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**DNA sequence of the control region of phage D108: the N-terminal amino acid sequences of repressor and transposase are similar both in phage D108 and in its relative, phage Mu**

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**ABSTRACT**

We have determined the DNA sequence of the control region of phage D108 up to position 1419 at the left end of the phage genome. Open reading frames for the repressor gene, ner gene, and the 5' part of the A gene (which codes for transposase) are found in the sequence. The genetic organization of this region of phage D108 is quite similar to that of phage Mu in spite of considerable divergence, both in the nucleotide sequence and in the amino acid sequences of the regulatory proteins of the two phages. The N-terminal amino acid sequences of the transposases of the two phages also share only limited homology. On the other hand, a significant amino acid sequence homology was found within each phage between the N-terminal parts of the repressor and transposase. We propose that the N-terminal domains of the repressor and transposase of each phage interact functionally in the process of making the decision between the lytic and the lysogenic mode of growth.

**INTRODUCTION**

The closely related heteroimmune temperate bacteriophages Mu and D108 function as transposons during their life cycle (for a review see ref. 1). During lytic growth, these phages replicate their DNA through cycles of replicative transposition, using multiple nonspecific sites on the host chromosome as the targets. At the end of the lytic cycle, transposed copies of the phage DNA with a piece of host DNA at each end are cut from the chromosome and packaged into phage particles. During the lysogenic cycle, the infecting phage DNA is inserted nonspecifically and without replication into the host chromosome. Further transposition of the prophage is rare during growth of the lysogenic cells. The genetic elements that control the choice between lytic and lysogenic growth, the repressor (or c) gene and operator-promoter sequences, are located in the immunity region at the left end of each phage genome.

The primary function of the repressor is to bind to the operator and turn down transcription of genes whose products are necessary for lytic growth. This includes gene A, which encodes transposase. However, the Mu repressor not only blocks transcription, but also seems to act as a direct inhibitor of

transposition (2). We have previously suggested that Mu repressor inhibits transposition by binding to the sites of action of transposase, which are located at the Mu DNA ends (2). The following argument supports this suggestion. We realized by inspection of the sequence of the Mu immunity region (3) that Mu repressor and Mu transposase have a similar amino acid sequence at their N-termini. This similarity hints at a functional interaction between the two proteins. The DNA sequence coding for the repressor and the N-terminal part of the transposase fall in the major area of sequence nonhomology between phage Mu and phage D108 (4, 5). If the repressor and transposase do indeed interact, and if the interaction is similar for each phage, then D108 repressor and transposase should also resemble each other.

We have sequenced the immunity region of phage D108 up to position 1419 in order to compare the genetic content of this regulatory region with that of phage Mu (3) and also to examine the amino acid sequence relationship between the D108 repressor and D108 transposase. We have indeed found the predicted resemblance between the two proteins.

### MATERIALS AND METHODS

#### Reagents

Restriction enzymes were obtained from New England Biolabs. T4 DNA ligase was from Boehringer-Mannheim Biochemicals and DNA polymerase (Klenow enzyme) was from International Biochemicals, Inc.

Oligonucleotide primers used for DNA sequencing corresponded to the Mu end sequences and were kindly synthesized by Dr. G. Zon (National Center for Drug and Biologics) using the Applied Biosystems 380A synthesizer using phosphoramidite chemistry and purified on a C-18 reverse phase column. The Mu left end primer had the sequence TTTCGTA<sup>CT</sup>CAAG and the Mu right end primer had the sequence TTTCGCATTTATCGTG. Other reagents for dideoxy chain terminator sequencing reactions were obtained from New England Biolabs. <sup>35</sup>S-labelled deoxyadenosine  $\alpha$ -thiotriphosphate (~ 1000 ci/mmol) was obtained from New England Nuclear.

