JOURNAL OF BACTERIOLOGY, Aug. 1993, p. 4712–4718
0021-9193/93/154712-07$02.00/0

Vol. 175, No. 15

Superinfection Exclusion (*sieB*) Genes of Bacteriophages P22 and λ

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Received 19 February 1993/Accepted 30 May 1993

The superinfection exclusion gene (*sieB*) of *Salmonella* phage P22 was mapped with phage deletion mutants. The DNA sequence in the region was reexamined in order to find an open reading frame consistent with the deletion mapping. Several discrepancies with the previously published sequence were discovered. The revised sequence revealed a single open reading frame of 242 codons with six likely translation initiation codons. On the basis of deletion and amber mutant phenotypes, the second of these six sites was inferred to be the translation initiation site of the *sieB* gene. The *sieB* gene encodes a polypeptide with 192 amino acid residues with a calculated molecular weight of 22,442, which is in reasonable agreement with that estimated from polyacrylamide gels. The transcription start site of *sieB* was identified by the use of an RNase protection assay. The *sieB* promoter thus identified was inactivated by a 2-base substitution in its −10 hexamer. The *sieB* gene of coliphage λ was also identified. The promoter for λ *sieB* was identified by homology to that of P22 *sieB*.

Lambdoid prophages encode systems that prevent the lytic growth of superinfecting phages. Thus, λ and P22 are unable to grow vegetatively in λ or P22 lysogens, respectively, because transcription from their major promoters (p_L and p_R) is sensitive to repression by *cI* (in the case of λ) or *c2* (in the case of P22) repressors present in the lysogen (for reviews, see references 8 and 13). P22 encodes an antirepressor which is capable of inducing any resident lambdoid phages by neutralizing the repressor already present in the cell. A P22 prophage itself is not induced by this antirepressor because it elaborates another repressor (Mnt) whose function is to prevent transcription of *ant* (which encodes the antirepressor) (for a review, see reference 25).

P22 and λ also have mechanisms to prevent the growth of superinfecting phages that are insensitive to repression. Thus, the λ *rex* genes prevent the growth of *rII* mutants of phage T4 (1). P22 has at least two genes which prevent superinfection by other phages: *sieA* (for superinfection exclusion) interferes with the DNA injection process (21), and *a1* causes conversion of the O antigen of the *Salmonella* host, thus preventing adsorption of some phages (reference 22 and references therein). Both λ and P22 possess another superinfection exclusion gene—*sieB*; its product aborts the lytic development of some superinfecting phages (23, 24). Perhaps surprisingly, these two genes have the same specificity in that they exclude the same *Salmonella* phages L, MG178, and MG40 (although *Salmonella typhimurium* is not a normal host for λ).

The *sieB* gene of P22 was found to map in the p_L operon between genes *c3* and *24* (19, 24). Sequencing studies by Franklin (7) revealed a rightward open reading frame (ORF) which was a likely candidate for *sieB*. Studies by Susskind and Botstein (23) showed that λ, too, had a *sieB* gene, and using *bio*-substituted λ phages, they showed that it mapped in an analogous position in the p_L operon of λ, between genes *cIII* and *N*. Sequencing studies by Ineichen et al. (9) revealed a rightward reading frame in the region which was later proposed by Court and Oppenheim (6) to be the λ *sieB* gene.

Data presented below serve to identify the two *sieB* genes and their respective promoters. The λ *sieB* gene is identical to the one proposed by Court and Oppenheim (6); however, the P22 *sieB* gene that we have identified differs substantially from the one proposed earlier (7).

MATERIALS AND METHODS

Bacteria. *Escherichia coli* W3110 *lacI*^{q}*L8* (4) was used for the propagation of plasmids. Strain CSR603 (*recA1 uvrA6*) (16) was used for maxicell analysis. *Salmonella typhimurium* LT2 strains MS1868 (*leuAam414* r$^-$ m$^+$) and MS1362 (*leuAam414 supD*) were provided by M. Susskind. Strain CV112 [*polA*(Ts)] was obtained from A. Wright.

Phages. Phages L *cII-101* and P22 *imm*C_L *esc*$^+{}_{P22}$ and P22 *sieA44 Ap2* were obtained from M. Susskind. P22 *sieA44 Ap2* is an oversized phage (it has a Tn*1* insertion in the *a1* gene) and hence is unable to form plaques on single infection (26). P22 *sieA44 a1-327* is a plaque-forming revertant of this phage, and its genome is approximately the same size as that of wild-type P22 (8a). Mutant alleles of *sieB* were introduced into P22 by crossing mutation-bearing plasmids with oversized kanamycin resistance-carrying P22 as described previously (19). For crossing deletions in *sieB* into P22, the oversized phage P22 *sieA44 m44 ral*::*Kn467* (19) was employed. This oversized phage has a kanamycin resistance gene inserted at the *Hin*dIII site in *ral*. Point mutations in *sieB* were crossed into P22 *sieA44 a1 sieB*::*Kn567*. In this phage, the 732 bp between the *Hin*dIII site (in *ral*) and the *Eco*RV site (in *sieB*) have been substituted by 5.04 kb of sequences from Tn*5*, including the kanamycin resistance gene, thus making it oversized. It was constructed by crossing pKR662 (see below) with P22 *sieA44 a1-327* as described previously (19).

