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Site-Specific Methylases Induce the SOS DNA Repair Response in *Escherichia coli*

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Expression of the site-specific adenine methylase *Hha*II (G^{me}ANTC, where me is methyl) or *Pst*I (CTGC^{me}AG) induced the SOS DNA repair response in *Escherichia coli*. In contrast, expression of methylases indigenous to *E. coli* either did not induce SOS (*Eco*RI [GA^{me}ATTC] or induced SOS to a lesser extent (*dam* [G^{me}ATC]). Recognition of adenine-methylated DNA required the product of a previously undescribed gene, which we named *mrr* (methylated adenine recognition and restriction). We suggest that *mrr* encodes an endonuclease that cleaves DNA containing N^6-methyladenine and that DNA double-strand breaks induce the SOS response. Cytosine methylases foreign to *E. coli* (*Msp*I [^{me}CCGG], *Hae*III [GG^{me}CC], *Bam*HI [GGAT^{me}CC], *Hha*I [G^{me}CGC], *Bsu*RI [GG^{me}CC], and M.*Spr*) also induced SOS, whereas one indigenous to *E. coli* (*Eco*RII [C^{me}CA/TGG]) did not. SOS induction by cytosine methylation required the *rglB* locus, which encodes an endonuclease that cleaves DNA containing 5-hydroxymethyl- or 5-methylcytosine (E. A. Raleigh and G. Wilson, Proc. Natl. Acad. Sci. USA 83:9070–9074, 1986).

Many species of bacteria make restriction and modification enzymes to destroy foreign DNA that enters the cell (1). In general, two enzymes compose a restriction-modification system: an endonuclease or restriction enzyme that cleaves a specific DNA sequence and a methylase that modifies the same sequence by transferring methyl groups from *S*-adenosylmethionine to either adenine (N-6 position) or cytidine (C-5 or N-4 position [13]). Methylation blocks cleavage by the restriction enzyme and thereby prevents the destruction of cellular DNA. A restriction-modification system thus enables the cell to selectively destroy unmethylated foreign DNA.

Restriction-modification systems from various bacterial species have been cloned in *Escherichia coli* (2, 9, 19, 36). We have studied the *Hha*II system of *Haemophilus haemolyticus*, cloned by Mann et al. (19), and have found that *Hha*II methylase expression drastically inhibits growth of several standard *E. coli* K-12 lab strains. In this report, we show that this results from insults to the DNA which elicit the SOS DNA repair response.

DNA-damaging agents (UV light and mitomycin C) or conditions which interfere with DNA replication (presence of nalidixic acid) induce a set of 20 or so genes responsible for a myriad of physiological effects termed the SOS response (for reviews, see references 39 and 40). Cell growth slows, the cellular replication machinery is altered, mutagenesis occurs, a number of DNA repair processes take effect, and λ prophages are induced in lysogenic strains. Both λ and the SOS genes are induced by a common cascade of steps initiated by the original DNA insult. RecA protein is activated to RecA* by damaged DNA, and RecA* then promotes the cleavage of the λ cI repressor and the LexA protein, the transcriptional repressor of the SOS genes. In this manner, both SOS and λ are induced by DNA damage.

In this report, we show that, in general, methylases foreign to *E. coli* induced the SOS response. Recognition of adenine-methylated DNA required the product of a hitherto undescribed locus, which we named *mrr* (methylated adenine recognition and restriction). We suggest that *mrr* encodes an endonuclease that cleaves adenine-methylated DNA and that DNA scission induces SOS. Similarly, the SOS response was induced by cytosine methylation, and this effect required the *rglB* locus (alternatively called *mcrB*), previously shown to encode an endonuclease specific for DNA containing 5-hydroxymethyl- (25) or 5-methylcytosine (24).

MATERIALS AND METHODS

Abbreviations. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) is a chromogenic substrate which, when cleaved by β-galactosidase, yields a blue dye. Isopropyl-β-D-thiogalactopyranoside (IPTG) is a gratuitous inducer of the *lac* operon.

Bacterial strains. The bacterial strains used in this study are shown in Table 1. Bacteriophage P1 transductions were performed by using P1 Cm^r *clr-100* as previously described (26). Strains harboring Tn*10* insertions were cured of tetracycline resistance by quinaldic acid selection (17). Tn*5* insertion mutants were obtained as described by Kleckner et al. (16), by using infection with λ^{467} (*c*I857 *b*221 *O*am29 *P*am80 *rex*::Tn*5*). For β-galactosidase assays, bacteria were grown in K120 minimal medium (8) supplemented with 0.2% glucose and 0.4% Casamino Acids (Difco Laboratories) and assayed as described by Miller (22). SOS induction in some experiments was assayed by growing colonies on yeast extract-tryptone plates supplemented with 35 μg of X-Gal per ml and scoring for blue color intensity. λ stocks were prepared as plate lysates.

Mu d(Ap^r *lac*) fusions provide a convenient assay of gene regulation (4). However, Mu d fusions confer temperature sensitivity, render host strains unstable owing to secondary transposition, and undergo zygotic induction and transpose when transduced by P1. To circumvent these problems, the *dinD2*::Mu d(Ap^r *lac*) fusion in GW1040 (14) was made transposition defective as follows. The Mu d(Ap^r *lac*) phage encodes a temperature-sensitive Mu *c* repressor; at the nonpermissive temperature, unrepressed replication and *kil* function are lethal to the host. We selected GW1040 survivors at 42°C and then screened for healthy colonies which still synthesized β-galactosidase in response to mitomycin C.

