Review

Flying tryps: survival and maturation of trypanosomes in tsetse flies

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Survival in and colonization of the tsetse fly midgut are essential steps in the transmission of many species of African trypanosomes. In the fly, bloodstream trypanosomes transform into the procyclic stage within the gut lumen and later migrate to the ectoperitrophic space, where they multiply, establishing an infection. Progression of the parasite infection in the fly depends on factors inherent to the biology of trypanosomes, tsetse, and the bloodmeal. Flies usually eradicate infection early on with both pre-existing and inducible factors. Parasites, in contrast, respond to these stimuli by undergoing developmental changes, allowing a few to both survive and migrate within the tsetse. Here we discuss parasite and fly factors determining trypanosome colonization of the tsetse, focusing mainly on the midgut.

Tsetse–trypanosome interactions: a matter of life and death

African trypanosomes alternate their life cycles between a vertebrate host and tsetse, *Glossina* spp. (see Glossary). These parasites cause human African trypanosomiasis (HAT, or sleeping sickness) and animal African trypanosomiasis (AAT). In addition to the considerable human morbidity caused by HAT, AAT has an estimated annual cost of $4.75 billion in lost agricultural gross domestic product [1]. The best-studied species, *Trypanosoma brucei* and *Trypanosoma congolense*, first have to establish an infection in the midgut and then migrate to the salivary glands (SGs) or the mouthparts of the fly, respectively, in order to mature into the mammalian infectious form (see Box 1 for a summary of the stages of infection in the tsetse fly). Despite the high incidence of trypanosomiasis in mammals in sub-Saharan Africa, it is puzzling that relatively few tsetse captured in the wild show midgut trypanosome infections, with estimates ranging from 2% to 20% in different tsetse species and sample sites [2,3]. Even within HAT foci the prevalence of *Trypanosoma brucei rhodesiense* in tsetse can be astonishingly low: for example, 0.0064% of individuals of the tsetse species *Glossina swynnertoni* in the Serengeti, Tanzania, were estimated to be infected [3]. In laboratory infection experiments, multiple colonization failures and bottlenecks in parasite survival imply that tsetse infection by trypanosomes is likely to be a strongly contested process. There is a balance between the ability of the tsetse to eliminate the infection and the capacity of the parasite to evade the hostile environment of the fly, physical defenses, and immune response in order to survive and develop. In this review, we discuss the recently discovered factors and the molecular events thought to control the success of *T. brucei* and *T. congolense* infections within the tsetse midgut and their respective maturation to the SGs or mouthparts. Other reviews cover specific aspects of these processes [4–6].

Glossary

Animal African trypanosomiasis (AAT): disease of mammalian livestock caused by infection with African trypanosomes including *T. b. brucei* and *T. congolense*, among others.
Antimicrobial peptides (AMPs): short peptides produced by the host in response to pathogens and parasites, which act directly on parasites to kill them.
Chitin: long chain polymers of β(1,4)-*N*-acetyl glucosamine residues found in many species, including insects.
Eclosion: emergence of a teneral fly from a pupal case.
Ectoperitrophic space (ES): the space between the peritrophic matrix and gut epithelium in the insect midgut.
Epimastigote: form of trypanosome in which the kinetoplast is anterior to the nucleus, found in both the PV and SGs of infected tsetse. Examples mentioned in the text include short epimastigotes (SEs), long epimastigotes (LEs), and attached epimastigotes.
Extrachromosomal susceptibility factor: a heritable factor determining the susceptibility of tsetse to trypanosome infection that is not present on the chromosomal DNA of the tsetse, instead showing cytoplasmic inheritance. Classical examples of extrachromosomal heritable factors include organelles such as mitochondria and maternally inherited bacteria.
Fat body: fat storing cells in the fly that store and secrete metabolites into the hemolymph.
Glutamic acid/alanine-rich protein (GARP): abundant glycoprotein expressed on the surface of epimastigote *T. congolense*.
Glycosaminoglycans (GAGs): large unbranched polysaccharides made of repeating disaccharide units (*N*-acetylhexosamine and uronic acid).
Glycosylphosphatidylinositol (GPI): conserved glycolipid particularly abundant on the plasma membrane of trypanosomatid parasites. In trypanosomes, it can occur as free or anchoring surface proteins (e.g., variant surface glycoproteins, VSGs).
Human African trypanosomiasis (HAT): human disease caused by infection with *T. b. rhodesiense* or *Trypanosoma brucei gambiense*.
Kinetoplast: a DNA network of kinetoplastids (including trypanosomes) containing the mitochondrial genome. The kinetoplast, also known as kDNA, is found adjacent to the basal body of the flagellum.
Long slender (LS): replicative *T. brucei* bloodstream trypomastigote form in which the kinetoplast is posterior to the nucleus and undergoes antigenic variation in the mammal. LS cannot transform to the PF in the tsetse midgut.
Maturation index (MI): the proportion of midgut infections, which go on to generate infected SGs (*T. brucei*) or mouthparts (*T. congolense*).
Metacyclogenesis: process by which trypanosomes transform into mammalian infective, metacyclic trypomastigotes.
Midgut: the middle portion of the tsetse gut, located between the foregut and hindgut, and lined with the PM. Digestion and absorption of the bloodmeal occur in the midgut, and it is in the midgut that both *T. brucei* and *T. congolense* first establish infection in the fly.

