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Genetics

Identification and characterization of a self-regulated repressor of translocation of the Tn3 element

(plasmid/DNA sequence/amber mutation/minicells/transposon)

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ABSTRACT **Gene fusions that bring expression of the *lacZ* gene under control of transcriptional and translational signals within the transposable element Tn3 have been used to study regulation of Tn3-specified proteins. A gene encoding a 21,355-M_r peptide that represses translocation of Tn3 and acts at the level of transcription to regulate its own synthesis has been identified and sequenced; amber, missense, and *cis*-dominant (operator-constitutive) point mutations in this gene have been isolated and characterized.**

Earlier investigations have shown that the transposable element Tn3 (1) encodes a function required for its own translocation (2, 3), as well as a β-lactamase gene that renders host cells resistant to the antibiotics penicillin and ampicillin (Ap). Translocation of Tn3 is regulated by a mechanism that prevents continued accumulation of Tn3 by the recipient plasmid population during prolonged growth of cells that concurrently carry donor and recipient plasmids (4).

Recent studies from our laboratory have utilized *lac* gene fusions to investigate the control of operons located within Tn3; such studies have shown that Tn3 encodes a *trans*-acting substance that inhibits expression of *lac* genes fused to Tn3 DNA sequences and that also affects the frequency of Tn3 translocation (ref. 5; unpublished results). Here we report experiments that elucidate the molecular characteristics and genetic control of a protein that we have identified as a repressor of Tn3 translocation. Our results indicate that the repressor is a 21,355-M_r peptide that regulates its own synthesis.

MATERIALS AND METHODS

Escherichi coli K12 strain MC1050 (unpublished results), which includes mutations in the *lac* and *trp* genes, and the pMC391 plasmid (ref. 5; unpublished results), which carries a Tn3-*lacZ* fusion have been described, as has the minicell-producing strain χ1488M [a Met$^+$ revertant of χ1488 (F$^-$, *strA*, *hsr*$^-$, *hsm*$^+$, *minA*$^-$, *minB*$^-$, *purE*$^-$, *pdxC*$^-$, *his*$^-$, *ilv*$^-$, *met*$^-$) obtained from R. Curtiss]. The pACYC999 plasmid is a ColE1::Tn3 isolate (unpublished data). pSC178 (6) is a pSC101::Tn3 isolate. Growth of minicells in a supplemented 4-morpholinepropanesulfonic acid (MOPS)-based minimal medium (7) and purification and radioactive labeling of minicells were as described (8). Viable cell counts were less than 1 in 10^5 minicells. The procedures used for plasmid DNA isolation (9), hydroxylamine mutagenesis (10), transformation of $CaCl_2$-treated bacteria with plasmid DNA (11), agarose (12) and polyacrylamide (13) gel electrophoresis, and restriction endonuclease mapping of plasmid DNA (14) have been reported. Antibiotics used were Ap (ampicillin at 25 μg/ml or carbenicillin at 250 μg/ml), Km (kanamycin at 10 μg/ml), and Tc (tetracycline at 10 μg/ml).

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RESULTS

Isolation of Tn3 Mutants Defective in Repressor Function. The pMC391 plasmid is a derivative of pMC81 (15), in which deletions have fused *lac* genes to Tn3 sequences contained on the plasmid. The site of fusion on pMC391 enables a promotor located within Tn3 to accomplish transcription and consequent expression of *lac*; earlier experiments have shown that decreased expression of the *lac* phenotype and decreased translocation of the fused Tn3 element occur when a wild-type Tn3 is introduced into the same bacterial cell *in trans* (ref. 5; unpublished results). The Tn3-*lac* fusion of pMC391 was introduced by homologous recombination onto the pSC217 plasmid (i.e., pSC105::Tn3) (16) and was then rendered incapable of translocation by *in vitro* deletion of a *Bam*HI-generated DNA fragment containing the Tn3 terminus adjacent to the β-lactamase gene, yielding the Ap^S, Tc^S, Km^R pMC823 plasmid. Because the replicon utilized by pMC823 is compatible with the ColE1 replicon (17), pMC823 can be maintained stably in cells that carry ColE1::Tn3 plasmids, such as pACYC999.

In order to isolate Tn3 mutants that fail to inhibit phenotypic expression of β-galactosidase encoded by the pMC823 Tn3-*lacZ* fusion *in trans*, the pACYC999 plasmid was treated with hydroxylamine and introduced by transformation into strain MC1050 carrying pMC823 under conditions that ensure that each transformant represents an independent clone. Transformants (frequency of 2.3×10^3 per μg of DNA) were selected on lactose MacConkey plates containing Ap and Km. Although most of the transformants showed reduced *lac* expression similar to that observed with pMC823 in the presence of the unmutagenized pACYC999 plasmid, approximately 0.6% of 16,000 colonies examined showed no reduction of *lac* expression; these 104 colonies were termed nonrepressing clones.

