Mendelian model of normal segregation.

Sub-tetraploid hybrids progressively lose DNA

Cytofluorometry indicates that the DNA content of C1 nuclei decreases with time, the mean genomic DNA content evolving from sub-tetraploidy (~170%) to near diploidy (~110%), for hybrids collected 66 days (Figure 4C) or 134 days (Figure 4D), respectively, after infection of the fly. Figure 6 shows the kinetics

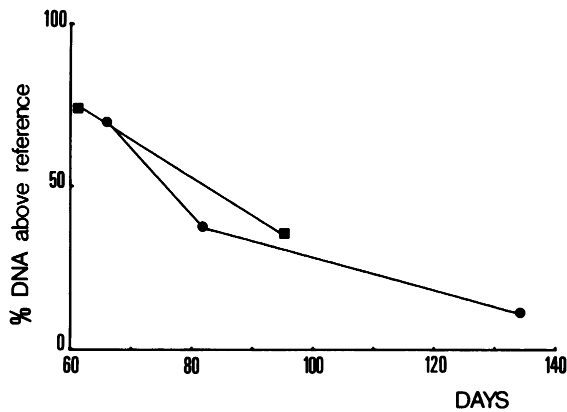

Fig. 6. Decrease of DNA content in hybrids during growth. The DNA content of hybrid trypanosomes from two different mixed-infections, C1 and C5 (● and ■ respectively), has been estimated by microfluorometry after different periods (in days) of growth. Since we ignore when exactly the cell fusion occurred, the moment of fly infection by the two parental stocks has been chosen as time 0. In ordinate, the reference is the normal diploid DNA content of the parents, P1 and P2.

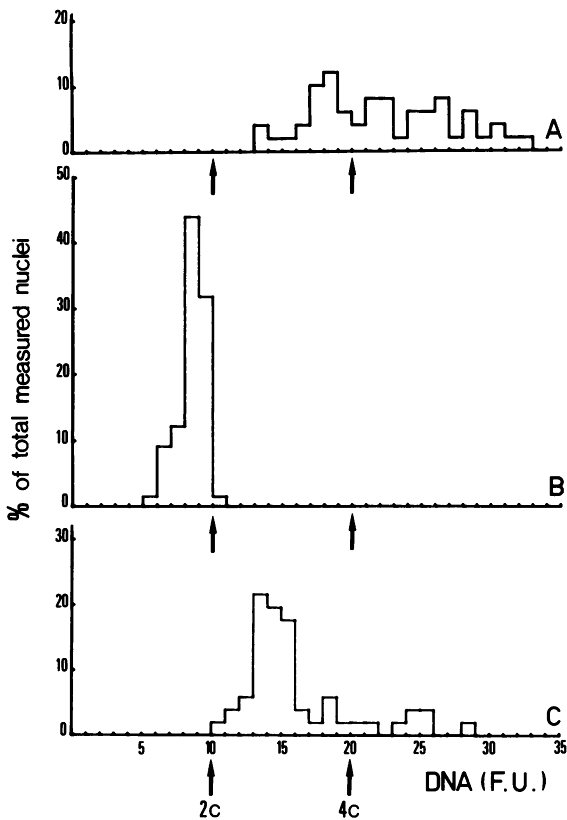

Fig. 7. Hybrid trypanosomes undergo meiosis upon passage through the fly, and subsequent syngamy. The nuclear DNA content of hybrid bloodstream (C5−61) and metacyclic forms of C5 (**A** and **B**), respectively) and of bloodstream forms of C5−95 hybrid trypanosomes (**C**) was measured as in Figure 3.

of this gradual decrease of DNA content in two independent hybrid clones. Accordingly, in Southern blots of calibrated amounts of genomic DNA from hybrid clones, the labelling intensity of DNA fragments specific to one of the parents increases in proportion to trypanosome growth (Figures 2 and 5B), as would be expected for sequences being maintained in nuclei whose total DNA content is decreasing. Data presented in Figure

