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# Isolation and characterization of mutants deleted for the *sulA-ompA* region of the *Escherichia coli* K-12 chromosome

(Outer membrane protein; SOS; gene fusion; phage lambda)

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## 1. SUMMARY

We have isolated mutants deleted for different segments of the *sulA-ompA* region of the *Escherichia coli* K-12 chromosome using gene fusion techniques. Genetic and physical analysis showed that the deletions ranged from 500 to more than 4000 base pairs (bp) Strains were found in which all, or part, of the *sulA* and *ompA* genes had been deleted.

## 2. INTRODUCTION

The tightly linked *ompA* and *sulA* genes are located at 21.5 minutes on the *E.coli* linkage map [1]. The LexA-regulated *sulA* gene encodes an inducible inhibitor of cell division, whereas *ompA* codes for a major polypeptide component of the outer membrane (for reviews, see [2,3]). Although both genes have been studied intensively in recent years, no well-defined deletions have been described in this region of the genome. The aim of this study was to isolate and characterize strains carrying such deletions, which should facilitate analysis of both *sulA* and *ompA*.

Induction of a lambda lysogen normally results in the killing of the bacterial host. However, rare surviving colonies can be detected that lack all or part of the lambda DNA and often also harbor deletions extending into adjacent chromosomal DNA of the *E.coli* host [4]. Since lac gene fusion techniques [5–8] allow a lambda prophage to be inserted almost anywhere in the *E.coli* chromosome, the above observation has recently been used to develop a generally applicable method to isolate chromosomal deletions [9]. In this technique, two selectable markers, the temperature-sensitive lambda repressor allele *c*Its857 and the *neo* gene, which confers kanamycin resistance ($Kan^R$), are crossed into a $Lac^+$ fusion strain by homologous recombination. The resulting temperature-sensitive lambda lysogen can then be used to select for temperature-resistant mutants, some of which carry chromosomal deletions extending into

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genes located in the vicinity of the lambda prophage. We have used this technique to isolate deletions in the *sulA-ompA* region.

## 3. MATERIAL AND METHODS

### *3.1. Bacterial strains and bacteriophage*

Bacterial strains and phage are listed in Table 1.

### *3.2. Media and chemicals*

L, M63 and MacConkey media were prepared as previously described [10,11]. To detect colonies showing a LacZ$^+$ phenotype, 0.1 ml of a 10 mg/ml solution of 5-bromo-4-chloro-3-indolyl-$\beta$-D-galactoside (XG) in dimethylformamide was spread on agar plates. Ampicillin (Ap), chloramphenicol (Cam) and Kan were added to media at 125 $\mu$g/ml, 25 $\mu$g/ml and 30 $\mu$g/ml, respectively.

### *3.3. Methods used with nucleic acids*

Isolation of plasmid and chromosomal DNA, digestion conditions for restriction enzymes, analysis of restriction fragments in agarose gels, and transfer of these fragments onto nitrocellulose filters were all done as previously described [11–13].

### *3.4. Genetic procedures*

Standard techniques were used for the growth of bacteria and bacteriophages, titering bacteriophages, lysogen formation of phage λ and generalized transduction with phage P1[10,11]. Mu immunity was detected by spotting serial dilutions of Mucts 62 (freshly prepared by temperature indication of a lysogen) onto lawns of cells at 30 and 42°C [14]. The method of Komeda and Iino [6] was employed to convert the Mu dl1 (Ap *lac*) prophage in strain BRE31 to a λ prophage by using λ pSG1 (Table 1). Homologous recombination between this λ prophage and λ p1081.1 (Table 1) was then used to cross the *c*Its857 allele and the *neo* gene into the genome of the prophage as described by Garrett et al. [9]. The resulting strain BRE34 was used for the isolation of *sulA-ompA* deletion derivatives.

