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Biochemistry

Nucleotide sequence of the recognition site for the restriction-modification enzyme of *Escherichia coli* B

(bacteriophage f1/spontaneous mutations/methylation/noncontiguous sequence)

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ABSTRACT **The nucleotide sequence of the recognition site for the restriction-modification enzyme of *Escherichia coli* B (SB site) has been determined. The recognition site is a 15-nucleotide sequence consisting of the trimer 5′TGA3′, followed by an 8-nucleotide domain of variable sequence, which in turn is followed by the tetramer 5′TGCT3′. The sequence has no 2-fold rotational symmetry. Single base changes in the constant nucleotide domains result in the loss of sensitivity to both restriction and modification. Our data are also consistent with modification occurring by methylation of two adenine residues per SB site: one on the adenine of the trimer 5′TGA3′ and the other on the complementary strand on the adenine complementary to the first thymine of the tetramer 5′TGCT3′. All nine independently isolated spontaneous mutants at the SB_1 site of bacteriophage f1 are caused by a G-to-T transversion. Mutations at the SB_2 site are caused by various single base changes.**

Bacteriophage f1, a small, filamentous, single-stranded DNA bacteriophage, is susceptible to host-controlled restriction and modification by *Escherichia coli* B (1). There are two genetic sites on the f1 phage genome that confer susceptibility to restriction by the B host (2). The sites have been mapped on the f1 genome (3, 4) and are called SB_1 and SB_2. Protection from restriction is afforded either by mutation of the SB sites (2) or by modification of the DNA through methylation of adenine residues (5, 6) that are presumably within the DNA sequence defining the SB sites (7). Two methyl groups are added for each SB site (5, 6), one on either strand (8).

The endo R·*Eco*B enzyme can function as either a restriction nuclease or a modification methylase (8–10), depending upon the nature of the SB site. *In vitro*, unmodified, wild-type (SB_1^+, SB_2^+) f1 replicative form DNA (RF), in the presence of Mg^{2+}, ATP, and *S*-adenosylmethionine, is rapidly broken once to yield full-length linear molecules (RFIII) by the action of this enzyme. This cleavage does not occur at the SB site (11); rather, there are many potential cleavage sites. Methylation will occur very slowly at unmodified sites in the presence of Mg^{2+} and *S*-adenosylmethionine. However, a DNA duplex in which one strand has a modified SB site and the other an unmodified SB site is not restricted (12, 13) but is rapidly methylated (10). When such DNA duplex molecules are used for transfection, they are not restricted (14).

The endo R·*Eco*B and K enzymes appear to be fundamentally different from the restriction endonucleases that have been isolated from a wide range of bacteria (for review see ref. 15). These two enzymes have complex cofactor requirements, are active DNA-dependent ATPases, and show modification activity. Moreover, their sites of cleavage are highly nonspecific. These properties are not found in the class II restriction enzymes, which have unique cleavage sites and less elaborate cofactor requirements. The recognition sites of most class II enzymes have 2-fold axes of symmetry and cleavage occurs at the recognition site or at some defined distance near it.

We have determined the nature of the endo R·*Eco*B recognition site (SB) through DNA sequence analysis of SB^o mutants of phage f1. The recognition site is a 15-nucleotide sequence consisting of the trimer 5′TGA3′, followed by an 8-nucleotide domain of variable sequence, which in turn is followed by the tetramer 5′TGCT3′.

MATERIALS AND METHODS

The bacterial strains have been described (3, 4). Bacteriophage f1 and its mutants were isolated in this laboratory. Some of the mutants have been described (3, 4). R119, R120, R121, and R144 are spontaneous SB_1^o mutants that were independently isolated from R86 (*amber* II_{60}), R21 (II_{20}), R21 (II_{20}), and R17 (IV_{17}), respectively, by the procedure described by Boon and Zinder (4). R132, R133, R146, and R163 are $SB_1^oSB_2^o$ mutants isolated similarly from R86 (II_{60}), R21 (II_{20}), wild-type f1, and R124 ($II_{124}SB_2^o$). With the exception of R146, all of the SB^o mutants used for sequence analysis in this paper were spontaneous revertants of the mutants described above, isolated by plating them on the nonsuppressing host K38. These revertants are denoted by the addition of an R to the parental phage designation, e.g., R119R.

