

Identification of *sbcD* Mutations as Cosuppressors of *recBC* That Allow Propagation of DNA Palindromes in *Escherichia coli* K-12

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The function of an open reading frame (*orf-45*) located upstream of the *sbcC* gene of *Escherichia coli* was investigated. Mutations that inactivate *sbcC* improve the ability to propagate λ red gam phage that carry a palindromic sequence in their DNA. They also act with *sbcB* mutations as cosuppressors of the defects in recombination, DNA repair, and cell viability associated with *recBC* mutations. A 1,282-bp cassette encoding resistance to kanamycin was used to disrupt *orf-45*. The mutation, which has a polar effect on the expression of *sbcC*, allowed stable propagation of palindromic λ phage even when the *sbcC* gene product was provided in *trans*. Additional nonpolar mutations in *orf-45* were isolated on the basis of their ability to improve the growth of *recBC sbcB* strains. These mutations also confer resistance to mitomycin C, allow efficient recombination in Hfr crosses, and facilitate stable propagation of palindromic phage. It is concluded that the products of *orf-45* and *sbcC* are functionally related. The *orf-45* gene is therefore renamed *sbcD*.

The *sbcC* gene of *Escherichia coli* was defined originally by mutations that arise spontaneously in *recBC sbcB* strains (16). These mutations act with mutations in *sbcB* to suppress the defects in recombination and DNA repair associated with the *recBC* genotype and are selected, therefore, during normal growth because of the improved viability. Subsequently, *sbcC* single mutants were shown to be good hosts for a λ red gam phage carrying a DNA sequence with palindromic symmetry (λ pal) (5). Phage of this type grow poorly in *sbcC*⁺ cells. Leach and Stahl (13) had observed this feature of the *sbcC* phenotype in their earlier work with *recBC sbcB sbcC* strains but, because isogenic strains were not constructed and used for comparisons, had attributed it to the absence of exonuclease V (RecBCD enzyme) and exonuclease I (the product of *sbcB*). This seemed reasonable since palindromes might form hairpins or cruciforms which could be recognized and cleaved by nucleases that normally resolve Holliday intermediates in genetic recombination. Kulkarni and Stahl (11) showed more recently that the Gam protein of λ allows palindrome-containing phage to form plaques on *sbcC*⁺ strains and suggested that Gam might interfere with the action of SbcC protein. Since Gam is known to be an inhibitor of the exonuclease activity of RecBCD enzyme, they went a step further to suggest that SbcC is a nuclease that attacks palindromic DNA. However, other studies indicate that palindromes can interfere with DNA replication and viability without directing cleavage of the carrier replicon (15).

The *sbcC* region of the chromosome has been cloned and sequenced, and the gene product has been identified as a protein of 118 kDa (23). The DNA encoding *sbcC* lies downstream of an open reading frame (*orf-45*) encoding a polypeptide of 45 kDa, with the TGA stop codon for *orf-45* overlapping the proposed ATG start codon for *sbcC*. This overlap and the absence of an obvious promoter sequence for the independent transcription of *sbcC* led Naom et al. (23) to conclude that expression of the two genes is coupled

at both a transcriptional and translational level. In this paper, we demonstrate that the two genes are also functionally related. We show that mutations in *orf-45* allow good growth of λ pal phage and function with *sbcB* mutations as cosuppressors of *recBC*.

MATERIALS AND METHODS

Bacterial strains and λ phages. The *E. coli* K-12 strains and λ phages used are described in Table 1. Phage stocks were prepared on strain JC7623, which allows stable propagation of the 571-bp palindrome carried by λ MMS1632.

Plasmids. Plasmid constructs carrying DNA inserts from the *sbcC-orf-45* region of the chromosome are shown in Fig. 1. pSB43, pJP77, and pIN510 are described in detail elsewhere (3, 23, 28). pIN507 corresponds to pJP77 *sbcC*::Tn1000 no. 5 described by Naom et al. (23). pFG103 was made by cloning a *Bam*HI-*Hind*III fragment of approximately 14.5 kb from pJP77 into pACYC184 (6). A 1,282-bp cassette (*kan*) encoding resistance to kanamycin was cloned from pUC4K (29) into the unique *Pst*I site of pFG103 to give pFG103K. pFG106 was constructed by removing the Km^r insertion from pFG103K by digestion with *Eco*RI and inserting it into the *Eco*RI site of the temperature-sensitive replicon pHSG415 (10). The *sbcC*⁺ construct, pFG101, was made by cloning a *Pst*I insert from pSM124 (23) into the *Pst*I site of the expression vector pKK223-3 (4).

