break of acute gastroenteritis in Norwalk, Ohio. Two milliliters of a 1 to 100 dilution of 8FIIa in tris-buffered saline (TBS) was administered orally to each individual with 80 ml of Milli-Q water (Millipore). Sodium bicarbonate solution was taken by each person 2 min before and 5 min after virus administration. The volunteer studies were approved by the Institutional Review Board for Human Research at Baylor College of Medicine and at the Clinical Research Unit. The virus was administered to the volunteers in the General Clinical Research Center where the volunteers were hospitalized and under medical care for 4 days. All stools were collected and kept at −70°C for later use.
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18. Norwalk virus from stool samples was purified by the following procedure. A 10% solution of stool samples in TBS was first clarified by low-speed centrifugation at 3000 rpm for 15 min. The resultant supernate was then extracted two or three times with Genetron in the presence of 0.5% Zwittergent 3-14 detergent (Calbiochem). Virus in the aqueous phase was concentrated by pelleting at 36,000 rpm for 90 min through a 40% sucrose cushion in a 50.2 Ti rotor (Beckman Instruments). The pellets were suspended in TBS, sonicated, and mixed with CsCl solution (refractive index 1.368) in TBS and centrifuged at 35,000 rpm for 24 hours in a SW50.1 rotor (Beckman). The CsCl gradient was fractionated by bottom puncture and each fraction was monitored for virus by examination with an electron microscope. The peak fractions containing Norwalk virus were pooled, and CsCl in the samples was diluted with TBS and removed by centrifugation at 35,000 rpm for 1 hour in a SW50.1 rotor. The purified virus was stored at −70°C.
19. The cDNA libraries were prepared from nucleic acid extracted from purified Norwalk virus. In order to extract nucleic acids, purified Norwalk virus from CsCl gradients was first treated with proteinase K (400 μg/ml) in 1× proteinase K buffer [0.1 M tris-HCl (pH 7.5), 12.5 mM EDTA, 0.15 M NaCl, 1% (w/v) SDS] at 37°C for 30 min. The samples were then extracted once with phenol-chloroform and once with chloroform. Nucleic acid in the aqueous phase was concentrated by precipitation with 2.5 volumes of ethanol in the presence of 0.2 M sodium acetate followed by centrifugation for 15 min in a microcentrifuge. The nucleic acid was then denatured with 10 mM CH_3HgOH, and cDNA was synthesized (from both DNA and RNA) by means of the cDNA synthesis kit with the supplied random hexanucleotide primer (cDNA Synthesis System Plus, Amersham). After synthesis of the second strand the reaction mixture was extracted once with phenol-chloroform and once with chloroform followed by ethanol precipitation. Amplification of DNA was performed with buffers as described in the Prime-a-Gene Labeling System (Promega). Eight cycles of denaturation (100°C for 2 min), reannealing (2 min of cooling to room temperature), and elongation (room temperature for 30 min) were performed after addition of the Klenow fragment of DNA polymerase I (Promega). A cDNA library was constructed in pUC13 with blunt-end ligation into the Sma I site. White colonies from transformed DH5α (Bethesda Research Laboratories) cells were picked, and both a master plate and minipreps of plasmid DNA were prepared for each clone. Clones containing inserts were identified after electrophoresis of the plasmid DNA in an agarose gel. The insert DNA was excised by digestion with restriction enzymes, and insert DNA that was resolved in low-melting temperature agarose gels was cut out and labeled with ^{32}P by random priming. Nucleic acids extracted from paired stool samples (before and after Norwalk infection) from two volunteers (543 and 544) were dotted onto Zetabind filters (AMF, Cuno, Meriden, CT). Replicate filter strips were prepared and hybridized with each labeled insert probe individually at 65°C without formamide. Potential positive clones were judged by their different reactions with the pre- and postinfection stools. Clones that reacted with post- (but not pre-) infection stools of volunteers were considered positive, and these clones on the master plates were characterized further.
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25. The knowledge that the Norwalk virus genome is single-stranded RNA has permitted the synthesis of additional cDNA clones from nucleic acid that was extracted from small amounts (~1 g) of stool samples. These preliminary results indicate that it will be possible to obtain cloned DNAs from many clinical specimens.
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27. We thank A. Opekun for help with the volunteer studies; T. Tanaka for establishing and performing the RIA assays; E. Calomeni for the electron microscopy; and J. Butel, L. Donehower, and J. L. Melnick for helpful discussions of the manuscript. Supported by Food and Drug Administration grant 223-88-2182. The General Clinical Research Center is supported by the Division of Research Resources of the NIH under grant RR 00350.

