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Mutants of *Escherichia coli* Defective for Replicative Transposition of Bacteriophage Mu

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We isolated 142 Hir⁻ (host inhibition of replication) mutants of an *Escherichia coli* K-12 Mu *cts* Kil⁻ lysogen that survived heat induction and the killing effect of Mu replicative transposition. All the 86 mutations induced by insertion of Tn*5* or a kanamycin-resistant derivative of Tn*10* and approximately one-third of the spontaneous mutations were found by P1 transduction to be linked to either *zdh-201*::Tn*10* or Tn*10-1230*, indicating their location in or near *himA* or *hip*, respectively. For a representative group of these mutations, complementation by a plasmid carrying the *himA*⁺ gene or by a lambda *hip*⁺ transducing phage confirmed their identification as *himA* or *hip* mutations, respectively. Some of the remaining spontaneously occurring mutations were located in *gyrA* or *gyrB*, the genes encoding DNA gyrase. Mutations in *gyrA* were identified by P1 linkage to *zei*::Tn*10* and a Nal^r *gyrA* allele; those in *gyrB* were defined by linkage to *tna*::Tn*10* and to a *gyrB*(Ts) allele. In strains carrying these *gyrA* or *gyrB* mutations, pBR322 plasmid DNA exhibited altered levels of supercoiling. The extent of growth of Mu *cts* differed in the various gyrase mutants tested. Phage production in one *gyrA* mutant was severely reduced, but it was only delayed and slightly reduced in other *gyrA* and *gyrB* mutants. In contrast, growth of a Kil⁻ Mu was greatly reduced in all gyrase mutant hosts tested.

Bacteriophage Mu is a temperate phage capable of growth on many enteric bacteria, including *Escherichia coli* K-12 (for a recent review, see reference 81). One of the most interesting aspects of Mu growth is its unusual mode of DNA replication, which requires integration of Mu into the host genome (82, 88). During replication, copies of the 38-kilobase (kb) Mu genome are inserted into new sites in the host genome in a process comparable in many respects to the transposition of other procaryotic transposable elements (4, 35, 52, 53). Like other transposable elements, transposition of Mu results in a duplication of host DNA at the target site of insertion (1, 47). Mu is also capable of promoting the types of chromosomal rearrangements generated by other elements, including inversions, deletions, duplications, and replicon fusions (reviewed in reference 49 and 81). The very high frequency of Mu transposition, approximately 100 events per cell per h during lytic growth (14), makes it an ideal system for both genetic and biochemical analyses of transposition. These analyses are facilitated by the use of temperature-sensitive mutations in the Mu repressor which permit induction of transposition in a lysogenic strain (39).

The phage-specified requirements for replicative transposition include the products of genes *A* and *B* and the *cis*-acting sites *attL* and *attR* located at the ends of the Mu genome (see Fig. 1) (30, 47, 82, 86–88). The *A* gene product, the transposase, is required for both replication and integration of the Mu genome; the *B* gene product is required for the high levels of replicative transposition observed during the lytic cycle (66, 88). Additional functions encoded downstream of gene *B*, though not essential for Mu growth in *E. coli* K-12, have been found to stimulate the rate of Mu DNA replication (28, 86; B. T. Waggoner, M. L. Pato, R. A. Forgie, M. T. Soltis, and M. M. Howe, submitted for publication).

A requirement for host-encoded functions in Mu transposition is indicated both by the properties of in vitro Mu transposition systems and by analysis of Mu growth in various mutant hosts. In vitro transposition of a plasmid carrying the attachment sites of Mu requires the Mu A and B proteins and a host-cell extract providing the HU protein and other, as yet unidentified, functions (8, 9, 63). The host-cell extract used is based on an extract developed to support *E. coli oriC*-dependent replication in vitro, thus suggesting the possibility that components of the host replication machinery may be involved in the transposition process. Like host DNA replication, Mu replication proceeds by a semiconservative, semidiscontinuous mechanism (35, 36).

A number of host mutations have been found to affect Mu phage production. Hosts with temperature-sensitive mutations in several functions required for host DNA synthesis, *dnaB, dnaC, dnaE, dnaG,* and *dnaZ,* produce a very low phage burst (79, 81) and do not replicate Mu DNA (71) at the nonpermissive temperature. Mutations in several other host genes, *uup* (38), *himB* (*gyrB*) (58), *tnm* (43, 44), and *himA* and *hip* (*himD*) (27, 57, 58), all result in a delay or an inhibition of Mu growth. However, among the latter group, only the *himA* mutations are known to inhibit Mu growth at the level of Mu DNA synthesis (R. K. Yoshida, Ph.D. thesis, University of Wisconsin, Madison, 1984). Mutations selected directly for inhibition of Mu replicative transposition have not previously been isolated.

Various models suggesting mechanisms for transposition have been proposed (22, 29, 34, 75). Central to these models is the creation of a new junction between the ends of the transposable element and host DNA ends generated by a staggered cut in the target site. In replicative transposition, a free 3′ end adjacent to this junction is proposed to serve as a primer for replication. In vitro the generation of a Mu transposition intermediate requires the host HU protein in addition to the Mu A and B products, and host functions are

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TABLE 1. Bacterial strains—general

Strain	Genotype	Source or derivation
CAG1574	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsdR rpsL recA56 srl*	C. Gross
CAG5055	HfrKL16 *zed*::Tn*10*[a] *thi-1 relA1 supD*	C. Gross
K37	*gal rpsL*	58
K634	*gal rpsL himA42*	58
KD1067	F⁻ *arg his mutD* Su⁺	11, 31
MH2874	F⁻ *supE42* T3ʳ (D108 cts10)	χ289 (D108 cts10); 42
MH2994	F⁺ *mel pro supF* (Mu cts62 *M*aml954)	QD5003 lysogen; Howe laboratory
MH4373	F⁺ *mel pro supF* (Mu cts62 Δ7701-1 Δ445-3)	QD5003 (Mu pf7701); Howe laboratory
MH4386	*gal rpsL himA42 zdh-201*::Tn*10*	K634 *zdh-201*::Tn*10*; Howe laboratory
MH4387	*gal rpsL himA42 zdh-201*::Tn*10* (P1 *clr*100 CM)	MH4386 (P1 *clr*100 CM); Howe laboratory
MH4939	F⁻ *supE thr leu fhuA lac*	Q1; 31
MH4945	F⁺ *mel pro supF*	QD5003; 31
MH5330	F′ *pro*⁺ *lacZ8305*::(Mu cts62)/Δ(*pro-lac*) *his met tyr rpsL* Muʳ	BU8305; 55
MH5709	Δ(*top-cysB*)*218 acrA12 gyrA224*	DM750; 12
MH5712	Δ*trpE63 pyrF287*	SD104; 12
MH5714	*galK2 recB rpsL gyrB203*(Ts) *gyrB221* (Couʳ)	N4178; 12
MH5715	Δ*lacX74 tna-7*::Tn*10*	KO635; 12
MH5768	HfrH *galT*::(λ Δ*int-F*II) Tn*10*-1230	RW1640; R. Weisberg
MH5769	F⁻ *pro his galK hip-115 lac supE*	NK115; 48
MH5778	HfrKL16 *zdh-201*::Tn*10 relA1 spoT1 thi-1* Δ(*xth-pncA*)DE90	BW9116; B. Weiss
MH5792	HfrH *galT*::(λ Δ*int-F*II) *hip-157* Tn*10*-1230	E79; R. Weisberg
MH5808	F⁻ *pro his galK hip-115* Tn*10*-1230 *lac supE*	MH5769 Tn*10*-1230; Howe laboratory
MH5816	*gal rpsL hip-115* Tn*10*-1230	K37 derivative; Howe laboratory
MH5818	*gal rpsL hip-157* Tn*10*-1230	K37 derivative; Howe laboratory
MH5830	EndoI⁻ *pro supE hsdR*(pNK290)	MM294(pNK290); N. Kleckner
MH5832	*leu*::IS*1 lacYZ*Δ*U169 nadA*::Tn*10 galE man*::IS*1 trp*::IS*1 dsdA argA21 rpsL mtl*::IS*1*	NK7140; N. Kleckner
MH5844	*leu*::IS*1 lacYZ*Δ*U169 man*::IS*1 trp*::IS*1 dsdA argA21 rpsL mtl*::IS*1*	MH5832 *gal*⁺ *nad*⁺; this work
MH5848	F⁺ *mel pro supF (Mu* cts62 Δ7701-1 *M*am1954)	QD5003 lysogen; this work
MH5861	MH5844 *gal*::(Mu cts62 Δ7701-1-Kanˢ3 *M*am1954)	MH5844 *gal*::(Mu Kil⁻); this work
MH5862	F⁺ *mel pro supF* (Mu cts62 Δ7701-1-Kanˢ3 *M*am1954)	QD5003 (Mu Kil⁻); this work
MH5886	MH5844 *himA42 zdh-201*::Tn*10*	This work
MH5893	MH5844 *gal*::(Mu cts62 Δ7701-1-Kanˢ3 *Mam*1954) *uraP*::?(Mu cts62 Δ7701-1-Kanˢ3 *M*am1954)	MH5861 *uraP*::?:(Mu Kil⁻); this work
MH5899	MH5893(pNK290)	This work
MH5918	MH5893 *leu*⁺	This work
MH6265	F⁻ *supE supF hsdR galK trpR metB lacY fhuA*	LE392; R. Weisberg
MH6292	*galK rpsL zei*::Tn*10*	RM101; M. Gellert
MH6902	F⁻ *supE thr leu lac fhuA hsdR hsdM* (λ) (p*himA*4)	K5626; H. Miller
MH6914	MH5893 *gyrA909*-Nalʳ	MH5893 Nalʳ; this work
MH6917	MH5844 *hip-115* Tn*10*-1230	This work
MH6918	MH5844 *hip-157* Tn*10*-1230	This work
MH6924	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsdR rpsL recA56 srl* (p*himA*4)	CAG1574(p*himA*4); this work
MH6943	MH5844 *gyrA912*-Nalʳ	MH5844 Nalʳ; this work

[a] The symbols :: and ::? indicate that the element which follows is known or presumed, respectively, to be inserted into the gene which precedes the symbol. The notations *zed*::Tn*10* and *zei*::Tn*10* do not represent genes but indicate the map positions of the Tn*10* insertions according to the conventions defined by Chumley et al. (6).

sufficient to convert this intermediate into cointegrate and simple insert products (8, 9). To identify host functions that are involved in Mu replicative transposition, we established a selection procedure for *E. coli* mutants defective in these functions. We report here the isolation of 142 Hir⁻ (host inhibition of replication) mutants, defective in Mu replicative transposition, and the properties of some of these mutants.

MATERIALS AND METHODS

Bacterial, bacteriophage, and plasmid strains. The bacterial strains used are listed in Tables 1, 2, and 3. Strain MH5844 is a Gal⁺ Nad⁺ transductant of MH5832; strain MH5918 is a Leu⁺ transductant of MH5893. Strain MH4386 was constructed by D. Lynn (personal communication) by transduction of K634 (*himA42*) to Tetʳ by P1 *clr*100 CM grown on MH5778 (*zdh-201*::Tn*10*). Tetʳ transductants retaining the *himA42* allele (<1% of the transductants) were detected by their inability to grow Mu *c*25 and Mu cts62 and their ability to grow Mu *nu*A1. Strain MH5886 was generated by P1 transduction of the linked *himA42* (missense) and *zdh-201*::Tn*10* alleles from MH4387 into MH5844 by selecting for Tetʳ and scoring for *himA42* by its inhibition of Mu growth in a cross-streak assay. Strain MH5808 was constructed by transduction of MH5769 (*hip-115*) to Tetʳ by P1 *clr*100 CM grown on strain MH5768 (Tn*10-1230*); the *hip-115* allele was detected by the inability of MH5808 to grow Mu cts62. Strains MH5816 (*hip-115*) and MH5818 (*hip-157*) were constructed by P1 transduction of the linked *hip* Tn*10-1230* alleles from strains MH5808 and MH5792, respectively.