Plasmids. Plasmids pTP83, pTP425, and pAS474 have been described previously (12, 19). pAS474 was used to make nested deletions in the *ral-sieB* region as follows. pAS474 was opened at the *Hin*dIII site in *ral* and digested with BAL 31 for various times, filled in, and religated in the

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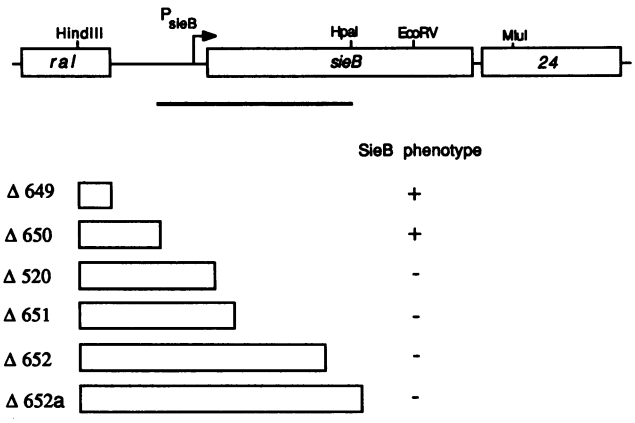

FIG. 1. Deletion mapping of the P22 *sieB* gene. Open boxes below the map represent sequences deleted from the phage. A plus sign indicates that the deletion mutant excludes phage L as well as does a wild-type (SieA$^-$) P22 prophage, and a minus sign indicates that L plates as well on the deletion mutant as it does on a nonlysogen. The solid line indicates the riboprobe used in the nuclease protection experiments.

presence of a *Hin*dIII linker or an 1,100-bp kanamycin resistance-encoding *Bam*HI (filled-in) DNA fragment with *Sal*I sites very close to both ends (19). This series includes pTP520, pTP649, pTP650, pTP651, pTP652, and pTP652a. To provide flanking homology for plasmid-by-phage crosses, these deletions were recloned into pTP425 by ligating the *Hin*dIII-*Eco*RI deletion-bearing fragment from pTP520 and pTP651 with the *Hin*dIII-*Eco*RI backbone of pTP425, or the *Sal*I (filled-in)-*Eco*RI deletion-bearing fragment from pTP649, pTP650, pTP652, and pTP652a with the *Hin*dIII (filled-in)-*Eco*RI backbone of pTP425. This subcloning step also ensures that all the deletions have a defined left endpoint at the *Hin*dIII site in *ral*. The extent of deletions is indicated in Fig. 1, and the deletion endpoints are indicated in Fig. 2. Plasmid pKR682 (which was used for maxicell analysis) was constructed by ligating a 969-bp *Hin*dIII-*Mlu*I *sieB*-containing fragment from pAS474 (Fig. 1) with the large *Hin*dIII-*Pvu*II *ori*-containing fragment from pBR322. A derivative of pKR682 was constructed by replacing the *Hin*dIII-*Eco*RV fragment with a similar fragment bearing an amber mutation at codon 4 of the *sieB* gene.

Mutant alleles of P22 *sieB* were constructed in plasmids and then introduced into P22 by recombination. To this end, the 969-bp *Hin*dIII-*Mlu*I fragment from pAS474 was cloned into the polylinker of M13mp19 and mutations were introduced as described previously (10). The mutagenic oligonucleotides (mismatched nucleotide(s) underlined) used were as follows:

P_{sieB} −35: 5′ AGGGGTACGCGAGAACC
P_{sieB} −10: 5′ GTGAATAGCGTTTCTCCGATTTCTCGC
Ser-4 to amber: 5′ CTGCCACCACTAGTTGTTCAT

(P_{sieB} −35 and P_{sieB} −10 refer to the −35 and −10 hexamers of the promoter for the P22 *sieB* gene; Ser-4 refers to the codon of the *sieB* gene that was changed to an amber). After confirmation by DNA sequencing that the region had only the desired mutation, a *Hin*dIII-*Eco*RV fragment (Fig. 1) carrying the mutation was used to replace the wild-type *Hin*dIII-*Eco*RV fragment in pTP83. These pTP83 derivatives were used to introduce the mutations into P22 by homologous recombination. A pTP83 derivative was also used to construct the oversized phage P22 *sieA44 ral sieB*::*Kn567*. This derivative, pKR662, was constructed by substituting the 732-bp *Hin*dIII-*Eco*RV (*ral-sieB*) segment with a 5.04-kb kanamycin resistance-encoding *Hpa*I fragment from Tn*5* (isolated from pPB20::Tn*5*-13 [19]).

A *sieB*-containing segment of the λ *cI-857 S-am7* chromosome extending from the *Xho*I site at 33,500 to the *Bgl*II site at 38,100 (17) was inserted between the *Xho*I and *Pvu*II sites of pTP83. In the process of construction, the *Bgl*II end was filled in with *E. coli* DNA polymerase I large fragment in the presence of deoxynucleoside triphosphates and the *Pvu*II and filled-in *Bgl*II ends were joined via an *Eco*RI linker; the resulting plasmid was designated pTP108. Plasmid pTP200

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                                                    Δ649
TTGCAGCCTTTGCATTTCACAAACGACTACACCATTGATTTGTATCAATAGTCGTAGTCATATGGGTAGTCCTGGTATTGTTCCATCACATCCTGAGGATG  101
                                                    Δ650
CTCTTCGAACTCTTCAAATTCTTCTTCCATATCTCATCTCAAATAGTGGATTGCGGTAGTAAAGATTGTGCCTGTCTTTTAACCACGTCAGGCTCGGTGG  201

