Genetic and Physical Mapping of the *mcrA* (*rglA*) and *mcrB* (*rglB*) Loci of *Escherichia coli* K-12

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ABSTRACT

We have genetically analyzed, cloned and physically mapped the *m*odified *c*ytosine-specific *r*estriction determinants *mcrA* (*rglA*) and *mcrB* (*rglB*) of *Escherichia coli* K-12. The independently discovered Rgl and Mcr restriction systems are shown to be identical by three criteria: 1) mutants with the RglA$^-$ or RglB$^-$ phenotypes display the corresponding McrA$^-$ or McrB$^-$ phenotypes, and vice versa; 2) the gene(s) for RglA and McrA reside together at one locus, while gene(s) for RglB and McrB are coincident at a different locus; and 3) RglA$^+$ and RglB$^+$ recombinant clones complement for the corresponding Mcr-deficient lesions. The *mcrA* (*rglA*) gene(s) is on the excisable element *e14*, just clockwise of *purB* at 25 min. The *mcrB* (*rglB*) gene(s), at 99 min, is in a cluster of restriction functions that includes *hsd* and *mrr*, determinants of host-specific restriction (*Eco*K) and methyladenine-specific restriction respectively. Gene order is *mcrB-hsdS-hsdM-hsdR-mrr-serB*. Possible models for the acqusition of these restriction determinants by enteric bacteria are discussed.

THE wide variety of restriction systems elaborated in different bacterial species allow bacteria to assess the origin of incoming DNA and determine its fate (for reviews see ARBER 1974; BICKLE 1982; MODRICH and ROBERTS 1982). In the most familiar type I and type II systems, exemplified by *Eco*K and *Eco*RI, endonucleases recognize specific unmodified nucleotide sequences and cleave the DNA into fragments, thus destroying its biological activity. DNA is protected from digestion when modified at the endonuclease recognition site by an associated modifying function, usually by methylation of a specific adenine (at N6) or cytosine [at C5 (SMITH and KELLEY 1984) or N4 (BUTKUS *et al.* 1985)] residue. In this way, endogenous DNA is immune to endonucleolytic cleavage, while foreign DNA is recognized and destroyed, providing protection from invading phages grown on heterologous hosts, as well as from foreign plasmids (ARBER 1974).

A less familiar type of restriction, aimed specifically at modified DNA in T-even phages, was historically the first restriction system to be described (LURIA and HUMAN 1952). Two functions specifying this restriction activity are present in *Escherichia coli* K-12 (REVEL 1967). Originally called R_6 and $R_{2,4}$, they were subsequently renamed RglA and RglB, for *r*estriction of *glucose*less phages (see review, REVEL 1983). During T-even phage replication the unusual base 5-hydroxymethylcytosine (HMC) completely substitutes for cytosine in phage DNA, and the hydroxymethyl group is subsequently glucosylated in phage-specific patterns at the polynucleotide level (LEHMAN and PRATT 1960). Wild-type T-even phage with the glucose modification are insensitive to Rgl restriction, as is a T4 triple mutant that synthesizes DNA with ordinary cytosine (TAKAHASHI, SAITO and IKEDA 1978). In contrast, T-even phage with HMC-DNA, but lacking the normal glucose modification, are restricted. These phage arise when the glucosyltransferase enzymes are defective, as in phage *gt* mutants (REVEL, HATTMAN and LURIA 1965; GEORGOPOULOS 1967), or when wild-type phage are grown on a mutant *galU* host, which cannot provide the glucosyl donor, UDPG (SHEDLOVSKY and BRENNER 1963; HATTMAN and FUKASAWA 1963). Evidence for limited cleavage of non-glucosylated HMC-DNA by RglB *in vivo* (DHARMALINGAM and GOLDBERG 1976) and *in vitro* (FLEISCHMAN, CAMPBELL and RICHARDSON 1976) suggests a role for recognition of sequence as well as of specific base modification. Nevertheless, it has been questioned whether the Rgl functions represent true restriction enzymes, because sequence specificity has not been clearly demonstrated, and because there is no cognate methylase activity (KRUGER and BICKLE 1983).

More recently, sequence-specific, modification-dependent restriction systems that attack DNA with the common base, 5-methylcytosine, were found in *E. coli* K-12 (RALEIGH and WILSON 1986; NOYER-WEIDENER, DIAZ and REINERS 1986). N^4-Methylcytosine also can confer sensitivity to one of these systems (BLUMENTHAL, GREGORY and COOPERIDER 1985; BUTKUS *et al.* 1987). These Mcr (for modified cytosine restriction) systems are able to restrict phage λ modified by some,

but not all, sequence-specific cytosine modification methylases foreign to *E. coli* K-12. Plasmid clones carrying the corresponding methylase genes are also restricted.

In the present communication we show, by comparative genetic and physical mapping and cloning, that the newly identified McrA and McrB restriction systems are genetically identical to the previously described RglA and RglB functions active against nonglucosylated HMC-DNA in T-even phages. The mapping reveals the interesting facts that the *mcrA* (*rglA*) locus is found on an accessory determinant, *e14*, and that the *mcrB* (*rglB*) locus is part of a cluster of restriction functions that may have been acquired from (an) accessory element(s).

MATERIALS AND METHODS

Nomenclature: The restriction systems described here were originally characterized independently by two different kinds of assays, using two different mnemonic designations, as described in the Introduction. A major point of this paper is that the two kinds of assays test the same underlying function, restriction of DNA modified at particular cytosine residues. Accordingly, we must initially retain a separate nomenclature for these two assays, but finally choose a single preferred designation for the loci. We will retain the original designations, RglA, RglB and *rglA*, *rglB* in discussing those experiments in which only T-even phages are at issue, and will use the terms McrA and McrB specifically to refer to restriction of methylated (not hydroxymethylated) plasmids and phages. In discussing transduction experiments, in some of which both assays were used, we will use both genotypic designations, placing first the mnemonic for the phenotype principally used in the experiment, and in parentheses that for the phenotype checked secondarily. In discussing physical mapping experiments, and in future work, we will use exclusively the mnemonic *mcr* for genotypic designations, since we believe that this more accurately reflects the function of these loci. The phenotypic designations, Rgl, will be employed when it is useful to distinguish restriction of T-even phage from restriction of methylated phages or plasmids.

Bacterial strains, plasmids and media: Strains used are given in Table 1. Plasmids used and their sources are shown in Table 2. All are based on the vector pBR322 or its relative pBR313, except for pGP325, pGP326 (+) and pGP326 (−), which are based on the vector pACYC184. Media were routinely Luria-Bertani medium (LB; SILHAVY, BERMAN, and ENQUIST 1984) or M9 minimal salts (MILLER 1972) supplemented with drugs (ampicillin, 100 μg/ml; tetracycline, 20 μg/ml; streptomycin, 100 μg/ml) or nutrients (L-amino acids, 40 μg/ml; bases, 20 μg/ml; or vitamins, 1 μg/ml) as required. Glucose was used in minimal media at 0.2%. In some cases, TG minimal medium (ECHOLS *et al.* 1961) was used instead of M9. Phage titrations and plate stocks were done on λ medium (KLECKNER *et al.* 1978) except for P1, for which LB with 5 mM $CaCl_2$ was used. Phages were diluted in λdil (10 mM Tris-HCl, pH 7.5, 10 mM $MgSO_4$).

Chemicals were from Sigma or Nutritional Biochemical Corporation, Cleveland, Ohio, except for streptomycin sulfate (E. L. Squibb and Sons, New York) and nitrosoguanidine (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; Aldrich Chemical Co., Inc., Milwaukee, Wisconsin).

Phage P1*vir* was used for transductions (MILLER 1972). λ*vir* or λcIb2 were used for assays for K and B restriction. T2H, T4D, and T6 (hereafter called T2, T4, and T6) and their derivatives T2*gt*2, T4*αgt*57*βgt*14 and T6*gt*41 [(REVEL, HATTMAN and LURIA 1965; GEORGOPLOULOS 1968; REVEL 1983), hereafter called T2*gt*, T4*gt*, and T6*gt*] were used to assay for Rgl phenotype. Nonglucosylated wild type T-even phages (T* phage) were prepared by growth on the *galU* host W4597 (HATTMAN and FUKASAWA 1963).

Mutant isolation: Isolation of RglA$^-$ and B$^-$ mutants was as described previously (REVEL 1967). Briefly, cells unable to restrict T6*gt* phage (RglA$^-$ cells) yield nibbled, irregular colonies when nitrosoguanidine-mutagenized wild-type cultures are spread onto plates seeded with about 10^8 T6*gt* phage. RglB$^-$ cells were selected similarly from mutagenized RglA$^-$ cultures using T2*gt* as the selective agent. Cells with the RglA$^+$ RglB$^-$ phenotype, not previously described, were selected from mutagenized RglA$^-$ RglB$^-$ cultures by screening for smooth, nonnibbled, *insensitive* colonies in the presence of T6*gt*. Alternatively, this phenotype could be selected, though less efficiently, from wild-type RglA$^+$ RglB$^+$ cells as nibbled colonies in the presence of phenotypically Glu$^-$ T4* phage that are obtained as above. HsdR$^-$ mutants were isolated in similar fashion, using unmodified λcIb2 (λ.0) as the screening agent.

McrB$^-$ mutants, spontaneous or induced by Tn*10* with λNK561 as described (KLECKNER *et al.* 1978), were isolated among the survivors of transformation of a restricting strain with p*Hae*II4-11 or pER82, plasmids that carry *haeIIM*, the gene for the *Hae*II methylase, M·*Hae*II.[1] Survival following transformation for spontaneous mutants was 1 to 5×10^{-5}. Of these, 20–35% had lost plasmid methylase activity, and 65–80% were host mutants permissive for the methylase plasmid. Tn*10* insertion with Tet^r selection yielded survivors at a frequency of 2×10^{-5} per infected cell with about 2% auxotrophs as reported in the literature (KLECKNER, ROTH and BOTSTEIN 1977; KLECKNER *et al.* 1978). Twenty independent pools of insertions were transformed with pER82, yielding survivors at a frequency of about 6×10^{-4}. All three independent survivors had insertions in *mcrB*.

To obtain plasmid-free mutants for further analysis, either the *mcrB* mutation was backcrossed to the original host by transduction, or the permissive mutant strains were cured of the *haeIIM* plasmid by growth in the presence of the incompatible Cm^r plasmid, pNK259, without Ap^r selection (SHEN, RALEIGH and KLECKNER 1987). pNK259 was then cured from the Cm^r Ap^s derivatives by penicillin selection (MILLER 1972) in the presence of chloramphenicol. Ampicillin kills the growing Cm^r Ap^s cells, but does not kill the nongrowing Cm^s Ap^s cells that arise by spontaneous plasmid loss. These plasmidless cells are not killed by the bacteriostatic agent chloramphenicol either, and will subsequently form colonies when washed cells are plated on drug-free plates.

Mcr assays: Three tests, a λ growth assay and two plasmid transformation assays, were used to measure Mcr activity.

I: The λ assay tested the ability of strains to restrict λ bearing a foreign modification pattern. λ was modified by growth on a host carrying a cloned methylase gene for M·*Hae*II, M·*Msp*I, or M·*Hpa*II. Modified λ titers were determined either by spotting 25 and 50 μl of 10-fold serial phage dilutions on a bacterial lawn in soft agar on λ medium agar and recording the endpoint for plaque formation (for λ·*Hae*II and λ·*Msp*I) or by full plate counts (λ·*Hpa*II). Plating efficency (EOP) is defined as the lysate titer on the

[1] M·*Hae*II, *Hae*II-specific modification methylase. This form of designation (SMITH and NATHANS 1973) will be used for all methylases cited here.

