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Molecular & Biochemical Parasitology 139 (2005) 163–172
MOLECULAR & BIOCHEMICAL PARASITOLOGY

The transcriptomes of *Trypanosoma brucei* Lister 427 and TREU927 bloodstream and procyclic trypomastigotes

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Received 2 September 2004; received in revised form 27 October 2004; accepted 3 November 2004
Available online 28 November 2004

Abstract

We describe developmentally regulated genes in two strains of *Trypanosoma brucei*: the monomorphic strain Lister 427 and the pleomorphic strain TREU927. Expression patterns were obtained using an array of 24,567 genomic fragments. Probes were prepared from bloodstream-form or procyclic-form trypanosomes. Fourteen procyclic-specific and 77 bloodstream-specific signals were obtained from sequences matching variant surface glycoprotein or associated genes, and a further 17 regulated sequences were repetitive or transposable-element-related. Two hundred and eighty-six regulated spots corresponded to mRNAs from other protein-coding genes; these spots represent 191 different proteins. Regulation of 113 different genes (79 from procyclic forms, 34 from bloodstream-forms) was supported by at least two independent experiments or criteria; of these, about 60 were novel. Only two genes – encoding HSP83 and an importin-related protein – appeared to be regulated in the TREU927 strain only. Our results confirmed previous estimates that 2% of trypanosome genes show developmental regulation at the mRNA level.

Keywords: Trypanosoma; Differentiation; Transcript; Regulation; Array

1. Introduction

The African trypanosome *Trypanosoma brucei* has two major replicative stages: the bloodstream-form, which grows in the blood and tissue fluids of the mammalian host, and the procyclic form found in the midgut of the Tsetse fly vector [1]. The two environments differ in temperature and nutrient availability: the abundance of glucose in the mammalian tissue fluids enables the parasites to synthesise ATP exclusively by glycolysis, whereas in the Tsetse fly amino acids are the principal energy source and mitochondrial metabolism is highly developed [2]. Host defences also differ: in the mammal, African trypanosomes evade the adaptive immune response by variation of the major surface protein, the variant surface glycoprotein (VSG), whereas in the insect, alternative surface proteins, the EP and GPEET procyclins, serve as a barrier against proteases and other invertebrate defences [3].

The control of gene expression in kinetoplastids is primarily post-transcriptional [4]. Expression of quite a large number of genes has been shown to be controlled at the levels of mRNA stability and translation, and nearly all the regulatory sequences investigated so far were mapped to the 3′-untranslated regions (3′-UTR) of the mature mRNAs [4]. We and others have previously suggested that specific regulatory signals in the 3′-UTRs could act as a code for particular regulatory patterns. Interactions of such sequences with specific protein factors could signal mRNA stabilisation or degradation or influence the translation efficiency. The existence of 3′-UTR signals would allow coordinate post-transcriptional regulation of specific sets of transcripts during the life cycle,

Abbreviations: ESAG, expression-site-associated gene; VSG, variant surface glycoprotein; 3′-UTR, 3′-untranslated region

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doi:10.1016/j.molbiopara.2004.11.004

rather as specific promoter elements allow coordinate regulation of transcription in other organisms. The most dramatic example of this so far is a 450-nt element found in the 3′-untranslated region (3′-UTR) of a large number of *Leishmania* mRNAs: this element enhances translation in the amastigote stage of the parasite [5]. In bloodstream-form *T. brucei*, a U-rich element depresses translation and stimulates rapid degradation of three otherwise unrelated mRNAs [6].

Genome-wide surveys will help us to understand the host-parasite relationship and the metabolic adaptations occurring in different life-cycle stages, and will also facilitate the identification of elements responsible for post-transcriptional regulation. We previously described the results of a limited survey of regulated trypanosome mRNAs. Genomic arrays were constructed using 21,000 independent random genomic clones from *T. brucei* TREU927. The arrays were hybridised with oligo d(T)-primed labelled cDNAs, generated using total bloodstream-form or procyclic-form RNA as the template. We found that between 1 and 2% of spots on the array showed regulated mRNA abundance, and described the sequences of 60 clones [7].

