C-Terminal Deletions Can Suppress Temperature-Sensitive Mutations and Change Dominance in the Phage Mu Repressor

Jodi L. Vogel,*[,1] Vincent Geuskens,[†] Lucie Desmet,[†] N. Patrick Higgins* and Ariane Toussaint[†,2]

**Department of Biochemistry, University of Alabama, Birmingham, Alabama 35294 and [†]Laboratoire de Génétique, Unité Transposition Bactérienne, Département de Biologie Moléculaire, Rhode Saint Genèse, Belgium*

Manuscript received July 27, 1995
Accepted for publication December 6, 1995

ABSTRACT

Mutations in an N-terminal 70-amino acid domain of bacteriophage Mu's repressor cause temperature-sensitive DNA-binding activity. Surprisingly, amber mutations can conditionally correct the heat-sensitive defect in three mutant forms of the repressor gene, *cts25* (D43-G), *cts62* (R47-Q) and *cts71* (M28-I), and in the appropriate bacterial host produce a heat-stable Sts phenotype (for *s*urvival of *t*emperature *s*hifts). *Sts* repressor mutants are heat sensitive when in *supE* or *supF* hosts and heat resistant when in Sup° hosts. Mutants with an Sts phenotype have amber mutations at one of three codons, Q179, Q187, or Q190. The Sts phenotype relates to the repressor size: in Sup° hosts *sts* repressors are shorter by seven, 10, or 18 amino acids compared to repressors in *supE* or *supF* hosts. The truncated form of the *sts62-1* repressor, which lacks 18 residues (Q179–V196), binds Mu operator DNA more stably at 42° *in vitro* compared to its full-length counterpart (*cts62* repressor). In addition to influencing temperature sensitivity, the C-terminus appears to control the susceptibility to *in vivo* Clp proteolysis by influencing the multimeric structure of repressor.

THE repressors of temperate bacteriophage are sensory proteins as well as operator-specific DNA-binding proteins. The function of the repressor is to integrate the biology of the virus with the biology of the host bacterium. For example, phage λ repressor is designed to detect DNA damage in the host (CRAIG and ROBERTS 1980; LITTLE 1984). When DNA damage leads to an increase in the abundance of intracellular single-stranded DNA intermediates, the activated RecA protein acts as a coprotease that stimulates repressor cleavage and eliminates the repressor's cooperativity in DNA binding. This proteolytic cleavage reaction places λ under the umbrella of the SOS regulatory network. Similarly, phage Mu repressor connects viral transposition to the physiology of its host. Insight into physiological control over Mu transposition comes from three lines of work. (1) Transposition patterns in growing colonies containing Mu*dlac* fusion elements can be visualized using the blue X-Gal cytological stain (SHAPIRO 1984; SHAPIRO and HIGGINS 1989; HIGGINS 1992). As colonies grow and age, the products of transposition are revealed in colonies as blue staining rings and wedge-like sectors; these patterns demonstrate that transposition is temporally and spatially regulated within the colony. (2) Since Mu's transposition pattern is characteristic in different strains, host mutants can be identified that change the pattern. Bacterial genes known to alter the frequency or timing of Mu transposition include *hns* (FALCONI *et al.* 1991), *fis* (FALCONI *et al.* 1991; BETERMIER *et al.* 1993), *clpP* and *clpX* (GEUSKENS *et al.* 1992; SHAPIRO 1993; MHAMMEDI-ALAOUI *et al.* 1994), *crp* (SHAPIRO 1994; A. MAENHAUT-MICHEL and J. A. SHAPIRO, personal communication) and DNA *polI* (SHAPIRO 1992). Two of the proteins encoded by this group of genes, H-NS and FIS, change their abundance in response to cell physiology and consequently alter the expression of numerous operons in *Escherichia coli* (BALL *et al.* 1992; FINKEL and JOHNSON 1992; DERSCH SCHMIDT and BREMER 1993). (3) In rare cases, Mu transposition activity produces chromosomal rearrangements that are selectable. For example, transposition of a Mu prophage situated between a defective *lac* operon and a promoterless *ara* operon can generate fusions that place the *ara* operon under control of *lac* regulatory sequences (SHAPIRO 1984). The probability of forming Mu-driven *ara-lac* fusions varies, being significantly higher in physiologically stressed cells compared to cells grown on a rich medium (SHAPIRO 1984; MITTLER and LENSKI 1990; FOSTER and CAIRNS 1994; MAENHAUT-MICHEL and SHAPIRO 1994). Whereas these observations show that physiology significantly alters Mu activity, little is known about the control circuits that modulate transposition.

Genetic and molecular studies of Mu repressor show that the protein has two domains that work together to establish and maintain repression at the Mu

Corresponding author: N. Patrick Higgins, Department of Biochemistry and Molecular Genetics, University of Alabama, 861-A Bevill Biomedical Research Bldg., Box 13, 845 19th St. South, Birmingham, AL 35294-2170. E-mail: nphiggins@bmg.bhs.uab.edu

[1]*Present address:* NIH-NIAID-LVD, Bldg. 4 Room 133, 9000 Rockville Pike, Bethesda, MD 20892.

[2]*Present address:* Laboratoire de Biochimie des Micro-organismes, CERMO, Université J. Fourier, PB 53X, 38041 Grenoble cedex 9, France.

operator: an N-terminal segment that binds operator DNA and a central region that contains a multimerization site.

The repressor headpiece: The N-terminal portion of repressor (amino acids M1–L77 and hereafter referred to as the headpiece) recognizes and binds operator DNA. Evidence for this conclusion stems from three observations. (1) Several changes in the headpiece alter DNA-binding activity of the protein. For example, 12 of 13 randomly selected temperature-sensitive mutants changed a single residue at one of four positions in the N-terminal domain: S18-L in *cts45*, M28-I in *cts71*, G43-D in *cts25* and R47-Q in *cts62* (VOGEL *et al.* 1991). These mutants are temperature sensitive for lysogenic growth *in vivo*, and the purified *cts62* repressor is temperature sensitive for DNA-binding activity *in vitro*. (2) A chimeric protein consisting of 63 amino acids of the headpiece fused to an enzymatically active β-galactosidase protein confers immunity *in vivo* and binds the operator DNA *in vitro* (VOGEL *et al.* 1991). (3) The Mu repressor headpiece is similar in sequence to the N-terminal domain of the Mu transposase, which also binds operator sites to enhance transposition efficiency (HARSHEY *et al.* 1985; LEUNG *et al.* 1989; MIZUUCHI and MIZUUCHI 1989). A recent NMR-derived structure of the N-terminal domain of Mu transposase proposes a novel "winged helix-turn-helix" DNA-binding motif for this region of the protein (CLUBB *et al.* 1994).

The multimerization domain: Mu repressor binds to nine sites in operator DNA through a cooperative mechanism (KRAUSE and HIGGINS 1986; VOGEL *et al.* 1991). Whereas the headpiece can function when appended to a large foreign tetramerization element like β-galactosidase, the repressor-*lacZ* chimera is much less effective at establishing repression *in vivo* and binding DNA *in vitro* compared to the wild-type or *cts* repressor (VOGEL *et al.* 1991). A leucine-rich segment in the central portion of the protein, which spans amino acids L121–L162 and is referred to here as the central domain, has been proposed to support efficient multimerization (VOGEL *et al.* 1991). Studies of the *cts4* mutation (L129-V), which affects the oligomeric structure of the repressor, is consistent with this hypothesis (J. E. Laachouch, P. ROUSSEAU, L. DESMET, R. ALAZARD, M. CHANDLER, and A. TOUSSAINT, unpublished results).

The operator: Bacteriophage Mu has three operator sites: O_1, O_2, and O_3 (Figure 1) KRAUSE and HIGGINS 1986; VOGEL *et al.* 1991). Two convergent promoters within the O_2 and O_3 operator sequences are the control points for lytic and lysogenic transcriptional regulation: the P_E promoter starts early lytic transcription and the P_c promoter starts transcription of the phage repressor gene *c* (Figure 1). A model for regulation of P_E and Pc transcription proposes that a DNA loop is formed by repressors bound to operator sites in O_1 and O_2 (VOGEL *et al.* 1991). The loop, which is stabilized by IHF binding and bending of the DNA at a position between O_1 and O_2, blocks RNA polymerase transcription from the P_E promoter but permits transcription from the P_c promoter (VOGEL *et al.* 1991; ALAZARD *et al.* 1992; GAMA *et al.* 1992). Repression of P_c transcription involves a larger loop with repressors bound at three sites, O_1, O_2, and O_3 (VOGEL *et al.* 1991).

