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Invited Review

The surface structure of trypanosomes in relation to their molecular phylogeny

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Abstract

Molecular phylogenetic analysis using genes coding for ribosomal RNA and proteins suggests that trypanosomes are monophyletic. Salivarian trypanosomes showing antigenic variation of the variant surface glycoprotein (VSG) diverged from non-Salivarian trypanosomes some 200–300 million years ago. Representatives of the non-Salivarian group, the mammalian parasite, *Trypanosoma cruzi,* and the fresh-water fish trypanosome, *T. carassii*, are characterised by surfaces dominated by carbohydrate-rich mucin-like glycoproteins, which are not subject to antigenetic variation. It is suggested that this latter surface structure is typical for non-Salivarian trypanosomes as well as members of the other Kinetoplastid suborder, the Bodonina. This would imply that at some point in time in the evolution of the Salivaria the highly abundant and comparatively poorly immunogenetic mucin-like molecules must have been replaced for equally abundant but highly immunogenic VSG-like molecules. While the selective advantage for such a unique transition is difficult to imagine, the subsequent diversification of VSG genes/molecules may have been comparatively straightforward because even the most limited form of antigenic variation would have extended the duration of infection in the vertebrate and thus would have increased the chance for transfer to the vector.

Keywords: Trypanosomes; Surface structure; Variant surface glycoprotein; Mucins; Molecular phylogeny; Antigenic variation

1. Introduction

Unicellular flagellates of the family Trypanomatidae are parasites found in all vertebrate classes. With the exception of *T. cruzi*, which is characterised by amastigotes dividing in the cytoplasm of mammalian cells and by extracellular, non-dividing trypomastigotes in the blood, trypanosomes are considered to thrive extracellularly in the vascular system of vertebrates. They are transmitted by insects or leeches to terrestrial and aquatic vertebrates, respectively. Their prevalence in a given vertebrate population is frequently high but the intensity of infection is generally low. Trypanosome infections in nature appear to be chronic but benign. Thus, there is a balance between the effectiveness of the innate and adaptive immune responses of the host to control the infection and properties of the parasite to evade or subvert these responses. Infections causing disease and mortality as observed for Salivarian trypanosomes in humans (sleeping sickness) or cattle (Nagana) are examples where such a balance has not or not yet been achieved.

One of the most fascinating aspects of trypanosome biology is the antigenic variation of the surface coat formed by the variant surface protein (VSG) in the Salivaria (Cross, 1996). Several years ago, we considered that this mechanism may not be unique to this group of parasites but that there may be relatives parasitising other vertebrate classes, which may show the same phenomenon possible in a less evolved form. In order to find such putative relatives we embarked on a phylogenetic analysis of trypanosomes, where just a few species had been studied at that time (Lake et al., 1988; Gomez et al., 1991; Fernandes et al., 1993; Maslov et al., 1994; Landweber and Gilbert, 1994; Berchtold et al., 1994; Marché et al., 1995). In a second approach, we decided to investigate as a model a trypanosome infecting non-mammalian vertebrates and chose *Trypanosoma carassii*, which is widely distributed in fresh-water fish (carp and other cyprinid and some non-cyprinid fishes). Infections by this trypanosome had been described as chronic and appeared to be controlled by antibodies (Lom and Dyková, 1992; Nazrui Islam and Woo, 1991). In the following, we will summarise some of our

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findings along both lines of investigation and pose some questions, which may stimulate comments by the participants of the Internet Conference.

2. Molecular phylogeny of trypanosomes

By sequencing the nuclear genes encoding the small rRNA of 11 species representative of trypanosomes of different vertebrates classes, we extended the existing data set for the Kinetoplastida (Haag et al., 1998; compare also recent studies Maslov et al., 1996; Lukes et al., 1997; Stevens et al., 1999; Wright et al., 1999). The derived tree (Fig. 1) led to the following conclusions:

- Trypanosomes are monophyletic.
- The tree reveals an early split containing Salivarian trypanosomes (Clade III) showing antigenic variation and all other trypanosomes (non-Salivarian trypanosomes, cf. Clades IV and V). Thus, we found no trypanosome in non-mammalian vertebrates that appeared to be closely related to the Salivaria. In particular, the fish trypanosome, *T. carassii,* grouped in Clade IV with other fish as well as amphibian trypanosomes.

Recently, this analysis has been extended to protein coding genes both by sequencing genes from representative vertebrate trypanosomes and by use of sequences available in databases (unpublished results, see also Hashimoto et al., 1995; Alvarez et al., 1996; Hannaert et al., 1998). Using several methods of phylogenetic reconstruction, we confirmed the monophyletic origin of the genus and the branching order shown by the ssrRNA tree. It may be noted that our results do not support a basal divergence of Clade IV termed 'aquatic' branch by Stevens et al., 1999.

