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Salmonella typhimurium SA Host Specificity System Is Based on Deoxyribonucleic Acid-Adenine Methylation

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We have determined the nature of the deoxyribonucleic acid (DNA) modification governed by the SA host specificity system of *Salmonella typhimurium*. Two lines of evidence indicate that SA modification is based on methylation of DNA-adenine residues. (i) The SA^+ locus of *Salmonella* was transferred into *Escherichia coli* B, a strain that does not contain 5-methylcytosine in its DNA; although the hybrid strain was able to confer SA modification, its DNA still did not contain 5-methylcytosine. (ii) the N^6-methyladenine content of phage L DNA was measured after growth in various host strains; phage lacking SA modification contained fewer N^6-methyladenine residues per DNA. We also investigated the possibility, suggested by others (32), that SA modification protects phage DNA against restriction by the RII host specificity system. Phages λ, P3, and L were grown in various SA^+ and SA^- hosts and tested for their relative plating ability on strains containing or lacking RII restriction; the presence or absence of SA modification had no effect on RII restriction. In vitro studies revealed, however, that *Salmonella* DNA is protected against cleavage by purified RII restriction endonuclease (R·*Eco*RII). This protection is not dependent on SA modification; rather, it appears to be due to methylation by a DNA-cytosine methylase which has overlapping specificity with the RII modification enzyme, but which is not involved in any other known host specificity system.

Deoxyribonucleic acid (DNA) restriction and modification (host specificity) is widespread in bacteria (1, 2, 4, 28). This phenomenon has been shown to be due to the action of two enzyme activities, viz., a modification methylase, which adds methyl groups to adenine or cytosine residues contained in a specific DNA sequence(s), and a restriction endonuclease, which makes double-stranded cleavages in any duplex DNA lacking the appropriate methylation. The methylated bases produced by modification are N^6-methyladenine (MeAde) or 5-methylcytosine (MeC). The restriction and modification activities may be associated together in a single large multifunctional complex (type I) or as separate, independent molecular species (type II) (6).

In *Salmonella typhimurium*, there are three known distinct restriction-modification systems: (i) LT, which maps near the *proC* locus (11); (ii) SA (previously designated S [9, 10]), which maps near the *serB* locus, and (iii) SB, which maps between SA and *serB* (10). The LT modification appears to be due to adenine-specific methylation (18). Since the SB modification system is allelic with the *Escherichia coli* B and K systems (34), it is likely that SB modification is based on adenine methylation. Slocum and Boyer (32) suggested that S- (or SA)-specific modification might be mediated by cytosine-specific methylation. This was based on their observation that after growth in an *E. coli* hybrid inheriting the *S. typhimurium* SA locus, phage λ was completely resistant to RII restriction. Since the RII modification enzyme is a DNA-cytosine methylase (5, 30), it was proposed that the SA modification enzyme might also methylate cytosine residues in sequences similar to those recognized by the RII enzyme; i.e., the SA methylase might have overlapping specificity with the RII enzyme (32). However, since RII modification did not protect phage λ against SA restriction, it was suggested that the RII enzyme could not modify SA sites (32).

We reported earlier that methylation by the *E. coli* DNA-cytosine methylase, controlled by the *mec* (or *dcm*) locus (21, 25), partially protects phage λ DNA against RII restriction (31). This observation suggested an alternative explanation to the above results, viz., that the hybrid strains studied by Slocum and Boyer (32) were able to protect λ against RII restric-

tion because they may have also inherited a *Salmonella mec*$^+$ gene, which is not involved in SA modification. In this communication, we show that the SA and RII modification-restriction systems are, in fact, independent of one another, and that SA modification is mediated by adenine-specific DNA methylation. We also show that *Salmonella* DNA is protected against degradation by the RII restriction endonuclease (R·*Eco*RII [33]); we propose that this protection is due to in vivo methylation by the *mec*$^+$ DNA-cytosine methylase.

