Involvement of Escherichia coli FIS Protein in Maintenance of Bacteriophage Mu Lysogeny by the Repressor: Control of Early Transcription and Inhibition of Transposition

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The Escherichia coli FIS (factor for inversion stimulation) protein has been implicated in assisting bacteriophage Mu repressor, c, in maintaining the lysogenic state under certain conditions. In a fis strain, a temperature-inducible Muc62 prophage is induced at lower temperatures than in a wild-type host (M. Bétermier, V. Lefrère, C. Koch, R. Alazard, and M. Chandler, Mol. Microbiol. 3:459–468, 1989). Increasing the prophage copy number rendered Muc62 less sensitive to this effect of the fis mutation, which thus seems to depend critically on the level of repressor activity. The present study also provides evidence that FIS affects the control of Mu gene expression and transposition. As judged by the use of lac transcriptional fusions, repression of early transcription was reduced three- to fourfold in a fis background, and this could be compensated by an increase in cts62 gene copy number. c was also shown to inhibit Mu transposition two- to fourfold less strongly in a fis host. These modulatory effects, however, could not be correlated to sequence-specific binding of FIS to the Mu genome, in particular to the strong site previously identified on the left end. We therefore speculate that a more general function of FIS is responsible for the observed modulation of Mu lysogeny.

Maintenance of the lysogenic state of the transposable bacteriophage Mu (see reference 52 for a review) is ensured by the phage-encoded c protein which acts both as a regulator of early gene expression and as an inhibitor of the transposition reaction. c represses transcription of the early genes encoding two essential transposition functions, the transposase, pA, and protein pB, by binding to two operator sites, O1 and O2 (Fig. 1A) (22, 39). An interesting feature of Mu repressor and transposase is their ability to recognize the same target sites on the phage genome. They both specifically interact with the ends of Mu DNA, attL and attR. In the case of pA, this occurs at three sites on attL (L1, L2, and L3) and on attR (R1, R2, and R3) (12, 24). The same regions have been shown to be targets for repressor binding (12). Both proteins also interact with an internal activating sequence (IAS), which overlaps the operator region and functions in cis (43, 47, 64) or in trans (63) to stimulate transposition 10- to 100-fold. These observations suggest that c could compete with pA for binding to the same sequences on the Mu genome. Indeed, several lines of evidence obtained in vitro and in vivo indicate that c inhibits Mu lytic growth at a posttranscriptional level by directly interfering with the transposition reaction (12, 47, 67a).

Host auxiliary DNA-binding proteins have been implicated in modulating the commitment to lysogeny or lytic growth at the level of transcription and of transposition. Integration host factor (IHF), which binds and strongly bends DNA at specific recognition sites, takes part in a number of site-specific recombination reactions and regulatory processes in Escherichia coli (see reference 19 for a review). Binding of IHF to the early operator region of Mu (39, 69) can influence transcriptional control in different ways. By stabilizing the interaction of the repressor with sites O1 and O2, it may favor lysogeny (19, 20), whereas in the absence of c (i.e., during lytic growth), it stimulates early transcription (23, 39). In addition, IHF binding to the IAS directly stimulates in vitro transposition of a substrate with suboptimal supercoiling levels (62, 64). A second related host factor, the heterodimeric histone-like protein HU, binds nonspecifically to DNA but induces curvature of the double-helix axis (13, 28). It contributes in vitro to the formation of an early intermediate in the transposition reaction, the synapic complex between Mu ends and pA (11, 46). While high concentrations of IHF can substitute for HU in vitro (62), the observation that mutants deficient in both HU subunits exhibit reduced Mu plating efficiency and transposition (31, 35) provides in vivo support for the crucial role of HU in Mu growth.

Another host protein, FIS (Factor for Inversion Stimulation), has been shown to be involved in the maintenance of Mu lysogeny (4). This homodimeric DNA-binding protein exhibits relatively low sequence specificity (30) and introduces a bend on binding (65). It enhances certain site-specific recombination reactions (2, 32, 36, 66) and acts as a transcriptional activator of rRNA and tRNA genes (49, 55). A modulatory role for FIS in Mu development was inferred from the observation that induction of a Muc62 prophage, encoding a thermosensitive repressor, occurs in vivo at lower temperatures in E. coli in the absence of FIS than in its presence (4). As pointed out above, Mu repressor acts at two levels to inhibit the onset of the lytic cycle, and FIS could intervene at either of these.

To further characterize the mechanism of FIS action, we have used lac transcriptional fusions to investigate the effect of the protein on the repression of Mu early transcription in vivo. The contribution of FIS to the inhibition of Mu
transposition by the repressor was also studied by using an in vivo mini-Mu transposition assay. The data presented here indicate that FIS positively modulates repressor activity in the control of Mu transcription and transposition. In addition, since a strong FIS binding site is located within the left end of Mu DNA (Fig. 1 (4)), we have addressed the question of the biological significance of FIS-specific binding to attL in both regulatory steps.

MATERIALS AND METHODS

Growth of phage and bacterial strains. Phage and E. coli strains used in this study are listed in Table 1. λ lysogens were prepared by UV induction of CSH50 lysogens as described elsewhere (45). Bacterial cultures were grown with agitation in Luria-Bertani (LB) medium, 2YT medium (45) (for growth of M13 derivatives), or medium E (70) supplemented with 0.4% glucose, 0.4% Casamino Acids (Difco), and 1 μg of thiamine per ml (for β-galactosidase assays). For plating, L agar or MacConkey agar base (Difco) supplemented with 1% lactose was used (45). Antibiotics (ampicillin, chloramphenicol, kanamycin [KM], nalidixic acid, rifampin, spectinomycin, streptomycin, and tetracycline) were purchased from Sigma. The fis::Cm' allele (kindly provided by H. Zikelloberger and R. Schmitt via C. Koch) was introduced into CSH50 and derivatives by P1 transduction (45).

β-Galactosidase assays. Strains CSH50 and Mi527, monolysogenic for the λ derivative carrying the transcriptional Mu-lac fusion to be analyzed, were grown overnight at 30°C in supplemented medium E with 12.5 μg of KM per ml. Cultures were then diluted 100-fold into fresh medium and shifted to 30, 35, 37, 38, 39, 40, or 42°C. The cells were grown at the desired temperature of induction for 90 to 120 min, until an AΔmax of 0.3 to 0.35 was reached, and β-galactosidase specific activities were monitored by the method of Miller (45) with some modifications (53). Measurement of β-galactosidase activities as a function of time after the temperature shift showed that new steady-state levels of synthesis were reached for all the cultures analyzed under these conditions (data not shown).