The repressor tail: The focus of this report is the C-terminal segment, which includes residues I170–V196 and is hereafter referred to as the tail. The tail influences two distinct repressor activities, DNA binding and protein stability. We discovered amber mutants at three glutamine codons in the repressor tail that shorten the repressors and suppress the heat sensitivity of three temperature-sensitive headpiece mutants: *cts25*, *cts62*, and *cts71*. This is the only case that we know of where temperature-sensitive defects are corrected by protein shortening, and it shows that the tail modulates DNA-binding activity of repressor. Second, the repressor tail determines repressor susceptibility to degradation through a proteolytic pathway catalyzed by the ATP-dependent ClpP/ClpX protease. Dominance of Clp proteolysis *in vivo* is mediated through the tail. These two behaviors suggest that the tail influences the physiological behavior of repressor and it may be part of a sensory input/output element that links Mu repression to the physiology of its host.

MATERIALS AND METHODS

Bacterial strains and plasmids: The bacterial strains, phages, and plasmids used in this work are listed in Table 1. Plasmids used in cloning were pUC19 (YANISCH-PERRON *et al.* 1985) and pRS551 (SIMONS *et al.* 1987). All cell growth was in Luria-Bertani medium (LB) prepared as described previously (MILLER 1972).

Bacteriophage: Mu *clr3* (*c*3213) was isolated as a spontaneous clear mutant from Mu c^+pAp1, which is a recombinant between Mu c^+ (TAYLOR 1963) and Mu *cts62pAp1* (LEACH and SYMONDS 1979). Mu *sts* mutants were isolated after *mutD* mutagenesis (in KD1079) by the method described by TOUSSAINT *et al.* (1987). The mutants *sts62-1* and *sts62-2* were derived from Mu *cts62pKn7701* (*kil*$^-$). The mutants *sts25-1* and *sts71-1* were derived from Mu *cts25pAp1* and Mu *cts71pAp1*, respectively, by direct selection for survival of lysogens at 42°. All the *cts* mutations used were described previously (VOGEL *et al.* 1991). The different Mu *ctspAp1* derivatives were constructed by recombination between a Mu *cts* phage and a cryptic prophage (Mu Δ*9ApAp1*) that carries a deletion removing the *c*, *ner*, and *A* genes (TOUSSAINT *et al.* 1987). Lysates of Mu *cts* phage grown on a strain carrying F'pro *lac::Mu* Δ*9ApAp1* were used to infect 594 selecting for Ap^R lysogens. To clone Mu repressor genes in single copy, we used the plasmid and λ phage system developed by SIMONS *et al.* (1987). Repressor genes were cloned into plasmid pRS551 and subsequently recombined into λ RS45, which inserts in single copy efficiently at *attB*.

Repressor cloning, purification, and assays: Preparation and analysis of Western blots has been described previously (GEUSKENS *et al.* 1991). Methods for cloning repressor mutants and for sequencing plasmid DNA were described previously (VOGEL *et al.* 1991). Purification of the short form of the *sts62-1* repressor was done using extracts of N99[pJV213]. Repressor synthesis was induced in cells grown in a 28L New

TABLE 1

Strains used

	Description	Reference
E. coli strain		
DH5α	*endo*A1 *hsdR*17 (*rk*$^-$, *mk*$^+$) *supE*44 *thi*-1 *recA*1 *gyrA*96 *relA1* D(*argF-lacZYA*) *U*169 ϕ80*dlac* Δ*am*15	BRL
N99	*rpsL galK* F$^-$	H. NASH
594	*rpsL galK lacZ*	APPLEYARD (1954)
KD1079	*mutD5 thr leu argD his spcA*	DEGNEN and COX (1974)
C600	F$^-$ *lacY*1 *tonA*21 *thr*-1 *leu*-6 *thi*-1 *supE*44	APPLEYARD (1954)
BHB2600	*hsr met gal lacY supE supF*	HOHN (1979)
XA100	*ara* Δ(*lac-pro*) *nalA metB argE-am rif*	COULONDRE and MILLER (1977)
XA102	*ara* Δ(*lac-pro*) *nalA metB argE-am rif supE*	COULONDRE and MILLER (1977)
XA103	*ara* Δ(*lac-pro*) *nalA metB argE-am rif supF*	COULONDRE and MILLER (1977)
MC4100	F$^-$ *araD* Δ(*argF-lacZYA*) *U*169 *rpsL relA1 flbB deoC ptsF rbsR*	COULONDRE and MILLER (1977)
NH710	MC4100 λJV300	VOGEL *et al.* (1991)
NH711	MC4100 λJV304	VOGEL *et al.* (1991)
NH712	MC4100 λJV313	This study
Bacteriophage		
Mu *c*$^+$		M. HOWE
Mu *cts62*pAp1	R47-Q	VOGEL *et al.* (1991)
Mu *sts62-1*	R47-Q, Q179-Am	This study
Mu *sts62-2*	R47-Q, Q187-Am	This study
Mu *cts25*pAp1	G43-D	VOGEL *et al.* (1991)
Mu *sts25-1*	G43-D, Q190-Am	This study
Mu *cts71*pAp1	M28-I	VOGEL *et al.* (1991)
Mu *sts71-1*	M28-I, Q187-Am	This study
Mu *vir3060*	*c*+, −1 frameshift in codon 186	GEUSKENS *et al.* (1991)
Mu *vir3061*	*cts62* (R47-Q), +2 frameshift at codon 171	GUESKENS *et al.* (1991)

Plasmids	Vector	Mu Insert	Reference
pJV200	pUC19	*attL-Hin*dIII Mu *c*$^+$	VOGEL *et al.* (1991)
pJV202	pUC19	*attL-Hin*dIII Mu *cts25*	VOGEL *et al.* (1991)
pJV204	pUC19	*attL-Hin*dIII Mu *cts62*	VOGEL *et al.* (1991)
pJV213	pUC19	*attL-Hin*dIII Mu *sts62-1*	This study
pJV214	pUC19	*attL-Hin*dIII Mu *sts62-2*	This study
pJV218	pUC19	*attL-Hin*dIII Mu *sts25-1*	This study
pJV304	pRS551	*attL-Hae*III Mu *cts62*	VOGEL *et al.* (1991)
pJV313	pRS551	*attL-Hae*III Mu *sts62-1*	This study

Brunswick fermentor at 37°. When the culture reached an A_{650} of 1, isopropyl thiogalactoside was added (1 mM), and incubation was continued for 3 hr at 37°. Purification of repressor followed the method of KRAUSE and HIGGINS (1986), and the purified protein (>95% pure as judged by SDS-polyacrylamide gel electrophoresis) was tested for contaminating nuclease activity. There was no detectable single- or double-stranded DNase activity using linear or supercoiled DNA substrates. Repressor binding reactions were carried out as described previously (VOGEL *et al.* 1991). Plasmid pHK09 was labeled at the *Eco*RI site by filling restriction enzyme-cut ends with α^{32}P-dATP and the Klenow large fragment of DNA PolI. Then, 10-μl reactions containing 5.5×10^{-9} M pHK09 DNA, the indicated amount of repressor, 50 ng/ml salmon sperm DNA and binding buffer were incubated for 1 hr at 30°. Five units of *Hin*dIII restriction enzyme were added to each mixture, and restriction enzyme cleavage was carried out for 1 hr by incubation at 30°, 37° or 41°. Products separated on a 7.5% polyacrylamide gel were visualized by autoradiography. The fraction of cut and uncut DNA was measured by densitometry.

RESULTS

Isolation of amber suppressors of *cts* repressors: An unexpected class of repressor mutant was isolated from Mu *cts62* lysogens using a selection designed to find amber mutations in phage transposition/replication genes. Mu derepression kills cells by two mechanisms: expression of the cytotoxic *kil* gene (PAOLOZZI and SYMONDS 1986) and transpositional scrambling of the host genome. The selection involves two manipulations. First, Mu *cts kil*$^-$ lysogens in a wild-type host are induced by shifting exponential cultures from 30° to 42°. This treatment induces viral replication and results in efficient killing of induced cells by replicative transposition. Rare survivors of the heat treatment are isolated, a suppressor tRNA gene is introduced, and the heat induction is repeated to recover phage that replicate only when a suppressor is present. This two-tiered selec-

TABLE 2

Mu *cts* and Mu *sts* lysogenization at 42° and 30°

Mu phage	XA100 (Sup°)	594 (Sup°)	XA102 (*supE*)	C600 (*supE44*)	XA103 (*supF*)
*cts62Kn*7701	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
*sts62-1Kn*7701	0.8	2	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
*sts62-2Kn*7701	0.5	0.8	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
*cts25*pAp1	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
*sts25-1*pAp1	0.6	0.75	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$	$<5 \times 10^{-4}$
*cts71*pAp1	ND	$<5 \times 10^{-3}$	ND	$<3 \times 10^{-5}$	$<2 \times 10^{-5}$
sts71-1	ND	1	ND	$<3 \times 10^{-5}$	$<10^{-4}$

The lysogenization frequencies were measured as described by VOGEL *et al.* 1991. The numbers are the ratio between the frequencies of lysogenization of each phage at 42° *vs.* 30°. ND, not determined.

tion was previously used to find amber mutants in the Mu *A* (transposase) and *B* genes (TOUSSAINT *et al.* 1987) and also to isolate mutants in nonessential host genes required for Mu transposition (ROSS *et al.* 1986). However, one additional mutant class with wild-type host and phage replication functions had lost thermo-inducibility in Sup° strains of bacteria. Such mutants had an intriguing Sts phenotype (for *s*urviving *t*emperature *s*hift); in strains with no amber suppressing t-RNA they were thermo-stable, but in amber suppressing strains they were thermo-inducible like the parental type.