To detect amber nonsense mutants among the nonrepressing clones, a bacteriophage λ derivative (λ*supF*) carrying an amber suppressor (18) was cross-streaked onto isolates of MC1050 carrying both pMC823 and a pACYC999-derived mutated plasmid; stable lysogens were identified by suppression of a *trp* amber mutation on the MC1050 bacterial chromosome by selection of Trp$^+$ cells on minimal media. Two of the 10–30 Trp$^+$ colonies appearing at each intersection were purified, and their Lac phenotype was compared with the phenotype of the nonlysogenized parent MC1050. Of the 104 original nonrepressing clones, 23 yielded colonies that showed at least partial suppression (19) by λ*supF* of the Tn3 mutation that had caused loss of repression activity, as indicated by reduced *lac* expression on lactose MacConkey plates. Two of these colonies and a clone carrying a nonsuppressible mutation were selected for further study; the coresident plasmids in each were separated by iso-

Abbreviations: Ap, ampicillin; Km, kanamycin; Tc, tetracycline; Ap^S, Ap-sensitive; Km^R, Km-resistant.

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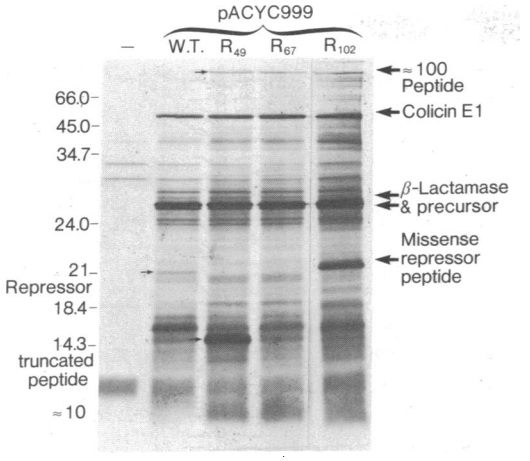

FIG. 1. One-dimensional autoradiogram showing ^{35}S-labeled polypeptides synthesized by minicells carrying either plasmid pACYC999 or its mutant derivatives. Purified minicells were resuspended at OD_{620} = 1 in 1.5 ml of growth medium containing the four DNA bases, pyridoxal, and a synthetic amino acid mixture (20) lacking methionine. After 25 min of incubation in the absence of label, 80 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{35}S]methionine (Amersham, 1295 Ci/mmol) was added and the incubation was continued at 30°C for 30 min. Minicells were harvested, resuspended in 50 μl of buffer (50 mM Tris·HCl, pH 6.8/10 mM $MgCl_2$), and frozen. Samples (7.5 μl) were suspended in sodium dodecyl sulfate-containing sample buffer (21), boiled for 2.5 min, and loaded onto 0.1% sodium dodecyl sulfate/15% polyacrylamide gels. Electrophoresis was at 6 W per gel for approximately 5.5 hr. Molecular weight standards were lysozyme (14,300), β-lactoglobulin (18,400), trypsinogen (24,000), pepsin (34,700), ovalbumin (45,000), and bovine plasma albumin (66,000). Arrows point to the 21,000-M_r polypeptide observed in extracts of wild-type Tn3 (W.T.) and to related peptides in the mutants -R_{49}, -R_{67}, and -R_{102}. The positions of colicin E1 and β-lactamase and its precursor (7, 22) are indicated. Nos. are × 10^{-3}. The track marked (–) is the minicell strain χ1488M alone.

lation of covalently closed circular plasmid DNA and subsequent transformation of strain MC1050. Tn3-containing plasmids carrying the amber-suppressible mutations were designated pACYC999-R_{49} and pACYC999-R_{67}. The nonsuppressible mutant was designed pACYC999-R_{102}.

Mutations that render expression of the Lac phenotype of the Tn3-*lacZ* fusion insensitive to repression by a coexisting wild-type Tn3 (i.e., *cis*-dominant) were isolated by procedures analogous to those described above. pMC823 DNA was mutagenized *in vitro* by hydroxylamine and introduced by transformation into strain MC1050 carrying the pACYC999 plasmid; colonies that showed greater *lac* expression than strains carrying pACYC999 plus the wild-type pMC823 were identified on lactose MacConkey plates. Thirteen such colonies were detected from a total of 10,000 colonies screened, and the mutagenized plasmids present in these clones were purified.

