DELETIONS GENERATED BY THE TRANSPOSON Tn10 IN THE
srl recA REGION OF THE ESCHERICHIA COLI K-12 CHROMOSOME

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ABSTRACT

A negative regulatory gene for the srl operon (srlR) was recognized by
the characteristics of an insertion mutation generated by the transposon Tn10
determining tetracycline resistance. This finding is discussed in light of previous
hypotheses on the regulation of the srl genes, which mediate metabolism of
glucitol (i.e., sorbitol). Mapping showed that the order of genes in this region
is: srlR srlD srlC recA alaS. Using two different methods, five mutations of
both srl and recA were detected. The phenotype conferred by these mutations,
UV sensitivity and extreme recombination deficiency, is characteristic of
standard recA point mutants. Three of the mutations were deletions that also
removed the genes for tetracycline resistance of the nearby transposon. A
fourth mutation ended at a distance from Tn10 sufficient to allow separation of
the two by recombination following P1 transduction; our tests did not allow
us to conclude whether this mutation was an inversion or a deletion. The fifth
mutation was a deletion that seemed to end immediately adjacent to the bound-
ary of Tn10, proximal to recA. Mechanisms for the generation of these srl recA
mutations are discussed.

IN Escherichia coli, the recA locus plays a central role in several aspects of
DNA metabolism, including recombination of homologous DNA elements,
It also plays a role in cell division (WITKIN 1976). In order to establish that the
recA gene is not essential for cell viability and to characterize the phenotype of
strains totally devoid of the recA product, we isolated mutants that had suffered
deletions of the gene.

In generating these deletions, we made use of the transposon Tn10, a trans-
posable genetic element coding for tetracycline resistance, which at a frequency
of 10^{-3} or 10^{-4} causes chromosomal deletions or inversions in the neighborhood
of its sites of insertion (Botstein and Kleckner 1977; Kleckner et al. 1978;
Kleckner and Ross 1978). Accordingly, we constructed strains harboring Tn10
in close proximity to recA and obtained mutant derivatives suspected to carry
deletions that affected recA function. To describe these mutants and the strains
used to detect them, we discuss three diverse subjects: (1) genetics of the srl
operon and a regulatory mutation generated by insertion of the Tn10 transposon,
(2) the isolation and phenotypic consequences of deletions that affect the Tn10 transposon as well as recA, and (3) the nature of two mutations affecting recA deletion mutants isolated independently by another method has already appeared (McEntee 1977b).

MATERIALS AND METHODS

Genetic nomenclature

We follow the recommendations of Demerec et al. (1966), Novick et al. (1976) and Campbell et al. (1976) for genetic nomenclature, with most of the genetic abbreviations defined by Bachmann, Low and Taylor (1976). In the case of deletion mutations, we follow a practice suggested by B. Bachmann. The three letter symbol "del" precedes parentheses in which the genes at the extremities of the deletion are listed to the best knowledge available. After the parentheses is a serial isolation number. Thus, del (srlR-recA)306 indicates that deletion 306 extends at least from srlR to recA. In one case, we were unable to demonstrate that a mutation was either a deletion or an inversion although it must be one or the other of these rearrangements. In consultation with B. Bachmann, we decided to introduce the symbol "crg," indicating that a mutation is a chromosomal rearrangement of unknown nature. Thus crg(srl-recA) 305 is such a rearrangement affecting at least one of the srl genes and recA.

At present there is a discrepancy concerning the symbols in use to refer to the genes of the srl operon. The operon, which functions in the initial steps of metabolism of D-glucitol (also known as sorbitol), has been designated srl by McEntee, Hesse and Epstein (1976) and by Jones-Mortimer and Kornberg (1976), and gut by Lengele (1975a). According to present understanding, D-glucitol (called simply glucitol hereafter) is taken up and phosphorylated by an enzyme II component of the phosphoenolpyruvate sugar-phosphotransferase system, encoded by the srlA (gutA) gene, and the product, glucitol-6-phosphate is converted to fructose-6-phosphate by glucitol-6-phosphate dehydrogenase encoded by the srlD (gutD) gene. Both gene products are specifically inducible by glucitol (Lengele 1975b, 1977). Three types of srl regulatory mutations have been described in the published literature. Lengele (1975a; cf., Lengele and Lin 1972) obtained one he designated gutC+, which resulted in constitutive expression of srlA and srlD and which may be an alteration of the operator-promoter region, for it was found to be cis-dominant to gutC+ in diploids (Lengele and Steinberger 1978). In the publication by Bachmann, Low and Taylor (1976), this gene was renamed srlC. A different set of regulatory mutations, including some that resulted in a temperature-conditional constitutive expression of the srl structural genes was isolated by Jones-Mortimer and Kornberg (1976). They called the relevant locus srlC and hypothesized that the operon was under negative control. McEntee (1977a) suggested that the operon is under positive control because mutations in a gene he called srlC abolished the expression of srlA and srlD, and wild-type expression was restored upon lysogenization by a λ specialized transducing phage that carried neither srlA and srlD, but did carry the wild-type allele of this proposed regulatory gene. At present, there is no available evidence to decide whether or not the regulatory mutations discovered by Jones-Mortimer and Kornberg are allelic to those found by McEntee, and it is possible that the designation "srlC" as used by Bachmann, Low and Taylor (1976), Jones-Mortimer and Kornberg (1976) and McEntee (1977a) actually refers to three different genes.