     C                    G   G                                                    Δ520
TTCTCGTGTACCCCTACAGCGAGAAATCGGATAAACTCTATTCACCCCTACAGAGAGTAAAAAGAGAATCGCCGATGAACAACTCATGGTGGCAGGAGTT  301
                                                                    M  N  N  S  W  W  Q  E  L
                                        Δ651
AATGCGTTTTTTCCTGCAAGGAATGACACTTAAACAGTTGATTCATATGCTAATCATCCTGATCGTATTGATTATTGTTATGCCGGTAAGCGTAAAAGAA  401
 M  R  F  F  L  Q  G  M  T  L  K  Q  L  I  H  M  L  I  I  L  I  V  L  I  I  V  M  P  V  S  V  K  E
TGGATAAACCTGCATAATCCAGAAATACTTCCTCATTACTGGATGTATTACATCCTGTTGTTCTGCGTTAGCTATGTGCTTAACGGTGTTGTTAATTCCG  501
W  I  N  L  H  N  P  E  I  L  P  H  Y  W  M  Y  Y  I  L  L  F  C  V  S  Y  V  L  N  G  V  V  N  S  V
                    Δ652
TTTATCACGCTGTTACTGAAAGAATTGAGGCATCAACTGCTCAGCGGCGTAAGGACAGAGAAGAAAAAGTCGTTCGGGATTTGTTTGATTCGTTAACTCT  601
  Y  H  A  V  T  E  R  I  E  A  S  T  A  Q  R  R  K  D  R  E  E  K  V  V  R  D  L  F  D  S  L  T  L
       Δ652a
TGGAGAAAGAGCGTATTTGGCATTCGCTGTAGCCGCTAATAACCAGCTAAAGACAGAAAAGGGAAGCCCTGAAGCAATTTCATTGCTCAAAAAAGGGATT  701
 G  E  R  A  Y  L  A  F  A  V  A  A  N  N  Q  L  K  T  E  K  G  S  P  E  A  I  S  L  L  K  K  G  I
ATCACTCGATTGCCTTCTGCTATTGGATATCCTGATATTGACCGTTTTATTATCCCGGAAAAGTATTTTAATGAGTGCTACATGAGATTTGCCGGGAAGT  801
I  T  R  L  P  S  A  I  G  Y  P  D  I  D  R  F  I  I  P  E  K  Y  F  N  E  C  Y  M  R  F  A  G  K  S
CAGACATTCTTATGAATGAACTTATTGTACAGGACGAACAGCTCAAAAAATAACGACTTAACCGACAAATACCTTACCTCGCTGTTATTTGTTTGCTCTT  901
  D  I  L  M  N  E  L  I  V  Q  D  E  Q  L  K  K  .
ACGATGACCAGCCGCGTAAAGTGCTACGCCTGGAAGAAGTACAGATCCTCCTTCAACTTCCTTCTGACGCGT                              973
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FIG. 2. Sequence of the P22 genome in the vicinity of the *sieB* gene. It was published first in reference 7; nucleotides 1 to 104 were reexamined in reference 19, and 104 to 973 were revised in this study (see text). Deletion endpoints are numbered and indicated by the capital letter delta; the underlined nucleotide is the last nucleotide that is absent in the deletion mutant. The six initiation codons (ATG) discussed in the text are underlined. The deduced amino acid sequence of SieB is shown; the serine whose codon was mutated to amber is shown in boldface and underlined. The −35 and −10 hexamers of the *sieB* promoter and the transcription start site are indicated in boldface; the P_{sieB} −35 and −10 mutations are indicated immediately above the hexamers.

was constructed by digesting pTP30 (2) with *Pvu*II and recircularizing the large fragment by ligation in the presence of *Nco*I linkers (5′-CCCATGGG-3′). Plasmid pTP442 was constructed by digesting pTP108 with *Aat*II and *Xho*I, filling in the ends, and recircularizing the large fragment by ligation in the presence of *Xba*I linkers. Plasmid pTP462 was constructed by digesting pTP442 with *Eco*RI and *Hpa*I, filling in the *Eco*RI ends, and recircularizing the large fragment by ligation in the presence of *Bgl*II linkers (CAGATCTG; this restores the *Eco*RI site at the junction). Plasmid pTP466 was constructed by joining the *lacUV5* promoter-containing fragment of pTP200 generated by digesting with *Nco*I, filling in the ends, and digesting with *Pst*I, to the origin-containing fragment of pTP462 generated by digesting with *Bam*HI, filling in the ends, and digesting with *Pst*I. Plasmid pTP482 was constructed by ligating the *sieB*-containing *Eco*RI fragment of pTP466 into the *Eco*RI site of pMC7 (the *lacI*^{q}-bearing version of the plasmids described in reference 5).

In order to synthesize large amounts of riboprobe, a 426-bp *Sal*I-*Hpa*I fragment from pTP650 was placed in the polylinker region of pGEM-3Z (Promega). The orientation of the insert in the resulting plasmid is such that *sieB* antisense RNA can be made by digesting with *Eco*RI and transcribing with T7 RNA polymerase.

RNase protection. RNA for nuclease protection experiments was isolated as follows. A 30-ml culture of the appropriate P22 lysogen was grown to approximately 2×10^8 cells/ml. The pelleted cells were washed with 1 ml of TE (10 mM Tris [pH 7.6] and 1 mM EDTA) and suspended in 500 μl of RNA lysis buffer (10 mM Tris [pH 7.6], 1 mM EDTA, and 0.2% sodium dodecyl sulfate [SDS]). An equal volume of phenol (equilibrated with water) was added, and the mixture was shaken at room temperature for 5 min. The aqueous supernatant was extracted two to three times with chloroform, and RNA was precipitated by adding a 1/10 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol. The pelleted RNA was dissolved in 200 μl of diethyl pyrocarbonate-treated water and precipitated again as above. The precipitate was washed with 70% ethanol, dried, and dissolved in 50 μl of diethyl pyrocarbonate-treated water. RNA was quantitated by measuring A_{260}, assuming that 1 A_{260} unit equals a concentration of 40 μg of RNA per ml. Riboprobe was synthesized as described by the supplier of plasmid pGEM-3Z (Promega).

To detect *sieB* mRNA, approximately 50 μg of total RNA was hybridized to 2 μl of riboprobe (approximately 10^6 Cerenkov cpm) in 50 μl of hybridization buffer (10 mM Tris [pH 7.2], 1 mM EDTA, and 1 M NaCl [3]). The RNA and riboprobe mixture was denatured at 85°C for 5 min and then hybridized at 65°C for 60 min. Excess riboprobe and unhybridized RNA were digested as described previously (15), with some modifications. The hybridization mixture was cooled to room temperature, and 450 μl of RNase digestion buffer (9.0 mM Tris [pH 7.5], 5 mM EDTA, and 120 mM NaCl) containing 30 μg of nuclease P1 per ml and 10 μg of RNase T_1 per ml was added. After incubation at 37°C for 60 min, the digestion mixture was extracted by adding 500 μl of phenol-chloroform-isoamyl alcohol (25:24:1). RNA was precipitated by adding 2 volumes of ethanol to the aqueous phase. The pelleted RNA was suspended in 8 μl of TE (pH 7.6) and 8 μl of Stop solution (United States Biochemical Sequenase kit). The protected riboprobe was denatured at 85°C for 5 min and fractionated on a 5% acrylamide–8 M urea gel. Urea was removed from the gel by soaking in cold 5% trichloroacetic acid for 20 min. The gel was then dried and placed on X-ray film (Fuji) at room temperature for 19 h.