#### Sequencing Strategy

The FnuDII-EcoRI fragment of interest was purified from phage DNA of D108 wild-type or D108 cts10 and cloned into M13 mp18 and M13 mp19. Details of the method for sequencing the cloned piece of DNA by mini Mu insertion will be described elsewhere (Adachi, T., Cellert, M. and Mizuuchi, K., manuscript in preparation). Briefly, a small mini Mu that carries the amp<sup>r</sup> gene was transposed into the M13 carrying the cloned fragment. The M13 phage lysate was

used to infect E. coli cells which were then plated onto ampicillin-containing agar plates. Each amp<sup>r</sup> colony carried an M13 with a copy of the mini Mu inserted at a random location on the cloned segment. Each colony was grown and phage particles were purified and DNA extracted as described by Sanger et al. (6). The primer extension method of Sanger et al. (7) involving a set of dideoxy chain terminators was used as the sequencing reaction. Both the Mu left-end primer and the Mu right-end primer were added together for each reaction. The reaction products were separated on a 0.5 mm thick buffer gradient 6% denaturing acrylamide gel (13 in x 16 in) according to Biggin et al. (8). The random segments of the sequence were assembled by making use of a computer program developed by Staden (9).

#### Promoter Search

Potential promoter sequences were identified by making use of a program developed by Mulligan et al. (10).

#### Amino Acid Sequence Homology Search

Amino acid sequences of a pair of polypeptides were compared with the "relate" program described by Orcutt et al. (11).

#### Construction and Testing of IHF Mutant Lysogens

The  $\Delta 82$ [himA]::Tn10 deletion mutation inactivates the  $\alpha$  subunit, and the  $\Delta 3$ [hip]::cat substitution mutation inactivates the  $\beta$  subunit of IHF (integration host factor) (12,13). These mutations were transferred to Mu cts62 or D108 cts10 lysogens of strain N99 by P1 transduction with selection for tetracycline or chloramphenicol resistance, as appropriate. The IHF-negative phenotype of the transductants was verified by resistance to infection with phage carrying the QSR region of  $\phi 80$  as described in reference (14). The fraction of temperature resistant cells of each strain was estimated by spotting serial dilutions of an overnight culture with capillary tubes on duplicate LB agar plates that were incubated overnight at 32° or 41°. The fraction of cells that could produce at least one phage particle was estimated by spotting dilutions of the same cultures onto LB agar plates containing a lawn of an indicator strain, and incubating the plates overnight at 41°.

### RESULTS AND DISCUSSION

#### Comparison of the Genetic Organization and the Amino Acid Sequences of Proteins of the Control Regions of Phages D108 and Mu

We have cloned and sequenced phage D108 DNA between the FnuDII site at position 125 and the EcoRI site at position 1414. (The sequence beyond the EcoRI site in Fig. 1 is from Toussaint et al. (5), and this part of the



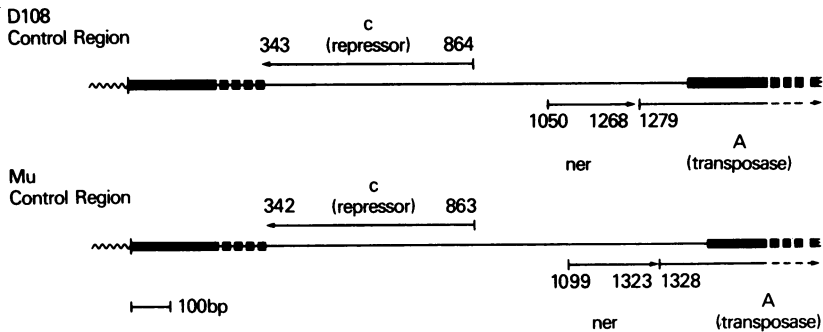


Fig. 2. Genetic organization of the regulatory region of phages D108 and Mu. Locations of the structural genes for the three proteins coded by this region are indicated with their starting and ending positions. The map of Mu genes is according to Priess *et al.* (3). Wavy lines indicate flanking non-phage sequences. Thick lines indicate the area with strong DNA sequence homology between D108 DNA and Mu DNA.

sequence matched within the area sequenced. Open reading frames for the three polypeptides expected to be coded by this region of the D108 genome were found in the sequence. The leftward open reading frame, starting with the methionine codon at position 864, corresponds to the structural gene for D108 repressor, as judged by the similarity of the direction and the position to those of the Mu repressor gene, the amino acid sequence homology to the Mu repressor (discussed below), and the position of the A to T transversion associated with the *cts10* mutation, which caused a Tyr to Asn amino acid substitution in the protein product. The rightward open reading frame, starting with the methionine codon at position 1050, is similar in size and location to the Mu *ner* gene and is assumed to code for the D108 Ner protein, as judged by its coding capacity for a polypeptide with strong amino acid sequence homology to the Mu *ner* gene product. The open reading frame which follows the *ner* gene and continues through the end of the sequenced area presumably corresponds to the D108 *A* gene which codes for D108 transposase, as judged by the similarity of its location and the amino acid sequence of the peptide it codes for, to those of the Mu *A* gene.

