Supporting Information

Kassem et al. 10.1073/pnas.1404873111

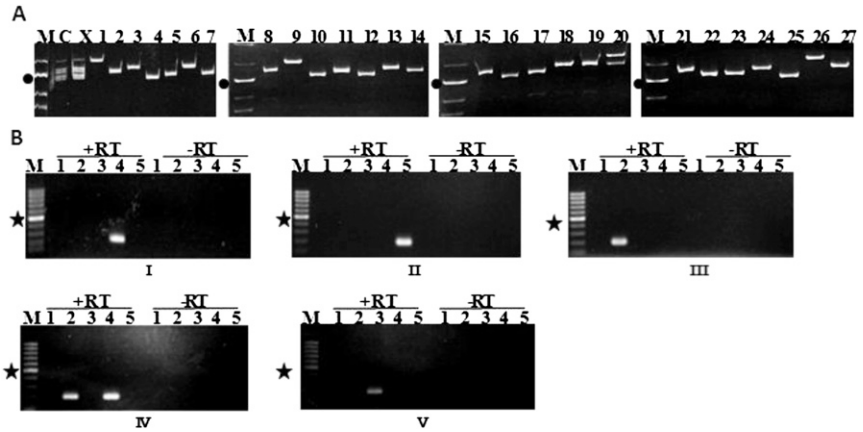

Fig. S1. Analysis of PCR and RT-PCR products by polyacrylamide and agarose gel electrophoresis. (*A*) PCR analysis of cloned populations. 328-114 bloodstream form (BF) trypanosomes were separately transfected with 10 different P2T7 plasmids containing different inserts. Equal numbers of trypanosomes selected from the 10 transfections were pooled, cultured for 10 d, and then subjected to a limiting dilution procedure to generate cloned populations. Genomic DNA was extracted from the control (containing a mix of the 10 transfected trypanosomes cell lines) (C) and from 27 different clones (1–27), subjected to PCR analysis using primers 1 and 2. The PCR product was analyzed by PAGE. X, mixture of the PCR products of the 10 different transfected trypanosomes cell lines. M, 1-kb DNA ladder. ● indicates the 1.5-kb marker. (*B*) Single-cell RT-PCR analysis. After limiting dilution of 328-114 BF or procyclic form (PF) trypanosomes to reach a concentration of one trypanosome per three tubes, RNA was extracted immediately from five tubes and subjected to RT-PCR in the presence (+RT) or absence (−RT) of RT. RT-PCR products were analyzed by agarose gel electrophoresis. Gels I– III and IV correspond to the first 149 bp of the expression site (ES) (PES1 PCR product) from BF and PF trypanosomes, respectively; gel V corresponds to the RT-PCR product of *ESAG6/7* amplified using primer pair 3 and 4. M, 100-bp ladder. ★ indicates the 500-bp marker.

