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Two Novel RNA Binding Proteins from *Trypanosoma brucei* Are Associated with 5S rRNA

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We have previously reported the identification of two closely related RNA binding proteins from *Trypanosoma brucei* which we have termed p34 and p37. The predicted primary structures of the two proteins are highly homologous with one major difference, an 18-amino-acid insert in the N-terminal region of p37. These two proteins have been localized to the nucleus based on immunofluorescence microscopy. To gain insight into their function, we have utilized UV crosslinking, coimmunoprecipitation, and sucrose density gradients to identify *T. brucei* RNA species that associate with p34 and p37. These experiments have demonstrated a specific interaction of both p34 and p37 with the 5S ribosomal RNA and indicate that other RNA species are unlikely to be specifically bound. This suggests a role for p34 and p37 in the import and/or assembly pathway of *T. brucei* 5S rRNA in ribosome biogenesis.

***Key Words: Trypanosoma brucei;* RNA binding proteins; 5S rRNA; RNA processing; RNP.**

Trypanosoma brucei are parasitic protozoans which cause disease in both humans (African sleeping disease) and livestock (nagana). The trypanosome life cycle is characterized by several morphologically and functionally distinct stages in mammalian and insect hosts (1). In the course of transmission between hosts, the primary surface antigen proteins (2) and various metabolic proteins (3–5) have been demonstrated to undergo developmental regulation. Genetic elements involved in this differential expression have been identified (6, 7), but the mechanisms of these processes have not been extensively characterized.

Nuclear RNA metabolism in *T. brucei* presents a number of interesting and unique features among eukaryotic organisms. For example, transcription of housekeeping genes by RNA polymerase II appears to be polycistronic (8, 9) and does not appear to be regulated at the level of transcription initiation. RNA polymerase I, or a molecule with similar properties, is responsible for transcription of the genes encoding the major surface glycoproteins; procyclic acidic repetitive protein and variant surface glycoprotein (10, 11). Each mRNA is processed in a *trans*-splicing event by addition of an identical 39 base RNA moiety [the spliced leader (SL) RNA] to its 5′ end (12, 13). Also, changes in RNA stability, mediated primarily by the 3′ UTR, are of key importance in developmental regulation of gene expression in these organisms (6, 7, 14, 15).

The major ribosomal processing and maturation events found in higher eukaryotes are conserved in *T. brucei.* However, the precise cleavage events that generate the mature rRNA species differ. The major rRNA precursor is transcribed by RNA polymerase I in the nucleolus and is processed to generate the 5.8S species, the mature 18S (small subunit or SSU in kinetoplasts) and the 28S rRNA (large subunit or LSU in kinetoplasts). The LSU is further processed via cleavage (16–19). The 5S rRNA gene of *T. brucei* is encoded in a separate tandem repeat unit of ~750 bp (20). This arrangement is similar to that found in metazoans, but is in contrast to yeast in which the 5S rRNA gene is present within the major rRNA unit.

A limited number of nuclear RNA binding proteins from *T. brucei* have been studied. A protein with homology to RNA binding proteins involved in *cis*-splicing, termed RRM1 (21), and proteins associated with SL RNP and the U2, U4/U6, and U5 snRNPs have been reported (22–26). The fibrillarin homologue has recently been cloned, and coimmunoprecipitation experiments have demonstrated its interaction with a number of box C/D snoRNA molecules including the U3 snoRNA homolog (27, 28). A family of nucleolar phos-

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phoproteins termed the NOPP44/46 proteins have also been identified (29, 30).

We have previously identified two nuclear RNA binding proteins from *T. brucei*, p34 and p37 (31, 32). These proteins contain a putative bipartite nuclear targeting sequence motif within their last 16 residues, and their nuclear localization has been demonstrated by immunofluorescence microscopy. Database searches indicate that these two proteins show no overall homology to other identified proteins in either *T. brucei* or other organisms, although smaller regions of homology (motifs) are present. However, p34 and p37 are highly homologous to one another, with the major difference being an 18-amino-acid insertion near the N-terminus of p37. Each protein contains an RNA binding region consisting of one complete RBD motif (containing RNP1 and RNP2 consensus sequences), a 45-amino-acid spacer, and a half RBD (containing only an RNP1 consensus sequence). Despite their high degree of homology to one another, the relative levels of these two proteins are developmentally regulated during the life cycle of *T. brucei*, the p34 protein being expressed predominantly in the procyclic form, while p37 is predominant in the bloodstream forms.