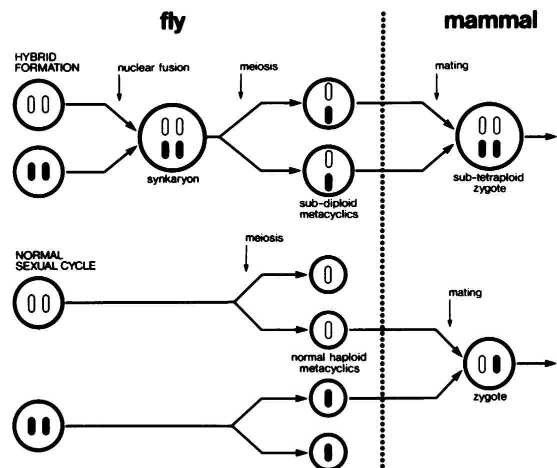

Fig. 8. Scheme of the processes probably involved in tetraploid hybrid formation, as compared to the putative normal sexual cycle of *T. brucei*.

5C give an estimate of a reduction from 178% to 107%, in good agreement with the estimates made by cytofluorometry.

It should be noted that, apart from the above-mentioned apparent exclusion of some DNA sequences from the hybrids, the progressive elimination of DNA shown in Figure 6 does not seem to be selective for any particular DNA fragment seen in our Southern blots (C1−66, C1−82 and C1−134 in Figure 2).

Sub-tetraploid hybrids can undergo meiosis in the fly salivary glands

Figure 7 shows the distribution of Feulgen-fluorescence intensity of individual nuclei of hybrid bloodstream C5−61 trypanosomes (A), of metacyclic forms in the saliva of C5-infected flies (B) and of bloodstream trypanosomes (C5−95) obtained after cyclic transmission of the same clone (C). The mean DNA contents are respectively 175%, 80% and 130% of the reference diploid value. These results are consistent with our previous observations, firstly, that the genome size is reduced to half the initial value when trypanosomes develop in the fly salivary glands, then is restored after transmission to the mammalian host (Zampetti-Bosseler *et al.*, 1986); secondly, that sub-tetraploid hybrids tend to lose DNA. In the particular case of hybrids, however, meiosis in the fly salivary glands appears to lead to near diploidy, sub-tetraploidy being restored upon mating following transmission to mice.

Two clones obtained at that stage (C5−95 and STIB 723 CAEA) have been analyzed by Southern blot hybridization and found to be identical to hybrids before cyclical transmission (data not shown). This suggests that in hybrids, homologs from each of the two parental complements do not segregate and/or re-assort completely randomly during the sexual cycle.

Discussion

Hybrids isolated following mixed cyclical transmission of two different trypanosome stocks by the same tsetse flies (Jenni *et al.*, 1986) have been analyzed. The main conclusions of this work may be summarized as follows. (i) These hybrids have a sub-tetraploid DNA content, suggesting that the mechanism generating the hybrids involves the fusion of diploid nuclei. Some particular parental DNA sequences appear to be selectively excluded from the hybrids. (ii) The genome of the cloned hybrid trypanosomes is unstable and progressively loses DNA, so that a near diploid DNA content is reached in a major part of the

population. (iii) Sub-tetraploid hybrids may undergo meiosis during cyclical transmission in the tsetse fly, resulting in near diploid metacyclic trypanosomes. After infection in blood, these hybrids restore their sub-tetraploid state.

The process of sub-tetraploid hybrids formation in trypanosomes is distinct from their normal sexual cycle

The recent finding that metacyclic forms of *T. brucei* are the haploid stage (Zampetti-Bosseler *et al.*, 1986) of an otherwise diploid organism (Borst *et al.*, 1982) strongly suggests that a sexual cycle exists in trypanosomes, with syngamy taking place in the mammal after cyclic transmission. Quite interestingly, a casual observation made in the present work seems to provide the first genetic evidence in support of these views. Indeed, we see no better explanation than meiotic segregation to account for the presence of a particular sequence (arrowed fragment in Figure 2) in clone C3 and its absence in clone C4. This sequence would be present as a single 'haploid' copy in the parental (P2) diploid genome. Examples of such 'haploid' sequences are frequent in variant-specific antigen gene families (Pays *et al.*, 1985), and are also illustrated in the present study by sequence A in stock STIB 247 L (P2, see Figure 5).

