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Biochemistry

RNA–protein complexes mediate *in vitro* capping of the spliced-leader primary transcript and U-RNAs in *Trypanosoma cruzi*

(RNA guanylyltransferase/*in vitro* capping/small nuclear ribonucleoprotein particle/trans-splicing)

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ABSTRACT **A 39-nucleotide spliced leader (SL) is joined to the 5′ ends of trypanosome mRNAs in a bimolecular or trans-splicing process. The SL in *Trypanosoma cruzi* is transcribed as an ≈110-nucleotide RNA (SL-RNA or SL primary transcript) bearing the 39-nucleotide SL at the 5′ end. The SL-RNA is 5′ capped by a guanylyltransferase activity prior to trans-splicing and trypanosome mRNAs thus obtain their mature caps from the SL by trans-splicing. We have previously characterized a guanylyltransferase activity from *T. cruzi* nuclear extracts and shown that this capping activity has an unusual ATP dependence and an apparent specificity for the SL-RNA and U-RNAs. Herein, we show that the capping activity sediments as a 12–15S particle during velocity sedimentation in glycerol gradients and fractionates as a >150-kDa particle during large-pore gel filtration chromatography. Moreover, the endogenous substrate RNAs—the SL-RNA and U-RNAs—consistently copurify with the capping activity, suggesting that the activity and the substrates form a ribonucleoprotein particle. The capping activity and substrate RNAs are not dissociated in isopycnic Cs_2SO_4 gradients and band at a density expected for an RNA–protein complex, confirming the existence of ribonucleoprotein particles bearing both the activity and its substrate RNAs. Finally, we partially purified these ribonucleoprotein particles and showed that the capping activity remains ATP dependent and highly specific for the SL-RNA and the U-RNAs. These observations are consistent with the hypothesis that one of the functions of trans-splicing is for mRNA capping.**

mRNA maturation in the kinetoplastid protozoa requires an unusual trans-splicing process in which a 39-nucleotide leader, the spliced leader (SL), from a small abundant transcript, the SL primary transcript (SL-RNA), is ligated to the 5′ ends of structural gene primary transcripts (1). The SL-RNA obtains an unusual 5′ cap that is transferred to the mature mRNA with the SL (2–5). In other eukaryotes, mRNA primary transcripts are capped shortly after transcription initiation by guanylyltransferases (GTases) that are probably associated with the RNA polymerase II (Pol II) initiation complex in a reaction requiring a 5′-tri- or -diphosphate substrate RNA (6). Although capping is thought to be mediated by the Pol II transcription initiation complex (6), soluble partially purified GTases lack substrate specificity and require only the energy from cleavage of the GTP substrate for activity. In the kinetoplastid protozoa, there is no evidence that mRNA primary transcripts are capped, many genes are transcribed as "multicistronic" RNAs that are processed into monocistronic mRNAs (cf. refs. 7 and 8), and some protein coding genes may not be transcribed by a normal α-amanitin-sensitive Pol II (9–11). Thus, it has been proposed that trans-splicing evolved in the kinetoplastid protozoa as a means of capping protein coding mRNAs (12).

We have established (13) nuclear extracts from *Trypanosoma cruzi* that cap the SL-RNA and at least two U-RNAs, U2 and an unassigned U-RNA, Ux. The GTase activity in these extracts exhibits remarkable specificity for the SL-RNA and these two U-RNAs and has an unexpected requirement for ATP. Herein, we report that the *T. cruzi* capping activity and the substrate RNAs cosediment as particles at 12–15 S in glycerol gradients, and fractionate as >150-kDa particles during large-pore gel filtration chromatography. Moreover, the activity and the substrate RNAs band together on isopycnic Cs_2SO_4 gradients at densities consistent with that expected for small nuclear ribonucleoprotein particles (snRNPs). Finally, the partially purified capping activity maintains both its apparent specificity for the SL-RNA and U-RNAs and its unusual ATP dependence.