The role(s) that p34 and p37 play in *T. brucei* RNA metabolism has not been defined. In this report we show that an examination of the molecular binding partners of p34 and p37 has revealed an interaction with the 5S rRNA of *T. brucei*, implicating these proteins in *T. brucei* ribosomal biogenesis.

MATERIALS AND METHODS

Cell culture. The procyclic form of *T. brucei brucei* strain TREU667 was grown essentially as described in Cunningham's semidefined medium containing 10% fetal calf serum plus 25 mM Hepes and 30 μg/ml gentamicin (32). Cells were harvested at a density of 1–2 $\times$ 10^7 trypanosomes per milliliter of medium. The bloodstream form of *T. brucei* strain TREU427 was grown and harvested essentially as described by Hirumi and Hirumi (33) in HMI-9 medium supplemented with 10% fetal calf serum plus 10 U/ml penicillin and 10 μg/ml streptomycin. Bloodstream cells were harvested at a density of 10^5–10^6 trypanosomes/ml of culture medium.

UV crosslinking studies. UV crosslinking experiments were performed essentially as described by Pinol-Roma *et al.* (34). Five hundred milliliters of procyclic *T. brucei* was washed 2$\times$ in PBS and resuspended in 5 ml PBS in 150 $\times$ 25-mm tissue culture dishes. Cells were irradiated for 90 s in a UV Strata-linker 2400 (Stratagene) in the autocrosslinking mode at a distance of 15 cm from the UV source. Cells were sedimented at 10,000*g* for 5 min, and resuspended in 3.5 ml RSB (10 mM Tris, pH 7.4, 10 mM NaCl, 1.5 mM $MgCl_2$) containing 1 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 0.5 mM PMSF, and 10 μl RNasin (Promega). After incubation on ice for 5 min, 100 μl 20% Triton X-100 and 400 μl MAGIK (10% Tween 20, 5% deoxycholate) were added and the cells homogenized in a Dounce homogenizer. Cells were then lysed by passage through a 25-gauge needle five times. Cell lysates were centrifuged briefly at 3000*g*. SDS, 2-mercaptoethanol, and EDTA 10 mM were added to the supernatant to final concentrations of 0.5% (w/v), 1% (v/v) and 10 mM, respectively. The pellet was resuspended in 500 μl RSB containing inhibitors. Five microliters of DNase I (Life Technologies) was added, and the extract was incubated at 37°C for 15 min. Following digestion, 3.5 ml RSB was added as were SDS, 2-mercaptoethanol, and EDTA to final concentrations of 0.5% (w/v), 1% (v/v), and 10 mM, respectively. The samples were then incubated at 65°C for 5 min and then quenched rapidly on ice. LiCl was added to a final concentration of 500 mM, and preparations were centrifuged to remove particulate material. Equivalent fractions were pooled and applied to poly(dT) columns (Life Technologies) and poly(A)$^+$ fractions were eluted according to the protocol of the manufacturer. Poly(A)$^+$ fractions were digested with 1 μg RNAse A (Sigma) and 25 U micrococcal nuclease (Sigma) for 30 min at 37°C. Western analysis was performed as previously described (31) using polyclonal antibodies directed against p34 and p37 or the poly(A) binding protein (PABP1) and detection was by chemiluminescence. Polyclonal antibodies directed against the *T. brucei* homologue of PABP1 were raised in rabbits using protein purified as described by Pitula *et al.* (35).