DNA sequencing. P22 DNA was sequenced as described previously (14). To confirm the DNA sequence between *ral* and *24*, BAL 31 deletions generated to map *sieB* (see above) were cloned into M13mp18 and M13mp19 and then sequenced (with the Sequenase kit from United States Biochemical) with the universal primer or special primers obtained from the in-house DNA synthesis facility. For obtaining the sequence of the *sieB* sense strand, *Sal*I-*Mlu*I fragments from pTP649 and pTP652 and a *Hin*dIII-*Cla*I fragment from pTP651 were cloned into M13mp18. The sequence of the antisense strand was obtained by cloning the *Sal*I-*Mlu*I and *Hin*dIII-*Cla*I fragments from pTP649 and pTP651, respectively, into M13mp19.

Maxicells. Maxicell analysis was performed essentially as described previously (20), except that *E. coli* CSR603 was used. Labelled proteins were fractionated on a Tricine–SDS–10% polyacrylamide gel (18).

Nucleotide sequence accession number. The revised nucleotide sequence presented here has been deposited in GenBank under accession number L18800.

RESULTS

Deletion mapping of P22 *sieB*. To precisely map the *sieB* gene, P22 mutants with deletions in the *ral-sieB* region were constructed. These phages carry deletions of varying lengths with a common left endpoint at the *Hin*dIII site in *ral* (Fig. 1). Deletion endpoints were identified by sequencing phage DNA; these are indicated in Fig. 2.

The SieB phenotype of deletion-bearing P22 *sieA44* prophages was determined by their ability to exclude phage L. In the absence of *sieA*, the only known P22 gene product that excludes L is SieB (24). As indicated in Fig. 1, lysogens of P22 *sieA44*, P22 *sieA44* Δ*649* and P22 *sieA44* Δ*650* plate L with the same low efficiency (10^{-2} to 10^{-3} relative to its efficiency of plaque formation on a nonlysogen). An L-P22 hybrid phage, which carries a P22 determinant, *esc* (for escape), that allows it to overcome SieB-mediated exclusion, has an efficiency of plating of approximately 1. Lysogens of P22 *sieA44* Δ*520*, P22 *sieA44* Δ*651*, P22 *sieA44* Δ*652*, and P22 *sieA44* Δ*652a* fail to exclude L, however; L plates as well on these lysogens as it does on a nonlysogen. Combined with previous studies (19, 24), these results indicate that sequences to the left of nucleotide 169 are not essential for *sieB* function, but a critical *sieB* determinant lies between 169 and 288 bp, in the numbering shown in Fig. 2.

Sequence and expression of the P22 *sieB* gene. The sequence of the *ral-sieB* region was reexamined in an attempt to identify a *sieB* ORF consistent with the mapping experiments described above. A number of discrepancies with the previously published sequence (7) were discovered. The corrections are as follows: there are two A's after position 278 (instead of three), there is no A after position 317, there is no T after 454, there is a T after 473, there are only two A's after 837 (as opposed to three) and a single A after 840 (instead of two), and finally there is a T after residue 922. The net effect of these revisions is to substantially alter the location of the putative *sieB* ORF; the correction at position 922 causes a frameshift in the 3′ end of gene *24*. The revised sequence shown in Fig. 2 revealed a candidate *sieB* ORF with the potential to encode a polypeptide of 242 amino acids.

This ORF has six likely initiation ATGs, and these are underlined in Fig. 2. P22 *sieA44* Δ*650* lacks the N-terminal 23 codons of this ORF but is still SieB$^+$; therefore, one can exclude the first ATG as the *sieB* initiation codon. An amber

TABLE 1. SieB phenotypes of cells expressing different alleles of P22 *sieB* or λ *sieB*[a]

Prophage or plasmid	Exclusion of phage[b]:	
	L	P22
P22 (SieB$^+$)	+	−
P22 P_{sieB} −35	+	−
P22 P_{sieB} −10	−	−
pKR682 (SieB$^+$)	+	−
pKR682a Ser4am in *sup*^{0}	−	−
pKR682a Ser4am in *supD*	+	−
pTP462 (λ *sieB*)	+	−
pTP482 (*lacI*^{q} P_{lacUV5}-λ *sieB*)		
Without IPTG	−	−
With IPTG	+	−
pTP466 (P_{lacUV5}-λ *sieB*)[c]	+	+

[a] *Salmonella* strain MS1868 (*sup*^{0}) was lysogenized with the appropriate P22 *sieA44 a1* phage or transformed with the appropriate plasmid; where indicated, the *supD* strain (MS1362) was used. Lysogens or plasmid-bearing cells were grown to late log phase in Luria-Bertani medium (LB) or LB supplemented with the appropriate antibiotic, and 0.1 ml of culture was used to make lawns on LB or LB-plus-antibiotic plates. Where indicated, IPTG was added to the bacterial lawn at a final concentration of approximately 1 mM. Tenfold dilutions of P22 *vir3* and L *cII-101* were spotted on such lawns. After the spots had dried, plates were incubated overnight at 30°C.

[b] A plus sign indicates that the phage plates 100- to 1,000-fold less efficiently on the particular strain compared with its plating efficiency on a prophage with a deletion in the *ral-sieB* region (the plating efficiency with respect to a nonlysogen is the same, although the plaque size is larger on a nonlysogen); a minus sign indicates that the efficiency of plating is close to 1.

[c] This plasmid, unlike pTP482, does not bear the *lacI*^{q} allele.