The present observation on the hybrid bloodstream trypanosomes isolated following mixed cyclical transmission indicate that these hybrids are sub-tetraploid, most probably generated by nuclear fusion. In addition, and in sharp contrast with trypanosome syngamy, this fusion must take place in the fly, since hybrid clones could be derived directly from the metacyclic populations that developed in the doubly infected flies. Finally, we have seen that some data on the DNA of the hybrids do not fit a Mendelian model. These observations allow the mechanism involved in the formation of these hybrids to be clearly differentiated from sexual mating, as illustrated by the tentative schemes in Figure 8.

About the loss of DNA from hybrids

The highest mean amount of DNA found in hybrids is 175% with respect to the genome of either parents, and some particular DNA sequences have never been detected in Southern blots of hybrids. It seems very unlikely that the absence of these DNA fragments could be due to a lack of cleavage expected from DNA modification at some restriction sites (Bernards *et al.*, 1984; Pays *et al.*, 1984, 1985), since it was observed with a variety of restriction enzymes (data not shown) and found in the DNA from both bloodstream and procyclic trypanosomes, which respectively do and do not exhibit DNA modification (Pays *et al.*, 1984, 1985). Moreover, this loss of DNA sequences is in accordance with the absence of at least one parental chromosome in all hybrid trypanosome preparations, as found by us (Figure 3), and the absence of at least two parental chromosomes as observed independently (Sternberg *et al.*, 1986). At what moment this 'early' loss of DNA occurs is not known. The shortest period of growth required for collecting an adequate number of trypanosomes was in this case 66 days after infection of the fly and, of these 66 days, 58 were spent in the fly and 8 in mice. Since these hybrids were cloned from the metacyclic population, the time between cell fusion and DNA analysis could thus be anywhere between 8 and 66 days. We conclude from the aforementioned data that the generation of sub-tetraploid trypanosomes involves the selective elimination of some DNA sequences, either at the moment of nuclear fusion or within a limited period of time thereafter. This conclusion is further supported by similar observations by another group, who analyzed the same material by flow cytofluorometry (R.W.F.Le Page, personal communication).

This early and selective DNA exclusion is followed by further losses of DNA, occuring during growth of trypanosomes. Curiously, as mentioned in the Results, we found that this substantial DNA elimination is not accompanied by the loss of any particular DNA fragments in the Southern blots of hybrid DNA. Although a larger battery of DNA probes should be used before drawing firm conclusions from these experiments, one may speculate that this further DNA loss would affect all DNA sequences more or less randomly, perhaps leading to a large proportion of defective cells, able to grow only through a limited number of cell divisions and whose DNA, examined at the population level, would still generate banding patterns in Southern blots very similar to the one found in the original sub-tetraploid hybrid. These views seem to be supported by some preliminary observations (data not shown), that the percentage of success in cloning the hybrids is much lower than for the parent trypanosomes, and that recloned hybrids have a DNA content per cell higher than that of the population from which they have been derived.

This loss of genetic material in *T. brucei* hybrids strikingly resembles that which occurs upon fusion of vertebrate somatic cells (references in Barski, 1970). Somatic cell hybrids lose chromosomes, during or very soon after fusion, and then upon extended cultivation (references in Gordon, 1975). It has been reported that the chromosomes which are preferentially lost are, in some cases, from the slow-growing parent (Kao and Puck, 1970). Interestingly, this appears to be also the case for hybrid trypanosomes since, when genetic material is lost in hybrids, it is the one inherited from P2 and P2 grows slower than P1 (P.Paindavoine, data not shown).

The physiological significance of sub-tetraploid hybrid production

It is worth noting that the fusion between diploid trypanosomes in the fly is clearly not an obligatory step in cyclical transmission. Among four clones derived from metacyclic trypanosomes in mixed infection experiments with the same pair of parental clones, two exhibit non-hybrid DNA patterns and do not appear to be sub-tetraploid.

