Supplementary material for

Di-leucine signal-dependent and AP-1-independent targeting of a lysosomal glycoprotein in *Trypanosoma brucei*

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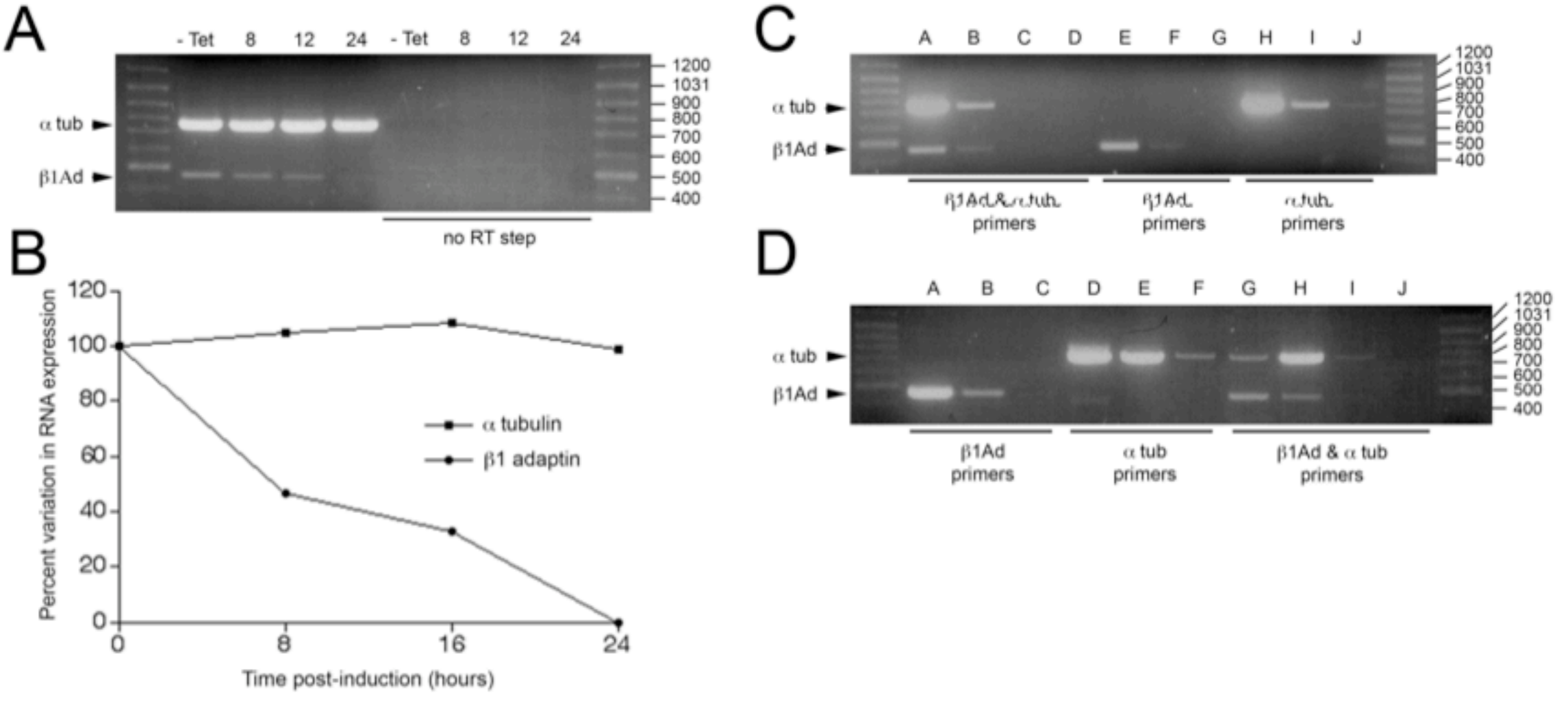
Methods and materials

