Poly(A)-Specific Ribonuclease (PARN-1) Function in Stage-Specific mRNA Turnover in *Trypanosoma brucei*†‡

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Deadenylation is often the rate-limiting event in regulating the turnover of cellular mRNAs in eukaryotes. Removal of the poly(A) tail initiates mRNA degradation by one of several decay pathways, including deadenylation-dependent decapping, followed by 5′ to 3′ exonuclease decay or 3′ to 5′ exosome-mediated decay. In trypanosomatids, mRNA degradation is important in controlling the expression of differentially expressed genes. Genomic annotation studies have revealed several potential deadenylases. Poly(A)-specific RNase (PARN) is a key deadenylation involved in regulating gene expression in mammals, *Xenopus* oocytes, and higher plants. Trypanosomatids possess three different PARN genes, PARN-1, -2, and -3, each of which is expressed at the mRNA level in two life-cycle stages of the human parasite *Trypanosoma brucei*. Here we show that *T. brucei* PARN-1 is an active deadenylase. To determine the role of PARN-1 on mRNA stability in vivo, we overexpressed this protein and analyzed perturbations in mRNA steady-state levels as well as mRNA half-life. Interestingly, a subset of mRNAs was affected, including a family of mRNAs that encode stage-specific coat proteins. These data suggest that PARN-1 functions in stage-specific protein production.

Regulation of gene expression in the protozoan parasite *Trypanosoma brucei* allows the organism to adapt and survive during its life cycle in two very different environments, the mammalian bloodstream and the tsetse fly. Expression of numerous protein-coding genes is regulated posttranscriptionally, particularly at the level of mRNA stability (4, 11, 25). For example, differential mRNA stability accounts for the stage-specific expression of procyclins, hexose transporters, and phosphoglycerate kinases (6, 20, 27, 28, 53).

In the well-studied *Saccharomyces* and mammalian systems, mRNA decay is a tightly controlled, multistep, and multipathway process. Various cis-acting elements, embedded in specific mRNAs, are recognized by RNA-binding proteins (7, 52, 55), which stabilize mRNAs or recruit RNases to carry out mRNA degradation and inhibit translation (8, 34, 39, 47). The removal of the mRNA 3′ poly(A) tail by 3′ to 5′ exoribonucleases (deadenylases) is often the rate-limiting step in mRNA degradation in vertebrates and thus a key point in regulation of mRNA turnover (19, 40).

A number of different deadenylases exist in eukaryotes, including poly(A)-specific RNase (PARN), the CCR4/CAF1/NOT complex, and the PAN2/PAN3 complex (reviewed in reference 23). The specific role of each of these proteins remains largely unknown, although evidence suggests that each enzyme may recognize a particular set of mRNA substrates (46). PARN functions in the targeted degradation of specific mRNAs in humans, *Xenopus*, and higher plants (1, 3, 22, 31, 32, 36, 58). To date, no PARN-encoding genes have been characterized in any single-celled eukaryote (56). In humans, PARN initiates decay of mRNAs containing AU-rich elements or nonsense codons. In *Xenopus*, PARN regulates oocyte maturation, whereas in *Arabidopsis*, PARN regulates embryogenesis.

The trypanosome genome encodes homologs to the deadenylation enzymes PARN, CAF1/NOT, and PAN2/PAN3, although a CCR4 homolog is absent (50, 51). Investigation of *T. brucei* CAF1/NOT1 showed that these two proteins were essential, whereas PAN2 depletion studies were less conclusive (50).

We set out to study PARN in *T. brucei* on the basis of our discovery of deadenylation activity in cytoplasmic extracts from this organism (42). *T. brucei* possesses three PARN homologs (*PARN-1, -2, and -3*), each of which is transcribed in both the procyclic (Pro) and bloodstream form (BF) stages of the parasite life cycle (30; this work). We chose PARN-1 to initiate our studies of PARN-dependent mRNA decay in trypanosomes. We verified that PARN-1 is a functional deadenylase, and we overexpressed PARN-1 in situ to identify transcripts that are targeted for degradation by this enzyme. A subset of mRNAs targeted for PARN-1-dependent degradation in *T. brucei* was identified using microarray studies and quantitative real-time PCR (qRT-PCR). Analysis of the genes coding for several of these mRNAs suggests that PARN-1 contributes to regulating differential gene expression.