Coimmunoprecipitation analysis of nuclear extracts. Extracts enriched in nuclear components were prepared essentially as described by Roberts *et al.* (36). For coimmunoprecipitation experiments, aliquots of nuclear extracts containing 500 μg of protein, as determined by Bradford analysis, were brought to a final volume of 350 μl. Then 50 μl of anti-p34/p37 antibodies (1 μg/μl, purified by protein A–Sepharose chromatography, Amersham Pharmacia) was added to the extracts and incubated at 4°C for 30 min. In all experiments 1 μl of the ribonuclease inhibitor RNasin (Promega) was included. The antigen–antibody complexes were collected with 100 μl of a 50% protein A–Sepharose/Buffer A slurry and washed three times with Buffer A. For negative control experiments, antibodies purified from preimmune serum were used. Western blot analysis was performed on 30 μl of the samples.

Precipitates for analysis of RNA species associated with p34 and p37 were washed and adjusted to a final volume of 500 μl containing 0.5% (w/v) SDS, 1 μl RNasin (Promega), and 50 μg/ml proteinase K (Sigma). Samples were digested for 15 min at 37°C and then phenol/chloroform extracted and ethanol precipitated using 1 μl of glycogen (Boehringer Mannheim) as a carrier.

Northern blot analysis. Northern blot analysis of precipitated RNA was performed essentially as described by Zhang *et al.* (31). Samples were resolved by 1.5% agarose/formaldehyde gel electrophoresis. RNA molecules were transferred to nylon membranes (Bio-Rad Zeta Blot) overnight. Membranes were prehybridized for 3 h at 42°C in Church Buffer (125 mM $NaPO_4$, pH 7.2, 7% SDS, 1% BSA, 1 mM EDTA), and then hybridized with 5′ ^{32}P-labeled oligonucleotide probes (specific activity $\sim 10^8$ dpm/μg) overnight at 42°C. Probes for Northern analysis were designed according to published sequences found in the NCBI database:

5S rRNA (5′-GCATTCGGCCAAGTATGGTC-3′)

LSU [28S] α (GTCCTGCCACACTCAGGTCTGA-3′)

SSU [18S] rRNA (5′-AAATGATCCAGCTGCAGGTTCACCTA-3′)

RNA B (5′-ATAAAACGATTCTGTTCAGAGTACGGTCTT-3′).

Prior to autoradiography, the blots were washed twice with 2$\times$ SSC (300 mM NaCl, 30 mM Na_3 citrate–$2H_2O$, pH 7.0)/0.1% SDS for 5 min, twice with 0.2$\times$ SSC/0.1% SDS, and finally once with 2$\times$ SSC, all at room temperature.

Sucrose gradient analysis. Nuclear extracts were dialyzed against Buffer A for 4 h and flash frozen in liquid nitrogen. One-half milliliter of nuclear extract was layered on a continuous 10–30% sucrose gradient in Buffer A containing 10 mM $MgCl_2$. Gradients were sedimented for 22 h at 150,000*g* at 4°C and 28 fractions (400 μl each) were collected. Fractions were subdivided into separate equal aliquots for Northern and Western blot analysis, respectively. *S* values were determined relative to bovine rRNA species (Sigma),

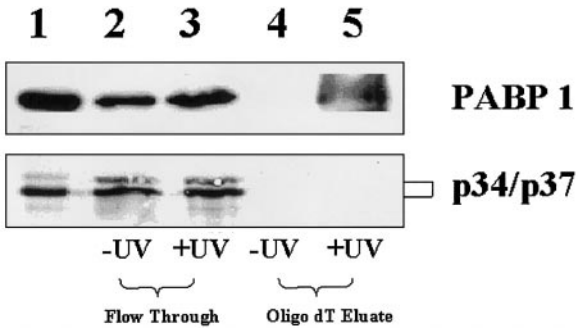

FIG. 1. UV crosslinking studies. *T. brucei* cells were irradiated *in vivo* with UV light, and poly(A)$^+$ RNA populations isolated from oligo dT columns as described under Materials and Methods. Western blot analysis was performed with antibodies specific to the indicated proteins. Lane 1, 1 μg whole cell lysate; lane 2, flowthrough negative control (−UV); lane 3, flowthrough experimental (+UV); lane 4, column eluate negative control; lane 5, column eluate experimental.