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TABLE 1. *E. coli* strains

Strain	Genotype	Comments and construction
GC3217	F⁻ *tif-1 sfiA11 thr leu pro his arg ilv*(Ts) *gal str*	Parent of the GW1000 strain series
GM1874	GW1040 *dam-4 mutS456*	From M. Marinus (6)
GW1000	GC3217 *pro*⁺ *lacΔU169*	From G. Walker (14)
GW1040	GW1000 *dinD2*::Mu d(Ap^r *lac*)	From G. Walker (14)
GW1060	GW1000 *uvrA215*::Mu d(Ap^r *lac*)	From G. Walker (14)
HB101	*hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44 mrr$_B$* (λ⁻)	(3)
JH5	GW1040 Ts⁺	GW1040 cured of temperature sensitivity
JH30	K91 *lexA3 malE*? *lacI^q lacZ*::Tn*5*	K91 P1 transduced to *lexA3* and *malE*::Tn*10* from JC13519,[a] cured of Tet^r, and transduced from GC2438[b]
JH35	JH5 *recA*⁺ *srl-300*::Tn*10*	JH5 P1 transduced to Tet^r and RecA⁺ from K590[c]
JH39	JH35 *srl*? Tet^s	JH35 cured of Tet^r
JH43	JH39/F′ *lacI^q lacZ*::Tn*5*	JH39 mated with GC2438[b]
JH59	JH39 *recA56 srl*?	JH39 P1 transduced to *srl*-300::Tn*10* and *recA56* from JC10240[d] (from A. J. Clark) and then cured of Tet^r
JH63	JH39 *hsdR2 rglB*/F′ *lacI^q lacZ*::Tn*5*	JH39 P1 transduced from ER1351,[e] cured of Tet^r, and mated with GC2438[b]
JH69	HB101 *recA*⁺ *srl*? *dinD2*::Mu d(Ap^r *lac*) Ts⁺	HB101 P1 transduced from K590,[c] cured of Tet^r, and P1 transduced from JH5
JH76	JH39 *mrr-2*::Tn*5*	Tn*5* insertion that blocks SOS induction by *Hha*II methylase
JH83	JH76 *zjj*::Tn*10-202*	JH76 P1 transduced from ER1351[e]
JH94	AB2740 *thyA748*::Tn*10*	AB2470 P1 transduced to *thyA748*::Tn*10* from X2904 (from B. Bachman); still carries *recB21*
JH100	K561 *recB21 thyA*::Tn*10*	K91 P1 transduced from JH94
JH104	JH43 *recB21 thyA*::Tn*10*	JH43 P1 transduced from JH94
JH119	JH76 *recB21 thyA*::Tn*10*	JH76 P1 transduced from JH94
JH120	ER21 *lacZ*::Tn*5 dinD2*::Mu d(Ap^r *lac*)	ER21 P1 transduced from GC2438[b] and JH5
JH125	AB1157 *rfa-550 lacZ alkA51*::Mu d(Ap^r *lac*)	Spontaneous *lacZ* derivative of MV1571 (from M. Volkert [35]); still *lac*⁺ when induced with MMS[f]
K38	HfrC *phoA*(Am) (λ)	(26)
K91	K38 (λ⁻)	K38 cured of λ (26)
K561	K38 *laci^q*	(7)
K871	K561 *recA56 srl-300*::Tn*10*	(7)

[a] Strain JC13519 carries *lexA3 malE*::Tn*10* (from A. J. Clark).
[b] Strain GC2438 (from S. Gottesman [12]) served as the donor, either in P1 transduction or mating, of the *lacI^q lacZ*::Tn*5* markers carried on its F′ episome.
[c] Strain K590 carries the *recA*⁺ allele linked to an *srl-300*::Tn*10* insertion (from M. Russel).
[d] Strain JC10240 carries the *recA56* allele linked to the *srl-300*::Tn*10* insertion (from A. J. Clark).
[e] Strain ER1351 carries the *hsdR2* and *rglB* markers linked to a Tn*10* insertion at 99 min (*zjj*::Tn*10*) (from E. Raleigh).
[f] MMS, Methyl methanesulfonate.

One of these mutants, JH5, contains a defective Mu d fusion which transposes at a greatly reduced frequency (10^{-4} to 10^{-5}) at 42°C. Strain JH5 served as the parent in further strain constructions (Table 1; see Results).

DNA manipulations and plasmid constructions. The plasmids used in this study are shown in Table 2. Plasmid DNA was prepared by the nondenaturing lysozyme-Triton method of Zinder and Boeke (41) or by alkaline lysis (18). DNA fragments were isolated from agarose gels and electroeluted. Other DNA manipulations were performed as described by Maniatis et al. (18). In vivo methylase activity was quantified by measuring the extent to which plasmid-borne restriction sites were protected from cleavage by the cognate restriction endonuclease (or an isoschizomer known to be inhibited by the methylase). *Hha*II methylation was tested by *Hin*fI digestion, *Pae*R7 methylation was tested by *Xho*I digestion, *Msp*I methylation was tested by *Hpa*II digestion, *dam* methylation was tested by *Mbo*I digestion, and *Bsu*RI, M · *Spr*, and *Bsp*RI methylation were tested by *Hae*III digestion. All methylase clones, except uninduced pJH49, as described in Results, rendered the appropriate plasmid-borne restriction sites completely resistant to cleavage.

Several plasmids conferred only ampicillin resistance. To study these plasmids in strains which carried a Mu d(Ap^r *lac*) fusion, we inserted a different antibiotic resistance gene. Shapira et al. (29) constructed plasmids in which the chloramphenicol or kanamycin resistance genes are flanked by polylinkers to form versatile gene cassettes. We used the chloramphenicol resistance gene cassette derived from pSKS114 to construct pJH1 by inserting the cassette into the *Pst*I site of pTP166 (20). Similarly, pJH51 was derived from pBamHI 2-50 (G. Wilson) by inserting the cassette at the *Sal*I site, pJH53 was derived from pHhaI 2-1 (G. Wilson) by inserting the cassette at the *Pst*I site, and pJH56 was derived from pME101 (38) by inserting the cassette at the *Eco*RI site.

The *Hha*II methylase gene was cloned under the control of the inducible *lacUV5* promoter in plasmid pGL101 (11) as follows. The 960-base-pair *Eco*RI fragment from pSK5 (28) carrying the *Hha*II methylase gene was isolated, filled in with the Klenow fragment of DNA polymerase I, and blunt-end ligated into pGL101, which had been digested with *Pvu*II and treated with calf intestinal phosphatase. The antibiotic resistance of the resulting plasmid (pJH40) was changed from ampicillin to chloramphenicol by inserting the chloramphenicol resistance gene cassette from pSKS114 into the *Pst*I site of pJH40, yielding pJH49.