**MATERIALS AND METHODS**

**Culturing and transfection of parasites.** *T. brucei* Lister 427 procyclic cells were cultured in SDM-79 medium containing 10% fetal calf serum (FCS) at 20°C in 5% CO₂. Procyclic 29-13 cells were cultured in the presence of 15 μg/ml G418 and 50 μg/ml hygromycin to maintain expression of T7 RNA polymerase and the tetracycline (Tet) repressor (57). Transfected parasites containing the integrated plasmid were selected for by the addition of 2.5 μg/ml phleomycin (2). Lister 427 BF cells were grown in HMI-9 medium containing 10% FCS and 10% Serum
Plus (SAFC Biosciences) at 37°C in 5% CO₂, (26). Single-marker BF cells (a gift from G. Cross) were cultured in the presence of 2.5 µg/ml G418 to maintain the T7 RNA polymerase and Tet repressor (57), and transfected parasites were obtained using an Amaxa system and selected for using 2.5 µg/ml phleomycin.

Plasmid constructs. The pSAP1 vector containing the streptavidin-binding protein-P protein A tandem affinity purification (TAP) tag was a generous gift from Larry Simpson. The 630-bp open reading frame (ORF) was PCR amplified to add HindIII and KpnI sites upstream and a BamHI site downstream of the tag. The product was ligated into the pLEW111 expression vector using the HindIII and BamHI sites to produce pLEW111-TAP. The pN1-1 ORF was PCR amplified, and KpnI sites were added to each end. PARN-I was ligated into pLEW111-TAP using KpnI to produce a T7-inducible, TAP-tagged PARN-1 protein expression vector.

RNA analysis. Total RNA was extracted from procyclic cells grown to 8 × 10⁶ cells/ml and BF cells grown to 1 × 10⁷ cells/ml using Qiagen’s DNeasy mRNA midikit. Poly(A)⁺ RNA was isolated from the total RNA using Qiagen’s Oligotex mRNA midikit.

For Northern blot analysis, 10 µg of total RNA was separated on a formaldehyde-12% agarose gel in morpholinepropanesulfonic acid (MOPS) buffer (49). RNA was protein expression vector.

pLEW111-TAP using KpnI to produce a Tet-inducible, TAP-tagged PARN-1 ORF was PCR amplified, and KpnI and BamHI sites to produce pLEW111-TAP. The product was ligated into the pLEW111 expression vector using the HindIII and BamHI sites to produce pLEW111-TAP. The pN1-1 ORF was PCR amplified, and KpnI sites were added to each end. PARN-I was ligated into pLEW111-TAP using KpnI to produce a T7-inducible, TAP-tagged PARN-1 protein expression vector.

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The reaction mixtures were incubated (1 h, 37°C), and the RNA was purified by phenol-chloroform extraction, followed by ethanol precipitation. Samples were resuspended in 10 μl TBE (Tris-borate-aceate)-urea loading buffer and separated on a 5% acrylamide–7 M urea gel. Radiolabeled RNA bands were excised from the gel and eluted in 400 μl HSCB buffer (50 mM Tris-HCl, pH 7.6, 400 mM NaCl, 0.1% SDS) with 50 μg proteinase K overnight at room temperature. Samples were phenol-chloroform extracted, ethanol precipitated, and resuspended in RNase-free water.