Media and general methods. The Luria-Burrows (LB) broth and agar media used for routine growth of bacterial strains contained sodium chloride at a concentration of 0.5 g/liter (16). Broth media were supplemented with 20 μ g of tetracycline per ml, 25 μ g of chloramphenicol or kanamycin per ml, or 50 μ g of ampicillin per ml for the selection of antibiotic-resistant strains. Mitomycin C was incorporated into LB agar at concentrations of 0.2 and 0.5 μ g/ml for plate sensitivity tests. The minimal medium was 56/2 salts supplemented as described by Low (19). 56/2 agar containing 40 μ g of 5-bromo-4-chloro-3-indolyl phosphate (Xp) per ml was

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TABLE 1. *E. coli* K-12 strains and λ phage

Strain or phage	Relevant genotype	Source or reference
Strain		
AB1157	F ⁻ <i>thi-1 his-4</i> Δ (<i>gpt-proA</i>)62 <i>argE3 thr-1 leuB6 kdgK51 rfbD1</i> (?) <i>ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31</i>	1
AB2480	Same as AB1157 but <i>recA13 uvrA6</i>	P. Howard-Flanders
JC7618	Same as AB1157 but <i>recB21 recC22 sbcB10 sbcC203</i>	12
JC7621	Same as AB1157 but <i>recB21 recC22 sbcB13 sbcC202</i>	12
JC7623	Same as AB1157 but <i>recB21 recC22 sbcB15 sbcC201</i>	1
χ 342	Hfr (Cavalli) <i>proC29 metB1 relA1</i>	16
KL226	Hfr (Cavalli) <i>relA1 tonA22 pit-10 spoT1</i>	K. B. Low
FG252	Same as AB1157 but <i>orf-45::kan (sbcD300)</i>	This work
N1116	Same as AB1157 but <i>thyA</i>	16
N1349	F ⁻ <i>recB21 sbcB15 sbcC207 thi-1 metE70 leuB6 proC32 cysC43</i> <i>lacZ36 ara-14 mtl-1 xyl-5 rpsL109 rpsE2015</i>	16
N2015	Same as AB1157 but <i>pro⁺ lac⁺</i>	KL226 \times AB1157 to Pro ⁺ (<i>rpsL</i>)
N2309	Same as JC7623 but <i>phoR79::Tn10 sbcC⁺</i>	16
N2315	Same as JC7621 but <i>phoR79::Tn10 sbcC⁺</i>	16
N2341	Same as N2015 but <i>thr⁺ leu⁺ ara⁺ proC29</i>	χ 342 \times N2015 to Thr ⁺ Leu ⁺ (<i>rpsL</i>)
N2353	Same as N1116 but <i>phoR79::Tn10 sbcC202</i>	P1.JC7621 \times N1116 to Tc ^r
N2364	Same as AB1157 but <i>sbcC201 phoR79::Tn10</i>	16
N2380	Same as AB1157 but <i>recB21 sbcB15 sbcC205</i>	16
N2381	Same as AB1157 but <i>recC22 sbcB15 sbcC206</i>	16
N2419	F ⁻ <i>thi-1 metE70 leuB6 proC32 lacI3 lacZ118 ara-14 mtl-1 xyl-5</i> <i>gyrA supD rpsL109 rpsE</i>	17
N2679	Same as AB1157 except <i>sbcC201 gpt⁺ proA⁺</i>	23
N3375	Same as N2419 but <i>pro⁺</i>	P1.FG252 \times N2419 to Pro ⁺
N3376	Same as N2419 but <i>pro⁺ orf-45::kan(sbcD300)</i>	P1.FG252 \times N2419 to Pro ⁺
N3506	Same as N2309 but <i>sbcD301^a</i>	— ^b
N3507	Same as N2309 but <i>sbcD sbcC^c</i>	— ^b
N3508	Same as N2309 but <i>sbcD sbcC^c</i>	— ^b
N3509	Same as N2309 but <i>sbcD302</i>	— ^b
N3510	Same as N2315 but <i>sbcD303</i>	— ^d
N3511	Same as N2315 but <i>sbcD sbcC^c</i>	— ^d
N3512	Same as N2315 but <i>sbcD304</i>	— ^d
N3513	Same as N2315 but <i>sbcC208</i>	— ^d
N3514	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD301</i>	P1.N3506 \times N2341 to Pro ⁺
N3515	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD sbcC</i>	P1.N3507 \times N2341 to Pro ⁺
N3516	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD sbcC</i>	P1.N3508 \times N2341 to Pro ⁺
N3517	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD302</i>	P1.N3509 \times N2341 to Pro ⁺
N3518	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD303</i>	P1.N3510 \times N2341 to Pro ⁺
N3519	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD sbcC</i>	P1.N3511 \times N2341 to Pro ⁺
N3520	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcD304</i>	P1.N3512 \times N2341 to Pro ⁺
N3521	Same as N2341 but <i>pro⁺ phoR79::Tn10 sbcC208</i>	P1.N3513 \times N2341 to Pro ⁺
N3522	Same as N2341 but <i>pro⁺ sbcC203</i>	P1.JC7618 \times N2341 to Pro ⁺
N3523	Same as N2341 but <i>pro⁺ sbcC205</i>	P1.N2380 \times N2341 to Pro ⁺
N3524	Same as N2341 but <i>pro⁺ sbcC206</i>	P1.N2381 \times N2341 to Pro ⁺
KL227	Hfr (PO3 of Hfr P4X) <i>metB1 relA1</i>	K. B. Low
KL548	F' (F128) <i>proAB⁺ lacI3 lacZ118/Δ(pro-lac)</i> χ 111	K. B. Low
Phages		
MMS885	<i>c1857 b1453</i> χ ⁺ D	27
MMS1632 ^e	<i>pal571 c1857 b1453</i> χ ⁺ 76	27

