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The transcriptional signatures of *Sodalis glossinidius* in the *Glossina palpalis gambiensis* flies negative for *Trypanosoma brucei gambiense* contrast with those of this symbiont in tsetse flies positive for the parasite: Possible involvement of a *Sodalis*-hosted prophage in fly *Trypanosoma* refractoriness?

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ABSTRACT

Tsetse flies, such as *Glossina palpalis gambiensis*, are blood-feeding insects that could be subverted as hosts of the parasite *Trypanosoma brucei gambiense*: initiated in the tsetse fly mid gut, the developmental program of this parasite further proceeds in the salivary glands. The flies act as vectors of this human-invasive parasite when their salivary glands sustain the generation of metacyclic trypomastigotes, the exclusive morphotypes pre-programmed to further develop in the human individuals. Briefly, once the metacyclic trypomastigotes have been deposited in the skin of humans from whom the parasite-hosting tsetse flies are taking their blood meals, the complex developmental program of this *Trypanosoma brucei* subspecies can result in a severe disease named sleeping sickness. Unveiling the processes that could prevent, in tsetse flies, the developmental program of *T. b. gambiense* could contribute reducing the prevalence of the disease.

Using a global approach, we were curious to extract transcriptional signatures of *Sodalis glossinidius*, a symbiont hosted by three distinct groups of tsetse flies. To meet this objective, the transcriptome of *S. glossinidius* from susceptible and refractory tsetse flies was analyzed at 3, 10 and 20 days after flies blood feed on *T. b. gambiense*-hosting mice. Within this temporal window, 176 trypanosome responsive genes were shown to interact in well-defined patterns making it possible to distinguish flies susceptible to the parasite infection from refractory flies. Among the modulated transcripts in the symbiont population of flies refractory to trypanosome infection many were shown to cluster within the following networks: "lysozyme activity, bacteriolytic enzyme, bacterial cytolysis, cell wall macromolecule catabolic process". These novel data are further delineated in the following questions: could the activation of prophage hosted by *S. glossinidius* lead to the release of bacterial agonists that trigger the tsetse fly immune system along a profile that no more allows the parasite developmental program?

1. Introduction

African trypanosomes, which cause human African trypanosomiasis (HAT) (sleeping sickness), are a challenge to global public health: 60 million people are estimated to be at risk of the disease (WHO, 2006). Tsetse flies are medically important vectors because they transmit African trypanosomes. Two of the subspecies, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, are responsible, respectively, for the chronic form of HAT in Western and Central Africa, and for the acute form of the disease in East Africa. Unfortunately, the drugs available to fight the disease remain very limited. To date, no vaccines have been developed for HAT, therapeutic treatments have serious side effects, and diagnostic tools are inadequate (Simarro et al., 2008). Thus, tsetse fly community control remains one of the only viable strategies for

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disease prevention, especially the reduction of tsetse fly populations which has been shown to be efficient in disease control (Simarro et al., 2008). However, although effective, such implementation in remote regions of Africa where the disease is endemic is difficult and expensive and relies on extensive community participation; thus it has not been widely used for human disease control (Leak et al., 1996). Mathematical models indicate that parasite infection prevalence in the tsetse host is a crucial parameter in HAT epidemiology and disease dynamics (Davis et al., 2010). Reducing the parasite transmission ability of flies could be an alternative approach in preventing disease emergence. Trypanosome transmission is totally dependent on: (1) the availability of a tsetse fly that will be susceptible to trypanosome infection following a blood feeding on an infected host (Van Den Abbeele et al., 1999), (2) the successful completion of the parasite life cycle in that infected fly (Sbicego et al., 1999; Van Den Abbeele et al., 1999) to produce the metacyclic form of the parasite, the only one that is infectious for humans and animals, and (3) the uptake of a novel blood meal by a fly that may then transmit the parasite to a new host. Consequently, critical stages include invasion of the midgut, establishment of infection and multiplication of the trypanosome in the midgut, and trypanosome maturation in the salivary glands (Vickerman et al., 1988; Maudlin and Welburn, 1994; Van Den Abbeele et al., 1999). This ability to acquire the parasite, favor its maturation, and transmit it to a mammalian host is called vector competence.

Understanding the basic mechanisms of how the tsetse fly successfully transmits trypanosomes is the first requirement in designing novel genetic control strategies. Molecular and cellular interactions occur during the tsetse fly infection by the parasite, which may offer opportunities for the development of fly-based molecular strategies to interrupt sleeping sickness transmission.

Natural populations of tsetse flies show extensive genetic diversity which may account for varying degrees of susceptibility to trypanosomes, since most flies are not infected (Frézil and Cuisance, 1994; Maudlin and Welburn, 1994). The physiological mechanisms that are involved in the fly's vector competence to trypanosomes are not well understood, and the genes that control these mechanisms remain unknown.