Table 1. Translocation frequencies of wild-type and mutant plasmids

	Translocation frequency	pSC178-R_{49} / pSC178
pSC178	7.5×10^{-6}	14
pSC178-R_{49}	1.1×10^{-4}	

pSC178 and a derivative of this plasmid into which the -R_{49} mutation had been introduced by recombination were introduced into a *recA* strain, EMC1916 (F′-Km/*recA*$_{56}$, *srl*::Tn10, *araD*139, Δ(*ara-ABIOC*, *leu*) 7697, Δ*lacx*74, *galUK*, *spcA*), by transformation. Cells were then mated at OD_{600} = 0.9 with a nalidixic acid derivative of strain M182 (F$^-$, Δ(*lacIPOZY*)χ74, *galK*, *galU*, *strA*), obtained from J. Beckwith. Transconjugants that had received an F′-Km plasmid carrying the wild-type or mutant Tn3 (Ap^R) element were scored by comparing the frequency of cells resistant to both Km and Ap with the frequency of Km resistance alone. The frequency shown reflects the particular assay system and host strains used.

Effects of Mutations on Tn3-Encoded Protein Synthesis. The pACYC999-R_{49}, -R_{67}, and -R_{102} plasmids and the parent pACYC999 plasmid were individually introduced by transformation into the minicell-producing strain χ1488M, and their ^{35}S-labeled polypeptide products were examined by electrophoresis on sodium dodecyl sulfate/polyacrylamide gels (Fig. 1). Several alterations were observed in the proteins specified by the mutant plasmids: (*i*) A peptide migrating at a position equivalent to an M_r of 21,000 and produced in small amounts by the wild-type Tn3 was lacking in both of the amber-suppressible mutants. pACYC999-R_{49} showed a (truncated) peptide produced in large amounts and migrating at the 14,000-M_r position, consistent with possible premature termination of the missing 21,000-M_r peptide as a consequence of amber mutation; -R_{67} shows no detectable truncated band. (*ii*) The nonsuppressible mutant -R_{102} shows an intense band migrating at the 21,000-M_r position; two-dimensional gel electrophoresis (data not shown) suggests that the isoelectric point of this peptide is slightly different from that of the wild-type protein. Thus, the pACYC999-R_{102} plasmid may contain a missense

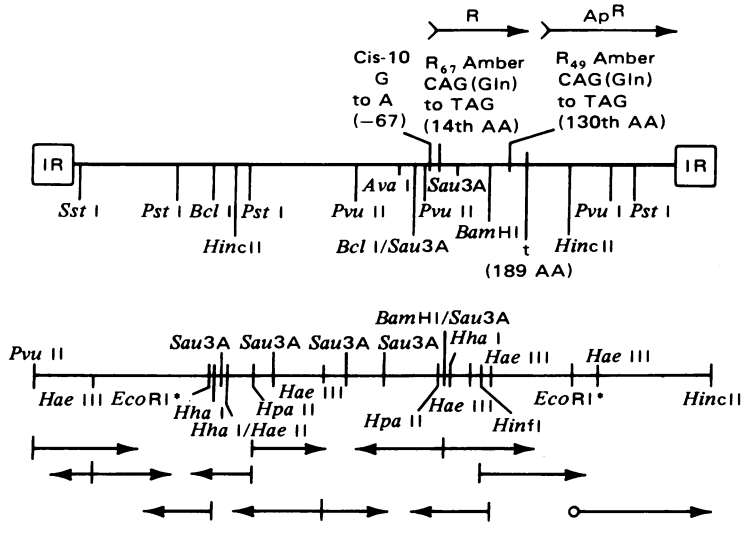

FIG. 2. (*Upper*) Restriction endonuclease cleavage map of Tn3 element showing the terminal inverted repeats (IR) and the locations of repressor (R) and β-lactamase (Ap^R) genes, -R_{49} and -R_{67} amber mutations, and the Cis_{10} operator-constitutive mutation. AA, amino acid. (*Lower*) Outline of sequence determination protocol employed for analysis of repressor gene. All DNA segments were labeled with [γ-^{32}P]ATP (≈3000 Ci/mmol, ICN) at the 5′ ends by using T4 polynucleotide kinase. Fragments were then cleaved at an internal endonuclease cleavage site, and segments containing radioactively labeled termini were separated by gel electrophoresis and analyzed as described by Maxam and Gilbert (25). Analysis of certain segments was overlapped as shown. The segment initiated by an open circle includes the region of Tn3 present on pBR322 (26).

