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Isolation and Genetic Characterization of *Escherichia coli* K-12 Mutations Affecting Bacteriophage T5 Restriction by the ColIb Plasmid

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A mutant derivative of *Escherichia coli* K-12 has been isolated which is permissive for bacteriophage T5 infection even when harboring a wild-type ColIb plasmid. The fully permissive phenotype was the result of two mutations that are located near the *rpsL-rpsE* region on the *E. coli* chromosome and are recessive to the wild-type alleles. These mutations had little or no effect on induction of colicin synthesis and did not affect the expression of antibiotic resistance by the resistance plasmids R64*drd*11 or R1*drd*19. Cells harboring the mutant alleles grew more slowly than isogenic wild-type derivatives in either minimal or complete media.

Strains of *Escherichia coli* K-12 harboring the conjugative plasmid ColIb (ColIb$^+$) acquire various properties not observed in non-colicinogenic (ColIb$^-$) strains. These include: the genetic potential to synthesize the antibiotic protein colicin Ib, along with specific immunity to the action of this colicin; the ability to conjugally transfer nonchromosomal genetic material; and an increased capability for repairing chromosomal deoxyribonucleic acid (DNA) damaged by ultraviolet irradiation (7). In addition, ColIb$^+$ cells are nonpermissive as hosts for bacteriophage T5 and the related phage BF23 (26).

Infection of a non-colicinogenic permissive host with T5 proceeds in the following manner. After adsorption of the phage particle, an initial 8% fraction of the phage genome is injected into the host (13). This first-step-transfer region of the genome contains genes for the synthesis of pre-early (class 1) phage proteins, two of which are requisite to the injection of the remaining portion of the phage chromosome (17). Phage proteins necessary for expression of early (class II) and late (class III) functions are encoded in the 92%, second-step-transfer fraction and are transcribed sequentially after secondary transfer in a calcium-dependent process (16, 25).

When cells harboring the ColIb plasmid are infected with T5, the phage injects its genetic material and expresses pre-early functions normally but is unable to transcribe genes for early or late functions. Phage DNA remains intact (22). The phage is thus restricted from the normal lytic cycle and the infection is aborted. Such infected cultures lyse prematurely, 15 min after infection, with no release of progeny phage (23). A thorough review of the growth of T5 in both restrictive and permissive hosts has recently been presented by McCorquodale (15).

Phage mutants able to grow in ColIb$^+$ cells have been isolated and lack a phage-specific class I protein (h) normally produced by wild-type phage (18). Herman and Moyer (8) have presented evidence to suggest that an early protein present in limited amounts after infection of a ColIb$^+$ host is also necessary for ColIb-mediated restriction. However, other explanations for their findings have been proposed (15).

Evidence for host cell involvement in the restriction process was first presented by Nisioka and Ozeki (23). They observed that the relative plaque-forming ability of BF23 on ColIb$^+$ strains was about 10^{-6} when phage were mixed with cells in soft agar and plated directly but that the plating efficiency increased to 10^{-2} when phage were preadsorbed to the cells and plated as infective centers. In the former experiment, plaques formed contained host range (h) mutants of BF23 which were subsequently able to plate with high efficiency on ColIb$^+$ cultures. Phage from plated infected centers, however, could not grow normally upon reinfection to ColIb$^+$ cells. They attributed this escaped growth to "certain physiological conditions of the host bacteria." In 1972, Moyer et al. (22) observed that certain *E. coli* strains were more

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restrictive than others even when harboring the same ColIb plasmid. They concluded that the degree of phage restriction was in some way dependent on the host bacterial strain harboring the plasmid.

In this study, we report the isolation of a mutant derivative of *E. coli* K-12 that is permissive for T5 infection even in the presence of a wild-type ColIb plasmid. The relative map positions of the genetic loci involved, along with their effect on cell growth rate and other plasmid functions, have also been determined.

MATERIALS AND METHODS

Media. The complete medium used was nutrient broth extract no. 2, purchased from Wilson Diagnostics. Mueller-Hinton agar was purchased from Difco. The complex medium is that described by Davis and Mingioli (5) and was supplemented with 0.5% glucose, 0.2% required amino acids, and 0.02% thiamine. Liquid medium was solidified for plates using 1.25% Davis New Zealand agar. For phage experiments, media were supplemented with 1 mM $CaCl_2$.