TABLE 2. Strains with *himA* and *hip* mutations

himA allele	Type of mutation	Original isolate[a]	MH5844 derivative[b]	*hip* allele	Type of mutation	Original isolate[a]	MH5844 derivative[b]
541	Tn*10*-K	MH6226	MH6247	*542*	Tn*10*-K	MH6233	MH6240
550	Tn*10*-K	MH6227	MH6248	*553*	Tn*10*-K	MH6234	MH6241
558	Tn*10*-K	MH6228	MH6249	*556*	Tn*10*-K	MH6936	MH6242
567	Tn*10*-K	MH6229	MH6250	*560*	Tn*10*-K	MH6235	MH6244
579	Tn*10*-K	MH6230	MH6251	*565*	Tn*10*-K	MH6236	MH6245
674	Spontaneous	MH6252		*575*	Tn*10*-K	MH6237	MH6246
677	Spontaneous	MH6253		*580*	Tn*10*-K	MH6937	MH6243
678	Spontaneous	MH6254		*672*	Spontaneous	MH6257	
686	Spontaneous	MH6255		*683*	Spontaneous	MH6258	
698	Spontaneous	MH6256		*691*	Spontaneous	MH6925	
699	Spontaneous	MH6919		*692*	Spontaneous	MH6259	MH6903
778	Spontaneous	MH6920		*781*	Spontaneous	MH6938	MH6904
785	Spontaneous	MH6921		*797*	Spontaneous	MH6939	MH6905
788	Spontaneous	MH6922		*798*	Spontaneous	MH6940	MH6906
790	Spontaneous	MH6923		*803*	Spontaneous	MH6941	MH6907
801	Spontaneous	MH6944		*813*	Spontaneous	MH6942	MH6908

[a] Original Hir$^-$ isolates from MH5893 (*leu*::IS1; *500* and *600* series alleles) or its *leu*$^+$ derivative MH5918 (*700* and *800* series alleles).
[b] Derivatives of MH5844 containing the Hir$^-$ mutant alleles which were moved into MH5844 by P1 transduction. For Tn*10*-K alleles the donor strains were the original Hir$^-$ mutant isolates and selection was for Kan^r; for spontaneous alleles the donor strains were Tn*10-1230* derivatives of the original isolates and selection was for Tet^r.

Strains MH6917 and MH6918 were made by P1 cotransduction of the *hip-115* and *hip-157* mutations with Tn*10-1230* from strains MH5816 and MH5818, respectively. Nal^r derivatives of MH5844 and MH5893, MH6943 and MH6914, respectively, were selected on LB-Nal plates. The Nal^r mutations did not inhibit growth of Mu on these strains.

The bacteriophage strains used are listed in Table 4. The Kil$^-$ deletion Δ7701-1 was derived from Mu pf7701 which was itself derived from Mu 7701 (C. J. Thompson and M. M. Howe, manuscript in preparation). Mu 7701 is a defective Mu *c*ts62 Δ445-3 prophage carrying a Tn*5* insertion approximately 4.4 kb from the left end of Mu DNA. The Tn*5* makes Mu 7701 DNA too long to be packaged into viable phage particles. Mu pf7701 is a plaque-forming derivative of Mu 7701 which is deleted for approximately 2.4 kb of the Tn*5* element and 2.8 kb of adjacent Mu DNA (see Fig. 1B; Thompson and Howe, in preparation). This deletion, which will be referred to as Δ7701-1, removes the *kil* gene of Mu and one terminal repeat of Tn*5* making it nontransposable. λ *hip*11 is a derivative of λD69 containing the *hip* structural gene and approximately 189 base pairs upstream and 1 base pair downstream of the gene (17). λ *hip*11 lysogens were isolated from lawns spotted with λ *hip*11 and were identified by their immunity to λ *hip*11 (*imm*^{21}) and sensitivity to λ *c b*2 (*imm*$^{\lambda}$).

Plasmid pBR322 (78) was obtained from R. Gourse. Plasmid p*himA*4 is a pBR322 derivative containing a 2-kb fragment that includes the *himA* gene, a portion of the *pheT* gene immediately upstream of *himA*, and approximately 700 base pairs of unknown function downstream of *himA* (H. Miller, personal communication). Plasmid pNK290 is a pBR333 derivative carrying IS*10*R (IS*10* right from Tn*10*) with the HH104 mutation (18). Usually they were transformed into the appropriate strains by the procedure of Mandel and Higa (54) selecting for ampicillin resistance. For transformation of pBR322 into MH5712 Hir$^-$ derivatives, a modification of the calcium shock method of Cohen et al. (7) was used (substituting LB for H1 medium, omitting the 10 mM NaCl wash,

TABLE 3. Strains with *gyrA* and *gyrB* mutations

Mutant allele	Original isolate[a]	Original isolate with linked Tn*10*[b]	Nonlysogenic MH5844 derivative[c]	Mu *c*ts62 derivative[d]	Mu pf7701 derivative[d]	MH5712(pBR322) derivative[e]
gyrA685	MH6263	MH6933	MH6293	MH7507	MH7519	MH7460
gyrA697	MH6915	MH6934	MH6294	MH7508	MH7520	MH7461
gyrA812	MH6916	MH6935	MH6295	MH7509	MH7521	MH7462
gyrA$^+$	MH5893		MH6296	MH7510	MH7522	MH7452
gyrB676	MH6260	MH6926	MH6297			MH7463
gyrB688	MH6913	MH6927	MH6304			MH7469
gyrB696	MH6264	MH6928	MH6298			MH7464
gyrB782	MH6274	MH6929	MH6299	MH7502	MH7505	MH7465
gyrB804	MH6280	MH6930	MH6300	MH7503	MH7506	MH7466
gyrB805	MH6281	MH6931	MH6301			MH7467
gyrB810	MH6283	MH6932	MH6302			MH7468
gyrB$^+$	MH5893		MH6303	MH7501	MH7504	MH7451

[a] Original Hir$^-$ isolates from MH5893 (*leu*::IS1; *600*-series alleles) or its *leu*$^+$ derivative MH5918 (*700*- and *800*-series alleles).
[b] Derivatives of the original isolates carrying *zei*::Tn*10* (from MH6292) linked to the *gyrA* alleles or *tna*::Tn*10* (from MH5715) linked to the *gyrB* alleles.
[c] Derivatives of MH5844 containing *gyr* alleles moved in by P1 transduction from original isolates containing linked Tn*10* alleles or MH6292 (for *gyrA*$^+$) and MH5715 (for *gyrB*$^+$) by selection for Tet^r.
[d] Mu *c*ts62 or Mu pf7701 lysogenic derivatives of the MH5844 derivative strains described in footnote *c* were constructed as indicated in Materials and Methods.
[e] pBR322 transformants of MH5712 derivatives containing the *gyr* and linked Tn*10* alleles constructed by P1 transduction as described for the MH5844 derivatives. Additional independent transformants for *gyrB810* (MH7470) nd *gyrB688* (MH7471) were also used.

TABLE 4. Bacteriophage strains

Strain	Genotype	Lysogen	Reference or source
Mu *c*25	Mu *c*25		40
Mu *c*ts62	Mu *c*ts62	MH5330	39
Mu *c*ts62 *M*am1954	Mu *c*ts62 *M*am1954	MH2994	65
Mu *vir*	Mu *vir*3057 *mom*		A. Toussaint; 84
Mu *nuA*1	Mu *c*ts62 *nuA*1		89
Mu *nuA*106	Mu *c*ts62 *nuA*106		89
Mu *nuB*104	Mu *c*ts62 *nuB*104		89
Mu pf7701	Mu *c*ts62 Δ7701-1 Δ445-3	MH4373	Howe laboratory
Mu Kil$^-$	Mu *c*ts62 Δ7701-1-Kan^{s}3 *M*am1954	MH5862	This work
D108	D108 *c*ts10	MH2874	42
λ *c b*2	λ *c b*2		Howe laboratory
λ *hip*11	λD69 (Bam1° srI(1,2)$^\Delta$ *imm*^{21} *nin*5)^a *hip*$^+$		17
λ939	λgt7 (*b*522 *nin*5) *hisG9424*::Tn*10* Δ*16* Δ*17*Kan^r *c*I857 *P*am80		18
λ::Tn*5*	λ *b*221 *O*am29 *P*am80 *rex*::Tn*5* *c*I857		46
P1 *vir*	P1 *vir*		A. Grossman
P1 *clr*100 CM	P1 *clr*100 CM		Howe laboratory 50

[a] Bam1° and SrI(1,2)$^\Delta$, mutation inactivating the first *Bam*HI site and deletion between first and second *Eco*RI sites, respectively.

substituting 0.1 M $CaCl_2$ for 0.03 M $CaCl_2$, and heat shocking for 1 min at 37°C before the 60-min 0°C incubation).

Construction of Mu Kil$^-$. To recombine the Kan^r-Δ7701-1 region from phage Mu pf7701 (see Fig. 1B) onto Mu *c*ts62 *M*am1954, a freshly saturated culture of the Mu *c*ts62 *M*am1954 lysogen MH2994 was infected with Mu pf7701 at a multiplicity of infection (MOI) of ~0.05. After 30 min of adsorption at 32°C, Kan^r derivatives were selected on LB-Kan plates at 32°C. The desired recombinants were identified by their release of only small-plaque-forming amber mutant phage upon heat induction. The ability of the phage released from one such recombinant, MH5848, to form kanamycin-resistant lysogens confirmed the presence of the 7701-1 deletion.

To obtain a Kan^s phage derivative, a population of KD1067 (*mutD*) Mu *c*ts62 Δ7701-1 *M*am1954 lysogens was obtained essentially as described previously (31) and mutagenized by growth in LB to 10^8 cells per ml, dilution 50-fold into SBPM, and growth to 3×10^8 cells per ml. The cells were heat induced by twofold dilution with 55°C SBPM, shaken for 45 min at 42°C and 2 h at 37°C, and treated with chloroform. The phage were plated on strain MH4945 at 32°C, and Mu lysogens were picked from 610 plaque centers and tested for kanamycin resistance by patching on LB and LB-Kan at 32°C. From the seven Kan^s plaque centers recovered, two (0.3%) released Kan^s phage when heat induced. One of these, MH5862 containing Mu *c*ts62 Δ7701-1-Kan^{s}3 *M*am1954, hereafter referred to as Mu Kil$^-$, was chosen for further use. The remaining five plaques were presumed to be derived from clear mutants since no Mu lysogens were recovered from the Kan^s cells.

Mu Kil$^-$ lysogen constructions. To construct strain MH5861 with Mu Kil$^-$ inserted into *gal*, MH5844 was grown to 10^9 cells per ml in LB containing 4 mM $MgSO_4$ and infected with Mu Kil$^-$ at 32°C at an MOI of ~2. After 30 min of adsorption, infected cells were diluted 10-fold with LB, grown at 32°C for 2 h, reinfected, and grown overnight at 32°C. (The second infection resulted in a substantially higher yield of Mu amber lysogens among surviving cells.) Dilutions were plated on appropriately supplemented minimal plates containing 1.0% glycerol and 0.13% 2-deoxygalactose (Sigma Chemical Co., St. Louis, Mo.) (2). Candidates for lysogens with Mu inserted in *gal* were identified among 2-deoxygalactose-resistant cells as strains that were Gal$^-$ on MacConkey-galactose plates and unable to grow at 42°C. A monolysogenic strain, MH5861, with Mu Kil$^-$ inserted in *gal* was detected as a strain whose Gal$^+$ transductants were able to grow at 42°C.

A strain with two (or more) copies of Mu Kil$^-$ (MH5893) was constructed by superinfecting the monolysogenic strain, MH5861, with Mu Kil$^-$ at an MOI of 2 at 32°C followed by fivefold dilution into LB and growth overnight at 32°C. 6-Azauracil-resistant colonies were selected by plating on appropriately supplemented minimal glucose plates containing citrate and 200 μg of 6-azauracil (Sigma) per ml. 6-Azauracil-resistant strains whose Gal$^+$ transductants were unable to grow at 42°C were considered to have one or more copies of Mu Kil$^-$ (in addition to the copy in *gal*), with one copy likely to be inserted in *uraP* conferring 6-azauracil resistance (61).

Mu *c*ts62 and Mu pf7701 lysogen construction. To construct Mu *c*ts62 and Mu pf7701 lysogens of the *gyrA*, *gyrB*, and control *gyr*$^+$ strains, strains MH6293-6296, MH6299, MH6300, and MH6303 were grown in LB containing 5 mM $MgSO_4$ and 5 mM $CaCl_2$ to a cell density of $\sim 3 \times 10^8$/ml and infected with phage at an MOI of 3 to 5. Mu *c*ts62-infected cultures were grown at 30°C for 2 h, reinfected, diluted fivefold into LB containing 2.5 mM $MgSO_4$, grown overnight at 30°C, and streaked for single colonies on LB plates at 32°C. Lysogens were detected by their reduced ability to grow at 42°C in a patch test. Mu pf7701-infected cultures were grown at 30°C overnight, and lysogens were isolated by streaking on LB-Kan plates at 32°C.

