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Efficient introduction of plasmid DNA into *Trypanosoma brucei* and transcription of a transfected chimeric gene

(transfection/variant surface glycoproteins/parasite/electroporation)

JOSIANE EID AND BARBARA SOLLNER-WEBB

Department of Biological Chemistry, The Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

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ABSTRACT **Electroporation induces efficient transient transfection of *Trypanosoma brucei*, and the introduced DNA can be transcribed into RNA. When we delivered a high-voltage electric pulse to cells mixed with radiolabeled pBR322, ≈15% of the plasmid DNA was taken up by the parasites. When transfecting DNA contained a segment of *T. brucei* ribosomal DNA that included the 5′ end of the rRNA gene, the introduced plasmid directed expression of RNA; this RNA expression was shown both by dot blots and by S1 nuclease protection assays carried out under conditions specific for probe hybridization to RNA. In the absence of the ribosomal region, analogous transcription did not occur. We optimized this trypanosomal expression system with regard to electric shock strength, concentration of input DNA, and incubation time after electric shock. This technique enabling specific trypanosome DNA expression *in vivo* should facilitate the molecular analysis of *T. brucei* gene expression.**

Trypanosoma brucei are unicellular flagellates that are a major cause of disease in Africa, being responsible for sleeping sickness in humans and nagana in cattle (1). Aside from creating a significant economic problem in the developing countries, these parasites exhibit curious characteristics, the elucidation of which is an important goal in molecular biology. *T. brucei* have the interesting property of sequentially varying their surface glycoprotein (VSG; ref. 2) through complex gene rearrangements (for review, see ref. 3), thereby preventing vaccination against the disease. Another surprising feature of trypanosomatid biology is the apparent trans-splicing (4, 5) of a common 35-nucleotide leader sequence onto the 5′ ends of all mature nuclear-encoded mRNA molecules examined to date (6–8), a result also suggesting that this spliced leader is involved in translation. Transcripts of certain trypanosome mitochondrial genes, on the other hand, evidently have small clusters of uracil residues inserted into the RNA by an unprecedented mechanism at precise positions in the coding region (9) and in the 5′ and 3′ noncoding regions (10). In contrast to better-understood eukaryotic systems, in trypanosomes the transcription of certain variant surface glycoprotein (11) and other genes appears to start far upstream from the coding region, and the 5′ portion of the primary transcript is removed during mRNA maturation. In addition, many trypanosome genes are organized as tandem repeats (12, 13), and the intragenic regions may also be transcribed. Thus to even identify transcription initiation sites of trypanosome genes has been challenging. Indeed, the analysis of many events in trypanosome molecular biology has proven fairly intractable—largely because no reproducible system has been available by which DNA can be introduced into a trypanosome and expressed *in vivo*.

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We report reproducible, high-level transfection of *T. brucei* by plasmid DNA using electroporation. Further, we show that a plasmid containing a DNA segment that includes the presumptive initiation region of a *T. brucei* rRNA gene is transcribed into RNA in transfected cells. This expression system should facilitate molecular analysis of gene expression in trypanosomes.

MATERIALS AND METHODS

DNA Constructs. Plasmid J1-Ig (diagrammed in Fig. 1*A*) consists of an *Eco*RI–*Hin*dIII fragment containing the presumptive initiation site of a *T. brucei* rRNA gene, a *Hin*dIII–*Pvu* II fragment containing sequences from an immunoglobulin gene (to serve as a tester segment), and (as vector) the large *Pvu* II–*Eco*RI fragment from pBR322. The ribosomal DNA (rDNA) fragment was isolated from plasmid pR4 (refs. 15 and 16; provided by P. Borst) and includes ≈1.5 kilobases (kb) of sequence encoding *T. brucei* 18S rRNA and ≈4 kb of adjacent upstream spacer. The *Hin*dIII–*Pvu* II tester fragment is a 355-bp segment from plasmid pV_H81X (Fig. 1*C*; provided by S. Desiderio) that consists of a 265-bp segment containing an intron from a human immunoglobulin region (14) plus 90 bp of pUC13 DNA.