Isolation of endo R·*Eco*B has been described (16). The restriction endonucleases endo R·*Hin*dII, endo R·*Hae* II, and endo R·*Hae* III were prepared as described (7). Endo R·*Hpa* was prepared by the procedure of Sharp *et al.* (17). Endo R·*Hha* I and endo R·*Alu* I were purchased from New England Biolabs. Preparation of covalently closed, circular, double-stranded DNA (RFI) of f1 phage has been described (7, 18). Marker rescue experiments were carried out as described (7, 19). *In vitro* methylation of DNA by endo R·*Eco*B has been described (10, 20). DNA sequence analysis was performed by the base-specific modification method of Maxam and Gilbert (21).

RESULTS

Analyses of genetic recombination in phage f1 have shown close linkage of the SB_1 site to genes *V* and *VII* and of the SB_2 site to the amber mutant site of R124 in gene *II* (3, 22). Our previous studies on the physical mapping of phage f1 have located the SB_1 and SB_2 sites on the restriction fragments *Hae* III-B1 (about 1500 base pairs) and *Hae* III-B2 (about 220 base pairs), respectively (ref. 7; see Fig. 1). Furthermore, the SB_1 site has been mapped to the larger of the two fragments produced by endo R·*Eco* RII cleavage of f1 RFI (23). To map the SB_1 site more precisely to a restriction fragment small enough for DNA sequence analysis, we tested fragments of f1 RFI produced by

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Abbreviation: RF, replicative form DNA.

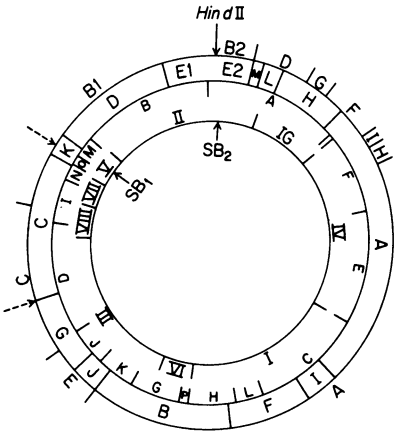

FIG. 1. Physical and genetic map of bacteriophage f1. The outer circle shows the location of *Hae* III fragments (letters on the outside), the middle circle the *Hpa* II fragments (letters on the outside) and the *Hha* I fragments (letters on the inside). The single cleavage site of endo R·*Hin*dII is denoted by an arrow marked *Hin*dII; the two endo R·*Eco*RII sites are denoted by dotted arrows on the outer circle (7, 23–25). The inner circle represents the genetic map (3), with the two SB sites indicated by arrows marked SB. Genes are indicated by Roman numerals. IG refers to the intergenic space between genes *IV* and *II* (23, 26).

endo R·*Hha* I for their ability to rescue markers upon transfection. The physical map of f1 (Fig. 1) suggested that the SB_1 site should be located on fragment *Hha*-B, *Hha*-M, or *Hha*-O.

The *Hha* fragments produced from nonamber RFI were isolated and hybridized to single-stranded viral DNA of various amber mutants and used to transfect Su^- cells. The results, shown in Table 1, indicate that the *Hha*-M fragment covers the V_{13} amber mutation site of gene *V*, which is carried by mutant strain R13, and that the *Hha*-B fragment covers the II_{86} amber mutation site of gene *II*, which is carried by mutant strain R132. Furthermore, the results show that the SB_1 genotype of the nonamber progeny phage produced as a result of marker rescue by the *Hha*-M fragment is identical to that of the RFI from which *Hha*-M was prepared, while the SB_1 genotype of the progeny phage produced by the *Hha*-B fragment is identical to that of the single-stranded viral DNA. These results indicate that the SB_1 site is located on the fragment *Hha*-M, which is about 130 base pairs long.

None of the amber mutants we tested was rescued by the fragment *Hha*-O. However, the following experiment (data not shown) indicated that the SB_1 site is found only on *Hha*-M and not on *Hha*-O or *Hha*-N. Single-stranded viral DNA of R140R ($SB_1^+SB_2^o$, nonamber) was subjected to denaturation and subsequent renaturation in the presence or absence of a 3-fold molar excess of fragments *Hha*-M, *Hha*-N, or *Hha*-O, which were prepared from RFI of R132R($SB_1^oSB_2^o$, nonamber). The DNA samples were used to transfect $CaCl_2$-treated bacteria K336B (r_B^+, m_B^+), and plated for infective centers. All the samples gave approximately equal numbers of plaques except the sample that was hybridized to the *Hha*-M fragment, which produced about 5 times as many plaques. Five individual plaques from each plate were picked and plated on K38 (r_k^+, m_k^+) [f1 is resistant to K-restriction (1)], and the individual plaques that were produced on K38 were tested for SB markers. All of the five tested progeny that were derived from DNA hybridized to *Hha*-M were SB_1^o SB_2^o. All of the other progeny phage were SB = 1, as is R140R, confirming the conclusion that the SB_1 site is located on the *Hha*-M fragment.