Bacteria and bacteriophage. All bacteria used in this study are derivatives of *E. coli* K-12 and are listed in Table 1. Bacteriophage T5 was obtained from the collection of D. Mount.

Mutagenesis. Cells from a 5-ml aliquot of an exponentially growing nutrient broth culture were collected by centrifugation at 5,000 × *g* for 5 min, washed once in 0.1 M sodium citrate (pH 5.0), and resuspended in 0.1 m sodium citrate (pH 5.0) with 100 μg of NTG (*N*-methyl-*N*-nitro-*N*-nitrosoguanidine) per ml (1). After 15 min of incubation at 37 C, the cells were washed twice with saline and streaked onto nutrient agar plates for single colonies. Survival after mutagenesis was about 50%.

Genetical methods. Bacterial matings using Hfr strains were performed as described earlier (21).

Transduction using P1*vir* were performed using the procedure previously described (9), except that a multiplicity of 1.0 was used for strain construction.

Merodiploid derivatives were prepared in the following manner. Fresh single colonies of strain KLF41/JC1553 were "patched" to a nutrient agar plate and allowed to grow for 3 h at 37 C. These were then replica plated to minimal agar plates which contained maltose as the sole carbon source and lacked methionine and which were spread with 10^9 cells from an overnight culture of strain UA490 *malT* (ColIb). After 48 h of incubation at 37 C, transconjugates from the patches were purified and tested for their ability to transfer *malT*$^+$, *argG*$^+$, *crp*$^+$, *rpsL*$^+$, and *aroE*$^+$ and for sensitivity to streptomycin. Isolates meeting these requirements were then grown to high density and stored at −20 C in 20% glycerol for later use.

Colicinogeny methods. Procedures for transfer of ColIb and resistance plasmids, as well as for determination of lacunae production and isolation of colicin Ib-resistant derivatives, are those described by Monk and Clowes (19, 20).

TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Source
DM506	F$^-$ *thr-1 leu-6 proA2 his-4 argE3 mtl-1 xyl-5 tsx-33 rpsL31 nalA* λ$^-$ *ara-14 lacY1 galK2 thi-1 sup-37*	D. Mount
UA35	As strain DM506, also *tonB*[b] ColIb$^+$	This paper
UA77	As strain UA35, also *cmrA2 cmrB2*[c]	This paper
UA493	F$^-$ *aroE353 rpsE* λ$^-$	Spectinomycin-resistant derivative of strain AB2828 from I. G. Young (24)
UA389	As strain DM506, also ColIb$^+$	Plasmid from UA77
UA482	As strain UA35, also *xyl*$^+$	This paper
UA483	As strain UA35, also *xyl*$^+$ *cmrA2 cmrB2*	This paper
UA 489	As strain DM506, also *xyl*$^+$	This paper
UA490	As strain DM506, also *xyl*$^+$ *cmrA2 cmrB2*	This paper
UA491	As strain UA489, also ColIb$^+$	This paper
UA492	As strain UA490, also ColIb$^+$	This paper
χ974	F$^-$ *thr leu ara minA tsx xyl minB dnaB malT rpsL thi mtl* λ$^-$	R. Curtiss III
HfrH	Hfr *thi-1* λ$^-$ *rel-1*	K. B. Low
KL14	Hfr *thi-1 rel-1* λ$^-$	K. B. Low
RA2	Hfr *mal-28* λ^r *sfa-4* λ$^-$	K. B. Low
PK191	Hfr *thi-1* Δ *(proB-lac)*X111 *sup-56* λ$^-$	K. B. Low
KL228	Hfr *thi-1 leu-6 sup-54 lacY1* or *lac-Z4 gal-6* λ$^-$	K. B. Low
P4X		K. B. Low
KLF41/JC1553	Hfr *metB1 rel-1* λ$^-$ F′ PO12 *argG*$^+$ *rpsL*$^+$ *malT*$^+$/*argG6 metB1 malT1 gal-6 lacY1 rpsL104 tonA2 tsx-1 supE44* λ$^-$ *recA*	K. B. Low (14)

[a] All gene symbols are from Bachmann et al. (2), except where otherwise noted.

[b] This was isolated as a mutant spontaneously resistant to colicin Ib.

[c] We propose to call the two chromosomal genes involved in ColIb-mediated restriction of bacteriophage T5 *cmrA* and *cmrB* in accordance with the proposals of Demerec et al. (6).

