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Review

Liaisons dangereuses: sexual recombination among pathogenic trypanosomes

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Abstract

Sexual recombination between pathogenic microbes has the potential to mobilise genes for harmful traits into new genetic backgrounds creating new pathogen strains. Since 1986 we have known that genetic exchange can occur in trypanosomes, but we are only now starting to unravel details of the process. In *Trypanosoma brucei* genetic exchange occurs in the tsetse vector, but is not an obligatory part of the life cycle. The process involves meiosis and production of haploid gametes, and thus appears to be true sexual reproduction. This review looks at the experimental evidence concerning genetic exchange and identifies current gaps in our knowledge.

Keywords: *Trypanosoma brucei*; Genetic exchange; Meiosis; Human African trypanosomiasis; Life cycle; Haploid gamete

1. Introduction

Trypanosomes are protozoan parasites with a single flagellum that are commonly found in the blood of vertebrates, typically appearing as elongated, writhing organisms among the red blood cells in a wet blood smear. Though some trypanosomes show tissue-tropism or have intracellular stages, it is these blood-dwelling parasites that are transmitted from one vertebrate to another by blood-sucking arthropods or leeches. The drastic change from the environment of the vertebrate bloodstream to the invertebrate gut must be successfully accomplished within seconds, and this transition usually initiates a complex cycle of differentiation and development within the invertebrate host before infective trypanosomes are ready for transfer back to another vertebrate.

Of the hundreds of trypanosome species described, few are known to be pathogenic to their vertebrate hosts, and only two cause human disease:

- *Trypanosoma cruzi* is the parasite responsible for Chagas disease in Latin America and is transmitted by blood-sucking triatomine bugs. Infective parasites are excreted in bug faeces and gain entry into the vertebrate host via contamination of abraded skin or mucosal surfaces such as the conjunctiva of the eye. A number of domestic (e.g. cats, dogs) and wild animals (e.g. opossums) have been implicated as reservoir hosts, allowing the disease to circulate in domestic or sylvatic transmission cycles where suitable triatomine vectors are present.
- *T. brucei* is the causative agent of sleeping sickness or human African trypanosomiasis (HAT) and is transmitted by the bite of blood-sucking tsetse flies, large dipteran flies found mainly in tropical Africa. Besides humans, *T. brucei* infects a wide range of mammals, both wild and domesticated, that serve as food sources for tsetse; some of these animals can act as reservoir hosts of HAT, if the parasites they harbour are infective to humans. However, only some *T. brucei* strains are human-infective and these are conventionally recognised as two subspecies: *T. b. rhodesiense* in East Africa and *T. b. gambiense* in West and Central Africa. *T. b. gambiense* is further divided by both

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phenotype and genotype into two groups; the majority of isolates from patients belong to type 1.

Trypanosomes are kinetoplastid flagellates, characterised by the unique conformation of the mitochondrial DNA, which is packaged into an organelle called the kinetoplast. Kinetoplastids belong to the eukaryote supergroup Excavata, which is considered to be an early diverging branch of the eukaryote tree [1,2]. Although biologists now believe that sex and meiosis were present in basal eukaryotes, evidence to support this contention has been lacking with respect to the excavate group. Some form of genetic exchange has been experimentally demonstrated in a few representative genera: the kinetoplastids, *Trypanosoma* [3,4], *Leishmania* [5] and *Crithidia* [6], and the diplomonad *Giardia* [7]; in addition, genetic recombination in *Trichomonas vaginalis* is suggested by population genetics analysis [8]. While genes associated with the mechanics of meiotic division have been identified in several excavate genera by phylogenomic analysis [9,10], experimental confirmation of function has been carried out only in *Giardia* [7] and *Trypanosoma brucei* [11].