MATERIALS AND METHODS

Culture of *T. cruzi* and Preparation of Nuclear Extract. *T. cruzi* epimastigotes were cultured in LIT medium at 28°C with gentle agitation, as described (14). Cells were harvested and homogenized, and nuclear extracts were prepared by the procedure of Dignam *et al.* (15) modified as described (14). Nuclear extracts were fractionated and stored at −70°C.

Capping Reactions and Analysis of the Products. Except where indicated, standard capping reactions were performed in 25 μl of 20 mM Hepes·KOH, pH 7.9/50 mM KCl/0.5 mM dithiothreitol/1 mM $MgCl_2$/1 mM ATP/30 μCi of [α-^{32}P]GTP (3000 Ci/mmol; 1 Ci = 37 GBq; ICN)/10% (vol/vol) glycerol. Reaction mixtures were incubated for 30 min at 30°C. Products were extracted once with phenol and once with chloroform, precipitated with ethanol in the presence of 10 μg of dextran carrier (42 kDa, Sigma), and electrophoresed in 8% polyacrylamide/7 M urea gels. Labeled (capped) products were detected by autoradiography.

RESULTS

Velocity Gradient Sedimentation of the *T. cruzi* Capping Activity. We have characterized (13) the *T. cruzi* GTase activity in crude nuclear extracts. To further examine the activity, we fractionated *T. cruzi* nuclear extracts by velocity sedimentation in 10–30% glycerol gradients (Fig. 1). Fractions removed from the gradients were assayed for protein concentration (Fig. 1*A*), and capping activity was measured directly in the presence of ATP and [α-^{32}P]GTP (Fig. 1*B*). Maximal capping activity of the endogenous SL-RNA and U-RNAs was observed at 12–15 S. In contrast, the free capped SL-RNA and U-RNAs partitioned at ≈5 S in identical

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Abbreviations: GTase, guanylyltransferase; v-GTase, vaccinia virus GTase; snRNP, small nuclear ribonucleoprotein particle; SL, spliced leader; SL-RNA, spliced-leader primary transcript; Pol, polymerase.
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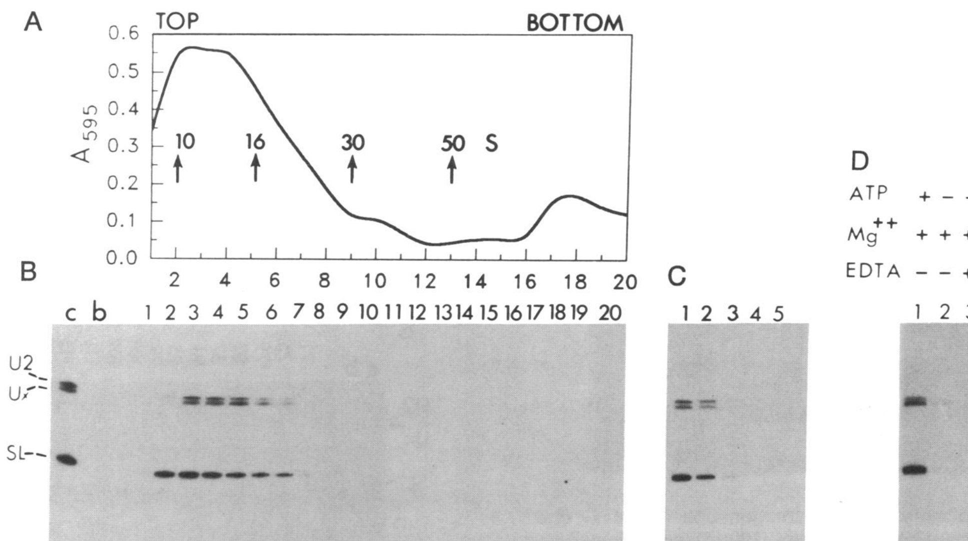