We follow, for the most part, the srl nomenclature used by McEntee (1977a); specifically by srlC we mean the gene designated by him rather than by Jones-Mortimer and Kornberg (1976) or Bachmann, Low and Taylor (1976). In a recent paper (Lengele and Steinberger 1978), gutC was changed to gut (P,O), but we retain gutC to refer to the gene containing the cis-dominant mutation conferring srl constitutivity, as elaborated by Lengele (1975a) and number that mutation gutC300. In addition, we introduce a new symbol, srlR, to denote a negative regulatory gene for srl, which we have discovered via a Tn10-induced mutation that resulted in constitutive expression of srlA and srlD. On the basis of mapping data, we shall argue that srlR is distinct from the gene called srlC by McEntee (1977a), and because of the cis-dominant nature
### TABLE 1

**Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>F plasmid</th>
<th>ColEI derivative plasmids</th>
<th>srl</th>
<th>gut</th>
<th>rec</th>
<th>Other genetic markers</th>
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<tr>
<td>A74</td>
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<td>+</td>
<td>+</td>
<td><em>alaS3</em> <em>his-1</em> <em>leu-6</em> <em>metB1</em> <em>argG6</em> <em>xyl-7</em> <em>mil-2</em> <em>malA1</em> <em>rpsL50</em></td>
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<td>AB1157</td>
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<td>+</td>
<td>+</td>
<td><em>thr-1</em> <em>leuB6</em> <em>proA2</em> <em>his-4</em> <em>argE3</em> <em>thi-1</em> <em>mil-1</em> <em>gatC7</em> <em>ara-14</em> <em>lacY1</em> <em>galK2</em> <em>xyl-5</em> <em>rpsL31</em> <em>tsx-33</em> <em>supE44</em></td>
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<tr>
<td>JC158</td>
<td>Hfr(P01)</td>
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<td>+</td>
<td>−</td>
<td>+</td>
<td><em>serA6</em> <em>lacI22</em> <em>thi-1</em> <em>rel-1</em></td>
<td><strong>CLARK 1963</strong></td>
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<td>JC2915</td>
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<td>+</td>
<td>+</td>
<td><em>cysC43</em> others same as AB1157</td>
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</tr>
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<td><strong>TEMPLIN et al. 1978</strong></td>
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<td>JC7221</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td><em>A56</em> <em>thr+</em>/<em>thr-1</em>, <em>leu+</em>/<em>leuB6</em>, <em>tonA2</em> <em>tsx-315</em> <em>lacY1</em>, <em>lacZ4</em> <em>argG6</em> <em>rpsL104</em> <em>malA1</em> <em>xyl-7</em> <em>mil-2</em> <em>pyrB</em></td>
<td><strong>M. GUER 1974</strong></td>
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<td>JC9894</td>
<td>F−</td>
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<td><em>D50</em></td>
<td>C300?</td>
<td>+</td>
<td>same as AB1157</td>
<td><strong>JC2915 × P1-L163 → Cys+</strong></td>
</tr>
<tr>
<td>JC9915</td>
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<td>pLC18-42</td>
<td><em>/C1</em></td>
<td><em>/+</em></td>
<td><em>/A1</em></td>
<td>same as KL386 (see below)</td>
<td><strong>L. CSONKA, A. TEMPLEN and A. J. CLARK, in preparation</strong></td>
</tr>
<tr>
<td>JC9918</td>
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<td>pJC597</td>
<td><em>/C1</em></td>
<td><em>/+</em></td>
<td><em>/A1</em></td>
<td>same as KL386 (see below)</td>
<td><strong>L. CSONKA, A. TEMPLEN and A. J. CLARK, in preparation</strong></td>
</tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td><em>alaS3</em> and markers of AB1157</td>
<td><strong>JC9894 × P1-A74 → Srl+</strong></td>
</tr>
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<td><em>D50</em></td>
<td>C300</td>
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<td><em>alaS3</em> and markers of AB1157</td>
<td><strong>JC10206 × P1-L163 → Mtl+</strong></td>
</tr>
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<td>+</td>
<td>C300</td>
<td><em>tlf-1</em></td>
<td>λ+ supE+ (?) and other markers of AB1157</td>
<td><strong>L. CSONKA, A. TEMPLEN and A. J. CLARK, in preparation</strong></td>
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<td><em>R301</em>: <em>Tn10</em></td>
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<td>+</td>
<td>same as AB1157</td>
<td><strong>This paper</strong></td>
</tr>
<tr>