Mating-out assays. Plasmid pOX38Km (7) was transferred into CSH50 by conjugation prior to transformation with pMi111 or pMi112. Before each experiment, the corresponding isogenic fis::Cm' strain was reconstructed by P1 transduction and mini-Mu donor plasmids were introduced into both male strains by transformation. Independent transformants were picked and grown overnight in LB medium plus 25 μg of KM, 50 μg of ampicillin, 12.5 μg of tetracycline, 20 μg of streptomycin, and 40 μg of spectinomycin per ml. Cultures were diluted into fresh LB medium without antibiotics and grown with agitation at 37°C to an AΔmax of 0.3 to 0.5. After 1 h at 37°C without agitation, each donor strain was crossed with strain XA103 for 1 h at 37°C, with a ratio of one donor for three recipient cells. Total exconjugants were plated on L agar plus 25 μg of KM, 20 μg of nalidixic acid, and 50 μg of rifampin per ml, and mini-Mu transposition onto pOX38Km was monitored on L agar plates containing 20 μg of streptomycin, 40 μg of spectinomycin, 20 μg of nalidixic acid, and 50 μg of rifampin per ml. Transposition frequencies were calculated as ratios of Nal' Rif' Sp' Sm' to Nal' Rif' Km' exconjugants.

DNA techniques. Large-scale preparation and minipreparation of plasmid DNA and of single-stranded or replicative forms of M13 DNA were performed as described elsewhere (56). T4 DNA ligase, E. coli DNA polymerase Klenow fragment, T7 DNA polymerase, bacterial alkaline phos-
phatase, T4 polynucleotide kinase, and all restriction enzymes were purchased from New England Biolabs or Applied Science and gene as recommended. Amersham’s DNA blotting kit was used to eliminate 3’ protruding ends of restriction fragments. [α-32P]dATP (3,000 Ci/mmol) and [32P]dATPαS (600 Ci/mmol) were purchased from Amersham, [γ-32P]ATP (7,000 Ci/mmol) was purchased from ICN, and nonlabelled nucleotides were purchased from Boehringer Mannheim. Oligonucleotides were synthesized with an Applied Biosystems 381A DNA synthesizer. Polymerase chain reaction amplification of DNA fragments was performed with a Techne PHC2 thermal cycler by using Tub DNA polymerase (Amersham) as described by Zerbib et al. (72). DNA fragments were purified from agarose gels by using the Gene Clean kit (Bio 101) or from low-melting-point agarose gels as described elsewhere (56). For cloning purposes, M13mi1 was constructed by inserting an NsiI restriction site by linker tailing (41) into the HindIII site of M13mp10 by using a single self-annealing oligonucleotide 5’AGCTATGCACTG3’ (by convention, restriction sites are underlined and specific motifs are shown in boldface). Site-directed mutagenesis of fragments cloned into M13mp10 or M13mi1 was performed on uracilated templates as described elsewhere (40) by using T7 DNA polymerase for the elongation step. The sequences of mutagenized fragments were systematically confirmed prior to subcloning by using the Sequenase kit (U.S. Biochemical).

Construction of the λ phages carrying the Mu-lac transcriptional fusions. Figure 3 shows the maps of the different phages used in this study.

(i) Insertion of the BamHI and ClaI sites between c open reading frame and attL. Plasmid pMi83 was constructed by inserting the HindIII fragment of pJ304 carrying Mu attL and the c open reading frame (71) into the unique HindIII site of pACYC177 (8), in which BamHI, Clal, and NsiI sites had been deleted by successive restriction, blunt-ending, and self-ligation steps. In parallel, the 547-bp EcoRI-NsiI fragment of pJV304 carrying attL was cloned into M13mi1 to give M13mi2. Site-directed mutagenesis was performed with oligonucleotide S’GGTGACAGCTTGAGATCCATGGAATTT (hybridizing with positions 212 to 245 of the Mu left end) to introduce the BamHI and Clal sites into M13mi2. The EcoRI-NsiI fragment of the resulting mutant phage, M13mi3, was subcloned between the EcoRI and NsiI sites of pMi83 to give pMi85.

(ii) Insertion of the p-independent transcription terminator. The unique HindIII site of plasmid pBB857 (54) was destroyed by restriction, Klenow treatment, and self-ligation to give pMi84, and the resulting BamHI-ClaI fragment carrying phage T4 gene 32 transcription terminator was inserted between the BamHI and Clal sites of pMi85 to give pMi86-1.

(iii) Mutagenesis of the FIS binding site on attL. Oligonucleotide S’GATTAAGCAGTAAAGATATCATACAGC C3’ (which hybridizes with positions 185 to 213 of the Mu left end) was used to introduce the FIS EcoRV mutation into M13mi3. The EcoRI-ClaI fragment of the resulting mutant phage, M13mi4, was subcloned between the EcoRI and Clal sites of pMi85 to give pMi91.

(iv) λMi4, λMi2, and λMi5. The HindIII fragments of pMi85, pMi86-1, and pMi91, which carry the mutated Mu left ends, were substituted for the corresponding fragment of pJV304 to give pMi90, pMi88, and pMi92, respectively. The λMi4, λMi2, and λMi5 phages carrying the corresponding Mu-lac fusions were obtained by growing AR545 on a lawn of CSH50 transformed with pMi90, pMi88, or pMi92, respectively, and selecting for Km primary lysogens on L agar plus 25 µg of KM per ml as described elsewhere (59). For each construction, a phage lysate prepared from one primary

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TABLE 1. Phage and bacterial strains

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<td>AR545</td>
<td>λ imm24 derivative carrying a Km' marker and a promoterless lac operon</td>
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</tr>
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<td>λMi4 with T4 gene 32 transcription terminator inserted between the BamHI and EcoRI sites</td>
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<td>λMi4 with FISEcorV mutation on Mu attL</td>
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E. coli

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<td>HfrK1 16P045 [ysi4(t1-62)] dut-1 ung-1 thi-1 relA1 zbd-279::Tnl0 recA1 supE44 (used to uracilate M13 DNA in vivo)</td>
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* BRL, Bethesda Research Laboratories.
lysogen was used to infect a lawn of CSH50 and purify each λ derivative from a single plaque as described elsewhere (56). CSH50 and Mi527 were lysogenized with the purified lysates.

Plasmid constructions. Figure 8 shows the maps of the plasmids carrying the mini-Mu’s and of the compatible plasmids providing the Mu transposition functions.

(i) IAS− mini-Mu (pMi55). pMm4 (kindly provided by C. Koch and R. Kahmann) carries the Tag 1 fragment of pTM2 (containing attL) (34) inserted into the ClaI site of pM1034 (33). A unique HindIII site was substituted for the ClaI site of pMm4 by first ligating the BamHI Ω-Cm’ fragment of pHP450-Cm (17) to ClaI-digested pMm4 (after Klenow treatment of both insert and vector fragments) and then deleting the Cm’ marker by HindIII digestion and religation. Residual host sequences adjacent to the Mu left end on the resulting plasmid, pMm44, were removed by subcloning the 772-bp HaeIII-HindIII fragment (carrying attL) between the EcoRI (treated with Klenow) and HindIII sites of pMm44 to give pMi45. The HindIII Ω-Sp/Sm’ fragment of pHP45 (17) was then inserted into the HindIII site of pMi45 to give pMi55; in this plasmid, transcription of the Sp/Sm’ gene is directed towards the Mu right end.