In addition to allowing lysogens to survive temperature shifts, *sts* mutants had a related lysogenization phenotype. For example, the parental Mu *cts62*pAp1 phage lysogenizes most strains of *E. coli* >1000-fold less efficiently at 42° compared to 30°. This behavior is due to the heat-sensitive DNA-binding activity of the *cts62* repressor protein (VOGEL *et al.* 1991). An *sts* mutant (*sts62-2*) lysogenized Sup° strains of *E. coli,* XA100 and 594, as efficiently at 42° as at 30°. Yet, on three amber-suppressing strains, XA102 (*supE*), C600 (*supE*44), or XA103 (*supF*), lysogenization of *sts62-2* was indistinguishable from Mu *cts62.* Five independent mutants (*sts62-1* to *sts62-5*) behaved identically. Data for the *sts62-2* mutant is shown in Table 2.

Phage production after thermal induction of Mu *sts* lysogens also responded to the amber genotype of the host. For example, a *supE, supF E. coli* strain (BHB2600) lysogenic for the Mu *sts62-1* prophage produced a titer of 5×10^{10} plaque-forming units per ml (PFU) after shift from 30° to 42°, but the *E. coli* Sup° strain (594) carrying that same prophage produced a titer of only 10^7 PFU. This is >1000-fold lower than the amount of phage produced in a 594 (Sup°) strain carrying the parental Mu *cts62* prophage (Table 3). When a plasmid with an amber suppressor t-RNA (RP4*SuA*1p2) was introduced into the strain, thermo-inducibility of the Mu *sts62-1* was restored.

Plaque morphology followed the pattern seen in induced liquid cultures. Each *sts* mutant made turbid plaques at 30° and 42° when plated on Sup° hosts but made clear plaques at 42° on *supE* or *supF* hosts. The Sts phenotype was stable, and phage retained their phenotype after lysogenizing Sup° or *supF* hosts or after lytic growth on strains with or without suppressors. Thus, *sts* mutants have conditional inducibility: in Sup° hosts they are pseudo-wild type and heat resistant like phage carrying a wild-type repressor, but in hosts with suppressor tRNAs they are heat inducible like *cts62.* These results suggested that *sts* phage retain the *cts* mutations.

All of the results described above were obtained with a single temperature-sensitive repressor allele, *cts62,* which carries an R47-Q substitution. This amino acid substitution might have exceptional properties since it accounts for half of all *cts* mutants isolated after EMS, nitrosoguanidine, or *mutD* mutagenesis (VOGEL *et al.* 1991). To find if other *cts* alleles behaved in a similar way, we looked for *sts* mutants, starting with phage carrying either *cts25* or *cts71* repressor genes. Strains lysogenic for Mu *cts25*pAp1, which has a G43-D substitution, or *cts71*pAp1, which has a M28-I substitution, were plated on LB at 42°. Survivors were purified and mated with a strain carrying RP4*SuA1p2.* Phage were induced from these cultures by heating to 42°, plaques were streaked to isolate individual phage, stocks were grown, and lysogens were isolated at 30°. Like the lysogens of Mu *sts62-1* and Mu *sts62-2,* lysogens of Mu *sts25-1* and Mu *sts71-1* were stable at 42° in Sup° and heat sensitive in amber-suppressing hosts. We conclude from these results that amber mutations can suppress three different heat-sensitive repressor mutations and that the Sts phenotype is not a unique allele-specific effect.

Mu *sts* mutants lack C-terminal amino acids: The amber suppression of heat sensitivity was difficult to reconcile with the fact that *cts62, cts25,* and *cts71* mutations all change the primary sequence of a DNA-binding domain at the N-terminal end of the protein (Figure 1). The *cts62* repressor protein has dramatically impaired DNA-binding activity at 37° and 42° (VOGEL *et al.* 1991). How could nonsuppressed amber mutations stabilize DNA-binding activity of three *cts* mutant proteins? To address this question, the *sts* repressor genes were cloned and sequenced. In each case, the *cts* mutations were retained and secondary amber mutations were

TABLE 3

Phage (PFU/ml) after thermoinduction of Sup° and *supE/F* lysogens

	Monolysogens			Dilysogens
Prophage	594 (Sup°)	594/RP4*SuA1p2* (*supF*)	Other *E. coli* (*supF* or *supE*, *supF*)	594/F′ *pro lac*::Mu *cts62* (Sup°)
cts62 Kn	$>10^9$	$>10^9$	$>10^9$ [a]	$>10^9$
sts62-1 Kn	3×10^6	$>10^9$	$>10^9$ [a]	4×10^7
sts25-1 Ap	$<10^4$	ND	$>10^9$ [b]	$<10^4$

[a] BHB2600.
[b] XA103. ND, not determined.

found in the repressor tail (Figure 1). Five independent *sts* mutants derived from *cts62*, one *sts* mutant derived from *cts25*, and one *sts* mutant derived from *cts71* had each gained an amber mutation at one of three CAG codons, Gln179 (*sts62-1* and *sts62-3*) Gln187 (*sts62-2*, *sts62-4*, *sts62-5*, and *sts71-1*) and Gln190 (*sts25-1*).

The DNA sequence analysis predicted that in a Sup° host, *sts* mutants would produce repressors shorter than the *cts62* (or c^+) repressor by seven, 11, and 18 amino acids, respectively. In *supE* or *supF* hosts we expect both the truncated and the full size forms because amber suppression is usually incomplete. Proteins expressed

