Data in Brief

Technical data of the transcriptomic analysis performed on tsetse fly symbionts, *Sodalis glossinidius* and *Wigglesworthia glossinidia*, harbored, respectively by non-infected, *Trypanosoma brucei gambiense* infected and self-cured *Glossina palpalis gambiensis* tsetse flies

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A B S T R A C T

Microarrays are a powerful and cheap method to identify and quantify gene expression in particular in a mix of total RNA extracted from biological samples such as the tsetse fly gut, including several organisms (here, the fly tissue and the intestinal microorganisms). Besides, biostatistics and bioinformatics allow comparing the transcriptomes from samples collected from differently treated flies, and thus to identify and quantify differential expressed genes. Here, we describe in details a whole microarray transcriptome dataset produced from tsetse flies symbionts, *Sodalis glossinidius* and *Wigglesworthia glossinidia*. The tsetse fly midguts were sampled at key steps of tsetse fly infection by trypanosomes, 3-day and 10-day sampling times to target differentially expressed genes involved, respectively, in early events associated with trypanosome entry into the midgut and with the establishment of infection; 20 days to target the genes involved in events occurring later in the infection process. We describe in detail the methodology applied for analyzing the microarray data including differential expression as well as functional annotation of the identified symbiont genes. Both the microarray data and design are available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48360; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48361; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55931.

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Direct link to deposited data


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Experimental design, materials and methods

Experimental design

Tsetse flies of the sub-species Glossina palpalis gambiensis were infected by trypanosomes of the sub-species Trypanosoma brucei gambiense. At key steps of flies’ infection, 3, 10, and 20 days, midguts of flies were dissected and total RNA was extracted in order to further analyze the transcriptome of tsetse fly symbionts, Sodalis glossinidius and Wigglesworthia glossinidia. The sampling days are chosen to target events associated respectively, i) with trypanosome entry into the midgut, ii) with the establishment of infection, and iii) with the late stages of the infection process. Fig. 1 shows the general experimental design.

Materials and methods

Experimental infection of G. p. gambiensis by T. b. gambiense

Insectary G. p. gambiensis flies from CIRAD, Montpellier, were T. b. gambiense infected experimentally according to the protocol reported by Geiger et al. [1] and Hamidou Soumana et al. [2-4]. Stabilate of T. b. gambiense S7/2/2 (isolated in 2002 from a HAT patient diagnosed in the sleeping sickness focus of Bonon, Ivory Coast [5]) was injected intraperitoneally into balb/cj mice. After the parasitemia has reached 15–25 × 10⁷ parasites/ml, teneral flies were fed on these infected mice. This group of flies was then separated into three sub-groups a, b, and c. Three days after feeding, four biological replicates, each of the seven flies, were randomly selected from the sub-group a; they were noticed “S3 for seven flies, and c. Three days after feeding, four biological replicates, each of the seven flies, were randomly selected from the sub-group a; they were noticed “S3 for “stimulated-sampled at day3”.” Ten days after feeding, the flies of the sub-group b were tested for the presence/absence of trypanosomes in their anal drop and separated into two “sub-sub-groups”, one noticed “I10” (flies fed on infected mice and that were shown to be infected, sampled at Day 10 post-feeding), the second noticed “NI10” (flies fed on infected mice and that were shown to be non-infected (= refractory flies), sampled at Day 10 post-feeding). Twenty days after feeding the sub-group c was processed as was the sub-group b; the corresponding “sub-sub-groups” were noticed “I20” and “NI20”. From each “S3”, “I20”, and “NI20”, 4 biological replicates were constituted each of 7 flies randomly sampled. For I10 and NI10: 4 replicates were constituted of 3 flies because of the low infection prevalence. Finally, a group of flies was fed on non-infected mice, of which four replicates, each of 7 flies, were constituted, three days after feeding, and noticed “NS3” (for non-stimulated = control flies).

Fly infection monitoring process

As mentioned, flies fed on infected mice and sampled at Day 10 and Day 20 were controlled for the presence or absence of trypanosomes in their anal drops. This was performed on chelex-extracted DNA [6] from the anal drops and the presence of trypanosomes was assessed by PCR using TBR1 and TBR2 primers [7]. When anal drops were PCR positive for the presence of trypanosomes, it indicates midgut infections. When PCR tests were negative, flies had self-cured the infection.