<td>Strain</td>
<td>F plasmid</td>
<td>ColE1 derivative plasmids</td>
<td>srl</td>
<td>gut</td>
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<td>Other genetic markers</td>
<td>Source or reference</td>
</tr>
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<td>$R301::Tn10, D50$</td>
<td>C300 (?)</td>
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<td>$R301::Tn10$</td>
<td>C300 (?)</td>
<td>$tif-1$</td>
<td>same as L163</td>
<td>This paper</td>
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<td>$R301::Tn10-79, D50$</td>
<td>C300 (?)</td>
<td>$A35$</td>
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<td>Tet$^R$ Srl$^-$ Rec$^-$ transductant of JC2922</td>
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<tr>
<td>JC10299</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>$cys-300::Tn10$ and markers of AB1157</td>
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<td>JC10301</td>
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<td>$D50$</td>
<td>C300</td>
<td>+</td>
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<td>Tet$^R$ Srl$^-$ Rec$^-$ transductant of L163 x P1. JC10299</td>
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<td>+</td>
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<tr>
<td>KL385</td>
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<td>$C1$</td>
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<td>+</td>
<td>$argA2$ $alaS5$ and other markers of KL385  (cf. below)</td>
<td>K. B. Low</td>
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<tr>
<td>KL386</td>
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<td>$C1$</td>
<td>+</td>
<td>$A1$</td>
<td>$leuB6$ $metE70$ $ilv-355$ $mlt-1$ $ara-14$ $lac13$ $lacZ18$ $xyl-5$ $malA5$ $rpsL109$ $supB32$</td>
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</tr>
<tr>
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<td>$D50$</td>
<td>C300</td>
<td>+</td>
<td>$metB4$ $mlt-2$ $gatC300$ $gatA50$ $malA1$ $xyl-7$ $rpsL104$ $sup^-$</td>
<td>J. Lengeler (see Lengeler 1975a for similar strains)</td>
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<td>SK362</td>
<td>F-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>$lacMS286$ $phy$ $dII$ $lacBK1$ $his4$ $argE3$ $thi-1$ $mlt-1$ $xyl-5$ $tsx-33$ $rpsL31$</td>
<td>Zieg and Kushner 1977</td>
</tr>
</tbody>
</table>

* In heterozygotes, the plasmid marker is indicated first and the chromosomal marker is indicated second. The two are separated by a slash mark. Question marks refer either to an unknown mutant allele number or to the possible presence of a mutant allele.

† The following phenotype abbreviations are used: Cys, cysteine; Srl, glucitol (trivial name sorb, tol); M1l, mannitol; Tet, tetracycline, Thr, threonine; Leu, leucine; Ilv, isoleucine and valine; Sm, streptomycin; Ura, uracil; Rec, recombination; UV, ultraviolet irradiation; superscripts + and −, independent and requiring, respectively, when used with the abbreviations of amino acids, and fermenting and nonfermenting, respectively, when used with abbreviations of sugars. $alaS^+$ and $alaS^{18}$ stand, respectively, for the resistance and sensitivity to 42° conferred by $alaS^+$ and $alaS3$ or $alaS5$. Superscripts R and S denote resistance or sensitivity to antibiotics, glucitol, UV, etc.
of gutC300, we feel that srlR is probably not allelic with it either. At present, we have no data to
decide whether or not the srlC mutations described by Jones-Mortimer and Kornberg (1976)
afect the same gene as our TnlO insertion.

Bacterial strains

The bacterial strains, all derivatives of E. coli K-12, are listed in Table 1. Construction of sev-
eral of the strains needs to be described in detail. The set of recA deletion mutants was made from
two strains that had in common a srl mutation generated by the insertion of the tetracycline-
resistance transposon, TnlO. This mutation had been detected in a two-step process that involved,
first, the production of a mixed culture of undefined TnlO mutants and then characterization of
that small fraction of mutations that were co-transduced with alas into a suitable recipient. One
srl mutation detected in this way (srlR3O1::TnlO) was then transduced to each of the two strains
that had properties making them advantageous for the detection of TnlO-generated deletions.
These steps are summarized in Figure 1 and are described in detail below.