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Homologous Recombination and Stable Transfection in the Parasitic Protozoan *Trypanosoma brucei*

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Development of methods for the manipulation of the genomes of parasitic protozoa will lead to enhanced understanding of parasite biology and host-parasite relationships. Efficient gene transfer and targeted integration by homologous recombination were achieved in the parasitic protozoan *Trypanosoma brucei*, the causative agent of sleeping sickness. An expression vector with the neomycin phosphotransferase gene (*neo*), under the control of a procyclic acidic repetitive protein (PARP) gene promoter, was targeted into an intergenic region in βα-tubulin–gene tandem array. Sixteen copies of *neo* were found in a tandem array in one of the transfectants where the PARP promoter controlled α-amanitin–resistant transcription of *neo*, whereas transcription of tubulin genes remained α-amanitin–sensitive.

TRYPANOSOMES ARE MEMBERS OF A large group of unicellular eukaryotic flagellates, causative agents of a wide spectrum of diseases in humans and livestock. The study of this group of protozoa has revealed many aspects of host-parasite interaction and novel biochemical control mechanisms, including precursor mRNA maturation by transsplicing, polycistronic transcription of protein coding genes, and RNA editing (*1*).

Transient expression of foreign genes has been achieved in several trypanosome species (*2–6*). Stable transfection of the protozoan *Leishmania* was also reported on the basis of the expression of *neo* from extrachromosomal elements. We had established transient DNA transfection of the protozoan *Trypanosoma brucei* by means of a plasmid construct in which the chloramphenicol acetyl transferase (CAT) gene was expressed from the promoter of one of the PARP genes (plasmid BNsp-CAT) (*6*). Positive selection was needed to identify trypanosomes in which exogenous DNA had been incorporated into chromosomes by targeted homologous recombination; therefore, the PARP promoter was used to direct expression of *neo* in the plasmid BNsp-Neo-T in *T. brucei* (Fig. 1A). To avoid disruption of essential trypanosome genes, we targeted the BNsp-Neo-T plasmid into the intergenic region of one of the tandem arrays of βα-tubulin genes. To mediate the homologous recombination at the tubulin intergenic regions, we inserted a 639-bp fragment derived from the intergenic region of the βα-tubulin genes (a blunt-ended Eco RI–Bgl II intergenic region fragment) (Fig. 1B) into the region immediately downstream of *neo*.

In *Saccharomyces cerevisiae*, the introduction of a double-stranded break in the region of homology between the transfected DNA and the target sequences drastically increases the efficiency of homologous recombination (*7*). Procyclic *T. brucei*, the form of the parasite found in its insect vector, the tsetse fly, was therefore transfected by electroporation with a BNsp-Neo-T plasmid, that had been linearized at a unique Mlu I site; this restriction site is located centrally in the 639-bp βα-tubulin intergenic region (see Fig. 1 legend). When the linearized plasmid was used, G418-resistant trypanosomes could be observed 2 weeks after transfection (in four individual experiments), whereas cells transfected with circular plasmids never developed G418 re-

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sistance. We have maintained the G418-resistant trypanosomes in medium containing G418 (25 μg/ml) for several months without noticeable changes in *neo* copy numbers.

The chromosomal location of the BNsp-Neo-T plasmid was determined in one G418-resistant population by pulsed-field gel electrophoresis (PFGE) and compared to the chromosomal location of βα-tubulin genes. Tubulin genes had previously been shown to be located on bands 11 (1.5 Mb) and 16 (3.0 Mb) by PFGE (*8*) (Fig. 2A). Nonspecifically trapped molecules also exist at the wells of these gels. As expected for integration events that mainly occurred by homologous recombination into the tubulin arrays, the *neo* coding region probe detected a hybridization signal on chromosomes 11 and 16 of the transfected trypanosomes and not in wild-type trypanosomes. This result indicates integration of the BNsp-Neo-T plasmid rather than the presence of *neo* on episomes. The existence of two hybridizing chromosomes indicates that at least two individual integration events occurred. The hybridization intensity of band 16 was, however, much stronger than that of band 11, and many copies of the BNsp-Neo-T plasmid may therefore have integrated in the chromosome (or chromosomes) at band 16. Alternatively, the trypanosome population might be heterogeneous, consisting of populations of cells with different integration events. Since these trypanosomes cannot be cloned readily, the presence of heterogeneous populations may confound their analysis.