The protein coding genes were also used to estimate divergence times (Haag et al., 1998 and unpublished results). The use of multiple protein loci should give a superior estimate of divergence times than estimates based on a single locus. Under the assumption of a molecular clock, calibrated using metazoan divergence times, the genus *Trypanosoma* diverged as a monophyletic clade from other Kinetoplastids some 400–600 million years (Myr) ago. The genus then split into Salivarian trypanosomes and non-Salivarian trypanosomes 200–300 Myr ago. The origin of the Salivarian trypanosomes therefore predated the appearance of placental mammals (<100 Myr ago), or their common vector, the tsetse fly (30–60 Myr ago), or even its ancestor, the protoglossina (140 Myr ago) as well as the split between the African continent from South America (80–100 Myr ago). This latter estimate was

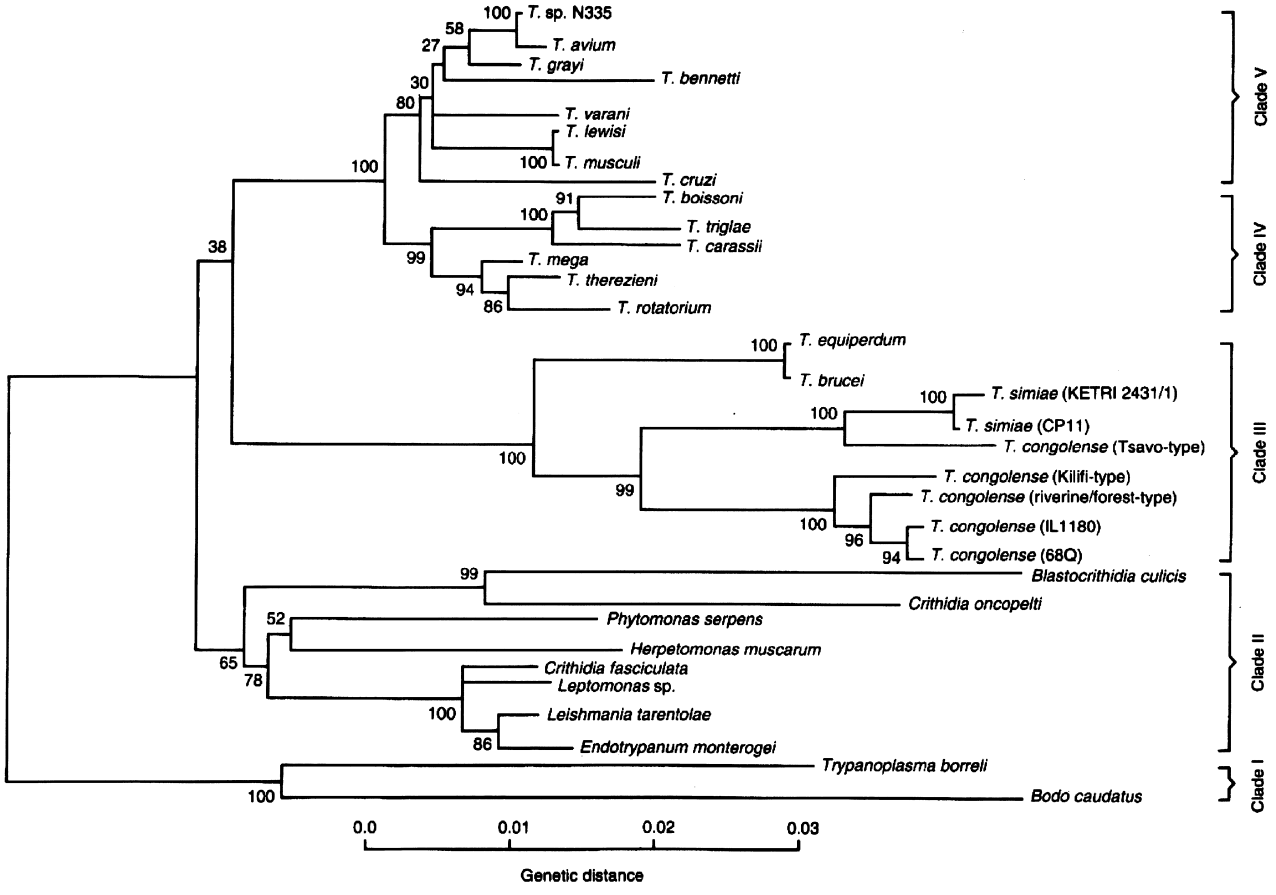

Fig. 1. Molecular phylogeny of trypanosomes based on the analysis of genes coding for the small subunit rRNA (cf. Haag et al., 1998 for details).

used by Stevens and Gibson (1999) to date the divergence of the Salivarian and non-Salivarian clades because *T. brucei* and its relatives are transmitted between mammals by tsetse flies exclusively in Africa.

3. Surface organization and immune response

It is firmly established that the surface of Salivarian trypanosomes is dominated by the coat formed by the VSGs. VSGs are highly immunogenic and parasites are readily eliminated by the humoral immune response against these molecules. Therefore, the successive expression of antigenically different variants is a necessary requirement for establishing a chronic infection. Without antigenic variation, Salivarian trypanosomes could only cause a transient infection.