Overall, the genetic organization of the control regions of D108 and Mu is very similar, except that the intercistronic operator-promoter area between the repressor gene and *ner* gene is slightly smaller in D108 than in Mu (Fig. 2).

The DNA sequence similarity between Mu and D108 at their left ends (2, 4) continues to about position 350. There is limited DNA sequence similarity

# Nucleic Acids Research

	-35	Spacing	-10	Location	Score
Consensus	AAATAATTC <b>TTGACA</b> T	17	TTTGG <b>TATAAT</b> ACC	-35 -10	100 %
Rightward D108	ACAGCTTT <b>CTTACAT</b>	17	ACAGG <b>GAAAT</b> ATG	354 378	58.0%
	TTATCTTT <b>CTGACAA</b>	17	AGTC <b>CTTAA</b> CCC	507 531	56.8%
	TATGGACGA <b>TTGATGT</b>	17	TATCAGAAAG <b>TGAG</b>	631 655	52.1%
	AAACCATT <b>CTTGTAT</b>	16	TCAT <b>TAAAA</b> CCT	845 868	56.2%
	CAAAATAAA <b>TTCATAT</b>	17	TGTTATATGAA <b>ATC</b>	902 926	50.9%
	AAATCACTA <b>TTGATT</b> T	17	CACAC <b>TACAT</b> GCA	958 982	71.0%
Leftward D108	CAACAATCT <b>TTCGCGT</b>	17	ACGGC <b>TAAAA</b> CTG	129 105	51.5%
	AAATGATGC <b>GTTGAAGT</b>	17	GTGTC <b>TTTAA</b> TGAA	184 160	56.2%
	GACATAAAC <b>TTCACTC</b>	17	ATATG <b>TAAGAT</b> ACC	311 287	55.6%
	AGAGAGATT <b>TTGTCTC</b>	18	AAGAA <b>CATAAT</b> TTC	407 382	50.9%
	TTTTAAATG <b>ATGTCTT</b>	18	AATGG <b>TTTAA</b> TGCG	860 835	52.1%
	AATAGTGA <b>TTGAAAG</b>	18	TTTCA <b>TATAAC</b> AGC	948 923	60.4%
	AAATCAATA <b>GTGATT</b> T	18	CATGA <b>TTTCAT</b> ATA	953 928	50.3%
Rightward MU	AACACCAA <b>TTGACTT</b>	16	TTCTT <b>TTCTAT</b> AAA	888 911	52.1%
	AACACCAA <b>TTGACTT</b>	19	TTTTCT <b>TATAAA</b> GTT	888 914	57.4%
	CAAAATGAC <b>TTTTCAG</b>	16	TTCTA <b>TAAAGT</b> TAC	893 916	50.3%
	TCAAAGGAA <b>TTTACCA</b>	17	TACAT <b>TAAGCT</b> TTT	978 1002	50.3%
	AAAAGCAGC <b>TTTACAT</b>	17	GTAAT <b>TATCT</b> TTT	994 1018	57.4%
	ACATTAAGC <b>TTTTCAG</b>	16	TTTAG <b>TAAGCT</b> AGC	1006 1029	52.1%
	AATTATCTT <b>TTTAGTA</b>	16	GTTTT <b>TACACT</b> TAG	1023 1046	54.4%
	AAGGAGAGA <b>TTGAAAC</b>	16	TATTT <b>TGAAAT</b> CGC	1531 1554	53.8%
Leftward MU	GTATTTTCA <b>ATGAAAC</b>	17	TTTAC <b>TATCTT</b> TGC	157 133	56.8%
	AAGCTGTTA <b>TTGAAAT</b>	16	GTTTT <b>TGTTTT</b> TGC	196 173	53.3%
	AAAACATAC <b>TTCCAGT</b>	17	ACATG <b>TAACAT</b> ACC	312 288	65.1%
	GTTTAAAT <b>TTGAAA</b> A	18	AAAAG <b>AATAAT</b> ACT	928 903	56.8%
	AATAAGGAG <b>TTTAAAT</b>	18	CTTTA <b>TAGAAA</b> AGA	936 911	51.5%
	CTGAAAAGC <b>TTAATGT</b>	16	TTTGG <b>TAAAT</b> CCT	1001 978	52.7%
	TGGAACACA <b>TTTAAA</b> A	17	TTTTG <b>TAATCT</b> ATA	1098 1074	48.5%