Media. LB, LB agar, soft agar, LBM, SBPM, SM, SMC, OM minimal medium, and TCMG plates were previously described (39, 40, 74). LBMC is LB broth containing 4 mM $MgSO_4$ and 1 mM $CaCl_2$. When appropriate, LB plates were supplemented with kanamycin at 65 μg/ml (LB-Kan), tetracycline at 10 μg/ml (LB-Tet), ampicillin at 50 μg/ml (LB-Amp), nalidixic acid at 40 μg/ml (LB-Nal), or streptomycin at 200 μg/ml (LB-Str). For transductions, plates also contained 10 mM sodium citrate (designated Cit). Minimal medium was supplemented with sugars at 0.2%, thiamine at 1 μg/ml, and amino acids (as needed) at 20 μg/ml.

Phage lysate preparation and titration. Lysates of Mu *c*ts62 and its derivatives and D108 *c*ts10 were prepared by heat induction (40) of the lysogens listed in Table 4. Mu *vir* was grown by infection (40). Mu *nu* lysates were received from

R. Yoshida. P1 *vir* lysates were prepared by infecting cells grown to a density of 3×10^8/ml in LBMC with P1 *vir* at an approximate MOI of 0.1. After 30 min of adsorption at 32 or 37°C, infected cells were diluted 10-fold in LBMC and incubated until lysis. λ939 and λ::Tn*5* were grown by infection of MH4939 and MH6265, respectively. Cells grown in LBM to 3×10^8 cells per ml were infected at an MOI of ~0.1, incubated for 15 min at 37°C, diluted 10-fold with LBM, and shaken at 37°C until lysis. All lysates were treated with chloroform and centrifuged to remove debris (40). Titrations were performed as described previously (40), using 3-ml soft agar and TCMG plates; for Mu pf7701 soft agar was supplemented with 0.04 M $CaCl_2$.

P1 transduction. Strains to be used as recipients in P1 transduction were grown to saturation in LBMC, infected with P1 *vir* at an MOI of ~0.1, and incubated for 40 min for adsorption. For construction of MH5844, the adsorption mixture was plated directly on appropriately supplemented minimal galactose plates containing citrate and 0.5 μM nicotinic acid. Tet^r transductants were selected by plating directly on LB-Tet-Cit plates; for Kan^r transductants the adsorption mixtures were diluted twofold in LB with 50 mM citrate and grown for 30 min before plating on LB-Kan-Cit plates.

Growth assays. Phage and cell growth assays were as follows. (i) Cross streak. Cells from single colonies were picked with a toothpick and drawn across streaks of phage lysate containing approximately 10^6, 10^7, and 10^8 phage per ml on LB plates which were incubated for 6 to 8 h at 37°C.

(ii) Spot test. For spot-test assays of Mu *c*ts62 or D108 *c*ts10 growth, 0.1-ml samples of freshly saturated cells grown in LB containing 5 mM $MgSO_4$ were plated in soft agar on either LB or TCMG plates. Ten-fold serial dilutions of phage lysate prepared in SM were spotted onto the plates which were incubated overnight at 37°C.

(iii) Patch test for cell death. Lysogenic cells from single colonies were patched onto LB plates and incubated at 42°C. For analysis of drug-resistant transductants the plates contained 10 mM citrate and the appropriate antibiotic. Under these conditions wild-type Hir$^+$ lysogens showed very sparse growth owing to prophage induction; however, Hir$^-$ mutant lysogens grew well.

(iv) *gyrB*(Ts) assay. To test Tet^r transductants for the *gyrB*(Ts) allele, they were suspended in 0.4 ml of LB, and a loopful (10 μl) was plated on LB-Tet-Cit agar and incubated at 42°C. Under these conditions, growth of cells with the *gyrB*(Ts) allele was not apparent; at higher cell density the temperature-sensitive phenotype of these cells was more difficult to detect.

(v) Δ*top* compensatory phenotype. Derivatives of MH5712 (Δ*trp pyrF*) containing gyrase alleles (*gyrA*$^+$, *gyrA685*, *gyrA697*, *gyrA812*, *gyrB*$^+$, *gyrB696*, *gyrB782*, and *gyrB804*) were constructed by P1 *vir* cotransduction with the linked Tn*10* from donor strains MH5715, MH6292, MH6933 to MH6935, and MH6928 to MH6930. These MH5712 derivatives were then transduced to *trp*$^+$ on minimal plates containing glucose, thiamine, cysteine, uracil, and citrate by using P1 *vir* grown on strain MH5709 (Δ*top-cysB*) and scored for inheritance of the linked *top-cysB* deletion by replica plating to similar media supplemented with 0.1% vitamin-free Casamino Acids (Difco Laboratories, Detroit, Mich.) and containing or lacking cysteine. High-frequency cotransduction of the *top-cysB* deletion with *trp*$^+$ indicates the presence of a mutation in the recipient strain able to compensate for the deleterious effects of the topoisomerase I mutation (12).

(vi) Antibiotic sensitivity. Cultures of strains MH6293 to MH6304 containing *gyr*$^+$, *gyrA*, or *gyrB* alleles were grown in LB at 32°C to 5×10^8 cells per ml. Drops (10 μl) of each culture were spotted onto a series of LB plates containing an antibiotic (all from Sigma). Cell growth was scored after ~24 h of incubation at 32°C. For each antibiotic six different concentrations were tested in a range from 0.5 to 20 μg/ml for tetracycline and kanamycin, 1 to 30 μg/ml for nalidixic acid and coumermycin A1, 5 to 100 μg/ml for rifampin, 10 to 150 μg/ml for streptomycin, and 10 to 600 μg/ml for novobiocin.

Isolation of Tn*5*- and Tn*10*-induced mutants. Strain MH5893 was used for mutagenesis with Tn*5*; a derivative of this strain, MH5899 carrying pNK290 (to provide Tn*10* transposase), was used for mutagenesis with the transposition-defective element Tn*10* Δ*16* Δ *17* Kan^r (which will be referred to as Tn*10*-K) (18). The cells were grown at 30°C to 4×10^8 cells per ml in LB containing 0.2% maltose or 10 mM $MgSO_4$ and 5 mM $CaCl_2$. Seven 50-ml cultures were infected at an MOI of 2 with the replication-defective λ phage λ::Tn*5* (one culture) or λ939 (six cultures). After 30 min at 30°C, the cells were diluted twofold in LB and grown for 60 min at 30°C. Appropriate dilutions were plated on LB containing 30 μg of kanamycin per ml to determine the transposition frequency (~10^{-4}) and the total number of insertion events (~10^6 per culture). After 10-fold dilution into LB containing 30 μg of kanamycin per ml and overnight growth at 30°C, 0.3 ml of each culture was diluted 35-fold further and grown to saturation at 42°C to select cells resistant to heat induction of the resident Mu Kil$^-$ prophage. To minimize the analysis of spontaneous mutations unlinked to the Tn insertions (and present at frequencies of 16 to 58% in different cultures; data not shown) and to separate the mutations from pNK290, the Tn insertions were moved by P1 transduction into strain MH5893. Samples (0.05 ml) of the 42°C cultures were inoculated into 5 ml of broth and infected with P1 *vir*. The resulting lysates were used to transduce strain MH5893 to Kan^r. After 40 min of adsorption and 30 min of growth, infected cells were plated on LB-Kan-Cit and incubated at 32°C for 6 h and then shifted to 42°C for 12 to 15 h. The 6-h outgrowth time at 32°C was chosen because: (i) transductants grown at 32°C and then tested at 42°C yielded mutants at the same frequency as the plate-shifting method, whereas direct plating at 42°C yielded no mutants; and (ii) when *himA42* was transduced into strain MH5893, a 6-h preincubation at 32°C was required to express the heat-resistant phenotype. Ninety Kan^r transductants were chosen for further study. With the exception of mutations in strains MH6241 and MH6242 and those in MH6243 and MH6246, each of those characterized in detail was isolated from independent cultures.

Isolation of spontaneously occurring mutations. A total of 30 single colonies, 10 from MH5893 and 20 from MH5918, were used to inoculate 30 independent cultures which were grown at 32°C and then used to inoculate cultures grown for 4 h at 42°C. Cells from the 42°C cultures were then streaked on LB agar and grown at 42°C. Three colonies (small, medium, and large) were picked from each of the 30 cultures and purified at 42°C, and a subset of 52 mutants which were clearly temperature resistant were chosen for further analysis. Mutant alleles with *600* series numbers were isolated from strain MH5893, and alleles with *700* to *800* series numbers were isolated in the Leu$^+$ derivative of MH5893, MH5918. All the alleles which were characterized in detail were isolated from different cultures except for the three pairs (*698* and *699*, *691* and *692*, and *804* and *805*) whose

members were isolated from the same culture. Derivatives of the spontaneous mutant strains containing linked Tn*10* elements (*zdh-201*::Tn*10*, Tn*10-1230*, *zei*::Tn*10*, and *tna*::Tn*10*) were identified among Tet^r transductants of the original mutant isolates as those able to grow at 42°C in patch tests. Mutant strains were maintained at −70°C in 9% dimethyl sulfoxide. This was particularly important for the *gyrA* mutants which were somewhat unstable.

Hfr mapping. Cultures of mutant and Hfr strains grown at 37°C in LB to a density of 3×10^8 cells per ml were mixed in a ratio of 1.8 ml of mutant with 0.2 ml of Hfr and incubated with gentle shaking at 32°C for 25 min. Mating pairs were disrupted by vortexing at high speed for 90 s, followed by immediate dilution. Str^r-Tet^r exconjugants were selected on LB-Str-Tet plates at 32°C and were tested for ability to support Mu growth by patching onto LB-Tet plates which were incubated at 42°C.

Growth of Mu in gyrase mutant strains. Growth of Mu in gyrase mutant hosts was assayed by measuring phage produced after heat induction of Mu *c*ts62 and Mu pf7701 lysogenic strains MH7501 to 7510 and MH7519 to 7522. Cells were grown to 0.8×10^8 to 1.6×10^8/ml in LB containing 2.5 mM $MgSO_4$ at 32°C, diluted 10-fold into similar medium, and shaken at 42°C. Samples were removed after 1, 2, and 3 h, and treated with chloroform, and titers were determined on strain MH4945.

Determination of levels of plasmid supercoiling. MH5712 derivatives carrying the Hir$^-$ mutant alleles were transformed with pBR322, and plasmid DNA from these strains was subjected to chloroquine-agarose gel electrophoresis (70). Duplicate transformants of each strain were grown at 37°C in LB containing 50 μg of ampicillin per ml to a cell density of 10^9/ml, and plasmid DNA was extracted by a rapid boiling procedure (37). Plasmid DNA was electrophoresed at 4°C for 14 h at 3 V/cm through horizontal 0.8% agarose gels in 50 mM Tris phosphate buffer (pH 7.2) containing different concentrations of chloroquine (Sigma) (1, 2, 5, 7, 10, or 20 μg/ml) (76). The gels were rinsed in water, stained in 0.5 μg of ethidium bromide per ml, and photographed with long-wave UV illumination with Polaroid type 57 film.

RESULTS

Our selection for host mutants defective for Mu replicative transposition is based on the observation that extensive replicative transposition of Mu results in killing of the host cell (85). Other Mu-encoded functions also contribute to host-cell killing during the Mu lytic cycle. They include *kil*, an early gene that can cause host-cell death in the absence of Mu DNA replication (85, 87) and *lys*, the gene required for host cell lysis (15). To select mutants defective for Mu DNA replication, it was necessary to establish conditions under which Mu replicative transposition was the primary source of cell killing. Since Mu late genes are not transcribed in the absence of Mu DNA replication (87; C. F. Marrs, Ph.D. thesis, University of Wisconsin, Madison, 1982), mutants defective for Mu DNA replication should not express *lys* or any other late functions that might contribute to cell death. To eliminate the effects of the early gene *kil*, we used a phage deleted for this gene in the selection procedure. We predicted that host mutants unable to support Mu DNA replication would survive heat induction of a Mu *c*ts62 Kil$^-$ lysogen.

The Kil$^-$ phage used in the selection was Mu *c*ts62 Δ7701-1-Kan^{s}3 *M*am1954 (referred to in this paper as Mu Kil$^-$). Deletion 7701-1, derived from Mu pf7701, removes the nonessential Mu DNA between approximately 4.4 and 7.2 kb from the Mu left end, including *kil*, and contains a substitution of part of Tn*5*, including the neomycin phosphotransferase gene (Fig. 1B). Although the deleted region is not essential for Mu growth in *E. coli*, it does include one and possibly two functions which are involved in stimulating the rate of Mu DNA replication (28, 86; Waggoner et al., submitted), as well as Gam and Sot, involved in inhibition of host exonuclease activity and stimulation of transfection (73, 83). The *M* amber mutation (*M*am1954) affects tail synthesis (32) and results in a severe reduction of infective phage particle production from Su$^-$ cells (burst size, $<10^{-6}$; W. Ross, unpublished observations). It was used to prevent possible high-multiplicity superinfection and Mu-induced mutagenesis of surviving host mutant cells by phage released from wild-type lysogens. The Kan^s mutation in the neomycin phosphotransferase gene was included to permit the use of Kan^r transposons as mutagens. Strain MH5844, a derivative of W3110 containing multiple mutations for use as markers in subsequent genetic mapping, was chosen as the host strain. To reduce the recovery of mutations in the Mu prophage, a lysogenic derivative of MH5844 with multiple Mu prophages was constructed; MH5893 carries at least two copies of Mu Kil$^-$, one inserted in *gal* and a second most probably inserted in *uraP* (see Materials and Methods). Heat induction of this Mu Kil$^-$ lysogen resulted in extensive cell killing (survival, $\sim10^{-5}$ at 42°C).