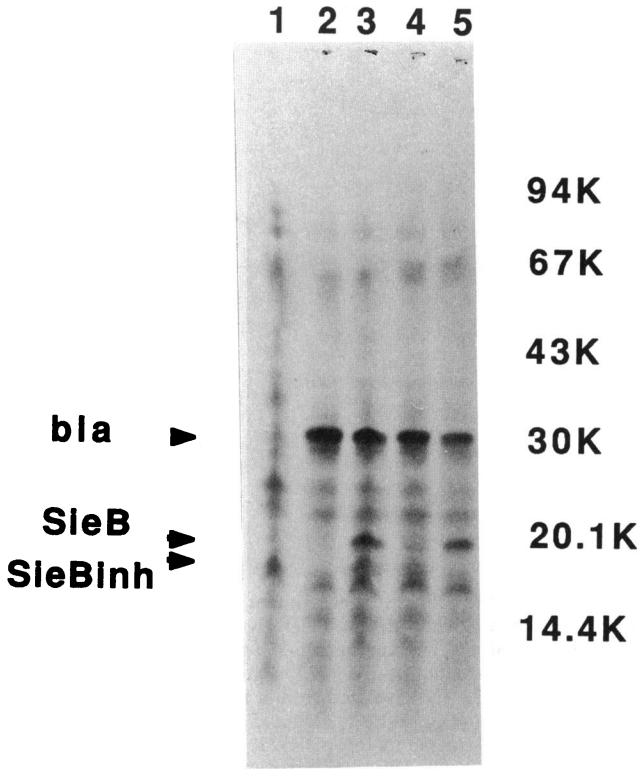

FIG. 3. Autoradiogram of ^{35}S-labelled proteins expressed in maxicells and separated by SDS–10% PAGE (Tricine system). Lanes: 1, no plasmid; 2, pBR322 with its tetracycline resistance-conferring gene deleted; 3, pKR682—a P22 *sieB*-expressing plasmid; 4, a derivative of pKR682 that has an amber allele (Ser4am) of *sieB*; 5, pTP462—a plasmid expressing λ *sieB*. Arrowheads indicate positions of β-lactamase, P22 and λ SieB, and SieBinh (SieB inhibitor). The numbers on the right indicate molecular size markers (Pharmacia) in kilodaltons.

mutation was introduced 4 codons after the second initiation codon, and its SieB phenotype was tested. As indicated in Table 1, *S. typhimurium* bearing this mutant allele of *sieB* plates L as efficiently as does a P22 prophage with a deletion of the *sieB* region. This observation suggests that the *sieB* ORF initiates at the second ATG (also see below). Whether the amber is suppressible in single copy could not be tested because the *supD* strain when lysogenized by this phage became resistant to phage infection—the control *esc*$^+$ P22 (which is insensitive to SieB-mediated exclusion) failed to grow on this strain (data not shown). The same *supD* strain bearing the *sieB* amber gene on a multicopy plasmid (pKR682a), however, excludes phage L with the same efficiency as does wild-type *sieB* (Table 1). The same plasmid in a *sup*^{0} strain, however, is completely innocuous for the growth of phage L.

The ORF thus identified (Fig. 2) as the *sieB* gene is read rightward in the conventional P22 prophage genetic map and has the potential to encode a polypeptide of 192 amino acids with a calculated molecular weight of 22,442.

Maxicells were used to analyze expression of proteins from this region of P22. To this end, a 969-bp *Hin*dIII-*Mlu*I wild-type or amber-carrying *sieB* (Fig. 1) fragment was subcloned between the *Hin*dIII and *Pvu*II sites of pBR322. These plasmids were analyzed with maxicells, and the results are presented in Fig. 3. As shown in lane 3, two polypeptides with apparent molecular weights of approximately 20,000 and 18,000 are synthesized from the wild-type *sieB* gene. We identify the larger protein as SieB because the N-terminal amber mutation at codon 4 (Ser4Am) confers a SieB$^-$ phenotype and prevents expression of this protein, but not the smaller, which is labelled SieBinh (Fig. 3, lane 4). The smaller polypeptide is due to internal initiation in the *sieB* ORF (13a).

Identification of the *sieB* promoter. The start site of *sieB* transcription was identified with RNase protection. A uniformly labelled *sieB* antisense riboprobe (Fig. 1) was digested with nucleases P1 and T_1 in the presence of RNA isolated from a SieB$^+$ or a SieB$^-$ lysogen. The wild-type *sieB* mRNA yields a protected fragment of approximately 350 nucleotides (Fig. 4, lane 1). A prophage which has the whole *ral-sieB* region deleted fails to give any protected fragment (Fig. 4, lane 2). An examination of the upstream DNA sequence in this region reveals a candidate σ^{70} promoter (Fig. 2).

To unequivocally identify the *sieB* promoter, mutations were made in the putative −10 and −35 sequences, and these are indicated in Fig. 2. The −10 hexamer was mutated at two sites; the highly conserved T resides at −7 and −12 were mutated to G, and the conserved T at position "−35" (which in this case is actually at −37) was mutated to a C. The mutation-bearing *sieA44 a1* prophages were tested for their ability to plate phage L. As indicated in Table 1, the prophage with a mutation at the −35 position excludes phage L just as well as wild type. The −10 mutant, however, plates phage L with an efficiency as high as that of a prophage which has its *sieB* gene deleted.