Table 2. Effects of Tn3 *in trans* on β-galactosidase synthesis.

	β-Galactosidase, units	Ratio
Cis_5	1597	
Cis_5 + pACYC999	557	2.87
Cis_6	1233	
Cis_6 + pACYC999	557	2.21
Cis_8	1470	
Cis_8 + pACYC999	772	1.90
Cis_{10}	663	
Cis_{10} + pACYC999	281	2.36
Cis_{13}	1020	
Cis_{13} + pACYC999	420	2.43
pMC823	656	
pMC823 + pACYC999	119	5.51

Strain MC1050 carrying the plasmids indicated was grown to proper cell density in minimal media and assayed for β-galactosidase as described (19). The ratios shown refer to units of enzyme from the cells that contain only the mutant plasmid vs. those that also include pACYC999.

mutation that results in production of a nonfunctional 21,000-M_r peptide having one amino acid alteration.

Extracts from minicells bearing wild-type Tn3 and the -R_{49} and -R_{102} mutant plasmids were run on two-dimensional gels (23) (data not shown), and appropriate peptide spots were cut out and assayed (24) for ^{35}S radioactivity. The counts (normalized to the β-lactamase spot in each extract) in the spots corresponding to the truncated -R_{49} peptide (24,081 cpm) and the putative -R_{102} missense peptide (6769 cpm) showed an increase over the wild-type peptide (216 cpm). In each case where a mutation yields a peptide unable to repress *lac* expression from the Tn3-*lacZ* fusion *in trans*, a concomitant increase in the amount of the nonfunctional peptide was seen, suggesting that the peptide is self-regulated. The basis for the quantitative differences in the amount of missense vs. nonsense peptide synthesized in minicells is not known.

Functional Analysis of Amber and *cis*-Dominant (Operator-Constitutive) Mutants. The -R_{49} amber mutant was observed to undergo translocation at a frequency 14-fold higher than wild-type Tn3 (Table 1). For these experiments, the -R_{49} point mutation was moved by homologous recombination from the ColE1-derived pACYC999 plasmid to the poorly mobilizable pSC101 plasmid in order to avoid the complicating effects of interbacterial transfer of the donor ColE1 plasmid by the conjugative F′ plasmid. Synthesis of the 14,000-M_r truncated peptide by the constructed plasmid was confirmed in minicells (data not shown).

The effects of wild-type Tn3 *in trans* on β-galactosidase synthesis encoded by *cis*-dominant mutants of the pMC823 plasmid are shown in Table 2. In the presence of a Tn3 element that synthesizes a functional repressor, β-galactosidase production by the unmutated pMC823 fusion plasmid was decreased to about one-sixth its control level, whereas enzyme synthesis encoded by Cis_{10} and other *cis*-dominant mutations was decreased approximately 50%, consistent with the postulated decreased binding of the Tn3 repressor to its operator in the mutants. Furthermore, some of the *cis*-dominant mutants showed a higher constitutive level of β-galactosidase synthesis than the wild type, suggesting that these mutations may lie within the transcriptional promotor region that overlaps the operator for the Tn3 repressor gene.

DNA Sequence Analysis of Mutant and Wild-Type Plasmids. The estimated size (i.e., ≈14,000 M_r) of the truncated peptide replacing the 21,000-M_r band in the -R_{49} mutation suggested that the amber-suppressible mutation responsible for