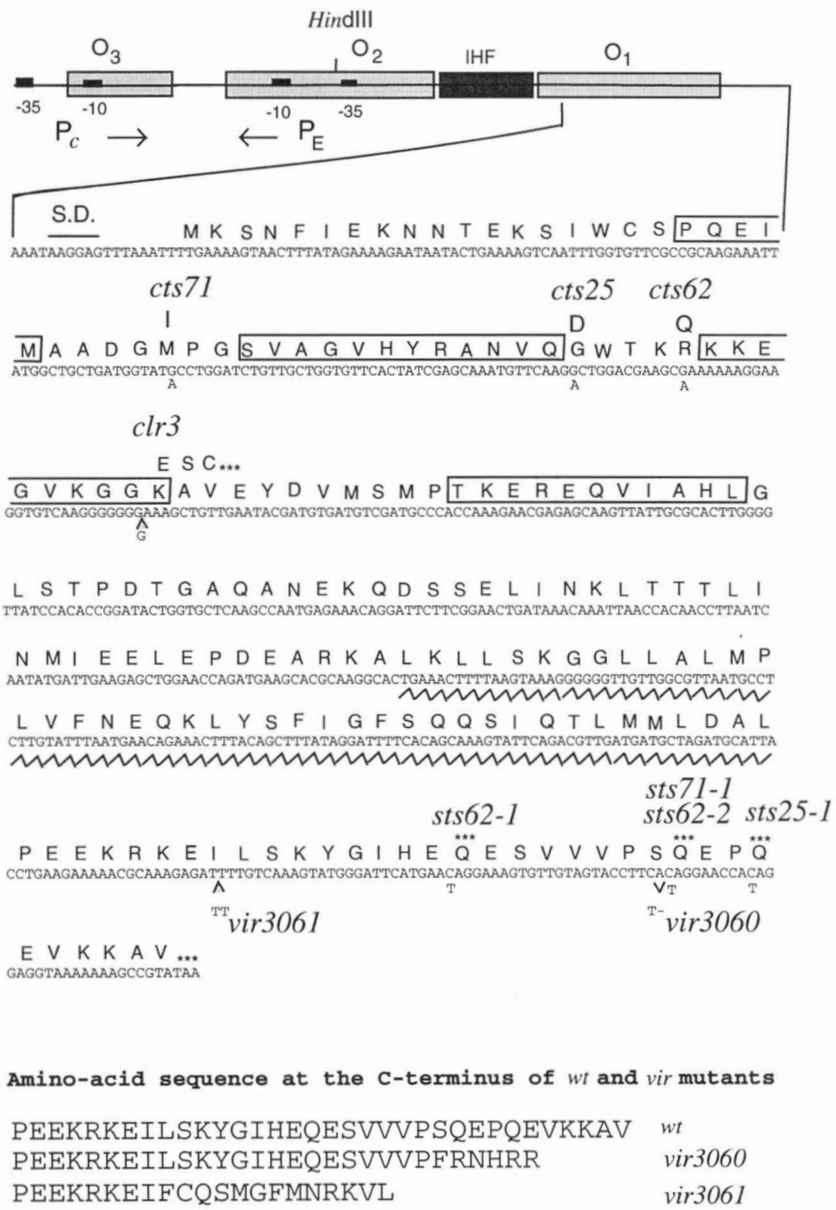

FIGURE 1.—Mu operator DNA and sequence of repressor mutations. Mu operator DNA has three repressor-binding segments: O_1, O_2, and O_3 indicated by ▭. Repressor bound at O_3 overlaps with the RNA polymerase −10 contact for the repressor P*c* promoter. Repressor bound at O_2 overlaps with RNA polymerase −10 and −35 contacts with the P_E promoter. An integration host factor binding site is shown as a dark stippled box between O_2 and O_1. The complete nucleotide sequence of c^+ repressor is shown, and *cts* and *sts* mutations are indicated below the c^+ sequence. Nucleotide insertions causing frame shifts in the *clr3* and *vir3061* mutations are indicated by a carat at the insertion point, and the nucleotide deletion of the *vir3060* mutation is indicated by an inverted carat. The positions of termination codons caused by mutations are shown with *, and amino acids related to the "winged helix-turn-helix" DNA-binding motif of Mu transposase (CLUBB *et al.* 1994) are boxed and correlated with the following landmarks: helix 1 (P19-M23), helix 2 (S31-Q42), the wing (K48-K54), and helix 3 (T67-L77). Coding changes associated with *cts*, *clr*, and *sts* mutations are given above the single letter code for c^+ protein. A potential leucine-rich dimerization interface is underlined with a jagged line from L121-L162. Single letter code for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr.

Amino-acid sequence at the C-terminus of *wt* and *vir* mutants

PEEKRKEILSKYGIHEQESVVVPSQEPQEVKKAV	*wt*
PEEKRKEILSKYGIHEQESVVVPFRNHRR	*vir3060*
PEEKRKEIFCQSMGFMNRKVL	*vir3061*

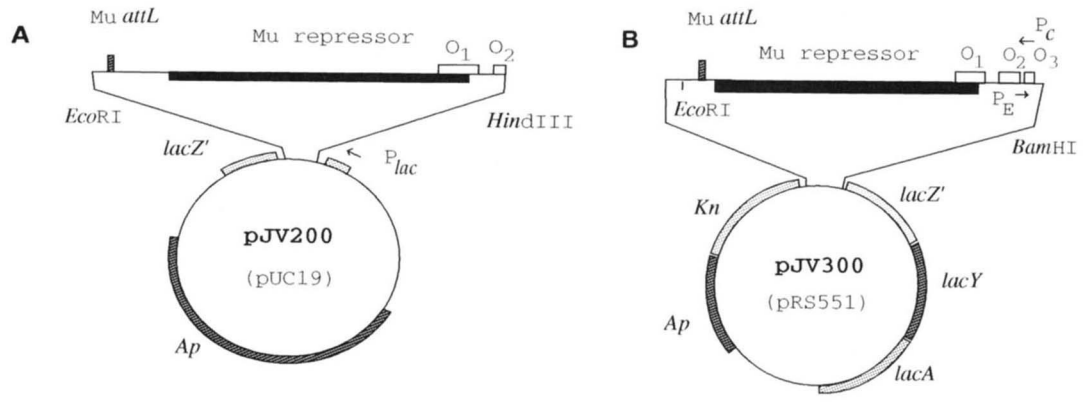

FIGURE 2.—Plasmid maps. (A) The pJV plasmids numbered in the 200s have Mu repressor genes inserted between *Hin*dIII and *Sma*I sites of the pUC19 polylinker with expression controlled by P_{lac}. (B) The pJV plasmids numbered in the 300s have repressor genes and Mu regulatory sequences cloned in pRS551 (SIMONS *et al.* 1987). Here, Mu repressor expression is controlled by P_c, and β-galactosidase expression is controlled by P_E.

from multicopy plasmids (Figure 2) carrying the wild-type and mutant *c* genes were displayed on SDS-containing polyacrylamide gels and then examined by Western blot analysis with rabbit antibodies prepared against the purified *cts62* protein (Figure 3). Each *sts* mutant made two polypeptides. One band comigrated with purified wild-type repressor, and each *sts* mutant made a shorter, more abundant, and faster-moving band. The sizes of the faster-moving bands correlate with the predicted mobility from the location of the *sts* amber mutations in the *c* gene. When plasmids were placed in Sup° backgrounds, only the faster-moving bands were found (data not shown). All repressor bands were stable for at least 90 min after addition of chloramphenicol (data not shown). Thus, repressors terminated at the amber codons were stable truncated proteins with sizes predicted by their DNA sequence.

Heat-stable DNA binding of *sts62-1* protein: The simplest explanation for the Sts phenotype is that truncated forms of *cts* repressors have heat-resistant DNA-binding activity. To eliminate complications that might involve other phage-encoded proteins, *sts* repressor genes were cloned into a system where all Mu genes except that for repressor were eliminated. We described previously the construction and use of the pJV300 series plasmids (Figure 2) (GEUSKENS *et al.* 1991; VOGEL *et al.* 1991). In these constructs, Mu repressor regulates its own transcription from the P_c promoter, but all other phage functions are replaced with the *lac* operon, which is under the control of the Mu early promoter P_E (Figure 2B, Table 4). The pJV300 series plasmids can recombine with a λ derivative that integrates into the chromosome at *attB* as a stable lysogen.

The *lacZ* expression levels were measured with strains lysogenic for the λ clones of c^+ repressor (λJV300), *cts62* repressor (λJV304), and *sts62-1* repressor (λJV313). Each strain exhibited different behavior. At 32°, cultures of NH711 (*cts62*) made 27 units of β-galactosidase, and after 20 min at 42°, expression rose 100-fold to ~2500 units. In agreement with the phage production and plaque morphology results explained above, the *sts* repression was efficient at low and high temperatures. At 32°, NH712 (*sts62-1*) produced only 4 units of β-galactosidase, which was tighter repression than the 27 units in NH711 (*cts62*). After 20 min at 42°, the β-galactosidase activity of NH712 cultures rose to only 44 units, or ~150% of the level of NH711 grown at 32°. By comparison, a wild-type repressor strain, NH710 (c^+), made 25 units of β-galactosidase at 32° or 42°. These results prove that the *sts62-1* repressor, which retains some thermo-sensitivity, confers efficient repression at 42° *in vivo*.

The heat-stable repression found with the *sts62-1* re-

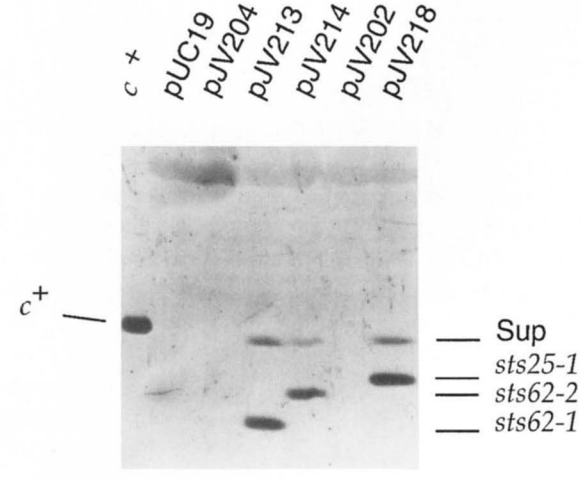

FIGURE 3.—Western blot analysis of mutant repressor proteins. Proteins from DH5α (*supE44*) transformants harboring pUC19, pJV204, pJV213, pJV214, pJV202 and pJV218 were transferred to a nitrocellulose filter, and the bands corresponding to Mu repressor were measured by reaction with repressor-specific rabbit antibodies as described in MATERIALS AND METHODS. Plasmids pJV204 (*cts62*) and pJV202 (*cts25*) made full-length repressor proteins, but in amounts that were below the detection limit of this Western blot (J. L. VOGEL, unpublished results).