Nucleotide sequence analysis of the restriction fragments *Hae* III-B2 and *Hha*-M were performed by the method of Maxam and Gilbert (21). Fig. 2*a* presents the detailed restriction map of the *Hha*-M fragment and schematically illustrates the experiments performed to determine the sequence of this fragment. The nucleotide sequence determined in this manner is shown in Fig. 3*a*. The sequence begins approximately five nucleotides to the right of the border between *Hha*-B and -M and continues to the border between *Hha*-M and -O. This fragment maps within gene *V*, the amino acid sequence of which is known (27, 28). The nucleotide sequence shown in Fig. 3*a* is consistent with the known amino acid sequence. Similarly, Fig. 2*b* presents the detailed restriction map and sequencing experiments for *Hae*-III-B2, whose sequence is shown in Fig. 3*b*. This sequence begins approximately five nucleotides to the right of the border between *Hae* III-D and -B2 and continues to approximately five nucleotides to the left of the *Hin*dII cleavage site. Only one reading phase is possible for the gene II protein sequence in this region; the other possible reading phases are blocked by in-phase termination codons.

The SB sequence within these restriction fragments was identified by nucleotide sequence determination of spontaneous

Table 1. Localization of SB_1 sites on *Hha* fragments of f1 RFI

Restriction fragment (nonamber)	Viral strand DNA	No. of am^+ phage/ plate	No. of SB^+ sites in am^+ phage
$SB_1^+SB_2^+$*Hha*-B	R132 ($II_{86}SB_1^oSB_2^o$)	200	SB = 0 (5/5)
$SB_1^+SB_2^+$*Hha*-B	None	0	—
None	R132	2	SB = 0 (1/1)
$SB_1^oSB_2^o$*Hha*-B	R13 ($V_{13}SB_1^+SB_2^+$)	5	SB = 2 (2/2)
$SB_1^oSB_2^o$*Hha*-B	None	0	—
$SB_1^oSB_2^o$*Hha*-L	R13	2	SB = 2 (1/1)
$SB_1^oSB_2^o$*Hha*-L	None	0	—
$SB_1^oSB_2^o$*Hha*-M	R13	104	SB = 1 (5/5)
$SB_1^oSB_2^o$*Hha*-M	None	0	—
$SB_1^oSB_2^o$*Hha*-N	R13	8	SB = 2 (2/2)
$SB_1^oSB_2^o$*Hha*-N	None	0	—
$SB_1^oSB_2^o$*Hha*-O	R13	9	SB = 2 (2/2)
$SB_1^oSB_2^o$*Hha*-O	None	0	—
None	R13	5	SB = 2 (2/2)

$CaCl_2$-treated cells of K38 ($Su^-r_k^+m_k^+$) were transfected with viral DNA of gene *II* and gene *V* amber mutants to which denatured *Hha* I fragments were hybridized. For each DNA sample that gave am^+ phage, plaques were picked and their plating efficiency (e.o.p.) on K336B ($r_B^+m_B^+$) was measured. SB = 2, $SB_1^+SB_2^+$ (e.o.p. = 0.001); SB = 1, $SB_1^oSB_2^+$ or $SB_1^+SB_2^o$ (e.o.p. = 0.03); SB = 0, $SB_1^oSB_2^o$ (e.o.p. = 1).

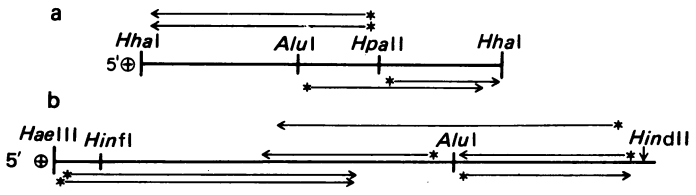

FIG. 2. Detailed physical map of the *Hha*-M (*a*) and *Hae* III-B2 (*b*) fragments and summary of nucleotide sequencing experiments. The sequencing experiments performed to determine the nucleotide sequence of each fragment is schematically illustrated by the light lines. An asterisk (*) indicates the 5′-terminal label. The length of the light lines indicates the portion of the restriction fragment whose sequence was determined in each experiment. Lines above the bold line denote sequences of the complementary strand (−); lines below the bold line denote sequences of the viral strand (+).

