



HAL
open science

Frameshift mutations in the bacteriophage Mu repressor gene can confer a trans-dominant virulent phenotype to the phage

V. Geuskens, J.L. Vogel, R. Grimaud, L. Desmet, N.P. Higgins, A. Toussaint

► To cite this version:

V. Geuskens, J.L. Vogel, R. Grimaud, L. Desmet, N.P. Higgins, et al.. Frameshift mutations in the bacteriophage Mu repressor gene can confer a trans-dominant virulent phenotype to the phage. *Journal of Bacteriology*, 1991, 173 (20), pp.6578-6585. 10.1128/jb.173.20.6578-6585.1991 . hal-01631825

HAL Id: hal-01631825

<https://hal.science/hal-01631825v1>

Submitted on 11 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Frameshift Mutations in the Bacteriophage Mu Repressor Gene Can Confer a *trans*-Dominant Virulent Phenotype to the Phage

VINCENT GEUSKENS,¹ JODI L. VOGEL,² RÉGIS GRIMAUD,³ LUCIE DESMET,¹
N. PATRICK HIGGINS,² AND ARIANE TOUSSAINT^{1,3*}

Laboratoire de Génétique, Université Libre de Bruxelles, B1640 Rhode Saint Genèse, Belgium¹; Department of Biochemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294²; and Laboratoire de Biologie Moléculaire Végétale, Université Joseph Fourier, 38041 Grenoble Cedex, France³

Received 17 January 1991/Accepted 20 May 1991

Virulent mutations in the bacteriophage Mu repressor gene were isolated and characterized. Recombination and DNA sequence analysis have revealed that virulence is due to unusual frameshift mutations which change several C-terminal amino acids. The *vir* mutations are in the same repressor region as the *sts* amber mutations which, by eliminating several C-terminal amino acids, suppress thermosensitivity of repressor binding to the operators by its N-terminal domain (J. L. Vogel, N. P. Higgins, L. Desmet, V. Geuskens, and A. Toussaint, unpublished data). *Vir* repressors bind Mu operators very poorly. Thus the Mu repressor C terminus, either by itself or in conjunction with other phage or host proteins, tunes the DNA-binding properties at the repressor N terminus.

Bacteriophage Mu is a complex viral transposon and a temperate phage. Like other such viruses, it has the ability to either enter the lytic cycle or lysogenize (see references 20 and 26 for reviews of Mu). However, Mu differs from the other temperate phages in that the first essential step in both the lytic and the lysogenic pathways is the random integration of Mu into the host genome, a step catalyzed by an essential viral lytic function, the *A* gene product (or transposase [pA]). As a consequence, Mu must synthesize at least some of its early proteins before it establishes immunity. Since early functions and immunity functions are expressed from convergent promoters, lysogenic development either requires a switch from one transcription mode to another or requires two copies of the Mu genome, one of which expresses repressor while the other expresses transposase. The Mu repressor clearly plays a central role in the lysogenic transcription switch.

The Mu repressor has been purified and studied *in vitro* (12, 13). Molecular recognition between Mu repressor and operator DNA is complex. The protein motif implicated may differ from the α -helix-turn- α -helix motif of the λ repressor which binds to a symmetrical DNA sequence as a dimer (8, 30). Mu repressor binds to nonsymmetrical 14-bp DNA consensus sequences located in three operators, *O*₁, *O*₂, and *O*₃ (13). At operator site *O*₂, four contiguous repressor consensus sequences overlap the -10 and -35 contacts of RNA polymerase at the early lytic promoter *P*_E (12, 13). As repressor concentration incrementally rises, *P*_E shuts off while the repressor promoter *P*_{CM} remains active. At the *P*_{CM} promoter, in operator site *O*₃, repressor binding at two weak consensus sequences blocks the -10 contact with RNA polymerase (13) so that repressor synthesis is negatively autoregulated. The repressor domain involved in operator binding has been defined as a 63-amino-acid sequence located at the N-terminal end of the protein (30). In this domain, one particular thermosensitive mutation, *cts62*, makes the protein RepTs62 thermosensitive for binding. Unexpectedly, amber mutations that truncate the protein by

7, 10, or 18 C-terminal amino acids suppress thermosensitivity (29). This strongly suggests that the C terminus plays an important role in DNA binding.

Virulent mutants of Mu were described several years ago (28). Selected for their ability to plaque on strains lysogenic for Mu, they also induced the development of the resident prophage (i.e., they were *trans*-dominant). Pseudorevertants that could not grow on Mu lysogens were isolated, and these mutated back to virulence at high frequency. One explanation was that Mu, like P22, made an antirepressor that was constitutively expressed in *Vir* mutants, enabling them to inactivate the repressor present in a lysogen (28). Accordingly, reversion of the *Vir* phenotype could be due to a mutation in the antirepressor structural gene. Subsequent reversion could be explained by a restored antirepressor (28). Sequence analysis of the early region of Mu (22) showed that Mu did not encode a separate antirepressor, which led us to reinvestigate virulence in Mu.

We have found that the antirepressor is the *Vir* repressor itself. Using the same selection as van Vliet et al. (28), we isolated new *Vir* mutants and mapped and sequenced the mutations. In each virulent mutant, a frameshift mutation was found near the 3' end of the repressor structural gene in a dispensable region that, when eliminated, suppresses thermosensitive binding of the RepTs62 protein. These results show that the C terminus of the Mu repressor is very important for its activity.

MATERIALS AND METHODS

Growth of bacteriophages, bacterial strains, and plasmids. Bacterial strains are listed in Table 1. MupAp1 contains about 1 kb of Tn3 DNA conferring ampicillin resistance, which is substituted for Mu DNA in the Mu G region (14). Bacteria were grown in LB (5 g of NaCl, 10 g of tryptone, 5 g of yeast extract [17]). Titers of phage were determined on a lawn of sensitive bacteria in LB plus 0.7% agar (LA) on LA plates. Bacterial strains were infected by Mu in LB as described by van Vliet et al. (28). Lysates of Mucts62 were prepared by thermal induction of a lysogen. Lysates of Muvir mutants were prepared by infecting C600 at a low

* Corresponding author.