RNA extraction

Flies from the different biological repeats (from “S3”, “NS3”,...) were then dissected separately and the midguts were collected in RNA latter (Ambion) for further RNA extraction.

RNA was extracted from the midguts of each biological replicate using TRizol reagent (Gibco-BRL, France). High quality of RNA sample was checked on an Agilent RNA 6000 Bioanalyzer and the RNA quantification was performed using the corresponding Nano kit (Agilent Technologies, France).

Fig. 1. General experiment design. Midgut of G. p. gambiensis was sampled at three times post-T. b. gambiense infected bloodmeal: 3, 10, and 20 days. For each time points, 4 biological replicates of seven or three (for the I10 and NI10 samples only) midguts were constituted and further analyzed for Sodalis or Wigglesworthia transcriptome. Total RNA was produced from each biological replicate, and reverse transcribed into cDNA that was then labeled and hybridized onto Sodalis or Wigglesworthia custom-made microarrays. Genes differentially expressed between the different conditions were further analyzed and annotated.
Custom-made 60-mers oligonucleotide microarrays

The tsetse fly symbiont custom-made density arrays (8 × 15 K format) were designed with 60-mer oligos specific to:

* For *Sodalis* [2,3]; genes of the *S. glossinidius* chromosome (NCBI RefSeq: NC_007712.1; GenBank accession number AP008232), and genes of the *Sodalis* four plasmids pSG1 (NCBI RefSeq:NC_007183.1), pSG2 (NCBI RefSeq: NC_007184.1), pSG3 (NCBI RefSeq: NC_007186.1), and pSG4 (NCBI RefSeq: NC_007187.1) [8,9]. Unique probe designs were chosen for each gene.

* For *Wigglesworthia* [4]; genes of the *W. glossinidia* chromosome (from *Glossina morsitans morsitans*) (NCBI Reference Sequence: NC_016893.1) [10]. Ten different probes were used for each gene.

To avoid cross-hybridization with non-target genes, for *Sodalis* and *Wigglesworthia* custom-microarrays, probes were selected only when they correspond to unique sequences.

The details of the *Sodalis* and *Wigglesworthia* array design, sample description, and expression data are available at Gene Expression Omnibus (GEO) under accession numbers respectively, GPL17347 and GSE48361 for *Sodalis*:


Preparation of cDNA and hybridization on *Sodalis* and *Wigglesworthia* custom-microarray

Microarray experiments were performed at the TAGC core facility (http://tagc.univ-mrs.fr/) for *Sodalis*, and at Hybrigenics platform (Clermont-Ferrand, France) for *Wigglesworthia*.

*Sodalis* cDNA labeling with Cy3 dCTP was done with 5 μg of total RNA using the ChipShot direct labeling and clean-up system kit (Promega). Samples were then hybridized onto the *Sodalis* custom-microarrays made from *S. glossinidius* Genome. Labeling of *Wigglesworthia* cDNA was performed with Cy3 dCTP and 100 ng of total RNA using the Low Input Quick Amp Labeling Kit One-Color (Agilent Technologies, France). cDNA samples were then hybridized onto the custom-microarrays made from *W. glossinidia* genome.

Hybridization was performed, for both custom-microarrays types, at 65 °C for 17 h at 60 rpm.

Microarray data analyses

Lowest normalization was used for within-array normalization. Quantile normalization was used to make the density distributions similar across arrays [11]. Only one expression value was then assigned to each biological replicate by averaging the normalized expression values through Cy3 signal intensities. The pictures of microarray data were selected for further analyses. In the case of *Sodalis* transcriptome analyses, statistics was performed using the TIGR MeV (MultiExperiment Viewer) v4.5 software (http://www.tm4.org/mev.html). A two-way ANOVA was formed to check the significance of the PCR reaction and to verify the amplification efficiency. Relative quantification was calculated with the 2−ΔΔCt method as described by Livak and Schmittgen [21].

Conflict of interest statement

There is no conflict of interest with respect to funding or any other issue.

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References


[2] I. Hamidou Soumana, et al., The transcriptional signatures of *Sodalis glossinidius* in the Glossina palpalis gambiensis flies negative for *Trypanosoma brucei gambienne*