TABLE 1

Bacterial strains

Strain	Background	*rgl/mcr* genotype[a] A	*rgl/mcr* genotype[a] B	Other relevant genotype[b]	Source[c]
1100		+	+	F⁻λ⁻*supE44 thi-1 rfbD1? spoT1? endA1*	I. R. LEHMAN (1)
C600		1	+		N. KLECKNER (1)
CH731		+	NT	*el4*⁺	H. BRODY (2)
CH1332	CH731	−	NT	*el4*⁰	H. BRODY (2)
CH1372	CH731	1272::Tn*10*	NT	*el4*-1272::Tn*10*	H. BRODY (2)
ER1370	NK7254	+	+	F⁻λ⁻*leu*⁺ *tonA2* Δ(*lacZ*)r1 *supE44 trp31 his-1 argG6 rpsL104 xyl-7 mtl-2 metB1 hsd*⁺ *serB28*	This work
ER1372	ER1370	+	1	*zjj*202::Tn*10*	This work
ER1378	ER1370	+	1	*hsdR2 serB*⁺	This work
ER1380	ER1370	+	+	*hsdR2 zjj*202::Tn*10 serB*⁺	This work
ER1381	ER1370	+	+	*hsdR2 serB*⁺	This work
ER1394	MM294	+	1	*hsd*⁺ *zjj*202::Tn*10 serB28*	This work
ER1398	ER1394	+	1	*hsdR2*	This work
ER1467	ER1370	+	4::Tn*10*	*hsdR2 serB*⁺	This work; Tn*10* induced
ER1468	ER1370	+	5::Tn*10*	*hsd*⁺ *serB*⁺	This work; Tn*10* induced
ER1469	ER1370	+	6::Tn*10*	*hsdR2 serB*⁺	This work; Tn*10* induced
ER1486	ER1370	+	Δ2	Δ(*mcrB-hsd-mrr*)2::Tn*10*	This work; spontaneous, from ER1380, containing *zjj*202::Tn*10*
ER1489	ER1370	+	3::IS	*hsdR2 serB*⁺	This work; spontaneous
ER1509	H680	−	NT	*zcg*2043::Tn*10 purB*51	This work; H680 from P. G. DE HAAN via B. BACHMANN
ER1516	ER1370	−	1	*zcg*2043::Tn*10 purB*51 *hsdR2 serB*⁺	This work
ER1564	ER1370	1272::Tn*10*	+	*hsdR2 serB*⁺	This work
ER1565	ER1370	1272::Tn*10*	1	*hsdR2 serB*⁺	This work
ER1576	JK268	−	NT	*zcf*2033::Tn*10 purB*58	This work; (JK268, see 3)
ER1648	ER1370	1272::Tn*10*	Δ2	Δ(*mrr-hsd-mcrB*)2::Tn*10 serB*⁺	This work; *arg*⁺
ER1656	ER1370	+	1	*hsdR2*	This work
HB101	K/B	+	B	*hsdS*20$_B$	J. BROOKS (4)
Hfr Cavalli	K12	+	1	*hsd*⁺	B. BACHMANN (1)
HR110	K12	+	+	K12 F⁺ (λ) hsd⁺	K-12 wild type, Stanford; (1)
HR111	HR110	2	+	P1^r	REVEL (5, 6); was K_{r6^-} or $K_{r6^-r2,4^+}$
HR112	HR111	2	8		REVEL (5, 6, 7); was $K_{r6^-r2,4^-} = K_{rgl}$
HR116	1100	*ts4*	+		This work, NG[d], T6*gt* selection
HR140	HR112	+	8	*sup*	REVEL (6, 7); was $K_{r6^+r2,4^-su^+}$
HR184	MM294	3	+	*hsdR17*	This work, NG[d], T6*gt* selection

[a] +: restricts modified DNA; −: does not restrict modified DNA; NT: not tested. Where assigned allele numbers have been assigned these are given in lieu of −; all alleles are defective for restriction. All *mcrB* alleles have been mapped. Not all *mcrA* mutations have been mapped, but we assume that they are allelic (see text). Not every strain has been tested for both Rgl and Mcr phenotypes; see text and RALEIGH *et al.* (1988) for those that have.

[b] Complete genotypes and details of strain construction are available from the authors upon request. Genotypes shown are complete for recipients used in strain construction. For strains prefixed ER and HR, the genotype is given only where it differs from the original strain (given under "Background"); *mcrA* and *mcrB* genotypes are given in the two columns so labelled. For strains not constructed here, only the genotype relevant to this paper is given.

[c] Numbers in parentheses refer to the following reports: (1) BACHMANN (1987b); (2) BRODY, GREENER and HILL (1985); (3) HUGHES, SIMONS and NUNN (1988); (4) BOYER and ROULLAND-DUSSOIX (1969); (5) REVEL (1967); (6) REVEL and GEORGOPOULOS (1969); (7) GEORGOPOULOS and REVEL (1971); (8) TAYLOR and TROTTER (1972); (9) HEITMAN and MODEL (1987); (10) BLUMENTHAL, GREGORY and COOPERIDER (1985); (11) ROBERTS *et al.* (1985); (12) BOLIVAR *et al.* (1977); (13) HATTMAN and FUKASAWA (1963); (14) WOOD (1966); (15) SIGNER, BECKWITH and BRENNER (1965); (16) BOYER (1966).

[d] NG, nitrosoguanidine-induced *rgl* mutation.

test strain divided by lysate titer on a permissive strain. McrB restriction reduced EOP to 0.02–0.001 for λ·*Hae*II and λ·*Msp*I; McrA restriction reduced EOP to 0.3–0.5 for λ·*Hpa*II.

IIA: Transformation assays tested the ability of strains to restrict transformation by plasmids bearing a foreign methylation pattern. Transformation was as described (MANDEL and HIGA 1970). Briefly, 50 μl of transformation mix was added to 1.5 ml LB broth and grown for 20 min at 37 °C to allow phenotypic expression prior to spreading aliquots on ampicillin selective medium to determine the number of transformed cells. In method IIA, pBR322 was methylated

TABLE 1—Continued

Strain	Background	*rgl/mcr* genotype[a] A	*rgl/mcr* genotype[a] B	Other relevant genotype[b]	Source[c]
HR186	AT2459	−	9	*hsd*$^+$ *serB22*	This work, (8) NG[d], T2*gt* selection
JH83	GW1040	NT	+	*hsd*$^+$ *mrr2*::Tn*5* *zjj*202::Tn*10*	J. HEITMAN (9)
JM107		−	+	*hsdR*17	R. M. BLUMENTHAL (10)
JM107MA2	JM107	−	7	*hsdR*17	R. M. BLUMENTHAL (10)
LCK8	WA802	1	1	*hsdR*2 *zjj*202::Tn*10*	L. COMAI via B. BACHMANN
MM294	1100	+	+	*hsdR*17	J. BROOKS (1)
NK7254	JC1552	+	+	F$^-$ *leu-6 ton*A2 Δ(*lac* Z)r1 *sup*E44 *trp*-31 *his*-1 *arg*G6 *str*-104 *xyl*-7 *mtl*-2 *metB*1 λ$^-$	N. KLECKNER (11)
RR1	HB101	+	B	as HB101 but *rec*A$^+$	G. WILSON (12)
W3110		+	+		N. KLECKNER (1)
W4597		+	+	*galU*	S. E. LURIA (13)
W6		+	+		B. BACHMANN (1)
WA802		1	1	*hsdR*2	N. MURRAY (14)
WA803		1	1	*hsdS*3	N. MURRAY (14)
X149		−	+	F$^-$ *lac*-2 *pur*B *pyr*F *trp* A *his tyr mal thi* λ^r	E. SIGNER (15)
E. coli B derivatives					
B		+	+		S. E. LURIA
AC2522		+	+	HfrB1 (unstable Hfr)	H. BOYER (16)
AC2519		+	+	F$^-$ *trp gal lac pro leu thr met his str*^{r}	H. BOYER (16)
HR45	AC2519	5	+		This work, T6*gt* selection
Shigella sonnei	SH	−	−		S. E. LURIA (12)

TABLE 2

Plasmids used

Plasmid	Functions carried	Vector	Source or construction
pBR322	Ap^r M·*Hae*II R·*Hae*II		BOLIVAR *et al.* (1977)
pHaeII 4-11	Ap^r M·*Hae*II R·*Hae*II	pBR322	R. CROFT and G. WILSON
pHhaII 2-13	Ap^r M·*Hha*II R·*Hha*II	pBR322	D. NWANKWO and G. WILSON
pHpaII	Ap^r M·*Hpa*II	pBR322	C. CARD and G. WILSON
pER82	Ap^r M·*Hae*II	pBR322	*In vitro* deletion of pHaeII 4-11
pMsp 1-30	Ap^r M·*Msp*I R·*Msp*I	pBR322	NWANKWO and WILSON (1988)
pPstI Reg1	Ap^r M·*Pst*I R·*Pst*I	pBR322	G. WILSON
pBg3	Ap^r HsdRMS′	pBR322	SAIN and MURRAY (1980)
pBg6	Ap^r Hsd′S McrB/Rg1B	pBR322	SAIN and MURRAY (1980)
pER105	Ap^r McrB/Rg1B	pBR322	This work; *mcrB Bam*HI clone, Figure 3
pER106	Ap^r McrA/Rg1A Pin	pBR322	This work; *mcrA Bam*HI clone, Figure 5
pER137	Ap^r McrA/Rg1A Pin	pBR322	This work; subclone of pER106, Figure 5
pER146	Ap^{r}Pin	pBR322	This work; subclone of pER106, Figure 5
pNK290	Ap^r IS10	pBR333	FOSTER *et al.* (1981)
pNK259	Cm^{r}Ap^s	pBR322	SHEN, RALEIGH and KLECKNER (1987)
pAG2	Ap^r 'e14	pBR313	GREENER and HILL (1980)
pHB106	Ap^r 'e14	pBR313	BRODY, GREENER and HILL (1985)
pHB107	Ap^r 'e14 Pur B	pBR313	BRODY, GREENER and HILL (1985)
pGP325	Tc^{r}Pin$^+$	pACYC184	PLASTERK and VAN DE PUTTE (1985)
pGP326 (−)	Tc^{r}Pin$^-$ (− orientation)	pACYC184	PLASTERK and VAN DE PUTTE (1985)
pGP326 (+)	Tc^{r}Pin$^-$ (+ orientation)	pACYC184	PLASTERK and VAN DE PUTTE (1985)

in vitro (designated, *e.g.*, pBR322·*Msp*I) to render it sensitive to restriction. Restriction was inferred when the fully methylated plasmid gave less than one-tenth as many transformants as the unmethylated plasmid (efficiency of transformation (EOT) ≤0.1). McrA$^+$ restricted pBR322·*Hpa*II with an EOT of 0.1; McrB$^+$ restricted pBR322·*Msp*I and pBR322·*Alu*I, with an EOT of 0.003–0.02.

IIB: In method IIB, a pBR322 derivative carries a gene for a methylase (the plasmid is designated, *e.g.*, p*HaeIIM* or pER82) that confers sensitivity to restriction. In this case, relative transformation efficiency (relative EOT) was used as the index of methylase-specific restriction. This was calculated as the ratio of the transformation efficiency (transformants per ml in test strain/transformants per ml in permissive strain) of the test plasmid to the transformation efficiency of the vector alone. This procedure controls for

effects on intrinsic transformability of strain background, plasmid size, and DNA concentration.

Mrr assays: Measurement of Mrr restriction activity was as for Mcr activity method I, except that the target λ phages were modified by M·*Hha*II or M·*Pst*I, as described by HEITMAN and MODEL (1987). Mrr$^+$ restricted λ·*Hha*II and λ·*Pst*I with an EOP of 0.1 to 0.3.

Rgl assays: Rgl restriction activity was measured by the growth properties of wild type and *gt* mutant T-even phages determined by spot titer, as described above (see Table 3) or by cross-streak tests. For the RglA cross-streak test, a toothpicked colony was streaked at right angles across a dried loop-streak of T6*gt* phages. RglB activity, in a strain known to be RglA$^-$, was assayed similarly by cross-streaking against T2*gt* or T4*gt*. In an RglA$^+$ host, RglB activity was assayed by full-plate titration assays of wild-type T2 EOP. RglA and RglB activity can also be measured by spot test analysis with phenotypically Glu$^-$ wild-type T-even phages (T*) that have grown on *galU* hosts (see RESULTS). The restriction patterns are the same but efficiencies of plating are greater (about 10^{-3} as compared to 10^{-6} for *gt* phages), since progeny phage, now modified with glucose, are no longer restricted.

Hsd assays: *Eco*K and *Eco*B restriction was assayed by cross-streak against several dilutions of unmodified λ or by spot titer, as described in Mcr assays method I. M·*Eco*K activity was determined by picking λ plaques grown on the strain of interest into 1 ml of diluent, and then spot titering the suspension on Hsd$^+$ and *hsdR* strains. Plaques from *hsdM* or *hsdS* strains, which lack M·*Eco*K, were restricted.

Pin assays: Pin function (see RESULTS) was tested using a *Pst*I digest. There is a *Pst*I site asymmetrically disposed within the invertible DNA of *e14*, yielding one pair of *Pst*I fragments when the invertible segment is in one orientation and another pair when it is in the other orientation. Pin is required to invert the segment. Preparations of a Pin$^+$ plasmid carrying the invertible segment will contain both orientations, yielding the sum of the two possible digest patterns. pGP326(+) and pGP326(−) are Pin$^-$ plasmids in which the invertible segment is frozen in different orientations unless complemented by a Pin$^+$ host. pGP325 is the Pin$^+$ parent of these. pGP325 (positive control), pGP326 (+) and (−) (negative controls), pAG2 (*e14* plasmid), pER106 (*mcrA* clone), pER137 (McrA$^+$ subclone) and pER146 (McrA$^-$ subclone), all prepared in Pin$^-$ CH1332, were analyzed with *Pst*I. Only one pair of diagnostic *Pst*I fragments or the other was present in the digests of pGP326 (+) or (−), verifying that CH1332 is Pin$^-$. pGP325, pAG2, pER106, pER137 and pER146 all displayed two pairs of bands, demonstrating that they all carry functional *pin* genes and invertible DNA segments (not shown).

Methylation reactions and restriction digests were done as described (RALEIGH and WILSON 1986). All enzymes were obtained from NEB and were used as recommended.

Mapping studies by transduction or mating were done essentially as described in MILLER (1972) except in Hfr crosses with *E. coli* B (BOYER 1966). In these crosses, the rich broth was washed out before plating the mating mixture for recombinants on minimal medium (since the efficiency of mating is lower). All recombinants were purified before scoring unselected markers.