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Monomorphic trypanosomes: bloodstream form (BSF) trypanosomes which do not appear to show morphological differences correlated with their ability to transform to the PF, that is, lacking separate ST and LS forms.
***N*-Acetyl glucosamine (GlcNAc) and glycosamine (GlcN)**: amino sugars differing by the presence of an acetyl group on the nitrogen-containing side chain.
Peptidoglycan recognition protein (PGRP): protein used in the innate immune response of insects to recognize surface peptidoglycans of pathogens and parasites.
Peritrophic matrix (PM): a sheath of acellular matrix separating the midgut lumen of some insects, including tsetse, from the gut epithelium. There are two types of insect PMs: type I, in which the PM is secreted directly from gut epithelial cells, sometimes in response to feeding, and type II (e.g., *Glossina* spp.), which continually secretes the PM from specialized cells in the PV, anterior to the midgut.
Peritrophins: proteins of the PM characterized by containing cysteine-rich CBDs, and usually modified with both *O*-linked and *N*-linked glycans.
Procyclic form (PF): trypanosome trypomastigote form in which the kinetoplast is posterior to the nucleus and which colonizes both the midgut and PV (as late procyclic) of infected flies.
Procyclins: GPI-anchored acidic glycoproteins expressed abundantly on the surface of procyclic trypanosomes of *T. brucei* and other insect stages of *T. congolense*.
Proventriculus (PV): portion of the gut at the foregut midgut boundary and connected to the crop, resembling a mushroom-shaped bulge. In tsetse, the PV may also act as a sphincter and contains specialized enlarged epithelial cells that secrete the PM. It may also be referred to as the cardia.
Sialic acids (SAs): negatively charged sugar residues widely distributed in nature. In trypanosomes, SAs can be found attached to surface GPIs and are essential for survival in the fly.
Short stumpy (ST): cell-cycle arrested *T. brucei* bloodstream trypomastigote form, which is preadapted for transformation to the PF in the tsetse midgut.
Teneral: young, immature adult fly that has not yet received a bloodmeal.
***Trans*-sialidase (TS)**: enzyme expressed by all African trypanosomes and *Trypanosoma cruzi*, and used to transfer host SAs onto trypanosome surface molecules.
Tricarboxylic acid (TCA) cycle: aerobic metabolic cycle used by procyclic but not bloodstream trypanosomes to release energy from amino acids.
Trypomastigote: form of trypanosome, including bloodstream, metacyclic, and procyclic stages, in which the kinetoplast is posterior to the nucleus.
Tsetse: obligate blood feeding viviparous flies of the genus *Glossina* in the superfamily Hippoboscidae. Found only in sub-Saharan Africa, tsetse are the only known cyclical vectors of the African trypanosomes *T. b. brucei* and *T. congolense*. On the basis of morphology, habitat, and phylogeny, *Glossina* has been divided into three sub-genera, here referred to as the morsitans, palpalis, and fusca groups. The best-studied laboratory species, *G. morsitans*, is in the morsitans group.
Variant surface glycoprotein (VSG): abundant GPI-anchored proteins, tightly packed on the surface of bloodstream and metacyclic trypanosomes, and responsible for antigenic variation in LS forms.