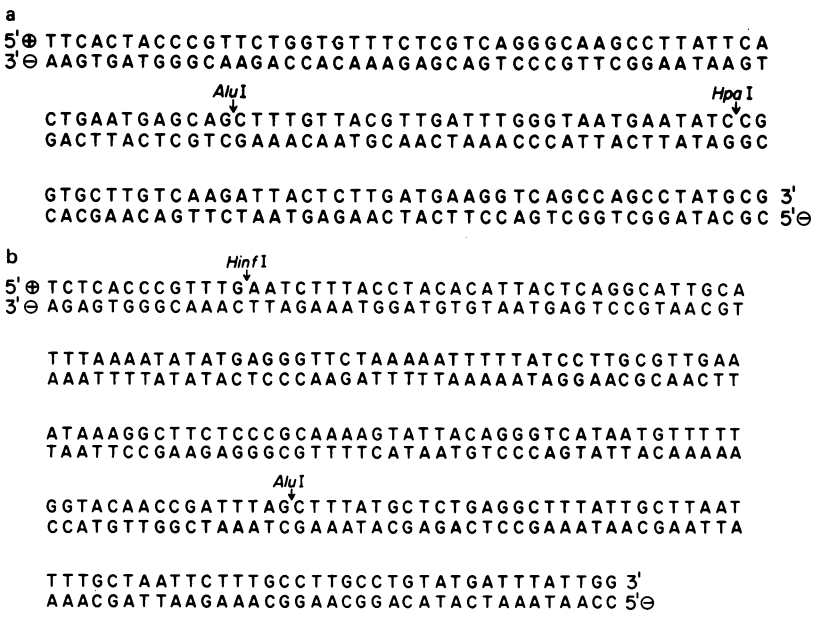

FIG. 3. Nucleotide sequences within the *Hha*-M (*a*) and *Hae* III-B2 (*b*) restriction fragments. The sequences were derived from the experiments schematically illustrated in Fig. 2. For some regions of the sequence, independent sequencing experiments were performed on both strands, while in other regions multiple sequencing experiments were performed on only one strand. The sequences of both strands are shown for convenience. +, The viral strand; –, the complementary strand.

SB mutants that had been isolated in this laboratory. The sequence of the entire *Hha*-M fragment for an SB_1^o mutant (R132R) was determined (data not shown) and, with the exception of a single G→T transversion, the sequence of R132R *Hha*-M is identical to the wild-type (SB_1^+) sequence (Table 2). Therefore, this G→T base change must represent the SB_1^o mutation in R132R. Seven other independently isolated SB_1^o mutants, as well as phage M13, which is closely related to phage f1 but lacks the SB_1^+ site, were analyzed for their base sequences in this region. All of these SB_1^o phages showed the identical mutation, the G→T transversion, at the same location (Table 2). The SB_2 site was determined by sequence analysis of the *Hae* III-B2 fragment from SB_2^o mutants. Fig. 4 shows two examples of nucleotide sequences in the relevant region of SB_2^o mutants compared to the SB_2^+ (wild type). Similar analyses were done for two other independently isolated SB_2^o mutants. The results of the base sequence analyses of the SB_1^o and SB_2^o mutants are summarized in Table 2.

Table 2. Nucleotide sequence changes for SB → SB° mutants of f1

Phage	SB mutation	Sequence of viral strand	Mutation
f1	SB_1^+	TGA ATATCCGG TGCT	—
R132R	SB_1^o	TGA ATATCCGG TTCT	G→T
R133R	SB_1^o	TGA ATATCCGG TTCT	G→T
R163R	SB_1^o	TGA ATATCCGG TTCT	G→T
R146	SB_1^o	TGA ATATCCGG TTCT	G→T
R120R	SB_1^o	TGA ATATCCGG TTCT	G→T
R121R	SB_1^o	TGA ATATCCGG TTCT	G→T
R144R	SB_1^o	TGA ATATCCGG TTCT	G→T
R119R	SB_1^o	TGA ATATCCGG TTCT	G→T
M13	SB_1^o	TGA ATATCCGG TTCT	G→T
f1	SB_2^+	TGA GGCTTTAT TGCT	—
R132R	SB_2^o	TCA GGCTTTAT TGCT	G→C
R133R	SB_2^o	CGA GGCTTTAT TGCT	T→C
R146	SB_2^o	TGA GGCTTTAT TACT	G→A
R163R	SB_2^o	TTA TGCTTTAT TGCT	G→T; G→T