(ii) IAS− mini-Mu (pMi58). pMm44 was digested by EcoRI, treated with Klenow, digested with HindIII, and ligated to the 1,014-bp HaeIII-HindIII fragment of pTM2, carrying the Mu left end. The HindIII Ω-Sp/Sm’ fragment was then inserted into the HindIII site of the resulting plasmid, pMi46-1, to give pMi58. In this plasmid, the orientation of the Sp/Sm’ gene was as in pMi55.

(iii) IAS++ mini-Mu (pMi117). The 176-bp EcoRI-Mul fragment of plasmid pHK9 (39) carrying partner operator sites O2 and O3 was inserted between the HindIII and MluI sites of pMi46-1 to reconstruct the whole Mu operator region (prior to MluI digestions, the EcoRI and HindIII ends were made blunt by Klenow treatment). In the resulting plasmid, pMi115, the represor gene was transcribed from its own transcription signals. To inactivate the Pc promoter, the small HindIII-Cacl fragment of pMi115 was inserted between the HindIII and SacI sites of M13mp10 to give M13mp7, and the -35 box of Pc was mutagenized by using oligonucleotide 5’-CTTAGGAGGGCCGGGTCTGTTCACAC G3’ (which replaces positions 1082 to 1111 of the Mu left end). The HindIII-SacI fragment of the mutant phage, M13mi8, was reintroduced between the HindIII and SacI sites of pMi115 to give pMi116. Inactivation of the Pc promoter was confirmed by the inability of pMi116 to repress transcription of the Mu-lac fusion present in a λJVS4 lysogen, while transcription was efficiently repressed in the presence of pMi115. The HindIII Ω-Sp/Sm’ fragment of pH45 treated with Klenow was then inserted in the SacI site of pMi116 (made blunt) to give pMi117. In this plasmid, the Sp/Sm’ marker is oriented as in pMi55 and pMi58.

(iv) FISCecoRV mutant mini-Mu’s. The EcoRI-NsiI fragment of pMi45 carrying Mu attL was inserted between the EcoRI and NsiI sites of M13mi to give M13mi5. Site-directed mutagenesis of M13mi5 was performed with oligonucleotide 5’-GATTACCGACTAAGATCATACAGG C3’ (hybridizing with positions 185 to 213 of the Mu left end) to give M13mi6. The EcoRI-NsiI fragment of M13mi6 was subcloned between the EcoRI and NsiI sites of pMi55, pMi58, or pMi117 to give pMi95, pMi96, and pMi119, respectively.

(v) Complementing plasmids pMi111 and pMi112. The construction was performed in three steps.

(a) Step 1: cloning the Mu A and B genes under the control of P_{Mu, LYSOGENY}. A 163-bp fragment carrying the P_{Mu, LYSOGENY} promoter and lac operator sites O1 and O3 was amplified by polymerase chain reaction from plasmid pBR793lacL8UV5 (carrying 789 bp of the lacL8UV5 promoter-operator region) (57) by using primers 5’-CCAGATCCGAGGAAAGCCGC AGTACGCCG3’ (phosphorylated at its 5’ end) and 5’-CCGAGA TTCGCCATATCGGTCTTCTGTCG3’.

In this fragment, the P_{Mu} promoter is flanked by BglII and XhoI sites upstream and EcoRV and EcoRI sites downstream. After EcoRI digestion, the amplified fragment was ligated between the NruI and EcoRI sites of pBR322 (5) to give pMi100. The EcoRV-EcoRI fragment of pLP105-6-3 carrying the Mu A and B genes (68) was then inserted between the EcoRV and EcoRI sites of pMi100 to give pMi106.

(b) Step 2: cloning the Mu c+ gene under the control of P_{Km}. A three-fragment ligation reaction was performed with the Klenow-treated HindIII-NsiI fragment of pJV300 (71) carrying the 5’ end of the c+ gene, the NsiI-ClaI fragment of pMi86-1 carrying the 3’ end of the c gene and T4 gene 32 transcription terminator, and vector pACYC177, digested with XhoI, treated with Klenow, and further digested with ClaI. In the resulting plasmid, pMi93, transcription of the c+ gene is driven from the pACYC177 P_km promoter. Plasmid pMi97, which does not carry the c+ gene, was constructed by inserting the BamHI-ClaI fragment of pMi84, containing the transcription terminator and made blunt at the BamHI site by Klenow treatment, into pACYC177 cut by XhoI, treated with Klenow, and cut again by ClaI.

(c) Step 3: assembly of pMi111 and pMi112. The HindIII-EcoRI fragment of pMJR1560 (60) carrying the lacF gene was treated with Klenow at the HindIII site and inserted between the EcoRI and SacI sites of pACYC184 (8) to give pMi98. The ClaI-XmnI fragment of pMi93 or pMi97, carrying the c gene under the P_{Km} or the P_{K} promoter alone, respectively, was treated with Klenow and ligated to pMi98 digested with AvaI (and treated with Klenow and bacterial alkaline phosphatase prior to the ligation step). In the respective resulting plasmids, pMi101 and pMi102, transcription initiated at P_{Km} is in the same direction as that of the lacI gene. Plasmid pMi106 was then digested with BglII, treated with Klenow, and digested again with EcoRI. The fragment carrying the Mu A and B genes under the control of P_{kpm, LYSOGENY} was inserted between the EcoRI and XmnI sites of pMi101 or pMi102 to give pMi111 and pMi112, respectively.

Southern blot analysis of chromosomal DNA. Total E. coli chromosomal DNA was prepared from overnight cultures grown in LB medium as described by Fayet et al. (16) and digested with EcoRI (for analysis of the Mu lysogens) or XhoI (for analysis of the λ lysogens). Restriction fragments were separated on 0.8% agarose gels and transferred onto nylon Biodyne B membranes (Pall) under alkaline conditions as described elsewhere (56). Purified probe Mu or λ DNA was labelled with [α-32P]dATP by using the Multiprime labelling kit (Amersham). Prehybridization, hybridization, and washing conditions of the membranes were essentially as described elsewhere (56). Hybridizing fragments were revealed by autoradiography of the membranes on Hyperfilm-MP films (Amersham). In particular, λ multisygos were identified by the appearance of an 8-kb XhoI fragment, characteristic for the junction between two tandem repeats of the prophage.