Single-step growth kinetics. Cells from an exponentially growing culture were collected by centrifugation and resuspended in nutrient broth at a titer of 10^9/ml. This culture was preincubated for 2 min with 1 mM potassium cyanide and then infected with phage at a multiplicity of 0.1. After 15 min of further incubation, a sample was diluted 10^5-fold into prewarmed nutrient broth and incubated at 37 C with vigorous aeration. Samples were withdrawn at various times, diluted appropriately, and plated for infective centers on nutrient agar. Strain DM506 was used as the T5-sensitive indicator strain.

RESULTS

Isolation of permissive ColIb$^+$ mutants. Strain UA35 was prepared by introducing a ColIb plasmid into a spontaneous colicin Ib-resistant mutant of strain DM506. An exponentially growing nutrient broth culture of strain UA35 was mutagenized with NTG and streaked on nutrient agar for single colonies. After overnight incubation at 37 C, single colonies were picked and tested for their ability to support growth of phage T5. This was done by cross-streaking T5 with each isolate on a nutrient agar plate. Isolates with wild-type restriction characteristics grow equally well before and past the phage streak, but growth of ColIb$^-$ or permissive ColIb$^+$ mutants is inhibited by the phage. Of 1,000 colonies tested, two, strains UA67 and UA77, were identified as permissive and were further purified. The strain UA67 mutant was subsequently found to be permissive as the result of a host mutation that reduced plasmid stability but did not affect phage restriction directly. Characterization of this derivative will be reported elsewhere. The plasmid from strain UA77 was transferred via an intermediate strain to an unmutagenized DM506 host. The plaque-forming ability of T5 on this strain, UA389, along with UA77 and appropriate parent derivatives, is shown in Table 2. The derivation of other bacterial strains presented in Table 2 is described below. These results show that the mutant defective in ColIb-*m*ediated *r*estriction (CMR) of T5 is nearly as permissive as the ColIb$^-$ parent and that the permissive character is not transferred when the plasmid from strain UA77 is moved to an unmutagenized strain DM506 host. ColIb$^+$ strains that allow T5 to plate with an efficiency similar to strain UA77 are defined as CMR$^-$.

To demonstrate that the CMR$^-$ phenotype is due entirely to host functions rather than the result of cooperative alterations to host and plasmid genes, it would be desirable to introduce an unmutagenized ColIb plasmid into a UA77 derivative, previously cured of ColIb. Attempts at this were frustrated by an inability to cure strain UA77 because of the extreme stability of the resident plasmid. Instead, the CMR$^-$ phenotype was transferred to unmutagenized ColIb$^+$ and ColIb$^-$ strains, UA35 and DM506, in the following manner. It was apparent from Hfr mapping data shown below that the genetic locus (loci) responsible for CMR$^-$ was linked to the *rspL* gene, which specifies synthesis of the 30S ribosomal subunit protein and determines resistance or sensitivity to streptomycin. Therefore, the streptomycin-sensitive Hfr strain KL14 was transduced to *rpsL31* (streptomycin resistance) using P1 phage grown on strain UA77. A ColIb plasmid was then introduced into each of these recombinants, and the colicinogenic derivatives were then scored for CMR. One of these KL14*rspL31* CMR$^-$ (ColIb$^-$) recombinants was then used as a donor strain in matings with UA35 and DM506. *xyl*$^+$*nalA* recombinants were selected and scored for inheritance of the CMR$^-$ phenotype. In this manner, strains UA482 and UA483 were constructed from strain UA35, and strains UA489 and UA490 were constructed from strain DM506. The relative plaque-forming ability of T5 on these two isogenic sets is also shown in Table 2. These results show that the permissive phenotype is preserved in the presence of an unmutagenized plasmid, confirming that the CMR defect is completely chromosomal in nature.