which were detected by ethidium bromide staining of 1.5% agarose gels.

pCp labeling. Fractions from sucrose gradients containing p34/p37 and 5S rRNA were used for immunoprecipitation. One hundred microliters of Buffer A was added to 100 μl of sample. Coimmunoprecipitation of RNA using anti-p34/p37 antibodies and preparation of RNA for analysis was then performed as described above. Labeling of RNA species was performed according to the protocol of Militello and Read (37). In brief, RNA pellets were resuspended to a final volume of 20 μl using 3 μl of glycerol, 2 μl DMSO, 2 μl 10× pCp buffer (25 mM Hepes, pH 8.3, 5 mM $MgCl_2$, 1.6 mM DTT, 0.25 mM ATP), 2 μl [5′ ^{32}P]pCp, and 2 μl T4 RNA ligase. Reactions were performed overnight at 4°C. The samples were phenol/chloroform extracted and ethanol precipitated. RNA analysis was performed using 6% acrylamide/8 M urea gels. Sizes of labeled RNAs were determined relative to RNA size markers (Ambion).

RESULTS

p34 and p37 Are Found in Association with Poly(A)$^-$ but Not Poly(A)$^+$ RNA

In *T. brucei*, posttranscriptional RNA processes play a major role in gene regulation. Since p34 and p37 are developmentally regulated, it was of interest to determine whether these proteins species were associated with poly(A)$^+$ RNA populations considering the differential stability of life cycle-specific mRNAs. We performed *in vivo* UV crosslinking studies using procyclic cell cultures, and isolated mRNA by oligo dT chromatography as described under Materials and Methods. As shown in Fig. 1, the *T. brucei* poly(A) binding protein 1 homologue (PABP1) was found in association with poly(A)$^+$ RNA by Western blot analysis, but only when the cells were irradiated by UV light (compare lane 4 with no signal and lane 5 with a strong positive signal). A portion of unbound PABP1 was also found in flow through fractions from both the control and experimental samples, indicating the presence of free protein (lanes 2 and 3). In contrast, no p34/p37 was found to be associated with poly(A)$^+$ RNA under these experimental conditions as seen by the absence of signal in both lanes 4 and 5. Under the experimental conditions employed, all of the protein was found in a fraction representing free protein or poly(A)$^-$ bound protein (lane 3). Both p34 and p37 are very abundant proteins in *T. brucei*, representing ~0.1% total cellular protein (31), similar in abundance to PABP1 and therefore should be detectable in these experiments if such an association exists.

5S rRNA Coimmunoprecipitates with p34 and p37

To specifically identify the RNA species that interact with p34 and p37, we tested for association of these proteins with poly(A)$^-$ species by immunoprecipitation. Nuclear enriched extracts were prepared in the presence of RNase inhibitors and used in coimmunoprecipitation studies. After immunoprecipitation with anti-p34/p37 antiserum, the presence of several specific RNA species in the precipitates was examined by Northern analysis. The results shown in Fig. 2 demonstrate that the *T. brucei* 5S rRNA was coprecipitated by the anti-p34/p37 antiserum (lane 3), but was not detected when preimmune antibodies were used (lane 2). In contrast, the *T. brucei* U3 snoRNA homologue, and the SSU rRNA molecule and its precursors were not detected in the immunoprecipitated complexes (lane 3 compared with total RNA in lane 1). Similar results were obtained when Northern analysis was performed using probes specific for LSU rRNA and 5.8S rRNA (data not shown). These results are consistent with other data showing that p34/p37 is/are found in association with 5S rRNA under native conditions