FIG. 1. Sedimentation analysis of *T. cruzi* capping activity. *T. cruzi* nuclear extract was dialyzed for two 3-hr periods at 4°C against 50 vol of buffer Dx [20 mM Hepes·KOH, pH 7.9/50 mM KCl/1 mM $MgCl_2$/0.5 mM dithiothreitol/10% glycerol (15)]. Samples (100 μl) of dialyzed extract were loaded onto 4-ml 10–30% glycerol gradients in buffer Dx at 4°C and centrifuged 3 hr (4°C at 40,000 rpm) in a Sorvall TST 60.4 rotor. Twenty 200-μl fractions were collected from the top of each gradient and 20-μl samples of each fraction were assayed for protein concentration using the Bio-Rad protein assay kit and measuring absorption at 595 nm, as suggested by the manufacturer. Molecular size standards [*Escherichia coli* 50S and 30S ribosome subunits, 16S and 23S rRNAs (Boehringer Mannheim), spleen apoferritin (17.6 S; 440 kDa, Sigma), bovine heart cytochrome *c* (5 S; 12.3 kDa, Sigma), and chicken egg white conalbumin (12.5 S; 43 kDa, Sigma)] were centrifuged in parallel to calibrate the gradients. (*A*) Protein concentration. Arrows 10, 16, 30, and 50 S indicate approximate positions of size standards on parallel gradients. (*B*) Capping activity assay. Samples (25 μl) from each fraction were transferred to tubes containing appropriate amounts of desiccated ATP and [α-^{32}P]GTP and incubated at 30°C for 30 min. Reaction products were isolated, concentrated, electrophoresed, and analyzed. Lanes: c, control capping reaction mixture with 10 μl of nuclear extract; b, blank reaction mixture, no extract; 1–20, samples from corresponding fractions of the gradient. (Note: no activity was observed in fraction 1 and peak activity was in lane 4.) The positions of U2, Ux, and SL-RNA (SL) are indicated. (*C*) Sedimentation of purified RNAs. SL-, U2, and Ux RNAs purified from crude nuclear extract after ^{32}P labeling in a capping reaction were fractionated in a glycerol gradient as described in *A*. Lane labels correspond to gradient fraction numbers. Almost all of the labeled RNA was found in fractions 1 and 2 (top of the gradient). No labeled RNA was observed in fractions 6–20 (data not shown). (*D*) ATP and Mg^{2+} requirements of partially purified capping activity. Fraction 4 from *A* was examined for capping activity in the presence (+) or absence (−) of 1 mM ATP, 1 mM Mg^{2+}, and 5 mM EDTA, as indicated, in 25-μl reaction mixtures as described in *B*.

parallel gradients (Fig. 1*C*). The fact that the capping activity and the endogenous RNA substrates comigrated in these gradients at 12–15 S suggests the possibility that the enzyme and the substrates may exist as part of a snRNP.

To determine whether the partially purified capping activity maintained its ATP and magnesium dependence, standard capping reactions were carried out with samples of the fraction with peak activity (fraction 4) in the presence or absence of ATP, Mg^{2+}, and EDTA (Fig. 1*D*). Clearly, very little capping was observed in the absence of exogenous ATP (lane 2), and chelation of Mg^{2+} by EDTA abolished activity (lane 3). Thus, the unusual ATP dependence was maintained after partial purification of the 12–15S particle(s) bearing the capping enzymes and the endogenous substrate RNAs.

Cs_2SO_4 Density Gradient Fractionation of the *T. cruzi* Capping Activity. The velocity sedimentation experiment suggested that both the *T. cruzi* capping activity and the substrate RNAs fractionate at 12–15 S. It has been shown (16) that the SL-RNA and U-RNAs of *Trypanosoma brucei* fractionate as snRNPs in isopycnic Cs_2SO_4 gradients. To examine the possibility that the *T. cruzi* capping activity and the substrate RNAs make up snRNPs, *T. cruzi* nuclear extracts were fractionated in Cs_2SO_4 gradients essentially as described (17, 18). Each fraction was dialyzed and the protein concentration and density were determined (Fig. 2*A*). Each dialyzed fraction was also tested directly for capping activity (Fig. 2*B*), and peak activity was found at a density of $\rho = \approx 1.26$ g/cm^3, significantly more dense than bulk protein. In fact, this is exactly the density observed for RNP complexes fractionated in very similar gradients (17). Thus, these observations argue that the *T. cruzi* capping activity and its endogenous RNA substrates are components of one or more specific snRNPs.