Growth of phage. Figure 1 depicts the results of one-step growth experiments of T5 on CMR$^+$ (ColIb$^+$) or CMR$^-$ (ColIb$^+$) and CMR$^+$ or CMR$^-$ (ColIb$^-$) cells. At zero time, the number of infective centers obtained on the CMR$^+$ (ColIb$^+$) derivative strain, UA482, is about 1% of that observed for strain UA489 (CMR$^+$ ColIb$^-$), a value similar to that previously reported. In contrast, the transmission coefficient of the CMR$^-$ (ColIb$^+$) culture, strain UA483, is similar to the ColIb$^-$ permissive culture. The average burst size per infective center was 200 for strain UA483. This is similar to the value for strain UA489 (180) and is increased somewhat over the burst size of 167 for the CMR$^+$ (ColIb$^+$) restrictive culture. The growth characteristics of T5 on strain UA490 (CMR$^-$ ColIb$^-$) are also shown to be similar to UA489, suggesting that the CMR$^-$ defect has no overt additional effect on phage production in the absence of ColIb. Finally, the latent period on all host strains was similar, between 35 and 40 min.

TABLE 2. *Efficiency of plating of bacteriophage T5 on CMR$^-$ E. coli derivatives*

Strain	CMR phenotype	Relative plaque no.
DM506	+	1
UA35 (ColIb)	+	9×10^{-7}
UA77 (ColIb)	−	0.8
UA389 (ColIb)	+	5×10^{-6}
UA482 (ColIb)	+	3×10^{-6}
UA483	−	0.8
UA489	+	1
UA490	−	1
UA491	+	1×10^{-6}
UA492	−	0.7

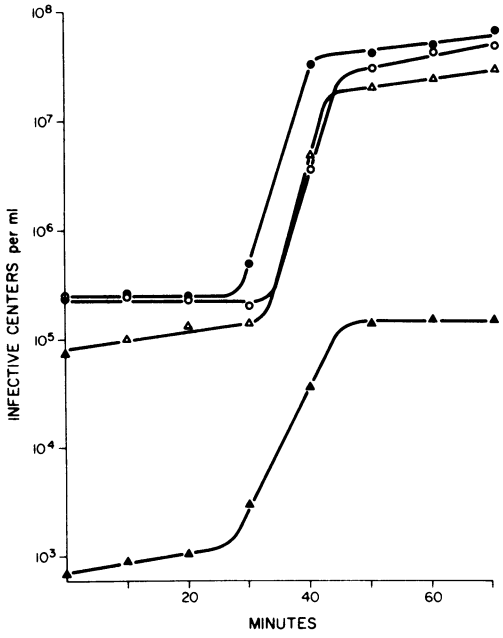

FIG. 1. *One-step growth kinetics of bacteriophage T5 on various E. coli derivatives. Experimental details are described in Materials and Methods. Symbols: (▲) UA482, CMR⁺ (ColIb⁺); (△) UA483, CMR⁻ (ColIb⁺); (●) UA489, CMR⁺ (ColIb⁻); (○) UA490, CMR⁻ (ColIb⁻).*

Growth of cells. The growth rate of the mutant and parent strains was studied to determine whether the CMR⁻ defect has an effect on cell growth. The kinetics of growth of cultures in nutrient broth was measured; cell doubling times calculated from these data are presented in Table 3. A small but readily reproducible difference in growth rate between the mutant and parent strains was noted. Growth of the mutant derivatives in minimal media was similarly found to be impaired (data now shown).

Effects of *cmr* mutations on plasmid expression. Since mutant ColIb plasmids that fail to arrest T5 development are also altered in their ability to synthesize colicin (unpublished observation), an attempt was made to determine whether *cmr* mutations might have a similar effect. A lacunae assay was used to measure the fraction of cells in CMR⁺ and CMR⁻ cultures spontaneously induced to synthesize colicin (Table 4). These results show little difference between the level of spontaneous induction in each culture.

Effects of *cmr* mutations on other plasmids. The influence of the defect in CMR⁻ cells on expression of antibiotic resistance by resistance plasmids was examined. Either the type I resistance plasmid R64*drd*11 (sm, tc) or the type F resistance plasmid R1*drd*19 (su, sm, sp, am, cm, km) was introduced into the CMR⁺ (ColIb⁻) and CMR⁻ (ColIb⁻) derivatives, strains UA489 and UA490. Cultures of strain UA489 R64*drd*11 and strain UA490 R64*drd*11 were then inoculated onto plates containing increasing amounts of tetracycline, and the efficiency of colony formation of the cultures at each concentration was measured. Derivatives carrying R1*drd*19 were similarly tested for resistance to penicillin. The results in Fig. 2 and 3 show that expression of antibiotic resistance by either plasmid is unaltered in the mutant strain. The small difference in tetracycline resistance apparent in Fig. 2 varied qualitatively upon several repeated experiments and is thus not considered an indication of altered resistance in the CMR⁻ derivative.