TABLE 1. Strains, plasmids, and phage used in this study

Strain, plasmid or phage	Characteristics, vector and insert, or source	Source or reference
Strains		
594	<i>rpsL galK lacZ</i>	1
MC4100	F ⁻ <i>araD</i> Δ(<i>argF-lacZYA</i>)U169 <i>rpsL relA1</i>	4
C600	F ⁻ <i>thr leu lacY supE</i>	3
JM101	<i>supE thi</i> Δ(<i>lacZ, proAB</i>)/F' <i>traD36 proAB lacZ⁺-ΔM15</i>	32
KD1079	<i>mutD5 thr leu argD his spcA</i>	6
MC4100 <i>dnaQ::mini-Tn10</i>		Shapiro
DH5α	<i>endA1 hsdR17</i> (r _k ⁻ m _k ⁺) <i>supE44</i>	BRL ^a
Plasmids		
pHK09	pSP65; contains the <i>Sau3A-HaeIII</i> (bp 838–1118) fragment of <i>Mucts62</i>	13
pRS551	pBR322	24
pJV200	pUC19; <i>attL-HindIII Muc</i> ⁺	30
pJV214	pUC19; <i>attL-HindIII Mucts62-2</i>	29
pJV220	pUC19; <i>attL-HindIII Muvir3051</i>	This study
pULB507	BluescribeM13 ⁺ ; <i>attL-HindIII Mucts62vir3061</i>	This study
pULB508	BluescribeM13 ⁺ ; <i>attL-HindIII Muvir3060</i>	This study
pJV300	pRS551; <i>attL-HindIII Muc</i> ⁺ , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	30
pJV304	pRS551; <i>attL-HindIII Mucts62</i> , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	30
pJV313	pRS551; <i>attL-HindIII Mucts62-1</i> , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	29
pJV320	pRS551; <i>attL-HindIII Muvir3051</i> , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	This study
pVG321	pRS551; <i>attL-HindIII Muvir3060</i> , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	This study
pVG322	pRS551; <i>attL-HindIII Muvir3061</i> , 117-bp <i>HindIII-EcoRI</i> fragment from pHK09	This study
Phages		
λRS45		24
λJV304	λRS45 <i>cts62pE::lacZ</i>	30
λJV300	λRS45 <i>c⁺pE::lacZ</i>	30
λJV313	λRS45 <i>sts62-1pE::lacZ</i>	29
λJV320	λRS45 <i>vir3051pE::lacZ</i>	This study
λVG321	λRS45 <i>vir3060pE::lacZ</i>	This study
λVG322	λRS45 <i>cts62vir3061pE::lacZ</i>	This study
<i>Muc</i> ⁺		27
<i>Mucts62</i>		11
MuGov3261		28
<i>Mucts62pAp1</i>		14
<i>Muvir3051</i>		28
<i>Muvir3060</i>		This study
<i>Mucts62vir3061</i> , <i>Mucts62vir3062</i> , <i>Mucts62vir3063</i>		This study
<i>Muvir3060rev42-1</i>		This study
<i>Mucts62vir3061rev43-1</i>		This study
<i>Mucts62vir3061rev45-2</i>		This study
<i>Mucts62vir3061rev46-1</i>		This study

^a BRL, Bethesda Research Laboratories.

multiplicity (0.02 phage per bacterium) in LB at 37°C. In both cases, the phage were concentrated and purified on CsCl gradients prior to DNA extraction as described previously (23). DNA manipulations and transformation of bacterial strains with plasmid DNA were as described previously (16).

Mutagenesis. *mutD* mutagenesis of *Muc*⁺ was performed by growing a plate lysate on lawns of KD1079 (6). For UV mutagenesis, phage grown on C600 were pelleted by centrifugation for 3 h at 8,000 rpm in a Sorvall GS3 rotor. They were resuspended in SM buffer (31) and irradiated with doses ranging from 150 to 250 J/m². Aliquots of a suspension of C600 irradiated at 75 J/m² were infected with the irradiated phage at a low multiplicity of infection, and the infected bacteria were plated on C600 (MuGov3261). Plates were incubated overnight at 37°C.

Plasmid construction. The repressor is encoded by the leftmost gene on the phage chromosome. Digestion of phage DNA with *HindIII* produces a fragment containing the left 1 kb of phage DNA and 50 to 150 bp of variable host DNA attached to the left end. The 1-kb *HindIII* fragments of *vir* phage DNAs were isolated and ligated into Bluescribe M13⁺ (Vector Cloning Systems) that had been digested with *HindIII* and *SmaI* for *vir3061* and only *HindIII* for *vir3060* (30). For *vir3051*, the pUC19 vector was digested with *HindIII* and *SmaI*. Transformation was carried out with JM101 made competent by the CaCl₂ method (16). The resulting plasmids, pJV220, pULB507, and pULB508, carry the left end of *Muvir3051*, *Mucts62vir3061*, and *Muvir3060*, respectively.

The three-way ligation strategy used to clone *Muvir* sequences from *attL* to position 1118 on the Mu map into the

vector, pRS551, that fuses their P_E promoter to *lacZ*, and the methods for moving these constructs into a specialized λ transducing phage were as described previously (30). In pULB508, the *HindIII* site downstream from the *c* gene was first removed by partial digestion with *HindIII*, and then the ends were filled with the Klenow fragment of DNA polymerase I and the plasmid was religated. The resulting λ phages were recovered either in MC4100 ($\Delta lacZ$) or in 594 (*lacZ*). β -Galactosidase assays were performed as described by Miller (17). The sequencing strategy was as described previously (30).