**In vitro deacylase assays.** Assays were adapted from the previously described protocol (17). For assays using S100 protein extracts, 14-ethylene precipitated, and resuspended in RNase-free water (50 mM Tris-HCl, pH 7.6, 400 mM NaCl, 0.1% SDS) with 50 μg bovine serum albumin (BSA), and 16 units RNase inhibitor (Amersham). All reaction mixtures were prepared. For assays using TAP tag-purified PARN-1 protein, 15 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.17 mM dTTP, 1 mM 5-(3-aminoallyl)-dUTP, 0.33 mM KPO₄, 80 U RNAseOUT (Invitrogen), and 800 U SuperScript III reverse transcriptase (Invitrogen). The steady-state levels of poly(A) tails on T. brucei mRNAs are 150 to 200 nt. The RNA in the cDNA-RNA mixture was hydrolyzed in 100 mM EDTA and 200 mM NaOH. The reaction mixture was incubated (15 min, 65°C), and then the pH was adjusted to ~7.0 with 333 mM Tris (pH 7.0). cDNA was purified using a QIAquick PCR purification kit (Qiagen) and dried to ~1 μl by a SpeedVac apparatus. cdNA was resuspended in 100 mM sodium carbonate buffer (pH 9.3) and labeled with Cy3 or Cy5 monoreactive dye (GE Healthcare). Two reaction mixtures were incubated for 2 h at room temperature and stopped with a 24- by 60-cm glass coverslip (Fisher), and placed in a chamber covered in aluminum foil, and incubated for 16 h. Slides were washed (2× SSC) and 0.1% SSC, 0.1% SSC, and 0.1% SSC, and dried by centrifugation. Samples were scanned using a microarray scanner (Axon Instruments) and MultiExperiment Viewer (version 4.1) software (the Institute for Genomic Research [TIGR]). Using this analysis, we determined that PARN-1 mRNA levels were increased ~27-fold following induction with tetracycline.

**Quantitative real-time PCR.** qRT-PCR experiments were carried out using an iScript one-step RT-PCR kit with SYBR green (Bio-Rad). The Primer sets are shown in Fig. S3 in the supplemental material. Briefly, 100 ng total RNA was added to a 25-μl reaction mixture containing 1× SYBR green reaction mixture, 300 nM forward and reverse primers, and 0.5 μl iScript reverse transcriptase. Reactions were run in a RotorGene 3000 real-time cycler (Corbett Research). Cycling conditions were as follows: 50°C for 10 min for cDNA synthesis, 5 min at 95°C for reverse transcriptase inactivation, and 40 cycles of 10 s at 95°C and 30 s at 55°C, followed by melt curve analysis from 55°C to 99°C. To assay for mRNA stability, cells were treated with 10 μg/ml actinomycin D, total RNA was extracted at 0, 30, 60, 120, and 180 min, and mRNA levels were analyzed by qRT-PCR. All mRNA levels were normalized to that of 7SL RNA, and the percentage of mRNA remaining from the starting amount (time zero) was calculated. For the decay kinetics, the percentage of mRNA remaining was plotted versus time. An exponential trendline was fit to each set of data, and the decay rate constant was calculated.

**Microarray analysis.** Proyclic cells were grown to a density of 8 × 10⁷ cells/ml. PARN-1 and TAP tag overexpression was induced for 48 h with 500 μg/ml phleomycin and 0.5 μg/ml amphotericin D. After 24 h, 1 μg/ml phleomycin was added to cultures to select transfected cells. After these single-knockout cell lines were verified by restriction digestion, cells were transfected with a NotI-digested neomycin resistance plasmid. After 24 h, 15 μg/ml G418 was added to the cultures to screen for cells containing deletions of both PARN-1 alleles. Double-knockout strains were confirmed by PCR of genomic DNA and Northern blotting.

**Microarray data accession number.** Microarray data were deposited into the NCBI GEO database (accession no. GSE20593).

**RESULTS**

*T. brucei* encodes three PARN homologs. The amino acid sequence of *Homo sapiens* PARN (*HsPARN*) was used to identify possible *PARN* homologs in the *T. brucei* genome (Fig. 1A) (5). Three genes were designated *T. brucei* PARN-1 (*TbPARN-1*), *TbPARN-2*, and *TbPARN-3* on the basis of their relative homology to *HsPARN*. PARN proteins are members of the DEDD exoribonuclease superfamily and the DEDDh family, characterized by (i) three exonuclease (Exo) motifs (Exo I, II, and III; Fig. 1A, gray boxes; see Fig. S1A in the supplemental material), (ii) four invariant acidic amino acids necessary for catalytic activity (Fig. 1A, asterisks; see Fig. S1A in the supplemental material), (iii) a fifth invariant acidic residue located between Exo II and III (DTK, where the boldface D indicates the fifth invariant acidic residue; Fig. 1A, triangle; see Fig. S1A in the supplemental material), and (iv) a conserved histidine within the Exo III motif (Fig. 1A, filled circle; see Fig. S1A in the supplemental material) (48, 59). Because the TbPARNs and HsPARN are highly divergent within their C termini, we restricted the comparison to the N termini. The N terminus of TbPARN-1 is 30% identical and 17% similar in amino acid sequence to the HsPARN sequence, TbPARN-2 is 27% identical and 15% similar, and TbPARN-3 is 18% identical and 11% similar.