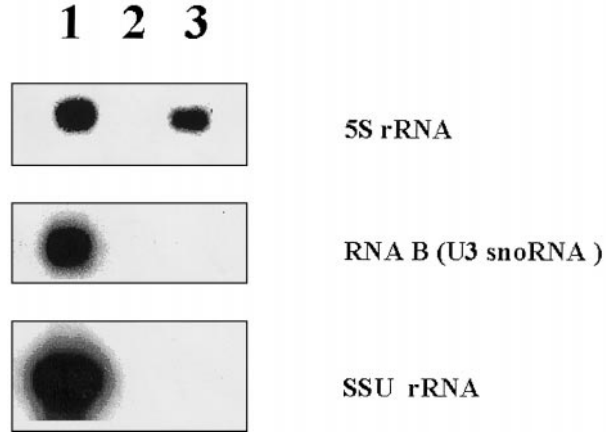

FIG. 2. Coimmunoprecipitation of 5S rRNA with p34 and p37. Procyclic form *T. brucei* nuclear extract containing 1 mg of protein was incubated with 50 μl of preimmune or polyclonal anti-p34/p37 antiserum. RNA species coimmunoprecipitated from nuclear enriched extracts were transferred to nylon membranes. Probes specific for the indicated rRNA molecules and the RNA B were used for Northern blot analysis. Lane 1, 20 ng of *T. brucei* whole cell RNA; lane 2, preimmune serum negative control coimmunoprecipitates; lane 3, anti-p34/p37 antiserum experimental coimmunoprecipitates.

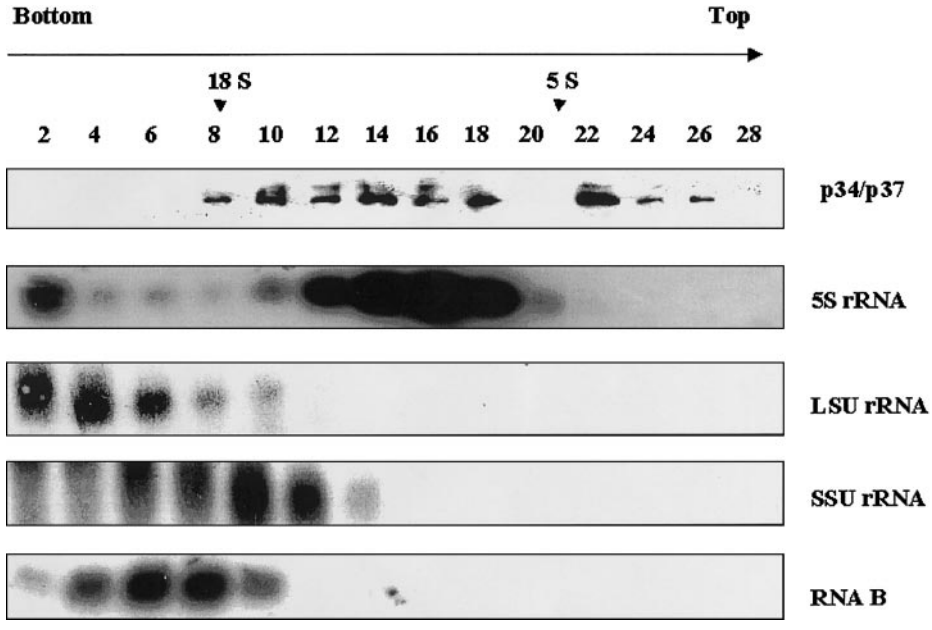

FIG. 3. Sucrose gradient analysis of nuclear extracts. Procyclic form nuclear extracts were sedimented on 10–30% sucrose gradients. The positions of the p34 and p37 were detected by Western blot analysis and the position of the indicated rRNA species and RNA B were detected by Northern hybridization. Fraction 2 designates the bottom of the gradient. The positions of bovine rRNA markers are indicated by arrowheads.

(see below), but not with the other ribosomal and nuclear RNAs tested.

p34 and p37 Cosediment with 5S rRNA

To extend our observations, we wished to determine whether p34 and p37 and 5S rRNA present in *T. brucei* nuclear extracts formed a complex that could be detected by sedimentation analysis and whether associations with other rRNA species might be detected under these experimental conditions. Nuclear extracts were prepared from procyclic cells as described under Materials and Methods and separated on 10–30% sucrose gradients. The results are presented in Fig. 3 and show that p34 and p37 were present in two major peaks, between fractions 8–18, and fractions 20–24. A significant portion of the total p34 and p37 in the sample is found to cosediment with the 5S rRNA fractions (fractions 12–18) and is well separated from the second peak of p34 and p37 at the top of the gradient (fractions 20–24). The distribution was consistent in five separate experiments.