T. brucei TREU 927 was adapted relatively recently to in vitro culture and can be transmitted readily by Tsetse [8]; it also shows limited resistance to human serum [9]. We were therefore interested in comparing its gene regulation with that of strain Lister 427, which has been subjected to in vitro culture or animal passage for over 30 years and is a standard strain for genetic manipulation [10]. Since comparisons of mRNAs do not detect translational regulation, we were also interested to find out whether additional information could be obtained from a polysomal RNA fraction.

2. Methods

2.1. Microarray analysis and Northern blotting

Microarray construction and hybridisation conditions have been previously described [7]. Briefly, independent random genomic clones from *T. brucei* 927 were used to create PCR products which were spotted onto glass slides. Seventy-five percent of insert sizes were within 1.8–2.5 kb. In the original experiments with the 927 strain RNA, 21,024 PCR products were used [7]. In the new experiments reported here, with 427 RNA, the arrays contained 24,567 spots. This array would be expected to cover at least 80% of the trypanosome genome if all fragments were present at equal probability.

Lister 427 procyclic- and bloodstream-form trypanosomes were harvested in the exponential phase of growth, and we prepared both total RNA and RNA from a 75,000 × *g* pellet. The RNA pellet fraction was made as follows, in a protocol adapted from [11]. After addition of cycloheximide (100 μg^{-1}), which freezes translating ribosomes on the mRNA, cells were collected by centrifugation at 2000 × *g* for 5 min at 4 °C and washed twice in ice-cold polysome buffer (120 mM NaCl, 20 mM Tris–HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM DTT, 10 $\mu g\,ml^{-1}$ leupeptin, 100 μg^{-1} cycloheximide). Cells were then resuspended in 0.5 ml of polysome buffer and lysed by the addition of NP-40. The cell suspension was homogenized and the lysate was cleared by centrifugation at 10,000 × *g* for 4 min at 4 °C. The supernatant was centrifuged again at 75,000 × *g* for 35 min at 4 °C in an ultracentrifuge. The pellet was resuspended in 1 ml Trizol per 10^6 cells and RNA was extracted following the manufacturer's protocol. Examination of the RNA after this procedure revealed that nearly all of the ribosomal RNA was recovered in the pellet fraction.

Fluorescent, oligo d(T)-primed cDNAs were prepared from bloodstream and procyclic-forms, mixed, and hybridised to the slides as before [7]. Exchanging of the dyes resulted in no significant differences. For total RNA, we obtained six slides giving a strong signal-to background ratio and for the pelleted RNA, four slides were used. Results were analysed using MCHIPS [12,13]. All ratios were recorded. To choose regulated clones for sequence analysis we selected only those with the following parameters—fitted intensities: at least 200,000; ratio: 2.5; min/max-separation: 0.2. All clones satisfying these criteria were sent for sequencing from one end.

For 73 of the regulated clones, a PCR product (pUC plasmids, M13 forward and reverse primers) of the entire insert was gel purified, labelled with ^{32}P dCTP (Amersham, Braunschweig) by random priming (Prime It II, Stratagene) and hybridised to Northern blots of total RNA. 15 μg per lane total RNA from bloodstream (cell culture densities from 5×10^5 to 1×10^6 cells ml^{-1}) and procyclic (cell culture densities from (2 to 4) × 10^6 cells ml^{-1}) was subjected to electrophoresis on formaldehyde gels and blotted onto Nytran membranes. Hybridization to the signal recognition particle (SRP) RNA [14] was used as a loading control. Blots were exposed to phosphorimaging plates for 48–72 h. Signals were measured using a phosphorimager and MacBas v2.0 software. Hybridisations were done only once unless otherwise stated.

2.2. Annotation

From the sequences obtained we identified all RNAs which would hybridise with the corresponding array spot. Prior to completion of the genome the sequences were used to search both GeneDB and the EMBL databases; some of the sequences which clearly matched *VSG*, *ESAG*s, repetitive elements or ribosomal RNA were not further analysed. Upon genome completion, the remaining sequences were used to search the trypanosome genome (mainly release no. 3) in GeneDB using the blastn algorithm. Matches of 96% or more were assumed to be identical. The sequence of the entire cloned fragment was then deduced, assuming a size of 2 kb unless the insert size was known, and predicted RNAs overlapping by at least 100 bp were annotated. To assign the likely position of polyadenylation sites, we estimated a distance of 200 bp between the polyadenylation site and the next downstream open reading frame (Benz et al., manuscript in