Southern blotting was done as described (MANIATIS, FRITSCH and SAMBROOK 1982) but using a mirror blotting procedure. In this method, DNA present in the gel is transferred to nitrocellulose in both directions normal to the plane of the gel, producing two blots. In this way, it is easier to be confident that a fragment hybridizing with one probe is identical in size with a fragment hybridizing with another probe, since the autoradiograms can be superimposed. Hybridization and the first wash were carried out at 65° (high stringency). Size standards were T7 digested with *Dra*III or with *Bgl*II. Probes were nick-translated (RIGBY *et al.* 1977) pBg6 or pNK290 (to detect chromosomal sequences) each mixed with nick-translated T7 (to detect the size standard).

Digested chromosomal DNA from twelve strains was probed with pBg6. Three different enzymes (*Hin*dIII, *Sal*I and *Apa*I) were used to digest chromosomal DNA of ER1380, ER1381, ER1489, ER1468, and W3110. DNAs from ER1370, ER1372, ER1378, ER1486, ER1467, ER1469 and LCK8 were digested with these and also with *Nco*I. These digests were probed with pBg6. The *Hin*dIII, *Nco*I, and *Apa*I digests were also probed with pNK290, which carries one of the inverted repeats of Tn*10* and will therefore detect fragments that contain Tn*10* ends.

Cloning of McrA and McrB: Donor DNA (about 25 μg; from ER1370) was prepared by the MARMUR (1961) procedure, digested with *Bam*HI (16 units for 2 hr in a total volume of 30 μl) and then precipitated with isopropanol in the presence of 2 M NH_4-acetate and resuspended in 10 mM Tris, 1 mM EDTA, 5 mM NaCl, pH 7.5. The DNA fragments were ligated to *Bam*HI-digested dephosphorylated pBR322 (about 5 μg; NEB) at a final DNA concentration of 25 μg/ml, with 10,000 units of T4 DNA ligase in the recommended buffer and incubated overnight at 16°. ER1516 was transformed (MANDEL and HIGA 1970) with the ligation mixture (5.25 ml total transformation volume) and the transformation mixture was subcultured 1:7 into LB with 40 μg/ml adenine (required to supplement the *purB* mutation in ER1516) and grown with vigorous aeration at 37° for 1.75 hr. T4*gt* was then adsorbed for 20 min at a multiplicity of about 1 phage per cell, and portions were plated on LB ampicillin plates. Survival was about 2×10^{-4} after overnight incubation, and 7 colonies, all clones of *mcrA* or *mcrB*, were obtained. Reconstruction experiments showed that RglA$^+$B$^-$ or RglA$^-$B$^+$ strains carried through a "mock" procedure in parallel (no DNA added during the transformation protocol) survived at a frequency of about 30%.

RESULTS

Phenotypes conferred by the Rgl and Mcr systems: RglA and RglB phenotypes are defined by the plating properties of T-even wild-type phages, and of their glucosyl transferase deficient mutant derivatives (*gt* mutants) that fail to add glucose to phage HMC-DNA (Table 3). In K-12 strains all four combinations of Rgl phenotypes can be distinguished with just T6*gt* and wild type T2. T6*gt* is restricted only by RglA (compare lines 1 and 2), while T2, with 25% of its HMC residues not modified by glucose (LEHMAN and PRATT 1960) is restricted only by RglB (lines 1 and 3). Since RglA strongly restricts T6*gt* growth, the RglA phenotype of a strain can be determined unequivocally. In contrast, the weak but reproducible RglB restriction of T2 is less definitive. However, in the absence of RglA function, both T2*gt* and T4*gt* provide a stringent assay for RglB function (compare lines 2 and 4). In K-12, T2*gt* and T4*gt* plate with an efficiency of 10^{-5}–10^{-7} when the wild-type allele of either *rglA* or *rglB* is present. Restriction by the two

TABLE 3

Growth of T-even phages and their *gt* mutants on strains carrying *rglA* or *rglB* mutations

Phenotype/genotype[a]		Plating efficiency of T-even phages[b]:			
RglA/*rglA*	RglB/*rglB*	T2	T2gt	T4gt	T6gt
Of K12 strain[c]					
+	+	0.1	10^{-7}	10^{-6}	4×10^{-6}
−	+	0.1	10^{-7}	10^{-6}	1.0
+	−	1.0	4×10^{-7}	10^{-5}	4×10^{-6}
−	−	1.0	1.0	1.0	1.0
Of B strain[d]					
+	+	1.0	10^{-6}	10^{-5}	10^{-6}
−	+	1.0	0.5	0.05	1.0

[a] We use the genotype designation because the mutations causing the RglA and RglB phenotypes have been mapped to distinct loci (see below). However, two or more closely linked genes could be required for each of these functions.

[b] Values represent the titer on a given strain/titer on the permissive host *Shigella sonnei* SH. The plating efficiency of wild-type T4 and T6 was 1.0 in all cases.

[c] Prototype *E. coli* K-12 strains were: HR110 *rglA*$^+$ *rglB*$^+$, HR111 *rglA2 rglB*$^+$; HR140 *rglA*$^+$ *rglB8*; HR112 *rglA2 rglB8*.

[d] Prototype *E. coli* B strains were: B *rglA*$^+$ *rglB*$^+$; HR45 *rglA*$^-$ *rglB*$^+$.

functions is not additive, because there always are *gt*$^+$ revertants in the phage stocks that determine a lower limit of plating efficiency. The RglA and RglB phenotypes can also be assayed using the growth properties of phenotypically Glu$^-$ T-even phages (T*), wild type phage that have been grown on *galU* hosts (data not shown). The restriction pattern is the same as for *gt* phages, but the EOPs are increased by a factor of 10^3–10^4, because the T* phages become modified with glucose during replication and are not restricted in subsequent growth cycles.

E. coli B also has two Rgl restriction functions. RglA restricts strongly like its K-12 counterpart (lines 5 and 3 in Table 3), but the RglB activity is very weak (line 6). As in K-12, T6*gt* is restricted only by RglA. In striking contrast to K-12, however, T2*gt* and T4*gt* are only slightly restricted by RglB (EOP of 0.5 and 0.05, respectively, compared with ~10^{-6} EOP for the K-12 RglB). This weak effect has prevented isolation of *rglB* mutants in *E. coli* B by procedures successful in K-12.

McrA and B restriction phenotypes are defined by the acceptance or rejection of DNA modified by sequence-specific methylases (RALEIGH and WILSON 1986). While the two phenotypes are readily separable and can be assayed by several procedures (see MATERIALS AND METHODS), the largest effect occurs when the restriction target is a cloned methylase gene (Table 4). A plasmid carrying the *Hpa*II methylase gene, *hpaIIM*, transforms McrA$^+$ cells at about 10^{-3} relative to transformation of McrA$^-$ cells (Table 4, lines 1 ane 3). Similarly, plasmids carrying *haeIIM* or *mspIM* transform McrB$^+$ cells less efficiently than

TABLE 4

Efficiency of transformation of strains carrying *mcrA* and *mcrB* mutations by plasmids bearing methylase genes

Phenotype/genotype[a] of K-12 strains[b]		Relative transformation efficiency[c] of plasmids		
McrA/*mcrA*	McrB/*mcrB*	pM·*Hpa*II	p*Hae*II 4-11	p*Msp*I 1-30
+	+	0.002	0.0005	0.001
−	+	0.8	0.0007	0.0009
+	−	0.001	0.3	0.4
−	−	(1)	(1)	(1)

[a] We use the genotype designation because the mutations have been mapped to distinct loci (see below). However, each Mcr function could be encoded by multiple closely linked genes.

[b] Mcr assay method IIB.

[c] The prototype recipient strains used were: ER1381 *mcrA*$^+$ *mcrB*$^+$; ER1378 *mcrA*$^+$ *mcrB1*; ER1564 *mcrA*1272::Tn*10* *mcrB*$^+$; ER1565 *mcrA*1272::Tn*10* *mcrB1*.

McrB$^-$ cells (Table 4, lines 1 and 2). The McrA and McrB functions act completely independently. The presence or absence of McrB has no effect on transformation with *hpaIIM*, and McrA has no effect on transformation with *haeIIM*. Assay of Mcr functions with *in vivo* methylated phage (method I) or *in vitro* methylated pBR322 (method IIA) yielded similar patterns, but restriction was reduced (not shown).

We have not characterized Mcr functions that may be present in *E. coli* B, but we have tested two K-B hybrid strains that carry the *mcrB* locus from *E. coli* B, HB101 and its RecA$^+$ derivative, RR1. We find no detectable McrB-dependent restriction in these strains (not shown). This observation is consistent with the attenuated RglB restriction phenotype of *E. coli* B. However, most of the methylase clones used to characterize McrB were isolated in RR1 (RALEIGH and WILSON 1986) and it is conceivable that the *E. coli* B McrB has a different specificity, not observable with our tests.

Characterization of mutant strains and coincidence of phenotype: The *rglA2* mutant, the *rglB8* mutant and the mutant alleles *mcrB1-mcrB7*, as well as Δ(*hsdS Hin*dIII 1.8) (SAIN and MURRAY 1980), were shown to display correlated Mcr and Rgl phenotypes (data not shown). The *mcrB* alleles tested included five isolated spontaneously or following Tn*10* mutagenesis (MATERIALS AND METHODS; tested in ER1467, ER1468, ER1469, ER1486 and ER1489), the *mcrB1* allele found in WA802 (see below) and the *mcrB7* allele of JM107MA2, isolated by BLUMENTHAL, GREGORY and COOPERIDER (1985; selection was for acceptance of *pvuIIM*). The allele Δ(*hsd*S *Hin*dIII 1.8) present in strain NM496 (SAIN and MURRAY 1980; N. MURRAY, personal communication) was constructed *in vitro* and transferred into the chromosome of C600 by recombination. The *rgl* and *mcr* mutations were isolated using three different selective methods (for

sensitivity to T-even *gt* phages, for M·*Hae*II acceptance, and for M·*Pvu*II acceptance) in a variety of strain backgrounds (HR110 (K-12 wild type), ER1370, W3110, and JM107). In addition, a large number of laboratory strains have been tested for Rgl or Mcr phenotypes (RALEIGH *et al.* 1988), and those tested by both methods (25 more strains) also yielded correlated phenotypes. Many laboratory strains are McrA$^-$ and a few are McrB$^-$.

We believe that McrA$^-$ laboratory variants are prevalent because a gene essential for McrA activity is carried by a UV-inducible prophage-like element, *e14* (GREENER and HILL 1980; BRODY, GREENER and HILL 1985; and see below) and that this has been cured on one or more occasions during construction of laboratory strains by mutagenic treatments. Three lines of evidence support this view. First, the loss or alteration of *e14* was correlated with the loss of McrA restriction. *e14*$^+$ CH731 is McrA$^+$, while isogenic strains cured of *e14* (CH1332) or carrying one of three transposon insertions in it (CH1372) are McrA$^-$ (RALEIGH *et al.* 1988). Second, RglA$^-$ strains can be derived from MM294 or ER1378 easily by a UV curing protocol (data not shown), consistent with the inducible nature of the *e14* element. Third, cloning experiments support this assignment (see below).

As far as we have determined by pedigree analysis, all McrB$^-$ laboratory K-12 strains (except the K/B hybrids RR1 and HB101) ultimately derive the 99-min *mcrB* region (see below) from the Cavalli Hfr or from its *hsd* descendants, WA802 (also known as K802) or WA803 (K803). W6, the parent of the Cavalli Hfr, is McrA$^+$McrB$^+$. The *mcrB1* mutation seems to have appeared in the Cavalli Hfr, which is McrA$^+$McrB$^-$. This strain was crossed with C600 (McrA$^-$McrB$^+$) to yield WA704 (WOOD 1966). The recombination event selected is consistent with an McrA$^-$RglA$^-$, McrB$^-$RglB$^-$ phenotype for WA704 (not tested), and thus for its spontaneous HsdR$^-$ derivatives WA802 and WA803, as is observed.

A spontaneous *mcrB* mutation selected from ER1380 (*hsdR2 zjj*202::Tn*10*) and now designated Δ(*mcrB*)2, deserves comment because it differs from its parent in four ways. Complementation (see Table 5) and transduction analysis (not shown) reveal that in addition to the selected loss of McrB activity, Δ(*mcrB*)2 lacks *Eco*K methylase and *mrr* gene activity, and the linkage between the nearby Tn*10* tetracycline-resistance marker and these phenotypes has increased from 30% to >98%. Simultaneous elimination of three functions and reduced frequency of recombination between two markers are properties of a deletion, and this has been confirmed by Southern analysis (see below).

Conjugational mapping of *rglA* and *B*: These two loci were initially mapped in K-12 using the Rgl phenotypes. The *rglA* gene was linked to *trp*, near 27 min; the *rglB* gene was linked to *thr*, around 0 min (data not shown). In *E. coli* B, *rglB* is in a similar location. However, the *E. coli* B *rglA* gene maps in a different position than in K-12, but we are unable to locate the position exactly. Except for *ts* mutations, the *rglA* mutants obtained in this background were simultaneously Thi$^-$ as previously noted (REVEL 1967). One of these apparent deletions was mapped by F$^+$ crosses to the vicinity of *his*. The F$^+$ donor, AC2322, was crossed with the *rglA thi* multiple auxotroph HR45 as described by BOYER (1966). RglA$^+$ was transferred at high frequency (63%) when His$^+$ was selected and was 100% linked to Thi$^+$ (400 scored), but was essentially unlinked to Trp$^+$ (0.5%) or Pro$^+$ (1%). In K-12, *his* is at 44 min, some 20 min away from *trp*, where *rglA* is found in K-12. A roughly mapped *thi* locus at 46 min in K-12 (BACHMANN, 1987a) might correspond to the site of *rglA* in *E. coli* B. The relative locations of *his*, *trp* and *thi* in *E. coli* B are not well known.