We determined whether each of the three *T. brucei* PARN-coding genes produces mRNAs in parasites using Northern blot analysis (Fig. 1B). DNA probes were targeted to the divergent, terminal 800 bp of each ORF to distinguish among the three *PARN* genes. Northern blot analysis showed that *PARN-1*, *PARN-2*, and *PARN-3* are transcribed in procyclic parasites, which is the life-cycle stage in the tsetse fly midgut, and in the bloodstream-form parasites, which is the replicating life-cycle stage in the mammalian host. In addition, the steady-state levels of each *PARN* mRNA do not differ in a stage-specific manner.
pared. We conclude that absolute levels of PARN-1, -2, and -3 proteins were not comparable with blot and qRT-PCR analyses (Fig. 1B and data not shown). The beta-tubulin mRNA quantities, as determined in the Northern blot analysis of the Arabidopsis thaliana PARN (AtPARN) to uncover similarities that might correlate with its predominantly cytoplasmic localization (9, 48). Thus, TbPARN-1 likely functions without a 5'-cap dependency and in the parasite’s cytoplasm.

**PARN-1 is nonessential for parasite viability.** To identify the role of PARN-1 in T. brucei, we deleted the two allelic copies of this gene using homologous recombination (see Fig. S2A in the supplemental material). Each allele was replaced with a drug resistance cassette. Northern blot analysis demonstrated the absence of PARN-1 mRNA in the double knockout (see Fig. S2B in the supplemental material). Western analysis demonstrated the absence of PARN-1 protein in the double knockout (see Fig. S2C in the supplemental material). Cell viability, growth rate, and microscopic analysis of two clonal cell lines showed no growth alterations compared to wild-type parasites (see Fig. S2D in the supplemental material). These results prove that PARN-1 is a nonessential gene in cultured procyclic T. brucei.

To explore the combined necessity of PARN-1, -2, and -3 in parasites, we simultaneously depleted all three PARNs using RNA interference in procyclic and bloodstream-form parasites (data not shown). PARN-1 and PARN-2 were shown to be decreased at the mRNA level by Northern analysis, and PARN-3 was shown to be decreased at the protein level by Western analysis using anti-PARN-3 antibody (data not shown). Neither growth rates nor gross morphology was affected in PARN-depleted procyclic forms, and growth rates were only slightly affected (~10% slower than control rates) in PARN-depleted BF parasites. Because decreased amounts of all three PARNs did not affect cell growth, we conclude that PARN proteins either are not essential for T. brucei viability or are sufficient to sustain cell viability at low levels.

**PARN-1 is a deadenylase in vitro.** Purified PARN-1 was used in RNase assays to assess its deadenylase activity. To obtain purified protein, the PARN-1 ORF was tagged with a TAP tag and transfected on a Tet-inducible expression vector to produce the PARN-1 OvEx cell line. TAP-tagged PARN-1 was expressed and purified from S100 extracts using a two-step affinity method that employed IgG and streptavidin chromatography (Fig. 3A). A synthetic, radiolabeled 60-nt poly(A) tail (RNA-A60) was used as substrate. As expected, RNA-A60 was trimmed, in an apparently distributive manner, in the presence of purified PARN-1 (fraction shown in Fig. 3A, lane 8) to produce an RNA lacking a poly(A) tail, RNA-A60, after 10 min (Fig. 3B, lanes 1 to 5). To ensure that we were specifically assaying PARN-1 activity, we tested eluate (Fig. 3A, lane 8, and C, lane 1) that was PARN-1 depleted using PARN-1-specific polyclonal antibody or mock depleted using nonspecific antibody (Fig. 3C, lanes 2 to 7). Following depletion of PARN-1 protein using PARN-1-specific antibodies, RNA-A60 degradation was abolished (Fig. 3D, lanes 6 to 10). This was not the case in the mock depletion of PARN-1 (Fig. 3D, lanes 1 to 5). Thus, PARN-1 has deadenylase activity.