RESULTS

***Hha*II methylase induced λ lysogens.** The *Hha*II restriction and modification genes from *H. haemolyticus* had previously been cloned in *E. coli* (19). Later, the two genes were cloned

TABLE 2. Plasmids

Plasmid	Genotype	Construction, reference, or source
pGL101	*lacUV5 bla*	(11)
pSKS114	Cm^r *bla*	(29)
Adenine methylase		
pJC1	M · *Eco*RI Cm^r	From S.-C. Cheng (5)
pJH1	*tacI-dam* Cm^r	Cm^r gene cloned in the *Pst*I site in pTP166 (from M. Marinus [20])
pJH40	*lacUV5*-M · *Hha*II *bla*	M · *Hha*II cloned in the *Pvu*II site in pGL101
pJH49	*lacUV5*-M · *Hha*II Cm^r	Cm^r gene cloned in the *Pst*I site of pJH40
pJH56	M · *Pst*I *bla* Cm^r	Cm^r gene cloned in the *Eco*RI site in pME101 (from R. Walder [38])
pPAOM.177	M · *Pae*R7 Km^r	From J. Brooks
pSK5	M · *Hha*II Tc^r	From H. Smith (28)
Cytosine methylase		
pES2	M · *Bsp*RI Tc^r *bla*	From A. Kiss (34)
pHaeIII 1-1	M · *Hae*III Tc^r *bla*	From G. Wilson
pJH51	M · *Bam*HI *bla* Cm^r	Cm^r gene cloned in the *Sal*I site of pBamHI 2-50 (from G. Wilson)
pJH53	M · *Hha*I Cm^r	Cm^r gene cloned in the *Pst*I site of pHhaI 2-1 (from G. Wilson)
pMER3	M · *Msp*I Tc^r *bla*	From R. Walder (37)
pR215	M · *Eco*RII Tc^r	From A. Bhagwat
pSU11	M · *Bsu*RI Tc^r *bla*	From A. Kiss (15)
pSU21	M · *Spr* Tc^r	From A. Kiss (23)

on separate compatible plasmids (28). We obtained these plasmids with the aim of conducting a mutational analysis of the restriction and modification enzymes. However, when several standard laboratory strains (K38, JM101, and JM109) were transformed with the plasmid that expressed only the *Hha*II methylase (pSK5), the resulting colonies were translucent, mottled, and grew poorly, giving rise to faster-growing mutants. When these transformants were plated on a medium containing X-Gal, a chromogenic substrate which yields a blue dye when cleaved by β-galactosidase, and IPTG, a gratuitous inducer of the *lac* operon, the K38(pSK5) colonies were ringed by blue haloes. Normally *lac*$^+$ strains do not leak β-galactosidase or the X-Gal cleavage product and thus yield blue colonies with distinct borders on X-Gal-IPTG plates. Therefore, the blue haloes induced by the *Hha*II methylase were indicative of cell lysis.

As strain K38 is a λ lysogen, we thought that this cell lysis might have been due to λ induction. Culture supernatants of K38 expressing the *Hha*II methylase contained 10-fold more phage than did K38 alone. Furthermore, when the *Hha*II methylase plasmid pSK5 was introduced into strain K91, an isogenic derivative of K38 cured of λ, the resulting colonies were no longer ringed by blue haloes. However, these colonies still grew slowly and were translucent and mottled. Thus, besides causing cell lysis through induction of λ in a lysogenic strain, *Hha*II methylase exerted a further deleterious effect on the cell.

***Hha*II methylase induced the SOS response.** λ lysogens can be induced by DNA-damaging agents, such as mitomycin C or UV light, which induce the SOS response. Induction of λ and the SOS genes results from RecA-stimulated cleavage of their respective repressors, cI and LexA. Kenyon and Walker (14) constructed a family of strains in which SOS-inducible promoters were fused to the *lac* operon by using the Mu d(Ap^r *lac*) phage (4). These strains make β-galactosidase in response to DNA damage and provide a convenient assay for SOS induction. Strain GW1060 carries a *uvrA*::Mu d(Ap^r *lac*) fusion. Transformation of this strain with plasmid pSK5 resulted in increased β-galactosidase expression (as measured by color intensity on X-Gal plates), indicating that the *Hha*II methylase does induce the SOS response. Strains GW1010, GW1030, GW1040, and GW1080 carry Mu d(Ap^r *lac*) fusions to other SOS-inducible promoters and gave similar results. Examination by microscopy showed that cells expressing *Hha*II methylase were filamented, another characteristic of SOS induction.

The effect of mutations in various DNA repair genes upon methylation-sponsored SOS induction was tested by introducing them into an appropriate derivative carrying a *dinD*::*lacZ* fusion. The resulting strains were transformed with plasmid pSK5 and plated on X-Gal indicator medium. Blue colonies were scored as induced for the SOS response, and white colonies were scored as uninduced. Mutations in either the *recA* (JH59) or *recB* (JH104) loci blocked SOS induction by *Hha*II methylation. In contrast, a mutation in the mismatch repair machinery (*mutS456*; strain GM1874) did not alter SOS induction by *Hha*II methylase, suggesting that methylation does not induce SOS by somehow disrupting mismatch repair. By the same criterion, *Hha*II methylase did not increase β-galactosidase expression in a strain (JH125) harboring a Mu d(Ap^r *lac*) fusion to one of the adaptive-response loci, *alkA*, which is induced by other alkylating agents (data not shown) (35; for a review, see reference 40).

Cloning the *Hha*II methylase under the *lacUV5* promoter. Transformation with a plasmid (pSK5) expressing the *Hha*II methylase causes most *E. coli* strains to grow poorly and give rise to faster-growing mutants. To avoid this selective pressure, the methylase gene was placed under the control of the *lacUV5* promoter, yielding plasmid pJH49 (see Materials and Methods). *Hha*II methylase expression was assayed by isolating plasmid DNA and testing its resistance to digestion by *Hin*fI, an isoschizomer of *Hha*II which is also inhibited by *Hha*II methylation (Fig. 1). pJH49 expressed a background level of enzyme sufficient to methylate roughly one-half of the plasmid *Hha*II sites under the conditions used (Fig. 1, lane 2). Some transcription from the uninduced *lacUV5* promoter probably accounted for the background level of expression from plasmid pJH49. IPTG induction rendered plasmid pJH49 almost completely resistant to *Hin*fI