TABLE 4

***lacZ* expression in λ lysogens**

Time after shift (min)	NH710 (λJV300 c^+)	NH711 (λJV304 *cts62*)	NH712 (λJV313 *sts62-1*)
0	25	27	4
5	—	180	4
10	—	840	7
20	25	2,450	44

Cultures were grown at 32° in LB to 50 Klett and then shifted to 42°. β-galactosidase assays were carried out as described (MILLER 1972).

pressor could be caused by intrinsic heat stability of the protein or by extrinsic factors in the host cell. For example, *sts* repressors might be more efficiently folded (or refolded) at elevated temperatures by host chaperones. To see whether an *sts* repressor is intrinsically heat-stable, the *sts62-1* protein was purified from a Sup° host. DNA-binding assays were done to compare activity of *sts62-1* repressor to both *cts62* (18 amino acids longer) and c^+ repressors after shifting the temperature from 30° to 37° or 41° (Figure 4). The assay for specific DNA binding was protection by repressor of a *Hin*dIII site within the O_2 operator from restriction enzyme cleavage (KRAUSE and HIGGINS 1984; VOGEL *et al.* 1991). When complexes were formed at 30° and challenged with *Hin*dIII at 30°, *cts62* and *sts62-1* repressor were similar; ~20 μg/ml of each protein protected half of the substrate from *Hin*dIII digestion. Both of these repressors were needed at higher concentration than the wild-type repressor (2.5 μg/ml) to achieve 50% protection. However, when complexes made at 30° were shifted to higher temperatures, significant differences were seen for *cts62* and *sts62-1* repressors. To protect 50% of the substrate from *Hin*dIII cutting after shift to 37°, 120 μg/ml of *cts62* repressor was needed, compared to 50 μg/ml of *sts62-1* repressor (Figure 4B). When complexes were shifted to 41°, the differences were larger; 50% protection required >300 μg/ml of *cts62* repressor and ~60 μg/ml of *sts62-1* repressor (Figure 4C). Wild-type repressor was not detectably affected by the temperature shift. Thus, *in vitro* the *cts62* repressor tail contributes significantly to the intrinsic heat-sensitive DNA-binding activity of the protein.

Truncated *sts* repressors resist Clp-mediated proteolysis: The C-terminal tail of one repressor can modify the behavior of a different repressor in the same cell. The clearest example of this effect is what happens when a Mu lysogen is superinfected with a mutant called *vir* (for *vir*ulent). Superinfection with a *vir* phage, which has a C-terminal mutation that changes the amino acid sequence of the tail (Figure 1), derepresses a wild-type Mu prophage. The protein causing this effect is the *vir* repressor, an efficient substrate for *in vivo* degradation by the ClpP/ClpX protease (GEUSKENS *et al.* 1992; MHAMMEDI-ALAOUI *et al* 1994). Expression of a *vir* repressor is dominant because it triggers Clp-dependent degradation of wild-type (or *cts*) repressor that is present in the same cell (GEUSKENS *et al.* 1992).

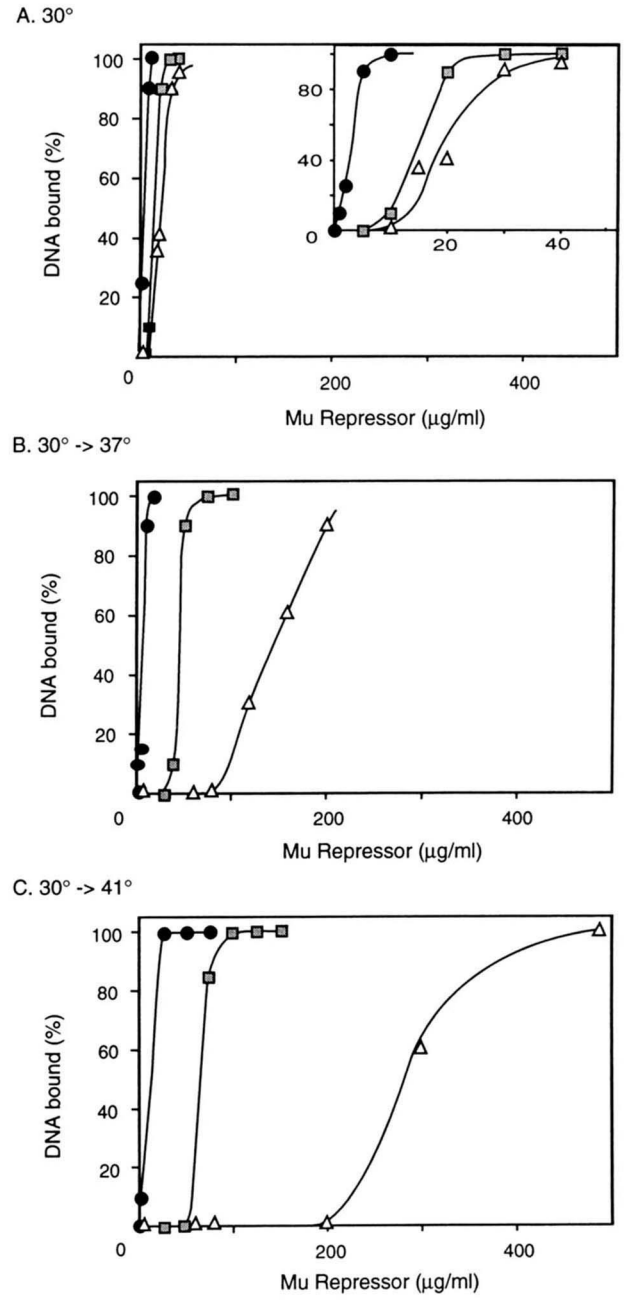

FIGURE 4.—Temperature dependence of c^+, *cts62*, and *sts62-1* repressor DNA binding. Plasmid pHK09 was labeled at the *Eco*RI site by filling in the cut ends with α^{32}P-dATP using Klenow fragment of DNA PolI. Reactions (20 μl) containing 5.5 × −11 M DNA, and the indicated amount of repressor were incubated for 1 hr at 30°. Five units of *Hin*dIII restriction enzyme were added to each mixture, and incubations were continued for 1 hr at 30° (A), 37° (B), or 41° (C). Products separated on a 7.5% polyacrylamide gel were visualized by autoradiography and quantitated by densitometry. The fraction of DNA bound to repressor (uncut at the *Hin*dIII site in the O_2 operator) was determined for the following: c^+ repressor, ●; *sts62-1* repressor, ▨; or *cts62* repressor, △.

Because *vir* dominance operates through the tail, we

TABLE 5

Phage production after superinfection with Mu *vir*

Strain	Phage burst	Phage types in burst
594	30	100% *vir*
594*mal*::Mu *cts62*Kn7701	16	40% *vir*, 60% *cts*62
594*mal*::Mu *sts62-1*Kn7701	0.1	100% *vir*

Phage bursts are given as phage produced per superinfected bacterium. Bacteria were infected at a MOI of 3–5 in LB at 30° as described in MATERIALS AND METHODS. Phage production was measured 120 min after infection by titration on C600. *vir* phages were identified as described previously (GEUSKENS *et al.* 1991).

tested *vir* phage for the ability to replicate in strains harboring an *sts* prophage. Significantly, Mu *sts62-1* lysogens were immune to Mu *vir* superinfection (Table 5). Superinfection of a Mu *sts62-1* lysogen with a MOI of three to five Mu *vir3061* yielded only 0.1 phage per infected bacterium. These phage were probably unabsorbed passengers rather than the products of replication because all were of the Mu *vir* phage type and the superinfection was, to first approximation, nonproductive. In the control experiment, Mu *vir3061* infection of a Mu *cts62* lysogen yielded a 100-fold larger titer of 14 PFU per infected bacterium. Of the viruses obtained, half were Mu *vir* and half were Mu *cts62* phage. These results suggest that transmission of a *vir* repressor's instability *in vivo requires* that the targeted repressor have the normal C-terminal tail.

Previously, we showed that expression of a *vir* repressor caused c^+ or *cts62* repressors to be degraded. To confirm the observation that an *sts* repressor was stable following Mu *vir* superinfection, we used λ phage constructs to express different repressor combinations in the same cell and measured protein abundance using Western antibody blots. A lysogen carrying λJV313 (*sts62-1*) was superinfected with homo-immune λ phage that expresses different forms of Mu repressor. Unlike c^+ and *cts62* proteins (GEUSKENS *et al.* 1991, 1992), the *sts62-1* protein was resistant to *vir*-induced ClpP/ClpX degradation (Figure 5). As seen in lanes 4–6 and 10–12 of Figure 5B, the band representing *sts* repressor (indicated by the open arrow) remained stable even after a *vir* repressor (indicated by filled arrows) was expressed in the same cell. That the *vir* repressors were unstable in cells expressing the *sts62-1* repressor can be seen from their very faint expression pattern compared to the expression of a *cts* repressor in the same experiment using the same number of superinfecting phage particles (compare filled arrows in lanes 9–14 of Figure 5A with filled arrows in lanes 4–6 and 9–12 of Figure 5B). We conclude from these results that the repressor tail, which in a mutant form makes Mu repressor sensitive to *in vivo* ClpP/ClpX proteolysis, also determines whether or not *vir* repressor instability can be transmitted to a preexisting repressor ensemble.