Genetic analysis of *cmr* using Hfr's. All genetic analysis except dominance testing was done in the original strain UA77 isolate. The approximate map position of the *cmr* defect relative to other loci on the *E. coli* genome was determined by using strain UA77 as a recipient in matings with various Hfr donor strains. Recombinants were selected for inheritance of auxotrophic markers and then scored for *cmr* by cross-streaking T5 on nutrient plates. These results, shown in Table 5, reveal that the *cmr* locus (loci) is located between the origins of KL14 and KL228. The origins and directions of transfer of Hfr strains used are depicted in Fig. 4. KL14 was again used as a donor with strain UA77, only the more proximal marker *xyl* was

TABLE 3. *Growth of E. coli CMR⁻ mutants in rich media*[a]

Strain	CMR phenotype	Cell doubling time (min)
UA482	+	21
UA483	−	31
UA489	+	23
UA490	−	31

[a] Exponentially growing nutrient broth cultures were diluted to a final concentration of 10^5/ml in nutrient broth and incubated with aeration at 37 C. At 30-min intervals, samples were withdrawn, diluted appropriately, and inoculated onto nutrient agar plates to determine the number of viable cells.

TABLE 4. *Production of lacunae by CMR⁺ and CMR⁻ cell cultures*

Strain	CMR phenotype	Expt	% Cells forming lacunae
UA482	+	1	18×10^{-5}
		2	3.8×10^{-5}
UA483	−	1	4.5×10^{-5}
		2	3.6×10^{-5}

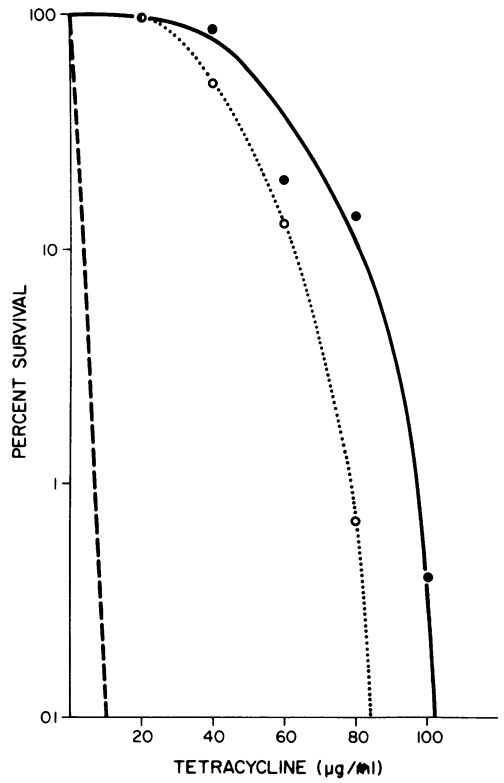

FIG. 2. *Tetracycline resistance of CMR⁺ and CMR⁻ E. coli K-12 derivatives carrying the R64drd11 (tc,sm) resistance plasmid. Dilutions of exponentially growing cultures were inoculated onto fresh Mueller-Hinton plates containing various concentrations of tetracycline. Resistance was determined by comparing the cell titer at each antibiotic concentration to the titer on control plates containing no antibiotic. Symbols: (●) UA489 (R64drd11) (CMR⁺); (○) UA490 (R64drd11) (CMR⁻); (- - -) typical sensitivity of R⁻ derivatives.*

selected, and *rpsL* and *cmr* were scored as unselected markers. Nalidixic acid was used to prevent growth of the donor. The results in Table 6 suggest that the *cmr* defect is closely linked to *rpsL*.

Genetic analysis by transduction. Bacteriophage P1 propagated on strain UA77 was used to transduce strain UA493 (ColIb⁺) to *aroE⁺*. Recombinants were then purified once on selective media and scored for *rpsL*, *rpsE*, and *cmr*. It was necessary to purify each recombinant away from background growth, since the recipient *cmr⁺* genotype is phenotypically dominant over the permissive donor marker. Initially, *cmr* was scored by cross-streaking T5 on nutrient plates as before. However, it was found that many of the recombinants that appeared as *cmr⁺* by this test were in fact somewhat permissive when tested by measuring relative plating efficiency of T5 on the isolate. These partial permissives had a plating efficiency for T5 that was about 10% lower than the relative plating efficiency on the fully *cmr* derivative. As a consequence, each *aroE⁺* recombinant was tested for its ability to plate T5. The results obtained are depicted in Fig. 4 and Table 7. The genetic locus responsible for the partially permissive phenotype has been designated *cmrA*. The *cmrA* allele must be present together with the *cmrB* mutation for the cells to be fully permissive. The recombinant classes for the three-point analyses shown in Table 7 are listed such that, given the gene order presented in Fig. 4, the recombinant class requiring four crossover events, and thus the least frequent, is listed last.