Isolation of a tetracycline-resistant culture with TnlO present at undetermined sites: (We are
indebted to Nancy Kleckner for suggesting some details of this procedure.) Strain SK362 (sup+)
was grown to a density of 5 × 10^8 cells per ml in L broth supplemented with maltose (4 mg per
ml). The cells from 10 ml of culture were sedimented and then resuspended in 1 ml of a lysate
of phage λ NK55 of titer 4 × 10^10 per ml. This phage is of the genotype b221 cI857 cIII167::TnlO
Oam29 (Kleckner et al. 1978). Because of the heat-sensitive repressor mutation, cI857, NK55
cannot be maintained in a lysogenic state at 42°C, thus incubation at 42°C of the tetracycline-
resistant survivors of the infection selects mutants in which TnlO has been translocated into the
E. coli chromosome (Kleckner et al. 1978). The phages were allowed to adsorb at room tempera-

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**Figure 1.—Summary of the isolation of recA deletion mutants.** This pedigree has been drafted
by analogy with those drawn by Bachmann (1972). Ancestral strains are listed to the left and
derived strains to the right of each arrow. A partial genotype is listed under each strain. A ques-
tion mark means that we are not certain whether a particular allele is present or not. When gen-
eralized transduction was employed, the symbol P1 (for phage P1) is included over the arrow and
the phenotype selected is included under the arrow. Following P1 is the name of the strain on
which the phage lysate had been obtained. Following the phenotype selected is any unselected
phenotype by which the derivative was recognized. JC10277 and JC10278 are lambda lysogenic.
ture for 15 min, and then the culture was diluted into 50 ml of L broth supplemented with glucose (4 mg per ml), sodium citrate (10 mm, pH 7.0) and incubated with aeration at 30°. There was an initial drop in optical density. After about one hr, when optical density began to increase, tetracycline was added to a final concentration of 1 µg per ml, and after another hr the drug concentration was increased to 15 µg per ml. The cells were grown to saturation at 30° over night, and then a 10 ml portion was diluted into 50 ml of the same growth medium and grown to saturation at 42°. Even though at this point 100 out of 100 single colonies obtained at 42° proved to be tetracycline-resistant clones, 10 ml of the culture were again diluted into 50 ml of the above medium, and the cells were grown once more to saturation at 42° before they were used for the next experiment.

Isolation of strains with Tn10 in the recA region: A set of Tn10 insertion mutations near recA was obtained by a type of localized mutagenesis in which the transposon was the mutagen (Kleckner, Roth and Botstein 1977). A P1 lysate was obtained from the culture consisting of tetracycline-resistant cells derived from SK362, as described above. It was used to transduce JC10206 (alaS3 mtl-1) to growth at 42° on L plates containing tetracycline. alaS3 is a mutation that confers heat lability to alanyl-tRNA synthetase, (cf., Kurzberg et al. 1974); hence, cells carrying alaS3 will not grow at 42°. In this, and other transductions selecting AlaS+ (i.e., 42° resistance), the plates were first incubated at 33° for 60 min after spreading of the transduction mixture; then they were transferred to 42° in order to allow the expression of the alaS3 allele. Since alaS and recA are about 85% linked, the double selection for AlaS+ and TetR was expected to yield strains in which Tn10 had been localized near recA. The transductants thus obtained were screened for UV sensitivity, recombination proficiency (for the identification of possible recA:: Tn10 insertions), for the acquisition of new auxotrophic markers, for growth on glucitol and for growth on mannitol. (The reason for testing growth on the latter hexitol will be explained below.)

From 332 AlaS+ TetR transductants thus obtained, six (not necessarily independent) strains were found that required cysteine, and three that were unable to grow on glucitol. There were no UV-sensitive or recombination-deficient isolates, or strains that had other auxotrophic requirements. One of the Cys- transductants was named JC10299, and its mutation was called cys-300:: Tn10. No tests were carried out to determine which particular cysteine gene had been affected in this or the other Cys- mutants. By phenotype on MacConkey indicator plates, all three Srl- strains were judged to be glucitol transport deficient; therefore they were either srlA or srlC mutants. The particular gene affected was not determined.