Transductional mapping of *rglA* (*mcrA*): P1 transduction of *rglA* revealed that it is linked only to *purB*, of four tested auxotrophic markers in the region of the *E. coli* chromosome near *trp* (23–28 min). In crosses a–c (Figure 1A), 67–75% of selected PurB$^+$ transductants received the donor RglA phenotype, while *trpA* and *pyrF* were unlinked. In a fourth cross (not shown) a *pyrC rglA* recipient was transduced with P1(HR110). No donor RglA$^+$ activity was detected among 200 PyrC$^+$ transductants. Since *purB* shows 4% linkage with *pyrC* (SIGNER, BECKWITH and BRENNER 1965) the data suggested, but did not prove, that the *rglA* locus is clockwise of *purB*. McrA restriction was unknown when these experiments were performed.

With the discovery of the McrA and McrB restriction systems (RALEIGH and WILSON 1986) and demonstration of coincidence of McrA/RglA and McrB/RglB phenotypes in more than 25 laboratory strains (RALEIGH *et al.* 1988) it was of interest definitively to localize the *rglA/mcrA* genes with respect to *purB* and to document a possible coincidence of the genetic loci. The analysis used strains with Tn*10* insertions at two loci in the *purB-trp* region (HUGHES, SIMONS and NUNN 1988). Tet^r Tn*10*-2 transductants showed weak linkage with *rglA* and *purB* (Figure 1A, crosses d–f). Though the number of recombinants exhibiting donor activity at these loci was low (18 total), the results consistently placed *rglA* on the *trp* side of *purB*, irrespective of strain background. RglA and McrA phenotypes, assayed as described in MATERIALS AND METHODS and illustrated in Tables 3 and 4, were coincident. In addition, the similarity of the results in the presence or absence of RglB$^+$ (McrB$^+$) confirms the genetic independence of the A and B restriction

A. Summary of P1 transduction crosses.

Donor strain	Recipient strain	#, Selected phenotype	Donor marker scored, %		Other phenotypes selected or scored
			rglA	*purB*	
a. P1(1100)	X149	150 Pur$^+$	67	100	Trp$^+$, Pyr$^+$; donor RglA unlinked
b. P1(HR110)	X149	108 Pur$^+$	75	100	Trp$^+$, Pyr$^+$; donor RglA unlinked
c. P1(HR116)	X149	150 Pur$^+$	75	100	
d. P1(ER1509)	ER1378	94 Tet^r	4	3	
e. P1(ER1509)	ER1381	200 Tet^r	2	1	
f. P1(ER1509)	ER1398	100 Tet^r	9	4	Donor Trp 8% linked
g. P1(ER1576)	ER1378	94 Tet^r	70	50	McrA, see legend

B. Genetic map of the *mcr/rglA* region.

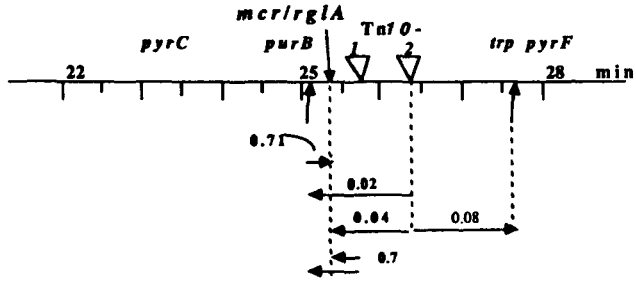

FIGURE 1.—P1 crosses mapping the *mcrA/rglA* region of *E. coli* K-12. (A) Summary of P1 crosses. Strains used with relevant genotype were as follows. Crosses (a–c), donors—1100: *purB*$^+$ *rglA*$^+$, HR110: *purB*$^+$ *rglA*$^+$, HR116: *purB*$^+$ *rglA*^{n}; recipient—*X149: pyrF purB trp rglA*. Crosses (d–g), donors—ER1509: *purB*51 *zcg*2043::Tn*10* (Tet^r) *rglA trp*45, ER1576: *purB*58 *zcf*2033::Tn*10* (Tet^r) *rglA trpA trpE*; recipients—ER1378: *rglB1 purB*$^+$ *rglA*$^+$ *trp*31 Tet^s, ER1381: *mcrB*$^+$ *purB*$^+$ *rglA*$^+$ *trp*31 Tet^s, ER1398: *rglB1 purB*$^+$ *rglA*$^+$ *trp*$^+$ Tet^s. Thirty transductants from the three recombinant classes in cross g (10 each of phenotypes Pur$^+$ RglA$^-$, Pur$^-$ RglA$^-$, and Pur$^+$ RglA$^-$) were tested for McrA phenotype; it coincided with the RglA phenotype. (B) Genetic map of the *mcrA/rglA* region of *E. coli* K-12. Arrows begin at the marker selected and point to the marker scored. Numbers are the average fraction of selected recombinants inheriting the donor allele at the unselected locus. Tn*10*-1 and Tn*10*-2 are *zcg*2043::Tn*10*, from ER1509, and *zcf*2033::Tn*10*, from ER1576, respectively. These insertions are described in HUGHES, SIMONS and NUNN (1988); the original strains containing them were RS3245 and RS3232, respectively.

activities. Using the Tn*10*-1 insertion, which is more closely linked to *rglA* (*mcrA*) (Figure 1A, cross g), we obtained greater numbers of Tet^r transductants bearing the donor alleles of *rglA* (*mcrA*) and *purB*. The ratio of transductants with the donor *rglA* allele to those with the donor *purB* allele confirmed the previous placement of *rglA* clockwise of *purB*. The RglA and McrA phenotypes of 30 recombinant transductants (10 of each type) were shown to be coincident (not shown).

We also showed that the *e14*-1272::Tn*10* insertion isolated by BRODY, GREENER and HILL (1985) is the direct cause of the McrA$^-$ phenotype of CH1372: Tet^r was >98% cotransducible with RglA$^-$ (not

A. Map position relative to *hsdR* and *serB*

	rglB	*hsdR*	*serB*	# of transductants
Donor	+	-	+	
Recipient	-	+	-	
			--------	309
	----------		----------	24
	----------		----------	112
	------		--------	5
			Total	450

B. Map position relative to *zjj202*::Tn*10* and *serB*

	mcrB	*zjj202*::Tn*10*	*serB*		
Donor	+	-	+		
Recipient	-	+	-		
			--------	67	(43)
		----------	----------	71	(54)
	----------		----------	24	(23)
	----------		--------	2	(2)
				164	(122)

C. Map position relative to *mrr2*::Tn*5* and *hsdR*

	mcrB	*hsdR*	*mrr*	
Donor	+	+	-	
Recipient	-	-	+	
			----	20
		----------		4
	----------			51
	-------		----	0
				75

FIGURE 2.—P1 crosses mapping the *mcrB/rglB* region of *E. coli* K-12. Representative crosses determining the positions of *mcrB*, *hsdR*, *mrr*, *zjj*202::Tn*10*, and *serB* are shown. Dashed lines represent donor DNA transferred to the transductant, and each row represents one class of recombinant, with the number of recombinants in that class given on the right. In cross (B), the numbers in parentheses are the number of recombinants in each class that were tested for RglB phenotype as well as McrB phenotype. In all cases the two coincided. Cross (A), P1(HR184) × HR186: selection was for Ser$^+$, the recipient was RglA$^-$; tests of restriction phenotype were: RglB, T2*gt*; HsdR, λ·0. Cross (B), P1(ER1381) × ER1394: selection was for Ser$^+$; the recipient was RglA$^+$; tests of restriction were: McrB, λ·*Msp*I; RglB, T2 wild type. Cross (C), P1(JH83) × ER1378: selection was for Kan^r; the recipient was Kan^s; tests of restriction were: McrB, λ·*Msp*I; HsdR, λ·0; Mrr, λ·*Pst*I. 5 Mrr$^+$ transductants were excluded from the analysis (BIEK and ROTH 1980).

shown), and all 10 transductants tested were also McrA$^-$. CH1372 and its parent CH731 are T6^r and thus could not be tested directly for RglA phenotype.

Transductional mapping of *rglB* (*mcrB*): Representative transductional crosses which establish that *mcrB* (*rglB*) is tightly linked to two other K-12 restriction systems, in the gene order *mcrB* (*rglB*)-*hsd-mrr-zjj*202::Tn*10-serB* proceeding clockwise on the host chromosome, are summarized in Figure 2.

The gene order *rglB* (*mcrB*)–*hsdR–serB* comes from the type of cross shown in Figure 2A. Here, Ser$^+$

transductants were selected and RglB and HsdR were scored using T2*gt* and λ·0 (MATERIALS AND METHODS). *rglB* and *hsdR* are tightly linked (as previously noted by REVEL 1983), in agreement with the report of RAVI, SOZHAMANNAN and DHARMALINGAM (1985). Of Ser^+ recombinants that received one of the two restriction loci, 79% received both (112/141). The recombinant class with the donor allele at *hsdR* in conjunction with the recipient allele at *rglB* was larger than its converse (24:5), suggesting that *hsdR* is in the middle. In five other crosses with the *rglB* (*mcrB*) and *hsd* markers in various configurations, Ser^+ recombinants were scored for HsdR and either RglB or McrB phenotypes (not shown). The results agreed with those shown, yielding a linkage of *rglB* (*mcrB*) to *serB* of 15–26% and to *hsdR* of 80–95%. All possible combinations of *hsdR* and *rglB* (*mcrB*) genotypes were obtained, confirming the independence of the two restriction systems.

The order *mcrB-zjj*202::Tn*10-serB* was from the type of cross represented in Figure 2B. The location of the transposon insertion agrees with a previous placement of *zjj*202::Tn*10* clockwise of *hsd* (FREITAG and EISENSTEIN 1983). The observed 58% linkage of Tet^r to *serB* when the Tn*10* was in the recipient was reduced slightly to 40% when the insertion was in the donor. All transductants were initially screened for McrB phenotype by method I. When a randomly selected large fraction of the recombinants were tested also for RglB phenotype by T2 plating efficiency (see Table 3), the two phenotypes coincided.

Crosses of the type shown in Figure 2C confirmed the gene order *mcrB-hsdR-mrr*, (HEITMAN and MODEL 1987) and showed the same tight linkage between *hsdR* and *mcrB* seen in Figure 2A. Of Kan^r Mrr^- transductants that received either unselected restriction locus, 90% received both; four received the donor *hsdR* allele only, but none received the donor *mcrB* allele only, placing *hsdR* in the middle.

Genetic organization and restriction map of the *mcrB-hsd-mrr* region: To verify this transductional order and to establish the close physical proximity of the three genes, we first cloned the *mcrB* gene *de novo*. We then carried out a comparative restriction mapping and complementation analysis of one *mcrB* clone, pER105, and of two previously characterized *hsd* clones that carry extra genomic sequences flanking the host-specific restriction loci, pBg3 and pBg6 (SAIN and MURRAY 1980; see Figure 3).

Clones of both *mcr* loci were selected using resistance to T4*gt* (Rgl^+ phenotype). Donor DNA was from ER1370 ($McrA^+$ $McrB^+$), while the recipient host was the double mutant strain ER1516 (*mcrA mcrB*1), which is completely sensitive to T4*gt* (Table 3). Since both single mutants are resistant to T4*gt* due to restriction by the remaining rgl^+ locus, acquisition by the double mutant of a clone complementing either *rgl* mutation should confer resistance. Accordingly, a population of transformed ER1516 cells carrying random *Bam*HI fragments of ER1370 DNA in pBR322 was grown for a generation, infected with T4*gt*, and plated on ampicillin plates (MATERIALS AND METHODS). Under these conditions, survival of isogenic singly mutant *rgl* strains transformed with vector alone was 30–50%. Of 23 survivors, seven retained a $T4gt^r$ phenotype upon purification. One of these, pER106, contained the *mcrA* gene, since it also conferred resistance to T6*gt* and displayed the $McrA^+$ phenotype. The other six, which retained sensitivity to T6*gt* and restored restriction of λ·*Msp*I and λ·*Hae*II, contained the *mcrB* gene. All six *mcrB* clones contained a common 8-kb fragment. The fragment in pER105 was mapped to a region that includes *hsdS* and about seven kb of downstream sequences (Figure 3). This fragment, as well as those in the other five *mcrB* clones, were in the same orientation with respect to the vector as in pBg6, with *hsdS* distal to the Tet promoter of pBR322. One of the six also contained a second *Bam*HI fragment from elsewhere in the genome.