If integration occurred by homologous recombination into the tubulin locus, new plasmid BNsp-Neo-T–derived restriction enzyme sites should have been generated in the regions flanking the β- and α-tubulin genes. When probes for either the β- or α-tubulin coding region were used, which do not cross-hybridize with the plasmid BNsp-Neo-T–derived sequences, additional

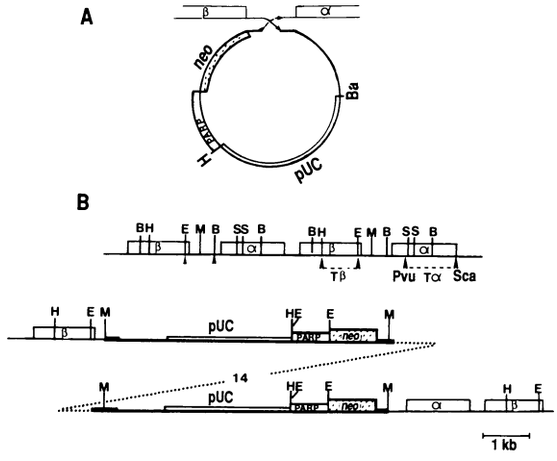

Fig. 1. (**A**) Construction of plasmid BNsp-Neo-T. The PARP promoter and 3′ splice acceptor site from the plasmid BNsp-CAT (*6*) were ligated to *neo* (Bgl II–Bam HI fragment) derived from the plasmid pSV2Neo (*12*). A blunt, 639-bp Eco RI–Bgl II fragment derived from the 3′ end of the βα-tubulin genes (*13*) was inserted into the Sma I site located downstream of the TAA translation termination codon of *neo*, in a 5′ to 3′ orientation. The BNsp-Neo-T plasmid was linearized at a unique Mlu I site located in the center of the tubulin intergenic sequence. The linearized BNsp-Neo-T plasmid was electroporated into the insect form of *T. brucei* stock 427-60, using 25 μF and 4000 V/cm as described (*6*). G418 was added 36 to 48 hours after transfection (final concentration, 25 μg/ml). The G418 concentration was slowly increased to 50 μg/ml over a 6-day period. Eight days after transfection, most cells had stopped dividing. Cells in the selection medium were spun down and resuspended into fresh medium containing G418 (25 μg/ml). (**B**) Top, the structure of tubulin locus in the wild-type trypanosomes. Bottom, the tubulin-*neo* locus in the transfected trypanosomes. Dotted lines below the top physical map represent the location of the probes. We used a probe for the β-tubulin coding sequence (Tβ), Hind III–Eco RI fragment, and a probe for the α-tubulin coding sequence (Tα), Pvu II–Sca I fragment; we also used the *neo* coding sequence (a 1-kb Bgl II–Sma I fragment derived from PSV2Neo, labeled *neo*) and plasmid pUC-18 (pUC) as probes. The solid lines in the bottom physical map indicate the inserted BNsp-Neo-T plasmid. Dots indicate the 14 additional plasmid copies. (Ba, Bam HI; B, Bgl II; E, Eco RI; H, Hind III; M, Mlu I; S, Sal I; and PARP, the PARP promoter and 3′ splice acceptor site.)