Plasmid ΔJ1-Ig (diagrammed in Fig. 1*B*) was constructed by ligating the 355-bp *Hin*dIII–*Pvu* II fragment from pV_H81X onto the large *Hin*dIII–*Pvu* II fragment from pBR322. This plasmid is virtually identical to plasmid J1-Ig except that the former lacks the rRNA gene region.

Transfection Procedure. *T. brucei* (clone Iltat 1.3) were grown in young adult female mice (Charles River Breeding Laboratories) and were purified on a DE 52 column equilibrated with bicine-buffered saline solution [50 mM bicine (pH 8.0), 50 mM NaCl, 5 mM KCl] plus 1% (wt/vol) dextrose (17). The isolated parasites were diluted with this buffer to 2–5 × 10^7 cells per ml and were incubated on ice with plasmid DNA (10–20 μg/ml) for 10 min. Electroporation was done by modification of the method of Potter *et al.* (18). Two successive electric pulses of 1 kV/cm each of ≈0.5 sec duration were delivered to the cell suspension using 1-cm disposable cuvettes with platinum electrodes and a Dan-Kar (Reading, MA) 3-kV power supply. After incubation on ice for 10 min, the cells were grown *in vitro* (ref. 19, using medium containing 10% horse serum, heat-inactivated at 56°C for 30 min) in a humidified atmosphere (5% CO_2) at 37°C. Cells were harvested after 15 hr, during which time two doublings had occurred.

Nucleic Acid Analysis. Preparation of cellular RNA (using guanidine thiocyanate/cesium chloride density gradient centrifugation) and of cellular DNA followed established procedures (20, 21). Generally, ≈1 μg of RNA is obtained per 10^7 transfected cells.

To free transfected trypanosomes of DNA that had not been taken up (for the experiment of Fig. 2), cells were pelleted, washed, and suspended in DNase I buffer [50 mM Tris·HCl (pH 7.5), 10 mM NaCl, 10 mM dithiothreitol, 6 mM

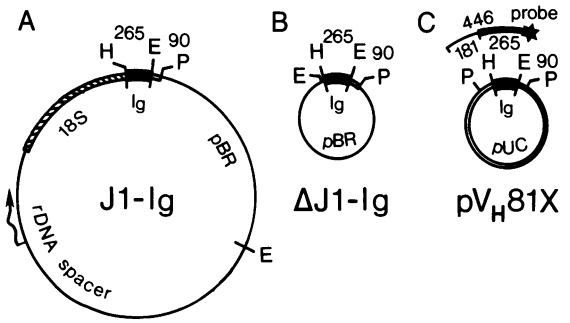

FIG. 1. Plasmid constructs. (*A*) J1-Ig was constructed as described in *Materials and Methods*. Direction and possible initiation site of rRNA transcription are shown as a wavy line. (*B*) ΔJ1-Ig is analogous to J1-Ig but lacks the rDNA segment. (*C*) pV$_H$81X contains a 256-bp *Pst* I–*Eco*RI fragment from the human immunoglobulin gene V$_H$81X(G) (14) inserted between the *Pst* I and *Eco*RI sites of pUC13. The *Hin*dIII site is 9 bp from the *Pst* I site, resulting in a 265-bp *Hin*dIII–*Eco*RI region. The *Pvu* II sites of pUC13 are 181 and 90 bp from the *Hin*dIII and *Eco*RI sites, respectively. The illustrated 446-bp S1 hybridization probe was prepared from this plasmid. The 265 bp proximal to the labeled terminus are contained in plasmid J1-Ig, whereas the distal 181 bp are not. H = *Hin*dIII, P = *Pvu* II, and E = *Eco*RI.

$MgCl_2$] at ≈5 × 10^7 cells per ml. After treatment with 0.1 mg of DNase I per ml for 20 min at 37°C, cells were washed once with bicine-buffered saline containing 0.5 M NaCl and then were washed extensively with bicine-buffered saline.