Complementation of mutations in the *mcrB-hsd-mrr* region by pER105 and by the two *hsd* clones pBg3 and pBg6 (SAIN and MURRAY 1980) is shown in Table 5 and correlated with the physical map in Figure 3B. In addition to supplying the missing *mcrB* gene function in the deletion strain ER1648 (*mcrB*Δ2) and the *mcrB1* point mutation in ER1656 (restriction of λ·*Msp*I, line 1), pER105 complemented all the remaining *mcrB* alleles in our collection (not shown). pBg3, which was known to include all of *hsdR* and *hsdM* and the proximal part of *hsdS* (Figure 3), complements the *hsdR2* mutation in ER1656 (Table 5, line 3). Although the overall effect of Mrr restriction is small (threefold), it is clear that pBg3 also complements the Mrr deficiency in ER1648 *mcrB*Δ2 (Table 5, compare lines 4 and 10). In contrast, pBg6, which includes the distal part of *hsdS* and at least 8 kb of added downstream sequences, complemented only the *mcrB* mutations. These data are consistent with the placement of *hsdR* in the middle of the *mcrB-hsd-mrr* gene cluster by transduction analysis (Figure 2C).

Combining our observed gene order *mcrB-hsd-mrr-serB* with the known orientation and physical positions of the *hsd* genes on pBg3 and pBg6 permits us to orient the *hsdRMS* genes relative to the chromosome as shown in Figure 3A. It is important to note that this orientation is *reversed* from all published versions of the *hsd* region. A consequence is that the *hsdRMS* genes (SAIN and MURRAY 1980; LOENEN *et al.* 1987) as well as the downstream genes putatively identified with *mcrB* (ROSS, ACHBERGER and BRAYMER 1987), are transcribed and translated in a counterclockwise direction on the *E. coli* map. Our independent deri-

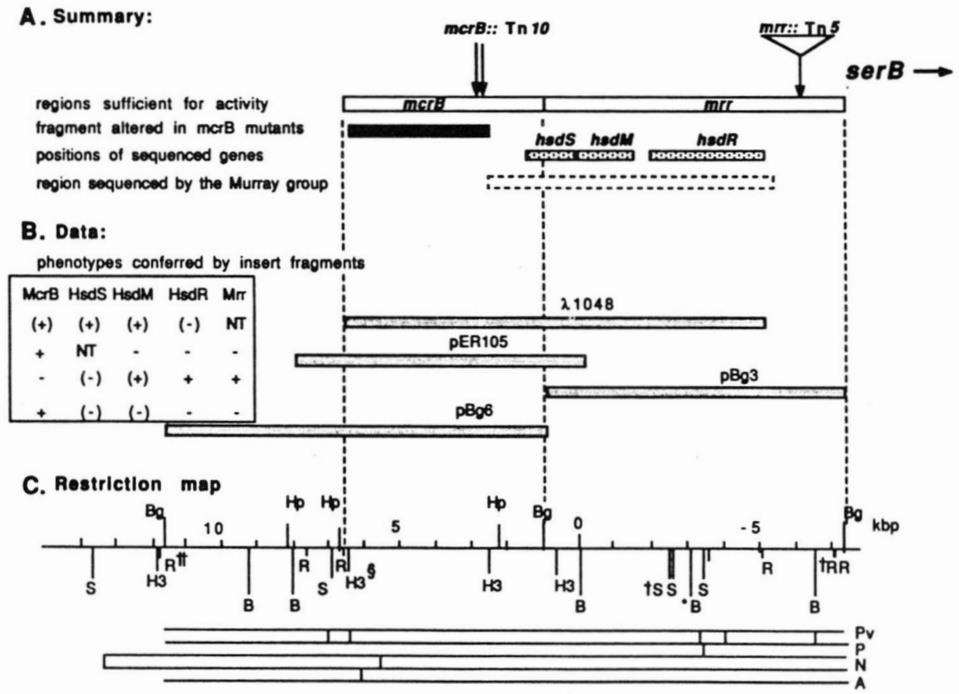

FIGURE 3.—Physical and genetic organization of the *mrr-hsd-mcrB* region. (A) Summary of the genetic organization of the region, aligned with the restriction map in part C. *serB* lies to the right of the figure, *uxu* to the left. Arrows: positions of transposon insertions (the Tn*10* insertions can be positioned within ±100 bp, Figure 4; the *mrr*::Tn*5* insertion isolated by HEITMAN and MODEL (1987) was mapped by them to a 2-kb *Eco*RI fragment upstream of *hsdR*, but the position of the *mrr*::Tn*5* insertion has not been mapped within the *Eco*RI fragment); open boxes: DNA shown to be sufficient but not necessarily wholly required for the indicated activities; filled box: *Hin*dIII fragment that is altered in the McrB mutants analyzed in Figure 4; hatched boxes: sequenced genes within the sequenced region; dashed box: sequenced region (GOUGH and MURRAY 1983; LOENEN *et al.* 1987). (B) Summary of genetic data. Stippled boxes indicate the DNA carried by the constructs named. Box at left summarizes the phenotypes shown to be conferred by each construct. Data in parentheses are from SAIN and MURRAY (1980) (*hsd* phenotypes) or ROSS and BRAYMER (1987) (*mcrB* phenotype). Other data are in Table 5 or not shown. The McrB$^+$ phenotypes of λ1048 and pBg6 allow us to limit the extent of DNA required for McrB activity (vertical dashed lines). (C) Restriction map. Scale is numbered in kilobasepairs. On the scale are placed sites for enzymes (B, H3, R, S, Hp, Bg) mapped by SAIN and MURRAY (1980) and confirmed by us, but reversed to conform with the orientation shown in Figure 2. Boxes below the scale show sites not mapped by SAIN and MURRAY (1980). B = *Bam*HI; H3 = *Hin*dIII; R = *Eco*RI; P = *Pst*I; Pv = *Pvu*II; S = *Sal*I; N = *Nco*I; A = *Apa*I; Hp = *Hpa*I; Bg = *Bgl*II. The restriction map was generated by sizing 30 (pBg3), 26 (pBg6) or 18 (pER105) fragments generated by single and double restriction digests of plasmid DNA, or from Southern blots of singly digested DNA from wild type cells, using pBg6 as probe. The scale is numbered to coincide with the numbering of the *hsdS* sequence (GOUGH and MURRAY 1983), with 0 at one end of the *Bam*HI fragment carried by pER105, and increasing in the direction of *mcrB*. (*) This *Bam*HI site mapped by us does not appear in SAIN and MURRAY (1980) but appears in LOENEN *et al.* (1987). (†) We did not attempt to confirm the *Eco*RI and *Sal*I doublets identified by SAIN and MURRAY (1980). (§) We did not order the *Hpa*I, *Eco*RI and *Hin*dIII sites between 6 kb and 7 kb. (††) We did not attempt to confirm the *Eco*RI site at 11.7 kb.

vation of this orientation of the *hsd* genes produces a restriction map that is in accord with that of a region between 98 and 99 min on the *E. coli* genome map obtained by KOHARA, AKIYAMA and ISONO (1987).

Southern blot analysis of *mcrB* mutants: To examine the stability of the *mcrB* region, to further characterize our *mcrB* mutations and to localize the gene(s), we used pBg6 and pNK290 (which carries Tn*10* homology) as hybridization probes to parental and mutant chromosomal DNAs. We first confirmed the restriction map of the insert DNA from K-12 strain CR63 in pBg3 and pBg6 (SAIN and MURRAY 1980) and located sites for four additional enzymes (Figure 3C). Labeled pBg6 was then hybridized to DNA of K-12 strains W3110, ER1370, LCK8 and ER1380 digested with *Sal*I, *Nco*I, *Hin*dIII, or *Apa*I. The pBg6 probe gave fragment patterns in each digest consistent with the restriction map developed from CR63 (data not shown). Thus we conclude that the *mcrB* region is conserved in four different K-12 backgrounds. In addition, it appears that the *mcrB1* mutation in LCK8 is either a point mutation or a very small deletion or rearrangement, since the fragment patterns were identical with those from strains carrying the wild type *mcrB*$^+$ allele.

When the pBg6 probe was hybridized to blots of our five newly-isolated spontaneous and Tn*10*-induced *mcrB* mutants (*mcrB2-mcrB6*), the fragment pattern was changed. Specifically, all mutations altered the 3.9-kb *Hin*dIII fragment just downstream from the 3′ end of *hsdS* (Figures 3 and 4, black box). This band was replaced by a new 5.1-kb fragment in *mcrB3*, by a 5.6-kb fragment in *mcrB5* and *mcrB6*, by two fragments of 5.4 and 2.6 kb in *mcrB4*, and was missing altogether in *mcrB2* (Figure 4). In addition, *mcrB2* showed no homology to the flanking 5.6- and 1.8-kb

TABLE 5

Complementation of *mcrB*, *hsdR* and *mrr*

Plasmid	Tester phage[a]	Efficiency of λ plating on host strain with indicated plasmid[b]		Inferred plasmid genotype		
		ER1648 Δ(*mcrB-hsdSMR-mrr*)	ER1656 HsdS$^+$ HsdM$^+$ *hsdR2 mcrB1*	*mcrB*	*hsdR*[c]	*mrr*
1. pER105[d]	λ·*Msp*I	**0.03**	**0.05**	+	NT	NT
2. pBg3	λ·*Msp*I	0.8	0.6	−		
3.	λ·0	0.6	**0.03**		+	
4.	λ·*Pst*I[e]	**0.27 ± 0.07**	NT			+
5. pBg6	λ·*Msp*I	**0.04**	**0.02**	+		
6.	λ·0	0.9	1.4		−	
7.	λ·*Pst*I[e]	0.85 ± 0.3	NT			−
8. pBR322	λ·*Msp*I	(1.0)	0.9	−		
9.	λ·0	(1.0)	0.6		−	
10.	λ·*Pst*I[e]	(1.0) ± 0.1	NT			−

[a] λ·*Msp*I tests the McrB phenotype of the host; λ·0 tests HsdR; λ·*Pst*I tests Mrr.

[b] Numbers (EOP) are the ratio of the titer of the phage on a given strain to the titer of the same phage on ER1648/pBR322. Numbers in boldface indicate positive restriction activity. NT = not tested.

[c] Note that *Eco*K restriction activity requires the presence of all three gene products: HsdR, HsdM, and HsdS. pBg3 carries R and M, but ER1648 does not provide HsdS.

[d] pER105 was tested in separate experiments; the hosts tested were ER1486 and ER1516 (Table 1). Numbers are normalized to the same strains with pBR322, as in other experiments.

[e] Mrr tests were a separate experiment. Four independent transformants for each plasmid were tested for Mrr; values are the average and standard deviation of EOP normalized to average titer on ER1648/pBR322.

*Hin*dIII fragments detected by pBg6 in the remaining mutants, consistent with its genetic behavior as a deletion. pNK290 hybridized to the same new bands as did pBg6 in the three Tn*10*-induced mutants *mcrB4*, *mcrB5* and *mcrB6*, as expected if the insertions caused the McrB$^-$ phenotype. The single exception, a 2.4-kb band in *mcrB5* and *mcrB6* detected by pNK290 but not by pBg6, probably has about 100 bp of sequence homologous to pBg6 that went undetected in the exposures used.

Combining the blot data from *Hin*dIII, *Apa*I and *Nco*I digests with the well-characterized Tn*10* restriction map (KLECKNER 1988) allowed us to locate the three Tn*10* insertions as shown in Figure 3A. The *mcrB4*::Tn*10* insertion is about 300 bp to the left of the first *Hin*dIII site located downstream of *hsdS*; both *mcrB5*::Tn*10* and *mcrB6*::Tn*10* insertions map about 200 bp closer to the same *Hin*dIII site. In view of the well-known site specificity of Tn*10* insertion (KLECKNER *et al.* 1979), the occurrence of two independent insertions at the same site is not surprising. The IS insert in *mcrB3*::*IS* is unknown, and its location within the 3.9-kb *Hin*dIII fragment in Figure 4 is arbitrary.

The observation that Tn*10* promotes loss of adjacent DNA (ROSS, SWAN and KLECKNER 1979), combined with an analysis of our Southern blot data (not shown) suggests that the deletion in Δ(*mcrB-hsd-mrr*)2 was probably mediated by the *zjj*202::Tn*10* insertion in the parental strain, ER1380. Specifically, we can conclude that *zjj*202::Tn*10* is some distance from *mcrB*, since its presence in LCK8 or ER1380 did not alter the fragments detected by the *mcrB*-specific probe. None of the *Hin*dIII, *Sal*I, or *Nco*I fragments homologous to pBg6 was disrupted. Further, two IS10-containing *Hin*dIII bands (4.9 and 4.1 kb) detected by pNK290 in the parental strain ER1380 did not hybridize with the *mcrB*-specific probe pBg6. The disappearance of the 4.9-kb band in the *mcrB2* deletion strain ER1486 suggests that one end of the deletion begins at the Tn*10* element.

The *mcrA* gene is on the *e14* excisable element: Our attention was drawn to the *e14* element by genetic observations. Transductional anomalies in some strains, suggesting the presence of a large deletion near *mcrA* and *purB*, led to the *E. coli* K-12 map (BACHMANN 1987a) and awareness of *att e14*. We then observed that strains known to lack *e14* (*e.g.*, C600) were McrA$^-$, while those known to carry it were McrA$^+$. As noted above (transduction), we showed that one of three strains with Tn*10* insertions in the element (BRODY, GREENER and HILL 1985) had also become McrA$^-$RglA$^-$. We designated the insertion *mcrA*1272::Tn*10* (Table 1). This mutation is complemented for both RglA and McrA phenotypes by our *mcrA* clone, pER106.