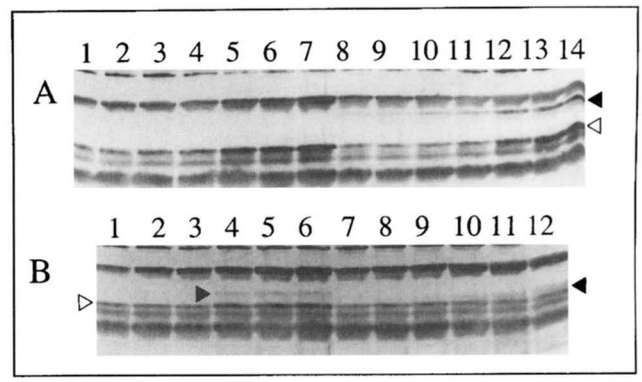

FIGURE 5.—Western blot analysis of the *sts62-1* protein in cells containing *cts62* and *vir* proteins. *E. coli* 594 (λJV313) that carries the Mu *sts62-1* repressor gene was grown and superinfected with a MOI of three to seven at 32° with λRS45, λJV300, λVG321, and λJV320, which carry respectively no Mu repressor gene, the *cts62*, *vir3060*, and *vir3051* repressor genes. Samples were withdrawn from the cultures at different times after superinfection and used to prepare extracts that were displayed on 15% acrylamide gels. Repressor assays were done by Western blotting as described in the MATERIALS AND METHODS section. In A strain 594 (λJV313) was infected with λRS45 (no repressor) for 0 min in lane 1, 5 min in lane 2, 10 min in lane 3, 15 min in lane 4, 30 min in lane 5, 45 min in lane 6, and 60 min in lane 7 and with λJV300 (*cts62*) for 0 min in lane 8, 5 min in lane 9, 10 min in lane 10, 15 min in lane 11, 30 min in lane 12, 45 min in lane 13 and 60 min in lane 14. The black arrow points to the *cts62* repressor and the open arrow to the *sts62-1* repressor. In B the strain was infected with λVG321 (*vir3060*) for 0 min in lane 1, 10 min in lane 2, 15 min in lane 3, 30 min in lane 4, 45 min in lane 5, and 60 min in lane 6 and with λVG322 (*vir3061*) for 0 min in lane 7, 10 min in lane 8, 15 min in lane 9, 30 min in lane 10, 45 min in lane 11, and 60 min in lane 12. Black arrows point to the *vir* repressors and open arrow to the *sts62-1* repressor. Bands above and below the repressor are host proteins unrelated to the repressor that react with repressor antiserum.

Since the C-terminal residues of a *cts* repressor influence the DNA-binding activity of the protein, it seemed possible that the *vir* repressor, which has an altered C-terminal tail, might have altered DNA-binding activity. To test this possibility, the *vir3060* protein, which was derived from a Mu c^+ prophage and carries a wild-type N-terminal DNA-binding headpiece (Figure 1), was purified and tested in the *Hin*dIII operator protection assay. The apparent k_D of *vir3060* repressor (Figure 6) was only slightly (twofold) higher than the wild-type protein (see Figure 4B). Thus, substitutions at the C-terminus affected only slightly the DNA-binding activity of a protein with the wild-type headpiece. This result shows that a *vir* C-terminal tail does not significantly destabilize the folded protein.

DISCUSSION

Amber mutations clustered near the C-terminus of Mu repressor produce the Sts phenotype, which changes two curious properties of the protein. One novel Sts trait is heat-resistant DNA binding in Sup°

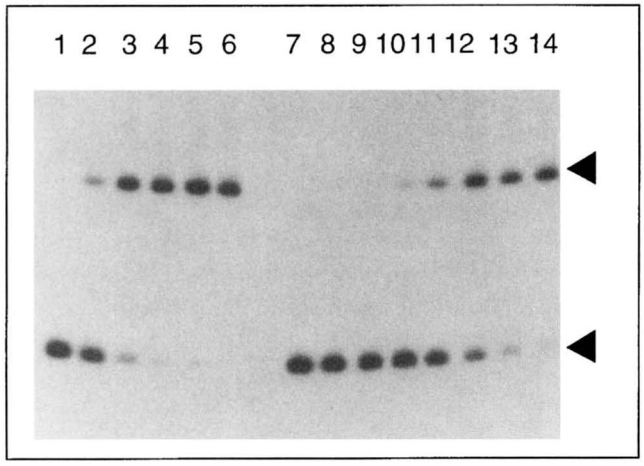

FIGURE 6.—DNA-binding activity of purified *vir* repressor protein. Reaction conditions were carried out at 37°, and the *Hin*dIII reaction was as described in Figure 4. The c^+ protein concentrations were as follows: none in lane 1, 1 ng/μl in lane 2, 2 ng/μl in lane 3, 3 ng/μl in lane 4, 5 ng/μl in lane 5, 10 ng/μl in lane 6. For *vir* protein, the concentrations were as follows: none in lane 8, 1 ng/μl in lane 9, 2 ng/μl in lane 10, 4 ng/μl in lane 11, 6 ng/μl in lane 12, 8 ng/μl in lane 13, 10 ng/μl in lane 14. The top and bottom arrows indicate respectively the positions of DNA fragments protected from *Hin*dIII cleavage by bound repressor protein and of DNA molecules cleaved by *Hin*dIII restriction enzyme.

hosts and heat-sensitive DNA binding in *supE* or *supF* hosts. Repressors missing the C-terminal seven to 17 amino acids of the protein are largely heat resistant in spite of the presence of the N-terminal headpiece mutations that initially caused temperature-sensitive DNA-binding activity. When a full-length protein is made from an *sts* mutant by *supE/supF*-mediated glutamine/tyrosine insertion at the amber codon, heat sensitivity is regained. The second novel Sts property is a change of genetic dominance. Unlike Mu c^+ or *cts* lysogens, which are induced after superinfection with a Mu *vir* phage, Mu *sts* lysogens remain immune to Mu *vir* superinfection. In this case, the absence of the tail makes the Mu repressor dominant and allows the protein to escape degradation by the ATP-dependent Clp protease. Together, these results demonstrate that the Mu repressor tail has an important, albeit dispensable, function.

Effect of the repressor tail on DNA binding: The dramatic heat sensitivity seen for DNA binding with Mu *cts25*, *cts62*, and *cts71* repressors stems from two sites in each protein: the DNA-binding headpiece and the C-terminal tail. For example, the *cts62* repressor, which has the R45-Q substitution *plus* the tail, was profoundly heat sensitive. Shift of cells containing a *cts62* repressor-regulated copy of *lacZ* from 30° to 42° for 20 min stimulated an increase from 25 to 2500 units of β-Gal activity *in vivo* (Table 4) (VOGEL *et al.* 1991). By comparison, the *sts62-1* repressor, which carries the R45-Q substitution but *lacks* the tail, showed two differences from the *cts62* repressor. First, the *sts62-1* protein acted as a superrepressor *in vivo* at 30°; the basal β-Gal activity dropped from 25 to 4 units. Second, the *sts* repressor showed mild heat sensitivity with a 10-fold derepression ratio from 4 to 40 units of β-Gal activity following shift for 20 min to 42° *in vivo* (Table 4). In absolute terms, *lacZ* expression at 42° in the *sts62-1* mutant was only marginally (50%) higher than the expression level of the wild-type strain (25 units). Therefore, the *sts62-1* protein is a superrepressor at low temperature, behaving as though it has lost sensitivity to a constitutive induction pathway. DNA-binding tests of the purified protein showed that the Sts phenotype is an intrinsic property of the mutant protein (Figure 4), although the superrepressor behavior was not evident *in vitro* as it was *in vivo* (Figure 4 and Figure 6).

How does the tail control heat-sensitive DNA-binding activity of the headpiece? Two explanations seem plausible. The tail could destabilize internal packing interactions so that the folded structure is lost at high temperatures. Alternatively, the tail could change the multimeric configuration of folded subunits so that a DNA-binding domain is concealed or repressor cooperativity is hindered. For several reasons, we favor the latter explanation.