RNA filter hybridization studies using dot blots were done as described (22). The probe DNA was the 355-bp *Hin*dIII–*Pvu* II fragment from plasmid J1-Ig containing immunoglobulin and pUC13 sequences and labeled with ^{32}P by nick-translation (23).

RNA analysis by S1 nuclease protection used formamide as well as aqueous hybridizing conditions. The hybridization probe (illustrated in Fig. 1*C*) was prepared from plasmid pV$_H$81X, linearized at the *Eco*RI site at the end of the immunoglobulin region, 5′ end-labeled with ^{32}P to ≈1.5 × 10^6 cpm/pmol, and then cleaved with *Pvu* II to generate a 446-bp fragment. The probe was strand-separated on an 8% acrylamide gel (24) and is only homologous to the J1-Ig template plasmid over the 265-bp *Eco*RI–*Hin*dIII immunoglobulin segment. The 20-μl hybridization reactions contained 0.01 pmol of probe and either 10–30 μg of cellular RNA or 0.01 pmol of "mock DNA" (see below). The aqueous hybridizations were done in a solution containing 0.3 M NaCl, 0.1 M Tris·HCl (pH 7.9), 2 mM EDTA, and 10 mM sodium phosphate, and were incubated at 65°C for 16–18 hr. Formamide hybridizations were done in a solution consisting of 80% formamide, 40 mM Pipes (pH 6.4), 0.4 M NaCl, and 1 mM EDTA; after heat denaturation at 85°C for 5 min, reactions were incubated at 48°C, or the indicated temperatures, for 16–18 hr. The hybridization mixtures were then brought to 300 μl with S1 nuclease buffer [0.2 M NaCl, 30 mM sodium acetate (pH 4.5), 5 mM $ZnCl_2$, and 30 μg of heat-denatured salmon sperm DNA] and were treated with S1 nuclease (0.15 unit/μl; Bethesda Research Laboratories) for 1 hr at 20°C. Samples were then ethanol precipitated, suspended in 95% formamide/10 mM EDTA, heated, resolved on a 4% polyacrylamide/8 M urea gel, and visualized by autoradiography.

Mock DNA was the double-stranded 355-bp *Hin*dIII–*Pvu* II fragment isolated from plasmid pV$_H$81X. In other experiments, mock DNA was prepared by linearizing plasmid pV$_H$81X at the *Hin*dIII site and treating with exonuclease III (Bethesda Research Laboratories) at 60 units per pmol of DNA in 60 mM Tris·HCl (pH 7.5)/5 mM $MgCl_2$ for 30 min at 37°C. Mock DNA prepared by both methods behaved identically under the various formamide hybridization conditions.

RESULTS

Plasmid DNA Is Efficiently Taken Up by Trypanosomes. To assess whether DNA could be introduced into trypanosomes by electroporation, cells were subjected to the electric shock as described in the presence of radiolabeled pBR322 at a DNA concentration of 10 μg/ml. After growth for two doubling periods, the percentage of radiolabeled DNA taken up by the cells was determined. To this end, the transfected trypanosomes were washed, treated with a high concentration of DNase I, and subjected to additional extensive washing, including a high-salt treatment to eliminate any plasmid DNA adhering to the exterior surface of the parasites. Control experiments showed that an equivalent amount of free pBR322 DNA was completely digested to an ethanol-soluble form by this DNase treatment. When DNA was extracted from the washed trypanosomes, resolved by agarose gel electrophoresis, and visualized by autoradiography, full-length, as well as partly degraded, pBR322 was obtained (Fig. 2, lane 3). Quantitated relative to aliquots of the radiolabeled pBR322 that had been added to the original transfection media (lanes 1 and 2), transfected cells took up >≈15% of input DNA. (In comparison, trypanosomes constitute <0.1% of the transfection-solution volume.) The DNA uptake is thus equivalent to an average of ≈5000 molecules of pBR322 per cell in the culture, about the same amount of DNA as originally contained in the untransfected trypanosomes.