RNA analysis. CH50 derivatives lysogenic for JXV304, ARS45, or KM2 were grown at 38°C to an A600 of 0.3 to 0.35 under the same conditions as those used for β-galactosidase assays, and 6-ml samples were removed for RNA extraction. Strain CH50 carrying plasmids pOX38Km and pMi112...
grown as for the mating-out assays, and 4-ml samples were removed. Total RNA was prepared by a rapid-extraction procedure (61). For each sample, approximately 5 µg of RNA was denatured for 10 min at 70°C in the presence of 1 M formamide and 50% formamide, electrophoresed in 1.5% agarose gels containing 1 M formamide, and transferred passively onto nylon Hybond N+ membranes (Amersham) as described elsewhere (56). Total amounts of RNA loaded on the gel were estimated by ethidium bromide staining followed by densitometer scanning. The 772-bp HindIII-BamHI fragment of pMi111 carrying the Mu c gene was labelled with [α-32P]dATP by using the Megaprime labelling kit (Amersham) and was used as a probe for c mRNA. To specifically identify c mRNA, oligonucleotide 5’CGCGTTAAATCAGTAATCAAAGG3’ (positions 953 to 974 on the Mu left end) was labelled at its 5’ end by using [γ-32P]ATP and T4 polynucleotide kinase. Prehybridization, hybridization, and washing of the membranes were performed as described elsewhere (56). Hybridizing species were identified by autoradiography on Hyperfilm-MP films (Amersham).

Detection of c protein by Western blotting (immunoblotting). CSH50 lysogenic for λVJ304, λMi2, or λRS45 and Mi527 lysogenic for λVJ304 were grown at 38°C as for the β-galactosidase assays. At an A600 of 0.3 to 0.35, 1.7 ml of culture was centrifuged, and the bacterial pellet was resuspended in 100 µl of lysis buffer (40 mM diethytohthiol, 400 mM Tris, 10% sodium dodecyl sulfate [SDS], 5% glycerol, and 0.04% bromophenol blue). CSH50 and Mi527 carrying plasmid pOX38Kn together with pMi112 or pMi111 was grown at 57°C as for the mating-out assays. For each strain, the bacterial pellet obtained after centrifugation of 1 ml of culture (A600 = 0.5) was resuspended in 100 µl of lysis buffer. Five microliters of each sample was denatured at 100°C for 5 min, electrophoresed in Tris-glycine–SDS–12% polyacrylamide gels (25), and transferred onto Hybond C Super membranes (Amersham) by using a semidy electrophoretic transfer apparatus (Touzard & Matignon) as described elsewhere (25). The membranes were blocked for 1 h at room temperature in Tris-buffered saline (TBS) buffer (10 mM Tris-HCl [pH 8], 150 mM NaCl) containing 1% bovine serum albumin and 0.2% Tween and were incubated overnight at 4°C with a 1:50,000 dilution of anti-c antibodies (kindly provided by P. Higgins. The membranes were washed in TBS-0.2% Tween buffer and then incubated with a 1:10,000 dilution of rabbit immunoglobulin G horseradish peroxidase-linked whole antibody (Promega) for 30 min at room temperature. After the membranes were washed, detection of the reacting bands was performed with a chemiluminescence assay by using the ECL detection reagents (Amersham) and following the supplier’s recommendations. In the range of protein concentrations used in this study, the ECL signal appeared proportional to the repressor amount loaded on the gel.

Gel retardation of DNA fragments in the presence of FIS. Plasmids pVL3 (4) and pHKO9 (39) were digested with EcoRI or EcoRI-BamHI, respectively, and labelled with [32P]dATP by using the Klenow fragment. To confirm the effect of the FIS EcoRV mutation, 277-bp fragments from M13mi3 or M13mi4 were polymerase chain reaction–labelled with [α-32P]dATP as described elsewhere (72) by using oligonucleotides 5’GTCAGGCGCTGAACATTGC3’ and 5’GCTGTTATTTGAATGTGATCCAGTG3’ as primers. Incubation of labelled DNA with purified FIS and gel electrophoresis of DNA–protein complexes were performed essentially as described previously (4).

RESULTS

Mucts62 induction in a fis mutant is facilitated by the presence of only one prophage. Cultures of a Mucts62 fis lysogen, MGC937, undergo substantial lysis after a temperature shift from 30 to 38 or 39°C, whereas growth of the parental fis+ lysogen, MGC936, is unaffected (4). The same preferential killing of the fis lysogen was observed on solid medium at 40°C. We used this property to screen for suppressors of the fis phenotype among survivors of a temperature shift. Cultures of MGC937, grown at 30°C in LB medium, were diluted and plated on L agar. After overnight incubation at 30°C, replicas made from each plate were incubated for an additional night at 30, 40, and 42°C. Like the wild-type control, MGC937 developed colonies at 30 but not 42°C. At 40°C, however, while the survival rate for the wild-type strain was 100%, only 1% survival was observed for the fis lysogen. The fis survivors selected in this way, like the wild-type lysogen, grew in liquid medium at 38.5°C but lysed at 42°C (data not shown).

The frequency of fis survivors at 40°C appeared too high to be explained simply by spontaneous mutation. It seemed possible that survival was linked to an increase in prophage copy number which can occur following partial induction (14). To determine whether this was the case, we used 32P-labelled Mu DNA to probe Southern blots of EcoRI-
λ derivatives carrying the Mu-lac fusions:

\[
\begin{align*}
\lambda JV304 & : FIS - cts62 - Pe - Pc - lacZYA \\ 
\lambda Mi4 & : C - Ba - terT4 - lacZYA \\ 
\lambda Mi2 & : RV - C - Ba - lacZYA \\ 
\lambda Mi5 & : RV - C - Ba - lacZYA
\end{align*}
\]

β-galactosidase activities (Miller units):

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FIG. 3. Activity of λ-Mu-lac fusion derivatives in wild-type and fis strains. The features of the different Mu-lac fusions inserted into the λ phage vector are shown on the left. Transcription of the Mu cts62 gene occurs to the left (arrow) from the repressor promoter, Pe. Transcription of the lac operon (lacZYA) is driven by the Mu early promoter, Pe. Relevant restriction sites: RI, EcoRI; N, NsiI; H, HindIII; C, Clal; Ba, BamHI; RV, EcoRV. The wild-type FIS binding site and its mutant derivative in the left end of Mu (attL) are represented by filled and open triangles, respectively. The phage T4 gene 32 transcription terminator (terT4) is shown as a stem-loop structure. The specific activities of β-galactosidase obtained in wild-type (wt) (CSH50) and fis::Cmr (fis) (Mi527) monolysogens for each of the derivatives at various temperatures of induction (see Materials and Methods) are shown on the right. These results are the averages of at least three independent measurements. The ratios of activities in the fis mutant to those in the wild-type strain (fis/wt) are also included. nd, not determined.

digested chromosomal DNA from independently isolated survivors. Figure 2B (lane 12) shows that DNA from the monolysogen MGC937 exhibits two Mu-specific bands representing the left junction fragment of about 5 kb and a doublet which migrates here at approximately 18 kb and which contains the central Mu EcoRI fragment and the right junction with host DNA (Fig. 2A). The presence of additional bands of various sizes in all 10 independent revertants analyzed (Fig. 2B, lanes 1 to 10) indicated insertion of new copies of the Mucst62 prophage at different loci (up to three in lane 5). Furthermore, introducing additional copies of the prophage into MGC937, by transfer of an F’ plasmid with an integrated Mucst62 pAp1 (Table 1), also resulted in a higher survival rate after a shift to 38°C but in normal lysis at 42°C (data not shown). Thus, increasing the copy number of a Mucst62 prophage improves repression of lytic growth in the absence of FIS.