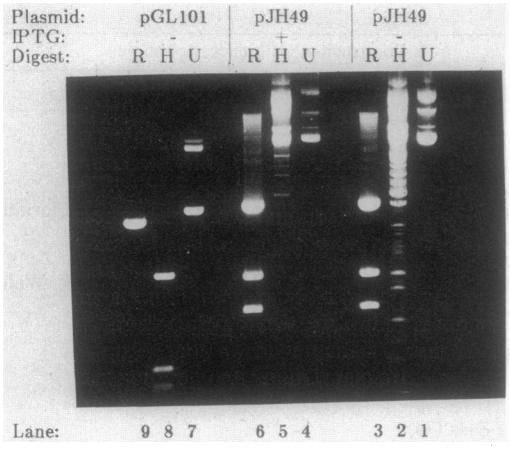

FIG. 1. *Hha*II methylase expression by plasmid pJH49. pJH49 DNA was prepared from the *lacI*^{q} strain K561 with and without IPTG induction of *Hha*II methylase expression. Samples were digested with *Hin*fI or *Eco*RI and run on a 0.6% agarose gel. pGL101 is the parent vector of pJH49 and served as a control. U, Undigested sample; H, *Hin*fI; R, *Eco*RI.

digestion (95% protection), indicating an increase in *Hha*II methylase (Fig. 1, lane 5).

To assay SOS induction by this inducible plasmid, we constructed strain JH43, a derivative of the Kenyon and Walker strain GW1040 (14). GW1040 carries a Mu d(Ap^r *lac*) fusion to a locus induced by DNA damage, *dinD* (*din* stands for damage inducible). Although the *dinD* gene product is unknown, we chose this fusion because it has a low basal level of β-galactosidase and the ratio of its induced/uninduced β-galactosidase levels is high. Strain JH43 was derived from GW1040 as follows. The Mu d fusion was rendered transposition defective as described in Materials and Methods. The resulting strain, JH5, was transduced with phage P1 grown on a *recA*$^+$ *srl-300*::Tn*10* strain to replace the temperature-sensitive *recA* allele (*tif-1*) originally carried by GW1040, cured of tetracycline resistance by selection for resistance to quinaldic acid, and mated with GC2438 to transfer in the F′ episome carrying *lacI*^{q}, to yield JH43.

The β-galactosidase levels in strain JH43 after mitomycin C treatment or expression of *Hha*II methylase from pJH49 are shown in Fig. 2. Without IPTG, plasmid pJH49 made enough *Hha*II methylase to modify about one-half of the plasmid-borne *Hha*II sites and to induce SOS to threefold higher than background. IPTG induction increased methylase expression so that nearly all plasmid *Hha*II sites were methylated and, correspondingly, SOS induction increased to sevenfold higher than background. The increase in SOS induction after IPTG addition was somewhat modest (due to the high basal level of methylase expression) but nonetheless reflected the increased methylation at *Hha*II sites from 50 to essentially 100%.

In control experiments, a dose of mitomycin C of 1 μg/ml induced SOS 21-fold higher than background. Greater SOS induction by mitomycin C than by *Hha*II methylase may reflect a difference in the nature of the inducing lesions (since mitomycin C causes DNA-DNA crosslinks, whereas we suggest that *Hha*II methylation leads to DNA scission) or a difference in their concentrations (a likely concern since mitomycin C can act at nearly any DNA sequence, whereas *Hha*II-induced lesions are probably no more frequent than *Hha*II sites). Other examples of partial or intermediate SOS induction have been described (39).

Does the cell need to turn on SOS in response to methylation? We next tested the effect of *Hha*II methylase on the growth of isogenic *recA*$^+$ (K561) and recA mutant (K871, *recA56* allele) strains. Growth (measured as the optical density at 600 nm) was mildly inhibited in strain K561, a λ lysogen (data not shown). Growth was greatly inhibited in the *recA56* strain: the culture doubling time increased by 40% within 6 h after IPTG induction of methylase expression. Strains carrying either a *lexA3* (JH30) or *recB21* (JH100) allele gave results similar to those for the *recA56* strain. These three mutations all block SOS induction by methylation, suggesting that SOS is required to overcome some ill effect of methylation.

Other adenine methylases induced SOS. Strain K38 carrying the *PstI* methylase plasmid pJH56 produced mottled, translucent colonies and grew poorly, giving rise to faster-growing mutants and blue haloes on X-Gal-IPTG medium. *Pst*I methylase caused the induction of β-galactosidase in the *dinD2*::Mu d(Ap^r *lac*) fusion-carrying strain JH43. The level of SOS induction by *Pst*I methylase was three- to fivefold, as measured by β-galactosidase assays (data not shown).

In contrast, expression of the *Eco*RI methylase from plasmid pJC1 did not induce SOS. K38 harboring plasmid pJC1 grew normally, and strain JH43 did not make increased β-galactosidase in response to *Eco*RI methylation. The *dam* methylase, overexpressed from the *tacI* promoter (pJH1), induced SOS to two- to fourfold higher than background, as measured with the *dinD2*::Mu d(Ap^r *lac*) fusion. Plasmid pPAOM.177 expressed the *Pae*R7 methylase (CTCG^{me}AG, where me is methyl) (9) at a level sufficient to fully methylate the single *Pae*R7 site on the plasmid but did not induce SOS.

How was the detrimental *Hha*II methylase originally cloned? The *Hha*II restriction-modification system (19) was originally cloned in HB101, a hybrid strain containing regions of both the *E. coli* K-12 and *E. coli* B genomes (3). We have shown that methylation inhibits growth most strongly

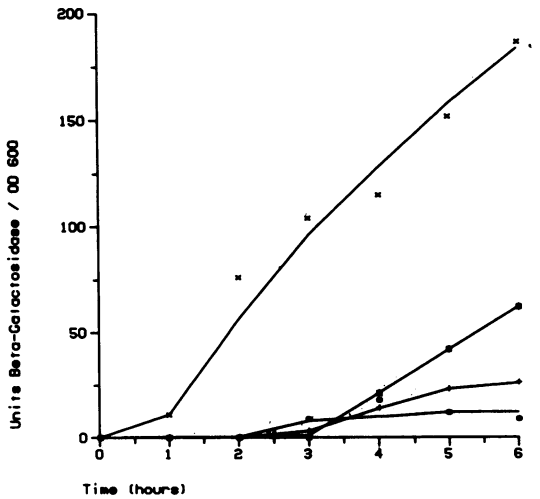

FIG. 2. Kinetics of β-galactosidase induction in strains JH43 [*lacI*^{q} *dinD2*::Mu d(Ap^r *lac*)] and JH43 (pJH49). Cells were grown at 30°C in K120 minimal medium supplemented with 0.2% glucose and 0.4% Casamino Acids. Mitomycin C (to 1 μg/ml) or IPTG (to 5 mM) was added to cultures at time zero. Samples (100 μl) were removed hourly, and β-galactosidase activity was assayed as described by Miller (22). Cell density was determined by measuring the optical density at 600 nm. Symbols: ○, untreated JH43; X, JH43 plus 1 μg of mitomycin C per ml; +, JH43(pJH49); *, JH43(pJH49) plus 5 mM IPTG.