When transfection was repeated under identical conditions except that the electric shock was omitted, a far smaller fraction of radiolabeled DNA was absorbed by the cells (Fig. 2, lane 4). Thus, although DNA is taken up in the absence of a shock, the shock protocol increases transfection efficiency >20-fold. We confirmed this efficient transfection by introducing unlabeled pBR322 into the parasites and similarly isolating the intracellular DNA; quantitation of the recovered pBR322 by Southern blot hybridization verified that >15% of the DNA was taken up by the shocked cells, whereas ≈1% of the DNA was taken up by untreated cells.

Transfected DNA Is Transcribed by the Recipient Parasites. To assess whether exogenous DNA introduced into *T. brucei* by electroporation could be expressed into RNA under direction of the parasite's transcription apparatus, plasmid J1-Ig (shown in Fig. 1*A*) was constructed to contain an active trypanosome promoter. In practice, because no promoter has yet been characterized in a trypanosomatid and because even the location of most trypanosome transcription initiation sites remains in question, we used a large segment of *T. brucei* ribosomal DNA thought to include the rRNA transcription

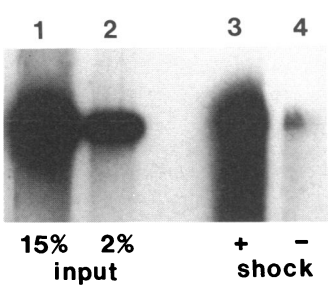

FIG. 2. Efficient DNA uptake by electroporation. Cells (5 × 10^7/ml) were mixed with radiolabeled pBR322 DNA at 10 μg/ml, subjected to electroporation, grown for 15 hr, washed extensively, and treated with DNase I as described (lane 3). A parallel culture was treated identically except that electric shock was omitted (lane 4). The protected intracellular DNA was then isolated, resolved by agarose gel electrophoresis, and visualized by autoradiography. Uptake was quantitated relative to aliquots of the initial transfection medium containing 15% (lane 1) and 2% (lane 2) of the nicked, radiolabeled DNA of the cell exposure medium.

initiation site. [This rDNA fragment contains 4 kb of sequence upstream from the 18S rRNA coding region, whereas the largest detected rRNA precursor begins only 1 kb upstream from the 18S coding region, near the middle of this fragment (15).] rDNA offers the added advantage of containing one of the most active eukaryotic promoters, so use of rDNA should facilitate detection of transcription. To selectively assess transcription directed by the transfected plasmid amid the transcripts from the ≈100 active endogenous rRNA genes, a tester segment of foreign DNA was positioned immediately downstream from the rDNA initiation segment. This tester DNA contains a 265-bp segment of intervening sequence from a human immunoglobulin gene, V_H81X, and its expression into RNA is diagnostic of transcriptional initiation on the transfected plasmid. The resultant J1-Ig plasmid was transfected into *T. brucei* by electroporation, as described above, and 15 hr post-transfection total cellular RNA was isolated using the guanidine thiocyanate/cesium chloride protocol (20).

Expression of the tester DNA was initially analyzed by RNA dot blot hybridization. The filters were probed with radiolabeled tester DNA segment and were then subjected to a stringent washing procedure [15 mM NaCl, 0.1 mM EDTA, 0.5% $NaDodSO_4$, 5 mM Tris·HCl (pH 8.0) at 65°C]. Typical data are shown in Fig. 3*A*. RNA from cells that received the electric shock in the presence of J1-Ig DNA (panel 1) exhibited a positive hybridization signal, indicating that the transfected plasmid was indeed transcribed. Cells that were treated with the same concentration of DNA but were not shock-treated (panel 2) yielded a hybridization signal lower by a factor of 5–10. Finally, RNA from cells that were shock-treated without template DNA (panel 3) or with pBR322 vector DNA (data not shown) gave no hybridization signal.