Preparation of protein extracts and analysis of repressor by Western blotting (immunoblotting). Protein extracts were prepared by solubilizing bacterial pellets (usually from 1-ml aliquots at 3×10^8 bacteria per ml) in 50 μ l of sample buffer (125 mM Tris-HCl [pH 6.8], 2% sodium dodecyl sulfate, 30% glycerol, 1 mM β -mercaptoethanol, 6 M urea) and boiling for 1 min. The proteins from one-third of the samples were separated on acrylamide gels (15% resolving gel with a 4% stacking gel) and electrotransferred to Amersham Hybond C nitrocellulose membranes. Transfer was performed with a Biometra transblot for 30 min in 25 mM Tris buffer, pH 8.3, containing 192 mM glycine and 20% (vol/vol) methanol at 5 mA/cm² of gel. Membranes were saturated with 1% bovine serum albumin (BSA) before overnight incubation with rabbit polyclonal antibody raised against purified repressor at a 1/5,000 dilution in phosphate-buffered saline buffer supplemented with 1% BSA and 0.1% Tween 80. The antibody response was amplified with a secondary biotinylated antibody raised against rabbit epitopes and a biotin-streptavidin complex coupled with peroxidase. The blot was developed using 3,3'-diamino-benzidine from Sigma at 0.2 mg/ml in 0.1 M Tris-HCl, pH 7.6.

Binding assays. Binding of the *vir3060* and *vir3061* repressors to Mu operators was tested in a gel retardation assay, as previously described (2). Protein extracts were prepared by gentle detergent lysis (5) and mixed with ³⁵S-ATP-labeled *BamHI-EcoRI* fragments of pHK09. The small fragment of 295 bp contains the three operator sequences O_1 , O_2 , and O_3 .

Isolation of nonvirulent revertants. Lysates of Muvir3060 pAp1 and Mucts62vir3061pAp1 grown as plate lysates on MC4100 *dnaQ::mini-Tn10* at 37°C were used to infect MC4100 (λ JV304). Ap^r colonies were selected on LA supplemented with 25 μ g of ampicillin at 30°C.

RESULTS

The Gov phenotype. As originally observed by van Vliet et al. (28), Mu lysogens can be arranged in two classes according to their capacity to plaque Mu Vir mutants. Strains in the first class do not allow Vir phage to plaque. Because all the lysogens (mono- or polylysogens) which contain the *Muc*⁺ originally isolated by Taylor (27) belong to this class, they were referred to as Gov⁺ (for wild type [wt]). Strains in the second class, when used in lawns, allow Vir phage to plaque. All of them are monolysoyngens and carry a particular *Muc*⁺ derivative which was named MuGov3261, and they were referred to as Gov (for growth of virulent). All Mu lysogens, whether of the Gov⁺ or Gov class, are induced upon infection with a Mu Vir mutant. However, they differ by the ratio of prophage and Vir type phage produced. The Gov⁺ lysogens produce more prophage-type phage than Vir phage, while the Gov lysogens produce about 50% each. This difference may explain the Gov phenotype because a Gov⁺ burst contains such a low proportion of Vir phage that they may be incapable of forming visible plaques. Gov

lysogens were used to isolate new Mu Vir mutants, and these were subsequently checked on both Gov and Gov⁺ lysogens.

Isolation of Vir mutants. *Muc3218* and *Mucts62* lysates were mutagenized by UV irradiation (150 to 250 J/m²), and *Muc*⁺ was grown on the *mutD5* KD1079 *Escherichia coli* strain. The lysates were plated on lawns of C600 (MuGov 3261). With *Mucts62*, plaques were found at frequencies ranging from 10⁻⁸ to 10⁻⁹, depending on the UV dose used. This provided *Mucts62vir3061*, *Mucts62vir3062*, and *Mu cts62vir3063*. No plaque with *Muc3218* (frequency, <10⁻¹⁰) could be found. One plaque from the mutagenized *Muc*⁺ lysate (Muvir3060) corresponding to a frequency lower than 10⁻¹⁰ was found. Three *vir* mutations, the old *vir3051* and the new *vir3060* and *vir3061*, were further analyzed.

Lysates of the Mu Vir mutants were used to infect different hosts. The proportion of Mu Vir and prophage-type phage in the burst was measured. The results obtained were similar to those described by van Vliet et al. (28). Upon infection of a Gov⁺ *Muc*⁺ lysogen, in a total burst of 50 to 100 phage per bacterium both *Muc*⁺ and Mu Vir phage were found in a ratio of 0.1 to 1 Vir for 10 *c*⁺. Upon infection of a Gov lysogen, the total burst was the same and *Muc*⁺ and Mu Vir phage were present in a 50% ratio. This led us to conclude that all three Vir mutants have the same *trans*-dominant phenotype.

We isolated λ RS45 phage (24) containing the left end of Muvir3051, Muvir3060, and Mucts62vir3061 (λ JV320, λ VG321, and λ VG322) in which *lac* expression is driven from the Mu P_E promoter (see Materials and Methods). These phage express constitutively high levels of β -galactosidase (2,000 to 4,000 U) at all temperatures tested. Under the same conditions, an equivalent construction with the *cts62* thermoinducible repressor mutation gives around 50 U at 30°C and 2,000 U at 42°C. This is consistent with the fact that Vir mutants are expected to be deregulated for the expression of the lytic functions.