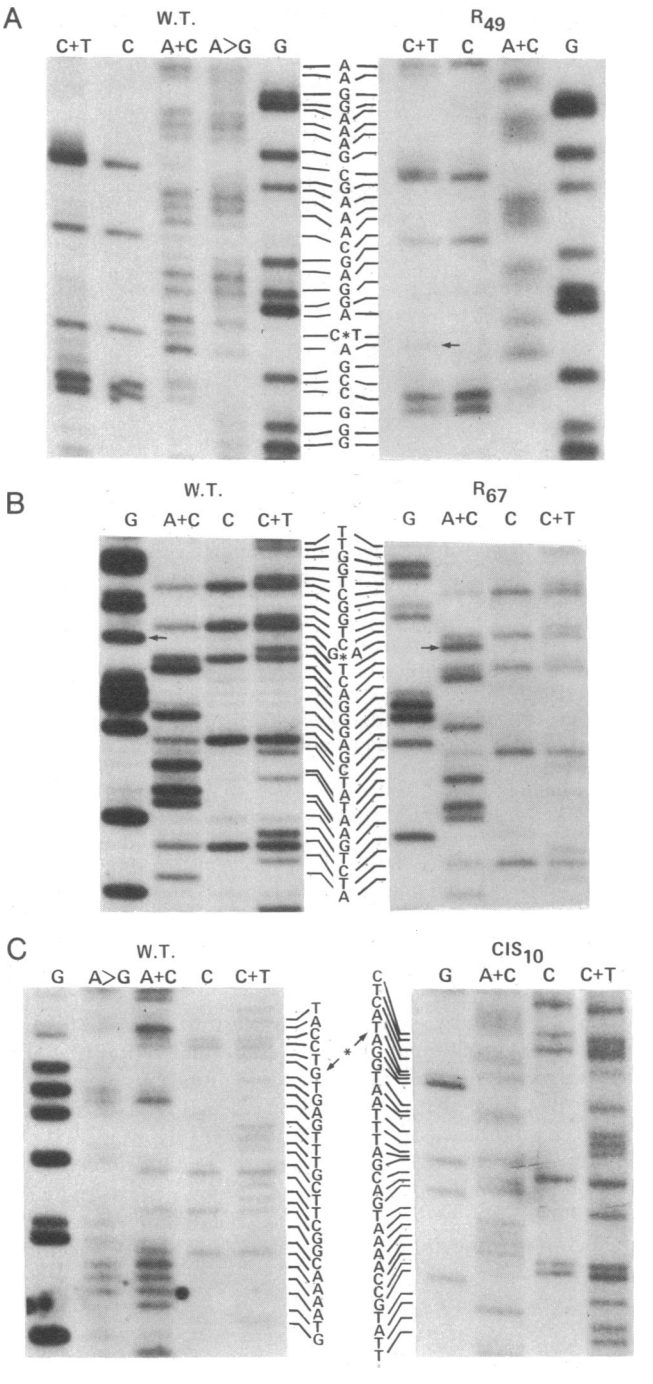

FIG. 3. DNA sequencing gels (25) that compare the wild-type (W.T.) Tn3 sequence with corresponding segments that carry the -R_{49}, -R_{67}, and Cis_{10} mutations. Arrows and * indicate locations of base changes corresponding to the mutations. In *C*, the sequences for the wild type and mutant were determined on different DNA strands and thus represent complements; the 5′ direction of both strands is "down." The locations of the sequences that appear here as excerpts are shown in Fig. 5.

premature termination of this peptide is located in a region about 370–420 nucleotides from the translational start codon. Similarly, our failure to observe any truncated peptide for the -R_{67} mutant suggested that this mutation might be located near the start of the gene, thus yielding a peptide fragment too short to be detected by gel electrophoresis. Endonuclease mapping followed by DNA sequence analysis (Fig. 2) of the appropriate corresponding segments of the wild-type Tn3 and the suppressible putative amber mutations -R_{49} and -R_{67} was carried

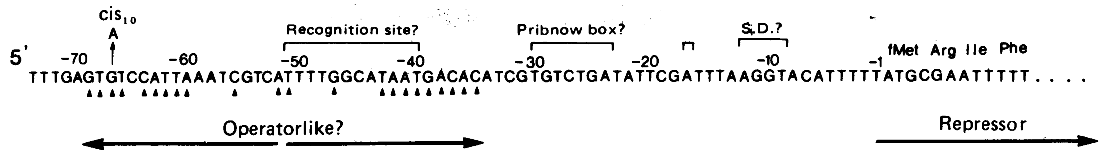

FIG. 4. Schematic diagram of putative transciptional and translational control regions of the Tn3 repressor of translocation. The principal features of the regions are noted in the figure and text. The numbers refer to the nucleotide distance preceeding the ATG (AUG) translational start codon. The complementary nucleotides within the palindromic operator-like sequence are indicated. S.D., segment partially complementary to the Shine and Dalgarno sequence.

out. The relevant sections of the sequencing gels are shown in Fig. 3: -R_{49} contains a single hydroxylamine-induced base change (C → T) in the codon for amino acid 130 (Gln), converting the CAG to an amber termination codon (TAG). -R_{67} showed a similar conversion in the codon for the 14th amino acid. In both instances, the translational reading frame inferred from the nucleotide sequence of the amber-suppressible mutation is seen to be the same as the frame observed for the hybrid protein within the Tn3-*lacZ* fusion sequence on pMC391 (5).