Dominance. Genetic dominance of the *cmr* loci was tested in the following manner. Thirion and Hofnung (28) have reported that *malT*

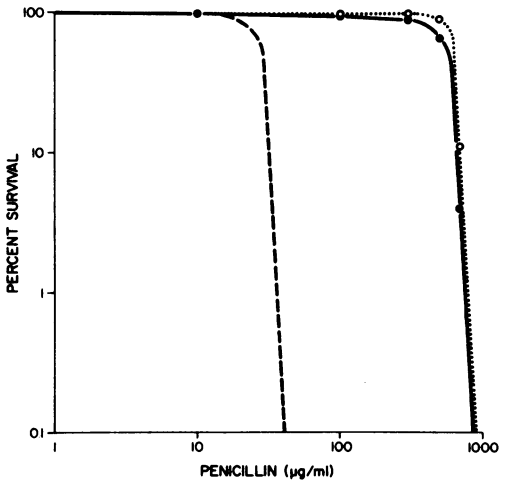

FIG. 3. *Penicillin resistance of CMR⁺ and CMR⁻ E. coli K-12 derivatives carrying the R1drd19 (sm,sp,am,cm,km) resistance plasmid. Dilutions of exponentially growing cultures were inoculated onto fresh Mueller-Hinton agar plates containing various concentrations of penicillin (1,550 U/mg). Resistance was determined as in Fig. 2. Symbols: (●) UA489 (R1drd19) (CMR⁺); (○) UA490 (R1drd19) (CMR⁻); (- - -) typical sensitivity of R⁻ derivatives.*

TABLE 5. *Hfr mapping of cmr*[a]

Donor	Selected markers	Fraction *cmr⁺*
KL14	*thr⁺ leu⁺ nalA*	13/51
KL228	*mtl⁺ nalA*	2/74
PK191	*arg⁺ rpsL*	0/83
P4X	*thr⁺ leu⁺ rpsL*	0/50
RA2	*thr⁺ leu⁺ rpsL*	0/126
H	*pro⁺ his⁺ rpsL*	0/14

[a] F⁻ recipient strain: UA77.