Chromatographic Fractionation and Partial Purification of the *T. cruzi* Capping Activity. Large-pore gel filtration chromatography has been applied in the purification of RNP components of mRNA splicing machinery from other eukaryotes (for example, see ref. 19). Thus, we fractionated the *T. cruzi* nuclear extracts by large-pore gel filtration chromatography using Sephacryl S-400 HR (Fig. 3). Protein concentration was determined for each fraction (Fig. 3*A*), and a sample of each was directly examined for capping activity (Fig. 3*B*). The capping activity eluted in a tight peak (fractions 111–115), indicating that this putative complex resolved very well in this chromatograph. The activity resolved between molecular mass standards apoferritin (440 kDa) and ovalbumin (44 kDa), and we estimate the size of the complex to be at least 150 kDa.

Fractions 108–118 from the Sephacryl S-400 HR column were pooled, concentrated to 350 μl in a Centricon-30 microconcentrator (Amicon), and fractionated on a column of Sepharose CL-6B (Fig. 4). Again, protein concentration was determined for each fraction (Fig. 4*A*), and samples were assayed directly for capping activity (Fig. 4*B*). Peak activity eluted between molecular mass standards apoferritin (440 kDa, 17.6 S) and conalbumin (43 kDa, 12.5 S), consistent with the above results suggesting a molecular mass of at least 150 kDa or 12–15 S.

The amount of total protein remaining in the pooled fractions containing the peak capping activity after the two chromatography steps represents <1% of the input. We estimate, based on radioactivity incorporated into RNA, that we lost roughly 50% of the activity during these fraction-

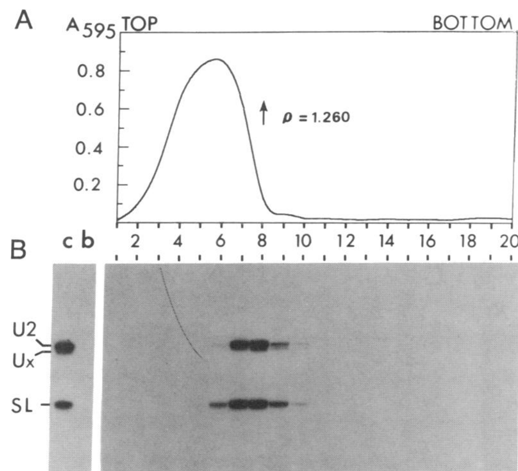

FIG. 2. Cs_2SO_4 density gradient fractionation of the *T. cruzi* capping activity. *T. cruzi* nuclear extract (100 μl) was adjusted to a density of 1.15 g/ml by addition of solid Cs_2SO_4 and loaded to the top of a 4-ml centrifuge tube containing 0.78-ml steps of Cs_2SO_4 at 1.60, 1.52, 1.40, 1.27, and 1.15 g/ml in buffer containing 20 mM Hepes (pH 7.9), 1 mM $MgCl_2$, 0.5 mM dithiothreitol, and 10% glycerol. The gradient was stored overnight at 4°C prior to loading the extract and centrifuged for 20 hr at 40,000 rpm at 4°C in a Sorvall TST60.4 rotor. Fractions were collected and protein concentrations were determined as described in Fig. 1. The density of each fraction was determined by weighing a 50-μl sample and confirmed by determining its refractive index. The fractions were dialyzed overnight at 4°C against buffer Dx (five 200-ml changes) and assayed directly for capping activity. (*A*) Protein concentration. Fraction numbers are indicated. ρ, Density at peak capping activity. (*B*) Capping activity. Lanes: c, control reaction mixture with unfractionated nuclear extract; b, blank reaction mixture with no extract. Other lanes are identified by fraction number. The positions of U2, Ux, and SL-RNA are shown. Similar gradients in which the crude nuclear extracts were brought to ρ = 1.35 g/ml in Cs_2SO_4 and centrifuged to equilibrium (73 hr, 40,000 rpm, 4°C) in a Sorvall TST60.4 rotor gave essentially identical results (data not shown).

