observations is centered at 6190 Å, with a 30 Å full width at half maximum (*5*).

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Expression of a Bacterial Gene in a Trypanosomatid Protozoan

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A simple and reproducible assay for DNA-mediated transfection in the trypanosomatid protozoan *Leptomonas seymouri* has been developed. The assay is based on expression of the *Escherichia coli* chloramphenicol acetyl transferase (CAT) gene flanked by *Leptomonas* DNA fragments that are likely to contain necessary elements for gene expression in trypanosomes. After electroporation of cells in the presence of plasmid DNA, CAT activity was detected in crude cell lysates. No activity was detected when the orientation of the *L. seymouri* mini-exon sequence (placed upstream of the CAT gene) was reversed, or in additional control experiments. This system provides a method for defining transcriptional control elements in trypanosomes.

MOLECULAR STUDIES HAVE REvealed many distinctive features of gene expression in the trypanosomatid protozoa. Mature mRNAs result from the joining of two exons, which are initially transcribed as separate RNAs. In *Trypanosoma brucei*, the 5′ exon common to all characterized mRNAs is transcribed as a short nonpolyadenylated RNA, the mini-exon donor RNA (medRNA), whose 5′ 39 nucleotides (nt) (the mini-exon) are spliced, in trans, onto an independently transcribed coding exon during mRNA maturation (*1, 2*). Trans-splicing is probably necessary to process polycistronic RNAs, whose existence in trypanosomes has been demonstrated (*3*) or implied (*4, 5*).

The medRNA genes of different trypanosomatids are mostly arranged in tandem arrays, and share sequence motifs within the mini-exon, at the medRNA exon-intron junction and at the presumed site of transcription termination (*6–8*). The identification of a complex methylated cap structure on the 5′ end of medRNA isolated from *T. brucei* indicates that medRNA transcription initiates at the conserved 5′ AACTAA sequence of medRNA (*9*), which is consistent with the results of transcription studies in isolated nuclei (*2*). Thus, it is likely that RNA polymerase promoter recognition sites are present within each reiterated unit, upstream of the 5′ end of medRNA. However, in the absence of an experimental genetic or in vitro system, it has been impossible to define the genetic elements that are essential for transcription initiation and for RNA maturation and stability.

We are developing *Leptomonas seymouri*, an insect trypanosomatid, as a model system for studying RNA transcription and splicing because large quantities can be grown axenically and individual cells can form colonies on agar plates (*4, 6*). Three laboratories have published their experiences in the pursuit of DNA-mediated transfection of trypanosomes. All of these studies relied on the detection of nucleic acid in potentially transfected cells, and were either unconvincing (*10*) or unrepeatable (*11*). To detect transfection, we utilized a plasmid (pAL5) incorporating the *Escherichia coli* chloramphenicol acetyltransferase (CAT) gene. Trypanosomes have no intrinsic CAT activity (Table 1). By measuring enzyme activity and not relying on the detection of nucleic acid as a measure of DNA uptake and expression, we were able to use large amounts of DNA without the danger of false positive results caused by DNA contamination of RNA preparations.

We surrounded the CAT coding region with *L. seymouri* sequences that we judged likely to contain control signals on the basis of available information concerning transcription of these sequences (*1, 4, 6, 7*). As all translated mRNAs in trypanosomes possess the mini-exon sequence at their 5′ end (*12*), we placed the *L. seymouri* mini-exon sequence and 304 nt of upstream sequence 5′ to the CAT coding region. To avoid the processing and potential loss of the mini-exon, which we intended to be fused directly to the 5′ end of CAT mRNA (rather than added by trans-splicing), we constructed pAL5 such that the dinucleotide sequence GT, normally found at the 5′ intron end of medRNA (*1, 6, 7, 8*), was absent from the region between the mini-exon sequence and the CAT coding sequence. To provide polyadenylation signals to the pAL5-derived mRNA, we placed the 3′ end and down-