in *recA* mutants, and surprisingly, HB101 carries a *recA* mutation. Nonetheless, we found that the viability of HB101 was apparently unaffected by the presence of pSK5. In JH69, a *recA*$^+$ HB101 derivative carrying the *dinD2*::Mu d(Ap^r *lac*) fusion, β-galactosidase levels were increased by mitomycin C but not by *Hha*II methylase.

HB101 is a genetic recombinant, and although most of its chromosome is from *E. coli* K-12, min 98 to 100 are derived from *E. coli* B (3). The ability of HB101 to tolerate the *Hha*II methylase is perhaps attributable to these *E. coli* B-specific sequences. A *lacZ*::Tn*5* *dinD2*::Mu d(Ap^r *lac*) derivative (JH120) of the *E. coli* B strain ER21 was constructed. As with JH69, β-galactosidase levels were increased by mitomycin C but not by the *Hha*II methylase. We next transduced JH69 and JH120 with phage P1 grown on a K-12 donor strain carrying a transposon insertion at 99 min (*zjj*::Tn*10*; strain ER1351). A K-12 marker that was 70% linked to the transposon enabled strains JH69 and JH120 to induce SOS in response to *Hha*II methylation. This marker was also very tightly linked to the *hsdRMS* loci at 98.5 min, which encode the *E. coli* host restriction system. This locus is distinct from both *hsdR* and the closely linked *rglB* locus, since mutations in either of these loci did not block SOS induction by adenine methylation. We called this locus *mrr* (methylated adenine recognition and restriction). We designated the *E. coli* K-12 allele *mrr*$_K$ or simply *mrr* and the *E. coli* B allele *mrr*$_B$.

Analysis of Tn*5* insertions that block induction of SOS by methylation. We sought Tn*5* insertions in strain JH39 which would block SOS induction by *Hha*II methylase but not by mitomycin C. A random library of 100,000 Tn*5* insertions in strain JH39 were pooled, transformed with plasmid pJH49, and screened on X-Gal-IPTG plates. Transposon insertions in genes needed to recognize adenine methylation should prevent induction of the Mu d(Ap^r *lac*) fusion and yield white colonies on X-Gal-IPTG medium. From a screen of 4,600, 18 white or light blue colonies were obtained; 12 were totally white, grew slowly on X-Gal-IPTG plates, and were presumably insertions in either *RecA*, *recBC*, or the *dinD2*::Mu d(Ap^r *lac*) fusion, and 6 were lightly blue and grew normally on X-Gal-IPTG plates. Mitomycin C still induced SOS in all of the latter six colonies. One insertion that cotransduced kanamycin resistance and the Mrr$^-$ phenotype at high frequency was further characterized (*mrr-2*::Tn*5*; strain JH76). This insertion also blocked SOS induction by the *Pst*I methylase, an adenine methylase, but not by several cytosine methylases. Cells carrying the Tn*5* insertion restrict λ grown on strains which do not provide *Eco*K modification; hence, the host *Eco*R$_K$ restriction system (*hsdRMS*) is unaffected by the *mrr-2*::Tn*5* insertion. Construction of a *mrr-2*::Tn*5* *recB21* double mutant (JH119) revealed that the *mrr-2*::Tn*5* insertion reversed the ill effects of adenine methylation in a *recBC* mutant. Similarly, the *mrr*$_B$ allele blocked the ill effects of adenine methylation in a *recA* mutant (HB101).

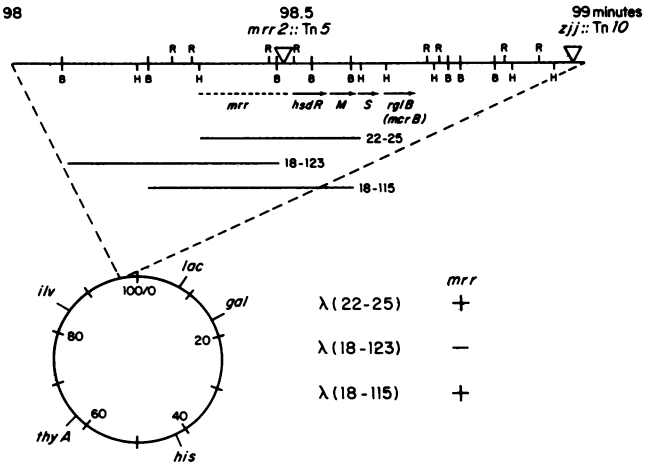

FIG. 3. Map location of the *mrr-2*::Tn*5* insertion and fine structure of the *hsd* region. λ transducing phages 22-25, 18-115, and 18-123 carry cloned segments that map in or near the *hsd* region (D. Daniels, personal communication). As described in Results, phages 22-25 and 18-115 carry the *mrr* gene. A limited restriction map of the *hsd* genes has been described (27). The more extensive and complete restriction map that appears here was constructed and generously provided by D. Daniels (personal communication).

TABLE 3. Restriction of methylated λ *vir* by Mrr

λ *vir* phage stock		Efficiency of plating on host strain (relative to plating on JH76):	
First growth cycle	Second growth cycle	JH39 (*mrr*$^+$)	JH76 (*mrr-2*::Tn*5*)
JH83		1	1
JH83(pJH49) + IPTG[a]		0.05–0.1	1
	JH83[b]	1	1
	JH39[c]	1	1
JH83(pJH56)[d]		0.1	1
	JH39[e]	1	1

[a] λ *vir* was methylated in vivo by growth on a strain expressing the *Hha*II methylase [JH83(pJH49) plus IPTG].
[b] *Hha*II-methylated λ *vir* was plated on strain JH83 to remove the methylation.
[c] *Hha*II-methylated λ *vir* that survived growth on the *mrr* strain JH39 was isolated and replated.
[d] λ *vir* was methylated in vivo by growth on a strain expressing the *Pst*I methylase [JH83(pJH56)].
[e] The methylated λ *vir* was passaged to remove the methylation.