Isolation of host mutations. To facilitate mapping and subsequent transfer of the host mutations, we chose to use transposons Tn*5* and Tn*10* as mutagens. A transposition-defective derivative of Tn*10*, lacking the transposase gene and conferring Kan^r (Tn*10* Δ*16* Δ*17* Kan^r, which we will refer to as Tn*10*-K;18), was used with transposase being provided from the plasmid pNK290. After infection of the Mu lysogen with replication-defective λ phage carrying either Tn*10*-K (λ939) or Tn*5* (λ::Tn*5*), Kan^r cells were selected during growth in liquid at 30°C. These cultures were then grown at 42°C to select for survivors unable to replicate Mu. To eliminate analysis of hosts with possible spontaneous mutations unlinked to the Tn*5* or Tn*10*-K elements, the mutations were moved by P1 transduction from the cultures selected at 42°C into the multiple lysogen MH5893 with selection of individual transductants able to grow on LB-Kan plates at 42°C. In addition to increasing the likelihood of linkage of the mutant phenotype with the transposon insertion, the transduction procedure accomplished transfer of the mutations into a strain lacking the pNK290 plasmid.

A total of 90 Tn*5* or Tn*10*-K insertion mutants, including 12 initially picked for their smaller colony size, were chosen for further study. These mutants were obtained from six independently Tn*10*-K-mutagenized cultures and one Tn*5*-mutagenized culture, each with $\sim10^6$ insertion events.

To permit recovery of mutations in genes essential for *E. coli* growth, spontaneously arising mutants (occurring at a frequency of $\sim10^{-5}$) were also selected from 30 independent cultures (10 from MH5893 and 20 from MH5918, a Leu$^+$ derivative of MH5893). From each culture two or three heat-resistant survivors with different colony sizes were chosen for further study.

Mutations in genes *himA* and *hip*. The *E. coli* genes *himA* and *hip* encode the two subunits of the integration host factor (IHF) required for lambda integration (17, 48, 60). Mutants defective in *himA* or *hip* cannot support Mu growth owing to inhibition of expression of the Mu replication functions A and B (27; Yoshida, Ph.D. thesis). We expected that host mutants defective in these genes would arise in our

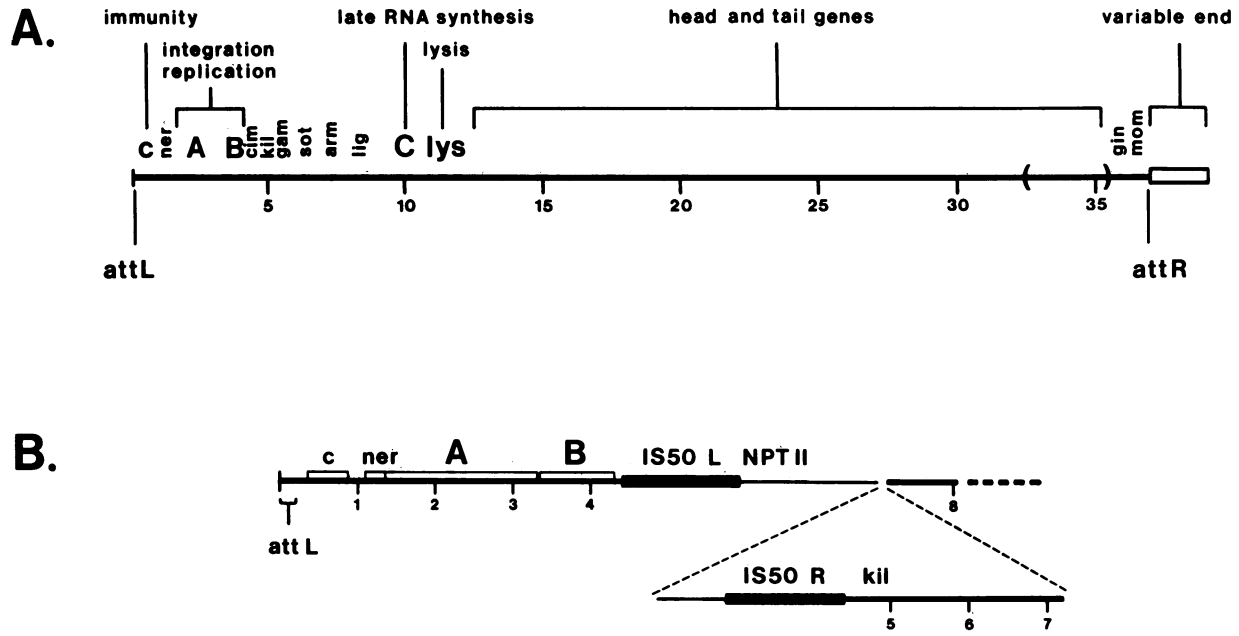

FIG. 1. Maps of the Mu genome (A) and the left end of the Mu Δ7701-1 genome (B). (A) The solid line represents Mu DNA with tick marks at 5-kb intervals representing distance from the left end. The vertical line at the left end and open box at the right end represent attached host DNA. The invertible G segment is indicated by parentheses; the attachment sites are designated *attL* and *attR*. This correlation of the genetic and physical map was prepared by Grundy and Howe (31) from previous findings; the locations of functions in the 4- to 10-kb region require confirmation. (B) The top line represents the left end of a phage containing Δ7701-1; the bottom line depicts the DNA removed from Mu 7701 by the 7701-1 deletion, as defined by heteroduplex and restriction analysis (Thompson and Howe, in preparation). Numbers below the line designate the distance (in kilobases) from the left end of wild-type Mu DNA lacking insertions or deletions. The approximate lengths of genes *c, ner, A,* and *B* derived from the DNA sequence (33, 62, 69) are indicated as open boxes. The thick line represents Mu DNA, the thin line indicates unique-sequence Tn*5* DNA, and the filled boxes represent the Tn*5* inverted repeats IS*50*L and IS*50*R. The vertical line at the left end represents attached host DNA. NPT II indicates the location of the neomycin phosphotransferase gene (72) which confers Kan^r on lysogens containing Mu Δ7701-1.

selection procedure and confirmed that transfer of the *himA42* missense mutation into the lysogenic strain MH5893 resulted in survival and growth of the strain at 42°C. Therefore, both the spontaneous and transposon insertion mutants were screened by P1 transduction for linkage of the mutations to Tn*10*-Tet^r elements linked to *himA* (*zdh-201*::Tn*10*, >99% linked, D. Lynn and M. M. Howe, unpublished observations) or to *hip* (Tn*10-1230*, 70% linked [48]). An average of 20 transductants was tested for each of the insertion mutant strains. The insertion mutations located near *zdh-201*::Tn*10* showed very high linkage to the Tn*10*-Tet^r element; in only one transductant was recombination between the mutation and this element observed (Table 5). For insertion mutations linked to Tn*10-1230*, a range of P1 linkage values from 5 to 80% was observed (see Table 6 for representative examples), with the majority being greater than 50%. These Tn*10*-K insertions were inseparable from the Hir$^-$ phenotype (Table 6), indicating that the Hir$^-$ mutations were caused by Tn*10*-K insertion. Of 90 insertion mutations tested, 44 and 42 were linked to *zdh-201*::Tn*10* and Tn*10-1230*, respectively, and are therefore located in or near *himA* or *hip* (Table 7). The mutations causing the Hir$^-$ phenotype in the remaining four insertion mutants (including one slow-growing mutant) were found to be unlinked to the Tn*5* or Tn*10*-K transposon used for mutagenesis. There were, therefore, no Tn*5*- or Tn*10*-induced Hir$^-$ mutations that mapped elsewhere in locations unlinked to *himA* or *hip*.

To determine whether these mutants were actually defective in *himA* or *hip*, rather than in nearby functions that might also be involved in Mu replication, a representative group of mutants was further analyzed by complementation. Each of five independently derived Tn*10*-K insertions linked to *zdh-201*::Tn*10* was complemented for Mu growth by the plasmid p*himA*4 as judged by growth of Mu *c*ts62 in spot-test assays on nonlysogenic Hir$^-$ strains (Table 5). Mu growth on the plasmid-containing mutant strains was comparable to that on a Hir$^+$ strain. Thus, these Tn-induced lesions occur within the *himA* gene or within the approximately 700 base pairs of unknown function downstream of *himA* also carried on the plasmid. In similar experiments, λ*hip*11 lysogens of strains containing each of the seven Tn*10*-K-induced insertion mutations mapping near Tn*10-1230* supported growth of Mu *c*ts62 to an extent comparable to that of a Hir$^+$ strain (Table 6). Thus, all seven mutations appear to affect the *hip* gene.

Each of the spontaneously occurring mutations mapping near *zdh-201*::Tn*10* or Tn*10-1230* that was tested (18 of 20) was also found, by complementation by either p*himA*4 or λ *hip*11, to be in either the *himA* or *hip* gene, respectively (Tables 5 and 6).

Mu growth in spot tests was reduced at least 100- to 1,000-fold in nonlysogenic strains carrying either the spontaneous or Tn-induced mutant alleles. There was no significant difference in Mu growth inhibition exhibited by these strains and those containing the previously characterized *himA42* and *hip115* alleles.

Mutants of Mu designated Mu *nuA* have been selected for their ability to grow on a *himA42* mutant strain (57, 89). Consistent with their identification as *himA* and *hip* mutants, the two representative *himA* insertion mutants (MH6247 and

TABLE 5. Identification of *himA* mutations

Allele no.	Type of mutation	Linkage to *zdh-201*::Tn*10*[a] (%)	Complementation with p*himA4*[b]	Growth of D108[c]
541	Tn*10*-K	>98	+	–
550	Tn*10*-K	98	+	–
558	Tn*10*-K	>98	+	–
567	Tn*10*-K	>98	+	ND[d]
579	Tn*10*-K	>98	+	ND
674, 677, 678, 686	Spontaneous	>93	+	–
698	Spontaneous	>93	+	ND
699, 801	Spontaneous	>93	ND	ND
778, 785, 788, 790	Spontaneous	>93	+	ND
himA42	Missense	ND	+	–

[a] Linkage to *zdh-201*::Tn*10* was tested by P1 transduction from the *him*$^+$ donor MH5778 into the original Hir$^-$ mutant isolates carrying the indicated alleles selecting for Tet^r. For Tn*10*-K mutant strains the percentage of the 48 Tet^r transductants tested which were Kan^s is indicated; for the spontaneous mutants the percentage of the 15 Tet^r transductants tested which died at 42°C in patch tests is indicated. Linkage for *himA42* is >99% (D. Lynn and M. M. Howe, unpublished observations).

[b] Complementation by p*himA4* for growth of Mu was tested in p*himA4* transformants of MH5844 derivatives carrying the indicated Tn*10*-K alleles or *himA42* (MH5886), or of the original spontaneous mutant isolates. For the Tn*10*-K mutant strains, complementation was assayed by spot-test plating of Mu *c*ts62 which grew equally well (efficiency of plating, ~1) on the p*himA4* mutant strains and the *him*$^+$ control. For the spontaneous mutants, complementation was reflected by death of the Mu Kil$^-$ lysogen at 42°C in patch tests.

[c] Growth of D108 *c*ts10 was determined in spot-test plating on MH5844 derivatives carrying the Tn*10*-K or *himA42* alleles and on the original spontaneous mutant isolates. A – indicates severe inhibition of growth (faint spots at 10^{-1} and 10^{-2} dilutions of lysates with titers of 10^9 to 10^{10} phage per ml).

[d] ND, Not determined.

MH6248) and four representative *hip* insertion mutants (MH6240 to MH6243) tested were found to support growth of Mu *nuA*1 and Mu *nuA*106 to levels comparable to those observed in the *himA42* strain in spot-test assays (data not shown).

In spot-test assays growth of D108 *c*ts10, a heteroimmune phage very closely related to Mu (25, 42), was reduced approximately 100- to 1,000-fold and was similar on all 17 *himA* and *hip* mutant strains tested (Tables 5 and 6). When plating of Mu *c*ts62 and D108 *c*ts10 was compared in a subset of nonlysogenic *himA* or *hip* mutant strains, growth of both phages was inhibited to a similar extent.