MATERIALS AND METHODS

Phage and bacterial strains. *Salmonella* bacteriophage Lc (3), a clear-plaque-forming mutant of phage L, was kindly provided by C. Colson. Phage P3c (29), a clear-plaque-forming mutant of phage P3, was donated by L. R. Bullas and R. L. Nutter; P3 is able to propagate in both *Salmonella* and *E. coli*. Table 1 lists most of the bacterial strains used in this study; the *Salmonella* strains and *Salmonella* (donor) × *E. coli* (recipient) hybrid strains were generously provided by C. Colson. The *E. coli* (donor) × *Salmonella* (recipient) hybrid, 4028 (15), was from N. Yamamoto. This strain has lost the SA, SB, and LT host specificity systems of the recipient and has inherited the K system of the *E. coli* donor (unpublished data). Additional strains used in this study have been described earlier (11, 21).

Analysis of methylated bases in DNA. Detailed procedures for the growth of labeled phage or cells, DNA isolation, and chromatographic analysis have been described previously (17-21).

Analysis of R·*Eco*RII degradation of DNA by agarose (tube) gel electrophoresis. The R·*Eco*RII enzyme preparation and the conditions for DNA digestion and agarose (tube) gel electrophoresis have been described (31).

TABLE 1. *Bacterial strains and relevant host specificity phenotypes*[a,b]

Strain	Host specificity phenotype	Abbreviation
Salmonella typhimurium		
4233	$r_{LT}^+m_{LT}^+r_{SA}^+m_{SA}^+$	LT^+SA^+
4253	$r_{LT}^-m_{LT}^-r_{SA}^+m_{SA}^+$	LT^-SA^+
4423	$r_{LT}^+m_{LT}^+r_{SA}^-m_{SA}^-$	LT^+SA^-
4296	$r_{LT}^-m_{LT}^-r_{SA}^-m_{SA}^-$	LT^-SA^-
4296 (RII)	$r_{LT}^-m_{LT}^-r_{SA}^-m_{SA}^-r_{RII}^+m_{RII}^+$	$LT^-SA^-RII^+$
S. typhimurium (donor) × *E. coli* (recipient) hybrids		
2027	$r_B^-m_B^-r_{SA}^+m_{SA}^+r_{SB}^+m_{SB}^+$	$B^-SA^+SB^+$
KS ser$^-$	$r_K^-m_K^-r_{SA}^+m_{SA}^+r_{SB}^+m_{SB}^+$	$K^-SA^+SB^+$
2.31	$r_K^-m_K^-r_{SA}^+m_{SA}^+r_{SB}^-m_{SB}^-$	$K^-SA^+SB^-$
3.21	$r_K^-m_K^-r_{SA}^-m_{SA}^-r_{SB}^+m_{SB}^+$	$K^-SA^-SB^+$

[a] The terminology for host specificity is that proposed earlier (2); r and m refer to restriction and modification ability, respectively. The various host specificity systems are as follows: LT, SA, and SB are *Salmonella* systems; B and K are in *E. coli*; RII is specified by the N-3 R-factor (20). The SB system is functionally complementary to the B and K systems (34).

[b] Strains 4233 (9), derived from *S. typhimurium* LT 7 *proC* 90 (8), served as the parental strain for derivation of the LT and SA mutants. Hybrid strains were made by mating Hfr *Salmonella* with F$^-$ *E. coli* recipients: 2027 was derived from *E. coli* Bc 251 *thr*$^-$ *leu*$^-$ *ilv*$^-$ *lac*$^-$ $r_B^-m_B^+$ after *thr*$^+$ *leu*$^+$ selection; KS *ser*$^-$ was derived from *E. coli* K-12 *thr*$^-$ *leu*$^-$ *thi*$^-$ *lac*$^-$ $r_K^-m_K^+$ after *thr*$^+$ *leu*$^+$ selection (*serB80* from the donor was also inherited). Both hybrids inherited the SA and SB systems of the *Salmonella* donor and lost the *E. coli* modification specificity; this was independently verified in our laboratory. Hybrids 2.31 and 3.21 were derived from KS *ser*$^-$.