Secondly, such fusion of diploid trypanosome nuclei seems to occur only between defined trypanosome stocks. Several attempts to generate hybrids by mixed cyclical transmission of other stocks have failed so far (Tait *et al.*, 1985). Moreover, tetraploids have not yet been found in cyclical transmissions of either of the parental clones taken separately. Although no estimate can presently be drawn with regards to its frequency, this process appears nevertheless to be reproducible, because it has been observed in several independent mixed infections (Jenni *et al.*, 1986).

Thirdly, DNA banding patterns similar to the hybrid one generated in the mixed infection experiments have been found in some wild stocks from the field (Jenni *et al.*, 1986; Richner and Jenni, 1986; P.Paindavoine, unpublished). It must be stressed, however, that the sexual cycle might lead to very similar patterns.

In conclusion, we think that this particular mode of genetic exchange, although probably rather infrequent, is not just a laboratory artefact and that it must occur in nature as well. Moreover, it is probably advantageous to the parasite to develop such a mechanism to increase its antigenic variation potential. In the particular example of the AnTat 1.8-specific antigen gene family illustrated in Figure 2, the fusion of nuclei led to an increase of the number of gene family members in the hybrids.

Further rearrangement and diversification could still subsequently occur upon syngamy of the near diploid metacyclics inoculated in the mammalian host. Since cyclical transmission of mixed trypanosomes populations is more likely to be the rule than the exception in natural conditions, the combination of these mechanisms may provide the parasite with a vast antigenic variation potential. Additional modifications of the antigen gene repertoire might be produced in the mammalian blood, following the alternate use of different mechanisms for antigen gene activation (see Pays, 1986).

Materials and methods

The mixed cyclical transmissions and cloning of trypanosomes have been described in detail elsewhere (Jenni *et al.*, 1986). Briefly, the STIB 723 VIL, STIB 723 VIM, STIB 723 G and STIB 723 H clones were directly derived from metacyclic forms after mixed transmissions of STIB 386 AA and STIB 247 L clones. The STIB 723 CA clone was derived from a bloodstream form after mixed transmission. This clone was retransmitted through flies to yield STIB 723 CAB, of which C5−95 is a derivative. STIB 723 CAEA is a derivative of STIB 723 CAE, which is a clone from a metacyclic form after cyclical transmission of STIB 723 CA. Figure 1 summarizes the origin of the cloned bloodstream trypanosomes used in this work. These were grown in mice and purified from blood components by anion exchange chromatography, as described by Lanham and Godfrey (1970). The metacyclic forms used for Figure 7B were obtained after an independent cyclical transmission of 723 CA. They were directly obtained from salivary probes of infected flies, as already reported (Zampetti-Bosseler *et al.*, 1986);

DNA isolation

The DNA isolation was carried out by lysis in 10 mM NaCl, 250 mM EDTA, 1% SDS, and 10 mM Tris−HCl (pH 8), followed by incubation with 100 μg/ml RNase A for 1 h at 37°C and an overnight incubation at the same temperature with 100 μg/ml proteinase K.

After dialysis against 10 mM NaCl, 0.2 mM EDTA, and 10 mM Tris−HCl (pH 8), the nuclear DNA was purified by CsCl gradient centrifugation and further dialysis. Alternatively, the DNA was extracted twice by phenol, then with phenol-chloroform (1:1), dialyzed against 10 mM Tris−HCl, 0.1 mM EDTA (pH 8) and precipitated in two volumes of ethanol at −70°C.

PFG gel electrophoresis

Lysis of trypanosomes and PFG gel electrophoresis were performed essentially as described by Schwartz and Cantor (1984) and Van der Ploeg *et al.* (1984).

Electrophoresis was in 1% agarose gel at a temperature of 20°C, alternating the electric fields (18.5 V/cm in the north/south and 8.5 V/cm in the west/east directions) every 63 s for 20 h. Size calibration is according to Guyaux *et al.* (1985).

Microfluorometry

DNA determinations were carried out by measuring the amount of fluorescence emitted by Feulgen-stained nuclei of trypanosome preparations, as already described by Zampetti-Bosseler *et al.* (1986).

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