For each phage shown, the nucleotide sequence of a restriction fragment containing the SB site was determined. The constant domains are indicated by the boxed-in sequences. Mutations are indicated by boldface type. Although the T at the seventh position of the sequence shown appears to be conserved, it is not present in the SB site of φX174 (see Fig. 6 and ref. 29).

Comparison of the nucleotide sequence of the two regions in which the SB_1^o and SB_2^o mutations were identified indicates the presence of a base sequence common to the SB_1^+ and SB_2^+ regions: TGA and TGCT, which are separated from each other by eight bases that are apparently nonspecific. Moreover, the results (Table 2) indicate that base substitution in either the TGA or the TGCT domain leads to the SB^0 mutation. All of the 14 independently isolated SB mutants tested have a base substitution within either the TGA domain or the TGCT domain. These observations strongly suggest that these two domains separated by eight bases constitute an SB site.

As shown in Table 2, the mutant R163R has two bases that differ from the wild type. R163R is a spontaneous revertant of a derivative of the amber mutant R124. R124 was isolated in this laboratory by selecting for an SB° mutant using bacterial strains that harbor an amber suppressor; it was found to be an SB_2^o mutant and at the same time a gene *II* amber mutant. Genetic recombination analyses have shown that the SB_2^o mutation and the II_{am} mutation are tightly linked (22). The base sequence of R163R (Table 2), when combined with the reading frame of the gene *II* protein described above, strongly suggests that R124 became SB_2^o and II_{am} by a single mutation, G→T, in the TGA domain. This gives rise to the amber triplet TAG. Reversion (R163R) of this amber mutation remained SB_2^o through a second G→T mutation outside of the constant domain of the SB site, giving rise to the triplet TAT, which codes for tyrosine. R124 is efficiently suppressed by SuIII, which substitutes tyrosine for the amber codon UAG. Thus, only the transversion that is within TGA domain of R163R is apparently related to the SB_2^o mutation.

We have previously shown that when DNA is modified *in vitro* with endo R·*Eco*B and is subsequently cleaved with site-specific endonucleases the methyl groups incorporated upon modification are found only in the DNA fragments that contain the SB^+ site (7). Those results suggested that methylation occurs at the SB site. If this is so, since only one adenine residue on each strand is methylated per SB site, we would ex-