Semi-quantitative RT-PCR: Total RNA was extracted using TRIzol reagent (Invitrogen) from BSFp2T7^{Ti}®1Ad parasite cells at the indicated times post-induction with 1µg ml^{-1} tetracycline. Semi-quantitative RT-PCR was performed using the Titan One Tube RT-PCR system (Roche). Each reaction was set up using 10ng of RNA template and the following primers; 5'-GCGTCACCACATTTATGCAC-3' and 5'-GATGCCATTTGCTGAGGAAT-3' to amplify a 457bp fragment of the Tb®1Ab gene (a region distinct from the fragment used for RNAi) and 5'-AAGTCCAAGCTCGGCTACAC-3' and 5'-CTCTTCCATACCCTCACCGA-3' to amplify a 756bp fragment of the Tb⟨Tub gene (as an internal control). The reverse transcription (RT) and PCR reactions were performed in the same tube according to manufacturer's instructions and including 2.5mM $MgCl_2$. The conditions for RT-PCR were; 1 cycle of 50˚C for 30mins, 1 cycle of 94˚C for 2 mins, 25 cycles of 94˚C for 10secs, 59˚C for 30secs and 68˚C for 45 secs, and 1 cycle of 68˚C for 7 mins. As both RT and PCR reactions were carried out in the same tube, to control for genomic DNA contamination of the RNA template duplicate reactions were set up omitting the RT step by heating the master mix for 2 minutes at 94^{o}C to inactivate the AMV reverse transcriptase, then the 50^{o}C incubation step was omitted, moving directly onto the PCR phase. WT BSF RNA was used for control reactions set up to identify the optimum number of cycles and RNA template concentration for amplifying the cDNA within the logarithmic phase. Amplified PCR products were separated on 1.2% agarose gels and identified by staining with ethidium bromide. Band intensity was quantified using ImageJ software.

Supplementary figure legends

Figure S1: Validation of Tb®1Ad RNAi knockdown by RT-PCR. Panel A: RT-PCR analysis of Tb®1Ad mRNA expression in induced BSFp2T7^{Ti}®1Ad cells. Total RNA was prepared from cells at indicated times post-induction. First strand cDNA was generated using 10ng total RNA template for each reaction, then multiplex PCRs were carried out using Tb⟨Tub and Tb®1Ad specific primers with 25 amplification cycles. RT-PCR products were separated by agarose electrophoresis and detected by ethidium bromide staining. Control experiments omitting the RT step confirmed the absence of significant genomic DNA contamination of the RNA template (the rightmost 4 lanes). Panel B: Analysis of relative expression levels of Tb®1Ad and Tb⟨tub mRNA in induced BSFp2T7^{Ti}®1Ad cells through quantitation of band intensities shown in panel A. The intensities of the ethidium bromide stained RT-PCR-amplified Tb⟨tub and Tb®1Ad products were measured using ImageJ software. Results are presented as a percentage of pre-induction levels for each product. Panel C: Optimization of RNA template concentration. To control for saturation of the RT-PCR reaction by excess RNA template a series of RT-PCR reactions were set up using the following range of BSF WT RNA template concentrations: Lanes A, D, E and H = 10ng; Lanes B, F and I = 1ng; Lanes C, G and J = 0.2ng. Lane D = no RT control. The primer pairs used in each reaction are shown below each lane. All reactions were performed using 25 cycles. Panel D: Optimization of cycle number. To optimize the RT-PCR conditions for semi-quantitative detection of mRNA expression a series of reactions were set up using the following range of amplification cycle numbers: Lanes A, D and G = 35 cycles; Lanes B, E, H and J = 25 cycles; lanes C, F and I = 20 cycles. Lane J = no RT control. The primer pairs used in each reaction are shown below each lane. All reactions used 10ng BSF WT RNA template. The scales on the right of panels A, C and D represent relative sizes in base pairs.


A
- Tet
8
12
24
- Tet
8
12
24
α tub
β1Ad
1200
1031
900
800
700
600
500
400
no RT step
B
Percent variation in RNA expression
120
100
80
60
40
20
0
α tubulin
β1 adaptin
0
8
16
24
Time post-induction (hours)
C
A
B
C
D
E
F
G
H
I
J
α tub
β1Ad
1200
1031
900
800
700
600
500
400
β1Ad & α tub primers
β1Ad primers
α tub primers
D
A
B
C
D
E
F
G
H
I
J
α tub
β1Ad
1200
1031
900
800
700
600
500
400
β1Ad primers
α tub primers
β1Ad & α tub primers