Under the sedimentation conditions used, the SSU and LSU rRNA and their precursor species were detected primarily at the bottom of the gradient in fractions 2–12. RNA B, the *T. brucei* U3 snoRNA homologue that is involved in processing of pre-SSU rRNA (27), was found to sediment in the region of the SSU rRNA though closer to the region of the SSU precursor and its external spacer. A portion of p34 and p37 overlaps with both SSU rRNA and RNA B in fractions 8–12, and might represent an association with these rRNA precursors under these experimental conditions. However, in conjunction with our observations from the coimmunoprecipitation studies, these results indicate that a substantial fraction of p34 and p37 is in a stable association with 5S rRNA in procyclic extracts.

pCp Labeling Studies

In the sucrose gradient experiment described above, 5S rRNA had the most significant overlap with p34/p37 compared to the other rRNA species identified. pCp labeling studies were performed to confirm that p34 and p37 are associated with 5S rRNA in these fractions, and to identify any other RNA species which might be present in these gradient fractions and associate with p34 and p37, but not be detected by the panel of probes used. Aliquots from procyclic form gradient fractions that contained p34/p37 and 5S rRNA were divided into two parts. In one half, all RNA species present within these fractions were 3′ end labeled with [^{32}P]pCp. In the other half, immunoprecipitates using anti-p34/p37 antibodies were obtained and the RNA species present in the precipitates were also labeled with [^{32}P]pCp. A parallel series of experiments was performed using preimmune serum. As shown in Fig. 4, preimmune antibodies did not precipitate any RNA (lane 2). Lane 4 shows those RNA species present in the entire (not yet immunoprecipitated) fraction including species migrating slightly above 100 nucleotides and between 50 and 75 nucleotides. Other major

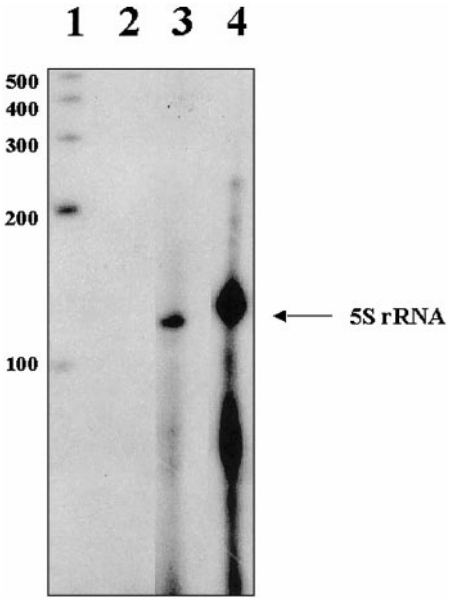

FIG. 4. pCp labeling of RNA. Gradient fractions containing p34/p37 and 5S rRNA (corresponding to fractions 16 and 18 in Fig. 3) were used in coimmunoprecipitation analysis using anti-p34/p37 antibodies. Bound RNA was analyzed by 3′ end labeling as described under Materials and Methods. Experimental fractions were compared to all RNA species labeled in the fractions and to a control coimmunoprecipitation using preimmune antibodies. Lane 1, RNA size standards in bases; lane 2, preimmune precipitate; lane 3, anti-p34/p37 immunoprecipitate; lane 4, total RNA in fraction.

RNA species are not present in these fractions. In lane 3 only a single species migrating at slightly above 100 bases was precipitated to a significant extent by the anti-p34/p37 antibodies. The size of the precipitated species is in good agreement with the known 120 nucleotide length of *T. brucei* 5S rRNA. It also migrates at a position comparable to a band present in an underloaded sample of the fraction equivalent to that in lane 4 which hybridizes with the 5S rRNA probe (data not shown). Quantification of immunoprecipitation experiments showed that 2–5% of the total cellular 5S rRNA was found in association with p34 and p37 comparable to the 5S rRNA found in association with the La protein in higher eukaryotes (see discussion below). Species migrating above 120 nucleotides were never detected in the experimental fractions upon longer exposures. There was not a significant coprecipitation of species in the range of 50–75 bases, which are present in abundant amounts in the whole RNA fractions (lane 4). Predicted RNA species of this size are tRNAs and the recently characterized box C/D snoRNAs (28). These data again indicate that of the RNA species found in these gradient fractions, the 5S rRNA is bound with the greatest selectivity.