To test whether PARN-1 was an adenosine-specific exonuclease, PARN-1 activity on RNA-A60 was evaluated in the presence of poly(A) or poly(C) competitor (Fig. 3E). Poly(A) competitor inhibited RNA-A60 deadenylation (Fig. 3E, lanes 2 to 5), whereas poly(C) competitor had no effect on RNA-A60 deadenylation (Fig. 3E, lanes 6 to 9). In addition, we tested PARN-1 activity using a 22-mer nucleotide sequence added 3' to the poly(A) tail of RNA-A60 (RNA-A60 + 22) as substrate.
Thus, PARN-1 appears to be exclusively a deadenylase.

The catalytic activity of most deadenylases requires divalent cations (13, 15, 59). To determine whether PARN-1 activity has this requirement, enzyme activity was tested in the presence and absence of Mg$^{2+}$. When Mg$^{2+}$ was absent, RNA-A$_{60}$ deadenylation was inhibited, demonstrating that Tb-PARN-1 activity is dependent upon a divalent cation, such as Mg$^{2+}$, for its deadenylation activity.

Cells overexpressing PARN-1 have increased deadenylase activity. To examine the role of PARN-1 in procyclic T. brucei, we determined whether overexpression of PARN-1 resulted in increased deadenylation. PARN-1 OvEx cells and a control culture expressing the TAP tag alone were induced, and S100 protein extracts were prepared (Fig. 4B). Both cultures grew at the same rate, indicating that there was no gross effect of PARN-1 overexpression on cell growth (data not shown).

Deadenylation rates were measured using the in vitro deadenylation assay (Fig. 4A). The PARN-1 OvEx cell extract rapidly deadenylated RNA-A$_{60}$, and nearly all RNA-A$_{60}$ substrate was converted to RNA-A$_0$ after 15 min (Fig. 4A, lanes 5 to 8). In contrast, control extracts deadenylated RNA-A$_{60}$ at normal rates, and 45 min was required to convert nearly all RNA-A$_{60}$ substrate to RNA-A$_0$ (Fig. 4A, lanes 1 to 4). A graphic representation of the data is shown in Fig. 4C. Other deadenylases were unaffected by PARN-1 overexpression; thus, their activities were the same in PARN-1 OvEx cell and control extracts. Therefore, these data indicate that induced overexpression of PARN-1 protein in parasites enhances deadenylase activity.

A subset of procyclic mRNAs is reduced in PARN-1 OvEx parasites. To determine the effect of PARN-1 overexpression on global mRNA steady-state levels, the mRNA expression profile of PARN-1 OvEx cells was examined by microarray analysis. Three clones each from the PARN-1 OvEx and control cell lines were used for analysis, and each experiment was run in duplicate, with dye labeling reversed between duplicates. Heat maps of the six arrays are shown (Fig. 5A). Twenty-nine protein-coding genes had their mRNAs decreased (Fig. 5B). Within this gene set, 4 genes encode T. brucei alanine-rich proteins (BARPs), 2 genes encode acidic phosphatases, 2 genes encode bona fide ribosomal subunits, and 12 genes encode hypothetical proteins. Eight protein-coding genes had their mRNAs increased. This set includes PARN-1, as expected. All of the misregulated mRNAs appear to be RNA polymerase II-dependent genes, except for Tb927.4.1200, a putative expression site-associated gene (ESAG), which is usually transcribed by RNA polymerase I. Thus, a limited number of mRNAs expressed in procyclic parasites are regulated, at least in part, by PARN-1.