Physical and genetic analysis of pER106 positions the *mcrA* gene at the right end of the *e14* excisable element, immediately adjacent to the *pin* gene and its associated invertible DNA, as summarized in Figure 5. We compared restriction digests of pER106 with digests of plasmids known to carry portions of *e14*. pHB106, pHB107 and pAG2 together carry most of *e14* and some flanking chromosomal sequences (BRODY, GREENER and HILL 1985), while pGP325

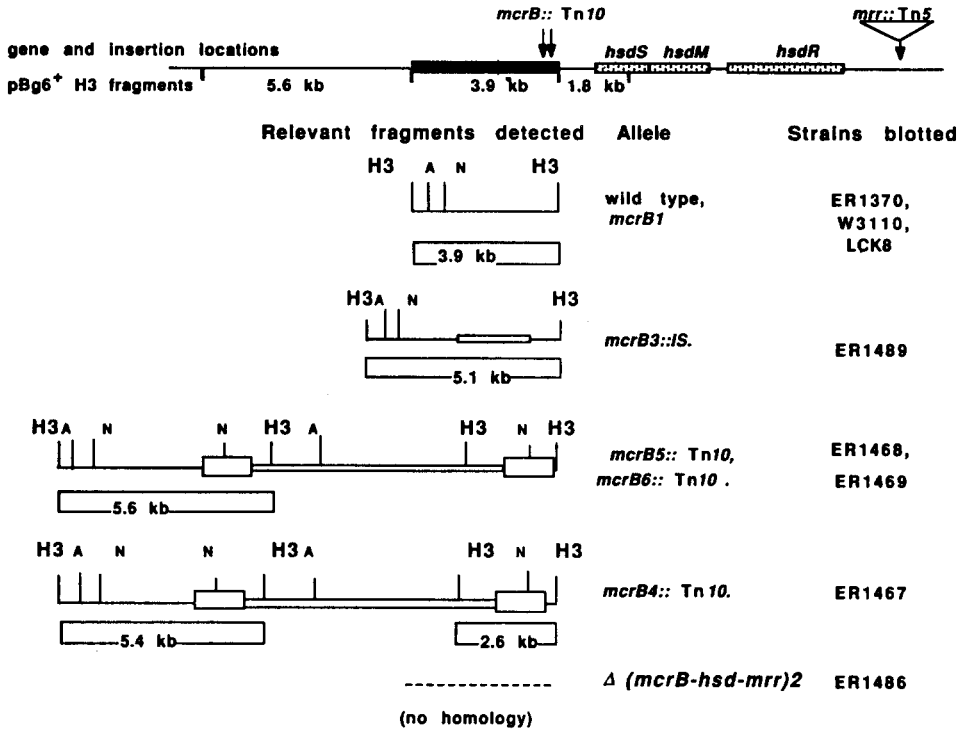

FIGURE 4.—Example of Southern blot analysis of parental and *mcrB* mutant DNA. *Hin*dIII digests of chromosomal DNA of the indicated strains were electrophoresed, blotted, and probed with labeled pBg6. At top is the organization of the *mcrB-hsd-mrr* region, redrawn from Figure 3. Ticks below the genetic map indicate the four *Hin*dIII (H3) sites that define fragments that hybridize to pBg6. The black box highlights the single *Hin*dIII fragment altered by all the mutations except *mcrB1*. The flanking 1.8- and 5.6-kbp *Hin*dIII fragments are conserved in all strains except in deletion strain, Δ(*mcrB-hsd-mrr*)2, which had lost all pBg6-homologous sequences (represented by the dotted line), as well as McrB, Hsd, and Mrr activity. Structures below the black box indicate the wild-type 3.9-kbp fragment and alterations in this fragment in insertion and deletion strains. Here thin lines represent chromosomal sequence; thin open boxes represent inserted DNA; thick open boxes represent the IS*10* inverted repeats of Tn*10*, which also hybridize with pNK290 as discussed in the text. Positions of restriction sites, abbreviated as in Figure 3, are shown. Shaded box below each schema are fragments detected by the pBg6 probe. This blot and similar blots to *Apa*I and *Nco*I digests (data not shown) allowed placement and orientation of the Tn*10* insertions. Placement of the unknown insertion in ER1489 (*mcrB*3::IS) is arbitrary, since no new sites for these restriction enzymes appeared.

carries a segment of *e14* encoding the site-specific recombination function, Pin, together with the invertible DNA on which it acts (PLASTERK and VAN DE PUTTE 1985). In *Bam*HI-*Hin*dIII digests, pER106 displayed a 4.1-kb fragment common to pHB107 and pHB106, and a 3.9-kb fragment in common with the right ends of the pAG2 and pGP325 inserts (Figure 5B). In the same digests, pAG2 and pGP325 shared a 1.7-kb fragment not present in pER106. pAG2 carried an additional 5.5-kb *Bam*HI-*Hin*dIII fragment, while pGP325 carried instead an additional 2.1-kb *Eco*RI-*Hin*dIII fragment. Thus, pER106 DNA includes the *Hin*dIII site at 13.9 kb (Figure 5A). The inference that pER106 carries part of the *e14* element was confirmed by functional complementation analysis (Figure 5B) and by further restriction digests (Figure 5C). These data show that pER106, like pAG2 and pGP325, carries a functional *pin* gene along with its associated invertible DNA segment. pHB107, which carries *att e14* and flanking chromosomal sequences, complemented only *purB* as expected, while pHB106, which retains the right end of *e14*, was inactive in all functional tests. Of the five plasmids, only pER106 complemented *mcrA* defects.

The *mcrA* gene must lie downstream of *pin*, straddling the *Hin*dIII site at 3.9 kb on the map of pER106 (Figure 5C), since pGP325 and pAG2 are McrA$^-$ due to truncation at this position, yet retain the site of the *mcrA*1272::Tn*10* insertion (triangle b) that inactivates the gene. We verified this hypothesis by analysis of subclones of pER106 that eliminated the DNA to the right of the *Hin*dIII site (pER146) or to the right of the *Eco*RI site in the flanking region (pER137); pER137 retained McrA activity but pER146 did not. Both plasmids retained Pin function and the invertible DNA.

DISCUSSION

***rgl* and *mcr* are genetically identical:** To provide a grounding for biochemical characterization, we went to considerable lengths to establish the identity of the Rgl and Mcr systems. In particular, we wished to know whether RglB is specific for hydroxymethylcytosine-containing DNA, because there are available biochemical data on RglB suggesting that at least two components are involved in restriction (FLEISHMAN,

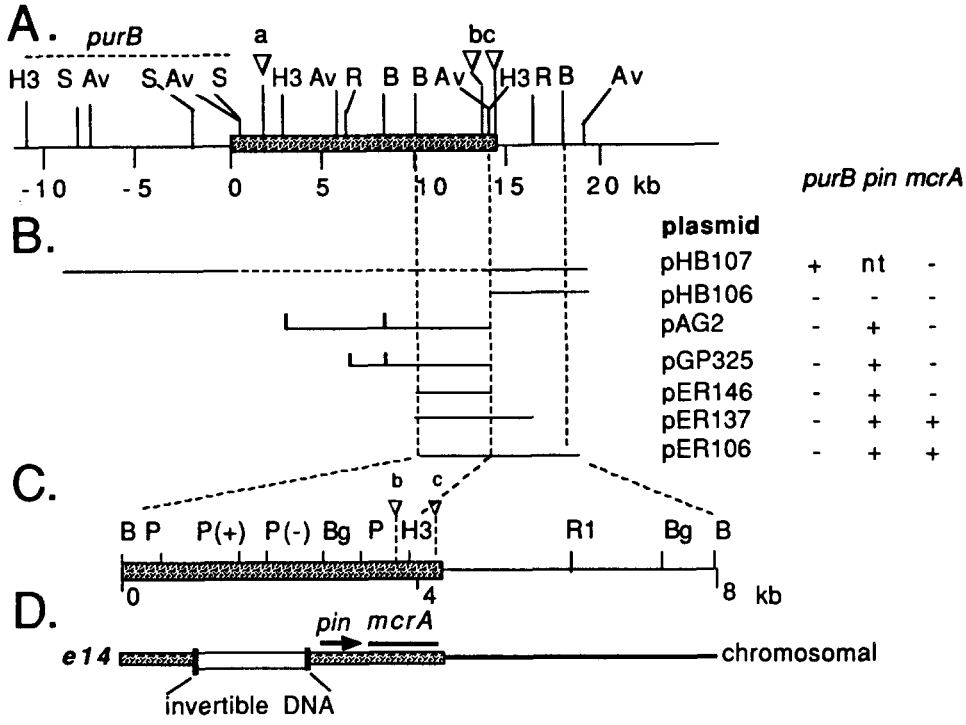

FIGURE 5.—Summary of the organization of the *mcrA* region. (A) Restriction map in kilobasepairs, redrawn from BRODY, GREENER and HILL (1985). The map shows the *e14* excisable element (wide filled box), the approximate position of *purB* (dashed line above the scale), positions of restriction enzyme sites (abbreviated as in Figure 3 and Av = *Ava*I) and positions of three *e14*::Tn*10* insertions (triangles: a = insertion 1279; b = 1272; c = 1290) as determined by BRODY, GREENER and HILL (1985). Note that these are not the same insertions indicated in Figure 1. (B) Parts of the region carried by various plasmids (Table 2) and the genetic loci associated with each, assayed as described in MATERIALS AND METHODS. pHB107 does not carry the DNA corresponding to the dashed line. Vertical dashed lines, and ticks on pAG2 and pGP325, indicate the restriction fragments used to correlate the various plasmid maps (see text). (C) Expanded map of the pER106 insert with *Pst*I (P) and *Bgl*II (Bg) sites added. P(+) and P(−) refer to *Pst*I sites in the two orientations of the invertible DNA. *Ava*I was not mapped. (D) Inferred positions of the various genetic elements on the 8.0-kb pER106 insert. The *mcrA*1272::Tn*10* insertion (triangle b) maps to the left of the *Hin*dIII site at 3.9 kb on the lower map (BRODY, GREENER and HILL 1985). The *e14*::1290 insertion (triangle c) maps to the right of the *Hin*dIII site, but is in *e14*, because it can be excised and reinserted with the element (BRODY, GREENER and HILL 1985); its precise position within these limits is not known. However, it does not inactivate *mcrA* (data not shown).

CAMPBELL and RICHARDSON 1976). We find that the RglA$^-$ and McrA$^-$ phenotypes always coincide, as do the RglB$^-$ and McrB$^-$ phenotypes, whether upon mutant isolation, upon genetic mapping (Figures 1 and 2), or by complementation analysis of the single mutants isolated so far. We will henceforth refer to these loci as *mcrA* and *mcrB*, since the mnemonic (*m*odified *c*ytosine *r*estriction) more accurately describes the observed restriction target than does the alternative (*r*estricts *g*lucose*l*ess phage).

These loci encode factors required for both Rgl and Mcr restriction activity to be expressed, not repressors or inhibitors of restriction activity that are activated by mutation, because presumed null alleles (insertions and deletions) at each locus abolish the restriction characteristic of that locus (Figures 3 and 5, and not shown). These required functions might be positive regulatory factors or the restriction functions themselves. Isolation of a thermosensitive allele at the *mcrA* locus (*ts*4 in HR116, Figure 1, cross c) suggests that the gene product is most likely a protein.

It is still possible that the Rgl and Mcr restriction phenotypes are mediated by nonidentical proteins. For example, the *mcrB* locus could encode a subunit(s) common to both an RglB enzyme and an McrB enzyme; or it could encode a positive regulator of two such enzymes; or all *mcrB* alleles isolated so far could be polar mutations eliminating expression of two enzymes simultaneously. Further genetic and biochemical analysis will be required to resolve this question.

The wandering restriction locus, *mcrA*: The *mcrA* gene is located near 25 min on the *E. coli* K-12 chromosome, tightly linked to *purB* (Figure 1), on the excisable prophage-like element *e14* (Figure 5). We suspect that the many *mcrA* mutations in laboratory strains originate as excisions of the *e14* genetic element, which is lost after DNA damage (BRODY, GREENER and HILL 1985; GREENER and HILL 1980), and these mutations may thus all be the same allele. The position of *mcrA* in *E. coli* B is consistent with an accessory element-mediated acquisition. It is not located near *trp*, as it is in K-12, but is linked with *his* in F$^+$ × F$^-$ crosses (conjugational mapping). There is no report of a test of *E. coli* B itself for *e14*; the B

derivative B/5 does not carry *e14* homology, but it does carry homology to the unoccupied (*e14*^{0}) K-12 attachment site (GREENER and HILL 1980; BRODY, GREENER and HILL 1985). Attachment of an *e14*-related element at a different position in *E. coli* B and imprecise excision from it could explain why *mcrA* mutants also acquire a non-revertible thiamine auxotrophy (REVEL 1967). It remains to be seen whether *E. coli* B carries *e14* at a different location (unlike B/5), or carries a related element at a different location, or carries *mcrA* in virtue of some other route of acquistion.