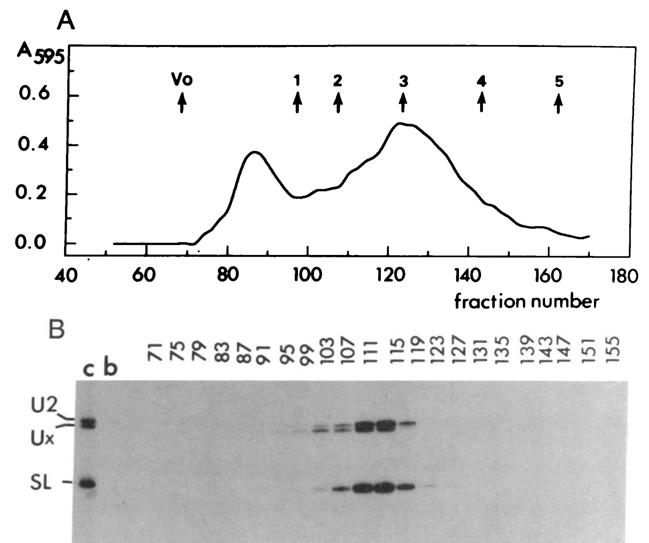

FIG. 3. Capping activity fractionated by Sephacryl gel filtration chromatography. *T. cruzi* nuclear extract (0.5 ml) dialyzed as described in Fig. 1 was loaded to a 1 × 50 cm Sephacryl S-400 HR (Pharmacia) column preequilibrated at 4°C in buffer Dx, and 230- to 240-μl fractions were collected at a flow rate ≈1.5 ml/hr. (*A*) Protein concentrations. Samples (20 μl) of each fraction were assayed for protein at A_{595} as described in Fig. 1. Arrows: V_o, void volume; 1–5, protein molecular mass standards [thyroglobulin (670 kDa, Bio-Rad), apoferritin (440 kDa), ovalbumin (44 kDa, Bio-Rad), cytochrome *c* (12.3 kDa), vitamin B_{12} (1.3 kDa, Bio-Rad), respectively]. (*B*) Capping activity. Samples (25 μl) of fractions 71–155 were assayed directly for capping activity. Activity peaks in fractions 111–115 represent an estimated molecular mass of >150 kDa. Lanes c and b show control reaction mixtures using crude nuclear extract or no extract, respectively, as described in Fig. 1. The positions of U2, Ux, and SL-RNAs are shown.

ations. Thus, in terms of specific enzyme activity per mg of protein, the partially purified fractions are ≈50-fold purified over the input material. SDS/polyacrylamide gel electrophoresis shows less than 10 major proteins remaining in these fractions (data not shown).

The capping activity in the pooled concentrated fractions from the two serial gel filtration steps was examined for its ATP and Mg^{2+} requirements (Fig. 4*C*). As expected from previous observations, very little activity was observed in the absence of ATP (lane 2), and activity was abolished in the presence of EDTA. Thus, the partially purified capping activity remains highly ATP dependent.

Specificity of the *T. cruzi* Capping Activity for the SL-RNA and U-RNAs. We have shown (13) that the *T. cruzi* activity in crude nuclear extracts is unable to recognize or cap exogenous heterologous transcripts; i.e., only the SL-RNA and U-RNAs are capped. This apparent specificity of the *T. cruzi* capping activity is consistent with the existence of a snRNP capping complex bearing the capping enzymes and the SL-RNA or one of the U-RNAs. If the GTase were specifically bound in snRNPs containing the substrate RNAs, it would be unable to recognize and cap other RNAs. Thus, we assessed the specificity of the partially purified capping activity for the SL-RNA and the U-RNAs (Fig. 5). RNAs generated by transcription of plasmid pGEM-2 (Promega) with T7 or SP6 RNA polymerase in the presence of [α-^{32}P]ATP (Fig. 5 *A* and *B*, lanes 1 and 2, respectively) were incubated in a standard capping reaction mixture with the partially purified *T. cruzi* capping activity and [α-^{32}P]GTP (lanes 4 and 5). The SL-RNA, U2, and Ux were labeled (capped) in these reactions, but the exogenous T7 and SP6 RNAs showed no mobility changes (Fig. 5*B* shows a shorter exposure of this gel that confirms this observation), suggesting that these RNAs were not capped. To verify this conclusion, the exogenous RNAs were excised from the gel and digested with P1 nuclease, and the products were analyzed by thin layer chromatography (TLC) in parallel with cap dinucleotide (GpppG) and AMP standards (Fig. 5*C*, lanes 4 and 5). No cap dinucleotide was observed even after extended autoradiographic exposures (data not shown), confirming the lack of activity of the exogenous RNAs in the *T. cruzi* capping reaction.