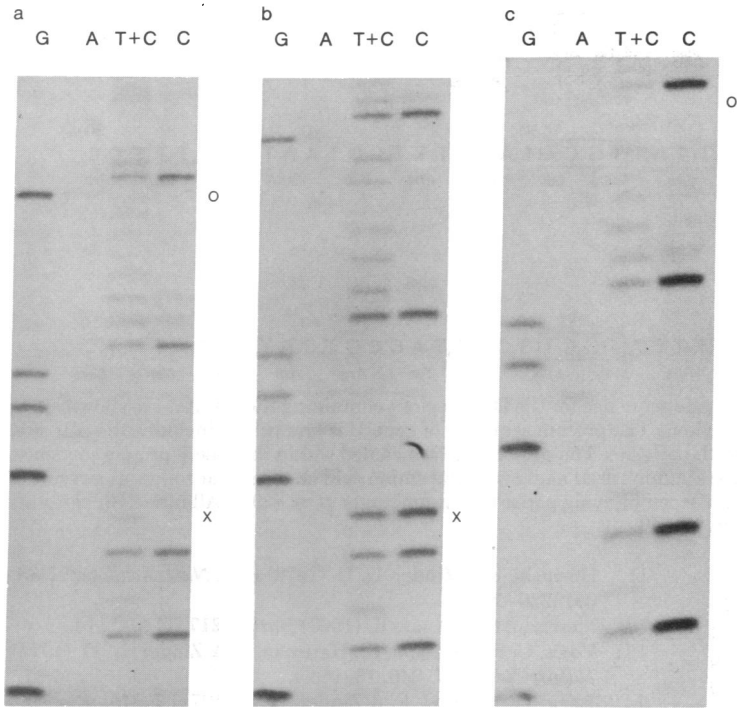

FIG. 4. DNA sequencing gels showing base substitution in SB_2^o mutants. Reaction conditions are those described by Maxam and Gilbert (21). G and A were methylated with dimethylsulfate for 25 and 30 min, respectively, at 20°. Bases modified at G were cleaved in 1 M piperidine at 90° for 30 min. Methylated adenosines were released by treatment with 0.1 M HCl at 0° for 120 min and the polynucleotides were cleaved by heating at 90° for 30 min in 0.1 M NaOH. T and C in H_2O and 2.5 M NaCl, respectively, were treated with 18 M hydrazine for 25 and 30 min, respectively, at 20°. Polynucleotides were cleaved by heating in 1.0 M piperidine for 1 hr at 90°. The cleavage products were fractionated on a 30 × 40 × 0.15 cm slab gel of 20% polyacrylamide/7 M urea. Electrophoresis was at 25 V/cm for 12 hr. (*a*) Cleavage products of SB_2^+; (*b*) cleavage products of R133R, SB_2^o; (*c*) cleavage products of R146, SB_2^o. X, The T-to-C transition of R133R; O, the G-to-A transition of R146.

pect that the A in the TGA domain would be methylated on the (+) strand and that an A complementary to one of the Ts of TGCT domain would be methylated on the (−) strand. The nucleotide sequence of the SB_1 site shown above (Table 2) indicates the presence of an endo R·*Hpa* II cleavage site (CCGG) between the TGA and TGCT domains. Thus, *in vitro* modification of SB_1^+ site with endo R·*Eco*B followed by endo R·*Hpa* II cleavage should generate fragments each containing a single methyl group. The result shown in Fig. 5 indicates that this is the case. Wild-type RFI, modified *in vitro* with S[^{3}H]adenosylmethionine and endo R·*Eco*B, was then cleaved with endo R·*Hpa* and analyzed by polyacrylamide gel electrophoresis. Fluorography (Fig. 5) and scintillation counting of the gel bands (data not shown) indicated that while the two methyl groups of the SB_2 site were found in fragment *Hpa*-E2, the methyl groups at the SB_1 site were found on two fragments, *Hpa*-D and *Hpa*-K (see Fig. 1). When endo R·*Hha* I was used instead of endo R·*Hpa*, the methyl groups were detected only in *Hha*-M (SB_1) and *Hha*-A (SB_2) fragments. We conclude that modification occurs on both sides of the variable domain that separates the two constant domains of the SB site.

DISCUSSION

Fig. 6 summarizes our sequence data for the SB sites of bacteriophage f1. It also includes the base sequence of the SB^+ site of the ϕX174 SB1 mutant (35), which is described in an accompanying paper by Lautenberger *et al.* (29). The constant domains, namely, TGA and TGCT, separated by eight unspecified nucleotides are common to the three sequences. Sequencing studies demonstrate that mutations of SB^+ to SB^o are reflected in nucleotide substitution in the constant domain of the recognition sequence. We conclude that this sequence represents the SB site, namely, the genetic site which confers upon DNA the susceptibility to host-specific restriction and modification by *E. coli B*.

In all the three cases shown in Figure 6 the sequence 5′TGA . . . TGCT3′ (and not its complement) is found on the viral (+) strand. The sequences are found in protein-coding regions of the DNA and are all in the same translational reading frame.

In the SB_1 site, all nine spontaneous, independently isolated mutants whose sequences have been determined show the same mutation, a G to T transversion (Table 2). This mutation to SB_1^o occurs in the third position of the codon GTG, giving rise to the synonymous codon GTT. Although several other synonymous mutations are possible in this region, they have not been observed. This suggests that the G to T transversion seen at this location is a highly favored event which occurs as the result of some unknown mechanism. The SB_2 site is contained within gene *II*, for which a potential amino acid sequence is shown in Fig. 6. Mutations to SB_2^o occur in either of the two constant domains, giving rise to both synonymous mutations and amino acid substitutions. With the data described by Lautenberger *et al.* (29), it can be seen that mutations to SB^o have been detected in all positions of the constant domain of the SB recognition sequence with the exception of the adenine of the conserved triplet TGA and the first thymine of the tetramer TGCT.