### *3.5. Isolation of Δ (sulA-ompA) mutants*

To isolate survivors following induction of the temperature-sensitive λ prophage, colonies of strain BRE34 were patched onto L agar and incubated at 42°C for 2 days. A single temperature-resistant colony from each patch was purified by restreaking twice at 42°C and tested for loss of the OmpA protein by cross streaking against the

Table 1
Bacterial strains

| Strain | Description | Reference or origin |
|---|---|---|
| *E.coli* | | |
| MC4100 | F− *araD*139 Δ(*argF-lac*)U169 *rspL*150 *relA*1 *deoC*1 *ptsF*25 *rbsR flbB*5301 | [5] |
| SG20070 | MC4100 *sulA*::Mu dl1(Ap *lac*) [Lac$^+$] *ilv*::Tn*5* *zai*::Tn*10* | S. Gottesman |
| BRE2[a] | MC4100 *ompA* | This work |
| BRE31 | MC4100 *sulA*::Mu dl1(Ap *lac*) [Lac$^+$] *ompA*$^+$ | P1 transductant from SG20070 into BRE2 |
| BRE32 | BRE31 *sulA*::λ*p*1(209) [Lac$^+$] | This work |
| BRE34 | BRE32 *sulA*::λ*p*1(209)*lacZ*:: *neo*1081.1 *c*Its857 [Lac$^-$]; | This work |
| Phage | see Fig. 1A | |
| λpSG1 | λ*p*1(209)*lacY*::Tn*9* | [11] |
| λp1081.1 | *lacZ*::*neo* 1081.1*int*am29 *c*Its857 *S*am100 | [9] |

[a] This *ompA*-containing strain was obtained by selecting for resistance against phage K3h1 [16].

OmpA-specific bacteriophages TuII*, K3 and K3h1 [15,16]. Strains resistant to these phages were scored at 37°C for growth on L agar containing Kan and, by cross-streaking, for sensitivity to phage λ *vir* and immunity to phage λ *cIh80* [11].

## 4. RESULTS AND DISCUSSION

We used a Mu dl1 (Ap *lac*) insertion [17] in the SOS-regulated [18–20] *sulA* gene to position a λ prophage next to *ompA*. However, because the β-galactosidase activity of this operon fusion in strain SG20070 (Table 1) is constitutively expressed and not induced coordinately with derepression of the SOS regulon (S. Gottesman, personal communication), the *lacZ* gene on the prophage cannot be aligned with the same polarity as the *sulA* gene [2,20]. It appeared, therefore, that *lac* expression resulted from transcription initiating at an uncharacterized promoter (Fig. 1A) located either in the intergenic region between *ompA* and *sulA* or at the 3 end of *sulA* [21–24]. Indeed, it was subsequently shown by physical analysis that the *sulA* promoter was adjacent to the Mu*c* end of the hybrid phage.

To allow further genetic manipulations, we transduced the *sulA*:: Mu dl1(Ap *lac*) insertion with phage P1 into strain BRE2 (*ompA*). $Lac^+$ transductants were selected on lactose M63 minimal plates at 30°C and all nine transductants tested were found to have acquired the expected temperature-sensitive, Ap-resistant phenotypes. The simultaneous acquisition of an $OmpA^+$ phenotype, shown by cross-streak tests with the OmpA-specific phages TuII*, K3 and K3h1 [15,16], confirmed the tight linkage of the *sulA*:: Mu dl1(Ap *lac*) insertion to the *ompA* gene. The Mu dl1(Ap *lac*) prophage in one of the transductants, strain BRE31, was then converted to a λ prophage as described previously [6,11]. The resultant temperature-resistant $Lac^+$ λ lysogen, strain BRE32, contained a λ prophage flanked by the Mu*S* end (about 200 bp; [25]) on one side and the Mu*c* region (about 3.1 kb; unpublished data) on the other (Fig. 1A). Homologous recombination between this prophage and λp1081.1 (Table 1) was used to cross the *c*Its851 allele and the *neo* gene into the genome of the prophage as described by Garrett et al. [9]. The resulting strain, BRE34, had the expected $Lac^-$, $Kan^R$ and temperature-sensitive phenotypes and contained a λ prophage located next to *ompA*. The presumed structure of the prophage is shown in Fig. 1A.