Similar experiments in which vaccinia virus GTase (v-GTase) was added to the capping reaction mixtures demonstrated that the exogenous transcripts were efficiently capped; i.e., their 5′ termini acted as substrate for v-GTase. As expected, under conditions optimal for v-GTase activity but in the absence of v-GTase and ATP, the partially purified *T. cruzi* capping activity did not cap the endogenous (Fig. 5, lanes 6) or the exogenous RNAs (lanes 7 and 8). However, when v-GTase was added to these reaction mixtures, the exogenous transcripts exhibited decreased mobility (lanes 9 and 10) and showed abundant cap dinucleotide after P1 digestion and TLC analysis (Fig. 5*C*, lanes 9 and 10); i.e., these RNAs were efficiently capped by v-GTase. Thus, these results confirm that the exogenous RNAs bear cappable 5′ termini but are not recognized and capped by the endogenous *T. cruzi* activity.

DISCUSSION

Several lines of evidence suggest that the *T. cruzi* capping activity and its substrate RNAs exist in snRNPs in our *T. cruzi* nuclear extracts. (*i*) The capping activity and the substrate RNAs copurify in velocity gradient sedimentation,

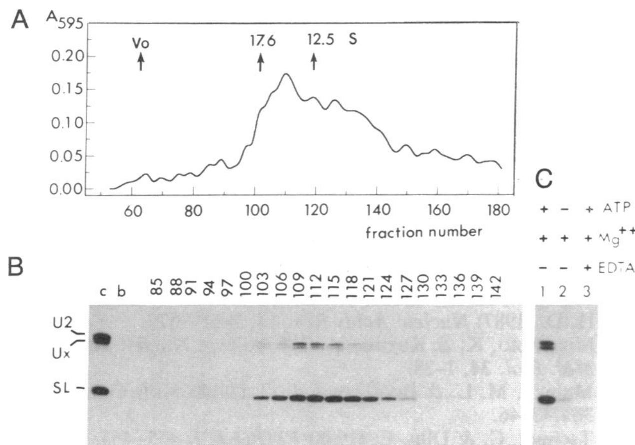

FIG. 4. Capping activity fractionated by Sepharose gel filtration chromatography. Fractions 108–118 from the Sephacryl column (Fig. 3) were pooled and concentrated to 350 μl in a Centricon-30 microconcentrator (Amicon) and loaded on a 1 × 30 cm column of Sepharose CL-6B (Pharmacia) preequilibrated at 4°C in buffer Dx and ≈150-μl fractions were collected at a flow rate of ≈1 ml/hr. (*A*) Protein concentration. Protein was determined in each fraction as described in Fig. 1. Arrows: V_o, void volume; 17.6 S, apoferritin (440 kDa); 12.5 S, conalbumin (23 kDa). (*B*) Capping activity. Samples (25 μl) of each fraction, as indicated, were assayed for capping activity. Lanes c and b show control reaction mixtures using partially purified pooled fractions from the Sephacryl column or no extract, respectively, as described in Fig. 1. The positions of U2, Ux, the SL-RNAs are shown. (*C*) ATP and Mg^{2+} dependence of partially purified capping activity. Fractions 107–116 containing the peak of capping activity were pooled (≈1 ml total), concentrated to 200 μl by Centricon-30 filtration, and stored at −70°C. Samples (5 μl) were assayed in the presence (+) or absence (−) of 1 mM ATP, 1 mM $MgCl_2$, and 5 mM EDTA in 25-μl reaction mixtures, as indicated.