To confirm that transfected DNA was, indeed, transcribed, RNA from cells transfected with plasmid J1-Ig was analyzed by a S1 nuclease protection assay. The 5′ end-labeled, single-stranded hybridization probe was prepared from plasmid pV_H81X after cleavage at the *Eco*RI site (Fig. 1*C*); its sequence diverges from that of plasmid J1-Ig beyond the 265-bp tester sequence. Transcripts of the transfected J1-Ig template would thus protect 265 bp of probe DNA (diagrammed in Fig. 3*B*). Fig. 3*C*, lane 1, shows the result. The S1 signal from RNA of trypanosomes that had been given a shock in the presence of plasmid J1-Ig (lane 1) was ≈10-fold higher than the signal obtained when the protocol was repeated without the shock treatment (lane 2). Transfecting the cells with pBR322 DNA using the shock treatment (lane 3) or delivering an electric shock in the absence of plasmid DNA (lane 4) gave no signal, confirming that S1 protection is diagnostic for sequences derived from the transfecting J1-Ig DNA.

To prove that the S1 protection observed was due to RNA from the transfected cells and not to a small amount of contaminating template DNA, the probe was allowed to associate with a mock DNA and with RNA prepared from the transfected cells under aqueous and formamide hybridization conditions of various stringencies. This mock DNA is a fragment from the noncoding strand of plasmid J1-Ig that includes the tester DNA segment; it therefore protects the same segment of probe from S1 digestion as does the transfected cell RNA (Fig. 4*A*; lanes 1, 2, and 4), confirming that the latter S1 product is of the expected size. However, because the mock DNA and probe will form a DNA·DNA duplex, whereas the cell RNA and probe should form a DNA·RNA duplex, these two hybrids should be favored under different hybridizing conditions, a prediction verified in Fig. 4*A*. The DNA·DNA duplex is formed in aqueous hybridizations (lanes 1 and 9) and in formamide hybridizations conducted at room temperature (lane 10), but it is abolished in formamide hybridizations at ≥40°C (lanes 3, 5, 7, 11, and 12). In contrast, the hybrid between the probe and the transfected cell RNA is favored at these higher temperatures (lanes 2, 4, 6, and 8). Thus, the material that hybridizes to the probe in the transfected-cell RNA preparation is an RNA transcript.

Further evidence that the S1 signal under study is due to RNA and not to plasmid DNA was obtained by nuclease digestion. Pretreatment of the RNA preparation from the transfected cells with RNase A abolished the signal (Fig. 4*B*, lane 2), whereas it was retained following pretreatment with DNase I. These data demonstrate that the S1 signal resulting from the transfected J1-Ig plasmid is, indeed, due to its transcription into RNA.

Transcription of the Transfected Plasmid Occurs Intracellularly. Although the transcription under study probably takes place within the transfected cells, it remained theoretically possible that the transcription was catalyzed extracellularly by polymerase molecules that had been released from cells that were damaged by the electric shock. *A priori*, this alternate scenario appears unlikely, because (*i*) the extracellular rNTP concentration should be too low to support transcription and (*ii*) the serum in which the cells are grown contains RNases that should degrade free RNA molecules. Nonetheless, to address the possibility of extracellular RNA synthesis occurring in the transfected trypanosome culture, the experiment shown in Fig. 4*C* was done. A *T. brucei* culture was transfected with plasmid J1-Ig, grown, and harvested as usual; RNA was then isolated from the growth medium as well as from the pelleted cells. S1 nuclease