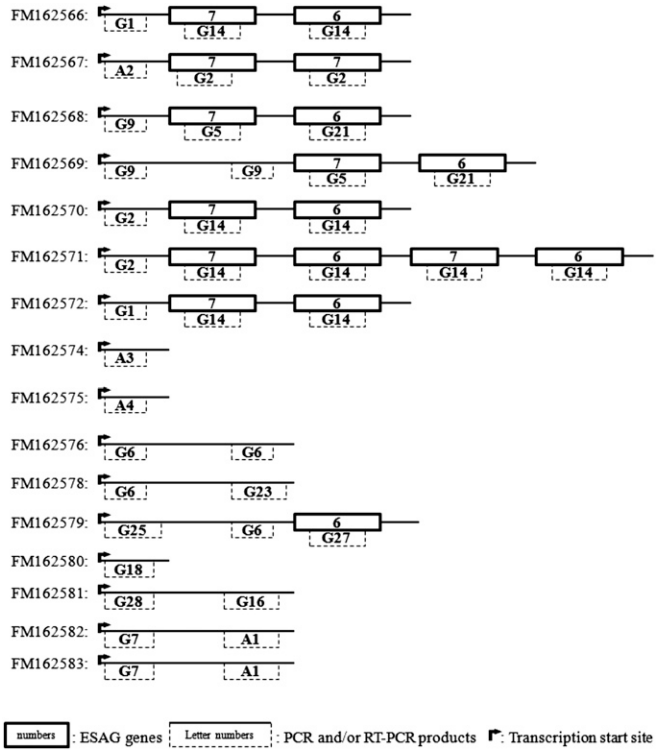

Fig. S2. Correspondence between names given to the ESs analyzed and sequenced by Hertz-Fowler et al. (1) and DNA fragments or RNA transcripts analyzed by PCR or RT-PCR by Vanhamme et al. (2) and in this paper. The beginning of the ESs identified and named (names on the left) by Hertz-Fowler et al. (1) is drawn, and the positions of the (RT)-PCR products sequenced by Vanhamme et al. (2) and in this paper are indicated in the boxes below the ES drawings. Not drawn to scale.

1. Hertz-Fowler C, et al. (2008) Telomeric expression sites are highly conserved in Trypanosoma brucei. *PLoS ONE* 3(10):e3527.
2. Vanhamme L, et al. (2000) Differential RNA elongation controls the variant surface glycoprotein gene expression sites of Trypanosoma brucei. *Mol Microbiol* 36(2):328–340.

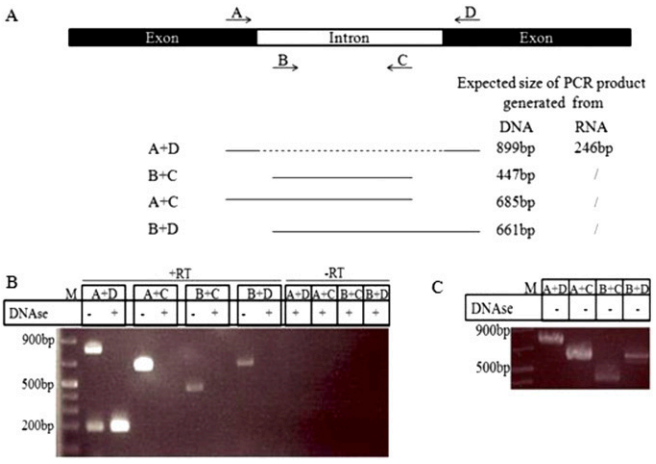

Fig. S3. Analysis of poly(A) polymerase (PAP) RT-PCR products by agarose gel electrophoresis. (*A*) Schematic of the exon–intron junction of the PAP gene. Lettered arrows indicate the PCR primers. Lines drawn under the gene structure stand for the putative (RT-)PCR products generated with the primer pairs indicated on the left; their expected sizes are shown on the right. The dotted line indicates the part of the PCR product removed after splicing. Not drawn to scale. (*B*) RT-PCR analysis. After limiting dilution of 328-114 BF trypanosomes, RNA was extracted immediately and subjected to RT-PCR analysis after (+) or without (–) DNase treatment with (+RT) or without (–RT) RT, using the indicated PCR primer pairs. RT-PCR products were analyzed by agarose gel electrophoresis. M, size markers. (*C*) PCR analysis. DNA was extracted and subjected to PCR analysis using the indicated PCR primer pairs. PCR products were analyzed by agarose gel electrophoresis. M, size markers.