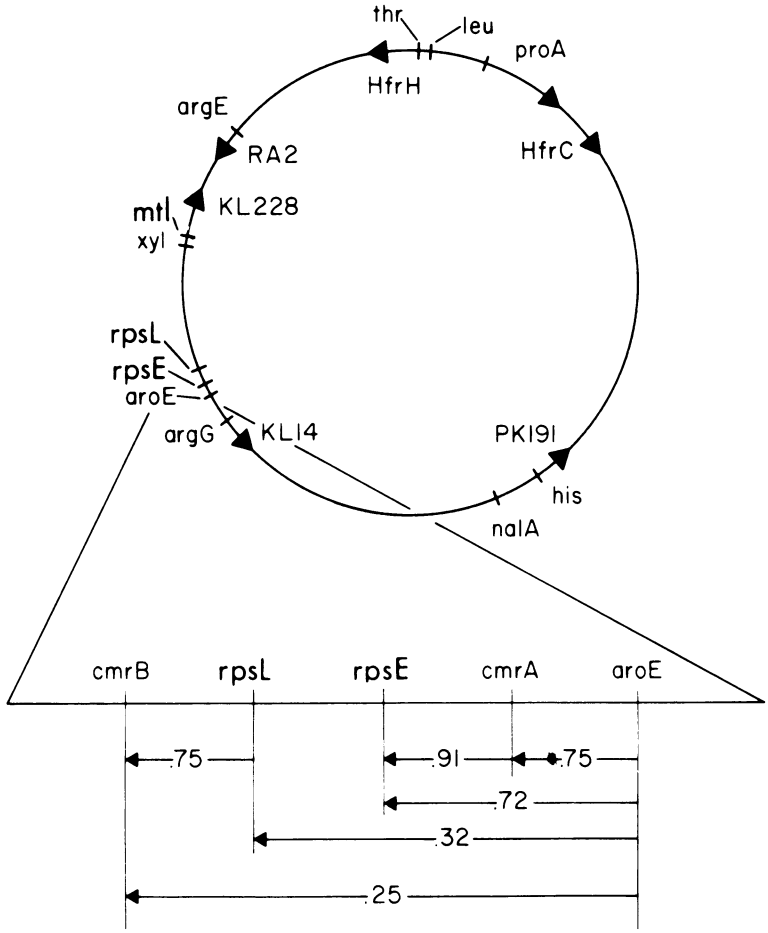

FIG. 4. *Location of the cmr loci on the circular linkage map of E. coli K-12. Reference loci are placed as described by Bachmann et al. (2). Origin and direction of transfer of relevant Hfr strains are also shown. At the bottom, co-transduction frequencies between markers near cmrA and cmrB are shown; values were derived from the data in Table 6. Arrows point to unselected donor markers. Distances are not drawn to scale.*

TABLE 6. *Linkage of cmr to the rpsL locus*[a]

Recombinant class	Fraction of total
rpsL$^+$ *cmr*$^+$	0.86
rpsL cmr	0.11
rpsL$^+$ *cmr*	0.02
rpsL cmr$^+$	0.01

[a] Hfr donor, KL14 (*cmr*$^+$ *rpsL*$^+$ *xyl*$^+$ *nal*$^+$); F$^-$ recipient UA77 (*cmr rpsL xyl nalA*); selected markers, *xyl*$^+$ *nalA*; number of recombinants scored, 100.

mutants of *E. coli* which are defective in the uptake and utilization of maltose are also resistant to infection by bacteriophage λ. Therefore, a *malT* derivative of UA490 was prepared by infecting UA490 with P1 transducing phage grown on χ974 and selecting for λ resistant recombinants. A ColIb$^+$ derivative of this strain (UA535) was then mated with strain KLF41/JC1553, and Mal$^+$ transconjugates with the genetic structure of KLF41 *cmr*$^+$ *rpsL*$^+$/UA535 were tested for ability to support growth of T5 (Table 8). Mating conditions and procedures used to verify relevant markers are discussed in Materials and Methods. These results show that the *cmrA cmrB* genotype is recessive to the wild-type alleles. The continued presence of the *cmr* alleles was confirmed in the manner described by Westling-Häggström and Normark (29) taking advantage of the phenotypic dominance of the *rpsL*$^+$ allele. Segregants of strain KLF41/UA535 which had become resistant to streptomycin, and had therefore presumably lost all or part of the episome, were tested for plating efficiency of T5; these were found to have regained the permissive phenotype. One

TABLE 7. *Ordering of the cmr alleles with respect to adjacent loci by three-point analysis*[a]

Recombinant class	No.
rpsL$^+$ *cmrA*[b]	189
rpsL$^+$ *cmr*$^+$	92
rpsL cmrA	134
rpsL cmr$^+$	0
rpsE cmrA	28
rpsE cmr$^+$	88
rpsE$^+$ *cmrA*	295
rpsE$^+$ *cmr*$^+$	4
rpsL cmr$^+$[c]	34
rpsL cmrB[c]	100
rpsL$^+$ *cmr*$^+$	278
rpsL$^+$ *cmrB*	3
rpsE$^+$ *cmr*$^+$	199
rpsE$^+$ *cmrB*	100
rpsE cmr$^+$	113
rpsE cmrB	3

[a] Donor, UA77 (*cmrA cmrB rpsL rpsE*$^+$ *aroE*$^+$); recipient, UA493 (ColIb) (*cmr*$^+$ *rpsL*$^+$ *rpsE aroE*); selected marker, *aroE*$^+$; number scored, 415.

[b] Recombinants that exhibited either full or partial permissiveness were scored as *cmrA*.

[c] When scoring the *cmrB* locus, both partially permissive *cmrA* single mutants and fully restrictive recombinants are defined as *cmr*$^+$. Only those recombinants expressing the fully permissive phenotype were scored as *cmrB* negative. These are of the genetic structure *cmrA cmrB*.