RESULTS

Effect of SA modification on the plating properties of various bacteriophages. Slocum and Boyer reported that phage λ was protected against RII restriction after growth in Hfr *Salmonella* × F$^-$ *E. coli* hybrids that had inherited the *Salmonella hspS* specificity locus (32); hereafter, *hspS* will be referred to as SA (10). We have reexamined this phenomenon using hybrids derived from F$^-$ *E. coli* K-12 and B recipient strains that had inherited the *Salmonella* SA and SB systems (Table 1). Phage λ, grown in these hybrids or in mutant derivatives, were assayed for their relative plating ability or various hosts. Under the conditions used here (phage growth and plating at 37 C), SA and SB restriction of unmodified λ was each approximately 100-fold (Table 2). The two systems acted independently and cumulatively; e.g., λ·$K^-SA^-SB^-$ was restricted to 10^{-4} on SA^+SB^+, but only to 10^{-2} on SA^+SB^- or SA^-SB^+. These data are in accord with those of Colson and Van Pel (10) and are included to illustrate the host specificity phenotype of the various strains.

Phage λ grown in *E. coli* B strains is strongly restricted by the RII system, whereas λ grown in most *E. coli* K-12 strains is partially protected against RII (21). Evidence that SA (or SB) modification does not protect λ against RII restriction is presented in Table 2: (i) λ grown in an SA^+SB^+ *Salmonella* × *E. coli* B hybrid is still strongly restricted by RII; (ii) λ grown in various *Salmonella* × *E. coli* K-12 hybrids is 10- to 100-fold more resistant to RII restriction, and this partial protection is not reduced when SA (or SB) modification is absent.

Additional evidence supporting this conclusion was obtained with phages L and P3. As can be seen in Table 3, the presence or absence of SA (or LT) modification has no influence on the susceptibility of phage L to RII restriction; e.g., phages L·SA^+ and L·SA^- are restricted to the same degree by the RII^+ host. Phage P3 is weakly restricted by the SA system (Table 3

TABLE 2. *Efficiency of plating of phage λ grown on various Salmonella × E. coli hybrids*[a]

Phage[b]	Indicator strain					
	$B^-SA^-SB^-$	$B^-SA^-SB^-RII^+$	$B^-SA^+SB^+$	$K^-SA^+SB^+$	$K^-SA^+SB^-$	$K^-SA^-SB^+$
$\lambda \cdot B^-SA^+SB^+$	1.0	3×10^{-4}	0.7	0.8	0.9	0.7
$\lambda \cdot K^-SA^+SB^+$	1.0	2×10^{-3}	0.4	0.4	1.0	0.4
$\lambda \cdot K^-SA^+SB^-$	1.0	4×10^{-3}	8×10^{-3}	8×10^{-3}	1.0	3×10^{-2}
$\lambda \cdot K^-SA^-SB^+$	1.0	2×10^{-2}	1×10^{-2}	6×10^{-3}	1×10^{-2}	0.5
$\lambda \cdot K^+SA^-SB^-$	1.0	4×10^{-2}	4×10^{-4}	1×10^{-4}	9×10^{-3}	1×10^{-2}

[a] Saturated overnight cultures were used as indicator bacteria. The efficiency of plating at 37 C on $B^-SA^-SB^-$ was defined as 1.0. Samples of suitable phage dilutions were mixed with indicator cells in 2.5 ml of melted soft agar at 45 C and poured on agar plates. Since restriction ability is temperature dependent (10), the time at which the indicator cells were at 45 C was kept as short as possible. After 10 min at room temperature, the plates were placed at 37 C and incubated overnight.

[b] Phage stocks were prepared by inoculating isolated-plaque suspensions of λ *vir* into log-phase broth cultures of the various *Salmonella* × *E. coli* hybrid strains; the growth temperature was 37 C. $\lambda \cdot K^+SA^-SB^-$ was grown in *E. coli* strain F^+ 1100 $r_K^-m_K^+$*mec*$^+$.