Mapping of the *vir* mutations. van Vliet et al. (28) mapped the *vir* mutations to the left of the Mu *B* gene. To find the exact location of *vir* mutations, we cloned the left end *HindIII* and *HindIII-PstI* fragments of Muvir3060, *cts62 vir3061*, and *vir3051* phage into the polylinker of either the pUC19 or the BluescribeM13⁺ plasmid (Fig. 1). These plasmids were transformed into JM101, and the strains obtained were superinfected with Mucts62pAp1. The phage released were plated on lawns of C600 and C600 (MuGov 3261). When infected with Mucts62pAp1, strains carrying the plasmids with the *HindIII* left end fragment of Mu Vir mutants produced virulent recombinants at frequencies ranging from 2×10^{-4} to 5×10^{-4} . These phage, Muvir3051pAp1, Muvir3060pAp1, and Mucts62vir3061pAp1, which carry the pAp1 substitution, exhibited the same phenotype as their respective progenitors. Mucts62vir3061 made slightly larger plaques than Muvir3060 and Muvir3051 on C600 (MuGov3261). From these results, we concluded that the mutation(s) necessary to confer virulence maps to the left end of the *HindIII* site, 1,001 bp from Mu's left end.

DNA sequence analysis of Muvir3060, Mucts62vir3061, and Muvir3051 showed that Mucts62vir3061 and Muvir3051, which resulted from UV mutagenesis, carried the same additional (+2) frameshift mutation caused by two additional T's in a run of four T's (Fig. 2). *vir3060*, which was found after *mutD5* mutagenesis, has an unusual double change; a (-1) frameshift caused by an AT base pair deletion is combined with an GC-to-AT transition. As expected, Mucts 62vir3061 retained the *cts62* mutation. No other change was

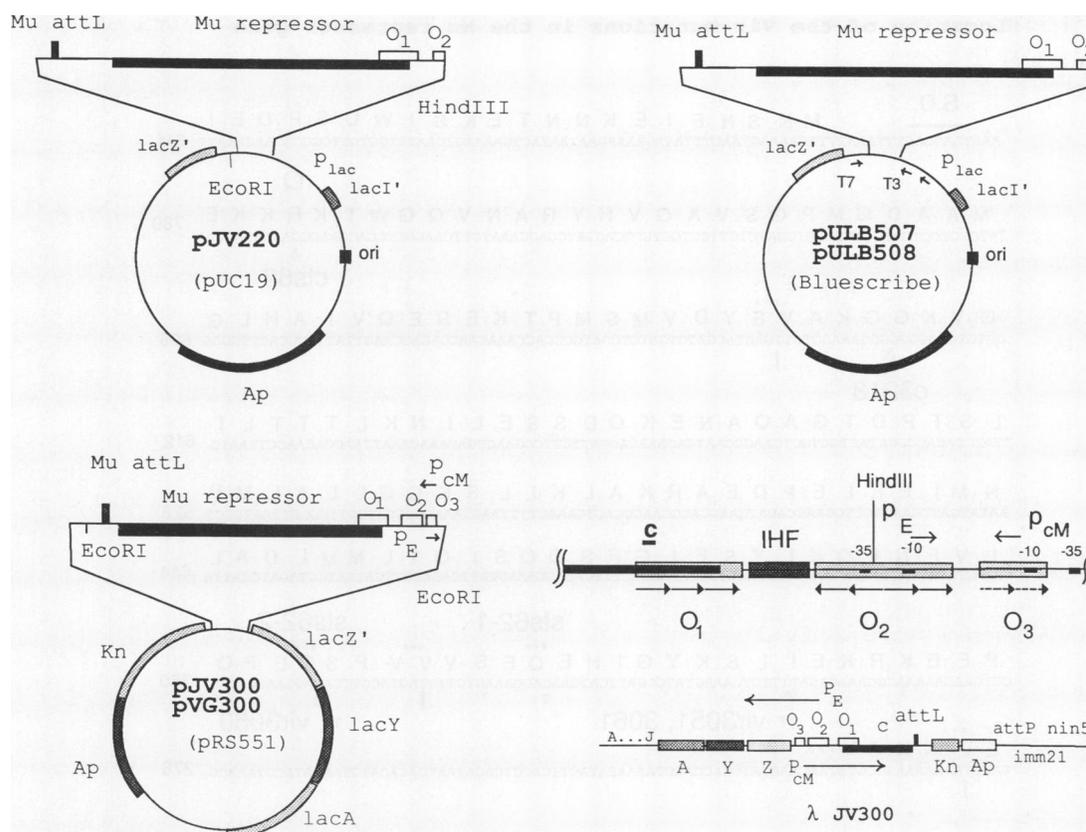


FIG. 1. Plasmids with Mu repressor genes originating from Muvir3051, -3061, and -3060. Muvir3060 DNA was digested with *Hind*III, and the 1-kb fragment was recovered from an agarose gel and introduced into BluescribeM13+ DNA digested with *Hind*III (pULB508). Muvir3051 and Muvir3061 DNAs were digested with *Hind*III, and the 1-kb fragment was recovered from an agarose gel and cloned in pUC19 (pJV220) or BluescribeM13+ (pULB507) digested with *Hind*III and *Sma*I. The resulting plasmids all carry the mutated Mu repressor gene under the control of the P_{lac} promoter. The *Hind*III-*Eco*RI fragments depicted in the top plasmids were isolated and ligated with a *Hind*III-*Eco*RI fragment from the plasmid pHK09 containing Mu sequences from 1001 to 1118 (illustrated at the top of λ JV300, right of the *Hind*III site), in pRS551 DNA cleaved with *Eco*RI and dephosphorylated. In the resulting constructs (plasmids of the series pJV300 and pVG300), *lacZ* expression is regulated by the mutated Mu repressors. Phages in the λ JV300 and λ VG300 series (bottom right) were isolated by in vivo recombination between λ RS45 and the pJV/VG300 plasmids, selecting for λ phages carrying Kn^r as recommended by Simons et al. (24).

found upon sequencing the entire left end of Mu DNA in clones containing these mutations. All *vir* mutations are at the 3' end of the *c* gene (Fig. 2) and bring a +2 change to the reading frame. Both mutations should shorten and modify a small segment of the repressor protein at its C-terminal end. The molecular masses calculated from the sequence data are 20.5 kDa for *vir*3051 and *cts62vir*3061 and 21.5 kDa for *vir*3060.