Effect of the fis mutation on the repression of Mu early transcription. The influence of prophage copy number on the effect of the fis mutation suggests that FIS may intervene if the level of active repressor in the cell reaches a critical lower threshold. One of the potential targets for FIS action in the maintenance of lysogeny could be the regulation of early transcription. This was tested with lysogens of phage λJV304 (Fig. 3) (71), which carries the leftmost 1,118 bp of Mucst62 DNA (fused to a promoterless lac operon) together with a Km' gene. Transcription of the lacZ gene is driven from the Mu early promoter, Pe, and is repressed by the thermosensitive cts62 protein. A striking difference was noticed on MacConkey lactose plates between primary Km' lysogens of isogenic wild-type (CSH50) and fis (Mi527) strains (Fig. 4A). At 30°C, colonies of CSH50 derivatives were white, indicative of repression of Pe transcription by cts62, while those of Mi527 lysogens appeared red, suggesting that Mu early transcription was not fully repressed in the absence of FIS. At 42°C, however, both strains exhibited the same red phenotype.

Variations in the lac phenotype observed during the construction of the fis λJV304 lysogens provided further support for the idea that an increase in cts62 gene dosage can partially overcome the need for FIS in the maintenance of repression of Mu early transcription. Most (80 to 90%) of the Km' survivors of λJV304 infection were red when grown at 30°C, while the others exhibited the white (wild-type) phenotype. Furthermore, in the rec' genetic background used in this experiment, the white lysogens were unstable and gave rise to red segregants when subcloned. Southern analysis (see Materials and Methods) demonstrated that this behavior was correlated with the number of λJV304 prophages present. The lighter unstable clones were polylysogens containing tandem repeats of the prophage, whereas the red colonies were monolysogens (data not shown). This is similar to the effect observed in the case of partial induction of Mucst62 described above.

To quantify the participation of FIS in repression, we assayed the β-galactosidase activities of wild-type and fis λJV304 monolysogens in exponentially growing liquid cultures following a shift from 30°C to different temperatures (see Materials and Methods). For both strains, increasing the temperature of the shift resulted in an increase in β-galacto-
FIG. 4. Effect of the fis mutation on the repression of Mu early transcription. (A) MacConkey lactose indicator plates showing the response of λJ304 to growth at 30 and 42°C in the fis mutant and its wild-type (wt) parent. (B) Graphic representation of the ratio of β-galactosidase levels of the various derivative phages in the fis mutant and its wild-type parent as a function of temperature (from the data in Fig. 3).
sidase activity (Fig. 3), although the induction pattern of the 

**FIG. 5.** Analysis of repressor production in the experimental systems used in this study. Western blot detection of c protein produced from the chromosomal Mu-lac fusions (lanes 1 to 4) or from multicopy plasmid pMi111 (lanes 5 to 8). Experimental conditions for cell growth, electrophoresis of protein samples, and immunoblotting are described in Materials and Methods. Lane 1, CSH50 lysogenic for λM12; lane 2, CSH50 lysogenic for λJV304; lane 3, Mi527 lysogenic for λJV304; lane 4, CSH50 lysogenic for λRS45. Lanes 5 to 8, proteins produced from control plasmid pMi112 (lanes 5 and 6) or from pMi111 (lanes 7 and 8) in strain CSH50 (lanes 5 and 7) or Mi527 (lanes 6 and 8).

This was tested by Western blot analysis. Within the limits of detection, repressor levels were not found to differ significantly (Fig. 5, lanes 2 and 3). These observations therefore suggest that FIS contributes to the action of the repressor in the control of Mu early transcription when c is partially inactivated.

**Search for FIS binding sites on Mu DNA involved in the control of transcription.** The effect on c-mediated repression of transcription from Pe may involve FIS sequence-specific binding to the Mu early operator region present in λJV304. However, gel retardation assays using a 291-bp fragment carrying Pe together with the three operator sites failed to demonstrate this. No specific complex was observed at low FIS concentrations (Fig. 6A, lanes 6 to 8), while a DNA fragment carrying the specific FIS binding site on Mu attL (4) gave rise to a specific retarded band resistant to challenge by nonspecific competitor DNA (Fig. 6A, lanes 1 to 3). Note in both cases that high FIS concentrations generate additional nonspecific complexes (Fig. 6A, lanes 4 and 5 and 9 and 10) (see also reference 4).

In the absence of detectable specific FIS binding in the Mu operator region, we investigated the potential role of the FIS site, previously identified on attL (4) and also present in λJV304 (Fig. 3), on early transcription. One possible way to do this is by deleting the attL region (i.e., the 221 leftmost bp of Mu DNA). However, because attL probably carries the natural transcription termination signals for the repressor gene (9), such a deletion would alter the 3' end of c mRNA and may affect its stability. As suggested by the above experiments, possible associated changes in repressor levels could interfere with the effect of the fis mutation. To ensure that the mRNA would be identical in the presence and

**FIG. 6.** FIS binding at attL and at the operator region. (A) Analysis by gel retardation of the binding of FIS to a 285-bp fragment carrying attL (lanes 1 to 5) and to a 291-bp operator fragment (lanes 6 to 10) radioactively labelled with [35S]dATP by using the Klenow fragment of DNA polymerase I. Seventy nanograms of digested plasmid DNA was used in each binding reaction. The quantity of purified FIS used in each binding reaction was 0 (lanes 1 and 6), 5 (lanes 2 and 7), 10 (lanes 3 and 8), 20 (lanes 4 and 9), or 50 (lanes 5 and 10) ng. (B) Effect of the FISEcoRV mutation on the binding of FIS to a 277-bp fragment carrying attL with (lanes 5 to 8) or without (lanes 1 to 4) the FISEcoRV mutation. In each case, the fragment was polymerase chain reaction-labelled with [a-32P]dATP. Four thousand counts per minute was used in each reaction mixture. The quantity of FIS used was 0 (lanes 1 and 5), 5 (lanes 2 and 6), 10 (lanes 3 and 7), or 20 (lanes 4 and 8) ng. In all cases, binding reactions were performed in the presence of 700 ng of sonicated calf thymus DNA and electrophoresis was on 5% acrylamide gels. wt, wild type.
the need for FIS to maintain repression, even at nonpermis-

sive temperatures.