Mutations in *gyrA*. A group of 32 spontaneous mutant strains contained mutations that were not linked to *zdh-201*::Tn*10* or Tn*10-1230*. To determine whether these strains were likely to contain host mutations rather than mutations in the Mu Kil$^-$ prophages, they were assayed for their ability to support the growth of two related phages capable of growth on a Mu lysogen: Mu *vir* (84) and the closely related heteroimmune phage D108 *c*ts10 (25, 42). Ten of these mutants plated both Mu *vir* and D108 less efficiently than the parent lysogen or nonlysogenic strains in spot-test assays. One mutant (with allele *812*) showed an inhibition of D108 growth comparable to that of a *himA42* strain (reduced at least 100- to 1,000-fold relative to the Hir$^+$ strain), whereas the others (with alleles *676, 685, 696, 697, 782, 804, 805*, and *810*) plated D108 to various degrees and somewhat better (inhibited approximately 10- to 100-fold relative to the Hir$^+$ strain). These 10 mutants were therefore considered to be host mutants and were chosen for further study.

Preliminary Hfr mapping with strain CAG5055 indicated that 3 of the 10 mutants mapped to a region which was defined by the origin of transfer at 61 min and *zed*::Tn*10* at 43 min (data not shown) and which thus contained the *gyrA* gene at 48 min. The observation that Mu grows poorly on *himB* (*gyrB*) host mutants (12, 21, 24, 58; S. H. [redacted]hore, Ph.D. thesis, University of Wisconsin, Madison, 1986) suggested the possibility that these three mutations might be located in the *gyrA* gene, encoding the other subunit of DNA gyrase. In P1 transduction the three mutations showed linkage to *zei*::Tn*10* of 8 to 24%, as compared with 12% for a control *gyrA* (Nal^r) allele (Table 8).

These three mutations were moved by P1 transduction from *zei*::Tn*10* derivatives of the original isolates into the parent lysogen, MH5893, and into two related nonlysogenic strains, MH5844 and MH5712. The resultant strains exhibited defects in Mu growth, as judged by cell growth at 42°C for MH5893 derivatives or by Mu plating in spot-test assays for MH5844 and MH5712 derivatives. The viability of the reconstructed MH5893 mutants at 42°C indicates that the mutations linked to *zei*::Tn*10* were sufficient to confer the Hir$^-$ phenotype originally selected.

To determine whether these mutations were located in *gyrA*, we took two approaches. In the first approach, linkage of the mutations to a *gyrA*-Nal^r allele was tested. Each showed 98% or greater linkage to this allele (Table 8), as would be expected for a *gyrA* mutation. In the second approach we asked whether the level of DNA supercoiling in MH5712 derivative strains carrying these mutant alleles was altered. The distribution of supercoiled species of pBR322 plasmid DNA isolated from the mutant strains was analyzed on a series of 0.8% agarose gels containing different concentrations of chloroquine. Plasmid DNA from two independent transformants of each of the three mutants was reproducibly less negatively supercoiled than that from the control Gyr$^+$ strain (see Fig. 2 for a representative gel). The decreased level of supercoiling observed was similar for each of the three mutants. On the basis of these experiments, we conclude that the three mutations are in *gyrA* (or are very near *gyrA* and affect its expression).

Nonlysogenic derivatives of strain MH5844 carrying the *gyrA* mutant alleles were tested for possible effects of the gyrase mutations on cell and phage growth. The growth rate, both in liquid LB and on LB plates, of MH6293 (*gyrA685*) was comparable to that of the *gyr*$^+$ control strain MH6296. The *gyrA* mutants MH6294 (*gyrA697*) and MH6295 (*gyrA812*) grew slightly more slowly at 32 or 37°C than the control strain MH6296, showing an approximately 10% longer generation time in liquid. On plates these strains reproducibly formed smaller colonies than strains carrying either the *gyrA685* or the wild-type allele. A mutant of Mu, Mu *nuB*104, selected for growth on a strain carrying a mutation in the *gyrB* subunit of DNA gyrase (89), was able to plate significantly better than Mu *c*ts62 on all three *gyrA* mutants (efficiency of plating, 0.2 to 0.8 with small plaques). P1 *vir* and λ *c b*2 both gave high efficiencies of plating on these three mutants, although they formed slightly smaller plaques on strains carrying the alleles *697* and *812*.

Mutations in *gyrB*. The seven remaining spontaneous mutations, which inhibited growth of Mu *vir* and D108 *c*ts10 but did not map in *gyrA*, were then tested by P1 transduction for possible location within *gyrB*, the gene for the other DNA gyrase subunit. They showed 58 to 99% linkage to *tna*::Tn*10* as compared with 89% linkage for a control *gyrB*(Ts) allele (Table 9), results consistent with a possible location in *gyrB*.

To determine whether these mutations were in *gyrB* or in

TABLE 6. Identification of *hip* mutations

Allele no.	Type of mutation	% Linkage to Tn*10-1230*[a]	% Linkage of Hir⁻ phenotype to Tn*10*-K[b]	Complementation by λ *hip*11[c]	Growth of D108[d]
542	Tn*10*-K	66 (21/32)	>99 (84/84)	+	−
553	Tn*10*-K	73 (19/26)	>99 (84/84)	+	−
556	Tn*10*-K	5 (1/20)	>99 (84/84)	+	−
580	Tn*10*-K	21 (5/24)	>99 (84/84)	+	ND
560	Tn*10*-K	55 (27/49)	>99 (90/90)	+	ND
565	Tn*10*-K	63 (15/24)	>99 (90/90)	+	ND
575	Tn*10*-K	60 (29/48)	>99 (86/86)	+	ND
672	Spontaneous	52 (13/25)	NR	+	−
683	Spontaneous	72 (18/25)	NR	+	−
691	Spontaneous	64 (16/25)	NR	+	ND
692	Spontaneous	32 (8/25)	NR	+	−
781	Spontaneous	47 (7/15)	NR	+	−
797	Spontaneous	67 (10/15)	NR	+	ND
798	Spontaneous	73 (11/15)	NR	+	ND
803	Spontaneous	53 (8/15)	NR	+	ND
813	Spontaneous	67 (10/15)	NR	+	ND
hip-115		ND	NR	+	−
hip-157		ND	NR	+	−

[a] Linkage to Tn*10-1230* was determined by P1 transduction from the *hip*⁺ donor strain MH5768 into the original Hir− mutant isolates selecting for Tet[r]. For Tn*10*-K mutations linkage is expressed as the percentage of Tet[r] transductants which were Kan[s]; for spontaneous mutations linkage indicated is the percentage of transductants which could support Mu growth as judged by death at 42°C in patch tests. Values in parentheses are the number of Tet[r] Hir⁺ transductants/total Tet[r] transductants tested. The value reported for *hip-157* is ~70% (48). ND, Not determined.

[b] For Tn*10*-K-induced mutations, linkage of Tn*10*-K to the Hir⁻ phenotype was determined by transducing the Mu lysogenic strain MH5893 to Kan[r] and scoring for cotransduction of the Hir⁻ phenotype (able to grow at 42°C). Linkage is expressed as the percentage of Kan[r] transductants which were also Hir⁻; numbers in parentheses indicate the number of Kan[r] Hir⁻ transductants/total Kan[r] transductants tested. NR, Not relevant.

[c] For all alleles except *672*, *683*, and *691*, complementation was assayed by spot-test plating of Mu *c*ts62 on λ *hip*11 lysogens of MH5844 derivatives carrying the indicated alleles. A + indicates that growth of Mu was comparable to that observed in Hip⁺ strains (efficiency of plating, ~1). For alleles *672*, *683*, and *691*, complementation was indicated by death at 42°C of λ *hip*11 lysogens of the original Hir⁻ mutants.

[d] Growth of D108 *c*ts10 was determined on the same strains used for complementation but without the λ *hip*11 prophage. The method used and representation of the results are the same as those described in Table 5, footnote *c*.

other genes located near *tna*::Tn*10*, we determined their linkage to a *gyrB*(Ts) allele and the level of plasmid DNA supercoiling in strains carrying these alleles. Linkage to the *gyrB*(Ts) allele was determined by P1 transduction of the *gyrB*(Ts) strain MH5714 to Tet[r] with P1 *vir* lysates grown on *tna*::Tn*10* derivatives of the original mutant isolates. Six alleles showed very tight linkage (95 to 98%) to the *gyrB*(Ts) locus (Table 9); however, the seventh, *696*, was found to confer temperature sensitivity at 42°C, making it impossible to distinguish *696* from the *gyrB*(Ts) allele. It is surprising that strains containing allele *696* are temperature sensitive, since it was originally selected by growth at 42°C. It is possible that an additional mutation was present in the original isolate, permitting its growth at 42°C.

Plasmid DNA isolated from each of these seven mutant strains was less negatively supercoiled than DNA from the parent strain (Fig. 2). DNAs from six of these strains showed similar levels of negative supercoiling, while DNA from the

TABLE 7. Distribution of mutations[a]

Type of mutation	No. of mutations		
	himA	*hip*	Other
Tn*10*-K induced	36	30	0
Tn*5* induced	8	12	0
Spontaneous	11	9	32

[a] Probable map positions in *himA*, *hip*, or other were assigned based on the observed linkage (in P1 transductions) to *zdh-201*::Tn*10* (99% linked to *himA42*; D. Lynn and M. M. Howe, unpublished observations) or to Tn*10-1230* (~70% linked to *hip-157*; 48). The four spontaneous mutations isolated from the Tn-mutagenized cultures are not included in the table.

TABLE 8. Identification of mutations located in or near *gyrA*

Mutant allele	% LInkage to *zei*::Tn*10*[a]			% Linkage to *gyrA*-Nal[r][b]
	Expt A	Expt B	Sum	
685	17 (18/104)	18 (15/83)	18 (33/187)	>98 (66/66)
697	24 (49/208)	8 (7/84)	19 (56/292)	98 (130/133)
812	20 (9/45)	16 (11/69)	18 (20/114)	99 (166/167)
912 (Nal[r])	12 (6/52)	ND[c]	ND	ND

[a] Linkage to *zei*::Tn*10* was determined in two experiments. In experiment A the original mutant isolates were transduced to Tet[r] with *zei*::Tn*10* from the Gyr⁺ strain MH6292, and transductants were tested for growth at 42°C. Linkage is expressed as the percentage of Tet[r] transductants which also became Hir⁺ as detected by death at 42°C. Numbers in parentheses indicate the number of Hir⁺ Tet[r] transductants/total Tet[r] transductants tested. For allele *912*-Nal[r] (MH6943), the values represent linkage of Nal[s] to *zei*::Tn*10* determined as described above, scoring for cotransduction of Nal[s] by inability to grow on LB-Nal plates. In experiment B derivatives of the original isolates carrying *zei*::Tn*10* were used as donor strains for P1 transduction of Tet[r] into the lysogenic strain. Linkage is expressed as the percentage (and number/total) of Tet[r] transductants acquiring the mutant allele as indicated by their ability to grow at 42°C. The column labeled Sum presents the linkages (expressed as above) determined from the sum of the results observed in experiments A and B.

[b] Linkage of the mutant alleles to the *gyrA*-Nal[r] allele was determined by transduction of MH6914 (*gyrA909*-Nal[r]) to Tet[r] with P1 grown on derivatives of the original isolates carrying *zei*::Tn*10* and scoring the transductants for loss of Nal[r] and acquisition of the Hir⁻ mutation. Colonies with recombinant phenotypes (i.e., Nal[r] Hir⁻ or Nal[s] Hir⁺) which could be recombinants or mixed colonies were reanalyzed after purification. The numbers represent the percent linkage of the Hir⁻ allele to the Nal[r] allele which was calculated as shown in parentheses by dividing the number of transductants which coinherited both donor Hir⁻ and Nal[s] alleles by the number inheriting at least one donor Hir⁻ or Nal[s] allele.

[c] ND, Not determined.