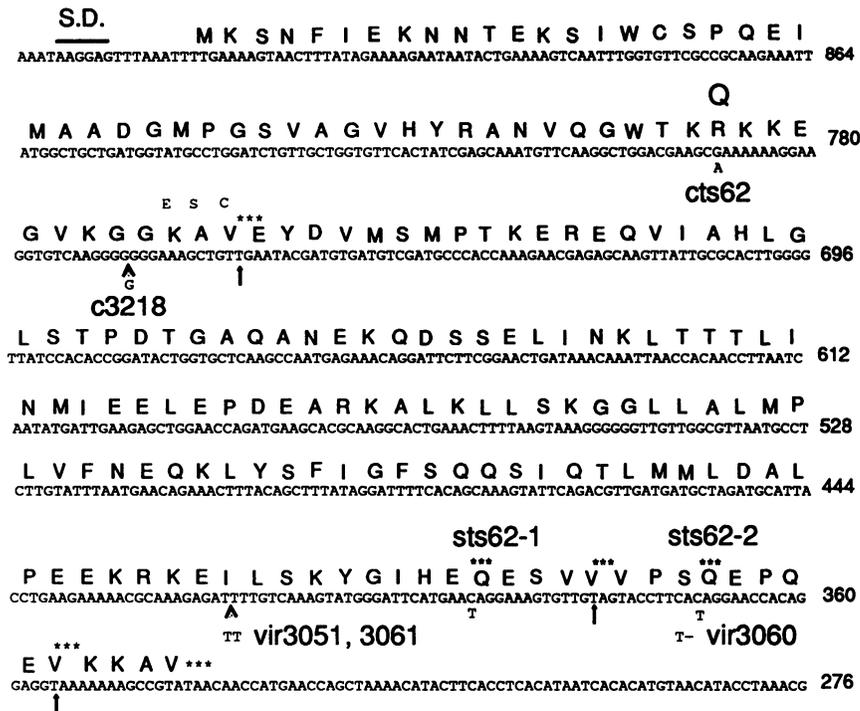
Western blot analysis of proteins extracted from strains carrying the *vir* genes cloned either on the high-copy-number plasmid or in the single-copy λ transducing phage showed proteins close to the expected sizes (Fig. 3). In JM101, which is *supE*, *cts62vir*3061 produces two truncated repressor proteins, a major form of the size expected for a polypeptide terminating at a TAG codon following the frameshift and a minor form of the size expected for termination at a subsequent TAA codon. The latter polypeptide has the same size as that produced by *vir*3060 since they both terminate at the same codon (Fig. 2). Two *Sts* repressor mutants (*sts62-1* and *sts62-2*) were used as controls in these experiments. From the gels in Fig. 3, we calculated molecular masses of 24.5, 22, 23, and 21.6 kDa for the WT, *sts62-2*, *vir*3060, and *cts62vir*3061 repressors, respectively.

As can be seen from the gel, all shortened proteins, *Sts*,

*vir*3060, and *cts62vir*3061, were overproduced on pUC-derived plasmids, in which expression of the *c* genes is under P_{lac} control. However, when present as a single copy on the λ RS45 derivatives, the *vir* genes produced very little repressor compared with the WT, *cts*, and *sts* genes. Overproduction may result from an increased mRNA stability due to the involvement of the repressor C terminus in its RNA degradation (29). The situation may be similar for the *Vir* mutants. This point clearly requires further investigation.

Binding of the mutated repressors to the Mu regulatory region. To have an idea of the binding capacity of the *Vir* repressor mutants to the phage regulatory region, we studied binding to operator DNA from pHK09 with a gel retardation assay. The two fragments generated by digestion of pHK09 with *Bam*HI and *Eco*RI were end labeled and mixed with protein extracts prepared from strains carrying high-copy-number plasmids that produced large amounts of the mutant repressors (Fig. 4B). The 300-bp fragment which contains the three operator sites was strongly retarded by the host protein integration host factor. This protein provokes DNA bending at its binding site located between O_1 and O_2 (7, 10) and is required for retardation of that fragment by repressor (6a). As shown in Fig. 4A, an additional band shift resulted from binding of either *c*⁺ or *sts62-2* repressor and the

Location of the Vir mutations in the Mu repressor gene



Amino-acid sequence at the C-terminal end of Sts and Vir mutants

QSIQTLMLLDALPEEKRKEILSKYGIHEQESVVVPSQEPQEVKKAV	wild type
QSIQTLMLLDALPEEKRKEILSKYGIHEQESVVVPS	sts62-2
QSIQTLMLLDALPEEKRKEILSKYGIHE	sts62-1
QSIQTLMLLDALPEEKRKEILSKYGIHEQESVVVPSFRNHR	vir3060
QSIQTLMLLDALPEEKRKEIFCQSMGFMNRKVL	vir3061, 3051

FIG. 2. Sequence of the Mu repressor gene. The base and amino acid changes corresponding to the *cts62*, *c3218*, *sts62-1*, *sts62-2*, and *vir* mutations are indicated. The asterisks show the termination codons for the normal stop and for amber mutations (*sts*), and asterisks with arrows underneath show the termination codons for frameshift mutations (*vir*).

retarded band is much stronger with the Sts repressor. This may be due both to the stronger binding of the Sts repressor to the operators (29) and to the difference in the protein concentration in the extracts. On the contrary, retardation by the Vir repressor, which was more abundant in the extract than the *c*⁺ protein (Fig. 4B), was negligible. Despite the fact that the DNA-binding N-terminal domain originates either from WT or from *cts62*, the Vir proteins are strongly impaired for DNA binding in vitro.