Since the behavior of λMi2 precluded its use in studying
the effect of deleting the FIS site, we chose to inactivate this
site without modifying the surrounding sequences. An
EcoRV recognition sequence was introduced into attL of
λMi4 (see Materials and Methods) by substituting three of
the four residues matching the left half of the consensus FIS
binding sequence (Fig. 1B) (30). Gel retardation assays
confirmed that this mutation abolished FIS-specific binding
in vitro to a linear DNA fragment carrying attL (Fig. 6B).
However, transcription of the corresponding Mu-lac fusion
introduced into prophage λMi2 was still preferentially
induced in a fts monoslyogen to the same extent as that
observed with the λMi4 and λJV304 derivatives (Fig. 3
and 4B). This strongly suggests that the contribution of FIS
to the maintenance of repression of Mu early transcription is
not transmitted through a specific interaction with its site
located in attL. Although we cannot exclude that FIS
binding to the Mu early regulatory region occurs in vivo or
under conditions different from those used in our in vitro gel
retardation experiments, we speculate that a more general
cellular function of FIS could be responsible for this phe-
nomenon.

Direct inhibition of the transposition of mini-Mu constructs
by the phage-encoded repressor. It has previously been
shown that the efficiency of in vitro transposition of a mini-Mu
 carrying an incomplete IAS extending to the
HindIII site (IAS<sup> HindIII </sup>mini-Mu) (Fig. 1A) was signifi-
cantly reduced in the presence of purified c (12). Thus, in addition
to repression of early transcription, an inhibitory action of c
on Mu transposition may also contribute to maintaining
lysozyme. FIS could be involved in this second regulatory
step. An in vivo mini-Mu transposition assay was designed
to test this hypothesis.

As a first step in our analysis, the transposition of mini-Mu
derivatives carrying various extents of the IAS was moni-
tored in the presence or absence of repressor by using the
conjugative plasmid pOX38Km as a transposition target in a
standard mating-out assay. Three pBR322-related mini-Mu
donor plasmids were constructed (see Materials and
Methods). These were composed of a fragment of variable length
from the left end of and the rightmost 1,024 bp of the Mu
genome (attR), flanking an Sp<sup> Sm </sup>m marker (Fig. 8A). The
IAS<sup>−</sup> (pMi55), IAS<sup>HindIII</sup> (pMi58), and IAS<sup>−</sup><sup>+</sup>
(pMi117) derivatives carried bp 1 to 739, 1 to 1006, and 1 to 1118 of
the left end, respectively. In addition, since pMi117 carries the c
gene together with its promoter, a mutation in the −35 box of
the Pc promoter was introduced to render the plasmid c
deficient (see Materials and Methods). Compatible comple-
menting plasmids provided the transposition functions pA
and pB in trans with (pMi111) or without (pMi112) the c<sup>+</sup>
repressor (Fig. 8B). As the regulation of Mu transposition from
Pe is affected by the fts mutation (see above), the A and
B genes were placed under control of the IPTG (isoprop-
ynyl-β-d-thiogalactopyranoside)-inducible P<sub>lac</sub>UV5
promoter. The c gene was also placed under control of hetero-
lolgous transcription signals: the weak constitutive P<sub>Km</sub>
promoter upstream and the independent T4 gene 32 tran-
scription terminator downstream (see Materials and
Methods). Preliminary experiments revealed that, in the presence
of pMi112, the IAS<sup>HindIII</sup> mini-Mu transposed at a frequency
of 10<sup>−2</sup> to 10<sup>−3</sup> without induction, presumably because of
complete repression of the P<sub>lac</sub>UV5 promoter. All subse-
quently mating-out assays were therefore performed without
IPTG.

Absence of attL, a BamHI-ClaI fragment carrying the phage
T4 gene 32 ρ-dependent transcription terminator (54)
was inserted into λJV304 just downstream of the c open reading
frame (i.e., upstream of attL) between suitable restriction
sites introduced by site-directed mutagenesis (see Materials
and Methods). Unexpectedly, the resulting phage, AMi2,
 exhibed much lower β-galactosidase activities in both
 genetic backgrounds and at all temperatures, while the
 intermediate phage containing only the BamHI and ClaI
 sites (λMi4) behaved like parental λJV304 (Fig. 3).
Furthermore, although the activities in the fts background
were significantly lower than in the wild-type strain, no preferen-
tial induction of Pe transcription occurred in a AMi2 fts
monoslyogen (Fig. 4B). This could result from stabilization
of cts62 mRNA by the hairpin structure introduced by the
 ρ-independent terminator at its 3′ end (48), leading to an
 increase in repressor synthesis. Northern (RNA) blot anal-
ysis confirmed this hypothesis and suggested that the c
 message was significantly more stable and at least 10-fold
 more abundant when produced from λMi2 (Fig. 7, lanes 5 to
 8) than from its parent λJV304 (Fig. 7, lanes 2 to 4).
In this experiment, the double-stranded DNA probe used for detec-
tion of the c transcript hybridized to three species (Fig. 7,
lanes 2 to 8 and 11). The two high-molecular-weight bands
were also detected in total RNA extracted from CSH50
lyogenic for the parental phage ARS45 (Fig. 7, lane 10) and
are therefore not Mu specific. Identification of the third,
 lowest-molecular-weight, species as c mRNA was confirmed
with an oligonucleotide probe specific for the c transcript
(Fig. 7, lane 1). The production of c<sub>ts62</sub> protein by the two
derivatives was assayed by Western blotting using anti-c
 antibodies. This experiment confirmed that c<sub>ts62</sub> was present
in great abundance in AMi2 relative to λJV304 lysogens (Fig. 5,
lanes 1 and 2). The properties of AMi2 provide further
evidence that an increase in the level of c<sub>ts62</sub> can suppress

FIG. 7. Analysis of repressor transcripts synthesised in the
λ-Mu-lac fusions. Northern blot analysis of RNAs produced by
λJV304, AMi2 (carrying the T4 gene 32 transcription terminator),
and ARS45 (parental phage with no Mu insert) lysogens of CSH50
is shown. Cultures were grown as for the β-galactosidase assays
performed at 38°C. At an A<sub>600</sub> of 0.3 to 0.35, rifampin was added to
a final concentration of 200 μg/ml. For total RNA extraction,
samples were taken after addition of rifampin at 0, 2, and 4 min
for λJV304 (lanes 2 to 4, respectively), at 0 (lanes 1, 5, and 11),
4 (lane 6), 15 (lane 7), and 30 min (lane 8) for λMi2, and at 0 min
for ARS45 (lane 10). Lane 9, an RNA sample extracted from strain CSH50
 carrying pOX38Km and pMi111 and grown as described in Materials
and Methods. A single-stranded oligonucleotide (lane 1) or the
high-specific-activity double-stranded pMi111 fragment (lanes 2 to
11) was used to probe for the c transcript (see text for details).
In the wild-type host, CSH50, and in the absence of repressor, transposition frequency increased with the size of the IAS fragment present in the mini-Mu (Table 2). The IAS\textsuperscript{His\textsubscript{III}} derivative (pMi58) exhibited a 2-fold increase compared with its IAS\textsuperscript{-} parent (pMi55), and an additional 11-fold increase was observed with the IAS\textsuperscript{++} mini-Mu (pMi58). This result confirmed the observations of Leung et al. (43). In the presence of c, transposition of all derivatives was reduced to similar low background levels: inhibition factors (i.e., the ratios of transposition frequencies in the absence of repressor to those in the presence of repressor) were found to be 4 for the IAS\textsuperscript{-}, 25 for the IAS\textsuperscript{His\textsubscript{III}}, and 130 for the IAS\textsuperscript{++} mini-Mu's, respectively (Table 2). This confirms and extends results demonstrating direct inhibition of transposition of an IAS\textsuperscript{His\textsubscript{III}} mini-Mu by repressor in vivo (67).