Temperature-resistant survivors of λ induction in strain BRE34 were then isolated at 42°C and were scored for loss of the OmpA protein by cross streaking against the most sensitive OmpA-specific bacteriophage, K3h1 [16]. Of 485 independent isolates, 22 strains were resistant to phage K3h1. These mutants were also $Kan^S$ and were no longer immune to phage λ. Since we had not selected for the resistance to phage K3h1, the simultaneous appearance of these phenotypes strongly suggested that a deletion had occurred in each of these 22 strains. This deletion most likely removed all or part of the prophage present in the parental strain and extended into the adjacent *ompA* gene (Fig. 1A). These mutant strains were not auxotrophs, as shown by their ability to grow on glucose-minimal plates.

To test whether the entire λ prophage had been removed by the deletion, the 22 strains were tested for temperature-sensitive immunity to phage Mu. Since the Mu*c* region (Fig. 1A) encodes a temperature-sensitive Mu repressor [7], the immunity against superinfecting Mu phages could be used as a genetic marker to probe for the loss of most of the Mu*c* DNA. 7 strains were sensitive to Mu*c* ts62 at both 30°C and 42°C, while the remaining 15 strains exhibited a temperature-sensitive Mu immunity. Thus, in the latter strains, some Mu DNA, and possibly some λ DNA, had not been removed by the deletion.

A physical study was performed to identify the end points of the *ompA* deletions more precisely. Chromosomal DNA was prepared from 11 randomly chosen mutants and digested with the restriction endonucleases *Eco*RI, *Bam*HI, *Hpa*I and *Pvu*II. The resultant fragments were subjected to agarose gel electrophoresis and DNA:DNA hybridization with two $^{32}P$-labeled probes [13,26]. Probe 1 (pTU302, Fig. 1B) was specific for *sulA*, the intergenic region, and about 60% of the coding sequence from the 5′ end of *ompA*; probe 2 (pTU1 *Sh*) includes the 3′ end of *ompA* plus *g48*, which is