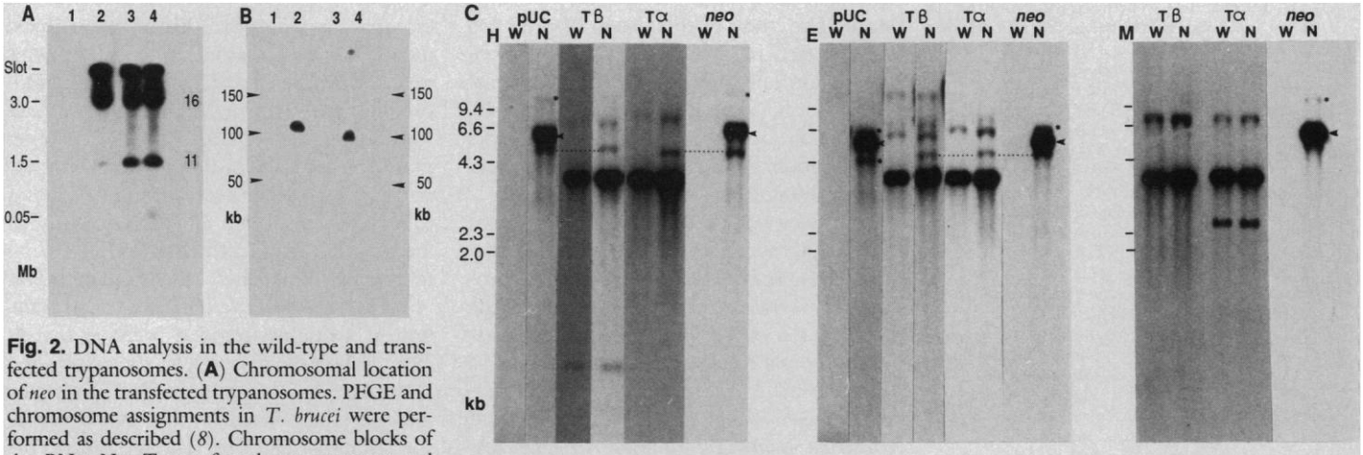

Fig. 2. DNA analysis in the wild-type and transfected trypanosomes. (**A**) Chromosomal location of *neo* in the transfected trypanosomes. PFGE and chromosome assignments in *T. brucei* were performed as described (*8*). Chromosome blocks of the BNsp-Neo-T–transfected trypanosomes and the wild-type insect form *T. brucei* stock 427-60 were separated in a 1% agarose PFGE gel for 7 days at 3 V/cm and a pulse frequency of 3000 s. Chromosome numbers were assigned as described previously (*8*). Parallel panels of PFGE blots were hybridized with the probe for the *neo* coding region (lane 1, wild type; lane 2, transfected) and probes Tα and Tβ (lane 3, wild type; lane 4, transfected). Post-hybridizational washing conditions were at 0.1× standard saline citrate (SSC) and 65°C. (**B**) PFGE. Chromosome blocks with DNA from transfected and wild-type trypanosomes were digested with the restriction enzymes Sal I (lane 1, wild type; lane 2, transfected) and Bgl II (lane 3, wild type; lane 4, transfected). DNA was separated in a 1% PFGE agarose gel for 30 hours at 16 V/cm with a pulse frequency of 10 s. The DNA was transferred to nitrocellulose filters, and the Southern blots were hybridized with the probe for the *neo* coding region (Bgl II–Bam HI fragment) or a probe for the tubulin coding region (*9*). Final post-hybridizational stringencies were at 65°C and 0.1× SSC. Multimers of phage lambda were used as size standards. (**C**) Physical mapping of the *neo* locus in transfected trypanosomes. Genomic DNA was isolated (*14*), digested with restriction enzymes, separated in 0.8% agarose gels, and transferred to nitrocellulose filters (*15*). After hybridization with ^{32}P-labeled probes, filters were washed to a final stringency of 0.1× SSC at 65°C. Dotted lines align the flanking fragments of the integration event; arrowheads indicate DNA fragments containing sizes predicted from a tandem array of BNsp-Neo-T plasmid; stars indicate an integration event that has not been further characterized. The band marked with the black dot, in the middle panel, represents the first copy of the array of pUC plasmids, flanking the β-tubulin gene. (H, Hind III; E, Eco RI; M, Mlu I; w, wild type; N, transfected. Abbreviations and probe locations are outlined in the Fig. 1 legend.)

restriction fragments were seen in genomic DNA from transfected trypanosomes (Fig. 2C). To confirm that these new bands resulted from the predicted homologous recombination, we isolated the polymorphic 4.6-kb and 5.0-kb Hind III fragments (which hybridized with α- and β-tubulin, respectively) from genomic libraries. Their DNA sequence analysis revealed that a β-tubulin gene was flanked by the pUC plasmid sequences derived from BNsp-Neo-T, whereas the α-tubulin gene was flanked by *neo*, as expected for a single integration event by homologous recombination (Fig. 3). Also, as predicted for the detection of these sequences in genomic DNA, the 4.6-kb Hind III fragment hybridized with *neo*, and the 5.0-kb band with the pUC probe (Fig. 2C). The DNA sequence also showed that the Mlu I site, used to linearize the plasmid, had been retained. Thus, as expected of integration by homologous recombination, an Mlu I digestion released the 6.2-kb plasmid (the size of the input BNsp-Neo-T plasmid) from the transfected DNA, while polymorphic restriction enzyme fragments could no longer be detected with probes for the tubulin coding region (Fig. 2C). However, the strong hybridization intensity of this linearized 6.2-kb plasmid band suggested that a tandem array of the BNsp-Neo-T plasmid might exist at the integration site.

To determine the number of individual integration events that had occurred, we generated a more detailed physical map of the regions surrounding the integrated BNsp-Neo-T plasmids. With both the pUC and *neo* probes, two additional hybridizing bands could be identified in a Hind III digestion of genomic DNA: a strongly hybridizing, 6.2-kb Hind III band and a weakly hybridizing, larger (12-kb) fragment (Fig. 2C). When restriction enzymes that do not cut the BNsp-Neo-T plasmid but that do cut the βα-tubulin genes were used, we were able to show that the strongly hybridizing 6.2-kb band could be mapped to a single strongly hybridizing Bgl II fragment of about 100 kb, or a Sal I fragment of 105 kb (Fig. 2B). Both the 100- and 105-kb bands also hybridized with the probes for the tubulin coding sequence and the size difference between these bands was as expected for the βα-tubulin physical map (Figs. 1B and 2B) (*9*). The relative intensity of the hybridizing BNsp-Neo-T bands and the size of the Bgl II and Sal I fragments thus indicated that they might consist of a tandem array of plasmid molecules. Indeed, as expected for a perfect tandem array of BNsp-Neo-T plasmid molecules, any of four different restriction enzymes that cut only once in the BNsp-Neo-T plasmid always released a 6.2-kb fragment from transfected genomic DNA (only Hind III and Mlu I are shown in Fig. 2C; Bam HI and Sca I were also tested). We conclude that a single 100-kb tandem array of plasmids is present at the βα-tubulin locus on PFGE band 16.