TABLE 3. *Efficiency of plating of phages L and P3 grown and tested on various host strains*[a]

Phage[b]	Indicator strain				
	LT^-SA^-	$LT^-SA^-RII^+$	LT^+SA^+	LT^-SA^+	LT^+SA^-
$L \cdot LT^+SA^+$	1.0	2×10^{-3}	1.0	1.0	1.0
$L \cdot LT^-SA^+$	1.0	3×10^{-3}	1×10^{-3}	1.0	1×10^{-3}
$L \cdot LT^+SA^-$	1.0	3×10^{-3}	5×10^{-4}	2×10^{-3}	1.0
$L \cdot LT^-SA^-$	1.0	3×10^{-3}	7×10^{-6}	2×10^{-3}	3×10^{-4}
$L \cdot LT^-SA^-RII^+$	1.0	1.0	4×10^{-6}	2×10^{-3}	4×10^{-4}
$P3 \cdot LT^+SA^+$	1.0	0.40	1.0	0.9	ND[c]
$P3 \cdot LT^-SA^+$	1.0	0.1	4×10^{-5}	1.0	ND
$P3 \cdot LT^-SA^-$	1.0	0.2	3×10^{-4}	0.6	ND
$P3 \cdot K^+LT^-SA^-$*mec*$^\pm$ (hybrid)	1.0	3×10^{-3}	1×10^{-6}	0.9	ND
$P3 \cdot K^+LT^-SA^-$*mec*$^+$ *(E. coli)*	1.0	0.5	1×10^{-6}	0.6	ND
$P3 \cdot K^+LT^-SA^-$*mec*$^-$ *(E. coli)*	1.0	5×10^{-4}	1×10^{-6}	0.7	ND

[a] Saturated overnight cultures were used as indicator bacteria. The efficiency of plating at 37 C on LT^-SA^- is defined as 1.0.

[b] Phage stocks were prepared by inoculating isolated-plaque suspensions of phage Lc or P3c into log-phase broth cultures (at 37 C) of various *Salmonella mec*$^+$ hosts (unless specified otherwise). $P3 \cdot K^+LT^-SA^-$*mec*$^\pm$ (hybrid) refers to P3 grown in the *E. coli* × *Salmonella* hybrid strain, WR4028. $P3 \cdot K^+LT^-SA^-$*mec*$^+$ *(E. coli)* and $P3 \cdot K^+LT^-SA^-$*mec*$^-$ *(E. coli)* refer to P3 phage grown in *E. coli* F^+1100 $r_K^-m_K^+$*mec*$^+$ and *mec*$^-$, respectively (21); *mec* refers to ability to produce DNA-cytosine methylase activity.

[c] ND, Not done; P3 does not form plaques on the LT^+SA^- indicator strain used here.

[7]) and, after growth in *E. coli mec*$^+$ or *Salmonella mec*$^+$ hosts, P3 is barely restricted by RII (*mec*$^+$ indicates ability to produce DNA-cytosine methylase). Absence of SA or LT modification does not render P3 sensitive to RII restriction (Table 3); however, if P3 is grown in *E. coli mec*$^-$ (lacks DNA-cytosine methylase), then RII restriction is more than 1,000-fold. Furthermore, after growth in an *E. coli* × *Salmonella mec*$^\pm$ hybrid (strain WR4028), P3 is sensitive to restriction by RII, albeit at a level intermediate between P3·*mec*$^-$ and P3·*mec*$^+$ (WR4028 is denoted as *mec*$^\pm$ since its DNA contains less than 50% the normal MeC level [unpublished data]). Taken together, these data indicate that P3 DNA is sensitive to restriction by RII, but that it can be protected by *E. coli mec*$^+$ and *Salmonella mec*$^+$ DNA-cytosine methylation, and not by SA (or LT) modification. This will be considered further below.

Influence of SA modification on the content of methylated bases in DNA. It is known that *E. coli* B strains are deficient in DNA-cytosine methylase activity (naturally occurring *mec*$^-$ strains) (12-16, 20, 23, 24), whereas K-12 strains contain this enzyme. We have shown that transfer of an RII factor into *E. coli* B leads to the appearance of MeC in cell DNA (20).