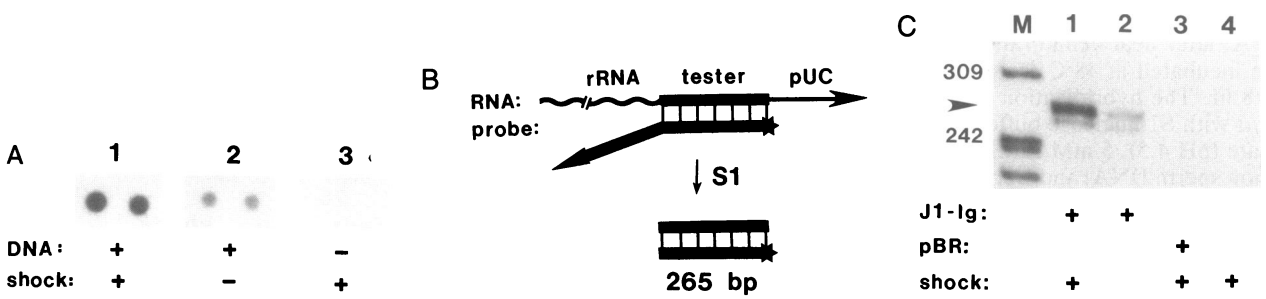

FIG. 3. Analysis of transfected cell RNA. (*A*) Dot blots were used to analyze 1 μg of RNA extracted from duplicate cultures of *T. brucei*, 15 hr post-transfection, that had been subjected to electric shock in the presence of J1-Ig DNA (panel 1), treated as in panel 1 except omitting the shock (panel 2), or treated as in panel 1 except omitting the J1-Ig DNA (panel 3). A 5-hr exposure is shown. (*B*) The S1 nuclease protection assay is diagrammed. Transcript RNA, upper line; 5′ end-labeled (*) probe, lower line. (*C*) S1 nuclease protection was used to analyze the RNA, 15 hr post-transfection, from cells that had been subjected to electric shock in the presence of J1-Ig DNA (lane 1), treated as in lane 1 except omitting the shock (lane 2), treated as in lane 1 except substituting pBR322 DNA for J1-Ig DNA (lane 3), or treated as in lane 1 except omitting the DNA (lane 4). (The minor band below the 265-bp-protected fragment is caused by S1 clipping the hybrid within an rU·dA region ≈15 nucleotides from the end of the duplex region.)

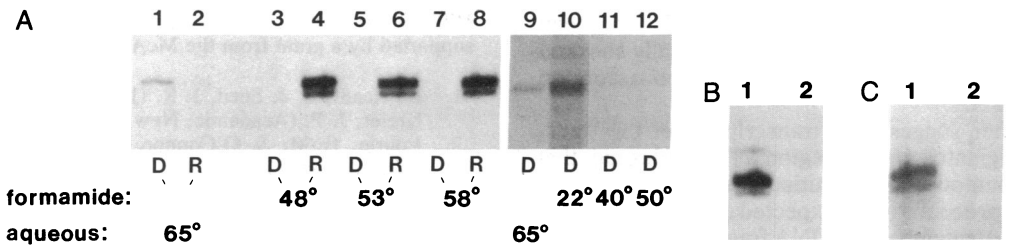

FIG. 4. S1 protection is derived from RNA transcribed intracellularly. (*A*) The probe was hybridized to DNA mock (D) or to transfected cell RNA (R), as described, in aqueous- and formamide-based solutions at the indicated temperatures. Hybrids were then assessed by S1 nuclease treatment. Lanes 1–8 were from one gel; lanes 9–12 were from a second gel. (*B*) Transfected cell RNA was left untreated (lane 1) or was treated with RNase A at 65°C in EDTA (lane 2) before S1 analysis. (*C*) RNA was isolated from pelleted transfected cells (lane 1) and from the extracellular growth medium (lane 2) in the presence of tRNA carrier, and it was detected by S1 nuclease analysis.

analysis confirmed that the J1-Ig transcript was found solely in the intracellular fractions (lane 1); no signal was detected from the extracellular compartment (lane 2). Thus, transcription of the transfected plasmid indeed occurs within the recipient cells.