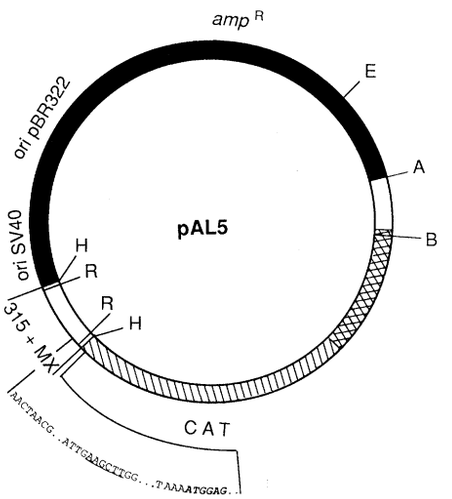

Fig. 1. Construction of plasmid pAL5 (5.9 kb). Plasmid pAL1 was constructed as follows: the 4.6-kb Apa I–Hpa I fragment from pSV2CAT (*14*) was ligated to a 700-bp Ava II–Hind III fragment from pST3 (*4*) (Ava II site filled in; Hind III site is present within the pUC12 polylinker region of pST3) and to the 290-bp Hind III–Apa I fragment from pM4 (*6*) in a single reaction. Consequently, pAL1 has, counterclockwise from the Apa I site: an ampicillin resistance gene, the pBR322 ori and the SV40 ori (black box); the CAT gene, small-t intron, and part of the SV40 early region lacking the polyadenylation signals (hatched box); the *L. seymouri* α-tubulin gene 3′ end and downstream region (cross-hatched box) and the *L. seymouri* mini-exon gene 3′ end and surrounding region (open box). pAL2, which contains a single Hind III site 36 bp upstream of the CAT gene, was constructed by deleting a Hind III site in pAL1 that is between the α-tubulin and 3′ medRNA regions. To generate pAL5, the 334-bp Rsa I fragment of pM4, which contains 304 bp of upstream sequence and the 5′ 30 bp of the mini-exon, was cloned into the Hind III site of pAL2 after ligation to an adaptor

5′ ACTTTATTGA
TGAAATAACTTCGA

that completes the mini-exon and provides Hind III ends. In pAL3, the orientation of this fragment (315 + MX; open box) is reversed. The sequence of the mini-exon–CAT region was confirmed by DNA sequencing. The three portions of sequence shown are, from left to right: the mini-exon 5′ end; the mini-exon 3′ end and Hind III site (underlined), generated by adaptor addition; and the translational start (boldface type) of the CAT gene. The two Hind III (H) and Rsa I (R) sites, the Apa I (A) site used in the construction of pAL1, the unique Bam HI (B) and Eco RI (E) sites, and the positions of the pBR322 ori, the SV40 ori, and the ampicillin resistance gene (*amp*) gene, are indicated.

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stream region of the *L. seymouri* α-tubulin gene (*4*) after the CAT gene. Finally, poly-T stretches that follow medRNA sequences and might serve as transcription termination signals (*1, 6, 7, 8*) were positioned after the α-tubulin region (Fig. 1).

Electroporation (*13*) of *L. seymouri* with pAL5 was successful, as judged by the detection of CAT activity in cell lysates (Fig. 2). The reaction products comigrated on thin-layer chromatographs (*14*) with those produced by authentic *E. coli* CAT. In contrast, electroporation in the presence of pAL3, pUC12, or sonicated and sheared salmon-sperm DNA (ssDNA) produced no detectable CAT activity.

CAT activity was dependent on the presence of pAL5 DNA during electroporation (Table 1). Thus, pAL5 is not passively internalized and transcribed. The amount of activity depended on DNA concentration and cell density. The fact that pAL3 did not give detectable CAT activity implies that the orientation of the 39-nt mini-exon and upstream sequences (Fig. 1; 315 + MX fragment) are important for CAT expression in *L. seymouri*. Thus, it is likely that this fragment contains the mini-exon promoter, although other interpretations are possible. Addition of pAL5 DNA to cell extracts did not result in CAT activity. In addition, enzyme activity in transfected cell extracts was unaffected by the addition of cycloheximide (*15*). Therefore, CAT activity was not dependent upon transcription and/or translation occurring in vitro after cell lysis.