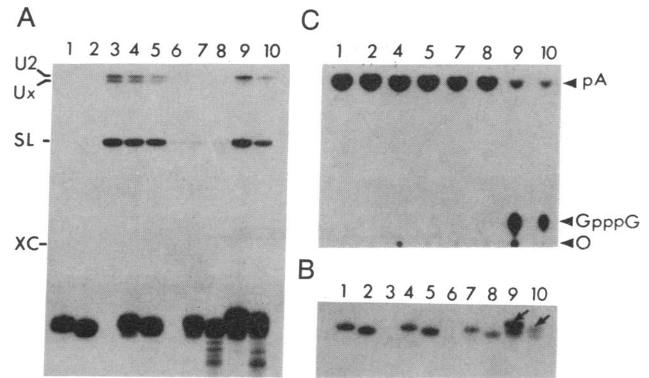

FIG. 5. Specificity of the partially purified capping activity. (*A*) Capping reactions in the presence of exogenous RNAs. Samples (5 μl) of the pooled concentrated fractions after Sephacryl and Sepharose chromatographies were assayed for capping activity on exogenously supplied RNA substrates in 25-μl reaction mixtures. Exogenous substrate was synthesized by transcription of *Eco*RI- or *Hin*dIII-linearized pGEM-2 (Promega) with T7 or SP6 RNA polymerase, respectively, in the presence of all four NTPs (each at 500 mM) and [α-^{32}P]ATP (2 mCi/ml; 800 Ci/mmol; NEN) as described (14). Lanes: 1 and 2, ≈20,000 cpm of the 59-nucleotide T7 or 58-nucleotide SP6 transcript, respectively; 3, control capping reaction mixture containing 5 μl of partially purified concentrated capping activity and 30 μCi of [α-^{32}P]GTP; 4 and 5, as for lane 3 except for the addition of ≈20,000 cpm of the T7 or SP6 transcript, respectively; 6, as for lane 3 except no ATP was added and incubation was at 37°C for 45 min; 7 and 8, as for lane 6 except T7 or SP6 transcript was added, respectively; 9 and 10, as for lanes 7 and 8 but 1 unit of v-GTase (BRL) was added. (*B*) Five-fold shorter exposure of *A*. Arrows denote exogenous transcripts extended by 1 nucleotide after incubation with v-GTase. (*C*) Nuclease P1 analysis of exogenous transcripts reisolated from *A*. The T7 and SP6 transcripts were excised and extracted from the gel (14) and digested with nuclease P1 (BRL), and the products were analyzed by one-dimensional thin layer chromatography in solvent a as described by Silberklang *et al.* (20) and detected by autoradiography. Positions of unlabeled cap dinucleotide GpppG (Pharmacia), 5′-AMP, and the origin of the chromatography plate (O) are shown. The lanes are labeled according to the lanes in *A* from which the transcripts were excised.

isopycnic density gradient centrifugation, and after two sequential chromatography steps. More recently, we have found that the activity and substrates also cofractionate in salt gradients on anion-exchange chromatography (data not shown). (*ii*) The activity consistently fractionates with a very large (>150 kDa) apparent molecular mass. Although some viral GTases may exceed 100 kDa (6), these are multisubunit multifunctional protein enzymes. Known cellular GTases are significantly smaller than viral GTases, ranging in size from ≈45 kDa for *Saccharomyces cerevisiae* GTase to ≈77 kDa for wheat germ GTase (6, 21). (*iii*) Finally, the density observed for the capping activity on the gradient of Cs_2SO_4 is consistent with that of an RNA–protein complex.

It is not surprising that the *T. cruzi* U-RNAs and SL-RNA fractionate as snRNPs since all other known U-RNAs exist in relatively small complex particles (22), and the SL-RNAs from nuclear extracts of *Leishmania enriettii* (23) and *T. brucei* (16) have been shown to fractionate in snRNPs. In higher eukaryotes, snRNPs are known to function in mRNA maturation (i.e., splicing, polyadenylylation, etc.). However, to our knowledge, this report is the first to suggest that GTases are associated with snRNPs in any organism.