Association of p37 with 5S rRNA in Bloodstream Form T. brucei

We also wished to determine whether a similar association of these RNA binding proteins with 5S rRNA occurs in bloodstream form parasites where there are significant differences in the potential binding partners. Previous results (31) showed that the two RNA binding proteins, p34 and p37, are developmentally regulated. Both proteins are expressed in the procyclic form with p34 being much more abundant than p37, while only p37 is expressed in bloodstream form parasites. As indicated in Fig. 5, the p37 protein sediments in the same region of the gradient as the 5S rRNA between fractions 14–20. In these experiments, the presence of a second peak of p37 at the top of the gradient (fraction 20), presumed to be free protein, was not observed as had been seen with extracts of procyclic forms, perhaps suggesting a stronger association with 5S rRNA in bloodstream forms. The presence of the LSU and SSU rRNAs was also assessed, and their distribution was similar to that seen in the procyclic parasites (data not shown).

DISCUSSION

Here we report the association of two nuclear RNA binding proteins from *T. brucei*, p34 and p37, with 5S

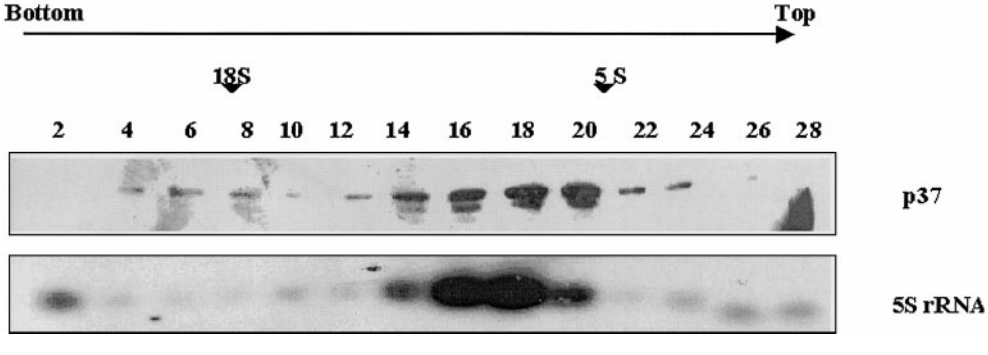

FIG. 5. Sucrose gradient analysis of bloodstream form nuclear extracts. Bloodstream form nuclear extracts were sedimented on 10–30% sucrose gradients. The position of the p37 protein was detected by Western blot analysis and the position of 5S rRNA was detected by Northern hybridization. Fraction 2 designates the bottom of the gradient. The position of bovine rRNA markers is indicated by arrowheads.

rRNA. UV cross-linking studies showed that p34 and p37 were not associated with poly(A)$^+$ RNA. Coimmunoprecipitation and sucrose gradient approaches were used to determine the poly(A)$^-$ RNA species with which p34 and p37 might associate. Separate anti-p34/p37 coimmunoprecipitation studies were performed under two distinct conditions: using a whole nuclear extract and a nuclear extract fractionated on sucrose gradients as the source of p34/p37 protein-RNA complexes. In both cases, the 5S rRNA was specifically coimmunoprecipitated with p34 and p37. Our experiments demonstrated that p34 and p37 did not coprecipitate with RNA B (U3 snoRNA) or SSU rRNA and its precursors. The pCp labeling studies of these gradient fractions confirmed that the only RNA species significantly associated with p34 and p37 is 5S rRNA. Thus the association of the p34 and p37 proteins with 5S rRNA in our experiments is quite specific in relation to several other RNAs involved in ribosomal biogenesis.