Trypanosome infection and variation between fly sexes and species

Susceptibility to *Trypanosoma brucei brucei* midgut infections and the maturation index (MI) vary between tsetse species. For example *Glossina morsitans morsitans* has a relatively weak barrier to infection with *T. brucei* strain J10, with 11.3% of flies becoming infected at age 24–48 h, but with a MI below 20%. By contrast, *Glossina pallidipes* has a huge barrier to midgut establishment, with only 1.3% of flies developing midgut infections under the same conditions, but a MI as high as 88% [7].

T. congolense susceptible and refractory lines selected in *G. m. morsitans* [8] and *Glossina morsitans centralis* [9] demonstrate that refractoriness is not an all-or-nothing phenotype; rather it affects the proportion of flies infected. Midgut susceptibility to *T. congolense* was maternally inherited, suggesting an extrachromosomal susceptibility factor. The refractory lines were more refractory to other trypanosome strains and species, including *Trypanosoma vivax*, which develops outside the midgut, suggesting a general effect of the factor on fly immunity [9,10].

The extrachromosomal susceptibility factor might be the genotype or density of rickettsia-like organisms (RLOs), later identified as *Sodalis glossinidius* [5]. In regions where *Sodalis* is variably present in the field, trypanosome-infected flies are more likely to harbor *Sodalis* than uninfected flies [11]. Consistently, selective elimination of *Sodalis* using streptozotocin (which did not kill the obligate tsetse symbiont *Wigglesworthia glossinidia*) led to almost 40% reduction in susceptibility to midgut infection in the progeny of treated flies [12]. Therefore, *Sodalis* appears not to be essential for trypanosome midgut infection, but increases the proportion of susceptible flies. How *Sodalis* influences vector competence remains to be elucidated.

The possible involvement of heritable factors has also been considered for the MI. The higher *T. brucei* MI in males led to the postulation that maturation is under the control of one or more non-dosage-compensated X-linked loci [7]. However, it is uncertain if tsetse have non-dosage-compensated X-linked genes [13], a question that should soon be answered using the genome and transcriptome data becoming available. Unlike *T. brucei*, *T. congolense* metacyclogenesis occurs in the proboscis of *G. morsitans* independently of fly sex [14], suggesting that independent mechanisms may determine *T. congolense* and *T. brucei* maturation. For *T. brucei*, the MI is higher for faster maturing strains [14], suggesting a limited time window for successful maturation after midgut infection. The mechanism governing this is still poorly understood: despite the increasing information available on trypanosome gene expression during different life stages in the fly [15], to date the only known *T. brucei* gene required specifically for maturation of infections (but not for midgut colonization) is *PSSA-2*, which encodes for a transmembrane glycoprotein of unknown function [16].

Variation between trypanosome species and strains

Tsetse transmissibility varies not only between trypanosome species but also between strains. Human pathogenic *T. b. rhodesiense* strains show lower MI in *G. m. morsitans* than closely related *T. b. brucei* strains [17]. *T. congolense* strains with a high virulence in mice showed higher tsetse midgut infection rates than strains with moderate or low virulence [18]. Isogenic clones of *T. congolense* differing only in the mutations underlying resistance to isometamidium chloride differed in the midgut infection rate in *G. m. morsitans*, with the highly resistant strain giving a higher infection rate [19]. *T. congolense* is believed to be monomorphic [lacking separate long slender and short stumpy (ST) forms], thus no variation in infectivity within a strain would be expected. However, significantly higher rates of tsetse midgut infection establishment have been observed in the acute than in the chronic phase of infection in mice, independent of parasitemia level [20]. Differences in the MI have also been observed between flies fed on infected mouse blood from days 4 to 10 post-mouse infection, also independent of parasitemia level [21]. These results suggest that either changes may occur in *T. congolense* itself during the mammalian infection or that the *T. congolense* infection causes changes in the blood that then impact on *T. congolense* midgut colonization.