From the relative hybridization intensities of the 4.6- and 5.0-kb Hind III junction fragments and the major 6.2-kb BNsp-Neo-T plasmid band, we predicted the presence of 15 copies of the BNsp-Neo-T plasmid. The 100-kb Bgl II restriction fragment in the PFGE blot confirmed this estimate (16 × 6.2 kb). Presumably, a single integration event occurred into the βα-tubulin intergenic region, located at PFGE band 16, after which amplification of *neo* as a result of G418 selection occurred. Alternatively, multiple integration events might have occurred in a single cell at the same locus, or ligation of the plasmids, preceding the integration event, could have generated the tandem array of BNsp-Neo-T plasmids.

A single, additional, faintly hybridizing fragment of about 12 kb can be detected with both the *neo* and pUC probes, in both the Hind III and Mlu I digestions (band marked with an asterisk in Fig. 2C). Our preliminary data indicate that it is present at PFGE band 11 in a minor population of trypanosomes (*10*).

The expression of *neo* results in the generation of a discrete 1.4-kb mRNA, present in transfected cells only (Fig. 4A). Steady-state RNA derived from pUC plasmid sequences was not detected. The amount of tubulin mRNAs was similar in the transfected and the wild-type cells, and the expression of tubulin genes was therefore not affected by the integration of BNsp-Neo-T plasmids. Analysis of a *neo* cDNA showed that the 39-nucleotide mini-exon had been transspliced onto the 5′ end of the *neo* precursor mRNA by means of the PARP-derived 3′ splice acceptor site that is located just downstream of the PARP promoter (Fig. 4B).

Transcription directed by the PARP promoter has previously been shown to be resistant to the drug α-amanitin and might be mediated by RNA polymerase I (*1, 6*). In contrast, transcription of the tubulin genes is inhibited by α-amanitin and is presumably mediated by RNA polymerase II (*11*). We analyzed the α-amanitin sensitivity of transcription at the neomycin and tubulin loci to determine whether the integration event had affected transcription. For most of the DNA samples used in the slot blot (Fig. 4C) we used purified restriction fragments. Plasmid sequences were excluded because transcription of the integrated plasmid copies, now present in the trypanosome genome, could obscure the specific signals derived from the other cloned genes. The nascent RNA analysis revealed that transcription of the *neo* coding region was highly resistant to α-amanitin and occurred at a relatively high level, as predicted for the function of the strong PARP promoter. The efficiency and α-amanitin sensitivity of transcription of control sequences [ribosomal RNA, RNA polymerase I; the mini-exon donor RNA (medRNA) clone, RNA polymerase II; a Hind III fragment from the coding region for the hsp 70 gene, RNA polymerase II] was unaffected as measured by comparisons between the wild-type and transfected cells.