It is curious to note that *mcrA* of *e14* and *mom* of bacteriophage Mu occupy similar positions. Both genes lie between *pin* or *gin*, respectively, and the end of the prophage. Mom is a DNA-modifying function active only late in lytic infection, and it acts to protect the infective stage from host restriction (KAHMANN and HATTMAN 1987); it represents the converse of McrA, which seems to act during lysogeny to protect the host from infective phage.

The identity of *mcrA*: There are four known functions encoded by *e14*. One, the site-specific inversion recombinase, *pin*, we have shown to be distinct from *mcrA* by subcloning experiments (Figure 5B). Another *e14* function, the product of gene *lit*, also interferes with growth of phage T4; but *lit* is not the same as *mcrA*, both because it maps at the other end of the element and because it must be activated by mutation before it will inhibit phage (KAO and SNYDER 1988). The *e14* integrase (BRODY, GREENER and HILL 1985; BRODY and HILL 1988) is carried by the element, has not been localized on it, and is presumably a DNA cutting-joining enzyme, as are restriction enzymes; but no requirement for cytosine modification of the attachment sites has been suggested and we think this unlikely to be *mcrA*. The gene *sfiC* has been mapped to the element but not localized (MAGUIN *et al.* 1986). It is involved in the coupling of cell division with replication and is thought to act by inhibiting the division protein FtsZ (MAGUIN, LUTKENHAUS and D'ARI 1986). Since nothing is known about mode the action of *mcrA*, we cannot rule out identity with *sfiC*. The region specifying *mcrA* is sufficient to encode one average-sized protein or two small ones. It must lie between the end of *pin* and the position of the *e14*-1290::Tn*10* (triangle c in Figure 5C), which did not inactivate *mcrA*. This leaves a maximum of about 1 kb of *e14* sequence in which *mcrA* might reside. We have not ruled out that some other part of our clone, including the *pin*-associated invertible DNA, might also be required but not be sufficient for McrA activity.

"Immigration control" in *E. coli*: The *mcrB (rglB)* gene(s) are found in a cluster of restriction genes near 99 min (Figures 2 and 3). The order of genes in this region is *mcrB-hsdS-hsdM-hsdR-mrr-zjj*202::Tn*10 serB*. The genes in the cluster are fairly tightly packed: *mcrB* straddles the *Hin*dIII site that lies 1 kb downstream of *hsdS* (Figure 3), since insertions to the left inactivate *mcrB*, and a deletion of the righthand fragment (SAIN and MURRAY 1980) also does so; and *mrr* must be contained in the ~2 kb between the beginning of the *hsdR* gene and the upstream end of the pBg3 insert (Table 5).

Our results are broadly consistent the data of others, except with respect to orientation relative to outside markers. Similar data have been reported in parts by other laboratories: RAVI, SOZHAMMANAN and DHARMALINGAM (1985) mapped *rglB* relative to *hsd*; ROSS, ACHBERGER and BRAYMER (1987) located *mcrB* downstream of *hsdS*; HEITMAN and MODEL (1987) derived the order *mcrB-hsd-mrr*; and KOHARA, AKIYAMA and ISONO (1987) reported a restriction map including this region. ROSS, ACHBERGER and BRAYMER (1987) infer the reverse orientation for reasons not stated. HEITMAN and MODEL (1987) infer the reverse orientation by reference to an unpublished restriction map of the *E. coli* chromosome. The orientation we show is consistent with both our own transducational data and those of RAVI, SOZHAMANNAN and DHARMALINGAM (1985), and also with the restriction map of KOHARA, AKIYAMA and ISONO (1987). On the other hand, we have made one contradictory observation. Hybridization of pBg3 to Southern blots of the *mcrB2* deletion suggested that this deletion spared some DNA homologous to pBg3 (E. A. RALEIGH and R. TRIMARCHI, unpublished data). If the deletion were a simple one promoted by the *zjj*202::Tn*10*, this fact would require the reverse orientation of the region. However, since Tn*10* may promote coupled multiple rearrangements (such as an inversion followed by a deletion:, RALEIGH and KLECKNER 1984), and because the autoradiogram was of poor quality, we consider these data unreliable. The agreement of two independent kinds of evidence (the restriction map of KOHARA, AKIYAMA and ISONO, and the transductional data of ours and of others) seems to us compelling.

Addition of *mrr* and *mcrB* to *hsd* (*Eco*K) increases the precision with which this locus can monitor the modification pattern of incoming DNA. One system recognizes an unmodified sequence, one recognizes a methyladenine-containing sequence, and one recognizes a methylcytosine-containing sequence. Recent data suggest even more complexity: the *mcrB* specificity of K-12 may be the sum of two different specificities encoded by adjacent genes (D. DILA and E. A. RALEIGH, unpublished observations). With this many restriction systems of different types in one region, an "immigration control" gene complex emerges. Strains might differentiate genetically in consequence of shuf-

fling the specificities at these loci, which could be a useful strategy (see below).

Is there evidence for gene shuffling in this complex? In the descent of K-12 strains, the structure of the region is quite stable, in contrast to the situation with the *mcrA* locus. Southern blot experiments (Figure 4 and text) tested four strain backgrounds representing three independent derivations from K-12 wild-type (BACHMANN, 1987b); all gave the same structure. The *mcrB1* allele, which is probably present in all common nonhybrid McrB$^-$ K-12 strains (see text), is a point mutation or a structural alteration too small to be detected by our method.

When different wild isolates are examined, however, there is considerable variability in structure (DANIEL *et al.* 1988). The three loci seem to vary independently, and there may be additional variation in the short region separating *hsd* and *mcrB*. For example, the organization of the *E. coli* B, C, and 15 T$^-$ regions (among others) has been examined by DANIEL *et al.* (1988). The center of the cluster, *hsd*, is at the same genetic location in *E. coli* B and 15T$^-$ as in K-12, but *hsdR* B hybridizes with *hsdR* K, while *hsdR* A (from 15T$^-$) does not; *E. coli* C has no such restriction system, and no homology. On the left flank (as in Figure 3), genetically there is a weak *mcrB* in the B strain (Table 3), and DNA in this region hybridizes with the K-12 *mcrB*-specific probe, pRH2. C and 15T$^-$ have not been tested genetically for *mcrB*, but no hybridization with this probe is observed; yet in between *mcrB* and *hsd*, 15T$^-$ hybridizes to K-12 (pRH3), as though *hsd* were inserted at the same position independently twice, once for K and B and once for A. C has no homology at all. On the right flank, *E. coli* B lacks *mrr* activity (HEITMAN and MODEL 1987); 15T$^-$ has homology with K-12 by hybridization with pBg3; and C lacks all homology. If more *E. coli* (GOUGH and MURRAY 1983; FULLER-PACE, COWAN and MURRAY 1985) and *Salmonella* (BULLAS, COLSON and NEUFELD 1980; BULLAS and COLSON 1975) data are added, even more genetic variation is seen. All of this is difficult to put into a simple tree, even if we assume that the cluster was assembled from accessory elements like transposons and phages. There could be an active shuffling process.

Why such a complex restriction cluster? Two types of pressures might maintain the high level of polymorphism described above: pressure for protection from invading phages, and regulation of gene flow between strains. In protecting against phage it would be advantageous for members of a sensitive population to carry multiple, and variable, restriction functions. Because phage survivors of restriction occur at biologically plausible levels (10^{-2}–10^{-6}), cells would do better with multiple restriction systems; and because restriction systems act only on the "first wave" of invading phage, a population of sensitive cells would do better if it were differentiated for restriction specificities, so that each phage burst faces a variety of restriction spectra and is not protected from most of them. Addition of restriction specificities recognizing methylated bases, as K-12 has done, both increases possibilities for differentiation and vitiates the phage strategy of incorporating modified bases in place of the normal ones, as does XP12 (HUANG *et al.* 1982).

One can also argue that restriction plays a role in the regulation of gene flow among bacteria. Continuing differentiation of restriction specificity within the population might promote genetic exchange where genetic differences exist but are small, and inhibit it where differences are large. If two strains differ in some restriction systems but not others, allowing some relatively low level of restriction upon DNA transfer, restriction would act to increase the levels of both the substrates and the enzymes required for recombination. Double strand breaks can be repaired by recombination (KRASIN and HUTCHINSON 1981; PICKSLEY, ATTFIELD and LLOYD 1984; SYMINGTON, MORRISON and KOLODNER 1985; STAHL 1986; THALER, STAHL and STAHL 1987); restriction must promote synthesis of some recombination functions, since the action of *mrr* and *mcrB* (HEITMAN and MODEL 1987; DHARMALINGAM and GOLDBERG 1980) as well as *hsd* (DAY 1977; THOMS and WACKERNAGLE 1982) induces the SOS response and some recombination functions (*recF*, *ruv*) are SOS-inducible (LOVETT and CLARK 1983); so restriction could promote its own recombination-dependent repair. However, if donor and recipient differed very much in restriction spectrum, incoming DNA would be degraded too rapidly or to pieces too small for recombination to occur, and restriction would become a barrier to genetic exchange. At some point the sequence divergence would become so great that lack of homology would limit genetic exchange, and restriction spectrum would no longer be particularly relevant. Thus, paradoxically, differentiation of restriction systems within a population would promote genetic homogenization in closely related strains, but it would reinforce genetic isolation of more distantly related strains.

There are difficulties with both of these models. If protection from phages is the primary role of restriction systems, it is hard to see why many of them have such small effects. On the other hand, the general consensus seems to be that genetic exchange via homologous recombination of chromosomal genes rarely occurs in nature (LEVIN 1981; CAMPBELL 1981; SELANDER, CAUGANT and WHITTAM 1988). Diversity of restriction spectra might contribute to the rarity of recombination, but then some other explanation for the diversity itself must be sought. Perhaps these two

potential roles—protection from phage infection and regulation of genetic exchange—work together to maintain the diversity of restriction systems in enteric bacteria. Further examination of the structure of the *mcrB-hsd-mrr* region should help us to understand how this cluster evolved.

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LITERATURE CITED

ARBER, W., 1974 DNA modification and restriction. Prog. Nucleic Acids Res. Mol. Biol. **14:** 1–37.

BACHMANN, B., 1987a Linkage map of Esherichia coli K-12, Ed. 7, pp. 807–876 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by J. L. INGRAHAM, K. BROOKS LOW, B. MAGASANIK, M. SCHAECHTER and H. E. UMBARGER. American Society for Microbiology, Washington, D.C.

BACHMANN, B., 1987b Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, pp. 1190–1219 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by J. L. INGRAHAM, K. BROOKS LOW, B. MAGASANIK, M. SCHAECHTER and H. E. UMBARGER. American Society for Microbiology, Washington, D.C.

BICKLE, T. A., 1982 The ATP-dependent restriction endonucleases, pp. 85–108 in *Nucleases*, edited by S. LINN, and R. J. ROBERTS. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

BIEK, D., and J. R. ROTH, 1980 Regulation of Tn*5* transposition in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **77:** 6047–6051.

BLUMENTHAL, R. M., S. A. GREGORY and J. S. COOPERIDER, 1985 Cloning of a restriction-modification system from *Proteus vulgaris* and its use in analyzing a methylase-sensitive phenotype in *Escherichia coli*. J. Bacteriol. **164:** 501–509.

BOLIVAR, F., R. L. RODRIGUEZ, P. J. GREENE, M. C. BETLACH, H. L. HEYNEKER, H. W. BOYER, J. H. CROSA and S. FALKOW, 1977 Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:** 95–113.

BOYER, H. W., 1966 Conjugation in *Escherichia coli*. J. Bacteriol. **91:** 1767–1772.

BOYER, H. W., and D. ROULLAND-DUSSOIX, 1969 A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:** 459–472.

BRODY, H., and C. W. HILL, 1988 Attachment site of the genetic element *e14*. J. Bacteriol. **170:** 2040–2044.

BRODY, H., A. GREENER and C. W. HILL, 1985 Excision and reintegration of the *Escherichia coli* K-12 chromosomal element *e14*. J. Bacteriol. **161:** 1112–1117.

BULLAS, L. R., and C. COLSON, 1975 DNA restriction and modification systems in *Salmonella*. III. SP, a *Salmonella potsdam* system allelic to the SB system in *Salmonella typhimurium*. Mol. Gen. Genet. **139:** 177–188.

BULLAS, L. R., C. COLSON and B. NEUFIELD, 1980 DNA restriction and modification systems in *Salmonella*: chromosomally located systems of different serotypes. J. Bacteriol. **141:** 177–188.

BUTKUS, V., S. KLIMASAUSKAS, D. KERSULYTE, D. VAITKEVICIUS, A. LEBIONKA and A. JANULAITIS, 1985 Investigation of restriction-modification enzymes from *M. varians* RFL19 with a new type of specificity toward modification of substrate. *Nucleic Acids Res.* **13:** 5727–5746.

BUTKUS, V., S. KLIMASAUSKAS, L. PETRAUSKIENE, Z. MANELIENE, A. LEBIONKA and A. JANULAITIS, 1987 Interaction of *Alu*I, *Cfr*6I, and *Pvu*II restriction-modification enzymes with substrates containing either N^4-methylcytosine or 5-methylcytosine. Biochim. Biophys. Acta **909:** 201–207.

CAMPBELL, A., 1981 Evolutionary significance of accessory DNA elements in bacteria. Annu. Rev. Microbiol. **39:** 55–83.