In addition to the parasite, tsetse flies may harbor three different symbiotic micro-organisms (Aksoy, 2000). Among them, *Sodalis glossinidius* (Cheng and Aksoy, 1999; Dale and Maudlin, 1999), a maternally transmitted secondary endosymbiont, was suspected of favoring the establishment of the parasite in the insect midgut through a complex biochemical mechanism (Maudlin and Ellis, 1985; Welburn and Maudlin, 1999; Dale and Welburn, 2001). In previous studies, we have shown that *Glossina palpalis gambiensis* (*palpalis* group) and *Glossina morsitans morsitans* (*morsitans* group) harbor genetically distinct populations of *S. glossinidius* (Geiger et al., 2005a), suggesting that fly vector competence might be linked to given genotypes of *S. glossinidius*. We also demonstrated that the ability of *T. b. gambiense* and *Trypanosoma brucei brucei* to establish in the *G. p. gambiensis* insect midgut was statistically linked to the presence of *S. glossinidius*-specific genotypes (Geiger et al., 2007). Finally, epidemiological studies conducted in two historical HAT foci in Cameroon validated the association between the presence of *S. glossinidius* and trypanosome infection (Farikou et al., 2010).

Since 2005, advances in *S. glossinidius* genomics (Darby et al., 2005; Toh et al., 2006) opened ways to further investigate the tripartite "blood-feeding *G. p. gambiensis*–*S. glossinidius*–*Trypanosoma* spp." interactions within the most demanding experimental conditions set up in our team. Using a global approach, we did compare transcriptional signatures of *S. glossinidius*, hosted by *G. p. gambiensis* colonies. The *G. p. gambiensis* blood-feeding flies were allowed to blood feed on either parasite-free mice or on mice hosting *T. b. gambiense*. From the latter flies, the anal drops were sampled at days 3, 10, 20 post the blood meal ingestion and further processed to detect parasite DNA. Two tsetse fly sub-groups were distinguished within this fly group (a) a sub-group of flies the anal drop of which were parasite positive and (b) a sub-group of flies the anal drop of which remains parasite negative. Within the temporal window that ended at day 20 post the blood meal, in contrast to the *S. glossinidius* hosted by the parasite positive tsetse flies, the symbiont population hosted by the parasite negative tsetse flies, display unique transcriptional signatures we were able to carefully extract and characterize. These novel data are discussed within the context of serial questions that will be further addressed in other experimental settings:

(1) could a *S. glossinidius* prophage be activated in tsetse flies the blood meal of which is loaded with *T. brucei* spp. unbalanced population – e.g., a parasite population where the slender parasite morphotypes dominate over the parasite stumpy morphotypes,
(2) could this activation lead to the release of *S. glossinidius* agonists that trigger the tsetse fly immune system along a profile that no more allow the developmental program of too rare parasite stumpy morphotypes to proceed?

2. Materials and methods

2.1. Ethical statement

All the experiments on animals reported in this article were conducted according to internationally recognized guidelines; the experimental protocols were approved by the Ethics Committee on Animal Experiments and the Veterinary Department of the Centre International de Recherche Agronomique pour le Développement (CIRAD), Montpellier-France.

2.2. Glossina species and trypanosome strains

G. p. gambiensis flies are progenies of field flies collected in different areas of Burkina Faso. Pupae were collected from these flies. Following adult emergence, the population was maintained in a level 2 containment insectary at 23 °C and 80% relative humidity (Geiger et al., 2005b) without any selection. Individuals used in the present work were randomly chosen for experimental infection. *T. b. gambiense* S7/2/2 was isolated in 2002 by rodent inoculation with blood from a HAT patient diagnosed in the sleeping sickness focus of Bonon, Côte d'Ivoire (Ravel et al., 2006). Cryostabilate of S7/2/2 was produced in the field and sent to our laboratory.

2.3. Anal drop and mid gut sampling procedures respectively from live and killed tsetse flies

A set of 100 randomly chosen *G. p. gambiensis* flies were fed on a noninfected blood meal, and a second set of 900 flies were fed on trypanosome-infected mice (Fig. 1). Midguts of the flies from the first set were recovered 3 days after feeding. Four biological replicates of seven randomly sampled noninfected flies was constituted and dissected to recover their midguts. The biological replicate samples were called, respectively, "NS 3 day sample 6, 8, 9, and 10" (NS for "nonstimulated" flies, since they had received a noninfected blood meal and were dissected 3 days post-blood feeding).

For the second set of flies, the experimental infection procedure was the following: a stabilate of *T. b. gambiense* S7/2/2 was thawed at room temperature and 0.2 ml was injected intraperitoneally into balb/cj mice as previously described (Geiger et al., 2007). The parasitemia was monitored by examining tail blood using a phase-contrast microscope at ×400 magnification. Teneral flies were fed