Mapping the *mrr-2*::Tn*5* insertion. Preliminary mapping of the *mrr-2*::Tn*5* insertion by P1 transduction-mediated three-factor crosses revealed that the Tn*5* insertion was 50% linked to a Tn*10* insertion at 99 min (*zjj*::Tn*10*) and very tightly linked to the *hsdR* and *rglB* loci, both near 98.5 min. However, these crosses gave conflicting results as to the gene order (data not shown). A chromosomal *Eco*RI fragment carrying the *mrr-2*::Tn*5* insertion was cloned into pBR322 by selection for kanamycin resistance. This fragment carries 2 kilobases of chromosomal DNA flanking Tn*5*, which, from restriction mapping, appears to lie adjacent and counterclockwise to the *hsdR* locus (Fig. 3). To further localize the *mrr* gene, we obtained a set of λ clones that carry inserts encompassing the region of the *E. coli* chromosome between 98 and 99 min (D. Daniels, personal communication). As discussed below, two of these λ transducing phages (18-115 and 22-25) carry the intact *mrr* gene, whereas one (18-123) does not. Phages 18-115 and 22-25 both carry regions of the chromosome that overlap the 2-kilobase *Eco*RI fragment to which the *mrr-2*::Tn*5* insertion was localized. Thus, the *mrr* gene maps just counterclockwise to the *hsdR* locus, at 98.5 minutes. A fine-structure map of this region is shown in Fig. 3, with the resulting gene order *mrr hsdRMS rglB zjj*::Tn*10*.

The *mrr* gene product restricted methylated λ in vivo. We suspected that the *mrr* gene encodes an endonuclease that

TABLE 4. SOS induction by cytosine methylases

Plasmid	Methylase	Specificity[a]	SOS induction[b]	
			In *rglB*$^+$ strain	In *rglB* mutant
pMER3	*Msp*I	^{me}CCGG	Y[c]	N
pHaeIII 1-1	*Hae*III	GG^{me}CC	Y[c]	N
pR215	*Eco*RII	C^{me}CA/TGG	N	N
pJH53	*Hha*I	G^{me}CGC	Y[c]	Y
pJH51	*Bam*HI	GGAT^{me}CC	Y	N
pSU11	*Bsu*RI	GG^{me}CC	Y[c]	N
pSU21	M · *Spr*	GGCC, CCGG, GGA/TCC	Y[c]	N
pES2	*Bsp*RI	GG^{me}CC	Y[c]	N

[a] ^{me}C, 5-Methylcytosine.

[b] SOS induction was assayed by plating transformants of strains JH43 and JH63 on X-Gal indicator medium. Blue colonies were scored as SOS induced (Y), and white colonies were scored as uninduced (N).

[c] Transformation efficiency decreased relative to the *rglB* mutant; slowly growing colonies sectored with faster-growing mutants.

cleaves DNA containing methyladenine. If so, the *mrr* locus should act, in vivo, as a classical restriction-modification system (1). To test this, a λ *vir* stock was prepared from a strain making the *Hha*II methylase [JH83(pJH49) plus 2 mM IPTG]. This methylated phage made 10- to 20-fold more plaques on a *mrr* mutant (JH76) than on a *mrr*$^+$ host (JH39) (Table 3). In contrast, unmethylated phage plated with equal efficiency on *mrr*$^+$ and *mrr* strains. Thus, *Hha*II-methylated λ is restricted on a *mrr*$^+$ host. Passaging this methylated phage on the *mrr* mutant JH83 to remove the methyl groups yielded phage which plated with equal efficiency on *mrr*$^+$ and *mrr* strains. Methylated phage that survived restriction by the Mrr protein (plaques picked from a JH39 lawn) also plated with equal efficiency. *Hha*II-methylated phage is therefore modified by passage on strains that do not express *Hha*II methylase. When methylated phage that survived Mrr restriction was methylated a second time, it again became sensitive to Mrr restriction (data not shown). *Pst*I-methylated λ gave similar results. Thus, the *mrr* gene behaves as a classical restriction-modification system that, rather than being inhibited by methylation, specifically restricts methylated DNA.

λp*mrr* transducing phages were restricted on hosts expressing *Hha*II methylase. Our finding that methylated λ was restricted by Mrr suggested that a λp*mrr* transducing phage might be restricted on hosts making the *Hha*II methylase. We tested three λ transducing phages carrying segments of the *E. coli* chromosome to which *mrr* mapped (D. Daniels, personal communication) for their ability to grow in the presence of *Hha*II methylase. Two of these, 22-25 and 18-115 (Fig. 3), plated with greatly reduced efficiency (5×10^{-6}) on strains making the *Hha*II methylase. We suggest that, upon infection, *mrr* is expressed at high levels, the *Hha*II-methylated host chromosome is degraded, and the subsequent cell death decreases or prevents the production of progeny phage. In addition, the infecting phage may also become *Hha*II methylated and suffer restriction by Mrr. λp*mrr* phages that survived growth in the presence of *Hha*II methylase plated with equal efficiency in the presence and absence of the methylase and presumably carry *mrr* alleles with reduced activity (data not shown).

Effects of cytosine methylases on *E. coli*. Many plasmids which express cytosine methylases cannot be introduced into standard *E. coli* strains (2, 24). The locus responsible for this effect, *rglB* (24), encodes a restriction enzyme that cleaves DNA containing 5-hydroxymethyl- (25) or 5-methylcytosine (24). We found that many cytosine methylases induced the SOS response and that the *rglB* locus was required for this effect (Table 4). In the same fashion as adenine methylation, *Hha*I-specific cytosine methylation in an *rglB*$^+$ background appeared to be lethal in combination with a *recA* (JH59) or *recB* (JH104) mutation, since no transformants could be obtained in the *recA* or *recB* background. The *rglB* mutation did not block SOS induction by the *Hha*II site-specific adenine methylase.