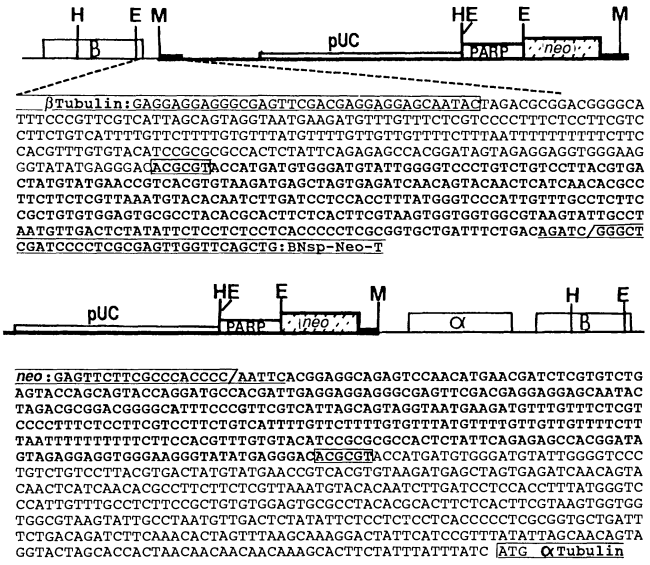

Fig. 3. DNA nucleotide sequence analysis of junction fragments. Two Hind III fragments of 4.6 and 5.0 kb were isolated from size-fractionated, Hind III genomic libraries made with DNA from transfected trypanosomes. The regions covering the βα-tubulin intergenic regions were subcloned into the phage M13mp18 and mp19 and their nucleotide sequence was determined by the Sanger dideoxy chain termination method (*16*). Physical maps made from the Hind III clones are indicated at the top of each sequence. The boxes in the DNA nucleotide sequence indicate the Mlu I sites. The underlined sequences (AGATC in the top sequence and AATTCA in the bottom sequence) represent the remainders of the blunted Bgl II and Eco RI restriction enzyme sites, respectively, which flanked the intergenic region (used for the construction of the BNsp-Neo-T plasmid). The plasmid BNsp-Neo-T sequences are printed in boldface. (Abbreviations are as indicated in the Fig. 1 legend.)

Transcription of tubulin genes in the BNsp-Neo-T–transfected trypanosomes remained sensitive to α-amanitin and occurred at a similar efficiency in wild-type trypanosomes. Therefore, the transcription of the tubulin locus was unaffected by its physical proximity to the *neo* locus.

The transcription at the *neo* locus is too abundant to enable us to comment on the potential read-through of α-amanitin–sensitive transcription that could have originated from the upstream tubulin array. However, as only α-amanitin–sensitive tubulin transcription was detected in the nascent RNA analysis, read-through or polycistronic transcription from the *neo* array into the downstream tubulin gene appears to have been negligible. In addition, transcription of plasmid pUC 18 occurred at a greatly (tenfold) reduced efficiency compared to *neo* transcription. The differences in the transcriptional efficiencies between the *neo* and pUC 18 sequences suggest that transcription of the tandem *neo* array may not be fully polycistronic. Alternatively, only few genes of the *neo* array might be active.

The integration event of plasmid BNsp-Neo-T into band 11 may also have contributed to the signal in the nascent RNA analysis. However, because the relative hybridization intensity of this plasmid shows that it is present at much less than 1% of total integrated plasmid, in a small population of G418-resistant cells (*10*), we expect its contribution to the nascent RNA signals to be limited.

We have shown that *T. brucei* can be efficiently transfected by means of homologous recombination, allowing the introduction and expression of foreign genes into the *T. brucei* genome. The efficiency of transfection when the linearized BNsp-Neo-T plasmid was used was about 5×10^{-6}. It is possible that an increase in the length of the homologous region of the plasmid and the target site will increase this efficiency. Because we have not been able to obtain transfectants using circular BNsp-Neo-T, the presence of a double-stranded break presumably increases the efficiency of the recombinational events. Our preliminary analysis indicates that comparable efficiencies of homologous recombination can also be obtained at other loci. The integration event can be explained by the double-strand–gap repair model involving gene conversion (*7*). However, this particular plasmid construct does not exclude integration events by a double crossover.

Homologous recombination can thus be used to stably introduce new genes, as well as to disrupt or delete known target genes for the study of trypanosome biology. Our results also indicate that these methods should be applicable to the production of auxotrophs that could be used as safe live vaccines for other protozoal infections.