Isolation of revertants of Muvir3060pAp1 and Mucts62vir3061pAp1. Knowing that the 63 amino acids near the N terminus are sufficient for DNA binding and that removing the C terminus of the *cts62* repressor improves its DNA-binding capacity, we reasoned that removal of the C-terminal end of a Vir repressor could eliminate virulence and possibly restore the ability to lysogenize. Therefore, we looked for nonvirulent revertants of Muvir3060pAp1 and Mucts62vir3061pAp1. Two phage lysates grown on MC4100 *mutD::mini-Tn10* were used to infect MC4100(ΔJV304), and phage that had lost the virulent phenotype were recovered as Ap^r lysogens. Ap^r colonies were recovered at a frequency of about 10⁻⁴. With nonmutagenized lysates, Ap^r colonies

were found at least 100-fold less frequently. The purified Ap^r colonies were stable for the Ap^r marker, and all strains released Mu phage by spontaneous induction. Four lysogens were further analyzed. The lysogen containing *rev42-1*, which originates from Muc⁺, grew at 42°C. The others, which contained *rev43-1*, *-45-2*, and *-46-1*, derived from Mucts62vir3061, were thermosensitive for growth and produced 10 to 50 phage per bacterium upon thermal induction. The phage produced by two of the thermoinducible lysogens could lysogenize C600 at 30°C with the same frequency as Mucts62pAp1 (data not shown). However, the third one (*rev46-1*) had an unexpected phenotype: whether produced at a low temperature as a result of spontaneous induction (0.5 phage per bacterium, compared with around 10⁻⁴ phage per bacterium for a *cts62* lysogen) or after the temperature shift (15 phage per bacterium), all the phage produced were virulent and unable to lysogenize. We are presently testing the interesting possibility that these lysogens carry a host mutation that neutralizes *vir* mutations.

Protein extracts prepared from these four lysogens were analyzed by Western blot with the repressor antibody. In all extracts, there is a band corresponding to the *cts62* repressor

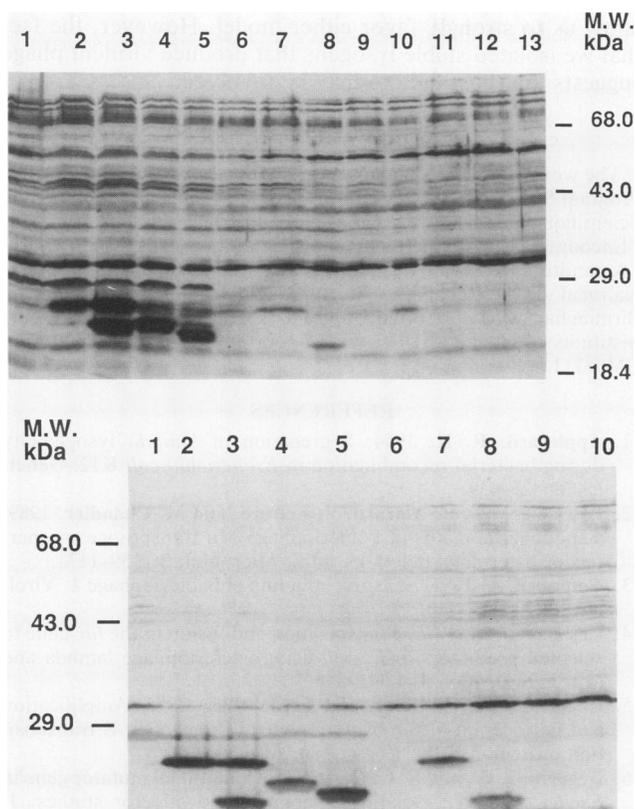


FIG. 3. Western blot analysis of the Vir repressors. Cells were grown at 30°C to the same optical density, corresponding to about 3×10^8 bacteria per ml. A 1-ml sample of each culture was centrifuged, and the pellet was resuspended in 50 μ l of lysis buffer. Extract (16 μ l) was loaded in each lane. (Top) Lanes: 1, JM101/BluescribeM13⁺; 2, JM101/pJV200, *c*⁺ repressor; 3, JM101/pJV214, *cts62sts62-2* repressor; 4, JM101/pULB508, *vir3060* repressor; 5, JM101/pULB507, *cts62vir3061* repressor; 6, MC4100; 7, MC4100 (λ JV300), *c*⁺ repressor; 8, MC4100 (λ JV313), *cts62sts62-1* repressor; 9, 594; 10, 594 (λ JV304), *cts62* repressor; 11, 594 (λ JV320), *vir3051* repressor; 12, 594 (λ VG321), *cts62vir3060* repressor; 13, 594 (λ VG322), *vir3061* repressor. (Bottom) Lanes: 1, 594/pRS551; 2, DH5 α /pJV300, *c*⁺ repressor; 3, DH5 α /pJV313, *cts62sts62-1* repressor; 4, 594/pVG321, (*vir3060* repressor); 5, 594/pVG322, *cts62vir3061* repressor; 6, 594 (λ RS45); 7, 594 (λ JV300), *c*⁺ repressor; 8, 594 (λ JV313), *cts62sts62-1* repressor; 9, 594 (λ VG321), *vir3060* repressor; 10, 594 (λ VG322), *cts62vir3061* repressor.

synthesized by the λ JV304 prophage. In addition, in the strains which produce nonvirulent Mu phage, there is a new band corresponding to a protein of smaller size (Fig. 5). In the lysogen that produces virulent phage, the size of the Vir repressor remains unchanged as expected. Considering the structure of the Mu regulatory region and the fact that these phage establish immunity only at a low temperature, it is likely that the N terminus is present and that the truncations involve (again) the C terminus.