An inference from these results is that the mutation at position −35 has little or no effect on transcription from the *sieB* promoter, but the double mutation at −10 and −7 is a severe "down." This is borne out by the RNase protection

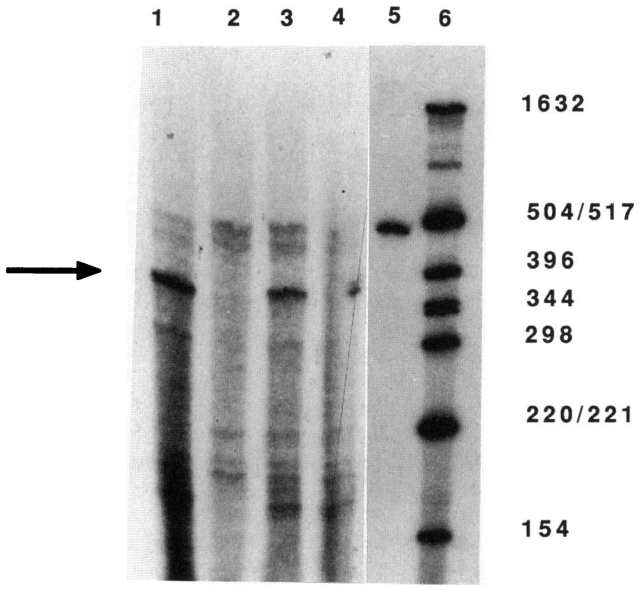

FIG. 4. RNase protection experiment to detect P22 *sieB* mRNA. Lanes: 1, RNA isolated from a SieB$^+$ lysogen; the arrow indicates the position of the approximately 350-nucleotide protected riboprobe; 2, RNA isolated from a P22 lysogen that has a deletion in the *ral-sieB* region; 3, RNA isolated from a P_{sieB} −35 lysogen; 4, RNA isolated from a P_{sieB} −10 lysogen; 5, untreated riboprobe; 6, markers—*Hin*fI fragments of pBR322, filled in by the Klenow fragment of *E. coli* DNA polymerase I in the presence of [α-^{32}P]dATP. The numbers on the side indicate the length of each fragment in nucleotides. Note that lanes 5 and 6 are taken from a shorter exposure of the same gel.

experiment whose results are shown in Fig. 4. RNA isolated from mutant lysogens was subjected to nuclease protection as before. As can be seen in Fig. 4, *sieB* mRNA is readily detectable in the −35 mutant (lane 3) but not in the −10 mutant (lane 4).

Identification of λ *sieB*. On the basis of genetic and sequencing studies by Susskind and Botstein (23) and Ineichen et al. (9), respectively, Court and Oppenheim (6) speculated that a rightward ORF between *cIII* and *N* is the *sieB* gene of λ. Data presented in Table 1 indicate that this is indeed the case. When the said ORF is expressed in *S. typhimurium*, it excludes phage L, but not wild-type P22. The relevant genetic structure of the p_L operon of λ is illustrated in Fig. 5. A *sieB*-containing fragment from the *Bam*HI site at 34,499 (17) to the *Hpa*I site at 35,261 (228 bp past the *sieB* termination codon) was used. The putative *sieB* ORF was fused at the initiating ATG by using the *Bam*HI site at 34,499 to the controllable *lacUV5* promoter; an in-frame initiation codon was provided by an *Nco*I linker used in the construction. In addition, this plasmid—pTP482—bears the *lacI*^{q} allele. As shown in Table 1, because of partial derepression of the *lacUV5* promoter in the presence of the inducer IPTG (isopropyl-β-D-thiogalactopyranoside), L plates with a very low efficiency on plasmid-bearing cells; however, the control *esc*$^+$ P22 plates with a high efficiency. In the absence of IPTG, both phages plate with a high efficiency. Thus, expression of the ORF is necessary and sufficient to confer a SieB$^+$ phenotype on wild-type *S. typhimurium*.

Another plasmid, pTP466, expresses λ *sieB* from the *lacUV5* promoter, but, unlike pTP482, it does not express the Lac repressor. As indicated in Table 1, cells bearing pTP466 exclude L and P22. The simplest interpretation of these results is that overexpression of λ *sieB* (in the absence of Lac repressor as opposed to partial derepression by IPTG) leads to exclusion of L and P22. That physiological levels of λ SieB are needed for the correct specificity of exclusion is further illustrated by the result with plasmid pTP462. As shown in Fig. 5, this plasmid bears the *ea10-ral-sieB* (and a part of *N*) region from λ; consequently, λ *sieB* is expressed, most likely, from its own promoter. As indicated in Table 1, cells bearing plasmid pTP462 exclude phage L but not P22.

An examination of the sequence of λ *sieB* revealed two potential internal initiation sites (not shown). To test whether these are functional, maxicell analysis was performed with plasmid pTP462. The result is shown in Fig. 3. As can be seen in lane 5, only one polypeptide is detected; its apparent molecular weight—20,000—is in good agreement with the calculated molecular weight—20,982—of λ SieB.

DISCUSSION

P22 *sieB*. Several lines of evidence support the notion that the ORF identified as *sieB* is indeed the *sieB* gene discovered by Susskind and coworkers (24). Deletion mutants of P22 which lack parts of this ORF are SieB$^-$. At least one amber mutation in this ORF confers a suppressible SieB$^-$ phenotype. The said ORF encodes a polypeptide with a calculated molecular weight of 22,442, which is in reasonable agreement with the apparent molecular weight of approximately 20,000 observed with SDS-polyacrylamide gel electrophoresis (PAGE).

It transpires that the ORF identified here as *sieB* encodes two polypeptides—a large one (SieB in Fig. 3, lane 3), which we have identified as SieB, and a truncated version of this

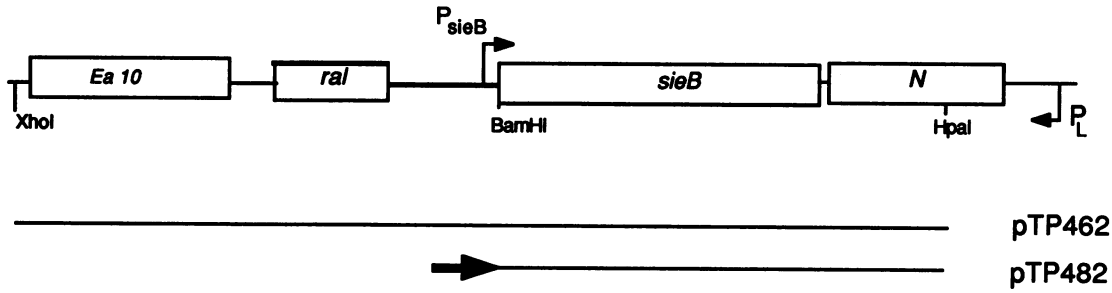

FIG. 5. Map of the λ genome in the vicinity of the *sieB* gene. Sequences present in the *sieB*-expressing plasmids constructed for these studies are indicated by solid lines below the map; the large bold arrow represents the *lacUV5* promoter.