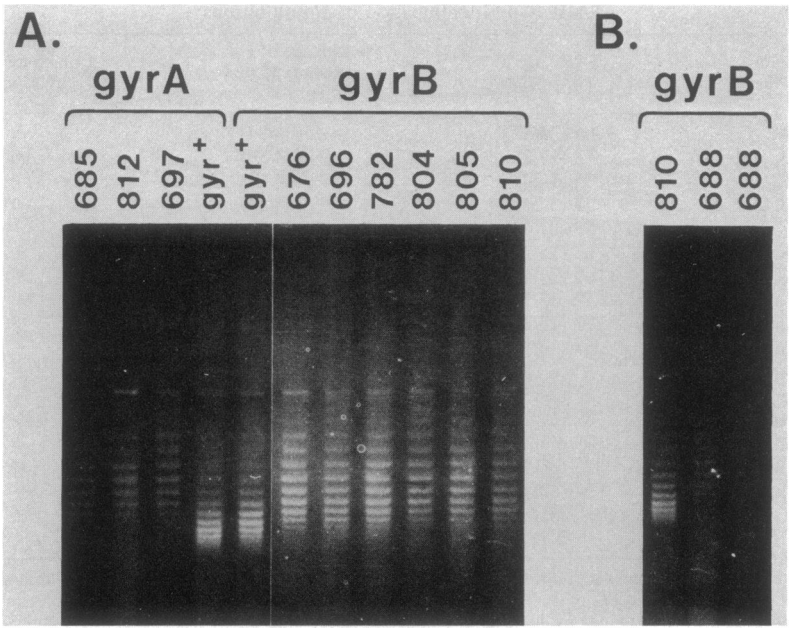

FIG. 2. Chloroquine-agarose gel electrophoresis of pBR322 DNA isolated from wild-type and mutant strains. Approximately equivalent amounts of pBR322 DNA isolated from strains carrying the mutant alleles indicated above each lane were electrophoresed on a 0.8% agarose gel containing either 10 μg of chloroquine per ml (A) or 7 μg of chloroquine per ml (B) for 14 h at 3 V/cm and 4°C. The species visualized on this gel are negatively supercoiled as judged by electrophoresis of these DNAs in a series of gels with different chloroquine concentrations (data not shown) (76). The strains used for DNA isolation were (from left to right): MH7460 (*gyrA685*), MH7462 (*gyrA812*), MH7461 (*gyrA697*), MH7452 (*gyrA*$^+$), MH7451 (*gyrB*$^+$), MH7463 (*gyrB676*), MH7464 (*gyrB696*), MH7465 (*gyrB782*), MH7466 (*gyrB804*), MH7467 (*gyrB805*), MH7468 (*gyrB810*), MH7470 (*gyrB810*), MH7469 (*gyrB688*), and MH7471 (*gyrB688*).

seventh, carrying allele *688*, was reproducibly less negatively supercoiled than the others (Fig. 2). These seven mutations are therefore believed to be in *gyrB* (or possibly very near *gyrB* affecting its expression).

Additional characterization of gyrase mutants. The results of two additional assays strengthened the identification of the latter two classes of Hir$^-$ mutants as *gyrA* and *gyrB* mutants. The first was the ability of these mutations to compensate for the deleterious effects on cell growth of a deletion of the topoisomerase I gene (12). Topoisomerase I introduces transient single-strand breaks into DNA, resulting in the relaxation of negative supercoils (13, 23). Mutations which inactivate topoisomerase I are deleterious to cell growth, presumably because the absence of topoisomerase I causes excess negative supercoiling in cellular DNA. Mutations in DNA gyrase which reduce the levels of negative supercoiling can compensate for the cell growth defect of a Δ*top* strain (12). Several of the *gyrA* and *gyrB* mutant strains were tested for their Δ*top* compensatory phenotype by assaying the frequency of cotransduction of a *top-cysB* deletion with a selected *trp*$^+$ marker. In the *gyrA*$^+$ and *gyrB*$^+$ control strains cotransduction was only 1.6%; in contrast, in the six *gyrA* and *gyrB* mutant strains tested cotransduction efficiencies were high, varying from 69 to 79%. These results clearly indicate that the Hir$^-$ mutations act as compensatory mutations for a topoisomerase I deletion, suggesting that they confer reduced levels of negative supercoiling upon the strain, consistent with the results of the plasmid supercoiling assays.

The second assay demonstrated that these mutant strains were altered in their degree of growth inhibition by various concentrations of the antibiotics nalidixic acid, novobiocin, and coumermycin A1 which inhibit gyrase (13, 23). When compared with the *gyr*$^+$ control strains, all 10 gyrase mutants showed either enhanced or decreased sensitivity to at least two and often all three of these antibiotics; they showed no difference in sensitivity to kanamycin or rifampin or in resistance to streptomycin or tetracycline. The patterns of the alterations in sensitivity were highly variable, exhibiting

TABLE 9. Identification of mutations located in or near *gyrB*

Mutant allele	% Linkage to *tna*::Tn*10*[a]			% Linkage to *gyrB*(Ts)[b]
	Expt A	Expt B	Sum	
676	58 (15/26)	74 (17/23)	65 (32/49)	98 (78/80)
688	87 (66/76)	ND[c]	ND	98 (43/44)
696	68 (53/78)	79 (19/24)	71 (72/102)	ND
782	99 (77/78)	70 (16/23)	92 (93/101)	97 (67/69)
804	73 (76/104)	95 (19/20)	77 (95/124)	97 (64/66)
805	95 (73/77)	60 (12/20)	88 (85/97)	95 (63/66)
810	97 (75/77)	90 (18/20)	96 (93/97)	97 (63/65)
gyrB(Ts)	89 (62/70)	ND	ND	ND

[a] In experiment A linkage to *tna*::Tn*10* was tested by P1 transduction from the *gyrB*$^+$ donor MH5715 into the original mutant isolates carrying the indicated alleles or into the *gyrB*(Ts) strain MH5714. The number of Tet^r transductants which acquired the Hir$^+$ allele (those lysogens which died at 42°C in patch tests) or the *gyrB*$^+$ allele (those MH5714 transductants which survived at 42°C in spot tests) is indicated as a percentage (and number/total) of Tet^r transductants tested. In experiment B, linkage was tested by P1 transduction from *tna*::Tn*10* derivatives of the original mutant isolates into MH5844. The percentage (and number/total) of Tet^r transductants acquiring the Hir$^-$ allele was determined in cross-streak assays. The sum of results from experiments A and B is also represented.

[b] Percent linkage of the mutant alleles to *gyrB*(Ts) was determined by P1 transduction from *tna*::Tn*10* derivatives of the original mutant isolates into the *gyrB*(Ts) recipient strain MH5714. The percentage (and number/total) of recombinants inheriting both the Gyr$^+$ (ability to grow at 42°C) and Hir$^-$ (inability to grow Mu *cts*62 in a cross-streak assay at 37°C) donor phenotypes among the Tet^r transductants acquiring at least one donor phenotype is indicated. [The *gyrB*(Ts) strain supports Mu growth at 37°C.]

[c] ND, Not determined.

no correlation between the alteration in sensitivity to different antibiotics. For example, one mutant might show increased sensitivity to one antibiotic, decreased sensitivity to the second antibiotic, and no alteration in sensitivity to the third antibiotic. Thus, the strains behaved as if different alterations in the gyrase subunits conferred different abilities of the enzyme to interact with and be inhibited by each antibiotic; the results could not easily be explained by alterations in the levels of wild-type gyrase protein. These results strengthen the identification of the Hir$^-$ mutations as *gyrA* and *gyrB* mutations and make it less likely that they are nearby mutations which affect gyrase expression.

Growth of Mu *c*ts62 and a Kil$^-$ Mu on gyrase mutants. On the basis of the premise of our mutant selection we did not expect to observe significant growth of Mu on our gyrase mutant strains; however, some of these mutants did support substantial growth of Mu *c*ts62 in spot-test assays. For one group of seven mutants (MH5844 derivatives carrying *gyrA* alleles *685* and *697* and *gyrB* alleles *676, 782, 804, 805,* and *810*), pinpoint plaques could be detected although not accurately quantitated when ~10^3 Mu *c*ts62 phage were spotted. A more severe growth inhibition was observed for the MH5844 derivative carrying the *gyrA812* allele; single plaques were not detectable, and cell lysis occurred only when ~10^5 phage were spotted. MH5844 derivatives carrying *gyrB696* and *gyrB688* alleles gave levels of Mu growth intermediate between those of the previous strains.

The degree of Mu growth exhibited by the first group of gyrase mutants appeared inconsistent with the basis for their selection. Nevertheless, for the alleles tested (*gyrA685* and *697*), P1 transduction experiments demonstrated that these alleles were sufficient to confer the Hir$^-$ phenotype on strain MH5893 (Table 8). We therefore compared the behavior of Mu pf7701 with that of Mu *c*ts62 on a subset of the DNA gyrase mutants (Table 10). Mu pf7701 is like the Mu Kil$^-$ phage used in our selection; its different markers (Δ445-3, Km^r, *M*$^+$) should not significantly alter phage growth. To quantitate the degree of inhibition of Mu *c*ts62 and Mu pf7701 growth in the gyrase mutant strains, we measured phage produced at various times after heat induction of lysogenic derivatives of these strains. Growth of Mu *c*ts62 was delayed in all the gyrase mutant hosts but varied considerably, reaching a level only twofold below that of the *gyr*$^+$ control for one mutant strain (Table 10). In contrast, growth of Mu pf7701 in the *gyr* mutant hosts was reduced at least 200-fold relative to that in the *gyr*$^+$ strain. Thus, the more extensive inhibition of Mu pf7701 growth was consistent with survival of the *gyr* Mu Kil$^-$ lysogens. As observed previously (Thompson and Howe, in preparation), growth of Mu pf7701 was somewhat delayed and reduced as compared with that of Mu *c*ts62 even in the *gyr*$^+$ host.

Other spontaneous mutants. An additional group of 22 spontaneous mutants showed an approximately 10-fold inhibition of growth of Mu *vir* in spot-test assays but normal growth of D108 which was comparable to that in a wild-type strain. These mutations have not yet been mapped or studied in detail.

TABLE 10. Phage release from gyrase mutant hosts[a]

Lysogen	*gyr* allele	Mu prophage	Phage titer after heat induction for:		
			1 h	2 h	3 h
MH7510	*gyr*$^+$	*c*ts	2.2×10^8	4.6×10^7	4.3×10^7
MH7507	*gyrA685*	*c*ts	2.0×10^7	9.3×10^7	7.6×10^7
MH7508	*gyrA697*	*c*ts	1.2×10^6	3.8×10^6	1.6×10^6
MH7509	*gyrA812*	*c*ts	8.4×10^3	~4×10^3	~1×10^3
MH7522	*gyr*$^+$	pf7701	1.7×10^6	3.2×10^7	3.0×10^7
MH7519	*gyrA685*	pf7701	2.6×10^5	1.4×10^5	6.9×10^4
MH7520	*gyrA697*	pf7701	2.9×10^4	8.6×10^4	9.2×10^4
MH7521	*gyrA812*	pf7701	1.3×10^2	$<1 \times 10^2$	~2×10^1
MH7501	*gyr*$^+$	*c*ts	2.7×10^8	3.6×10^8	4.5×10^7
MH7502	*gyrB782*	*c*ts	1.2×10^6	2.7×10^7	1.9×10^7
MH7503	*gyrB804*	*c*ts	4.8×10^6	2.4×10^7	4.2×10^7
MH7504	*gyr*$^+$	pf7701	~5×10^4	2.4×10^7	6.9×10^7
MH7505	*gyrB782*	pf7701	~6×10^1	~4×10^1	~6×10^1
MH7506	*gyrB804*	pf7701	2.4×10^2	6.6×10^2	3.4×10^3

[a] Lysogens were grown and heat induced as described in Materials and Methods. The free phage titer present after 1, 2, and 3 h of incubation at 42°C is given as phage per milliliter.

DISCUSSION

We developed a selection procedure for isolating *E. coli* mutants defective in replicative transposition of bacteriophage Mu. The selection is based on the finding that in the absence of the Mu Kil function, host-cell death occurs as a consequence of Mu replication (85). Thus, we expected that cells surviving induction of a Mu *c*ts Kil$^-$ lysogen would carry either phage or host mutations which would inhibit Mu replication.

The 142 Hir$^-$ mutants chosen for analysis were screened initially for P1 linkage to Tn*10*-Tet^r elements located near *himA* or *hip*, since mutations in these genes were known to inhibit Mu replication (Yoshida, Ph.D. thesis), or expression of Mu replication functions (27). All 86 of the Tn*5*- and Tn*10*-K-induced mutations and approximately one-third of the spontaneous mutations showed linkage to these Tn*10*-Tet^r elements and were, therefore, located in or near the *himA* or *hip* genes. Location within *himA* or *hip* was confirmed for a subset of these mutations by complementation of these strains for Mu growth by a plasmid carrying the *himA* gene or a lambda transducing phage carrying the *hip* gene, respectively.