Thus, if SA or SB modification were due to cytosine-specific methylation, we would expect to find MeC in the DNA of SA^+SB^+ *Salmonella* × *E. coli* B hybrids. This possibility was tested directly by growing such a hybrid in medium containing [*methyl*-^{3}H]methionine and analyzing purified DNA for its content of ^{3}H-labeled, methylated bases. We observed that DNA from the hybrid strain, 2027, contained [^{3}H]MeAde but lacked [^{3}H]MeC (MeC/MeAde <0.01); in contrast, the DNA from the *Salmonella* × *E. coli* K-12 (KS *ser*$^-$) hybrid contained both methylated bases (MeC/MeAde = 0.59). Since both strains are SA^+SB^+, the absence of MeC in strain 2027 indicates that SA (or SB) modification does not involve cytosine-specific DNA methylation.

To obtain more direct evidence on the nature of SA modification, we determined the MeAde content in phage L DNA labeled with [2-^{3}H]adenine during growth in various hosts. We observed that the loss of SA (or LT) modification led to a reduction of approximately 20 (or 50) MeAde residues per phage DNA (Table 4); these values are calculated from the observed moles percent of MeAde and the assumption that phage L has the same molecular weight and base composition as phage P22 DNA (see footnote *c*, Table 4). When both SA and LT modifications were lost, a cumulative reduction in the MeAde content was observed (Table 4); however, the RII factor did not affect the MeAde content. It should be noted that loss of LT modification also leads to a reduced MeAde content in phage P22 DNA (18). Additional experiments showed that SA and LT modification had no influence on the MeC content of phage L DNA (data not shown), whereas RII modification resulted in a two- to threefold increase in MeC content. We conclude that adenine-specific DNA methylation is the chemical basis for SA (and LT) modification.

***S. typhimurium mec*$^+$ strains can protect DNA against RII restriction.** Phage P3 is almost totally insensitive to RII restriction if the phage is grown in a *mec*$^+$ host (Table 3). This suggested that the *Salmonella mec*$^+$ DNA-cytosine methylase may have overlapping sequence specificity with the RII system, as has been shown for the *E. coli mec*$^+$ enzyme (26, 27, 31). To investigate this possibility, we examined a variety of *Salmonella* strains for the susceptibility of their DNA to in vitro cleavage by R·*Eco*RII. As can be seen in Fig. 1 (a–h), all the *mec*$^+$ DNAs were resistant to cleavage by R·*Eco*RII, and this was independent of SA and LT modification. In contrast, MeC-deficient DNA from *E. coli* × *Salmonella* hybrid, strain WR4028, was extensively degraded by R·*Eco*RII (Fig. 1i and j); phages P3 and λ grown in this strain are susceptible to RII restriction in vivo (Table 3; unpublished data). Taken together, these results suggest that *Salmonella* contains a DNA-cytosine methylase that has overlapping specificity with the RII modification enzyme.

TABLE 4. *Analysis of methylated bases in phage L DNA as a function of host specificity*[a]

Phage	Mol% MeAde[b]	Calculated avg no.[c] MeAde per DNA
Lc·LT^+SA^+	0.76	171
Lc·LT^-SA^+	0.53	119
Lc·LT^+SA^-	0.67	151
Lc·LT^-SA^-	0.42	95
Lc·$LT^-SA^-RII^+$	0.42	95

[a] Phage were grown at 37 C on various hosts in medium containing [2-^{3}H]adenine (17, 18); the labeled phage was purified by alternate cycles of high- and low-speed centrifugation and by sedimentation equilibrium centrifugation in a CsCl gradient. Isolation of the DNA and determination of the MeAde content was as described earlier (17, 18).

[b] Moles percent of MeAde = counts per minute MeAde/(counts per minute Ade + counts per minute/MeAde) × 100. The values presented are the means of duplicate chromatographic analyses of three independently labeled DNA preparations; an exception is the RII$^+$-modified DNA, where only two separate preparations were analyzed. The range of values was less than ±10% of the mean value.

[c] The number of MeAde per DNA was calculated assuming 25% Ade content and 9 × 10^4 total nucleotides per DNA. There is no published data on the size and composition for phage L DNA; however, since phage L is serologically, morphologically, and genetically related to phage P22 (3), we have assumed phage L and P22 DNAs to have the same molecular weight and base composition.