In T4 lysozyme, destabilization of internal packing interactions is an accepted explanation for many heat-sensitive mutants. Most amino acid substitutions that cause a temperature-sensitive loss of function are located in the solvent-inaccessible interior of the protein where they interfere with residues that stabilize the folded structure (ALBER *et al.* 1987; HEINZ *et al.* 1992). Similar results have been demonstrated for amino acid substitutions within the internal core of temperature-sensitive mutants of the λ repressor (LIN and SAUER 1991). This explanation does not account for the behavior of the temperature-sensitive Mu repressor mutants studied here. The temperature-sensitive headpiece mutations are not in a solvent inaccessible hydrophobic core, rather, they are in or near charged residues on the surface of a helix-turn-helix motif. The N-terminal domain of Mu transposase and repressor are similar (HARSHEY *et al.* 1985) and both proteins bind to the same DNA sequences (CRAIGIE *et al.* 1984). The NMR structure has been determined for this region of transposase, and it represents a new class of helix-turn-helix protein that contains a "wing" between helices 2 and 3 (CLUBB *et al.* 1994). Contacts with DNA are thought to involve helix 2, which may bind in the major groove of DNA, and the wing between helix 2 and helix 3 (Figure 1). The *cts71* M28-I substitution is located in a turn between helices 1 and 2, and both the *cts25* G43-D substitution and *cts62* R47-Q substitutions are near a strand of β-sheet that lies between helix 2 and the wing. These residues are likely to be in a solvent exposed area of the protein. Since the headpiece domain functions independently of the

rest of the molecule, the tail is not likely to be directly involved in its folding.

A more probable explanation is that the repressor tail alters the cooperativity of repressor or interferes with DNA binding by occluding the site. This explanation is attractive for two reasons. First, Mu repressor has the ability to form complexes that vary in cooperativity number from 2 to ~10. Wild-type repressor binds operator DNA and regulates the P_E promoter with a cooperativity number of 2, but it regulates the P_c promoter with a cooperativity number of >6 (VOGEL *et al.* 1991). Significantly, at 30° the *cts62* repressor regulates the P_E promoter with a cooperativity value near 6 (VOGEL *et al.* 1991). Thus, the *cts62* repressor is cooperatively different from the wild-type protein even at a permissive temperature. Second, the only heat-sensitive mutant known that involves an amino acid substitution outside of the headpiece (*cts4*) is in the putative leucine-rich multimerization domain (L129-V). This heat-sensitive mutant shows a temperature-dependent altered dimer crosslinking pattern *in vitro* (J. E. LAACHOUCH and A. TOUSSAINT, unpublished results). Flexible conformational behavior may underpin the heat-sensitive response of mutants affecting the head, the tail, and the central segment of the protein.

Effect of the repressor tail on dominance: A role for the repressor tail in changing the structure of a multimeric form of repressor is also suggested by the dominance pattern. Superinfection with a Mu *vir* mutant causes induction of a wild-type (or *cts*) prophage (VAN VLIET *et al.* 1978). Early genetic evidence suggested that *vir* mutants make an anti-repressor, which is reminiscent of the P22 Ant protein (SUSSKIND and YOUDERIAN 1983). The prediction was prescient, but the Mu anti-repressor turned out to be a mutant form of the *c* repressor rather than a product of a separate gene (GEUSKENS *et al.* 1991). Repressor virulence works through a cellular protease composed of two subunits, ClpP and ClpX (GEUSKENS *et al.* 1992; MHAMMEDI-ALAOUI *et al.* 1994). The *vir* repressors have a frameshift mutation that changes the tail (Figure 1) and that makes the resulting protein hypersensitive to the Clp proteolysis. Moreover, *vir* repressors transfer their instability to wild-type (or *cts*) repressors present in the same cell. This transfer is thought to occur when a *vir* repressor invades a complex with wild-type (or *cts*) repressor molecules. The close connection between virulence and the tail is supported by two additional observations. (1) All unstable repressors do not have a virulent phenotype: the *clr3* clear plaque mutant has a frameshift mutation early in the gene (Figure 1) and makes an unstable protein that is not virulent (GEUSKENS *et al.* 1992). 2) Deletion of a *vir* repressor's tail at several positions eliminates virulence (J. E. LAACHOUCH and A. TOUSSAINT, unpublished results).

We've shown here that the dominant transmission of instability from a *vir* repressor to a *cts* repressor requires the presence of a normal C-terminal tail on the *cts* repressor; truncated *sts* repressors are resistant to *vir*-induced proteolysis (Table 4 and Figure 5). The repressor tail might either facilitate a *vir* repressor's invasion of a preformed ensemble by stimulating subunit exchange, or the C-terminal tail of *cts* (or wild-type) subunits in a *vir*-invaded complex might attract the Clp protease. Either way, the repressor tail is crucial in transmitting virulence to cooperative preformed repressor complexes.

Sensory transduction: We are unaware of another case in which temperature-sensitive mutations are suppressed by protein shortening. We suggest that the C-terminal domain of Mu repressor is a sensory element that, although not required for function, connects the repressor's activity to changes in cellular physiology. According to this hypothesis, the Mu and λ repressors are strikingly similar in organization, though they differ substantially in structure and mechanism. The similarity is in the general protein organization. The λ repressor, like Mu repressor, has an N-terminal DNA-binding domain and a C-terminal tail that controls cooperative DNA-binding behavior and induction. However, the mechanisms of induction are quite distinct. λ induction is stimulated by the interactions between RecA protein and repressor. A protease encoded by the C-terminal domain of the repressor is activated by RecA to cleave (either in *cis* or *trans*) a single site that frees the N-terminal DNA-binding domain from the tail (SAUER *et al.* 1990; LITTLE 1993). Cleavage of the Mu repressor involves the ATP-dependent host Clp protease (GEUSKENS *et al.* 1992), and degradation probably yields short polypeptides.

Another striking difference is the sensory input connected to derepression. λ responds exclusively to the SOS pathway. The induction frequency of λ lysogens drop to the mutation frequency of *cI* clear plaque mutants when the repressor is made nonresponsive to RecA-stimulated cleavage (MUSTER and SHAPIRO 1981; SHAPIRO personal communication). For Mu, there may be more than one induction mechanism and the strongest cellular signals for derepression appear to be complex and linked to starvation biology or stationary phase. Significantly, three of the five host genes that are known to alter the frequency of Mu derepression change in abundance or activity during the shift from exponential to stationary phase; these genes are *hns* (FALCONI *et al.* 1991), *fis* (FALCONI *et al.* 1991; BETERMIER *et al.* 1993), and *crp* (SHAPIRO 1994). The C-terminal tail of Mu repressor seems to modulate repressor activity by influencing the multimeric structure and cooperativity of DNA binding. Deciphering the molecular signals connected to Mu derepression could be an important step in understanding the mechanisms of gene regulation during stationary phase.

This work was funded by a grants GM-33143 from the National Institutes of Health (NIH) and INT-8821706 from the National Science Foundation. J.V.L was supported by NIH training grant GM-08111. Work in Rhode St. Genese was sponsored by the European Economic Community Science program SCI 0143-C(GDF) and the Belgian Fonds de la Recherche Scientifique Medicale. V.G. is a fellow from the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. A.T. is Maitre de Recherche from the Fonds National de la Recherche Scientifique (Belgium).

LITERATURE CITED

ALAZARD, R., M. BETERMIER and M. CHANDLER, 1992 *Escherichia coli* integration host factor stabilizes bacteriophage Mu repressor interactions with operator DNA *in vitro*. Mol. Microbiol. **6:** 1707–1714.

ALBER, T., S. DAO-PIN, J. A. NYE, D. C. MUCHMORE and B. W. MATTHEWS, 1987 Temperature-sensitive mutations of bacteriophage T4 lysozyme occur at sites with low mobility and low solvent accessibility in the folded protein. Biochem. **26:** 3754–3758.

APPLEYARD, R. K., 1954 Segregation of lambda lysogenicity during bacterial recombination in *Escherichia coli* K12. Genetics **39:** 429–439.

BALL, C., R. OSUNA, K. FERGUSON and R. JOHNSON, 1992 Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. J. Bacteriol. **174:** 8043–8056.

BETERMIER, M., I. POQUET, R. ALAZARD and M. CHANDLER, 1993 Involvement of *Escherichia coli* FIS protein in maintenance of bacteriophage Mu lysogeny by the repressor: control of early transcription and inhibition of transposition. J. Bacteriol. **175:** 3798–3811.

CLUBB, R. T., J. G. OMICHINSKI, H. SAVILAHTI, K. MIZUUCHI, A. M. GRONENBORN *et al.*, 1994 A novel class of winged helix-turn-helix protein: the DNA-binding domain of Mu transposase. Structure **2:** 1041–1048.