Optimization of the Transfection Conditions. Conditions of electroporation were optimized for maximal expression of the transfecting plasmid (Fig. 5). As concentration of plasmid is increased from 1 to 20 μg/ml (*A*), resultant RNA amount increases until a plateau is reached at ≈10 μg of plasmid DNA per ml. Fig. 5*B* illustrates the effect of varying the voltage of the electric pulse from 0 to 2 kV/cm; a shock of 0.1 kV/cm affords ≈5-fold more expression than is obtained in the absence of shock, and the level of expression increases with higher-strength impulses. Above 1 kV/cm, cell survival decreases markedly, but the application of two successive 1 kV/cm pulses was found to allow good (≈50%) survival and a somewhat higher overall level of expression than that obtained with a single pulse; therefore, this is used in our standard protocol. Other studies (data not shown) demonstrated that the transcript increased until ≈15 hr post-transfection, when it leveled off, and hence transfected cells are grown for this length of time before harvesting.

Transcription of the Transfected Plasmid Is Dependent on the Presence of the rDNA Initiation Region. Although the J1-Ig plasmid was constructed to assess transcription driven by the rDNA promoter, the resultant RNA still could have initiated within the pBR322 vector sequences at a site that fortuitously acts as a eukaryotic promoter (25). To determine whether the transcriptional signal from trypanosomes transfected with plasmid J1-Ig was dependent on the rDNA initiation region, plasmid ΔJ1-Ig (Fig. 1*B*) was constructed: it contains all the pBR322 vector sequences and the same tester DNA sequences as are present in J1-Ig, but it lacks the entire trypanosome rDNA region. Fig. 6, lane 1, shows that cells transfected with ΔJ1-Ig exhibit only very low amounts of tester transcript. This result shows that the rDNA initiation region is important in expression of the transfected DNA and suggests that the *T. brucei* rDNA promoter is, indeed, contained within 4 kb upstream of the 18S coding region and is active in the transfected cells.

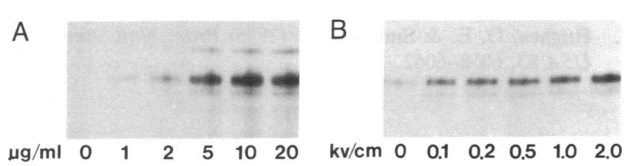

FIG. 5. Optimization of transfection conditions. (*A*) Electroporation was done in the presence of the indicated concentrations of J1-Ig DNA, using two successive 1 kV/cm shocks, and the resultant RNA was quantitated by S1 nuclease analysis. (*B*) Transfections were done by applying the indicated strengths of electric shock in the presence of J1-Ig DNA (15 μg/ml), and RNA was assessed as in *A*.

DISCUSSION

We provide evidence of successful *in vivo* transfection of *T. brucei* with plasmid DNA using electroporation. Besides showing high-efficiency uptake of foreign DNA (Fig. 2), we document transcription of a transfected template into RNA. These studies used a plasmid that contained an rDNA fragment bearing the putative transcription initiation region attached to a tester sequence not found in untransfected trypanosomes. Transcription of the tester sequence in the transfected cells was demonstrated both by dot blot and S1 nuclease analyses (Figs. 3 and 4). Compared per μg of RNA isolated from *T. brucei* cells transfected with plasmid J1-Ig, the transcript amount is approximately the same as that obtained from Chinese hamster ovary cells that have been transfected (using an optimized DEAE-Me_2SO protocol) with a plasmid construct in which a rodent rDNA promoter drives transcription of a tester DNA segment.

Because of the biological importance and potential medical relevance of developing a transformation/expression system for *T. brucei*, unequivocally verifying the reliability of the technique was essential. To this end, the tester sequences were first shown to be unique to the transfecting plasmid, for they were found neither in untransfected cells nor in cells transfected with only vector DNA (Fig. 3). That the signal under study was due to an RNA transcript of the transfected plasmid, and not to a small amount of contaminating plasmid DNA, was demonstrated by hybridizing the probe—both to the isolated RNA and to the corresponding region of plasmid DNA under various formamide and aqueous hybridizing conditions; in the 80% formamide-containing reactions incubated at ≥40°C, the probe hybridized to the RNA, but the probe did not hybridize to the DNA sample (Fig. 4*A*). Furthermore, when the transfected cell RNA was treated with RNase prior to analysis, no S1-protected fragment was observed (Fig. 4*B*), confirming that the S1 signal is, indeed, due to RNA transcribed from the transfected template. Finally, analysis of RNA obtained within and external to the transfected cells showed that the plasmid transcript occurs intracellularly (Fig. 4*C*), ruling out the *a priori* unlikely