DISCUSSION

By three separate criteria [λ prophage induction, cellular filamentation, and increased expression of Mu d(Ap^r *lac*) fusions to DNA damage-inducible loci], we found that many site-specific methylases induced the SOS DNA repair response when expressed in *E. coli*. In general, methylases foreign to *E. coli* (*Hha*II, *Pst*I, *Msp*I, and others) induced the SOS response, whereas methylases indigenous to *E. coli* induced SOS to a lesser extent (*dam*) or not at all (*Eco*RI and *Eco*RII). Methylases that induced the SOS response also caused the cell to grow somewhat slowly. This slow growth may have arisen from unrepaired methylation-dependent DNA lesions or from SOS induction itself, since one of the SOS gene products is the SfiA protein, an inhibitor of cell division (12). When SOS induction was blocked (by using mutations in either *recA*, *recB*, or *lexA*), the cell grew much more poorly or died in response to methylation. Clearly, SOS induction counteracts some detrimental effect of methylation. On the other hand, we showed that certain mutations completely blocked the ill effects of methylation and circumvented the need for SOS induction. Thus, a mutation in the *rglB* locus allowed normal cell growth without SOS induction in the presence of cytosine methylation, and, likewise, a *mrr* mutation blocked the deleterious effects of adenine methylation. In the absence of RglB and Mrr activity, methylation itself had little or no effect on cell growth.

The *rglB* locus was originally described as a restriction system that cleaves DNA containing 5-hydroxymethylcytosine, which is present in unglucosylated T-even phages (25). Recently, *rglB* was shown to also restrict DNA containing 5-methylcytosine (24). We found that many cytosine methylases induced the SOS response and that this effect required *rglB*. It seems likely that DNA double-strand breaks, caused by *rglB* action, induce SOS. In separate experiments, we found that a temperature-sensitive allele of the *Eco*RI endonuclease induced the SOS response at the nonpermissive temperature (unpublished results); therefore, DNA scission does induce the SOS response. We also found that the adenine methylases *dam*, *Hha*II, and *Pst*I induced SOS. With *Hha*II and *Pst*I, this effect required the *mrr* locus, whereas SOS induction by *dam* was independent of *mrr* (unpublished results). By analogy with RglB and *Eco*RI and

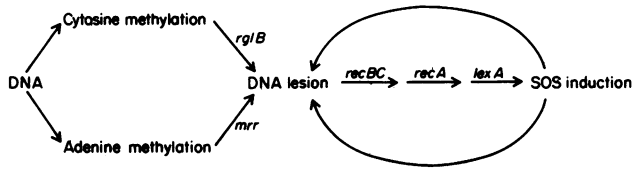

FIG. 4. Model of the pathway from methylation to SOS induction. Mrr and RglB are shown causing lesions in methylated DNA that induce, and are subsequently repaired by, the SOS response. See the text for further discussion.

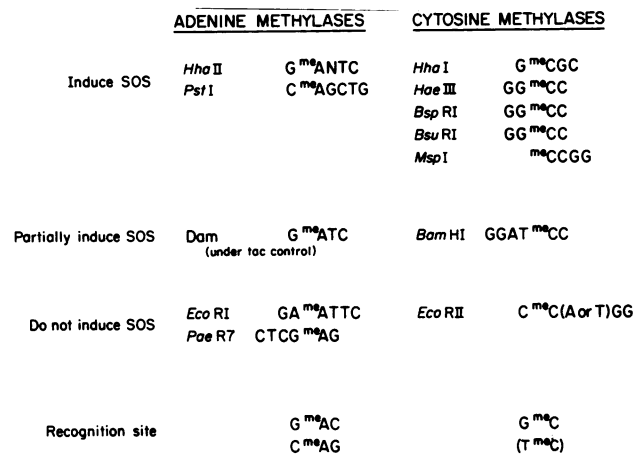

FIG. 5. Effects of methylation on SOS induction. Putative recognition sites for Mrr and RglB are shown. Methylase recognition specificities are from Smith and Kelly (31).

on the basis of our in vivo evidence that *mrr* functions as a restriction system, we suggest that Mrr cleaves adenine-methylated DNA.

Our model of the pathway from methylation to SOS induction is shown in Fig. 4, in which RglB and Mrr are depicted introducing lesions in methylated DNA. We suggest that these lesions are DNA double-strand breaks which are processed by the RecBCD exonuclease or helicase activity to yield the SOS inducing signal, most likely single-stranded DNA. Repair by SOS functions then allows normal cell growth to continue. The role RecBCD plays may be analogous to other known functions it performs including degradation of restricted DNA (30), activation of SOS by other inducing agents (21), and recombinational activity at Chi sites (32). DNA double-strand breaks can be repaired by homologous recombination with an intact copy of the severed DNA (33). If methylation-dependent lesions are repaired in this way, the RecA and RecBCD proteins may be required not only to induce the SOS response but also to participate directly in recombination-mediated repair.

Our findings that *Hha*II- or *Pst*I-methylated λ was restricted on *mrr*$^+$ strains and λp*mrr* transducing phages were restricted on strains expressing the *Hha*II methylase clearly show that Mrr can restrict adenine-methylated DNA. This raises two interesting points. First, the *mrr* locus maps adjacent to other loci, *hsdRMS* and *rglB*, which also encode restriction systems. This clustering may be functional, to ensure that *E. coli* infrequently divulges its means of marking its DNA, to increase the region of the chromosome that can be transferred without donating a potentially lethal gene, or to perhaps in some way coordinate the cellular defense system. Second, Mrr activity is found in *E. coli* K-12 but not in *E. coli* B. Similarly, the RglB activity in *E. coli* B is weaker than and differs in specificity from that found in K-12 (25), and the *hsdRMS* loci have diverged to different specificities from a common ancestor (10). Mutations of this sort, which inhibit or promote genetic exchange, were perhaps responsible for the divergence of *E. coli* K-12 and B and may represent a common event in bacterial speciation.

As to how Mrr acts biochemically, our simplest hypothesis is that the Mrr protein is an endonuclease specific for DNA containing N^6-methyladenine residues. A comparison of methylated sequences that resulted in SOS induction suggested that Mrr may recognize the methylated trinucleotides G^{me}AC and C^{me}AG (Fig. 5). We are presently cloning *mrr* with the aim of characterizing the in vitro enzymatic activity of its encoded product.