We do not know how the capping enzymes in the partially purified *T. cruzi* extracts recognize and bind to the substrate RNAs. The enzyme that adds the γ-monomethyl phosphate cap to mammalian U6 RNA recognizes a specific sequence on the U6 primary transcript (24). This system may be functionally analogous to the *T. cruzi* capping system; i.e., the capping enzymes may require a common primary or secondary structure of the RNA. If so, common sequences or structural motifs would be expected on the different RNA substrates, the SL-RNA and the U-RNAs.

The *T. cruzi* capping activity is also unique in its requirement for ATP; all other known GTases require only the energy provided by cleavage of the GTP substrate. In contrast, assembly and maintenance of snRNPs that participate in mRNA splicing is known to require ATP (25). Thus, it is possible that ATP in the *T. cruzi* capping reaction is required for assembly or stabilization of the putative snRNPs containing the substrate RNAs and the capping enzyme. However, the capping complexes survive several purification steps in the absence of exogenous ATP and the capping reaction remains ATP dependent; i.e., preassembled partially purified complexes are not competent for capping in the absence of ATP. Alternatively, the ATP may mediate a conformational change that is required in the snRNP for GTase activity. We have not yet determined if ATP is hydrolyzed during the capping reaction.

The apparent specificity of the partially purified *T. cruzi* GTase activity for the SL-RNA and U-RNAs is also unusual. Purified GTases isolated from other sources cap 5′-tri- or -diphosphate RNAs indiscriminately, and the specificity of the capping reaction for Pol II transcripts *in vivo* is thought to reside within the Pol II transcription initiation complex (6). However, since it has been argued that the SL-RNA is transcribed by Pol III (10), it is unlikely that the capping activity in the kinetoplastid protozoa is limited to the Pol II initiation complex. It is particularly interesting that the apparent specificity of the *in vitro T. cruzi* activity closely reflects the *in vivo* capping requirements of the kinetoplastid protozoa. In these organisms "multicistronic" pre-mRNAs

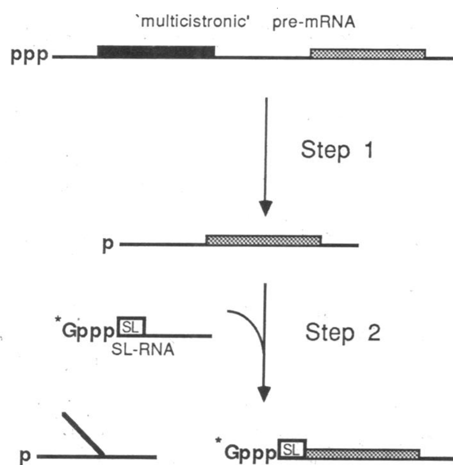

FIG. 6. Trans-splicing as trans-capping. In kinetoplastid protozoa, "multicistronic" pre-mRNA is shown being cleaved into discrete units in step 1 producing RNAs lacking 5′-di- or -triphosphates. Such RNAs are not substrates for classical GTases and can only be capped by trans-splicing of a capped SL onto their 5′ termini to produce the mature mRNAs and the expected "Y" form intermediates (step 2). Thus, trans-splicing can be considered trans-capping. The apparent specificity of the *in vitro* capping reactions in *T. cruzi* for the SL-RNA and the U-RNAs closely reflects the *in vivo* capping requirements and fits the model described. It is not known that step 1 precedes the trans-splicing event as depicted here; it is equally possible that cleavage of separate units of the multicistronic transcript occurs during trans-splicing.

are processed by trans-splicing events that join the capped SL to the 5′ ends of the mature nuclear mRNAs (Fig. 6). The only other RNAs that are known to require 5′ caps are the U-RNAs. Thus, an activity that caps only the SL-RNA and U-RNAs—as does the *T. cruzi* activity—would provide all of the requirements for capping in these organisms. These observations are consistent with the hypothesis that the SL-RNA and trans-splicing evolved in the kinetoplastid protozoa to provide a mechanism for capping (trans-capping) otherwise uncappable RNAs (Fig. 6).

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