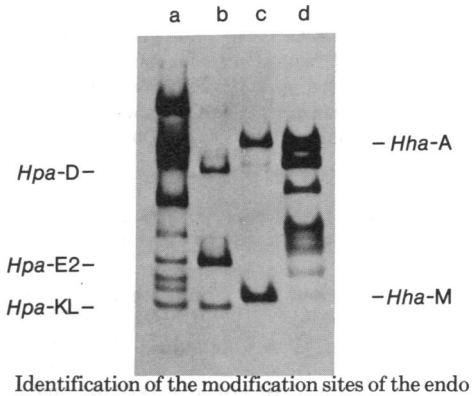

FIG. 5. Identification of the modification sites of the endo R·*Eco*B enzyme. f1 RFI methylated *in vitro* with *S*-[^{3}H]adenosylmethionine by the action of the endo R·*Eco*B enzyme was digested with endo R·*Hpa* (a mixture of *Hpa* I and *Hpa* II) (lane b) and endo R·*Hha* I (lane c). Uniformly ^{3}H-labeled f1 RFI DNA digested with endo R·*Hpa* (lane a) and endo R·*Hha* I (lane d) are shown in the adjacent lanes to identify the fragments. The fragments were fractionated on a 2.4–7.5% polyacrylamide gradient gel as described (7). Bands were visualized by fluorography (30, 31).

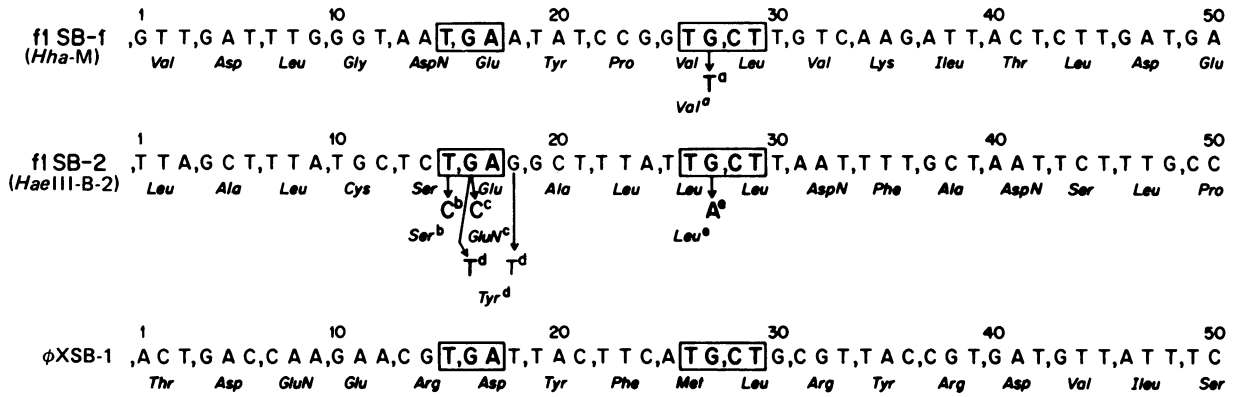

FIG. 6. Comparison of the SB sites of f1 and φX174. The nucleotide sequence for the DNA regions containing the SB sites are shown along with the amino acid sequence for the proteins coded for by these regions. The protein sequence of gene *V* is known and includes the SB_1 site. A proposed amino acid sequence is shown for gene *II*, which includes the SB_2 site. The φX-SB_1 site is included within the gene F protein sequence, which is known (32–34). The sequencing data for the SB^o mutant are summarized along with the amino acid changes that follow as a result of these mutations. The sequence of the φX-SB_1 site is presented in an accompanying paper by Lautenberger *et al.* (29). [a] All nine SB_1^o mutants show this same mutation (see Table 2). [b] R133R. [c] R132R. [d] R163R. [e] R146.

Van Ormondt *et al.* (36) have analyzed ^{3}H-labeled oligonucleotides produced by the action of pancreatic DNase from phage fd RF that had been methylated *in vitro* with ^{3}H-labeled *S*-adenosylmethionine by the action of the modification methylase of *E. coli* B. Their oligonucleotide data, combined with our nucleotide sequence of the SB_1 and SB_2 sites of f1, strongly suggest that methylation occurs on the adenine residue in the triplet TGA and the adenine residue that is complementary to the first thymine of the tetramer TGCT. The experiment in Fig. 5 is consistent with and supports this notion.