The genetic properties of the *cis*-dominant or operator-constitutive type mutations, which render the Tn3-*lac* fusion insensitive to repression *in trans* by a wild-type Tn3 element, suggested that these mutations were located in the operator-promotor control region of the Tn3 gene fused to *lac* on pMC823. DNA sequence analysis of *Pvu* II endonuclease-generated DNA fragments that include this region from mutant and wild-type plasmids (Fig. 3C) shows a hydroxylamine-induced single base change (G → A) at a position 67 nucleotides before the putative AUG translational start codon of the same gene that contains the -R_{49} and -R_{67} amber mutations. The nucleotide changed by the mutation lies within an 18-base pair incomplete palindrome of the type found previously to be associated with bacterial operators (Fig. 4) (27, 28). This DNA sequence, which includes a dA-dT rich region (at base pairs −40 to −50 relative to the AUG) characteristic of an RNA polymerase recognition site (29), is followed by other elements characteristic of a transcriptional and translational control region: a candidate Pribnow box (30) and a segment (S.D.) partially complementary to the sequence at the 3′ end of 16S ribosomal RNA proposed by Shine and Dalgarno (31) to be involved in the binding of mRNA to ribosomes.

The entire sequence of the 567-base pair long Tn3 repressor gene is shown in Fig. 5. The AUG triplet at position 1 was de-