^a *sbcD* is *orf-45*.

^b Fast-growing segregant of N2309.

^c The precise nature of the mutation responsible for the defect in both *sbcC* and *sbcD* is unknown.

^d Fast-growing segregant of N2315.

^e *pal571* is a 571-bp palindrome (5).

used to score the alkaline phosphatase constitutive phenotype of *phoR* mutants.

Hfr and F' matings and transductions with phage P1vir have been described elsewhere, as have methods for measuring recombination in Hfr crosses and sensitivity to UV light (16, 18, 21). Preparation and titration of stocks of phage λ were according to the methods and media described by Silhavy et al. (26). Recombinants or transductants carrying mutations in *sbcC* or in *orf-45* (*sbcD*) were recognized by

their poor growth on LB agar spread with approximately 10⁷ PFU of phage λ MMS1632 (5). DNA manipulations and the radiolabeling of probes were according to the recipes and protocols described by Sambrook et al. (24).

Identification of plasmid-encoded proteins. The procedure used was adapted from that described by Sancar et al. (25). Strain AB2480 transformed with the relevant plasmid was grown to an *A*₆₅₀ of 0.5 in 5 ml of M9 medium (25) supplemented with ampicillin (100 μ g/ml), irradiated with 45

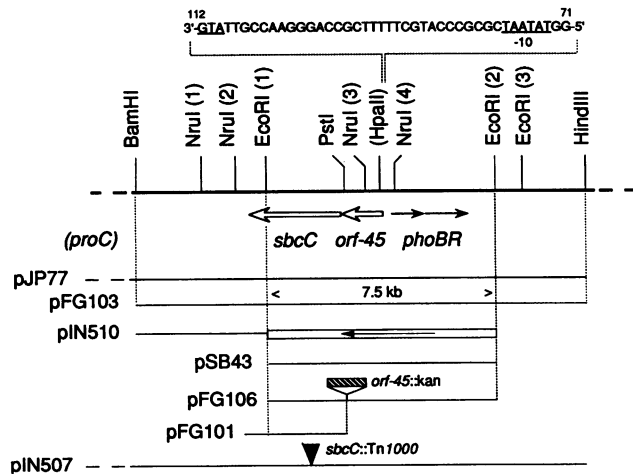


FIG. 1. Map of the *sbcC* region of the *E. coli* chromosome showing relevant restriction sites and DNA inserts cloned in recombinant plasmids. The *HpaII* site in parentheses is at bp 246 in the sequence described by Naom et al. (23) and is not unique. *NruI*(4) lies within *phoB* (20), 372 bp upstream of the *HpaII* site. The boxed and arrowed 7.5-kb *EcoRI* fragment in pIN510 is inverted with respect to the rest of the insert. The nucleotide sequence for the promoter region is numbered according to Naom et al. (23). The -10 sequence and the proposed ATG translational start codon are underlined.

J/m² of UV light, and then incubated in the dark for 1 h at 37°C. Cycloserine was added to a final concentration of 200 µg/ml, and the incubation was continued for a further 16 h. The cells were pelleted, washed, and resuspended in 1 ml of M9 medium lacking MgSO₄ and amino acids. L-[³⁵S]methionine (10 µCi) was added, and the incubation continued at 37°C before the cells were washed to remove unincorporated label, resuspended in 50 µl of sodium dodecyl sulfate (SDS) gel loading buffer (2% SDS, 20% [vol/vol] glycerol, 5% β-mercaptoethanol, 0.2% bromophenol blue), and heated for 3 min at 100°C. Samples (10 to 20 µl) were then electrophoresed through a 10% polyacrylamide gel containing 0.2% SDS, and the labeled proteins were visualized by fluorography. ¹⁴C-labeled molecular weight markers were from Amersham.