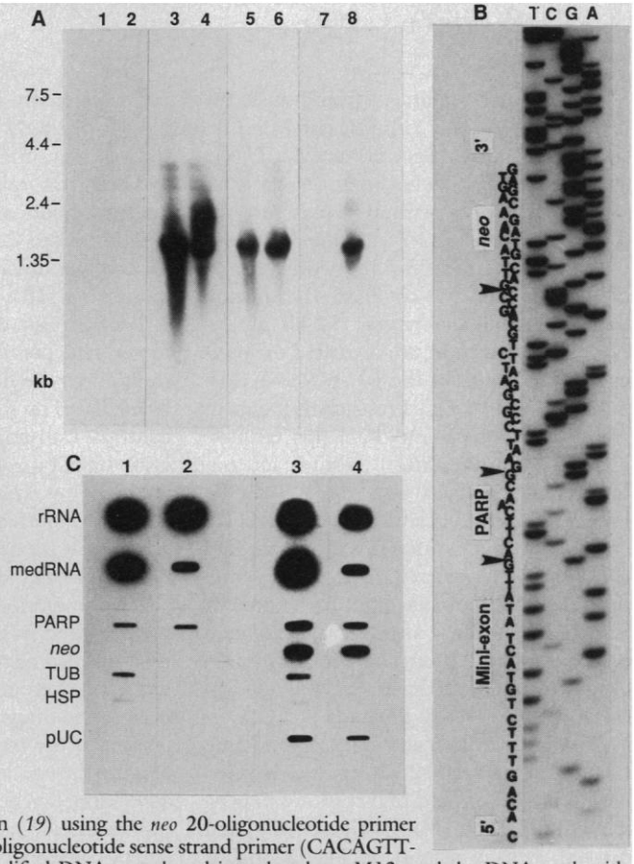

Fig. 4. (**A**) Analysis of steady-state mRNA from the tubulin-*neo* locus in transfected trypanosomes. Total RNA was isolated from transfected and wild-type trypanosomes as described (*17*). Total RNA was separated in a 1% formaldehyde agarose gel, transferred onto nitrocellulose filters and hybridized with the following probes: lane 1 (wild type) and lane 2 (transfected), pUC-18; lane 3 (wild type) and lane 4 (transfected), β-tubulin; lane 5 (wild type) and lane 6 (transfected), α-tubulin; and lane 7 (wild type) and lane 8 (transfected), the *neo* coding sequence. Post-hybridizational washes were performed at 0.1× SSC and 65°C. (**B**) Analysis of the nucleotide sequence at the 5′ end of *neo* mRNA. The cDNAs spanning the 5′ end of *neo* transcripts were obtained by primer extension (*18*) with a 20-oligonucleotide primer (CCATCTTGTTCAATCATGCG) complementary to the 5′ end of *neo*. The cDNAs were amplified by the polymerase chain reaction (*19*) using the *neo* 20-oligonucleotide primer and a mini-exon–derived 21-oligonucleotide sense strand primer (CACAGTTTCTGTACTATATTG). Amplified DNA was cloned into the phage M13; and the DNA nucleotide sequence was determined by the dideoxy chain termination method (*16*). Arrowheads separate, from 5′ to 3′, the mini-exon sequence, PARP-derived sequences that immediately follow the 3′ splice acceptor site, plasmid linker sequences, and *neo*-derived sequences. (**C**) Nascent RNA analysis. Nuclei preparation and nuclear run-on were performed as described (*11*). The left panel (lanes 1 and 2) shows nascent RNA analysis with RNA from wild-type trypanosomes and the right panel (lanes 3 and 4) shows nascent RNA analysis with RNA from transfected trypanosomes. Nuclear run-on analysis was performed without α-amanitin (lanes 1 and 3) or with α-amanitin (lanes 2 and 4) added to a final concentration of 1 mg/ml. Probes used are the *T. brucei* ribosomal transcription unit clone pR4 (rRNA) (*20*); the mini-exon clone, CL103 (medRNA) (*21*); a PARP coding sequence clone, CPT4 (PARP) (*11*); the coding region of *neo* (*neo*); the coding region of tubulin genes (TUB) (*13*); a 1.8-kb Hind III fragment from the coding region of the hsp 70 gene (HSP) (*22*); and plasmid pUC-18 (pUC). Final post-hybridizational washing stringency was at 0.1× SSC and 65°C. Hybridization signals were quantitated using a Beta Scope scintillation counter.

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Spontaneous Neurodegeneration in Transgenic Mice with Mutant Prion Protein

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Transgenic mice were created to assess genetic linkage between Gerstmann-Sträussler-Scheinker syndrome and a leucine substitution at codon 102 of the human prion protein gene. Spontaneous neurologic disease with spongiform degeneration and gliosis similar to that in mouse scrapie developed at a mean age of 166 days in 35 mice expressing mouse prion protein with the leucine substitution. Thus, many of the clinical and pathological features of Gerstmann-Sträussler-Scheinker syndrome are reproduced in transgenic mice containing a prion protein with a single amino acid substitution, illustrating that a neurodegenerative process similar to a human disease can be genetically modeled in animals.

GERSTMANN-STRÄUSSLER-SCHEINKER syndrome (GSS) is a rare human neurodegenerative disease that is vertically transmitted, apparently as an autosomal dominant trait (*1, 2*), and can often be horizontally transmitted to primates and rodents through intracerebral inoculation of brain homogenates from patients with the disease (*3*). Patients with GSS develop ataxia and dementia in the third to seventh decades of life and deteriorate until they die, in 1 to 10 years. The protease-resistant isoform of prion protein (PrP) is implicated in the pathogenesis and transmission of GSS and in scrapie, a similar animal disease (*4*). Enriching scrapie-infected hamster brain fractions for infectivity led to the discovery of PrP 27-30 (*5*), which was later recognized to be derived from a larger protein, PrP^{Sc}, by limited proteolysis (*6*). PrP^{Sc} is derived from cellular PrP (PrP^{C}) by a post-translational process; both PrP isoforms are encoded by a single copy gene (*7*).