Fig. 3. Potential promoter sequences in the regulatory region of phage D108 and Mu. The D108 sequence reported here and the Mu sequence described by Priess *et al.* (3) were searched for potential promoter sequences with the computer program of Mulligan *et al.* (10). Potential promoters with a spacing of 16 to 19 nucleotides between the -10 and -35 regions and a promoter score above 50% were listed. The Mu leftward promoter at position 1098 to 1074 had a lower score but has been identified as one of the promoters for the repressor gene (16). The -10 region and -35 region are boxed and nucleotides in these regions that form part of the consensus sequence are shown in large letters.

between the two phages within the area corresponding to the C-terminal part of the repressor gene, *ner* gene, and the 5' portion of the *A* gene. Strong sequence similarity for the remaining part of the *A* gene starts after the EcoRI site at position 1414, based on the published sequence of Toussaint *et al.* (5).

We searched for potential promoter sequences by making use of the program developed by Mulligan *et al.* (10). The operator-promoter area contains a cluster of potential promoter sequences in both orientations. Examples of the potential promoters are shown in Fig. 3 together with their McClure promoter

score. Potential promoters are also found elsewhere in the sequenced region. These potential promoters are listed in the figure together with those found in the corresponding area on the Mu DNA sequence of Priess *et al.* (3). The biological significance of each of these potential promoters remains to be clarified. In the case of phage Mu, the rightward promoter at position 994 to 1018 has been shown to be the major early promoter (15), and the leftward promoter at position 1098 to 1074 has been identified as one of the promoters for the *cI* repressor (16).

Potential integration host factor (IHF) binding sequences found in the control region of D108 are underlined in Fig. 1. IHF, which is required for the phage lambda integrative recombination reaction, is a site-specific DNA binding protein made up of two polypeptides (17) and could also be involved in the control of some promoters. The Mu early promoter seems to be such a promoter (18, 19) and *E. coli* mutants that lack IHF (*himA*<sup>-</sup> or *hip*<sup>-</sup>) fail to grow phage Mu (20). The spatial relationship between the potential IHF binding sequence of position 911 and the potential promoter sequence at position 958 to 982 in D108 is exactly the same as the IHF binding sequence (position 947) and the early promoter (position 994 to 1018) of phage Mu. We therefore examined the ability of phage D108 to grow in *himA* and *hip* mutants. We found that the plating efficiency of phage D108 on such mutants was  $< 10^{-4}$  relative to the parental strain (strain N99; data not shown). We then constructed *hip*<sup>-</sup>, *himA*<sup>-</sup>, and *hip*<sup>-</sup> *himA*<sup>-</sup> derivatives of D108 *cts10* and Mu *cts62* lysogens (see Material and Methods). The proportion of cells in cultures of these mutant lysogens that were capable of releasing at least one infective phage after thermal inactivation of the repressor was between  $10^{-3}$  and  $10^{-5}$  for both phages (data not shown). This shows that the presence of IHF is essential for intracellular phage growth and not simply for virion adsorption or DNA injection. However, the absence of IHF did not prevent cell killing after thermal induction of D108: relative survival of the single and multiple mutants at 41° was between  $10^{-4}$  and  $10^{-5}$  than at 32°, similar to the survival for the *hip*<sup>+</sup> *himA*<sup>+</sup> parental strain (data not shown). In contrast, IHF mutant derivatives of Mu *cts* lysogens formed colonies with near unit efficiency at 41°, as has been previously reported (20). We conclude that D108, like Mu, requires IHF for intracellular growth. The difference in survival of mutant lysogens of the two phages could reflect a higher basal level of D108 early gene expression in the absence of IHF. Alternatively, or in addition, IHF may be required for a later stage of D108 development.

The remarkable correspondence between the location of the genes and sites



Fig. 4. Amino acid sequence homology between the corresponding polypeptides of phages D108 and Mu. Conserved amino acids are indicated by reverse contrast letters. Only the N-terminal segment of A protein sequences are shown. The entire A gene sequence is now available for Mu (23) but not for D108. However, the remaining parts of the A genes of the two phages are expected to be quite similar (4, 5).

in the control regions of D108 and Mu and the absence of substantial nucleotide sequence conservation (except at the ends) is reminiscent of the lambdoid family of phages. Like the lambdoid family, the Mu family appears to have a common genome that is nearly but not quite independent of nucleotide sequences (21).