Table S1. Analysis of PCR and RT-PCR products amplified from the beginning of the ES using primer pair 1 and 2 (product PES1 in Fig. 1) and *ESAG 6* and *7* genes using primer pair 3 and 4 (product PES6/7 in Fig. 1)

Sequence name of PES6/7*	No. of PCR products of PES6/7 (genomic population)	No. of RT-PCR products of PES6/7 (single cell)
G14	10	77
G2	2	2
G5	3	—
G27	3	—
r39	2	—
r47	2	—
r59	13	—
A1	5	—
A2	5	—
A3	5	—
A4	3	—
A5	3	—
No. of additional unique sequences	32	11
Total number	88	90

PCR (genomic) and RT-PCR analyses were performed on the indicated materials. The PCR products were cloned, and individual inserts were sequenced. The total number of analyzed sequences is given at the bottom of each column. The number of times each sequence was recovered is indicated. Sequences were generated by PCR on DNA extracted from a 328-114 population and by RT-PCR on RNA extracted from a single cell.
*Source: Vanhamme et al. (1) (sequences beginning with "G" and "r") and this paper (sequences beginning with "A").

1. Vanhamme L, et al. (2000) Differential RNA elongation controls the variant surface glycoprotein gene expression sites of Trypanosoma brucei. *Mol Microbiol* 36(2):328–340.

Table S2. Correspondence between names given to the ESs analyzed and sequenced by Hertz-Fowler et al. (1) and their fragments or RNA transcripts analyzed by PCR or RT-PCR by Vanhamme et al. (2) and in this paper

Sequence name of PES1			Sequence name of PES6/7
Hertz-Fowler et al. (1)	Vanhamme et al. (2)	This paper	Vanhamme et al. (2)
FM162566	G1	—	Duplicated (G14)
FM162567	—	A2	Duplicated (G2)
FM162568	G9	—	G5 and G21
FM162569	Duplicated (G9)	—	G5 and G21
FM162570	G2	—	Duplicated (G14)
FM162571	G2	—	Quadruplicated (G14)
FM162572	G1	—	Duplicated (G14)
FM162574	—	A3	—
FM162575	—	A4	—
FM162576	Duplicated (G6)	—	—
FM162578	G6 and G23	—	—
FM162579	G25 and G6	—	G27
FM162580	G18	—	—
FM162581	G28 and G16	—	—
FM162582	G7	A1	—
FM162583	G7	A1	—

Names designating the ESs in Hertz-Fowler et al. (1) are indicated in the left-most column. The names of PCR products with identical sequences generated in Vanhamme et al. (2) (beginning with "G" in columns 2 and 4) and this paper (beginning with "A" in column 3) are given in the corresponding rows. Because Vanhamme et al. (2) named the products before the ESs were sequenced, PES1 and PES6/7 products carrying the same number often do not originate from a common ES. PES1, PCR products obtained using primer pair 1 and 2 (Fig. 1). PES6/7, PCR products obtained using primer pair 3 and 4 (Fig. 1).

1. Hertz-Fowler C, et al. (2008) Telomeric expression sites are highly conserved in Trypanosoma brucei. *PLoS ONE* 3(10):e3527.
2. Vanhamme L, et al. (2000) Differential RNA elongation controls the variant surface glycoprotein gene expression sites of Trypanosoma brucei. *Mol Microbiol* 36(2):328–340.

Dataset S1. Sequences of inserts cloned in the TOPO vector from the single-cell PES1 PCR and RT-PCR, PES6/7 PCR and RT-PCR, and VSG RT-PCR analyses

Dataset S1

Plasmids were extracted from individual bacterial colonies, and inserts were sequenced.

Dataset S2. Sequences of inserts cloned in the TOPO vector from the hypothetical material coming from PES1 RT-PCR analyses performed without reverse transcriptase and appearing negative by gel electrophoresis analysis

Dataset S2

Plasmids were extracted from 3 × 100 bacterial colonies obtained in three independent experiments giving rise to 89, 81, and 87 inserts, respectively. The 5′ and 3′ TOPO cloning sites are highlighted in light gray. Sequences found to originate from primers 1 or 2 (inserted in either forward or reverse orientation) used for the PCR are underlined. Differences from the expected sequences are in bold and are highlighted in dark gray. Other sequences did not give any significant result when used as query for BLAST analysis. *, undetermined nucleotide.