DANIEL, A. S., F. V. FULLER-PACE, D. M. LEGGE and N. E. MURRAY, 1988 Distribution and diversity of *hsd* genes in some enteric bacteria, particularly *Escherichia coli*. J. Bacteriol. **170:** 1775–1782.

DAY, R. S., 1977 UV-induced alleviation of K-specific restriction of bacteriophage lambda. J. Virol. **21:** 1249–1251.

DHARMALINGAM, K., and E. B. GOLDBERG, 1976 Phage-coded protein prevents restriction of unmodified progeny T4 DNA. Nature **260:** 454–456.

ECHOLS, H., A. GAREN, S. GAREN and A. TORRIANI, 1961 Genetic control of repression of alkaline phosphatase in *E. coli*. J. Mol. Biol. **3:** 425–438.

FLEISCHMAN, R. A., J. L. CAMPBELL and C. C. RICHARDSON, 1976 Modification and restriction of T-even bacteriophages. In vitro degradation of DNA containing 5-hydroxymethylcytosine. J. Biol. Chem. **251:** 1561–1570.

FOSTER, T. J., M. A. DAVIS, D. E. ROBERTS, K. TAKESHITA and N. KLECKNER, 1981 Genetic organization of Tn*10*. Cell **23:** 201–213.

FREITAG, C. S., and B. I. EISENSTEIN, 1983 Genetic mapping and transcriptional orientation of the *fimD* gene. J. Bacteriol. **156:** 1052–1058.

FULLER-PACE, F. V., G. M. COWAN and N. E. MURRAY, 1985 *Eco*A and *Eco*E: alternatives to the *Eco*K family of type I restriction and modification systems of *Escherichia coli*. J. Mol. Biol. **186:** 65–75.

GEORGOPOULOS, C. P., 1967 Isolation and preliminary characterization of T4 mutants with non-glucosylated DNA. Biochem. Biophys. Res. Commun. **28:** 179–184.

GEORGOPOULOS, C. P., and H. R. REVEL, 1971 Studies with glucosyltransferase mutants of the T-even bacteriophages. *Virology* **44:** 271–285.

GOUGH, J. A., and N. E. MURRAY, 1983 Sequence diversity among related genes for recognition of specific targets in DNA molecules. J. Mol. Biol. **166:** 1–19.

GREENER, A., and C. W. HILL, 1980 Identification of a novel genetic element in *Escherichia coli* K-12. J. Bacteriol. **144:** 312–321.

HATTMAN, S., and T. FUKASAWA, 1963 Host-induced modification of T-even phages due to defective glucosylation of their DNA. Proc. Natl. Acad. Sci. USA **50:** 297–300.

HEITMAN, J., and P. MODEL, 1987 Site-specific methylases induce the SOS DNA repair response in *Escherichia coli*. J. Bacteriol. **169:** 3243–3250.

HUANG, L.-H., C. M. FARNET, K. C. EHRLICH and M. EHRLICH, 1982 Digestion of highly modified bacteriophage DNA by restriction endonucleases. Nucleic Acids Res. **10:** 1579–1591.

HUGHES, K. T., R. W. SIMONS and W. D. NUNN, 1988 Regulation of fatty acid degradation in *Escherichia coli: fadR* superrepressor mutants are unable to utilize fatty acids as the sole carbon source. J. Bacteriol. **170:** 1666–1671.

KAHMANN, R., and S. HATTMAN, 1987 Regulation and expression of the *mom* gene, pp. 93–109 in *Phage Mu*, edited by N. SYMONDS, A. TOUSSAINT, P. VAN DE PUTTE and M. M. HOWE. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

KAO, C., and L. SYNDER, 1988 The *lit* gene product which blocks bacteriophage T4 late gene expression is a membrane protein encoded by a cryptic DNA element, *e14*. J. Bacteriol. **170:** 2056–2062.

KLECKNER, N., 1988 Transposon Tn*10*. In *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

KLECKNER, N., D. BARKER, D. ROSS and D. BOTSTEIN, 1978 Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics **90:** 427–450.

KLECKNER, N., J. ROTH and D. BOTSTEIN, 1977 Genetic engineering *in vivo* using translocatable drug resistance elements: new methods in bacterial genetics. J. Mol. Biol. **116:** 125–159.

KLECKNER, N., D. A. STEELE, K. REICHARDT and D. BOTSTEIN, 1979 Specificity of insertion by the translocatable tetracycline-resistance element Tn*10*. Genetics **92:** 1023–1040.

KOHARA, Y., K. AKIYAMA and K. ISONO, 1987 The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:** 495–508.

KRASIN, F., and F. HUTCHINSON, 1981 Repair of DNA double-strand breaks in *Escherichia coli* cells requires synthesis of proteins that can be induced by UV light. Proc. Natl. Acad. Sci. USA **78:** 3450–3453.

KRUGER, D. H., and T. A. BICKLE, 1983 Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of their hosts. Annu. Rev. Microbiol. **47:** 345–360.

LEHMAN, I. R., and E. A. PRATT, 1960 On the structure of the glucosylated hydroxymethylcytosine nucleotides of coliphages T2, T4 and T6. J. Biol. Chem. **235:** 3254–3258.

LEVIN, B. R., 1981 Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. Genetics **99:** 1–23.

LOENEN, W. A., A. S. DANIEL, H. D. BRAYMER and N. E. MURRAY, 1987 Organization and sequence of the *hsd* genes of *Escherichia coli* K-12. J. Mol. Biol. **198:** 159–170.

LOVETT, S. R., and A. J. CLARK, 1983 Genetic analysis of regulation of the RecF pathway of recombination in *Escherichia coli* K-12. J. Bacteriol. **153:** 1471–1478.

LURIA, S. E., and M. L. HUMAN, 1952 A nonhereditary, host-induced variation of bacterial viruses. J. Bacteriol. **64:** 557–569.

MAGUIN, E., J. LUTKENHAUS and R. D'ARI, 1986 Reversibility of SOS-associated division inhibition in *Escherichia coli*. J. Bacteriol. **166:** 733–738.

MAGUIN, E., H. BRODY, C. W. HILL and R. D'ARI, 1986 SOS-associated division inhibition gene *sfiC* is part of excisable element *e14* in *Escherichia coli*. J. Bacteriol. **168:** 464–466.

MANDEL, M., and A. HIGA, 1970 Calcium dependent bacteriophage DNA infection. J. Mol. Biol. **53:** 159–162.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MARMUR, J., 1961 A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3:** 208–218.

MILLER, J. W., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MODRICH, P., and R. J. ROBERTS, 1982 Type II restriction and modification enzymes. pp. 109–154. In *Nucleases*, edited by S. LINN and R. J. ROBERTS. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

NOYER-WEIDNER, M., R. DIAZ and L. REINERS, 1986 Cytosine-specific DNA modification interferes with plasmid establishment in *Escherichia coli* K-12: involvement of *rglB*. Mol. Gen. Genet. **205:** 469–475.

NWANKWO, D. O., and G. G. WILSON, 1988 Cloning and expression of the *Msp*I restriction and modification genes. Gene **64:** 1–8.

PICKSLEY, S. M., P. V. ATTFIELD and R. G. LLOYD, 1984 Repair of DNA double-strand breaks in *Escherichia coli* K-12 requires a functional *recN* product. Mol. Gen. Genet. **195:** 267–274.

PLASTERK, R. H. A., and P. VAN de PUTTE, 1985 The invertible P-DNA segment in the chromosome of *Escherichia coli*. EMBO J. **4:** 237–242.

RALEIGH, E. A., and N. KLECKNER, 1984 Multiple IS10 rearrangements in *Escherichia coli*. J. Mol. Biol. **173:** 437–461.

RALEIGH, E. A., and G. WILSON, 1986 *Escherichia coli* K-12 restricts DNA containing 5-methylcytosine. Proc. Natl. Acad. Sci. USA **83:** 9070–9074.

RALEIGH, E. A., N. E. MURRAY, H. REVEL, R. M. BLUMENTHAL, D. WESTAWAY, A. D. REITH, P. W. J. RIGBY, J. ELHAI and D. HANAHAN, 1988 McrA and McrB restriction phenotypes of some *E. coli* strains and implications for gene cloning. Nucleic Acids Res. **16:** 1563–1575.

RAVI, R. S., S. SOZHAMANNAN and K. DHARMALINGAM, 1985 Transposon mutagenesis and genetic mapping of the *rglA* and *rglB* loci of *Escherichia coli*. Mol. Gen. Genet. **198:** 390–392.

REVEL, H. R., 1967 Restriction of non-glucosylated T-even bacteriophages: properties of permissive mutants of *Escherichia coli* B and K-12. Virology **31:** 688–701.

REVEL, H. R., 1983 DNA modification: glucosylation, pp. 156–165 in *Bacteriophage T4*, edited by C. K. MATHEWS, E. M. KUTTER, G. MOSIG and P. BERGET. American Society for Microbiology, Washington, D.C.

REVEL, H. R., and C. P. GEORGOPOULOS, 1969 Restriction of nonglucosylated T-even bacteriophages by prophage P1. Virology **39:** 1–17.

REVEL, H. R., S. HATTMAN and S. E. LURIA, 1965 Mutants of bacteriophages T2 and T6 defective in α-glucosyltransferase. Biochem. Biophys. Res. Commun. **18:** 545–550.

RIGBY, P. W. J., M. DIECKMANN, C. RHODES and P. BERG, 1977 Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. J. Mol. Biol. **113:** 237–251.

ROBERTS, D., B. C. HOOPES, W. R. MCCLURE and N. KLECKNER, 1985 IS*10* transposition is regulated by DNA adenine methylation. Cell **43:** 117–130.

ROSS, T. K., and H. D. BRAYMER, 1987 Localization of a genetic region involved in McrB restriction in *Escherichia coli* K-12. J. Bacteriol. **169:** 1757–1759.

ROSS, T. K., E. C. ACHBERGER and H. D. BRAYMER, 1987 Characterization of the *Escherichia coli* modified cytosine restriction (*mcrB*) gene. Gene **61:** 277–289.

ROSS, D. G., J. SWAN and N. KLECKNER, 1979 Physical structures of Tn*10*-promoted deletions and inversions: role of 1400 bp inverted repetitions. Cell **16:** 721–731.

SAIN, B., and N. E. MURRAY, 1980 The *hsd* (host specificity) genes of *E. coli* K-12. Mol. Gen. Genet. **180:** 35–46.

SELANDER, R. K., D. A. CAUGANT and T. S. WHITTAM, 1987 Genetic structure and variation in natural populations of *Escherichia coli*, pp. 1625–1648 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by J. L. INGRAHAM, K. BROOKS LOW, B. MAGASANIK, M. SCHAECHTER and H. E. UMBARGER. American Society for Microbiology, Washington, D.C.

SHEDLOVSKY, A., and S. BRENNER, 1963 A chemical basis for the host-induced modification of T-even bacteriophages. Proc. Natl. Acad. Sci. USA **50:** 300–305.

SHEN, M. M., E. A. RALEIGH and N. KLECKNER, 1987 Physical analysis of Tn*10* and IS*10*-promoted transpositions and rearrangements. Genetics **116:** 359–369.

SIGNER, E. R., J. R. BECKWITH and S. BRENNER, 1965 Mapping of suppressor loci in *Escherichia coli*. J. Mol. Biol. **14:** 153–166.

SILHAVY, T. J., M. L. BERMAN and L. W. ENQUIST, 1984 *Experiments with Gene Fusions*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SMITH, H. O., and S. V. KELLEY, 1984 Methylases of type II restriction-modification systems, pp. 39–71 in *DNA Methylation:*

Biochemistry and Biological Significance, edited by A. RAZIN, H. CEDAR and A. D. RIGGS. Springer-Verlag, New York.

SMITH, H. O., and D. NATHANS, 1973 A suggested nomenclature for bacterial host modification and restriction systems and their enzymes. J. Mol. Biol. **81:** 419–423.

STAHL, R. W., 1986 Roles of double-strand breaks in generalized genetic recombination. Prog. Nucleic Acids Res. Mol. Biol. **33:** 169–194.

SYMINGTON, L. S., P. MORRISON and R. KOLODNER, 1985 Intramolecular recombination of linear DNA catalyzed by the *Escherichia coli recE* recombination system. J. Mol. Biol. **186:** 515–525.

TAKAHASHI, H., H. SAITO and Y. IKEDA, 1968 Viable T4 bacteriophage containing cytosine substituted DNA (T4dC phage). I. Behavior towards the restriction modification systems of *Escherichia coli* and derivation of a new T4 phage system (T4dC) having the complete T4 genome. J. Gen. Appl. Microbiol. **24:** 297–306.

TAYLOR, A. L., and C. D. TROTTER, 1972 Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36:** 504–524.

THALER, D. S., M. M. STAHL and F. W. STAHL, 1987 Double-chain cut sites are recombination hot spots in the Red pathway of phage λ. J. Mol. Biol. **193:** 75–87.

THOMS, B., and W. WACKERNAGEL, 1982 UV-induced alleviation of lambda restriction in *Escherichia coli* K-12: Kinetics of induction and specificity of this SOS function. Mol. Gen. Genet. **186:** 111–117.

WOOD, W. B. 1966 Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16:** 118–133.

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