Construction of a chromosomal disruption of *orf-45*. Chromosomal disruption of *orf-45* was based on a procedure described by Hamilton et al. (8). The temperature-sensitive replicon pFG106, which carries a *kan* cassette inserted in *orf-45* at the *PstI* site (Fig. 1), was transformed into strain AB1157, and Km^r transformants arising from recombination between the plasmid insert and homologous sequences in the chromosomal *orf-45-sbcC* region were selected at 42°C. A single Km^r (and Ap^r) colony was then inoculated into LB broth supplemented with kanamycin, and after two cycles of growth at 30°C to encourage the segregation of plasmid-free cells, the Km^r Ap^s clone, FG252, was identified as a putative *orf-45* insertion mutant.

Isolation of fast-growing variants of *recBC sbcB* strains. Eight colonies each of the *phoR::Tn10* strains N2309 (*recBC sbcB15*) and N2315 (*recBC sbcB13*) were inoculated into 5 ml of LB broth, grown to saturation at 37°C, diluted 100-fold in fresh broth, and regrown to approximately 10⁹ cells per ml before samples were streaked onto LB agar. Fast-growing variants were identified by their large-colony morphology, as described by Lloyd and Buckman (16). One variant was saved from each culture for further analysis.

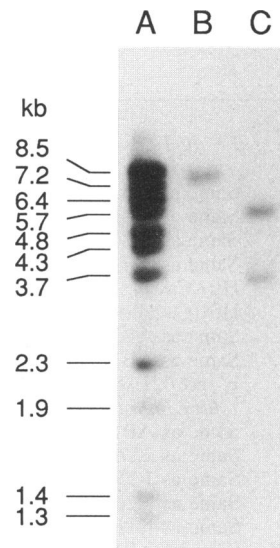


FIG. 2. Autoradiograph showing Southern analysis of chromosomal DNA from AB1157 (lane B) and FG252 (lane C). DNA was digested with *EcoRI* and *HindIII*, separated by electrophoresis on a 0.8% agarose gel, and transferred to a Hybond N filter (Amersham). The filter was probed with the 7.5-kb *EcoRI* fragment from pSB43 labeled with [α -³²P]dCTP by using random primers (Pharmacia LKB) and Klenow polymerase as described by Sambrook et al. (24). The filter was then washed, blotted dry, and exposed to X-ray film. Molecular weight markers (lane A) are ³²P-labeled, *BstEII* fragments of λ DNA.

RESULTS AND DISCUSSION

Strain FG252 carries a disruption of *orf-45*. A 1,282-bp cassette encoding resistance to kanamycin was directed into the chromosome of strain AB1157 as described in Materials and Methods. Chromosomal DNA extracted from the putative *orf-45* insertion mutant, FG252, and the AB1157 parent was digested with *EcoRI* and *HindIII* and probed with the 7.5-kb *EcoRI*(1)-*EcoRI*(2) fragment covering *orf-45* (Fig. 1). Given that the 1,282-bp *kan* cassette has a single *HindIII* site located centrally, the probe was expected to hybridize to fragments of approximately 3.5 and 5.3 kb in the FG252 digest, as opposed to a single fragment of 7.5 kb in the AB1157 digest. The results (Fig. 2) revealed the expected pattern.

Disruption of *orf-45* allows growth of λ pal phage. The palindrome-containing phage, λ MMS1632, forms plaques on strain FG252 with a high efficiency (data not shown). To confirm that this is due to the *kan* insertion, phage P1vir grown on strain FG252 was used to transduce strain N2419 to a Pro⁺ phenotype. Of 100 transductants tested, 78 were Km^r. This cotransduction value is as expected for insertion of the *kan* cassette in *orf-45*, since earlier studies had shown 70 to 80% cotransduction of the adjacent *sbcC* gene with *proC* (16). All the Km^r transductants were good hosts for λ MMS1632, as judged by their very poor growth on LB agar spread with $\sim 10^7$ PFU of the phage. The Km^s transductants were resistant to λ MMS1632. The efficiency of plaque formation by λ MMS1632 on representative Km^s (N3375) and Km^r (N3376) transductants is shown in Table 2. A similar cross was conducted with N1349 as the recipient. With this *recB sbcB sbcC* strain, 77 of 100 Pro⁺ transductants tested were Km^r. All 100 Pro⁺ transductants grew λ MMS1632, and all remained resistant to mitomycin C. Since the gene order