PARN-1 affects the steady-state level and decay rate of at least four different procyclic mRNAs. To confirm the microarray data indicating that the levels of specific mRNAs were reduced in PARN-1 OvEx parasites, qRT-PCR was performed on a subset of genes (Fig. 5B, asterisks, and 6A). Primer sets and amplified RNA regions are shown in Fig. S4 in the supplemental material. BARP mRNA levels were reduced ~3.2-fold in cells overexpressing PARN-1, determined using a primer set that recognized sequences common to all BARP...
isoforms (Fig. 6A). mRNA levels of the BARP isoform Tb09.244.2520 were reduced ~5.9-fold in cells overexpressing PARN-1. The mRNA levels of two conserved hypothetical proteins (designated p28 and p16 to reflect their molecular masses) were reduced ~2-fold in PARN-1 OvEx cells. The acidic phosphatase (Acid phos) mRNA transcribed from Tb927.5.630 was reduced ~2-fold in PARN-1 OvEx cells. Dihydroxyacetone phosphate acyltransferase (DHAPAT) mRNA steady-state levels, which were unchanged in the microarray data set, were the same in PARN-1 OvEx and control cell lines. Thus, the qRT-PCR data and the microarray data are consistent with each other and confirm that a subset of mRNAs is regulated by PARN-1.

To determine whether the reduced levels of BARP, p28, p16, and Acid phos mRNAs in PARN-1 OvEx cells were caused by increased rates of mRNA decay, we measured mRNA amounts at 0 and 2 h following RNA synthesis inhibition by actinomycin D (Fig. 6B). DHAPAT mRNA was used to
represent the mRNAs that were unaffected by PARN-1 overexpression in the microarray study. As expected, the amount of DHAPAT mRNA remaining was similar in the PARN-1 OvEx and control cell lines (30% versus 31%). The amount of mRNA remaining from all BARP isoforms was lower in PARN-1 OvEx cells (8%) than control cells (18%) after 2 h. Similarly, the amount of mRNA remaining from the BARP isoform Tb09.244.2520 was also decreased in PARN-1 OvEx cells (2%) relative to control cells (6%). In addition, the amount of p28 mRNA remaining was lower in PARN-1 OvEx cells (8%) than control cells (21%). Unexpectedly, the p16 mRNA amounts remaining in PARN-1 OvEx cells (23%) were close to those in control cells (19%), and the Acid phos mRNA amounts remaining in PARN-1 OvEx cells (37%) was greater than those remaining in control cells (27%). A direct measure of mRNA half-life was done for BARP (using the primers specific for the isoform Tb09.244.2520), p28, and control message DHAPAT (Fig. 6C). The degradation kinetics were exponential, as expected, and are shown for BARP mRNA (see Fig. S4 in the supplemental material). The half-life of BARP mRNA was decreased from 22 min to 15 min and the half-life of p28 mRNA was decreased from 60 min to 33 min after PARN-1 overexpression. DHAPAT, the control mRNA, was unaffected by PARN-1 overexpression, maintaining a half-life of 100 min under the two different conditions. In summary, the overexpression of PARN-1, as analyzed by qRT-PCR and mRNA decay profiles, resulted in an increase in the mRNA decay rates of BARP and p28 mRNAs.

DISCUSSION

Herein we show that trypanosomatids possess three different PARN genes, PARN-1, -2, and -3. Each PARN gene is expressed at the mRNA level in two life-cycle stages of the human parasite *Trypanosoma brucei*. PARN-1 is an active deadenylase and appears to regulate a subset of mRNAs, including a family of stage-specific coat proteins, the BARPs.