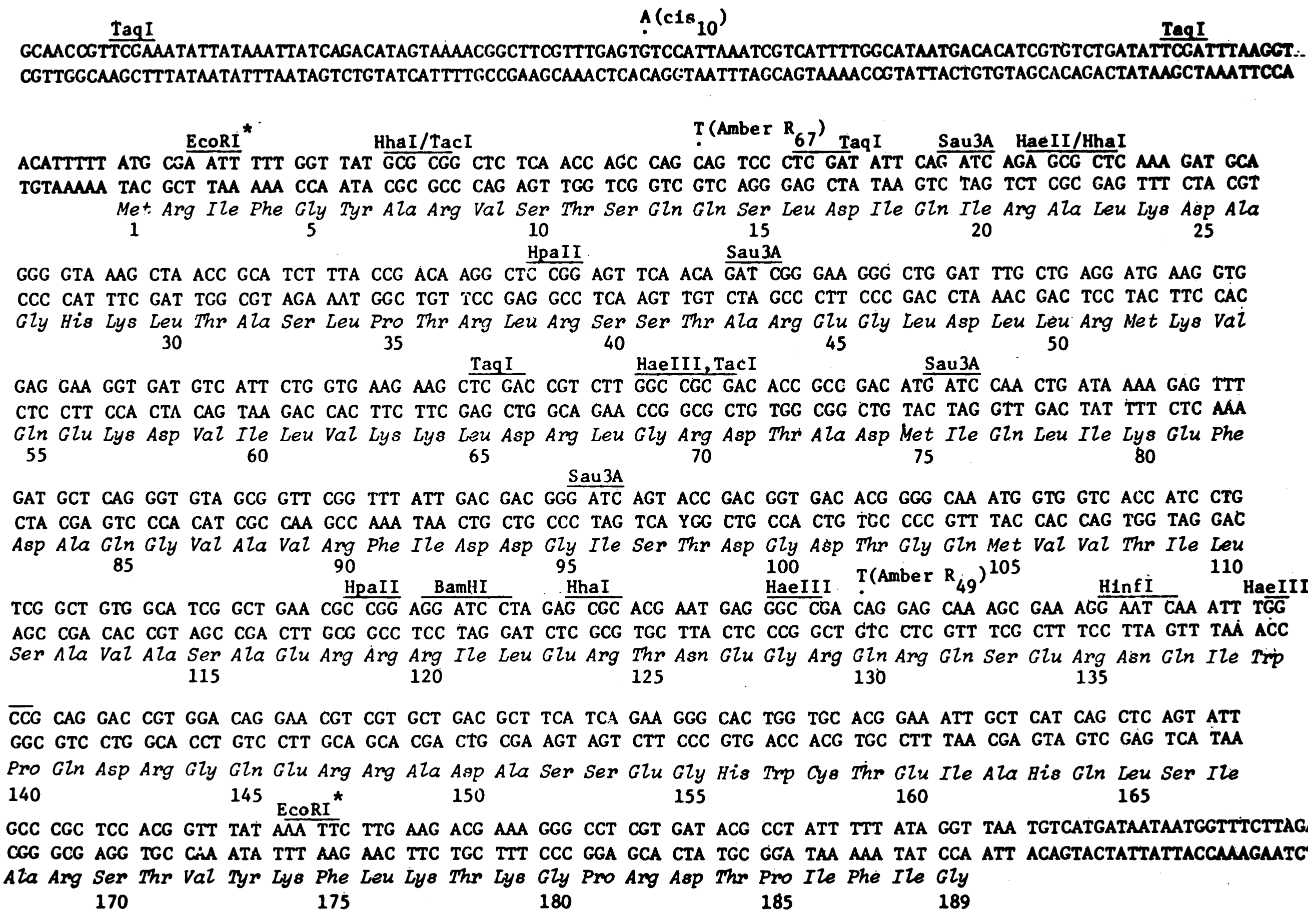
TaqI A(cis10) TaqI
GCAACCGTTCGAAATATTATAAATTATCAGACATAGTAAAACGGCTTCGTTTGAGTGTCCATTAAATCGTCATTTTGGCATAATGACACATCGTGTCTGATATTCGATTTAAGGT
CGTTGGCAAGCTTTATAATATTTAATAGTCTGTATCATTTTGCCGAAGCAAACTCACAGGTAATTTAGCAGTAAAACCGTATTACTGTGTAGCACAGACTATAAGCTAAATTCCA
EcoRI* HhaI/TacI T(Amber R67) TaqI Sau3A HaeII/HhaI
ACATTTTT ATG CGA ATT TTT GGT TAT GCG CGG CTC TCA ACC AGC CAG CAG TCC CTC GAT ATT CAG ATC AGA GCG CTC AAA GAT GCA
TGTAAAAA TAC GCT TAA AAA CCA ATA CGC GCC GAG AGT TGG TCG GTC GTC AGG GAG CTA TAA GTC TAG TCT CGC GAG TTT CTA CGT
Met Arg Ile Phe Gly Tyr Ala Arg Val Ser Thr Ser Gln Gln Ser Leu Asp Ile Gln Ile Arg Ala Leu Lys Asp Ala
1 5 10 15 20 25
HpaII Sau3A
GGG GTA AAG CTA ACC GCA TCT TTA CCG ACA AGG CTC CGG AGT TCA ACA GAT CGG GAA GGG CTG GAT TTG CTG AGG ATG AAG GTG
CCC CAT TTC GAT TGG CGT AGA AAT GGC TGT TCC GAG GCC TCA AGT TGT CTA GCC CTT CCC GAC CTA AAC GAC TCC TAC TTC CAC
Gly His Lys Leu Thr Ala Ser Leu Pro Thr Arg Leu Arg Ser Ser Thr Ala Arg Glu Gly Leu Asp Leu Leu Arg Met Lys Val
30 35 40 45 50
TaqI HaeIII,TacI Sau3A
GAG GAA GGT GAT GTC ATT CTG GTG AAG AAG CTC GAC CGT CTT GGC CGC GAC ACC GCC GAC ATG ATC CAA CTG ATA AAA GAG TTT
CTC CTT CCA CTA CAG TAA GAC CAC TTC TTC GAG CTG GCA GAA CCG GCG CTG TGG CGG CTG TAC TAG GTT GAC TAT TTT CTC AAA
Gln Glu Lys Asp Val Ile Leu Val Lys Lys Leu Asp Arg Leu Gly Arg Asp Thr Ala Asp Met Ile Gln Leu Ile Lys Glu Phe
55 60 65 70 75 80
Sau3A
GAT GCT CAG GGT GTA GCG GTT CGG TTT ATT GAC GAC GGG ATC AGT ACC GAC GGT GAC ACG GGG CAA ATG GTG GTC ACC ATC CTG
CTA CGA GTC CCA CAT CGC CAA GCC AAA TAA CTG CTG CCC TAG TCA YGG CTG CCA CTG TCC CCC GTT TAC CAC CAG TGG TAG GAC
Asp Ala Gln Gly Val Ala Val Arg Phe Ile Asp Asp Gly Ile Ser Thr Asp Gly Asp Thr Gly Gln Met Val Val Thr Ile Leu
85 90 95 100 105 110
HpaII BamHI HhaI HaeIII T(Amber R49) HinfI HaeIII
TCG GCT GTG GCA TCG GCT GAA CGC CGG AGG ATC CTA GAG CGC ACG AAT GAG GGC CGA CAG GAG CAA AGC GAA AGG AAT CAA ATT TGG
AGC CGA CAC CGT AGC CGA CTT GCG GCC TCC TAG GAT CTC GCG TGC TTA CTC CCG GCT GTC CTC GTT TCG CTT TCC TTA GTT TAA ACC
Ser Ala Val Ala Ser Ala Glu Arg Arg Arg Ile Leu Glu Arg Thr Asn Glu Gly Arg Gln Arg Gln Ser Glu Arg Asn Gln Ile Trp
115 120 125 130 135
CCG CAG GAC CGT GGA CAG GAA CGT CGT GCT GAC GCT TCA TCA GAA GGG CAC TGG TGC ACG GAA ATT GCT CAT CAG CTC AGT ATT
GGC GTC CTG GCA CCT GTC CTT GCA GCA CGA CTG CGA AGT AGT CTT CCC GTG ACC ACG TGC CTT TAA CGA GTA GTC GAG TCA TAA
Pro Gln Asp Arg Gly Gln Glu Arg Arg Ala Asp Ala Ser Ser Glu Gly His Trp Cys Thr Glu Ile Ala His Gln Leu Ser Ile
140 145 150 155 160 165
EcoRI*
GCC CGC TCC ACG GTT TAT AAA TTC TTG AAG ACG AAA GGG CCT CGT GAT ACG CCT ATT TTT ATA GGT TAA TGTCATGATAATAATGGTTTCTTAGAC
CGG GCG AGG TGC CAA ATA TTT AAG AAC TTC TGC TTT CCC GGA GCA CTA TGC GGA TAA AAA TAT CCA ATT ACAGTACTATTATTACCAAAGAATCTG
Ala Arg Ser Thr Val Tyr Lys Phe Leu Lys Thr Lys Gly Pro Arg Asp Thr Pro Ile Phe Ile Gly
170 175 180 185 189