In yeast and metazoans the 5S rRNA transcription units are transcribed in the nucleoplasm by RNA polymerase III (38, 39) separate from the rDNA repeat units, which serve as the nucleolar organizer regions and are transcribed by RNA polymerase I. In *T. brucei*, the arrangement of the genome is similar to that found in higher eukaryotes, with distinct rDNA transcription units, and polymerase II and III transcribed genes closely interspersed throughout the rest of the genome (8, 40, 41). Therefore it is likely that the 5S rRNA of *T. brucei* is also transcribed in the nucleoplasm. In higher eukaryotes, 5S rRNA metabolism begins with association of the primary transcript with the La autoantigen (42, 43). The La protein possesses an enzymatic activity that removes 3′ end U residues resulting in the mature 5S species (43). This association is transient, and only about 1–2% of free 5S rRNA containing particles contain the La protein (44, 45). The 5S rRNA is then bound by the ribosomal protein L5 (homologue to yeast L3) as part of an ~7S RNP particle. In higher eukaryotes, this interaction with L5 involves ~95% of the bound 5S rRNA in the cell (44). This complex migrates to the nucleolus where ribosomal assembly proceeds (38, 44, 45, 46).

Homologues to both of the L5 and La proteins have been found in *T. brucei* (47, 48) and an interaction of 5S rRNA with an L5 homologue has been demonstrated (47). Quantification of the 5S rRNA coimmunoprecipitated with p34 and p37 from nuclear extracts demonstrated that 2–5% of the total 5S rRNA is associated with p34 and p37 (data not shown), comparable to the fraction of 5S rRNA bound by the La protein in higher eukaryotes. Thus the fraction of 5S rRNA bound by p34 and p37 is comparable to the amount of 5S rRNA bound by another protein involved in ribosomal biogenesis.

In yeast and eukaryotes, 5S rRNA metabolism is believed to serve a central role in regulating ribosomal biogenesis. It has been hypothesized that the 5S rRNA gene has been separated from the major rRNA unit for this purpose (40). The majority of 5S rRNA in the nucleus is not found in association with pre-rRNA particles in the nucleolus, but rather is present as free RNP particles in the nucleoplasm (49, 50). The rate of incorporation of 5S rRNA into pre-rRNA during processing is believed to determine the rate of ribosomal biogenesis (51). Consistent with these observations, the sucrose gradient data show that the majority of 5S rRNA in nuclear extracts is found as free RNP particles separate from the major rRNA species. This suggests that, in *T. brucei*, a similar role in regulating ribosomal levels may be played by those proteins found in association with 5S rRNA. Corroborating evidence for such a fundamental role in the *T. brucei* life cycle comes from recent RNA interference experiments which indicate that these proteins are required for normal growth (52; J. Li, unpublished data).

We currently do not know the function of the interaction of p34 and p37 with 5S rRNA. It is possible that the association occurs during the transit of 5S rRNA from the nucleoplasm to the nucleolus based on the localization of these proteins and this RNA species to the entire nucleus. Transcription of 5S rRNA in the nucleoplasm and the subsequent transport of this species into the nucleolus favors p34 and p37 as serving a bridging role between these cellular compartments. It should also be noted that the sucrose gradient data also show that a portion of both p34 and p37 do not cosediment with the 5S rRNA. Thus their association with the 5S rRNA does not rule out other important functions for these highly abundant RNA binding proteins.

Another important issue concerns how this complex between p34 and p37 and 5S rRNA may function in ribosomal biogenesis. In higher eukaryotes it is known that rRNA processing occurs within very large multicomponent complexes (46, 53). The interactions that were detected in this study may thus be stable associations within a much larger but more loosely associated complex. A portion of p34 and p37 was found in regions of the sucrose gradient experiment that also contained the SSU rRNA and RNA B. However, the coimmunoprecipitation experiments did not show any specific interactions between p34 and p37 and these species. This, however, does not rule out the possibility of transient associations between these RNAs and the components of the p34/p37 complexes during rRNA processing. We are currently undertaking experiments to identify those *T. brucei* proteins that interact with p34 and p37 and may participate in these RNA biogenesis events.

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