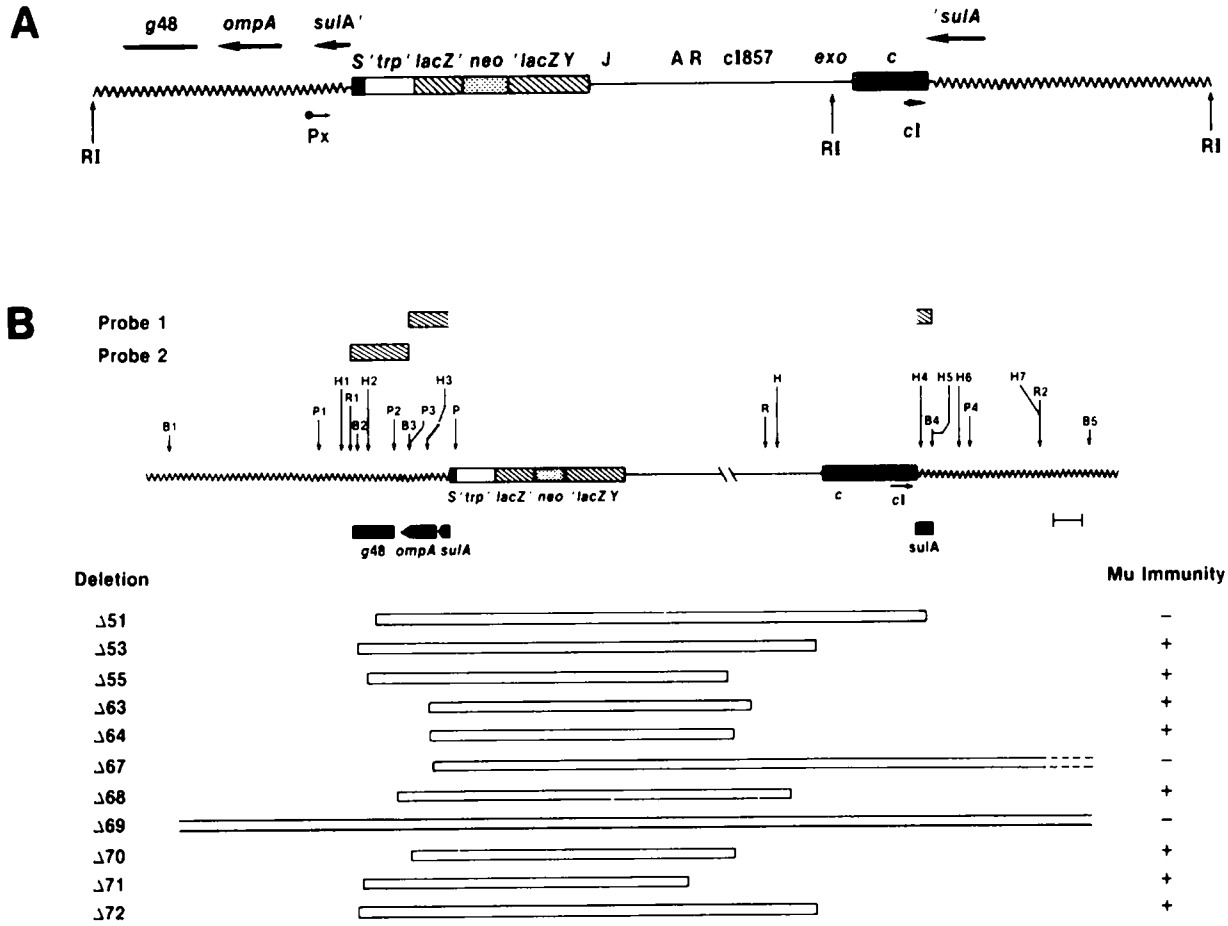


Fig. 1. (A) Organization of the *sulA-ompA* region in strain BRE34. Bacterial DNA is shown as a zig-zag line, Mu sequences are represented by black boxes, λ DNA by a thin line, *lacZ* and *lacY* sequences by striped boxes, *trp* DNA by an open box, and the $Kan^R$ gene (*neo*) by the stippled box. Incomplete or interrupted genes are denoted prime. The right Mu attachment site is designated *S* and the left attachment site is designated *c*. The positions of the chromosomal genes *g48 ompA*, and *sulA* and of the *c* ItsMu repressor are shown, together with their directions of transcription (where known). The inferred position of an unknown promoter (Px) directing transcription of *lacZ* in the original fusion strain is indicated, as are the relevant *Eco*RI restriction sites (RI). (B) Hybridization analysis of deletion mutants in the *sulA-ompA* region of the *E.coli* chromosome. Hybridization probes 1 and 2 are indicated above by striped boxes. Restriction sites are indicated by arrows: B, *Bam*HI; H, *Hpa*I; *Pvu*II; R, *Eco*RI. Sites outside the *Eco*RI fragment R1-R2 [27] were inferred from the hybridization data. The symbols representing the physical and genetic organization of the λ prophage are as in (A). The positions of the *sulA*, *ompA* and *g48* structural genes are indicated by solid boxes, and the extent of the various deletions by open boxes. The deduced deletion endpoints are accurate with approx. 200 bp. Open-ended boxes indicate deletions extending beyond the last tested restriction site. The allele numbers for the Δ(*sulA-ompA*) deletions correspond to the strain numbers given in the legend to Fig. 2. The immunity of the various deletion strains to Mucts62 is indicated. The scale marker (lower right) represents 1000 bp.

an unidentified gene located downstream from *ompA* (Fig. 1A). The *g48* gene encodes a 48 000-Da polypeptide [22]. A representative example of this analysis is shown in Fig. 2. Taken together with the known restriction maps of the *sulA-ompA* region [21,22,27,28] and the inserted hybrid phage [9,29], these results enabled us to deduce the extents of the deletions. This information is summarized in Fig. 1B.