FIG. 5. Nucleotide sequence of the Tn3 repressor of translocation, showing endonuclease cleavage sites and the amino acid sequence predicted from the determined DNA sequence. The fragment proceeding rightward from the *Eco*RI* site at amino acid 174 of the repressor gene is contained on the pBR322 plasmid, and its DNA sequence has been independently determined (26). Sequence determination was carried out as outlined in Fig. 2.

termined to be the translational start signal for the protein by the reading frame of the *lacZ* sequence in the pMC391 fusion (5) (Fig. 4), and also by the translational reading frame of the nonsense condons identified by the $-R_{49}$ and $-R_{67}$ amber mutations; no other AUG in the correct reading frame was seen for more than 250 base pairs in the NH_2-terminal direction from the point of the fusion. The last of the 189 amino acids of the gene defined by the reading frame shown is followed by a TAA translational termination codon. The amino acid composition predicted from the experimentally determined DNA sequence indicates an M_r of 21,355 for the peptide, which is in agreement with the size estimated for the repressor protein by gel analysis. In addition, the high content of lysine and arginine (17%) predicted from the DNA sequence is consistent with the relatively basic isoelectric focusing point at pH 7.6 that we have observed for the repressor peptide in gels.

DISCUSSION

Our structural and functional analysis of amber, missense, and *cis*-dominant (operator-constitutive) mutations that affect translocation frequency leads us to conclude that the gene we have identified (5) and determined the sequence of does in fact encode a repressor of Tn3 translocation. Premature termination of translation by nonsense mutations located in amino acid 14 or 130 of the repressor peptide results in an increased translocation frequency, loss of repression activity *in trans* on β-galactosidase produced by *lac* genes fused to the NH_2-terminal segment of the repressor, and (in one of the cases) elevated synthesis of a truncated peptide. This suggests that the Tn3 repressor gene is autogenously regulated. The observed increase in production, as a result of nonsense mutations and a presumed missense mutation, of either truncated or altered full-length peptides that do not repress translocation is consistent with this interpretation. Moreover, identification of operator-constitutive mutations, one of which has been mapped in a palindromic-like segment that preceeds the DNA sequence encoding the repressor protein, further supports the view that the Tn3 repressor controls transcription of its own gene. Our findings explain the origin of low molecular weight polypeptides observed by Dougan *et al.* (22) to be associated with deletion mutations in the segment shown here to contain the self-regulated repressor gene and also explain the increased translocation frequency associated with such deletions (2, 3, 32).

In the absence of a functional repressor, there is a prominent increase in the synthesis of certain other Tn3-encoded proteins, one of which has an approximate M_r of 100,000 (see Fig. 1). Deletion studies and analysis of fusions in which the *lac* genes have been joined to the NH_2 terminal segment of this peptide suggests that the 100,000-M_r peptide is produced in increased amounts in the absence of functional repressor and is a major protein required for translocation (unpublished results); the origin and function of shorter peptides produced in elevated amounts in repressor-minus mutants have not been determined. Increased synthesis of these various proteins by mutants that do not make a functional repressor should facilitate their biochemical analysis. Moreover, the availability of *cis*-dominant mutants potentially enables constitutive production of the wild-type repressor.

The methods employed here offer a general approach to the analysis of genes of previously undefined structure and function within transposable elements. Our findings provide a genetic and structural basis for regulation of Tn3 translocation, and further indicate that such regulation occurs by an active process that involves control of gene expression in Tn3 at the level of RNA synthesis.

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