DISCUSSION

Several types of virulent mutants of temperate phage have been isolated and characterized. Phage λ and 434 from *E. coli* and P22 from *Salmonella typhimurium* are the best-documented cases of two different paradigms. λ and 434 control early promoters having two operator regions with several repressor binding sites each. Typically, virulent

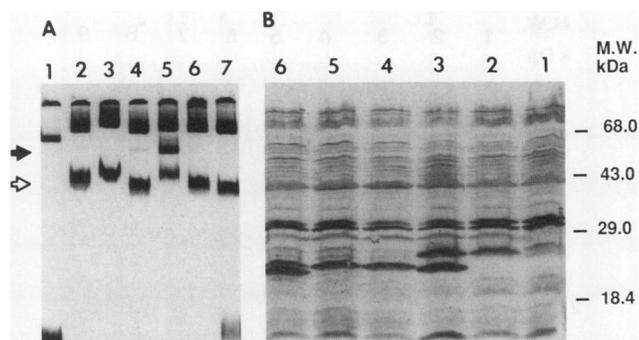


FIG. 4. Binding of Mu Vir repressors to the operators. Protein extracts were prepared as described in Materials and Methods ("Binding assays"). (A) The amount of extract indicated was mixed with pHK09 DNA digested with *Bam*HI and *Eco*RI and end labeled with ³⁵S-ATP. For other details, see Materials and Methods. The open arrow points to the integration host factor retarded band, and the filled arrow points to the band further retarded by repressor. Lanes: 1, no extract; 2, JM101/BluescribeM13⁺ (1.5 μ l); 3, JM101/BluescribeM13⁺ (3 μ l); 4, JM101/pJV200 (*c*⁺) (1.5 μ l); 5, JM101/pJV214 (*cts62sts62-2*) (1.5 μ l); 6, JM101/pULB508 (*vir3060*) (1.5 μ l); 7, JM101/pULB507 (*cts62vir3061*) (1.5 μ l). (B) The presence of repressor in the extracts was checked by Western blot analysis. Lanes: 1, JM101/BluescribeM13⁺ (5 μ l); 2, JM101/pJV200 (*c*⁺) (5 μ l); 3, JM101/pJV214 (*cts62sts62-2*) (3 μ l); 4, JM101/pULB508 (*vir3060*) (3 μ l); 5, JM101/pULB508 (*vir3060*) (5 μ l); 6, JM101/pULB507 (*vir3061*) (5 μ l).

mutations are located in the operator sequences and the changes prevent repressor binding. Consequently *vir* mutations are *cis*-dominant. λ virulence can also result from an imbalance between the amount of active repressor and operator sites; λ cI,c17 double mutants constitutively express the phage replication functions and cannot synthesize any repressor. When these double mutants infect a lysogen, they amplify enough copies of operator to titrate the repressor and induce the resident prophage (for a review, see reference 9).

P22 has a second regulatory region involved in the lysogenic-lytic balance that includes one gene encoding an antirepressor protein (Ant). Ant inactivates all lambdoid repressors. In the lysogenic state, Ant expression is repressed by the Mnt protein. Hence, P22 virulent mutants can result from mutations in the *mnt* operator leading to constitute Ant expression. These mutants are *trans*-dominant because Ant inactivates the repressor (25).

No virulent mutants of Mu affecting operator sites have been found. This may be due to the fact that the O₁ and O₂ operators, which would be the targets of such mutations, overlap with the internal activating sequence, a transposase binding site (15, 18) that is essential for the phage replication. Mutations that impair repressor binding might impair transposase binding as well and, as a result, produce a defective phage that would not have been recovered in our selection.

Both recombination analysis and DNA sequence analysis show that Muvir mutations are near the 3' end of the repressor gene. The nature of the mutations (a double T insertion in one case, a single base pair deletion plus a substitution in the second case) explains their low frequency of occurrence, which is around 10⁻¹⁰. No Vir mutants could be isolated from the c3218 clear mutant, which synthesizes a short inactive repressor (29). This fact is consistent with the notion that virulence requires expression of a functional protein that induces replication of a lysogenic prophage in

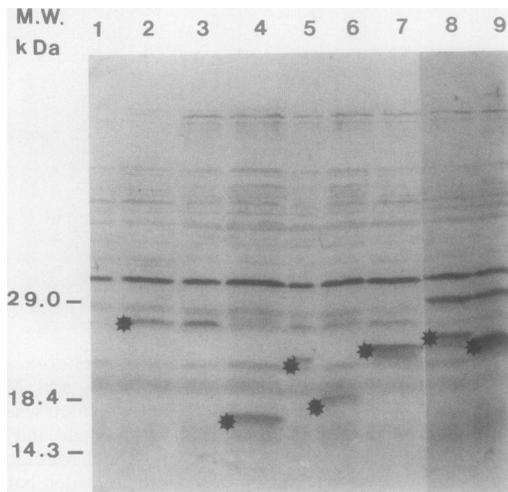


FIG. 5. Western blot analysis of proteins produced in MC4100 (λ JV304) lysogenic for pseudorevertants of Muvir3060 and Mucts62vir3061pAp1. The experimental protocol was the same as for Fig. 3. Lanes: 1, C600; 2, C600 (Mu Gov3261); 3, MC4100 (λ JV304) *cts62* repressor; 4, MC4100 (λ JV304) (Mu *cts62vir3061rev42-1*); 5, MC4100 (λ JV304) (Mu *cts62vir3061rev43-1*); 6, MC4100 (λ JV304) (Mu *cts62vir3061rev45-2*); 7, MC4100 (λ JV304) (Mu *cts62vir3061rev46-1*); 8, C600/pVG321 (*vir3060*); 9, C600/pVG322 (*cts62vir3061*). The strong band which migrates around 29 kDa comes from the pRS551 plasmid (Fig. 3). The asterisks mark the bands corresponding to the mutated repressors.

trans. The virulent mutants have to inactivate repressor to replicate in a lysogen which contains active repressor, and we conclude that the antirepressor is a mutant repressor.

How does a repressor turn into an antirepressor? Given the biochemical properties of WT, Ts, and Sts repressors, we can suggest two models. Model 1 is that the Mu repressor is an allosteric protein in which an N-terminal DNA-binding domain alternates between a high-affinity state and a low-affinity state. High- and low-affinity conformations are controlled by long-range interactions that involve the C terminus of the protein. Vir repressors are assumed to be locked into the low-affinity conformation and they must form mixed complexes that shift WT repressors to the low-affinity form, causing both proteins to come off DNA and allow transcription of the prophage.