PARN proteins are members of the DEDD RNase superfamily, characterized by three exonuclease motifs that contain four invariant acidic amino acids. Most eukaryotes possess a single PARN gene. The three *T. brucei* PARNs, PARN-1, -2, and -3, each contain all three exonuclease motifs. Moreover, each possesses the conserved acidic amino acids, suggesting that all three PARNs are active in deadenylation. Other members of the trypanosomatid family also possess three PARN...
FIG. 5. Overexpression of PARN-1 decreases the steady-state mRNA levels of a subset of genes in T. brucei. (A) Heat map showing the change in steady-state mRNA levels after induction of PARN-1 deadenylase. Data were determined by microarray analysis. The data are arranged by the systematic gene name, which is based on chromosomal gene location. Columns 1 to 6 present the results for the 6 independent experiments. RNAs are represented as lines colored relative to their expression levels, as indicated in the heat map key on the left of the map. Green indicates an increase in mRNA levels in PARN-1 OvEx cells, red indicates a decrease in mRNA levels in PARN-1 OvEx cells, and black indicates no change. Each gene on the microarray was represented in duplicate; thus, for each experiment, mRNA levels were obtained from both points and averaged. In the case of dye flips, values were multiplied by \( \frac{1}{2} \) to allow numerical comparisons of all six arrays. Heat maps were generated in the MultiExperiment Viewer (MeV) program (version 4.1; TIGR), with genes arranged by identifier. The locus on chromosome (Chr) 9 containing the BARP genes is indicated with an arrow. (B) Summary of the gene loci encoding the mRNAs that were decreased or increased in PARN-1 OvEx cells. Column 1, systematic gene name, as designated in GeneDB; column 2, description of the gene product; column 3, number of independent experiments (\( n/6 \)) in which the gene-encoding mRNA was decreased at least 1.5-fold; column 4, average fold decrease in PARN-1 OvEx cells among the six experiments; rows 1 to 24, genes located on the locus on chromosome 9 between \( Tb90.244.2400 \) and \( Tb90.244.2860 \); asterisks, genes further analyzed by qRT-PCR experiments; shaded gray, genes with reduced mRNA levels in procyclic parasites relative to other life-cycle stages (30, 44, 54). Microarray data were deposited into the NCBI GEO database (accession no. GSE20593). Eleven of the 14 BARP ORFs were present in triplicate on the microarray slide.
homologs, indicating multiple roles for PARNs in mRNA regulation during the complex life cycle of these organisms.

The sequences at the C terminus of all three T. brucei PARNs and that of the active AtPARN are highly divergent from the sequence at the C terminus of human PARN. In human PARN, the C terminus binds cap 0 (m7GpppG) of mRNA during deadenylation (14, 21, 38). Trypanosome mRNAs contain a cap 4 (a hypermethylated form of m7GpppAACU). Thus, the divergence from the human PARN in the C termini of the three T. brucei PARNs may reflect an interaction between at least one of them and the unique trypanosome cap structure.

Deletion of PARN-1 was not lethal to cultured procyclic
parasites. This result was not surprising, as functional PARN is also not essential for viability in cultured HeLa cells, *Xenopus* oocytes, or *Saccharomyces pombe* (10, 31). However, PARN may be required for cellular processes involved in development. For example, embryonic development is stymied in *Arabidopsis* lacking PARN (48).

*T. brucei* PARN-1 participates in regulating specific mRNAs, as determined by our microarray and qRT-PCR data. Overexpression of PARN-1 affected BARP and p28 mRNA abundance and decay. In humans, PARN plays a role in regulating specific mRNAs via targeting to AU-rich element-containing mRNAs (35). In *Xenopus* and *Arabidopsis*, PARN plays a role in embryogenesis, targeting different subsets of mRNAs at specific stages of development (31, 48). Similarly, PARN-1 may regulate BARP in a single-cell eukaryote. PARN-mediated degradation of mRNA will be interesting to see if the set of three PARN deadenylation targets contributes to mRNA instability in procyclic parasites (54).

Other mRNA decay studies in trypanosomes have begun to characterize CAF1, PAN2, DHH1, XRNA, DCP1/2, and DCPS-like activities and the exosome in *T. brucei* (16, 24, 33, 37, 41, 50, 51). CAF1 and PAN2 appear to affect the decay of stable mRNAs since depletion of either protein resulted in an increased poly(A) tail length in bloodstream-form parasites (50, 51). In addition, depletion of the CAF1, PAN2, or XRNA protein decreased the decay rate of the unstable EPI procyclin mRNA in bloodstream-form cells. Also, recent studies of DHH1 show that this RNA helicase has a selective role in modulating levels of developmentally regulated mRNAs (33).