The deletions removed from about 500 bp

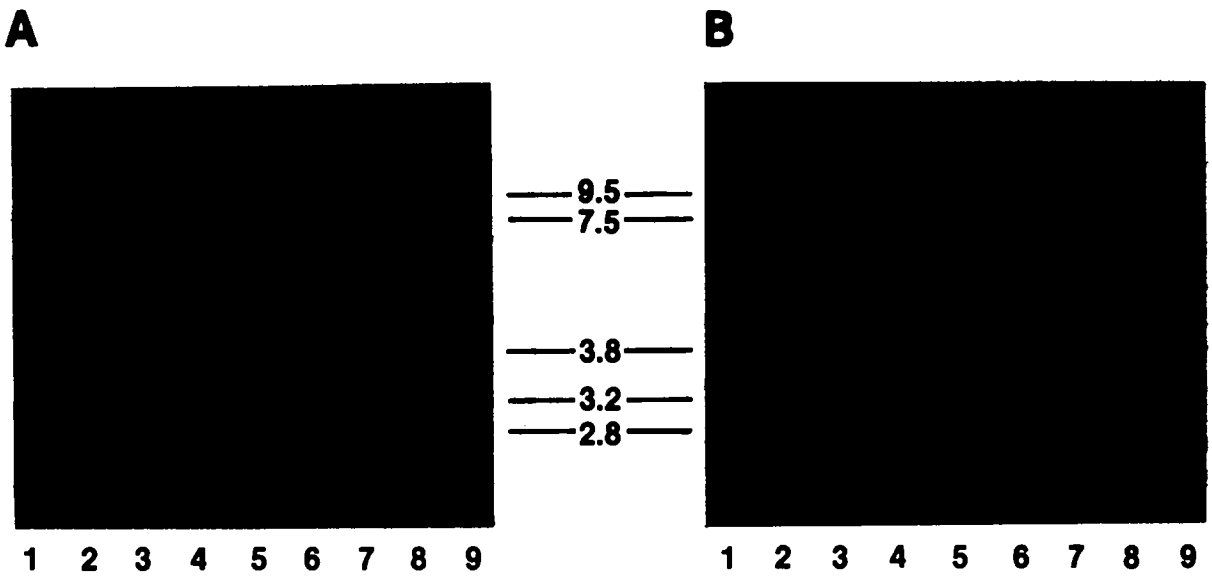


Fig. 2. DNA hybridization analysis using probe 1 (pTU302) (panel A) or probe 2 (pTU21 *Sh*) (panel B) of *Eco*RI restriction fragments of genomic DNA from strains MC4100 (lane 1), BRE51 (lane 2), BRE53 (lane 3), BRE55 (lane 4), BRE63 (lane 5), BRE64 (lane 6), BRE67 (lane 7), BRE68 (lane 8), and BRE69 (lane 9). The approximate sizes of selected fragments in kb are indicated. Due to the low-stringency washes employed, we often detected an additional band caused by hybridization between vector sequences and specific chromosomal fragments (asterisk).

(BRE63, 64) to more than 4000 bp (BRE67, 69) of chromosomal DNA. Several mutants completely lack *sulA, ompA* or *g48*. The existence of strains missing *g48* indicates that, like the SulA and OmpA proteins, the 48000-Da polypeptide encoded by this gene is not an essential product for *E.coli*. In addition, a number of mutants possessing deletion endpoints within the *ompA* gene have been obtained. As expected for *ompA* mutants obtained by this procedure, no DNA from the Mu*S* end of the hybrid phage (Fig. 1A) remained at the deletion endpoint. This was determined by using plasmid pMLB1010 [30], which bears *trp-lac* DNA as a hybridization probe (not shown).

It should be noted that this method for isolating the *sulA-ompA* deletions did not yield inversions or other rearrangements. This finding is in contrast to the results obtained when deletions are isolated using the transposable element Tn*10* [31–33].

The Δ(*sulA-ompA*) mutations described here will be useful for further analysis of the *sulA* and *ompA* genes. Mutants deleted for the entire *sulA-ompA* region can be used as host strains to screen clones of these genes from other organisms either by genetic complementation or DNA:DNA hybridization. These strains should be particularly useful in cases where the DNA:DNA hybridization must be performed under conditions of low stringency, since the Δ(*sulA-ompA*) mutation should strongly reduce the background hybridization. Isolation of mutations in cloned *sulA* and *ompA* genes and their maintenance will be facilitated by using strains that are deleted for these genes. Such a chromosomal mutation can not revert; therefore, complications that arise when there is recombination between the cloned genes and their homologous region in the chromosome can be avoided. The mutant strains possessing deletion endpoints within *ompA* are valuable tools for mapping uncharacterized *ompA* lesions. We have already used such deletions to analyse mutations isolated on a specialized lambda transducing phage carrying an *ompA-lacZ* gene fusion. By crossing these mutations into a strain deleted for a small segment at the 5′ end of *ompA*, we have dem-

onstrated that these mutations occurred very early in *ompA* and most likely affect initiation of transcription or translation.

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