TABLE 2. Effect of *sbcC* and *orf-45* on the ability of λ MMS1632 to form plaques

Strain	Plasmid ^a	Relative titer ^b	
		λ MMS885	λ MMS1632
AB1157 (wt ^c)		1.0	0.00018
N2364 (<i>sbcC201</i>)		0.92	0.93
N3375 (wt)		1.85	0.00017
N3376 (<i>orf-45::kan</i>)		2.69	2.37
AB1157	pACYC184	0.45	0.00047
	pKK223-3	0.92	0.0001
	pBR322	0.75	0.00006
N2679 (<i>sbcC201</i>)	pACYC184	0.45	0.12
	pJP77 (<i>sbcC</i> ⁺ <i>orf-45</i> ⁺)	0.92	0.00002
	pKK223-3	0.67	0.97
	pFG101 (<i>sbcC</i> ⁺)	0.69	0.00043
FG252 (<i>orf-45::kan</i>)	pACYC184	0.77	0.27
	pJP77 (<i>sbcC</i> ⁺ <i>orf-45</i> ⁺)	0.85	0.00005
	pKK223-3	0.77	0.93
	pFG101 (<i>sbcC</i> ⁺)	0.66	0.28
	pBR322	0.65	0.4
	pIN510 (<i>orf-45</i> ⁺)	0.46	0.4
	pSB43 (<i>orf-45</i> ⁺)	0.63	0.28

^a pACYC184, pKK223-3, and pBR322 are the vector controls for pJP77, pFG101, and pIN510, respectively.

^b Values shown are relative to the titers of the phage stocks on the *recBC sbcBC* strain, JC7623, which were 1.3×10^9 PFU/ml for MMS885 and 3.0×10^{10} PFU/ml for MMS1632. The titers of the phages were determined on agar plates supplemented with the appropriate antibiotic where required to maintain plasmid selection.

^c wt, wild type.

is *proC sbcC orf-45*, most of the Km^r transductants would be expected to have lost the recipient *sbcC207* allele. Their resistance to mitomycin C shows that the *orf-45* disruption is also an efficient cosuppressor of *recB*.

The ability of λ MMS1632 to grow well in strain FG252 could be due to the inactivation of *orf-45* or to a polar effect of the insertion on the downstream *sbcC* gene. To try and distinguish between these possibilities, we made use of pFG101 to express *sbcC*⁺ in *trans*. The data in Fig. 3, lane a, show that the 118-kDa SbcC protein is expressed quite well in maxicells harboring pFG101. This construct was introduced into strain FG252 and the *sbcC* mutant, N2679. λ MMS1632 was titrated on the strains made and on the relevant controls transformed with the appropriate vector plasmids or with pJP77, which carries wild-type alleles for both *orf-45* and *sbcC*. The palindrome-free λ MMS885 was used to control for the efficiency of infection.

The results obtained are summarized in Table 2. As expected, pFG101 severely reduces the ability of λ MMS1632 to form plaques on the *sbcC201* strain, N2679. However, it does not affect growth of the phage on strain FG252. pJP77 prevents growth on both strains. From these data, it is quite clear that growth of λ pal phage is improved in strain FG252 as a result of the inactivation of *orf-45*, regardless of any polar effect of the *kan* insertion on the downstream *sbcC* gene. We decided, therefore, to investigate whether the similarity between *sbcC* and *orf-45* mutations extends to other properties.