On the basis of our findings that PARN-1 is a nonessential protein for cultured procyclic parasites and appears to regulate the steady-state levels of a subset of cellular mRNAs, we propose that PARN-1 operates as a regulatory enzyme to control the levels of a subset of mRNAs during the parasite life cycle. The paradigm for differential deadenylation targeting of specific mRNAs includes RNA-binding proteins that recruit deadenylases and the exosome in mammals (26), *Saccharomyces cerevisiae* (34), and *Arabidopsis* (54), and we predict that PARN-1-mediated decay of BARP mRNA is decreased in this life-cycle stage.

Procyclic parasites. This result was not surprising, as functional PARN is also not essential for viability in cultured HeLa cells, *Xenopus* oocytes, or *Saccharomyces pombe* (10, 31). However, PARN may be required for cellular processes involved in development. For example, embryonic development is stymied in *Arabidopsis* lacking PARN (48).

*T. brucei* PARN-1 participates in regulating specific mRNAs, as determined by our microarray and qRT-PCR data. Overexpression of PARN-1 affected BARP and p28 mRNA abundance and decay. In humans, PARN plays a role in regulating specific mRNAs via targeting to AU-rich element-containing mRNAs (35). In *Xenopus* and *Arabidopsis*, PARN plays a role in embryogenesis, targeting different subsets of mRNAs at specific stages of development (31, 48). Similarly, PARN-1 may regulate BARP in a single-cell eukaryote. PARN-mediated degradation of mRNA will be interesting to see if the set of three PARN deadenylation targets contributes to mRNA instability in procyclic parasites (54).

Other mRNA decay studies in trypanosomes have begun to characterize CAF1, PAN2, DHH1, XRNA, DCP1/2, and DCPS-like activities and the exosome in *T. brucei* (16, 24, 33, 37, 41, 50, 51). CAF1 and PAN2 appear to affect the decay of stable mRNAs since depletion of either protein resulted in an increased poly(A) tail length in bloodstream-form parasites (50, 51). In addition, depletion of the CAF1, PAN2, or XRNA protein decreased the decay rate of the unstable EPI procyclin mRNA in bloodstream-form cells. Also, recent studies of DHH1 show that this RNA helicase has a selective role in modulating levels of developmentally regulated mRNAs (33).

On the basis of our findings that PARN-1 is a nonessential protein for cultured procyclic parasites and appears to regulate the steady-state levels of a subset of cellular mRNAs, we propose that PARN-1 operates as a regulatory enzyme to control the levels of a subset of mRNAs during the parasite life cycle. The paradigm for differential deadenylation targeting of specific mRNAs includes RNA-binding proteins that recruit deadenylases and the exosome in mammals (26), *Saccharomyces cerevisiae* (34), and *Arabidopsis* (54), and we predict that PARN-1-mediated decay of BARP mRNA is decreased in this life-cycle stage.

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On the basis of our findings that PARN-1 is a nonessential protein for cultured procyclic parasites and appears to regulate the steady-state levels of a subset of cellular mRNAs, we propose that PARN-1 operates as a regulatory enzyme to control the levels of a subset of mRNAs during the parasite life cycle. The paradigm for differential deadenylation targeting of specific mRNAs includes RNA-binding proteins that recruit deadenylases via *cis*-acting elements in specific mRNAs. Interestingly, Roditi and colleagues have shown that the BAR 3’ untranscribed region (UTR) contains *cis*-acting elements that contribute to mRNA instability in procyclic parasites (54). PARN-1 may participate in the regulation of BARP mRNA turnover by interacting with 3’ UTR-protein complexes. Thus, it will be interesting to see if the set of three PARN deadenylases, recruited by different RNA-binding proteins, serves to modulate stage-specific mRNA decay during the parasite life cycle.

In summary, we present herein the first characterization of PARN in a single-cell eukaryote. PARN-mediated degradation of stage-specific BARP messages suggests a role for PARN in *T. brucei* development much like the developmental role of PARN in multicell eukaryotes. Interestingly, *T. cruzi* and *Leishmania* spp. also encode PARN homologues. We speculate that these human-infective trypanosomes, and possibly other pathogenic single-cell eukaryotes, utilize PARN to regulate gene expression during development.

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