To identify those strains most likely to contain other host mutations, the remaining mutants were further screened for their ability to support Mu *vir* and D108 growth in spot-test assays. Ten spontaneous mutants showing inhibition of D108 and Mu *vir* growth were found to contain mutations in *gyrA* or *gyrB*. These locations were based on both genetic and biochemical criteria: (i) P1 linkage to Tn*10* elements cotransducible with *gyrA* (*zei*::Tn*10*) or *gyrB* (*tna*::Tn*10*), (ii) very close P1 linkage to known alleles of *gyrA* (Nal^r) or *gyrB* [*gyrB*(Ts)], (iii) altered levels of supercoiling of plasmid DNA in mutant strains, (iv) ability to serve as compensatory mutations to prevent the deleterious effects on cell growth of a topoisomerase I mutation, and (v) altered sensitivity to the gyrase inhibitors nalidixic acid, novobiocin, and coumermycin A1. Consistent with the basis for their selection, Mu-specific DNA synthesis in hosts containing these mutations is inhibited or delayed (S. H. Shore, W. Ross, and M. M. Howe, manuscript in preparation).

An additional group of spontaneous mutants supported normal growth of phage D108 but showed an approximately 10-fold inhibition of growth of Mu *vir*. These mutants have not yet been further characterized. Since the Mu and D108 transposase proteins and *att* site sequences differ somewhat in specificity and sequence (5, 10, 80), these mutations might affect host functions that interact with these phage-specific sites or functions. Alternatively, they may be phage muta-

tions that are either dominant, thus inhibiting superinfecting Mu *vir* growth, or slightly leaky, such that a low level of transposition has occurred in these strains. Since the extent of Mu *vir* growth on Mu lysogens is dependent on the number of prophages present (84; W. Ross, unpublished observations), the higher prophage copy number in leaky mutants could result in the inhibition of Mu *vir* growth that was observed.

The detection of only *himA*, *hip*, *gyrA*, and *gyrB* mutations among those conferring a severe inhibition of Mu and D108 growth does not rule out the possibility that there are other host functions involved in transposition. Viable mutations in essential genes, specifically altering their function in Mu transposition, might occur at a much lower frequency than mutations in the IHF or DNA gyrase genes and thus have escaped our detection.

Mutations in IHF. Our selection procedure proved to be a useful way to isolate large numbers of mutations affecting the *himA* and *hip* genes; both Tn*5* and Tn*10*-K insertion mutations and spontaneous mutations were isolated. The isolation of insertion mutations in *himA* and *hip* corroborates previous results with deletion mutations, indicating that these genes are not essential for cell viability (48, 56). Nevertheless, IHF encoded by these two genes plays a role in many different systems in *E. coli*: the λ site-specific recombination system (48, 57, 58); packaging of certain lambdoid phage DNAs (3, 16); growth of phages with the *QSR* region of φ80 (64); and expression of a number of different genes, including *himA* (59), λ *c*II (41, 68), *ilv* and *xyl* (19, 20), and the Mu early operon (27; Yoshida, Ph.D. thesis; H. Krause and N. P. Higgins, personal communication). It is not yet known whether IHF plays a mechanistically similar or different role in these diverse systems. Our collection of IHF mutants, selected for inhibition of Mu replicative transposition, may be useful in further study of the multiple functions of IHF.

Growth of the closely related heteroimmune phage D108 is also inhibited in the *himA* and *hip* mutant strains. In Mu IHF interacts specifically with the early regulatory region (H. Krause and N. P. Higgins, personal communication) and exerts a positive regulatory effect on Mu *Pe* expression (27; H. Krause and N. P. Higgins, personal communication). Phage mutations permitting growth on *himA* or *hip* strains are also located in this region (26, 89; Yoshida, Ph.D. thesis). Although Mu and D108 share extensive homology (25), they are not homologous within this early regulatory region. Thus, it will be of interest to ascertain whether IHF regulation of D108 occurs by a similar mechanism and whether IHF also exerts a regulatory influence at additional sites shared by these two phages.

All the Tn*5*- and Tn*10*-K-induced mutations isolated by our selection procedure mapped in or near only two genes, *himA* or *hip*. It is unlikely that this is due to transposon insertion site preference, since similar results were obtained with both transposons, and both are known to utilize many target sites. We believe that if other nonessential *E. coli* functions were required for Mu transposition, the number of insertion events ($\sim 10^6$ events per culture) was sufficiently large to have permitted their detection. Although the steps involved in our selection procedure (selection at 42°C in liquid medium, and P1 transduction of the inserted transposons) could have eliminated certain kinds of mutants, our results suggest the possibility that there are no other nonessential genes required for Mu transposition.

Mutations in DNA gyrase. Of the 52 spontaneous mutations chosen for further analysis, 10 were identified as being in one of the genes encoding the two subunits of DNA gyrase, *gyrA* (3 mutants) or *gyrB* (7 mutants). No insertion mutations were found in these genes, as was expected, since they are known to be essential for cell growth (51, 67).

Mutations in DNA gyrase were previously reported to inhibit Mu growth (12, 21, 24, 58), but the mechanism of growth inhibition was not known. The premise of our selection, that cells surviving Mu *c*ts Kil$^-$ induction are unable to support Mu transposition, strongly suggests that Mu growth is inhibited in our gyrase mutants at the level of Mu DNA replication. Analysis of Mu-specific DNA synthesis demonstrated an inhibition of Mu replication commensurate with the degree of growth inhibition (Shore et al., in preparation). Consistent with the conclusion that DNA gyrase is required in some capacity for Mu transposition is the observation that addition of novobiocin (an inhibitor of the *gyrB* subunit) to host-cell extracts inhibits Mu transposition in vitro (63).

The isolation of mutations in both DNA gyrase subunits suggests a requirement for DNA gyrase itself, rather than independent roles for each subunit in transposition; however, the specific role of gyrase in Mu transposition is not yet understood. DNA gyrase activity is required for cellular DNA replication and recombination, and the levels of DNA supercoiling produced by gyrase action also influence the efficiency of transcription initiation from many promoters (for reviews, see references 13, 23, and 77). Thus, DNA gyrase may be required directly in the Mu DNA replication process, or its role may be indirect, exerted through maintenance of a required level of DNA supercoiling. DNA supercoiling could affect any of several possible functions, for example, (i) transcription of necessary host functions or the Mu transposition functions A and B, or (ii) generation of the appropriate DNA conformation of the donor and target sites for Mu transposition. A role for DNA gyrase other than, or in addition to, transcription of Mu functions is suggested by the requirement for a supercoiled Mu DNA donor molecule (8) and functional activity for at least the *gyrB* subunit (63) in an in vitro transposition system in which Mu functions A and B are provided. Similarly, the inhibition of Tn*5* transposition with coumermycin A1 without apparent alteration of expression from the Tn*5* transposase promoter indicates that in this system there is also a requirement for DNA gyrase at a level other than transcription initiation (45).

Growth of the Kil$^+$ phage, Mu *c*ts62, on the gyrase mutant strains was delayed and somewhat reduced but in most cases was not blocked. The delay and slight reduction are sufficient to account for the failure of Mu *c*ts62 to form readily distinguishable plaques on these strains. It is interesting to note that the Mu growth properties of strains carrying the *gyrA* alleles differ and do not strictly correlate with the effects of these mutations on cellular growth rates. Alleles *697* and *812* confer comparable, though small, increases in cell doubling time, yet differ dramatically in their ability to support Mu growth. We do not yet know whether possible small differences in the levels of DNA supercoiling, not readily detectable in our gel assays, are sufficient to explain these different phenotypes or whether the mutants are affected in other possible functions that gyrase may carry out.

Although the extensive growth of Mu *c*ts62 on some of these mutant strains appeared inconsistent with the basis for the mutant selection, the significantly reduced growth of Mu pf7701 (which carries the same Kil$^-$ 7701-1 deletion as the phage used in the selection) explains the survival of the *gyr* mutant lysogens. It appears, therefore, that the nonessential region of Mu DNA deleted by Δ7701-1 (4.4 to 7.2 kb from the

Mu left end) becomes more important for growth under the conditions of limiting or modified DNA gyrase activity in these gyrase mutant strains. This suggests the possibility that this region, which is nonessential for growth in *E. coli*, might be required for Mu growth in other species.

Although not lethal to Mu in wild-type *E. coli*, Δ7701-1 does influence the kinetics of Mu growth. Mu pf7701 forms very small plaques, and lysis after induction of a Mu pf7701 lysogen is delayed by approximately 1 to 1.5 h (Thompson and Howe, in preparation; W. Ross, unpublished data). Similarly, in Mu 13[4] (28), another Mu phage that is unable to express functions downstream of gene *B* owing to a polar IS1 insertion, Mu DNA replication is delayed and phage growth is severely inhibited. Both of these phage may be defective in expression of the two regions involved in stimulation of Mu DNA replication and enhancement of levels of active Mu A and B proteins (Waggoner et al., submitted). The functions of these two regions are not yet understood; however, one possibility is that they lead to greater stability or increased production of the A protein. Thus, the severe inhibition of Mu pf7701 growth in the gyrase mutant strains might reflect a limitation in the concentrations of A protein in these strains in the absence of these functions. Alternatively, DNA gyrase and these functions might carry out similar roles in the transposition reaction and partially substitute for one another. An understanding of the basis of Mu pf7701 growth inhibition in the gyrase mutant strains must await further definition of both the nature of these replication-enhancing functions and the requirement for DNA gyrase for normal growth of wild-type Mu.

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LITERATURE CITED

1. **Allet, B.** 1979. Mu insertion duplicates a 5 base pair sequence at the host inserted site. Cell **16**:123–129.
2. **Alper, M. D., and B. N. Ames.** 1975. Positive selection of mutants with deletions of the *gal-chl* region of the *Salmonella* chromosome as a screening procedure for mutagens that cause deletions. J. Bacteriol. **121**:259–266.
3. **Bear, S. E., D. L. Court, and D. I. Friedman.** 1984. An accessory role for *Escherichia coli* integration host factor: characterization of a lambda mutant dependent upon integration host factor for DNA packaging. J. Virol. **52**:966–972.
4. **Bukhari, A. I.** 1976. Bacteriophage Mu as a transposition element. Annu. Rev. Genet. **10**:389–412.
5. **Bukhari, A. I., J. R. Lupski, P. Svec, and G. N. Godson.** 1985. Comparison of left-end DNA sequences of bacteriophages Mu and D108. Gene **33**:235–239.
6. **Chumley, F. G., R. Menzel, and J. R. Roth.** 1979. Hfr formation directed by Tn*10*. Genetics **91**:639–655.
7. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
8. **Craigie, R., D. J. Arndt-Jovin, and K. Mizuuchi.** 1985. A defined system for the DNA strand-transfer reaction at the initiation of bacteriophage Mu transposition: protein and DNA substrate requirements. Proc. Natl. Acad. Sci. USA **82**:7570–7574.
9. **Craigie, R., and K. Mizuuchi.** 1985. Mechanism of transposition of bacteriophage Mu: structure of a transposition intermediate. Cell **41**:867–876.
10. **Craigie, R., M. Mizuuchi, and K. Mizuuchi.** 1984. Site-specific recognition of the bacteriophage Mu ends by the Mu A protein. Cell **39**:387–394.
11. **Degnen, G. E., and E. C. Cox.** 1974. Conditional mutator gene in *Escherichia coli*: isolation, mapping and effector studies. J. Bacteriol. **117**:477–487.
12. **DiNardo, S., K. A. Voelkel, R. Sternglanz, A. E. Reynolds, and A. Wright.** 1982. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. Cell **31**:43–51.
13. **Drlica, K.** 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. **48**:273–289.
14. **DuBow, M. S., and A. I. Bukhari.** 1980. Effect of prophage Mu induction on expression of adjacent host genes. Mol. Biol. Rep. **6**:229–234.
15. **Faelen, M., and A. Toussaint.** 1973. Isolation of conditional defective mutants of temperate phage Mu-1 and deletion mapping of the Mu-1 prophage. Virology **54**:117–124.
16. **Feiss, M., S. Frackman, and J. Sippy.** 1985. Essential interaction between lambdoid phage 21 terminase and the *Escherichia coli* integrative host factor. J. Mol. Biol. **183**:239–249.
17. **Flamm, E. L., and R. A. Weisberg.** 1985. Primary structure of the *hip* gene of *Escherichia coli* and of its product, the β subunit of integration host factor. J. Mol. Biol. **183**:117–128.
18. **Foster, T. J., M. A. Davis, D. E. Roberts, K. Takeshita, and N. Kleckner.** 1981. Genetic organization of transposon Tn*10*. Cell **23**:201–213.
19. **Friden, P., K. Voelkel, R. Sternglanz, and M. Freundlich.** 1984. Reduced expression of the isoleucine and valine enzymes in integration host factor mutants of *Escherichia coli*. J. Mol. Biol. **172**:573–579.
20. **Friedman, D. I., E. J. Olson, D. Carver, and M. Gellert.** 1984. Synergistic effect of *himA* and *gyrB* mutations: evidence that Him functions control expression of *ilv* and *xyl* genes. J. Bacteriol. **157**:484–489.
21. **Friedman, D. I., L. C. Plantefaber, E. J. Olson, D. Carver, M. H. O'Dea, and M. Gellert.** 1984. Mutations in the DNA *gyrB* gene that are temperature sensitive for lambda site-specific recombination, Mu growth, and plasmid maintenance. J. Bacteriol. **157**:490–497.
22. **Galas, D. J., and M. Chandler.** 1981. On the molecular mechanisms of transposition. Proc. Natl. Acad. Sci. USA **78**:4858–4862.
23. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
24. **Ghelardini, P., J. C. Liebart, C. Marchelli, A. M. Pedrini, and L. Paolozzi.** 1984. *Escherichia coli* K-12 *gyrB* gene product is involved in the lethal effect of the *lig*ts2 mutant of bacteriophage Mu. J. Bacteriol. **157**:665–668.
25. **Gill, G. S., R. C. Hull, and R. Curtiss III.** 1981. Mutator bacteriophage D108 and its DNA: an electron microscopic characterization. J. Virol. **37**:420–430.
26. **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.
27. **Goosen, N., M. van Heuvel, G. F. Moolenaar, and P. van de Putte.** 1984. Regulation of Mu transposition. II. The *Escherichia coli* HimD protein positively controls two repressor promoters and the early promoter of bacteriophage Mu. Gene **32**:419–426.
28. **Goosen, T., M. Giphart-Gassler, and P. van de Putte.** 1982. Bacteriophage Mu DNA replication is stimulated by nonessential early functions. Mol. Gen. Genet. **186**:135–139.
29. **Grindley, N. D. F., and D. J. Sherratt.** 1978. Sequence analysis at IS1 insertion sites: models for transposition. Cold Spring