TABLE 8. *Efficiency of plating of bacteriophage T5 on a cmr*$^+$*/cmr merodiploid E. coli derivative*

Strain	Genotype	Relative plating efficiency
UA489	*cmr*$^+$	1
UA535 (ColIb)	*cmr*	0.75
KLF41/UA535 (ColIb)	*cmr*$^+$*/cmr*	5×10^{-6}
UA535 (F$^-$) (ColIb)	*rpsL* segregant	0.80

of these streptomycin-resistant segregants is included in Table 8.

DISCUSSION

Mutations in *E. coli* K-12 have been identified which permit T5 to grow efficiently on ColIb$^+$ cells. The presence of these mutations confirms the prospect of host cell influence on the phage arrest mechanism. The collective effect of these mutations on host cell metabolism is a slight reduction in growth rate in either minimal or complete media.

A genetic analysis of the CMR$^-$ defect revealed that what was originally thought to be a single mutation was actually two, one in the *cmrA* gene, 91% co-transducible with *rpsE* and proximal to *aroE*, and one in the *cmrB* locus, 75% co-transducible with *rpsL* and distal to *aroE*. Based on the formula suggested by Wu (30) for calculation of gene distance from frequency of co-transduction, the *cmrA* locus is only about 2,700 base pairs from *rpsE*, the gene for the S5 protein of the 30S ribosomal subunit, and may possibly be a mutation in ribosome structure. Chromatographic analysis of ribosomal proteins from these strains is currently underway. Alternately, *cmrA2* may be an allele of the structural gene for the α subunit of *E. coli* ribonucleic acid (RNA) polymerase, *rpoA*, which has recently been positioned near *rpsE* (12). By analogy to the rifamycin-resistant mutants (*rpoB*) affecting the β polymerase subunit, one might expect that permissive *cmr* mutants altered in RNA polymerase structure would exhibit intermediate dominance in the heterozygous state. For example, bacteriophage T2, like T5, requires functional host RNA polymerase for normal development. When T2 growth was measured on *rpoB*/*rpoB*$^+$ merodiploids in the presence of rifampin, intermediate resistance of phage development to the antibiotic was observed (10). Still, although *cmr* mutations are completely recessive, one cannot exclude the possibility that their recessive nature is the result of subunit mixing.

The phenotype of the *cmrB* locus in the absence of the *cmrA2* allele has not been determined, but preliminary results suggest that it may be responsible for the slow growth of the mutant strain. In that event, it would be phenotypically similar to and possibly identical with the *sloB* gene reported to be linked to *rpsL* (29).

The initial observations concerning ColIb-mediated restriction of phage T5, and of the related phage BF23, support molecular mechanisms involving transcriptional regulation, perhaps through alteration of host RNA polymerase activity or phage DNA template structure. However, Szabo et al. (27) have shown that the RNA polymerase from T5-infected ColIb$^+$ cells is capable of efficiently transcribing all classes of T5 RNA in vitro.

Herman and Moyer (8) examined the effect of phage DNA template structure on ColIb-mediated restriction and presented evidence to suggest that T5 restriction is due to an inability of "h$^+$" phage to transcribe class II messenger RNA (mRNA) from a sealed phage DNA template in a ColIb$^+$ host. Studies on the template structure of the phage chromosome during infection of *cmrA cmrB* (ColIb$^+$) cells may be useful in evaluating this model.

The isolation of host *cmr* mutations closely linked to *E. coli* K-12 genes, which are known

to specify synthesis of ribosomal proteins, suggests that regulation at the post-transcriptional level may have a role in the plasmid-mediated restriction of T5. Post-transcriptional control of phage T4 development has recently been proposed (4). ColIb plasmid mutants that are permissive for T5 infection are also altered in their ability to direct the synthesis of colicin (unpublished observation). This, along with the observation that the T5 phage arrest property is limited to colicin or resistance plasmids that produce the Ib type colicin, suggests that the restriction phenomenon is closely associated with the regulation of colicin synthesis. Isaacson and Konisky (11) have presented evidence consistent with a model for regulation of colicin Ib synthesis at the post-transcriptional level. However, other models involving transcriptional control are also compatible with their findings.

Impairment of proper translation of phage mRNA by the F sex plasmid-specified product has also been implicated as a mechanism for F-mediated restriction of phage T7 (3). However, since all classes of T7-specific mRNA can be detected in abortive infections, the nature of this restriction process must be different from that involving ColIb and T5.

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