DISCUSSION

SA modification is based on adenine-specific DNA methylation. The results presented in this paper demonstrate that *Salmonella* SA restriction-modification is unrelated to the RII specificity system (and that SA modification is based on specific methylation of DNA-adenine residues). In support of this is the observation that susceptibility to in vivo restriction by RII is independent of whether or not an infecting phage carries SA specificity (Tables 2 and 3); in addition, RII modification does not protect against SA restriction. Furthermore, the presence or absence of SA modification does not affect the resistance of *Salmonella* DNA to in

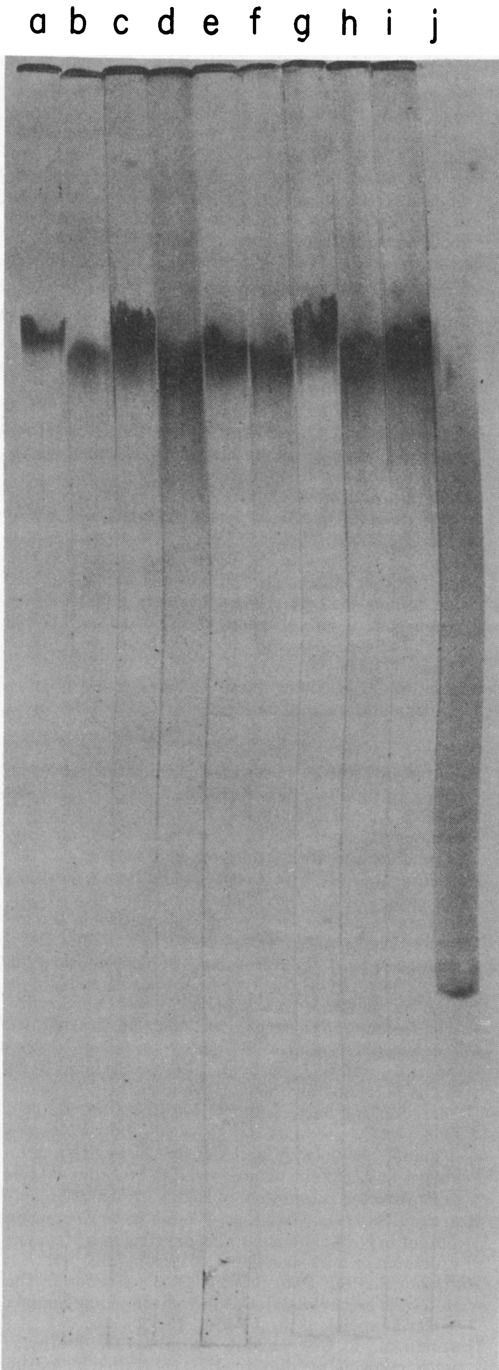

FIG. 1. *Agarose (tube) gel electrophoresis of Salmonella DNA treated with R·EcoRII. DNA isolated from various bacterial strains was incubated in the presence or absence of purified R·EcoRII and subjected to agarose gel electrophoresis (see Materials and Methods). After electrophoresis, the gels were illuminated from below with short-wavelength ultraviolet light and photographed (using TRI-X Pan film and a 25A Hoya red filter). The fluorescent DNA bands would normally appear white against a dark-background; however, a copy-negative was first made and the resulting print has black and white reversed. The following bacterial DNAs tested: (a, b) LT^+SA^+; (c, d) LT^-SA^+; (e, f) LT^+SA^-; (g, h) LT^-SA^-; (i, j) WR4028. Tracks a, c, e, g, and i were the controls not treated with R·EcoRII.*

vitro cleavage by purified R·*Eco*RII restriction nuclease (Fig. 1). These results are not in agreement with those of Slocum and Boyer (32), who reported that λ grown in SA^+SB^+Hfr *Salmonella* × F^- *E. coli* hybrids is fully resistant to RII restriction. The hybrids used in our experiments are stable, haploid recombinants; it is possible that Slocum and Boyer used hybrids that were partially diploid for the *mec*$^+$ gene, and that λ grown in such a strain is fully protected against RII. For the present, this remains an open question.