Why is it important to find out more about the mechanisms of genetic recombination used by the excavates? This will increase understanding of the evolution of sex in eukaryotes, because of the assumed early divergence of this group and its basal position in eukaryote trees [1]. Furthermore, as several important human and animal parasites are found among the Excavata, it is imperative to find out if and how virulence genes can be transferred between different pathogen strains and whether new pathogen strains are generated by genetic exchange. For example, two of the six recognised genetic lineages (or discrete typing units, DTUs) of *T. cruzi* are hybrids that have combined genetic material from other DTUs; these hybrid DTUs occur with high prevalence in patients with Chagas disease in southern countries of South America such as Bolivia, Paraguay, Chile and Argentina [12]. Regarding human African trypanosomiasis the virulence gene, *SRA*, is responsible for human infectivity in *T. b. rhodesiense* [13]. In the laboratory transfer of this single gene can convert a strain of *T. b. brucei* to human infectivity [13] and evidence from the field suggests that this has occurred through genetic recombination between *T. b. rhodesiense* and *T. b. brucei* in East Africa [14]. These two examples serve to demonstrate how genetic recombination between pathogen strains can have profound epidemiological consequences and hence is of more than academic interest.

2. Genetic exchange in trypanosomes

Genetic exchange has been studied in depth in *Trypanosoma brucei* and *T. cruzi* by performing experimental crosses in the laboratory. Results to date suggest that the process is quite different in the two species. *T. brucei* mates in its tsetse fly vector rather than the mammalian host [3], whereas *T. cruzi* appears to mate in the mammalian host rather than the insect vector, since hybrids appeared in cultures of mammalian cells infected with two different trypanosome strains [4]. *T. cruzi* hybrids appear to result from fusion of parental trypanosomes with subsequent random loss of DNA [4]. While early experiments suggested that *T. brucei* hybrids were also produced by fusion, because hybrid progeny had raised DNA contents [15,16], subsequent results contributed to the present consensus that Mendelian inheritance and diploid progeny are the norm [17–24]. To date only a single *T. cruzi* cross has resulted in production of hybrids [4], whereas many successful *T. brucei* crosses have been carried out (Table 1), and consequently more is known about genetic exchange in *T. brucei*, which is therefore the focus of the rest of this review.

That said, analysis of genetic exchange in *T. brucei* is not without challenges. In contrast to other parasitic protists such as *Plasmodium*, where sexual reproduction in the mosquito vector is an obligatory part of the transmission cycle, genetic exchange in *T. brucei* appears to be a non-essential event in the trypanosome life cycle. As mating takes place in the tsetse fly among life cycle stages that are not amenable to *in vitro* culture, experimental crosses require access to specialist facilities for tsetse fly transmission. Tsetse are relatively refractory to trypanosome infection [25], with an extensive arsenal of immune defences that counter each stage of the trypanosome's developmental cycle in the insect [26–28]. This severely restricts the number of infected flies that are produced, and on top of this, genetic exchange can, of course, only occur in flies infected with not just one, but two *T. brucei* strains, further reducing the likelihood of finding flies containing hybrids.

The development of approaches to overcome these obstacles has been crucial to progress on elucidating the mechanism of genetic exchange in *T. brucei*. For example, methods to enhance trypanosome infection through inhibition of tsetse immune defences [29–32] have greatly increased the numbers of infected flies available for analysis, while techniques to facilitate the identification of hybrids have diminished effort wasted on analysis of parental genotypes. In the first *T. brucei* crosses, hybrids were found by isolating trypanosome clones at random, a labour-intensive and time-consuming “needle in a haystack” approach [3,18,33]. With the advent of techniques to genetically engineer trypanosomes in the 1990's, it became possible first to select hybrids by double drug resistance [22,34], and subsequently to identify trypanosome hybrids directly inside the tsetse fly by the use of fluorescent proteins to visualize the living cells [35–37]. Using parental lines distinguishable by fluorescence had the additional advantage that visual inspection could detect co-infected flies. This overturned the belief that genetic exchange was an infrequent event in the *T. brucei* life cycle, because hybrids were almost invariably found in tsetse flies with a mixed infection of the two parental trypanosomes in the salivary glands [37].

In addition to these advances, progress in understanding the developmental cycle of *T. brucei* in the tsetse fly, particularly the role of the foregut migratory stages, has been crucial to interpretation [38–40]. The various developmental stages of *T. brucei* are shown in Fig. 1. While it has taken many years of research effort to put all these individual pieces in place, research is now able to move forward rapidly.