Modulatory effects of FIS on the control of mini-Mu transposition. To study the potential involvement of FIS in the observed inhibition of Mu transposition by c, similar mating-out assays were performed in a fis background (Mi527) with the same mini-Mu plasmids. Fluctuations were consistently obtained in the transposition frequencies measured in this strain. Since this could be due to an accumulation of compensatory mutations, the fis::Cm\textsuperscript{r} allele was always

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**Figure 8.** Structure of the mini-Mu phages and of plasmids used to supply transposition functions. Only relevant restriction sites are shown: A, AvaiI; B, BgII; Ba, BamHI; C, CiaI; H, HindIII; M, MluI; N, NsiI; RI, EcoRI; RV, EcoRV; S, SacI; Sc, ScaI; T, TaqI; Xh, XhoI; X, XmnI. Destruction of a restriction site in the construction is indicated by an asterisk. (A) The mini-Mu derivatives are inserted between the EcoRI and BamHI sites of pBR322. They carry identical 1,024-bp fragments of the right end of Mu (attR) together with a 2.1-kb cassette carrying a streptomycin (Sm) and spectinomycin (Sp) resistance gene, whose direction of transcription is indicated by the arrow. The derivatives differ only in the extent of the left Mu end (attL) included. The operator-IAS region is represented by a filled box. Plasmid designations for derivatives carrying a wild-type (wt) or mutated FIS site within attL are listed on the right. (B) Plasmids used to supply transposition functions. The A, B, and c\textsuperscript{+} genes of Mu and their directions of transcription are indicated. Transcription of the A and B genes is driven by promoter P\textsubscript{lac} (filled arrow), whose activity is modulated by the presence of a lacI\textsuperscript{g} gene driven by its own promoter, while that of the c\textsuperscript{+} gene is driven by a constitutive promoter, P\textsubscript{Km}, from the Km\textsuperscript{r} gene of pACYC177 (open arrow). The tetracycline resistance gene (Tc) and phage T4 gene 32 transcription terminator (stem-loop structure) are indicated. The vector plasmid backbone is a derivative of pACYC184.

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**A**

![Diagram](http://jb.asm.org/)
TABLE 2. Inhibition by c of mini-Mu transposition in wild-type and fis strains

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<th>c&lt;sup&gt;c&lt;/sup&gt;</th>
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<th>f&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Inhibition factor</th>
<th>Transposition frequency</th>
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<td>(4.7 ± 2.6) x 10^-4</td>
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<td>(3.0 ± 0.7) x 10^-4</td>
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<td>13</td>
<td>(1.8 ± 0.5) x 10^-4</td>
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<td>pMi119</td>
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<sup>a</sup> Mating-out assays were performed as described in Materials and Methods by using the CSH50 (wild-type host) or Mi527 (fas::Cm<sup>R</sup>) mutant genetic backgrounds for the donor strain.

<sup>b</sup> Plasmids pMi55, pMi58, and pMi117 carry the wild-type <i>attL</i> sequence, while pMi95, pMi96, and pMi119 carry the FISEcoRV mutation.

<sup>c</sup> The complementing plasmids providing the transposase were pMi112 (without c [-]) and pMi111 (with c [+]).

<sup>d</sup> The data are averages of at least six independent experiments.

<sup>e</sup> Ratio of the transposition frequency without c to that with c.

reintroduced into the different wild-type donor strains by P1 transduction immediately before the mating-out experiments (see Materials and Methods). In the absence of repressor, no significant effect of the fis mutation was detected for any mini-Mu derivative (pMi55, pMi58, and pMi117 in Table 2). The fis mutation itself thus does not seem to affect transposase activity. However, in the presence of c, the transposition frequencies of all mini-Mu's dropped as already described, but for all derivatives a two- to fourfold stronger inhibition was observed for the wild-type host compared with the fis strain. Control Western blots did not reveal any significant difference in the levels of repressor produced from plasmid pMi111 between the wild-type and the fis strains (Fig. 5, lanes 7 and 8). Thus, FIS appears to enhance the effectiveness of c in inhibiting transposition.

In order to determine whether this effect involves the specific FIS binding site on <i>attL</i>, the behavior of a set of related mini-Mu derivatives carrying the FISEcoRV mutation was analyzed. While this mutation abolishes FIS binding, it does not modify the overlapping PA binding site, L2, in <i>attL</i> (Fig. 1B). In the absence of repressor, all FISEcoRV mini-Mu's transposed at the same frequencies as their wild-type parents in both genetic backgrounds (Table 2 [compare pMi95 with pMi55, pMi96 with pMi58, and pMi119 with pMi117]). Thus, this mutation has no effect on transposase activity. In the presence of repressor, inhibition of transposition was observed for all FISEcoRV derivatives (pMi95, pMi96, and pMi119 in Table 2) in the fis<sup>+</sup> host. Intriguingly, it was two- to threefold stronger than for the parental wild-type mini-Mu's (compare the inhibition factors for pMi95, pMi96, and pMi119 with those for pMi55, pMi58, and pMi117; see also Discussion). Inhibition was, however, still more pronounced (10-fold) in the wild-type than in the fis negative background. Therefore, the FISEcoRV mutation inactivating FIS binding to <i>attL</i> does not abolish, and may even amplify, the enhancement of repressor action by FIS.

**DISCUSSION**

**FIS favors the maintenance of Mu lysogeny.** The experiments presented here were designed to explore the way in which FIS intervenes in the maintenance of Mu lysogeny. The results obtained indicate that this involves a subtle balance of repressor and FIS and operates both at the level of control of early transcription and on transposition itself. Repression of Mu early transcription by the thermosensitive repressor C<sub>ser62</sub> is three- to fourfold weaker in the absence of FIS at partially inducing temperatures (37 to 38°C) in vivo. Inhibition of mini-Mu transposition in the presence of wild-type repressor is reduced two- to fourfold in the absence of FIS. These effects are small compared with the 600- to 800-fold higher yield in viral particles observed after partial induction of a fis strain monolysogenic for Mu(ser62 relative to a wild-type isogenic host (4). The disparity may reflect an amplification during lytic growth of the effects of the fis mutation on early transcription and on transposition or an additional inhibitory action of FIS at other stages of Mu growth. The magnitudes of the effects of the fis mutation on the control of transcription and transposition cannot, however, easily be compared directly. Indeed, as discussed below, there might be differences in the relative levels of repressor and FIS activity from one type of experiment to the other.

