Analysis of a cross between green and red fluorescent trypanosomes

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Abstract

*Trypanosoma brucei* undergoes genetic exchange in its insect vector, but the mechanism is unknown and no one has yet seen the process. By crossing genetically engineered red and green fluorescent trypanosomes, we have been able to pinpoint the location of genetic exchange in the fly and search for intermediate stages. In experimental crosses of red and green parental trypanosomes, yellow hybrid trypanosomes first appeared in the fly salivary glands as early as 13 days after infection and were observed only in flies with a mixture of red and green trypanosomes in one or both salivary glands. Despite high numbers of flies with mixed infections, yellow trypanosomes were not detected in the fly midgut or proventriculus. The hybrid nature of yellow trypanosomes was confirmed by analysis of molecular karyotypes and microsatellite alleles. As well as yellow hybrids, hybrid trypanosomes with red, green or no fluorescence were also recovered from fly salivary glands. Analysis of microsatellite alleles in parental and progeny clones showed Mendelian inheritance. Our findings are consistent with the hypothesis that mating takes place between trypanosomes in the salivary glands of the fly before they attach to the salivary gland epithelium.

Introduction

Trypanosomes are pathogenic protozoa of medical and veterinary importance. In particular, *Trypanosoma brucei* is responsible for human African trypanosomiasis and is transmitted by bloodsucking tsetse flies. During its developmental cycle in the insect, *T. brucei* may undergo genetic exchange [1], but details of the mechanism remain elusive and the frequency of genetic exchange in Nature is controversial [2]. Understanding how gene flow works in these pathogens clearly has relevance to epidemiology and control strategies for trypanosomiasis; for example, the single gene responsible for human infectivity in *T. b. rhodesiense* could be transferred to non-human infective *T. b. brucei* strains by genetic exchange [3]. Elucidation of the mechanism of genetic exchange in these divergent lower eukaryotes [4] may also inform studies on the evolution of sex.

Our current knowledge of genetic exchange in *T. brucei* has been gleaned by comparison of parental and hybrid genotypes from a series of experimental crosses, but the process has never been observed directly. Thus we do not know about the intermediate stages involved or where the process takes place in the fly. Despite the fact that haploids have not been observed, three lines of evidence point to the occurrence of a meiotic division: firstly, extensive analysis of genetic markers [isoenzyme, RFLP (restriction-fragment-length polymorphism) and mini- and micro-satellite markers] has shown a Mendelian pattern of segregation and reassortment, e.g. [5]. Secondly, hybrid clones show a high frequency of chromosomal recombination relative to parental clones by PFGE (pulsed field gel electrophoresis) analysis, as expected for products of meiotic division [6,7]. Thirdly, hybrids with a DNA content of either 2n or 3n, but not intermediate

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Abbreviation used: PFGE, pulsed field gel electrophoresis.

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Figure 2 | Genetic analysis of parental and progeny clones

(A) PFGE. RP, red parent; GP, green parent. The hybrid progeny clones appear to have a combination of chromosomal bands from the parents. M, marker chromosomal DNAs from Hansenula wingei. (B) PCR amplification of phospholipase C microsatellite alleles. Both parental trypanosomes are heterozygotic at this locus. Lanes 3–10 show alleles amplified from individual hybrid progeny clones from three flies SG1, SG3 and SG22. Each progeny clone appears to have inherited one allele from each parent and all possible reassortments of the parental alleles are represented.

amounts, have been found in several crosses [6,7], suggesting that DNA is inherited in packets of \( n \). There is also convincing evidence that cell fusion is involved, because hybrid progeny have hybrid kinetoplast (mitochondrial) DNA networks, implying that the mitochondria, and hence cell bodies, have fused [2]. Thus there is evidence that both meiosis and fusion occur during trypanosome mating, but the order of these events is uncertain.

The difficulty of identifying hybrids and their apparent rarity in infected tsetse flies have been major hindrances in the study of genetic exchange in trypanosomes, and to overcome these problems, we have developed approaches based on detection of fluorescent hybrids directly in the fly [8]. In recent experimental crosses, a red fluorescent parental line carrying the \( mRFP \) gene [9] was mated with another trypanosome line carrying \( GFP \), producing abundant yellow hybrid trypanosomes that were easily visualized as live cells in dissected fly organs by fluorescence microscopy (Figure 1). Yellow hybrids were detected as early as 13 days after fly infection and were only found in flies with a mixture of red and green trypanosomes in one or both salivary glands. Although some flies had a pure infection of green trypanosomes in one salivary gland and red trypanosomes in the other of the pair, such mixed infections did not produce hybrids. Neither could yellow hybrids be detected in the fly midgut or proventriculus, despite very high numbers of flies with mixed infections, and only red or green trypanosomes were seen among migratory developmental stages recovered from saliva samples. These results are consistent with the hypothesis that mating takes place as soon as both parental trypanosomes reach the same salivary gland and involves the epimastigote lifecycle stage.

Progeny from four independent crosses were cloned and examined in detail. Whether they exhibited red, green, yellow or no fluorescence, all progeny had hybrid genotypes, as revealed by PFGE and microsatellite analysis (Figure 2). This result was unexpected, as parental genotypes in addition to hybrids were recovered in previous crosses, e.g. [1]. Inheritance of microsatellite markers followed a Mendelian pattern of inheritance. Identifying potential intermediate stages such as haploid cells will now be easier thanks to our new knowledge of where and when genetic exchange occurs during the developmental cycle of trypanosomes in the tsetse fly.

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References


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