Mutations in *orf-45* improve the viability of *recBC sbcB*

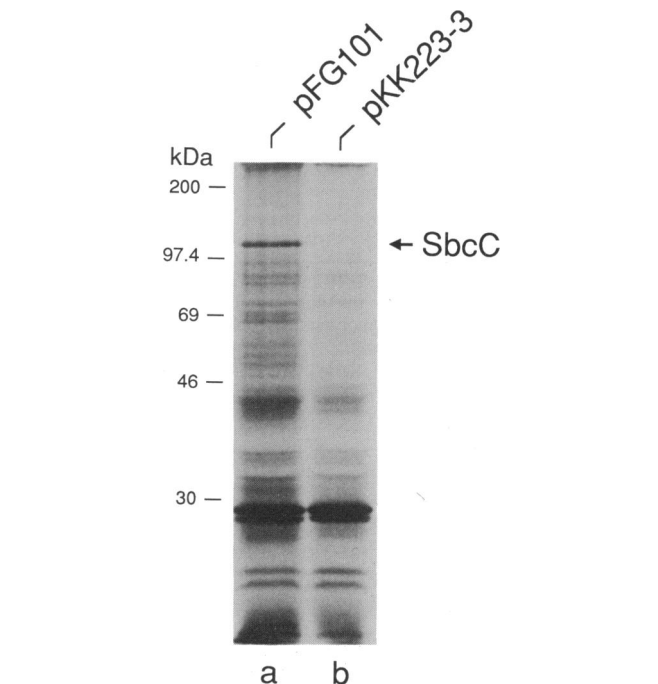


FIG. 3. Fluorograph showing plasmid-encoded proteins labeled in maxicells with [³⁵S]methionine and separated by SDS-polyacrylamide gel electrophoresis. The positions of molecular mass markers are indicated on the left. The plasmids used are identified above each lane.

strains. Mutations in *sbcC* were identified initially because they improve the viability of *recBC sbcB* strains and increase their resistance to the DNA-damaging agent mitomycin C (16). If inactivation of *orf-45* were to have the same effect, we reasoned that a collection of fast-growing variants of a *recBC sbcB* strain ought to contain some strains with mutations in *orf-45*. Eight independent, fast-growing variants of both N2309 and N2315 were isolated as described in Materials and Methods. These variants were also resistant to mitomycin C (data not shown) and behaved generally very much like the *recBC sbcBC* strains JC7623 and JC7621 (see Table 5) which were isolated as mitomycin C-resistant revertants of a *recBC* strain (2, 12). Eight of the 16 isolates proved sensitive to tetracycline. They were assumed to carry deletions or inversions affecting the *orf-45-sbcC* region that resulted from the imprecise excision of Tn10 in *phoR*. These strains were not studied any further. To investigate the genetic basis for the improved growth of the eight Tc^r strains (N3506 to N3513), phage P1 was used to transduce the *proC* region to strain N2341. The Pro⁺ transductants selected were tested for sensitivity to λ MMS1632 and for inheritance of the linked *phoR::Tn10* marker. All eight crosses gave very similar results. The data from four of these are summarized in Table 3. Each of the eight fast-growing variants strains was found to carry a mutation linked to *proC* that confers sensitivity to the λ pal phage in a *recBC*⁺ *sbcB*⁺ background. From the pattern of inheritance of the unselected markers, it is clear that these mutations are located in the *orf-45-sbcC* region between *proC* and *phoR*.

Three further crosses were conducted with P1 from strains JC7618, N2380, and N2381. The first of these is a mitomycin C-resistant derivative of a *recBC* strain (2, 12), while N2380 and N2381 are mitomycin C-resistant derivatives of *recB*

TABLE 3. Transductional analysis of mutations conferring sensitivity to the λ pal phage^a

P1 donor	No. of colonies tested	No. (%) of colonies with the following phenotype:			
		λ pal ^s Tc ^r	λ pal ^s Tc ^s	λ pal ^r Tc ^s	λ pal ^r Tc ^r
N3506	126	30 (23.8)	2 (1.6)	92 (73.0)	2 (1.6)
N3507	172	43 (25.0)	7 (4.1)	120 (69.7)	2 (1.2)
N3512	124	38 (30.6)	5 (4.0)	78 (62.9)	3 (2.4)
N3513	180	42 (23.3)	30 (16.7)	107 (59.4)	1 (0.6)

^a Donor strains are all *phoR79::Tn10* and sensitive to λ MMS1632, while the N2341 recipient is resistant to the phage and sensitive to tetracycline. Selection was for the Pro⁺ phenotype. In control platings, the recipient *proC29* allele was found to revert to Pro⁺ at a significant frequency. Cotransduction values for donor markers are likely, therefore, to be underestimated.

sbcB and *recC sbcB* strains, respectively, that were selected initially as fast-growing variants (16). Lloyd and Buckman (16) showed that all three strains carry mutations linked to *proC* and assigned these mutations to *sbcC*. As expected, between 40 and 66% of the Pro⁺ transductants of N2341 selected in these new crosses proved sensitive to λ MMS1632.