Harbor Symp. Quant. Biol. **43**:1257–1261.
30. **Groenen, M. A. M., E. Timmers, and P. van de Putte.** 1985. DNA sequences at the ends of the genome of bacteriophage Mu essential for transposition. Proc. Natl. Acad. Sci. USA **82**:2087–2091.
31. **Grundy, F. J., and M. M. Howe.** 1984. Involvement of the invertible G segment in bacteriophage Mu tail fiber biosynthesis. Virology **134**:296–317.
32. **Grundy, F. J., and M. M. Howe.** 1985. Morphogenetic structures present in lysates of amber mutants of bacteriophage Mu. Virology **143**:485–504.
33. **Harshey, R. M., D. L. Baldwin, E. D. Getzoff, J. L. Miller, and G. Chaconas.** 1985. Primary structure of phage Mu transposase: homology to Mu repressor. Proc. Natl. Acad. Sci. USA **82**:7676–7680.
34. **Harshey, R. M., and A. I. Bukhari.** 1981. A mechanism of DNA transposition. Proc. Natl. Acad. Sci. USA **78**:1090–1094.
35. **Higgins, N. P., P. Manlapaz-Ramos, R. T. Gandhi, and B. M. Olivera.** 1983. Bacteriophage Mu: a transposing replicon. Cell **33**:623–628.
36. **Higgins, N. P., D. Moncecchi, P. Manlapaz-Ramos, and B. M. Olivera.** 1983. Bacteriophage Mu DNA replication in vitro. J. Biol. Chem. **258**:4293–4297.
37. **Holmes, D. S., and M. Quigly.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
38. **Hopkins, J. D., M. Clements, and M. Syvanen.** 1983. New class of mutations in *Escherichia coli* (*uup*) that affect precise excision of insertion elements and bacteriophage Mu growth. J. Bacteriol. **153**:384–389.
39. **Howe, M. M.** 1973. Prophage deletion mapping of bacteriophage Mu-1. Virology **54**:93–101.
40. **Howe, M. M., J. W. Schumm, and A. L. Taylor.** 1979. The *S* and *U* genes of bacteriophage Mu are located in the invertible G segment of Mu DNA. Virology **92**:108–124.
41. **Hoyt, M. A., D. M. Knight, A. Das, H. I. Miller, and H. Echols.** 1982. Control of phage λ development by stability and synthesis of *c*II protein: role of the viral *c*III and host *hflA*, *himA* and *himD* genes. Cell **31**:565–573.
42. **Hull, R. C., G. S. Gill, and R. Curtiss III.** 1978. Genetic characterization of Mu-like bacteriophage D108. J. Virol. **27**:513–518.
43. **Ilyina, T. S., E. V. Nechaeva, Y. M. Romanova, and G. B. Smirnov.** 1981. Isolation and mapping of *Escherichia coli* K12 mutants defective in Tn*9* transposition. Mol. Gen. Genet. **181**:384–389.
44. **Ilyina, T. S., Y. M. Romanova, and G. B. Smirnov.** 1981. The effect of *tnm* mutations of *Escherichia coli* K12 on transposition of various movable genetic elements. Mol. Gen. Genet. **183**:376–379.
45. **Isberg, R. R., and M. Syvanen.** 1982. DNA gyrase is a host factor required for transposition of Tn*5*. Cell **30**:9–18.
46. **Johnson, R. C., J. C. P. Yin, and W. S. Reznikoff.** 1982. Control of Tn*5* transposition in *Escherichia coli* is mediated by protein from the right repeat. Cell **30**:873–882.
47. **Kahmann, R., and D. Kamp.** 1979. Nucleotide sequences of the attachment sites of bacteriophage Mu DNA. Nature (London) **280**:247–250.
48. **Kikuchi, A., E. Flamm, and R. A. Weisberg.** 1985. An *Escherichia coli* mutant unable to support site-specific recombination of bacteriophage λ. J. Mol. Biol. **183**:129–140.
49. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
50. **Kondo, E., and S. Mitsuhashi.** 1964. Drug resistance of enteric bacteria. IV. Active transducing bacteriophage P1CM produced by the combination of R factor with bacteriophage P1. J. Bacteriol. **88**:1266–1276.
51. **Kreuzer, K. N., and N. R. Cozzarelli.** 1979. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. J. Bacteriol. **140**:424–435.
52. **Ljungquist, E., and A. I. Bukhari.** 1977. State of prophage Mu DNA upon induction. Proc. Natl. Acad. Sci. USA **74**:3143–3147.
53. **Ljungquist, E., and A. I. Bukhari.** 1979. Behavior of bacteriophage Mu DNA upon infection of *Escherichia coli* cells. J. Mol. Biol. **133**:339–357.
54. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
55. **Marrs, C. F., and M. M. Howe.** 1983. *Ava*II and *Bgl*I restriction maps of bacteriophage Mu. Virology **126**:563–575.
56. **Miller, H. I.** 1984. Primary structure of the *him*A gene of *Escherichia coli*: homology with DNA-binding proteins HU and association with the phenylalanyl-tRNA synthetase operon. Cold Spring Harbor Symp. Quant. Biol. **49**:691–698.
57. **Miller, H. I., and D. I. Friedman.** 1980. An *E. coli* gene product required for lambda site-specific recombination. Cell **20**:711–719.
58. **Miller, H. I., A. Kikuchi, H. A. Nash, R. A. Weisberg, and D. I. Friedman.** 1979. Site-specific recombination of bacteriophage λ: the role of host gene products. Cold Spring Harbor Symp. Quant. Biol. **43**:1121–1126.
59. **Miller, H. I., M. Kirk, and H. Echols.** 1981. SOS induction and autoregulation of the *himA* gene for site-specific recombination in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:6754–6758.
60. **Miller, H. I., and H. A. Nash.** 1981. Direct role of the *himA* gene product in phage lambda integration. Nature (London) **290**:523–526.
61. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 229. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
62. **Miller, J. L., S. K. Anderson, D. J. Fujita, G. Chaconas, D. L. Baldwin, and R. M. Harshey.** 1984. The nucleotide sequence of the *B* gene of bacteriophage Mu. Nucleic Acids Res. **12**:8627–8638.
63. **Mizuuchi, K.** 1983. In vitro transposition of bacteriophage Mu: a biochemical approach to a novel replication reaction. Cell **35**:785–794.
64. **Mozola, M. A., and D. I. Friedman.** 1985. A φ80 function inhibitory for growth of lambdoid phage in *him* mutants of *Escherichia coli* deficient in integration host factor. I. Genetic analysis of the Rha phenotype. Virology **140**:313–327.
65. **O'Day, K., D. Schultz, W. Ericsen, L. Rawluk, and M. Howe.** 1979. Correction and refinement of the genetic map of bacteriophage Mu. Virology **93**:320–328.
66. **O'Day, K. J., D. W. Schultz, and M. M. Howe.** 1978. Search for integration-deficient mutants of bacteriophage Mu, p. 48–51. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
67. **Orr, E., N. F. Fairweather, I. B. Holland, and R. H. Pritchard.** 1979. Isolation and characterization of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K12. Mol. Gen. Genet. **177**:103–112.
68. **Peacock, S., H. Weissbach, and H. A. Nash.** 1984. In vitro regulation of phage lambda *c*II gene expression by *Escherichia coli* integration host factor. Proc. Natl. Acad. Sci. USA **81**:6009–6013.
69. **Priess, H., D. Kamp, R. Kahmann, B. Bräuer, and H. Delius.** 1982. Nucleotide sequence of the immunity region of bacteriophage Mu. Mol. Gen. Genet. **186**:315–321.
70. **Pruss, G. J., S. H. Manes, and K. Drlica.** 1982. *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. Cell **31**:35–42.
71. **Résibois, A., M. Pato, P. Higgins, and A. Toussaint.** 1984. Replication of bacteriophage Mu and its mini-Mu derivatives, p. 69–76. *In* U. Hübscher and S. Spaderi (ed.), Proteins involved in DNA replication. Plenum Publishing Corp., New York.
72. **Rothstein, S. J., R. A. Jorgensen, J. C.-P. Yin, Z. Yond-di, R. C. Johnson, and W. S. Reznikoff.** 1981. Genetic organization of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45**:99–105.
73. **Schaus, N. A., and A. Wright.** 1980. Inhibition of *Escherichia coli* exonuclease V by bacteriophage Mu. Virology **102**:214–217.
74. **Schumm, J. W., D. D. Moore, F. R. Blattner, and M. M. Howe.** 1980. Correlation of the genetic and physical maps in the central region of the bacteriophage Mu genome. Virology **105**:185–195.
75. **Shapiro, J. A.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable ele-

ments. Proc. Natl. Acad. Sci. USA **76**:1933–1937.
76. **Shure, M., D. E. Pulleyblank, and J. Vinograd.** 1977. The problems of eukaryotic and prokaryotic DNA packaging and *in vivo* conformation posed by superhelix density heterogeneity. Nucleic Acids Res. **4**:1183–1205.
77. **Smith, G. R.** 1981. DNA supercoiling: another level for regulating gene expression. Cell **24**:599–600.
78. **Sutcliff, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
79. **Toussaint, A., and M. Faelen.** 1974. The dependence of phage Mu-1 upon the replication functions of *E. coli* K12. Mol. Gen. Genet. **131**:209–213.
80. **Toussaint, A., M. Faelen, L. Desmet, and B. Allet.** 1983. The products of gene *A* of the related phages Mu and D108 differ in their specificities. Mol. Gen. Genet. **190**:70–79.
81. **Toussaint, A., and A. Résibois.** 1983. Phage Mu: transposition as a life style, p. 105–158. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
82. **van de Putte, P., M. Giphart-Gassler, T. Goosen, A. van Meeteren, and C. Wijffelman.** 1978. Is integration essential for Mu development?, p. 33–40. *In* P. Hofschneider and P. Starlinger (ed.), Integration and excision of DNA molecules. Springer-Verlag KG, Berlin.
83. **van de Putte, P., G. C. Westmass, and C. Wijffelman.** 1977. Transfection with Mu DNA. Virology **81**:152–159.
84. **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.
85. **Waggoner, B. T., C. F. Marrs, M. M. Howe, and M. L. Pato.** 1984. Multiple factors and processes involved in host cell killing by bacteriophage Mu: characterization and mapping. Virology **136**:168–185.
86. **Waggoner, B. T., M. L. Pato, A. Toussaint, and M. Faelen.** 1981. Replication of mini-Mu prophage DNA. Virology **113**:379–387.
87. **Wijffelman, C., M. Gassler, W. F. Stevens, and P. van de Putte.** 1974. On the control of transcription of bacteriophage Mu-1. Mol. Gen. Genet. **131**:85–96.
88. **Wijffelman, C., and B. Lotterman.** 1977. Kinetics of Mu DNA synthesis. Mol. Gen. Genet. **151**:169–174.
89. **Yoshida, R. K., J. L. Miller, H. I. Miller, D. I. Friedman, and M. M. Howe.** 1982. Isolation and mapping of Mu *nu* mutants which grow in *him* mutants of *E. coli*. Virology **120**:269–272.