**The need for FIS to maintain lysogeny can be compensated by an increase in repressor activity.** Bacteriophage Mu lysogeny is principally ensured by interactions of the c protein with sites on the Mu genome, and its maintenance depends critically on the levels of active repressor in the cell. The thermosensitive C<sub>ser62</sub> protein is less active than the wild-type repressor, even at low temperatures (71). In vitro, the affinity of c<sup>+</sup> for its operator sites at 30°C (<i>K<sub>a</sub></i> = 2.5 x 10<sup>-9</sup> M) is 20-fold higher than that of C<sub>ser62</sub> (5 x 10<sup>-8</sup> M). This difference is reflected in a 1.5-fold-higher repression of early
transcription at 30°C by c+ compared with cتس2 (71) and a 10-fold-higher spontaneous phage yield in cultures of MuCts62 relative to Muc c+ lysogens at this temperature (3a). However, several lines of evidence indicate that the intrinsically lower activity of cتس2 can be enhanced by an increase in the level of the protein. Thus, when the cتس2 gene is expressed from a multicopy plasmid (1, 71) or when a stabilizing stem-loop structure is introduced at the 3' end of cتس2 mRNA (see λM12 in Fig. 3), repression is improved, even at 42°C.

FIS contributes to the maintenance of lysogeny only when repressor activity in the cell is low. Our search for outside suppressors of the fis mutation in MucCts62 monolysogens led to isolation of multilysogens carrying one to three additional copies of the prophage. These probably survived the temperature shift in the absence of FIS because of an increase in c تس2 levels, even though the copy number of the operator region was raised concomitantly. Such gene dosage effects could be due to the cooperative binding of c تس2 to the operator which has been demonstrated in vitro (71). Similarly, the fis phenotype of a JY304 monolysogen, which harbors a MucCts62-lac fusion, is suppressed when the prophage copy number is increased. In addition, the need for FIS to maintain repression is completely abolished with the λM12 fusion which overproduces c تس2 (Fig. 3). Finally, a small effect of the fis mutation has been observed at 42°C on MacConkey lactose plates with a fusion controlled by the wild-type repressor (3a). This is consistent with the observation that c+ operator interactions are less stable at 42°C (Kc = 2.5 × 10^-8 M) than at 30°C (2.5 × 10^-9 M) in vitro (71) and suggests that the fis phenotype does not depend specifically on the c تس2 allele but, rather, is conspicuous when the DNA-binding activity of the repressor is lowered.

**FIS affects c action at the Mu operator and Mu ends.** The data presented here indicate that the fis mutation results in a reduction of Mu repressor activity in vivo. Because two types of c binding sites have been identified, the operator region (high affinity) and the ends (low affinity) (12, 38), FIS could influence c activity at either or both. The results obtained with the Mu-lac transcriptional fusions (Fig. 3) indicate that, when active repressor is limiting, repression of early transcription, which results from binding to the operator sites, is weaker in the absence of FIS. In the mini-Mu transposition assays, c+ is overexpressed from heterologous transcription signals on a multicopy plasmid and binding to the operator region is presumably complete. Under these conditions, it appears that FIS may modulate repressor interactions with the ends since, in the presence of FIS, a two- to fourfold increase in inhibition by c was observed independently of the presence or absence of the IAS-operator region (Table 2).

**Potential modes of action of FIS.** In view of the various effects of FIS on repressor activity and because gel retardation assays did not permit the detection of a specific FIS site in the operator region, it was important to ascertain whether the specific FIS binding site in attL intervenes in any way. This site plays no role in the FIS effect on early transcription, as demonstrated by transcriptional fusions in which it was destroyed (XM15). Nor is it required for FIS-mediated strong inhibition of transposition by c. Indeed, when the inactivating mutation was introduced in the series of mini-Mu transposons, inhibition was still stronger in the wild-type relative to the fis host. Moreover, in the presence of FIS, the inhibition factors for the mutant mini-Mu were two- to threefold higher than those for their wild-type parents, although they were similar in the fis mutant strain. This small but consistent difference could indicate that the mutation in attL directly affects the binding of c or prevents FIS from competing with c for binding to this region. Further experiments are needed to test these possibilities.

The mechanism of FIS-induced stimulation of Mu repressor activity still remains an open question. FIS could control the amounts of repressor present in the cell. However, no significant difference in c levels could be detected by Western blotting between fis and wild-type cells in the various expression systems used in this study. Alternatively, FIS could stimulate repressor binding activity to the operator region and to the ends of Mu DNA. The possibility of a specific in vivo interaction of FIS with Mu early regulatory region, which could not be detected under the experimental conditions used in this study, cannot be completely ruled out at this stage. It should be noted, however, that a similar stimulating role of FIS has been demonstrated in establishment and maintenance of bacteriophage λ lysogeny (3). This does not involve specific binding to any known site on λ DNA. These results suggest that FIS could play a more general regulatory role in the cell, either by changing the topological state of the DNA, which could affect the binding of Mu repressor or other regulatory proteins (17), or by regulating the expression of other DNA-binding host factors, such as IHF or the histone-like protein H-NS, which have both been implicated in the stabilization of Mu repressor-DNA complexes (1a, 15, 20). In this regard, host mutations which stimulate the transposition of a MucCts62 phage have been recently isolated. Significantly, some of these are located in the fis gene (26a) while others map to the hns gene (15). This could indicate some interplay between these auxiliary DNA-binding proteins.

In assessing the biological significance of the regulation of Mu growth by FIS, it should be noted that the intracellular level of FIS varies during the bacterial growth cycle as a result of complex transcriptional regulation (51). Consequently, FIS is specifically accumulated after a nutritional shift-up or during early exponential growth in rich medium (50, 66). FIS has been implicated in cellular processes, such as trans activation of stable RNA operons (49, 50, 55) and chromosome replication (18, 21), which could allow rapid growth of E. coli. Our observation that Mu lysogeny is stabilized by FIS provides support for the idea that spontaneous induction of Mu transposition could occur preferentially during periods of restricted growth (e.g., stationary phase), when FIS levels are low (26, 58).

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**REFERENCES**

3a. Bétermin, M. Unpublished data.
27. Higgins, N. P. Personal communication.
tion requires interaction of transposase with a DNA sequence at the Mu operator: implications for regulation. Cell 58:399–408.


67a. van de Putte, P. Personal communication.