A phage-sensitive transductant from each of the 11 crosses (strains N3514 to N3524) was transformed with a series of plasmids that carry wild-type alleles for *sbcC* (pFG103K), *orf-45* (pIN507), both (pJP77 and pFG103), or neither (pACYC184 vector). Strain N2353, which carries a mutation from JC7621 that had been assigned to *sbcC* (16) and which confers sensitivity to λ MMS1632 (this work), was also transformed with the same plasmids. The Cm^r colonies selected were tested for sensitivity to phage λ MMS1632 in plate tests. The results allowed the unambiguous assignment of the 12 strains into one of three categories: (i) strains that are deficient in *orf-45* (N3514, N3517, N3518, and N3520), (ii) strains deficient in *sbcC* (N3521, N3523, N3524, and N2353), and (iii) strains that are deficient in both *orf-45* and *sbcC* (N3515, N3516, N3519, and N3522). The identity of the three *sbcC* single mutants was confirmed by using pFG101, which prevented growth of the λ pal phage (data not shown). To check the results of the plate tests, phage λ MMS1632 was titrated on the Cm^r transformants of representative strains from each category. Similar transformants of strains N2341 and FG252 were included as controls. The data obtained (Table 4) confirm that there are indeed three categories of mutants. Analysis of the data from P1 transductions (Table 3; data not shown) revealed that the *sbcC* alleles are slightly more closely linked to *proC* than are those in *orf-45*, which agrees with the known order of these genes (Fig. 1).

TABLE 5. Effect of *orf-45* mutations on viability, recombination, and sensitivity to UV light in *recBC sbcB* strains

Strain	Relative viability ^a	Relative yield of transconjugants ^b		UV survival (40 J/m ²) ^c
		\times KL548	\times Hfr KL227 ^d	
AB1157 (<i>rec⁺ sbc⁺</i>)	1.0	1.0	1.0	0.83
<i>recBC sbcB sbcC</i> strains				
JC7623 (<i>recBC sbcB15 sbcC201</i>)	0.89	1.24	2.24	0.47
JC7621 (<i>recBC sbcB13 sbcC202</i>)	0.95	1.10	1.19	0.15
<i>recBC sbcB orf-45</i> strains ^e				
N3506 (<i>recBC sbcB15 orf-45</i>)	0.85	1.13	1.08	0.38
N3509 (<i>recBC sbcB15 orf-45</i>)	0.75	1.18	1.07	0.34
N3510 (<i>recBC sbcB13 orf-45</i>)	1.29	1.22	1.04	0.18

^a Values are relative to the CFU/ml in a culture grown in LB broth to an A_{650} of 0.4 as measured on 56/2 glucose minimal agar supplemented with the required amino acids. A viability of 1.0 was based on 1.66×10^8 CFU/ml.

^b Selection was for Pro⁺ (*rpsL*); mating was for 30 (KL548) or 40 (KL227) min. Values are relative to those for AB1157, which were 1.97×10^7 (\times KL548) and 2.04×10^7 (\times Hfr KL227) transconjugants per ml.

^c Survival is relative to unirradiated controls.

^d The *orf-45-sbcC* region is transferred very late.

^e *orf-45* mutations are identified as *sbcD* alleles in Table 1.

A further conclusion that can be drawn from the data in Table 4 is that the *kan* insertion in strain FG252 not only inactivates *orf-45* but also exerts a polar effect on *sbcC*. This follows from the fact that pIN507, which carries an intact *orf-45* region, fails to prevent the growth of phage λ MMS1632 on this strain. The same is true for pSB43 and pIN510 (Table 2). Presumably, expression of the chromosomal *sbcC* gene in FG252 is reduced below that needed to prevent phage growth. However, the polarity is not absolute, since the multicopy plasmid construct pFG103K is able to express *sbcC⁺* well enough to prevent the λ pal phage from growing in strains identified as *sbcC* mutants.

From these data, it is clear that mutations that inactivate *orf-45*, but which do not have a polar effect on *sbcC*, are able to function with a mutation in *sbcB* to improve the growth of a *recBC* mutant. These mutations allow efficient recombination in the *recBC sbcB* background (Table 5; data not shown). Cell viability and resistance to UV are also both high. So far, the only phenotype we have associated with

TABLE 4. Complementation of sensitivity to λ MMS1632 by *orf-45* and *sbcC* plasmids

Strain	Relative titer of phage λ MMS1632 on strain with plasmid ^a					Deduced genotype
	pACYC184 (vector control)	pJP77 (<i>orf-45⁺ sbcC⁺</i>)	pIN507 (<i>orf-45⁺ sbcC</i>)	pFG103 (<i>orf-45⁺ sbcC⁺</i>)	pFG103K (<i>orf-45 sbcC⁺</i>)	
N2341	0.00015	0.000087	0.00011	0.000038	0.000066	(<i>orf-45⁺ sbcC⁺</i> control)
N3514	0.52	0.000065	0.000098	0.00003	0.23	<i>orf-45 sbcC⁺</i>
N3515	0.82	0.000054	0.87	0.000019	0.52	<i>orf-45 sbcC</i>
N3520	0.69	0.000043	0.00015	0.000043	0.83	<i>orf-45 sbcC⁺</i>
N3521	0.59	0.000054	1.24	0.000036	0.000065	<i>orf-45⁺ sbcC</i>
FG252	0.35	0.00087	0.74	0.000013	0.83	<i>orf-45 sbcC</i>

^a Titers are given relative to that on the *recBC sbcB sbcC* strains JC7623, which measured 9.2×10^9 PFU/ml. Titers were also determined for a stock of phage λ MMS885 on all of the transformants tested; the values obtained relative to the titer on JC7623 (1.8×10^9 PFU/ml) were between 0.2 and 1.2. Phage titers were determined on agar plates supplemented with chloramphenicol.