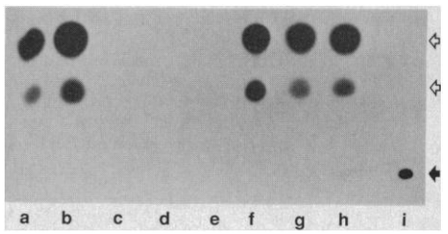

Fig. 2. Thin-layer chromatography of the butyryl [^{3}H]chloramphenicol (Cm) produced by CAT activity in *L. seymouri*. Cells were transfected with either 60 μg (lanes a and g) or 150 μg (lanes b and h) of pAL5; 60 μg (lane c) or 150 μg (lane d) of pAL3; or 150 μg (lane e) of pUC12. Lane f contains products obtained after addition of 0.1 U of *E. coli* CAT (Pharmacia) to a crude lysate of *L. seymouri* that had been transfected with ssDNA. Open arrows point to the 3-butyryl and 1-butyryl [^{3}H]Cm products. Lane i contains a portion of the [^{3}H]Cm substrate (closed arrow) remaining in the aqueous phase of the reaction shown in lane a after xylene extraction. Cells in late log phase (3×10^7 to 5×10^7 per milliliter) grown as described (*6*) were harvested by centrifugation at 21°C and washed once in sterile Zimmerman Postfusion medium (ZM) (American Sterilization Co.), which contains 132 m*M* NaCl, 8 m*M* KCl, 8 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, 0.5 m*M* $MgOAc_2$ and 90 μ*M* $CaOAc_2$, adjusted to *p*H 7.0 with acetic acid. Cell pellets were resuspended in ZM to 5×10^7 cells per milliliter and set at 21°C for up to 1 hour. Aliquots (0.5 ml) were placed into wells of a 24-well polystyrene plate (Corning Co.) and 150 μg of CsCl-banded test DNA, in 70 μl of H_2O, was added. A flame-sterilized single ring electrode (PG120-10, Hoefer Scientific Instruments) was inserted into the well and cells received five pulses from the Hoefer Progenetor I, set to deliver 450 V for 99 mS from the Hoefer PS 500XT power supply. Five minutes after pulses were delivered, cells were transferred to 10 ml of SDM-79 (*17*) supplemented as described (*18*) and containing 100 U each of penicillin and streptomycin per milliliter. Cultures were incubated for 18 to 22 hours at 27°C before harvesting for CAT assays. Then each 10-ml culture (5×10^7 live cells) was harvested by centrifugation, washed once in phosphate-buffered saline, and resuspended in 160 μl of 0.1*M* tris-HCl *p*H 7.8, 1 m*M* EDTA, and 1 m*M* dithiothreitol. Cells were broken by two cycles of freezing and thawing and debris was removed by centrifugation (4°C, 3 min) in a Microfuge. Supernatants were heated (65°C, 6 min) and cooled on ice before CAT activity was assayed as described (*19*), except that 50 μ*M* Cm was used at a final specific activity of 100 mCi/mmole ([^{3}H]Cm, Dupont), and assays were for 3 hours at 37°C. After xylene extraction and two aqueous extractions of the xylene phase to remove contaminating free [^{3}H]Cm (*18*), the final xylene fractions were dried, resuspended in 20 μl of ethyl acetate, spotted onto silica G plates, and chromatographed (*14*).

Table 1. Requirements for CAT activity in *L. seymouri*. Values shown are from one-tenth of the xylene-extracted product from 3-hour CAT assays from transfections with 7×10^6 (column A) or 2.5×10^7 cells (column B). Background (not subtracted), resulting from [^{3}H]Cm contamination in the final xylene fraction, was approximately 50 count/min. Chromatographic analysis of the remaining nine-tenths of the samples demonstrated that the products were butyryl[^{3}H]Cm. All values are the average of at least two experiments. Values differed by ≤15% from the average. DNA amounts were 150 μg unless otherwise specified.

Additions	Butyryl-[^{3}H]Cm (count/min)	
	A	B
pAL5 + DNase I	54	
pAL5 + RNase A	758	
pAL5 60 μg	200	2259
150 μg	305	4565
250 μg	544	
pAL5 + ssDNA (50 μg)	300	
pAL5, no pulse		30
pAL5, added 1 min after pulse		50
pAL5, added to crude cell lysate		28
pAL3 60 μg		46
150 μg		44
pUC12		36
ssDNA	26	37
Crude cell lysate + *E. coli* CAT		
0.1 U		10,000
0.01 U		3500
0.001 U		950

Because pAL5 probably does not contain a trypanosome origin of replication, CAT expression was transient. Activity peaked between 20 and 40 hours after electroporation and fell to background levels by 100 hours, although the cells were still viable (*15*). To quantitate CAT activity in transiently transfected cells, we mixed known amounts of *E. coli* CAT with crude lysates of *L. seymouri* transfected with ssDNA. Since reactions were linear for up to 4 hours when 10^{-5} to 10^{-1} U of enzyme were used, we obtained rate measurements in 3-hour assays (Table 1). A log-log plot of butyryl [^{3}H]chloramphenicol production versus *E. coli* CAT concentration, linear between 10^{-4} and 5×10^{-2} U of CAT, was used to determine that approximately 0.02 U of CAT (approximately 200 pg) was produced from the electroporation of 2.5×10^7 cells and 150 μg of pAL5 DNA. This level of expression represented about 200 molecules of CAT per cell, but the proportion of cells expressing CAT has not been determined.

Transient transfection assays with the *E. coli* CAT gene in mammalian cells can result in the accumulation of CAT activity in the media (*16*). In our electroporation protocol, approximately 50% of the input cells were lysed immediately after electric shock. To confirm that CAT activity was associated with living cells, pAL5-transfected cultures were harvested, washed, and fractionated by centrifugation (27,000*g* for 15 min at 21°C) on an 11-ml, 60% Percoll (Pharmacia) density gradient containing phosphate-buffered saline and 0.4% (w/v) glucose. More than 90% of input activity was associated with live cells and no activity was found in fractions containing cell debris (*15*). This indicates that the CAT gene was being transcribed and translated from pAL5 DNA that had entered live cells.