A leucine substitution at codon 102 of the human PrP gene (*PRNP*) (*2, 8*) on the short arm of chromosome 20 (*9*) is genetically linked to GSS (*2*) and is present in families with GSS from different ethnic backgrounds (*2, 10, 11*). Other mutations in *PRNP* segregate with disease in families with other varieties of prion diseases (*11, 12*), providing evidence for a strong but not absolute correlation between specific PrP mutations and different clinical forms of familial prion diseases (*13*). Thus, the codon 102 mutation in *PRNP* is more than a linked genetic marker and may cause the disease or at least directly influence its pathogenesis.

Studies showing that Syrian golden hamster (Ha) PrP transgenes modify virtually all aspects of experimental scrapie in mice suggested that the dominantly inherited GSS phenotype might be manifest in transgenic mice expressing mutant PrP (*14, 15*). To test this hypothesis, we microinjected mouse (Mo) PrP genes containing a codon 101 leucine substitution (homologous to codon 102 in *PRNP*) into fertilized oocytes to produce transgenic mice (*14*). The microinjected DNA consisted of a chimeric murine cosmid constructed by exchanging the Bam HI–Sal I fragment containing the open reading frame (ORF) from a recombinant NZW genomic clone (*16*) for the corresponding fragment in an I/Ln mouse cosmid (*17*) (Fig. 1). We modified a ~100-nucleotide region flanking the codon 101 leucine to yield DNA sequence homology to HaPrP while retaining amino acid homology to MoPrP, thus enabling tail DNA of weanling mice to be screened with a ~60-nucleotide probe derived from this region. We detected mice containing $MoGSSPrP_{Leu^{101}}$ transgenes by comparing intensities of the radiolabeled probe hybridized to approximately 20 μg of denatured tail DNA from weanling mice or control hamsters.

Three transgenic founder lines were initially created—Tg(GSSPrP)174, Tg(GSSPrP)180, and Tg(GSSPrP)196. All animals were housed in a room in which no animals inoculated with scrapie prions had ever been kept; all second- and third-generation transgenic mice were kept in new cages and drank from new water bottles not previously exposed to scrapie-infected animals. The haploid transgene copy numbers in each line were determined by comparison of the intensities of a radiolabeled 0.6-kb Bst EII–Eco RI fragment near the 3′ end of exon III (containing the ORF) (*16, 17*) hybridized to mouse DNA of varying dilutions. Tg(GSSPrP)174, 180, and 196 mice harbored approximately 64, 9, and 6 haploid transgene copies, respectively. The male Tg(GSSPrP)174 founder mouse readily produced offspring, but the Tg(GSSPrP)180 founder is sterile and the female Tg(GSSPrP)196 founder has only recently produced progeny. PrP^{C} expression in Tg(GSSPrP)174 brains was > eightfold higher than in controls as determined by intensities of immunoblots on nitrocellulose of varying dilutions of brain extracts. The ~60-nucleotide hamster-biased probe (Fig. 1A) hybridized to 0.1 μg of transgenic brain RNA but not to 20 μg of nontransgenic brain RNA, thus confirming expression of the transgene. The mutant transgenes were inherited in a manner consistent with a single autosomal site of insertion, because this site was present in approximately half (87/176) of Tg(GSSPrP)174 progeny and could be transmitted by males.

Thirty-five Tg(GSSPrP)174 mice appeared healthy until symptoms of ataxia, lethargy, and rigidity developed between 7 and 39 weeks of age (Fig. 2). Mouse 0.1 in Fig. 2 is the nontransgenic littermate of the transgenic founder 0.2 and is now more

Table 1. Transgenic mice expressing *Prn-p*^{b} mouse and wild-type Syrian hamster PrP do not develop spontaneous neurologic dysfunction.

Mouse	Transgene	Copy number	*n*	Mean age (days)
NonTg*		0	24	225 (175–312)‡
Tg 94†	MoPrP-B	>10	16	468 (332–589)
Tg 117†	MoPrP-B	>10	4	488 (438–542)
Tg 71†	HaPrP	~4	15	364 (303–389)
Tg 81†	HaPrP	~30	26	400 (395–415)
Tg 7†	HaPrP	>60	14	313 (284–351)

*A representative sample of nontransgenic mice that are littermates of Tg(GSSPrP)174 mice. †Tg 94 and Tg 117 brain PrP^{C}, relative to nonTg, are fourfold higher. Tg 71, Tg 81, and Tg 7 brain $HaPrP^{C}$ are one- to twofold, two- to fourfold, and four- to eightfold higher, respectively, as determined by ELISA and immunoblot dilutions of brain extracts (*14, 15, 17, 20*). ‡The range is given in parentheses.

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