An SB site, as described here, consists of two nonequivalent constant domains separated by an eight-base variable domain. There is no 2-fold axis of symmetry. Since the two constant domains are separated by eight bases and since a DNA double helical structure has the periodicity of ten bases (37), the two constant domains are found on the same side of the DNA duplex. Moreover, the two methyl groups that are introduced upon modification onto the adenine residues described above also face the same side of the duplex (in the major groove) at orientations of approximately 70° from each other. This would suggest that the endo R·*Eco*B enzyme may interact with only one side of the DNA duplex. The enzyme would have to span at least 50 Å to cover the distance of the 15 nucleotide pairs involved in the SB recognition site.

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1. Arber, W. (1966) *J. Mol. Biol.* **20,** 483–496.
2. Arber, V. W. & Kühnlein, U. (1967) *Pathol. Microbiol.* **30,** 946–952.
3. Lyons, L. B. & Zinder, N. D. (1972) *Virology* **49,** 45–60.
4. Boon, T. & Zinder, N. D. (1971) *J. Mol. Biol.* **58,** 133–151.
5. Smith, J. D., Arber, W. & Kühnlein, U. (1972) *J. Mol. Biol.* **63,** 1–8.
6. Kühnlein, U. & Arber, W. (1972) *J. Mol. Biol.* **63,** 9–19.
7. Horiuchi, K., Vovis, G. F., Enea, V. & Zinder, N. D. (1975) *J. Mol. Biol.* **95,** 147–165.
8. Vovis, G. F. & Zinder, N. D. (1975) *J. Mol. Biol.* **95,** 557–568.
9. Haberman, A., Haywood, J. & Meselson, M. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3138–3141.
10. Vovis, G. F., Horiuchi, K. & Zinder, N. D. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3810–3813.
11. Horiuchi, K. & Zinder, N. D. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3220–3224.
12. Meselson, M. & Yuan, R. (1968) *Nature* **217,** 1110–1114.
13. Vovis, G. F., Horiuchi, K., Hartman, N. & Zinder, N. D. (1973) *Nature New Biol.* **246,** 13–16.
14. Enea, V., Vovis, G. F. & Zinder, N. D. (1975) *J. Mol. Biol.* **96,** 495–509.
15. Roberts, R. J. (1976) *CRC Crit. Rev. Biochem.* **4,** 123–164.
16. Horiuchi, K., Vovis, G. F. & Zinder, N. D. (1974) *J. Biol. Chem.* **249,** 543–552.
17. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12,** 3055–3063.
18. Model, P. & Zinder, N. D. (1974) *J. Mol. Biol.* **83,** 231–251.
19. Edgell, M. H., Hutchison, C. A., III & Sclair, M. (1972) *J. Virol.* **9,** 574–582.
20. Lautenberger, J. A. & Linn, S. (1972) *J. Biol. Chem.* **247,** 6176–6182.
21. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
22. Enea, V. & Zinder, N. D. (1976) *J. Mol. Biol.* **101,** 25–38.
23. Vovis, G. F., Horiuchi, K. & Zinder, N. D. (1975) *J. Virol.* **16,** 674–684.
24. Ravetch, J., Horiuchi, K. & Model, P. (1977) *Virology* **81,** 341–351.
25. Ravetch, J. V., Horiuchi, K. & Zinder, N. D. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4219–4222.
26. Enea, V., Horiuchi, K., Turgeon, B. G. & Zinder, N. D. (1977) *J. Mol. Biol.* **111,** 395–414.
27. Nakashima, Y., Dunker, A. K., Marvin, D. A. & Konigsberg, W. (1974) *FEBS Lett.* **40,** 290–292, erratum **43,** 125.
28. Cuypers, T., van der Ouderaa, F. J. & deJong, W. W. (1974) *Biochem. Biophys. Res. Commun.* **59,** 557–563.
29. Lautenberger, J. A., Kan, N. C., Lackey, D., Linn, S., Edgell, M. H. & Hutchison, C. A., III (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2271–2275.
30. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
31. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
32. Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., III, Slocombe, P. M. & Smith, M. (1977) *Nature* **265,** 687–695.
33. Air, G. M. (1976) *J. Mol. Biol.* **107,** 433–444.
34. Air, G. M., Blackburn, E. H., Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* **96,** 703–719.
35. Schnegg, B. & Hofschneider, P. H. (1969) *J. Virol.* **3,** 541–542.
36. van Ormondt, H., Lautenberger, J. A., Linn, S. & deWaard, A. (1973) *FEBS Lett.* **33,** 177–180.
37. Watson, J. D. & Crick, F. H. C. (1953) *Nature* **171,** 737–738.