The observation that CAT activity was low, gave rise to concern that the activity could be attributed to efficient transfection of a minor contaminant organism. However, cultures incubated with tetracycline before pAL5 electroporation, and with penicillin, streptomycin, tetracycline, and rifampicin after transfection, still produced CAT activity. Enzyme activity was abolished when electroporated cells were incubated in medium containing cycloheximide; thus, activity was dependent upon eukaryotic translational machinery. No contaminants were visible upon careful microscopic examination or when electroporated cells, DNA preparations, and solutions were plated on agar that supports fungal and bacterial growth (*15*).

The ability to introduce reproducibly a reporter gene into live trypanosomes provides us with a simple experimental system to explore transcriptional regulatory signals

in these organisms. Further experiments will be necessary to identify the pAL5 sequences responsible for CAT expression.

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Rhenium-Osmium and Samarium-Neodymium Isotopic Systematics of the Stillwater Complex

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Isotopic data for the Stillwater Complex, Montana, which formed about 2700 Ma (million years ago), were obtained to evaluate the role of magma mixing in the formation of strategic platinum-group element (PGE) ore deposits. Neodymium and osmium isotopic data indicate that the intrusion formed from at least two geochemically distinct magmas. Ultramafic affinity (U-type) magmas had initial ε_{Nd} of −0.8 to −3.2 and a chondritic initial $^{187}Os/^{186}Os$ ratio of ~0.88, whereas anorthositic affinity (A-type) magmas had ε_{Nd} of −0.7 to +1.7 and an initial $^{187}Os/^{186}Os$ ratio of ~1.13. These data suggest that U-type magmas were derived from a lithospheric mantle source containing recycled crustal materials whereas A-type magmas originated either by crustal contamination of basaltic magmas or by partial melting of basalt in the lower crust. The Nd and Os isotopic data also suggest that Os, and probably the other PGEs in ore horizons such as the J-M Reef, was derived from A-type magmas. The Nd and Os isotopic heterogeneity observed in rocks below the J-M Reef also suggests that A-type magmas were injected into the Stillwater U-type magma chamber at several stages during the development of the Ultramafic series.

MAFIC LAYERED INTRUSIONS ARE the world's major repositories of nickel, chromium, and PGEs. Much of the petrologic and geochemical information concerning the evolution of mantle-derived magmas in large crustal-level magma chambers is in part a result of the intensive study of these mineral deposits in intrusions such as the Bushveld Complex of South Africa and the Great Dyke of Zimbabwe. The Stillwater Complex is an Archean mafic layered intrusion exposed in a belt approximately 45 km long and 6 km wide in the Beartooth Mountains, Montana. Magmas parental to the complex intruded metamorphic rocks of the Wyoming craton having ages of 3200 to 2750 Ma (*1*). The age of the Stillwater Complex is constrained to 2701 ± 41 Ma by several isotopic techniques [reviewed in (*2*)]. Five major stratigraphic subdivisions of the complex have been recognized (*3*): the Basal series (BS), the Ultramafic series (UMS), and the Lower, Middle, and Upper Banded series (LBS, MBS, and UBS, respectively) (Fig. 1). Magnesium-rich olivine appears as a cumulus phase only in five zones throughout the LBS, MBS, and UBS. These have been referred to as Olivine-bearing zones [OBZs (*3, 4*)] or Troctolite-Anorthosite zones (*3, 5*). The PGE-rich horizon of the Stillwater Complex, informally named the J-M Reef (*5*), occurs as a relatively thin (1 to 3 m) layer along nearly the entire 45-km length of the intrusion, approximately 400 m above the top of the UMS in OBZ I (Fig. 1).

Recent models for the formation of PGE deposits in mafic layered intrusions have emphasized the role of magma mixing (*5–8*). In the Stillwater Complex, two geochemically distinct magma types have been proposed: U-type magmas that were enriched in MgO and SiO_2 [similar to modern olivine boninites (*9, 10*)], and A-type magmas that were enriched in Al_2O_3 [similar to modern high-Al tholeiitic basalts (*9*)]. Phase equilibria studies have demonstrated that both A-type magmas and mixtures of U- and A-type magmas have a paragenetic sequence appropriate for OBZ I and the J-M Reef, which is olivine followed by plagioclase (*6, 9*). This sequence is different from that in the UMS, which is olivine followed by orthopyroxene (*5*). Mixing of compositionally disparate magmas may also have had a profound influence on the degree of sulfur saturation (*6, 7*). Because sulfide melt–silicate melt partition coefficients for the PGEs are large [$>10^5$ for the J-M Reef (*7*)], an immiscible sulfide melt that formed as a result of magma mixing would act as a collector of the PGEs (*7*). Hence, mixing of magmas with contrasting major and trace

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