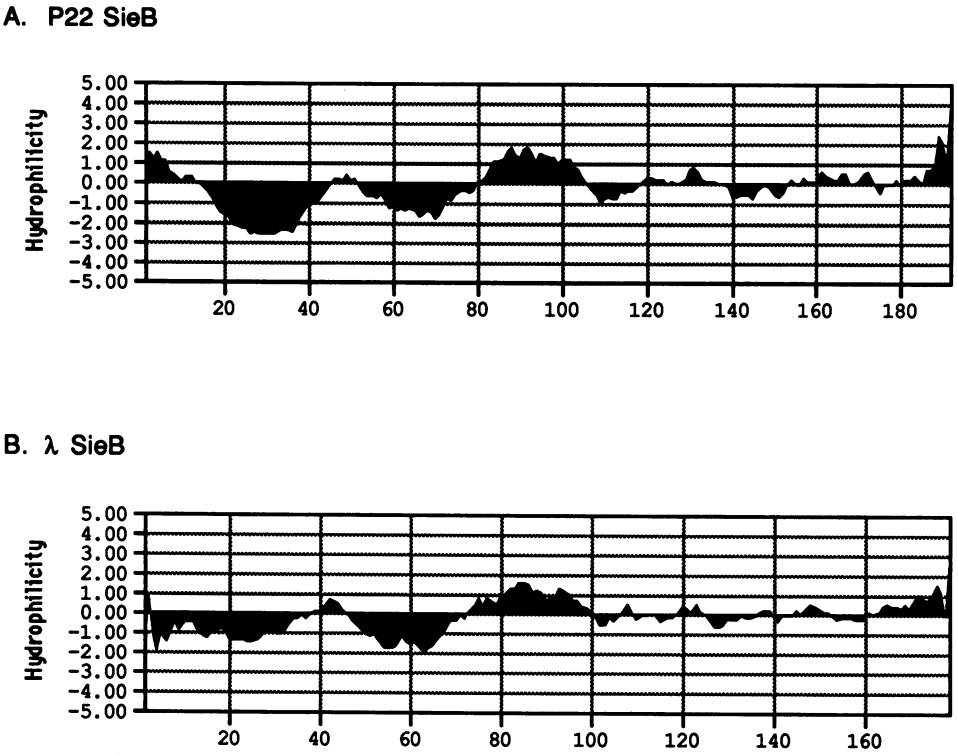

FIG. 6. Hydrophilicity profiles of P22 (A) and λ (B) SieB. Hydrophilicity was analyzed with the MacVector protein analysis program. A window size of 20 residues was used.

protein (SieBinh in Fig. 3). The shorter protein is a product of internal initiation (at the fifth ATG in Fig. 2) in *sieB*, and in fact is an inhibitor of SieB (13a). It is clear that the larger protein is responsible for conferring a $SieB^+$ phenotype because an amber mutation in its fourth codon confers a $SieB^-$ phenotype but still directs synthesis of the shorter protein, at least in maxicells and presumably in P22 lysogens. The formal possibility that the shorter polypeptide contributes to SieB-mediated exclusion in some way is not ruled out by the data presented in this paper.

λ *sieB*. Ineichen et al. (9) identified a rightward ORF (*git*) in the p_L operon of λ. On the basis of the deletion mapping experiments of Susskind and Botstein (23), Court and Oppenheim (6) speculated that *git* is *sieB*. Data presented above confirm this idea. When *git/sieB* is placed under the control of *lacI* and P_{lac}, it excludes phage L (but not P22) in an IPTG-dependent manner. Data shown in Table 1 indicate that expression of λ *sieB* from the *lacUV5* promoter, in the absence of any Lac repressor, causes exclusion of L and P22. This observation reveals the basis of the super-sieB phenotype described by Susskind and Botstein (23). They observed that some λ *bio*-substituted prophages excluded L and P22; in light of the result with pTP466, this is most likely due to overexpression of λ *sieB* by these prophages. The λ *sieB* ORF encodes a polypeptide with a molecular weight of 20,982, which is in good agreement with the apparent molecular weight of 20,000 estimated from SDS-PAGE.

There is no appreciable homology between the *sieB* genes of the two phages, although the two genes exclude the same *Salmonella* phages. As shown in Fig. 6, however, they have remarkably similar hydrophilicity profiles. Both proteins appear to have a hydrophilic N terminus followed by two hydrophobic regions that are of sufficient length to be transmembrane domains. These hydrophobic regions are separated by a small stretch of hydrophilic amino acid residues. In both proteins, the second hydrophobic domain is followed by a region rich in hydrophilic residues. Finally, both proteins have a hydrophilic C terminus which is relatively rich in charged amino acids. Whether this similarity reflects a common exclusion mechanism remains to be determined. The idea that P22 SieB may be a membrane protein is consistent with the physiological studies of Susskind et al., which suggested that damage to the cell membrane may be involved in P22 SieB-mediated exclusion (24). A comparison of the deduced sequences of λ SieB with those of λ RexA and RexB (which exclude some *rII* mutants of T4) revealed two regions of partial homology between RexB and λ SieB; they share the sequences GLLLLS and VFVFAL (except that the RexB sequence differs from the latter in that it has an I instead of F at the second position). There is no comparable homology between RexB and P22 SieB.

***sieB* promoters of P22 and λ.** The transcription start site of P22 *sieB* was mapped by RNase protection. Point mutations served to confirm that the sequence upstream of this site is indeed the *sieB* promoter. The double mutation in the −10 hexamer conferred a $SieB^-$ phenotype; moreover, *sieB* mRNA was undetectable in mutant lysogens. The mutation at −35 had no apparent effect on SieB phenotype or mRNA levels. This lack of effect is almost certainly due to the context of the *sieB* promoter since a similar mutation in another P22 promoter (P_{ant}) had a severe effect (11).