Since SA specificity is unrelated to the RII modification-restriction system, this left open the question as to the chemical basis for SA modification. As a first approach we analyzed the content of methylated bases in Hfr *Salmonella* × F^- *E. coli* hybrids, derived from either *E. coli B mec*$^-$ or *E. coli* K-12 *mec*$^+$ recipients, which had inherited the SA and SB systems of the *Salmonella* donor. The DNA of the B hybrid, as well as the DNA from phage λ grown in this strain, was devoid of MeC (unpublished data). We conclude that SA (or SB) modification does not involve methylation of DNA-cytosine residues.

To demonstrate that SA modification affects methylation of DNA-adenine residues, we determined the content of MeAde in the DNA of phage L grown in various host strains in the presence of [2-^{3}H]adenine. We observed a specific reduction in phage L DNA MeAde content after loss of SA or LT modification; and loss of both LT and SA modifications led to a cumulative reduction in the MeAde content (Table 4). Approximately 20 and 50 MeAde residues per phage DNA are associated with SA and LT modification, respectively. We do not know if all the MeAde is located exclusively at "recognition sites" for the corresponding restriction enzyme.

***S. typhimurium* has a DNA-cytosine methylase(s) that protects DNA against the RII restriction nuclease.** We have shown that the *E. coli mec*$^+$ DNA-cytosine methylase has overlapping specificity with the RII restriction enzyme (26, 27, 31). The results presented in this paper indicate that the same is true of a *Salmonella* DNA-cytosine methylase: (i) *Salmonella*

mec$^+$ DNA is resistant to R·*Eco*RII, but MeC-deficient DNA from the *E. coli* × *Salmonella* hybrid, WR4028, is extensively degraded; (ii) P3·*mec*$^+$ phage (grown in *Salmonella* or *E. coli*) is barely restricted by RII, but P3·*mec*$^-$ is restricted by RII. Furthermore, fd·*mec*$^+$ RFI (covalently closed double-stranded circular DNA) from infected *E. coli* or *Salmonella* is resistant to R·*Eco*RII (31; unpublished data), but fd·*mec*$^-$ RFI is cleaved at least twice (31). That cellular DNA, phage P3 DNA, and fd·RFI DNA are protected by the *Salmonella mec*$^+$ methylase seems to be inconsistent with the fact that phages λ·*mec*$^+$, L·*mec*$^+$, and P22·*mec*$^+$ are restricted by the RII system. We believe that the differences in susceptibility to R·*Eco*RII result from differences in the extent of methylation of these DNAs. For example, *mec*$^+$ bacterial DNA has almost the same MeC content as DNA from RII-containing strains (20, 21; unpublished data); thus, it appears that the *mec*$^+$ methylase can modify almost all RII sites on host DNA. In contrast, DNA isolated from λ·*mec*$^+$, L·*mec*$^+$, and P22·*mec*$^+$ mature phage have less than 50% of MeC level of the RII-modified phage (19, 21). Thus, at the time of maturation, there are still unmodified sites on λ, P22, and L phage DNA that are susceptible to RII cleavage; however, it is known that λ·*mec*$^+$ DNA is partially resistent to R·*Eco*RII, compared with λ·*mec*$^-$ DNA (22). Incomplete in vivo methylation of λ DNA is not likely to be due to inability of the *mec*$^+$ enzyme to recognize all RII sites. We favor the notion that there is a lower rate of methylation in *mec*$^+$ versus RII$^+$ hosts; this is supported by the observation that in vitro *mec*$^+$ and RII$^+$ cell extracts methylate λ·*mec*$^-$ and λ·*mec*$^+$ DNAs to the same MeC extent (25). Moreover, in vitro methylation by partially purified *mec*$^+$ enzyme modifies λ·*mec*$^-$ and λ·*mec*$^+$ DNAs to complete resistance to cleavage by R·*Eco*RII (S. Hattman, manuscript in preparation).

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