Infections by *T. carassi* in carp turned out to be profoundly different but also showed some similarities:

- This trypanosome divides in the trypomastigote form in the blood and tissue spaces. One of two closely related types found in fresh-water fishes can also be readily adapted as bloodstream forms for growth in culture in a similar medium as that used for *T. brucei* but with carp serum as a supplement (Overath et al., 1998).
- Infection of specified pathogen-free carp in the laboratory leads to an initial rise in blood parasitamia followed by a sharp decline in all fish (acute phase). Thereafter, in some carp, parasites become undetectable both in the blood and in internal organs while, in others, low numbers can be found in the blood for at least 1 year (chronic phase). Thus, *T. carassi* infections do not show the fluctuating parasitamia that is characteristic for infections with Salivarian trypanosomes (Overath et al., 1999).
- Fish that have controlled an acute infection with a trypanosome clone are not only protected against a homologous challenge infection, but also against infection with parasite lines derived from carp in the chronic phase of infection. This observation provides a clear argument against the occurrence of antigenic variation (Overath et al., 1999).
- When IgM isolated from the serum of carp that have controlled an infection is transferred to naive carp, these animals are resistant to infection (Overath et al., 1999).
- The surface of *T. carassii* is covered by mucin-like carbohydrate-rich glycoproteins ($6.0 \pm 1.7 \times 10^6$ molecules per cell), which are attached to the membrane by glycosylphosphatidylinositol residues. The polypeptide backbone of these glycoproteins is dominated by threonine, glycine, serine, alanine, valine and proline residues. On the average each polypeptide carries carbohydrate chains composed of about 200 monosaccharide units (galactose, *N*-acetylglucosamine, xylose, sialic acid, fucose, mannose and arabinose), which are most likely O-linked to hydroxy amino acids (Lischke et al., 2000). The surface of the fish trypanosome is therefore very similar to that of the mammalian *T. cruzi*, where GPI-anchored mucin-like molecules and their function have been described in great detail (Frasch, 2000; Pereira-Chioccola et al., 2000)

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Taken together, these properties are consistent with the deduction that *T. carassii* belongs to the non-Salivarian branch of the phylogenetic tree. It is also clear that a high prevalence (up to 100%) and intensity of trypanosome infections observed, in particular, in farmed fish populations is no indication of an immune evasion mechanism akin to antigenic variation. Because under defined laboratory conditions carp can control the infection very well, the high prevalence in farmed fish is most likely a reflection of the poor immune status of the fish due to co-infections with other pathogens or a general stress response (intra- and interspecific competition for space and nutrients, threat by predators etc.).

4. Conclusions and speculations

Although information on the surface organisation of non-Salivarian trypanosomes is limited to one mammalian and one fish species it may be assumed that carbohydrate-rich mucin-like proteins occur in many, perhaps all members of this lineage. Furthermore, we may speculate that ancestors of the Trypanosomatidae had a carbohydrate-dominated surface coat. In this context, it would be interesting to characterise surface molecules of members of the other Kinetoplastid suborder, the Bodonina, such as the free-living *Bodo saltans*, which may be the closest relative to the trypanosomatids (Simpson et al., 2000), or the parasititic species *Trypanoplasma borreli*, a flagellate, which, like *T. carassii*, thrives extracellularly in the vascular system of freshwater fish. Thus, it appears that during evolution, Kinetoplastids with a relatively poorly immunogenic carbohydrate-rich surface could adapt to a parasitic life style in vertebrates starting either from a free-living stage or from parasitic stages in the alimentary tract of blood-sucking insects or leeches.

In this scenario, the origin of a protein dominated surface coat characteristic for the Salivaria is a unique event, i.e. at some point in time there must have been a selective advantage for replacing highly abundant and comparative poorly immunogenic mucin-like molecules for equally abundant but highly immunogenic VSG-like molecules. A candidate for a pre-VSG molecule could, for example, be the transferrin receptor, a proteins present in very low abundance in bloodstream form *T. brucei* (Steverding et al., 1995; Salmond et al., 1997). The subsequent diversification of VSG genes/molecules may have been comparatively straightforward because even the most limited form of anti-

genic variation will extend the duration of infection in the vertebrate and this in turn increases the chance for transfer to the vector. Establishment of switching mechanisms and expansion of the VSG repertoire is thus considered to require cycling of trypanosomes between vertebrate and vector over evolutionary times.

It is possible that the Salivaria originally arose in reptiles, which were the dominant vertebrates at the time of their divergence from other trypanosomes and that transfer to mammals occurred when tsetse flies or their ancestors had evolved. In fact, tsetse flies are known to frequently take blood meals on reptiles such as monitor lizards (Okoth and Kapaata, 1988; Clausen et al., 1998) and it has recently been shown that *T. brucei* can survive and perhaps even multiply in these reptiles (Njagu et al., 1999). Therefore, a systematic search for relatives of the Salivaria in reptiles could be rewarding.

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