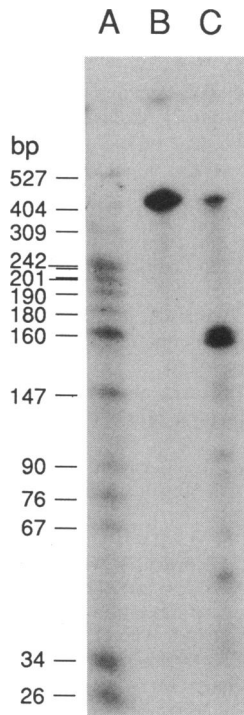


FIG. 4. Autoradiograph showing nuclease protection analysis of the 5' end of the *orf-45* transcript. Total RNA from AB1157 carrying pJP77 was extracted as described by Chomczynski and Sacchi (7) and annealed to the 372-bp *HpaII-NruI*(4) DNA fragment of *orf-45* (Fig. 1) as described by Murray (22). The probe DNA was labeled at the *HpaII* end with [γ - 32 P]ATP by using T4 polynucleotide kinase (24). After digestion with mung bean nuclease (Bethesda Research Laboratories, Inc.), the protected fragment was separated on a denaturing 6% polyacrylamide gel. Lanes: A, 32 P-labeled, *HpaII*-digested pBR322 DNA; B, 372-bp probe DNA; C, protected probe fragments.

these *orf-45* mutations in a *recBC*⁺ *sbcB*⁺ strain is the ability to propagate palindromic λ phage. Overall, the mutations in *orf-45* appear identical in their effect to mutations that inactivate *sbcC*.

Four of the fast-growing variants isolated carry mutations that prevent the expression of both *orf-45* and *sbcC*. These strains carry *phoR*::Tn10 and form blue colonies on minimal agar plates supplemented with the alkaline phosphatase substrate, Xp. They must therefore carry the *phoB* gene located between *phoR* and *orf-45* (16, 28). We conclude that these four strains do not have deletions extending into the *orf-45-sbcC* operon from the Tn10 insertion in *phoR*. None of the mutations is 100% cotransduced with Tn10 in *phoR* (Table 3; data not shown), which suggests they are not transposon-linked inversions with an endpoint in *orf-45*. They are most likely polar mutations in *orf-45* that prevent the expression of *sbcC*, or they are deletions directly affecting both genes.

Identification of the *orf-45-sbcC* promoter. During these studies, we assumed on the basis of the DNA sequence that *sbcC* is transcribed from a promoter located just upstream of *orf-45* (Fig. 1) (23). We used a 372-bp *HpaII-NruI*(4) fragment covering the putative promoter (Fig. 1) in a nuclease protection assay to probe for the initiation of transcription in this region. The autoradiograph shown in Fig. 4 reveals that RNA extracted from strain AB1157 carrying pJP77 protected

two probe fragments of around 160 bp in length. The size of these fragments is consistent with transcription initiating in the region around 7 bp downstream of the 3' end of the proposed -10 region (Fig. 1), which is typical of *E. coli* promoters (9). We conclude that the promoter sequence identified by Naom et al. (23) is functional.

To conclude, the data presented show that the products of the *orf-45* and *sbcC* genes are functionally related and expressed from the same promoter. The inactivation of either of these two genes allows for the stable propagation of λ pal (*red gam*) phages and helps *sbcB* to suppress the *recBC* mutant phenotype. We propose, therefore, that *orf-45* should be designated as *sbcD*. Naom et al. (23) drew attention to the fact that the products of the *sbcCD* genes have features in common with certain phage nucleases and with the *recBC* products. A more recent analysis of the data base has also shown a relationship between the putative nucleotide-binding domain of SbcC and that of membrane-associated ATPases (14). It is possible that SbcC and SbcD are components of a single enzyme. This conclusion would be consistent with the observation made here that the phenotype of strains deficient in both *sbcC* and *sbcD* is no different from that of the single mutants, a feature shared with the *recBC* genes.

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