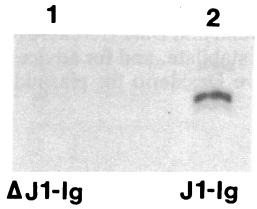

FIG. 6. Dependence of transcription on the rDNA initiation region. Cells were transfected using: plasmid J1-Ig (lane 1) or plasmid ΔJ1-Ig (lane 2), an analogous construct lacking the rDNA region. Equal aliquots of RNA were subjected to the S1 nuclease protection assay.

scenario that the observed transcription could have been catalyzed by polymerases released from electrically shocked cells. These studies confirmed that transcription occurs on the transfected DNA.

Our data further suggest that transcription of the transfected DNA originates in the region of the plasmid that contains the presumptive rRNA initiation site. The transcription under study proceeds in the expected direction (Fig. 4A), and it requires the trypanosome rDNA fragment that contains the presumptive initiation site (15) for RNA polymerase I transcription (Fig. 6). The very small amount of tester transcript obtained from an analogous plasmid that lacks the rDNA region presumably initiated fortuitously within the prokaryotic vector (as can occur in transfected mammalian cells; ref. 25). Where transcription initiates on the cellular *T. brucei* rRNA genes and whether this process is faithfully mimicked on the transfected rDNA plasmid await definitive answers.

From our data, limited transcription was clearly detected even when the electric shock was omitted from the electroporation protocol (Fig. 3). This transcription signal intensity relative to that obtained from the shock-treated cells was comparable to the relative amount of DNA taken up by nonshock treated versus shock-treated cells (Fig. 2). DNA uptake in the absence of electrical shock could be due to endocytosis (26), which occurs in the bloodstream form of trypanosomes used in our experiments should some of the DNA molecules escape lysosomal digestion and reach the nucleus.

Electroporation appears to be the transfection method of choice for introducing DNA into *T. brucei*. Alternate methods have proven unsuccessful due to a great loss of viability of the treated *T. brucei* cells and/or a failure of viable cells to take up or express input DNA. Relative to protocols used successfully for transfecting mammalian cells (27–30), *T. brucei* do not survive even quite-mild treatments with calcium phosphate or with DEAE-dextran plus Me_2SO. Neither do they survive electroporation at reduced ionic strength. On the other hand, attempts to introduce DNA using DEAE-dextran alone, hypotonic permeabilization, or chloroquine yielded no measurable transfection.

In summary, this communication describes a reproducible system for introducing DNA into *T. brucei* and for expressing such DNA into RNA. An earlier publication of transfection/expression studies in trypanosomatids involved an attempt to stably introduce the neomycin-resistance gene into *Crithidia* using a sorbitol/polyethylene glycol transfection protocol, but although initial results were promising, the authors reported these results not to be reproducible (31). In contrast, our transfection/expression system worked in 50 out of 50 separate experiments, using three different *Trypanosome* stabilate preparations and approximately 20 different plasmid preparations.

We thank Dr. Piet Borst for plasmid pR4 and for providing information about *T. brucei* rDNA before publication. To Dr. John Boothroyd we are indebted for helpful discussion and for making available a subclone of *T. brucei* rDNA. We very gratefully acknowledge Drs. Paul Englund and Dale Hereld for encouragement, for the initial trypanosome stabilate, and for advice on its propagation. We also thank Dr. Steve Desiderio for plasmid pV_H81X and Ms. Sue Millionie for help in preparing the manuscript. This work was supported by a grant from the McArthur Foundation.

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