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LITERATURE CITED

1. **Arber, W.** 1974. DNA modification and restriction, p. 1–37. *In* W. E. Cohn (ed.), Progress in nucleic acid research and molecular biology. Academic Press, Inc., New York.
2. **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.
3. **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.
4. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
5. **Cheng, S.-C., and P. Modrich.** 1983. Positive-selection cloning vehicle useful for overproduction of hybrid proteins. J. Bacteriol. **154**:1005–1008.
6. **Craig, R. J., J. A. Arraj, and M. G. Marinus.** 1984. Induction of damage inducible (SOS) repair in *dam* mutants of *Escherichia coli* exposed to 2-aminopurine. Mol. Gen. Genet. **194**:539–540.
7. **Davis, N. G., and P. Model.** 1985. An artificial anchor domain: hydrophobicity suffices to stop transfer. Cell **41**:607–614.
8. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
9. **Gingeras, T. R., and J. E. Brooks.** 1983. Cloned restriction/modification system from *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **80**:402–406.
10. **Gough, J. A., and N. E. Murray.** 1983. Sequence diversity among related genes for recognition of specific targets in DNA molecules. 1983. J. Mol. Biol. **166**:1–19.
11. **Guarente, L., G. Lauer, T. M. Roberts, and M. Ptashne.** 1980. Improved methods for maximizing expression of a cloned gene: a bacterium that synthesizes rabbit β-globin. Cell **20**:543–553.
12. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
13. **Janulaitis, A., S. Klimasaukas, M. Petrusyte, and V. Butkus.** 1983. Cytosine modification in DNA by *Bcn*I methylase yields N^4-methylcytosine. FEBS Lett. **161**:131–134.
14. **Kenyon, C. J., and G. C. Walker.** 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:2819–2823.
15. **Kiss, A., and F. Baldauf.** 1983. Molecular cloning and expression in *Escherichia coli* of two modification methylase genes of *Bacillus subtilis*. Gene **21**:111–119.
16. **Kleckner, N., R. K. Chan, B.-K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97**:561–575.
17. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:

1110–1112.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Mann, M. B., R. N. Rao, and H. O. Smith.** 1978. Cloning of restriction and modification genes in *E. coli*: the *Hha*II system from *Haemophilus haemolyticus*. Gene **3**:97–112.
20. **Marinus, M. G., A. Poteete, and J. A. Arraj.** 1984. Correlation of DNA adenine methylase activity with spontaneous mutability in *Escherichia coli* K-12. Gene **28**:123–125.
21. **McPartland, A., L. Green, and H. Echols.** 1980. Control of *recA* gene RNA in *E. coli*: regulatory and signal genes. Cell **20**:731–737.
22. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–359. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Posfai, G., F. Baldauf, S. Erdai, J. Posfai, P. Venetianer, and A. Kiss.** 1984. Structure of the gene coding for the sequence-specific DNA-methyltransferase of the *B. subtilis* phage SPR. Nucleic Acids Res. **12**:9039–9049.
24. **Raleigh, E. A., and G. Wilson.** 1986. *Escherichia coli* K-12 restricts DNA containing 5-methylcytosine. Proc. Natl. Acad. Sci. USA **83**:9070–9074.
25. **Revel, H. R.** 1983. DNA modification: glucosylation, p. 156–165. *In* C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget (ed.), Bacteriophage T4. American Society for Microbiology, Washington, D.C.
26. **Russel, M., and P. Model.** 1983. A bacterial gene, *fip*, required for filamentous bacteriophage f1 assembly. J. Bacteriol. **154**:1064–1076.
27. **Sain, B., and N. E. Murray.** 1980. The *hsd* (host specificity) genes of *E. coli* K-12. Mol. Gen. Genet. **180**:35–46.
28. **Schoner, B., S. Kelly, and H. O. Smith.** 1983. The nucleotide sequence of the *Hha*II restriction and modification genes from *Haemophilus haemolyticus*. Gene **24**:227–236.
29. **Shapira, S. K., J. Chou, F. V Richard, and M. J. Casadaban.** 1983. New versatile plasmid vectors for expression of hybrid proteins coded by a cloned gene fused to *lacZ* gene sequences encoding an enzymatically active carboxy-terminal portion of β-galactosidase. Gene **25**:71–82.
30. **Simmon, V. F., and S. Lederberg.** 1972. Degradation of bacteriophage lambda deoxyribonucleic acid after restriction by *Escherichia coli* K-12. J. Bacteriol. **112**:161–169.
31. **Smith, H. O., and S. V. Kelly.** 1984. Methylases of the type II restriction-modification systems, p. 39–71. *In* A. Razin, H. Cedar, and A. D. Riggs (ed.), DNA methylation, biochemistry and biological significance. Springer-Verlag, New York.
32. **Stahl, F. W., and M. M. Stahl.** 1977. Recombination pathway specificity of Chi. Genetics **86**:715–725.
33. **Stahl, M. M., I. Kobayashi, F. W. Stahl, and S. K. Huntington.** 1983. Activation of Chi, a recombinator, by the action of an endonuclease at a distance. Proc. Natl. Acad. Sci. USA **80**:2310–2313.
34. **Szomolanyi, E., A. Kiss, and P. Venetianer.** 1980. Cloning the modification methylase gene of *Bacillus sphaericus* R in *Escherichia coli*. Gene **10**:219–225.
35. **Volkert, M. V., and D. C. Nguyen.** 1984. Induction of specific *E. coli* genes by sublethal treatments with alkylating agents. Proc. Natl. Acad. Sci. USA **81**:4110–4114.
36. **Walder, R. Y., J. L. Hartley, J. E. Donelson, and J. A. Walder.** 1981. Cloning and expression of the *Pst*I restriction-modification system in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:1503–1507.
37. **Walder, R. Y., C. J. Langtimm, R. Chatterjee, and J. A. Walder.** 1983. Cloning of the *Msp*I modification enzyme. J. Biol. Chem. **258**:1235–1241.
38. **Walder, R. Y., J. A. Walder, and J. E. Donelson.** 1984. The organization and complete nucleotide sequence of the *Pst*I restriction-modification system. J. Biol. Chem. **259**:8015–8026.
39. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
40. **Walker, G. C.** 1985. Inducible DNA repair systems. Annu. Rev. Biochem. **54**:425–457.
41. **Zinder, N. D., and J. D. Boeke.** 1982. The filamentous phage (Ff) as vectors for recombinant DNA—a review. Gene **19**:1–10.