Amino Acid Sequence Comparison Between Corresponding Peptides of D108 and Mu

Although the nucleotide sequences of the two control regions are rather dissimilar, the amino acid sequences they encode are more closely related. The primary structures of the two repressors are quite similar toward the C-terminal halves but less similar toward the N-terminal halves, where conservation is apparent only when "conservative" amino acid changes as well as amino acid identities are taken into account (Figs. 4 and 5A; see legend to Fig. 5). The Ner proteins of the two phages are even more similar than the repressors even though significant divergence has occurred here too (Fig. 4 and Table 1). Some sequence divergence between the two repressors and between the two Ner proteins is expected because repressor and probably Ner protein as well, help to determine immunity specificity, and D108 is heteroimmune to Mu.

The A genes of the two phages code for transposases. Each transposase, in addition to its normal activity, promotes transposition of the other phage to a significant extent, and the ends of the two phage chromosomes, which are the sites of transposase action, are quite similar in nucleotide sequence (2,



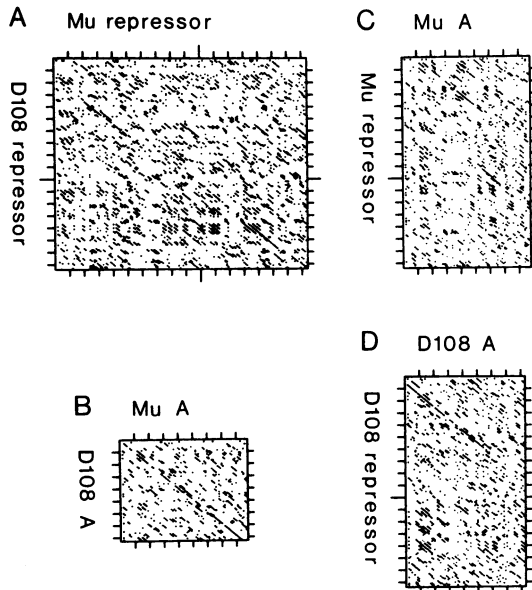


Fig. 5. Dot matrix diagrams of sequence similarities between repressors and the N-terminal segment of transposases (26). One sequence is displayed left to right, N- to C-terminus, and the second is displayed top to bottom, N- to C-terminus. All possible segments of 3 amino acids in one protein are compared to all segments of the same length in the other, and a score is computed based on the evolutionary relatedness of the amino acids at each position (using the "Mutation Data Matrix" of Dayhoff *et al.* (25)). Whenever the score exceeded 4, a dot was placed in the appropriate position on the diagram. A straight line with a slope of -1 indicates sequence similarity. Panel A: Mu repressor (horizontal) compared to D108 repressor (vertical); Panel B: Mu transposase (horizontal) compared to D108 transposase (vertical); Panel C: Mu transposase (horizontal) compared to Mu repressor (vertical); Panel D: D108 transposase (horizontal) compared to D108 repressor (vertical).

22). Therefore, we expect that the two transposases will be structurally related. Indeed, sequence conservation is substantial from amino acid 61 to the C-termini (Fig. 4; also see ref. 5). However, the sequences of the first 60 amino acids are less closely related, although a resemblance is nevertheless apparent when conservative changes are taken into account (Fig. 5B). In fact, the N-termini of the transposases appear to have diverged from each other to the same extent as have the repressor and Ner proteins. (Nonhomology at the N-termini of the two *A* polypeptides was first reported by Toussaint *et al.* (5). However, their published nucleotide sequences differ from ours at several points. We feel confident about our sequence because the same region

Table 1

	Mu A	D108 Ner	Mu Ner	D108 rep	Mu rep
D108 A	13.923	-0.041	-0.125	15.160	3.505
Mu A		0.340	0.739	6.220	13.443
D108 Ner			15.260	-0.095	0.017
Mu Ner				0.265	-0.255
D108 rep					12.269