In model 2, the C terminus interacts with cellular machinery that inactivates or alters the DNA-binding site at the N terminus. Again, this model requires that Vir interact with the WT repressor in order to induce lytic transcription by the prophage. The cellular machinery that alters DNA binding of the repressor could be a variety of things. Vir repressor might activate proteolytic enzymes that subsequently degrade both Vir and the WT repressor located at the same site. Interestingly, Pakula and Sauer recently showed that changes at the C terminus of the P22 Arc repressor dramatically alter susceptibility of this protein to proteolytic degradation *in vivo* (19). A second possibility would be unfolding enzymes that change the conformation of repressor when they are bound (e.g., heat-shock chaperonins). Model 2 in this version is similar to that described by Picard and Yamamoto for the mechanism of glucocorticoid regulation in eukaryotes (21), and it predicts that specific host proteins will be necessary for expression of the Vir phenotype.

What we currently know about Mu repressor does not

allow us to strongly favor either model. However, the fact that we isolated stable lysogens that produce virulent phage suggests that host factors may be involved.

ACKNOWLEDGMENTS

The work in Rhode St Genèse was supported by the EEC Science Program SCI-0143-C(GDF) and the Belgian Fonds de la Recherche Scientifique Medicale. V.G. is fellow from the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA). A.T. is Maître de Recherche from the Fonds National de la Recherche Scientifique (Belgium). The work in Birmingham was supported by grant GM33143 from the National Institutes of Health. J.L.V. was supported by NIH training grant GM08111.

REFERENCES

- Appleyard, R. K. 1954. Segregation of *lambda* lysogenicity during bacterial recombination in *Escherichia coli* K12. *Genetics* **39**:429-439.
- Bétermier, M., R. Alazard, V. Lefrère, and M. Chandler. 1989. Functional domains of bacteriophage Mu transposase: properties of C-terminal deletions. *Mol. Microbiol.* **3**:1159-1171.
- Campbell, A. 1961. Sensitive mutants of bacteriophage λ . *Virology* **14**:22-32.
- Casadaban, M. 1976. Transposition and fusion of the *lac* gene to selected promoters in *E. coli* using bacteriophage *lambda* and Mu. *J. Mol. Biol.* **104**:541-555.
- Chaconas, G., G. Gloor, and J. L. Miller. 1985. Amplification and purification of the bacteriophage Mu encoded B transposition protein. *J. Biol. Chem.* **260**:2662-2669.
- Degnen, G. E., and E. C. Cox. 1974. Conditional mutator gene in *Escherichia coli*: isolation, mapping, and effector studies. *J. Bacteriol.* **117**:477-487.
- Gama, M. J., A. Toussaint, and N. P. Higgins. Unpublished data.
- Goosen, N., and P. van de Putte. 1984. Regulation of Mu transposition. I. Localization of the presumed recognition sites for *himD* and *ner* functions controlling bacteriophage Mu transcription. *Gene* **30**:41-46.
- Harrison, S. C., and A. K. Aggrawal. 1990. DNA recognition by proteins with helix-turn-helix motif. *Annu. Rev. Biochem.* **59**:933-969.
- Hendrix, R. W., J. W. Roberts, F. W. Stahl, and R. A. Weisberg. 1983. *Lambda* II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Higgins, N. P., D. A. Collier, M. W. Kilpatrick, and H. M. Krause. 1989. Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage Mu. *J. Biol. Chem.* **264**:3035-3042.
- Howe, M. M. 1973. Prophage deletion mapping of bacteriophage Mu-1. *Virology* **54**:93-101.
- 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 *E. 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 *Tn3* conferring ampicillin resistance. *Mol. Gen. Genet.* **172**:179-184.
- 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 (London)* **338**:656-658.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Miller, J. H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 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.
19. Pakula, A. A., and R. T. Sauer. 1989. Genetic analysis of protein stability and function. *Annu. Rev. Genet.* **23**:289–310.
 20. Pato, M. L. 1989. Bacteriophage Mu, p. 23–52. *In* D. E. Berg and M. M. Howe, *Mobile DNA*. American Society for Microbiology, Washington, D.C.
 21. Picard, D., S. J. Salser, and K. R. Yamamoto. 1988. A movable and regulable inactivation function within the steroid binding domain of the glucocorticoid receptor. *Cell* **54**:1073–1080.
 22. Priess, H., C. Schmidt, and D. Kamp. 1987. Sequence of the left end of Mu, p. 277–296. *In* N. Symonds, A. Toussaint, P. van de Putte, and M. M. Howe (ed.), *Phage Mu*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 23. Résibois, A., A. Toussaint, F. van Gijsegem, and M. Faelen. 1981. Physical characterization of mini-Mu and mini-D108. *Gene* **14**:103–113.
 24. 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.
 25. Suskind, M. M., and P. Yourderian. 1983. Bacteriophage P22 antirepressor and its control, p. 251–277. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), *Lambda*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 26. Symonds, N., A. Toussaint, P. van de Putte, and M. M. Howe. (ed.). 1987. *Phage Mu*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 27. Taylor, A. L. 1963. Bacteriophage-induced mutation in *E. coli*. *Proc. Natl. Acad. Sci. USA* **50**:1043–1051.
 28. 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.
 29. Vogel, J. L., N. P. Higgins, L. Desmet, V. Geuskens, and A. Toussaint. Unpublished data.
 30. Vogel, J. L., Z. J. Li, 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 site. *J. Bacteriol.* **173**:6568–6577.
 31. Weigle, J. M., M. Meselson, and K. Paigen. 1959. Density alterations associated with transducing ability in the bacteriophage λ . *J. Mol. Biol.* **1**:379–386.
 32. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.