As shown in Fig. 7, the sequence in λ of the region upstream of its *sieB* gene is identical to that in P22. We suspect, therefore, that λ uses the same sequence as a promoter for its *sieB* as the one used by P22. Another feature in the sequence around P_{sieB} stands out. In P22 and λ, there

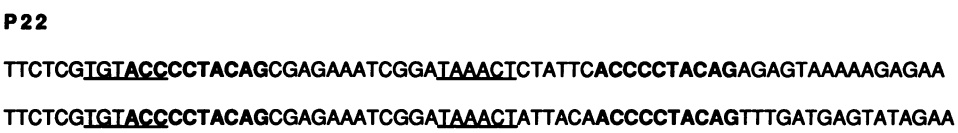

FIG. 7. The *sieB* promoter region. The DNA sequence around the transcription start site of P22 *sieB* is shown at the top; the −35 and −10 hexamers of the P22 *sieB* promoter are underlined; the homologous sequence in λ upstream of its *sieB* gene is shown below. The likely λ *sieB* promoter is underlined as well. The 10-nucleotide direct repeat referred to in the text is shown in boldface.

is a 10-nucleotide direct repeat around P_{sieB} (indicated in boldface in Fig. 7)—one overlapping the putative −35 of P_{sieB} and one after +1. This leads us to speculate that this sequence may be involved in regulating *sieB* expression.

One of the sequence corrections mentioned above (see Results) causes a frameshift in the 3′ end of gene *24* resulting in an increase in the length of gene *24* protein by 4 amino acid residues; its new calculated molecular weight is 10,969. The revised deduced amino acid sequence of the C terminus of gene *24* protein (from residues 86 to 101) reads as follows: LYAAGHRKSKQITAR.

ACKNOWLEDGMENTS

We thank M. Marinus for the RNA extraction protocol and for the use of his UV meter.

This research was supported by a grant from the NIH, AI18234.

REFERENCES

1. **Benzer, S.** 1955. Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41**:344–354.
2. **Berget, P. B., A. R. Poteete, and R. T. Sauer.** 1983. Control of phage P22 tail protein expression by transcription termination. J. Mol. Biol. **164**:561–572.
3. **Berk, A. J.** 1989. Characterization of RNA molecules by S1 nuclease analysis. Methods Enzymol. **180**:334–347.
4. **Brent, R., and M. Ptashne.** 1981. Mechanism of action of the lexA gene product. Proc. Natl. Acad. Sci. USA **78**:4204–4208.
5. **Calos, M. P.** 1978. DNA sequence for a low-level promoter of the *lac* repressor gene and an "up" promoter mutation. Nature (London) **274**:762–765.
6. **Court, D., and A. B. Oppenheim.** 1983. Phage lambda's accessory genes, p. 251–277. *In* R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. **Franklin, N. C.** 1985. Conservation of genome form but not sequence in the transcriptional antitermination determinants of bacteriophages λ, φ 21, and P22. J. Mol. Biol. **181**:75–84.
8. **Gussin, G., A. D. Johnson, C. O. Pabo, and R. T. Sauer.** 1983. Repressor and cro protein: structure, function and role in lysogenization, p. 93–121. *In* R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

8a. **Hardy, L., and A. Poteete.** Unpublished observations.

9. **Ineichen, K., J. C. W. Shepherd, and T. A. Bickle.** 1981. The DNA sequence of the phage lambda genome between P_L and gene *bet*. Nucleic Acids Res. **9**:4639–4653.
10. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154**:367–382.
11. **Moyle, H., C. Waldburger, and M. M. Susskind.** 1990. Hierarchies of base-pair preferences in the P22 *ant* promoter. J. Bacteriol. **173**:1944–1950.
12. **Poteete, A. R.** 1982. Location and sequence of the erf gene of phage P22. Virology **119**:422–429.
13. **Poteete, A. R.** 1988. Bacteriophage P22, p. 647–682. *In* R. Calendar (ed.), The bacteriophages, vol. 2. Plenum Press, New York.

13a. **Ranade, K., and A. R. Poteete.** A switch in translation mediated by an antisense RNA. Genes Dev., in press.

14. **Rennell, D., S. E. Bouvier, L. W. Hardy, and A. R. Poteete.** 1991. Systematic mutation of bacteriophage T4 lysozyme. J. Mol. Biol. **222**:67–88.
15. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
16. **Sancar, A., and C. S. Rupert.** 1978. Determination of plasmid molecular weights from ultraviolet sensitivities. Nature (London) **272**:471–472.
17. **Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Petersen.** 1982. Nucleotide sequence of bacteriophage λ DNA. J. Mol. Biol. **162**:729–773.
18. **Schagger, H., and G. von Jagow.** 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. **166**:368–379.
19. **Semerjian, A. V., D. C. Malloy, and A. R. Poteete.** 1989. Genetic structure of the bacteriophage P22 P_L operon. J. Mol. Biol. **207**:1–13.
20. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 213–214. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Susskind, M., A. Wright, and D. Botstein.** 1974. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. III. Failure of superinfecting phage DNA to enter *sieA*$^+$ lysogens. Virology **62**:350–366.
22. **Susskind, M. M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. Microbiol. Rev. **42**:385–413.
23. **Susskind, M. M., and D. Botstein.** 1980. Superinfection exclusion by λ prophage in lysogens of *Salmonella typhimurium*. Virology **100**:212–216.
24. **Susskind, M. M., A. Wright, and D. Botstein.** 1974. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. IV. Genetics and physiology of *sieB* exclusion. Virology **62**:367–384.
25. **Susskind, M. M., and P. Youderian.** 1983. Bacteriophage P22 antirepressor and its control, p. 347–363. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Weinstock, G. M., M. M. Susskind, and D. Botstein.** 1979. Regional specificity of illegitimate recombination by the translocatable ampicillin-resistance element Tn*1* in the genome of phage P22. Genetics **92**:685–710.