COULONDRE, C., and J. H. MILLER, 1977 Genetic studies of the *lac* repressor III. Additional correlation of mutational sites with specific amino acid residues. J. Mol. Biol. **117:** 525–575.

CRAIG, N. L., and J. W. ROBERTS, 1980 *E. coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. Nature **283:** 26–30.

CRAIGIE, R., M. MIZUUCHI and K. MIZUUCHI, 1984 Site-specific recognition of the bacteriophage Mu ends by the Mu A protein. Cell **39:** 387–394.

DEGNEN, G. E., and E. C. COX, 1974 Conditional mutator gene in *Escherichia coli:* isolation, mapping and effector studies. J. Bacteriol. **117:** 477–487.

DERSCH, P., K. SCHMIDT and E. BREMER, 1993 Synthesis of the *Escherichia coli* K-12 nucleoid-associated DNA-binding protein H-NS is subjected to growth-phase control and autoregulation. Mol. Microbiol. **8:** 875–889.

FALCONI, M., V. MCGOVERN, C. GUALERZI, D. HILLYARD and N. P. HIGGINS, 1991 Mutations altering chromosomal protein H-NS induce mini-Mu transposition. New Biol. **3:** 615–625.

FINKEL, S., and R. JOHNSON, 1992 The Fis protein: it's not just for DNA inversion anymore. Mol. Microbiol. **6:** 3257–3265.

FOSTER, P. L., and J. CAIRNS, 1994 The occurrence of heritable Mu excisions in starving cells of *Escherichia coli*. EMBO J. **13:** 5240–5244.

GAMA, M. J., A. TOUSSAINT and N. P. HIGGINS, 1992 Stabilization of bacteriophage Mu repressor-operator complexes by the *Escherichia coli* integration host factor protein. Mol. Microbiol. **6:** 1715–1722.

GEUSKENS, V., J. L. VOGEL, R. GRIMAUD, L. DESMET, N. P. HIGGINS *et al.* 1991 Frameshift mutations in the bacteriophage Mu repressor gene can confer a trans-dominant virulent phenotype to the phage. J. Bacteriol. **173:** 6578–6585.

GEUSKENS, V., A. MHAMMEDI-ALAOUI, L. DESMET and A. TOUSSAINT, 1992 Virulence in bacteriophage Mu: a case of trans-dominant proteolysis by the *Escherichia coli* Clp serine protease. EMBO J. **11:** 5121–5127.

HARSHEY, R. M., E. D. GETZOFF, D. L. BALDWIN, J. L. MILLER and G. CHACONAS, 1985 Primary structure of the phage Mu transposase: homology to Mu repressor. Proc. Natl. Acad. Sci. USA **82:** 7676–7680.

HEINZ, D., W. BAASE and B. MATTHEWS, 1992 Folding and function of a T4 lysozyme containing 10 consecutive alanines illustrate the redundancy of information in an amino acid sequence. Proc. Natl. Acad. Sci. USA **89:** 3751–3755.

HIGGINS, N. P., 1992 Death and transfiguration among bacteria. Trends Biochem. Sci. **17:** 207–211.

HOHN, B., 1979 In vitro packaging of λ and cosmid DNA. Methods Enzymol. **68:** 299–343.

KRAUSE, H. M., and N. P. HIGGINS, 1984 On the Mu repressor and early DNA intermediates of transposition. Cold Spring Harbor Symp. Quant. Biol. **49:** 827–834.

KRAUSE, H. M., and N. P. HIGGINS, 1986 Positive and negative regulation of the Mu operator by Mu repressor and *Escherichia coli* integration host factor. J. Biol. Chem. **261:** 3744–3752.

LEACH, D., and N. SYMONDS, 1979 The isolation and characterization of a plaque-forming derivative of bacteriophage Mu carrying a fragment of Tn*3* conferring ampicillin resistance. Mol. Gen. Genet. **172:** 172–179.

LEUNG, P. C., D. B. TEPLOW and R. M. HARSHEY, 1989 Interaction of distinct domains in Mu transposase with Mu DNA ends and an internal transpositional enhancer. Nature **338:** 656–658.

LIN, W. A., and R. T. SAUER, 1991 The role of internal packing interactions in determining the structure and stability of a protein. J. Mol. Biol. **219:** 359–376.

LITTLE, J. W., 1984 Autodigestion of *lexA* and phage l repressors. Proc. Natl. Acad. Sci. USA **81:** 1375–1379.

LITTLE, J., 1993 LexA cleavage and other self-processing reactions. J. Bacteriol. **175:** 4943–4950.

MAENHAUT-MICHEL, G., and J. A. SHAPIRO, 1994 The roles of starvation and selective substrates in the emergence of *araB—lacZ* fusion clones. EMBO J. **13:** 5229–5239.

MHAMMEDI-ALAOUI, A., M. L. PATO, M.-J. GAMA and A. TOUSSAINT, 1994 A new component of bacteriophage Mu replicative transposition machinery: the *Escherichia coli* ClpX protein. Mol. Microbiol. **11:** 1109–1116.

MILLER, J. H., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MITTLER, J. E., and R. E. LENSKI, 1990 New data on excisions of Mu from *E. coli* MCS2 cast doubt on directed mutation hypothesis. Nature **344:** 173–175.

MIZUUCHI, M., and K. MIZUUCHI, 1989 Efficient Mu transposition requires interaction of transposase with a DNA sequence at the Mu operator: implications for regulation. Cell **58:** 399–408.

MUSTER, C. J., and J. A. SHAPIRO, 1981 Recombination involving transposable elements: on replicon fusion. Cold Spring Harbor Symp. Quant. Biol. **40:** 239–242.

PAOLOZZI, L., and N. SYMONDS, 1986 The SE region, pp. 53–62 in *Bacteriophage Mu*, edited by N. SYMONDS, A. TOUSSAINT, P. VAN DE PUTTE and M. M. HOWE. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

ROSS, W., S. H. SHORE and M. M. HOWE, 1986 Mutants of *Escherichia coli* defective for replicative transposition of bacteriophage Mu. J. Bacteriol. **167:** 905–919.

SAUER, R. T., S. JORDAN and C. O. PABO, 1990 λ repressor: a model system for understanding protein-DNA interactions and protein stability. Adv. Protein Chem. **40:** 1–61.

SHAPIRO, J. A., 1984 Observations on the formation of clones containing *araB-lacZ* cistron fusions. Mol. Gen. Genet. **194:** 79–90.

SHAPIRO, J., 1992 Differential action and differential expression of DNA polymerase I during *Escherichia coli* colony development. J. Bacteriol. **174:** 7262–7272.

SHAPIRO, J. A., 1993 A role for the Clp protease in activating Mu-mediated DNA rearrangements. J. Bacteriol. **175:** 2625–2631.

SHAPIRO, J. A., 1994 Pattern and control in bacterial colonies. Sci. Prog. **76:** 399–424.

SHAPIRO, J. A., and N. P. HIGGINS, 1989 Differential activity of a transposable element in *Escherichia coli* colonies. J. Bacteriol. **171:** 5975–5986.

SIMONS, R. W., F. HOUMAN and N. KLECKNER, 1987 Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:** 85–96.

SUSSKIND, M. M., and P. YOUDERIAN, 1983 Bacteriophage P22 antirepressor and its control, pp. 251–277 in *Lambda II*, edited by R. W. HENDRIX, J. W. ROBERTS, F. W. STAHL and R. A. WEISBERG. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

TAYLOR, A. L., 1963 Bacteriophage-induced mutation in *E. coli*. Proc. Natl. Acad. Sci. USA **50:** 1043–1051.

TOUSSAINT, A., L. DESMET, M. FAELEN, R. ALAZARD, M. CHANDLER *et al.* 1987 In vivo mutagenesis of bacteriophage Mu transposase. J. Bacteriol. **169:** 5700–5707.

VAN VLIET, F., M. COUTURIER, L. DESMET, M. FAELEN and A. TOUSSAINT, 1978 Virulent mutants of temperate phage Mu-1. Mol. Gen. Genet. **160:** 195–202.

VOGEL, J. L., J. L. ZHU, M. M. HOWE, A. TOUSSAINT and N. P. HIGGINS, 1991 Temperature sensitive mutations in the bacteriophage Mu *c* repressor locate a 63-amino-acid DNA-binding domain. J. Bacteriol. **173:** 6568–6577.

YANISCH-PERRON, C., J. VIERA and J. MESSING, 1985 Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M12mp18 and pUC19 vectors. Gene **33:** 103–119.

Communicating editor: N. L. CRAIG