Amino acid sequence similarity among polypeptides coded by the regulatory regions of phage D108 and Mu. Amino acid sequence similarity scores were calculated for pairwise combinations of the peptides with the "RELATE" computer program of Orcutt *et al.* (11). Only the N-terminal segment shown in Fig. 4 was used for this analysis for A proteins. This program compares all possible segments of a given length of one protein to all possible comparable segments of the other. We chose a segment length of 25 amino acids. For each comparison, a score is computed based on the evolutionary relatedness of the amino acids at each position (the "Mutation Data Matrix" of Dayhoff *et al.* (25)). The computer also calculates scores for randomly permuted sequences of the same proteins. The similarity of two proteins is determined by comparing the means of the top groups of scores for the real and the permuted sequences: the difference between the means increases with increasing similarity. The table presents the differences, in standard deviation units, between the means for each pair of proteins. The statistical significance of these differences can be judged by consulting a table of the cumulative normal distribution.

was sequenced several times, and also because the material from two sources,  $c^+$  and  $cts$ , match exactly except for a one-base-pair substitution within the repressor gene.) This finding suggests that this portion of the A polypeptide might be involved in a function other than, or in addition to, the transposase activity of the protein. This possibility is further supported by the observation described below.

#### Amino Acid Sequence Comparison Between Heterologous Polypeptides

Next, we compared the sequences of functionally different proteins encoded by the control regions of Mu and D108 (Table 1 and Fig. 5C and D). Although the sequences of the two Ner polypeptides closely resemble each other, neither resembles that of any other polypeptide. However, the repressors are clearly similar to the N-terminal segment of the transposases. The similarity is substantially stronger between the pair of polypeptides of the same phage than between those of different phages, and it is concentrated in the N-terminal regions (Figs. 5C, 5D and 6). It is interesting to recall that we found the N-terminal regions to be the least well conserved when we compared proteins of analogous function.

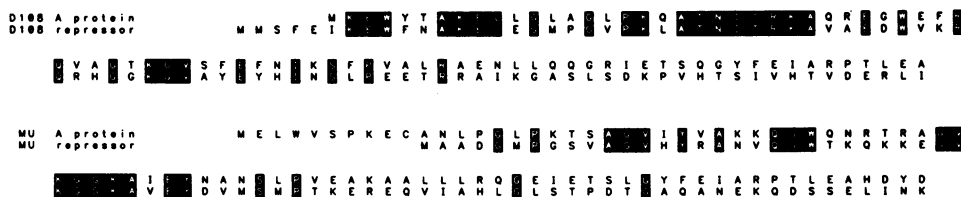


Fig. 6. Amino acid sequence homology between the N-terminal segment of transposase and repressor of phage D108 or Mu. Conserved amino acids are indicated by reverse contrast letters.

The nonuniform distribution of the sequence homologies between the N- and C-terminal segments of the two repressors and transposases is a strong argument that these proteins are divided into distinct functional domains with different constraints on their rates of evolutionary divergence. The resemblance between the N-terminal domains of repressor and transposase from the same phage suggests that these domains have a similar function. Finally, the greater extent of sequence divergence between the N-terminal domains of repressor and transposase from different phages than between those of the same phage leads us to surmise that the proteins from the same phage interact functionally.

At this point, the functions or the interactions are not clear. We offer two possibilities. First, the N-terminal domain of each protein might bind directly and specifically to similar or identical sequences in phage DNA. A similar possibility has already been suggested by Harshey *et al.* (23). The ability of repressor and transposase to bind to similar sequences could allow one protein to inhibit the primary function of the other by hindering its access to its substrate. In support of this hypothesis, our own previous work has shown that Mu transposase binds the Mu operators, although less tightly than the Mu ends, and that Mu repressor binds the Mu ends, although less tightly than the Mu operators (2). In a second model, the structural similarity between the N-terminal domains of repressor and transposase might promote their association into an inactive hetero-oligomer. A similar hypothesis has been proposed to account for the inactivation of phage P22 repressor by anti-repressor (24). Thus, when the ratio of repressor concentration to that of transposase within a cell exceeds some limit, transposition will be inhibited even before transcriptional repression reduces the concentration of transposase. Similarly, a sufficiently high relative concentration of transposase will lift transcriptional repression by the repressor. Such inhibition,

whether it occurs by a protein-DNA or a protein-protein interaction, is likely to be biologically important because it will sharpen the transition between the lytic and lysogenic states and thereby help avoid indecision between the two modes of growth.

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