Isolation of JC10275 carrying srlR301::Tn10: JC10206, the strain used as the recipient in the above transduction to yield derivatives with Tn10 in the recA region, was unable to grow on mannitol due to the mtl-1 mutation, which abolishes the activity of the mannitol transport system (B. Bachmann, personal communication). Lengeler (1975a) found that mannitol transport deficiency can be suppressed by mutation to srl constitutivity, because mannitol is a substrate, but not an inducer, of the srlA gene product. In the hope of discovering Tn10-induced mutations that might have conferred constitutive expression to the srl operon, we tested the mannitol-utilizing phenotype of the 332 AlaS+ TetR transductants of JC10206 and found one which had gained the ability to grow on this sugar. The strain was called JC10275. According to the rationale presented above, it was assumed to be constitutive for the expression of the srl operon. Assays of glucitol-6-phosphate dehydrogenase were carried out as described by Lengeler (1975a). The srlR mutant JC10275 showed 0.110 and 0.100 units of dehydrogenase activity per mg protein when grown in glycerol and glucitol minimal media, respectively. An srlR+ comparison strain (JC10299) carrying cys-300::Tn10 showed 0.007 and 0.049 units per mg protein when grown under the same conditions. This bears out the expectation that the srlR mutation derepresses the srlD gene in the srl operon. Since Tn10 insertions cause strongly polar mutations with concomitant loss of function of the genes that are interrupted (Kleckner et al. 1975), the most plausible explanation of this mutation is that the insertion has been into a regulatory gene that is a repressor for the operon. We call this mutation srlR301::Tn10 and the corresponding wild-type allele srlR+, even though we recognize the possibility that it may be the same gene as the one called srlC by Jones-Mortimer and Kornberg (1976). Joseph Lengeler (personal communication) found that
srIR301::Tn10 was recessive to srIR+ in partial diploids, which is consistent with the hypothesis that the srl structural genes are under negative control and that the srIR301::Tn10 mutation abolishes the functioning of the repressor.

Methods of detecting recA deletions

Since Tn10 acts as a deletion-generating mutagen (Botstein and Kleckner 1977; Kleckner, Roth and Botstein 1977; Kleckner et al. 1978), we isolated strains harboring Tn10 near recA. We then constructed derivatives that permitted two alternative methods of enriching deletion mutant of recA. The first method permitted enrichment of mutants carrying deletions on the recA side of Tn10; the second method permitted enrichment of mutants carrying deletions affecting recA.

Method I: Selection of glucitol-resistant derivatives of a srID50 strain: The basis for the first method of enriching for recA deletion mutants is that mutants lacking glucitol-6-phosphate dehydrogenase (the second enzyme in the glucitol catabolic pathway) are inhibited by glucitol. This inhibition can be circumvented by a mutation to glucitol-transport deficiency (srIA- or srIC-) (cf., Lengeler 1975a). The srIR301::Tn10 allele was transduced from JC10275 into L163 (srID50 gutC300), selecting tetracycline resistance, and one TetR SrlD- transductant, JC10279 (recognized by glucitol sensitivity), was isolated. This strain may have retained gutC300 from L163 because the gene order is srlR srlD gutC as inferred from the result of Lengeler (1975a) and our own results to be presented below. JC10279 was used for the production of recA deletion

(a) JC10279

(b) JC10276

Figure 2.—The srl to alas portion of the genetic map of each of two strains is represented: (a) JC10279 and (b) JC10276. The map is not drawn to scale. Genotype of each strain, respectively, is listed immediately above or below the line representing the genetic map. The question mark after gutC300 indicates two uncertainties: (1) position of gutC relative to srlC and (2) identity of the allele (i.e., the allele may be gutC+). The question mark after gutC+ indicates only positional uncertainty. The boxes that include arrows are the inverted repeat sequences at each end of Tn10. Above the map of JC10279 and below the map of JC10276 are drawn two parallel lines, partly solid and partly dashed. Above or below these, respectively, are listed particular phenotypes. These parallel lines are joined to the map by dashed lines and thus are divided into segments according to the position of particular genes. The parallel lines represent the majority classes of deletion mutation described by Kleckner and Ross (1978). The solid segments of the parallel lines represent those sequences deleted by all TetR or TetB majority class mutations. The dashed segments of the parallel lines represent regions within which any deletion may end to produce the phenotype indicated. alasS is a vital gene, so that all mutants carrying deletions extending into or beyond alasS will be inviable.
mutants. On the basis of the gene order: srlR3Ol::TnlO srlD srlA srlC recA (see Figure 2A), we expected that some mutations to glucitol resistance would be deletions that removed srlA, srlC, and all or part of recA.

The selection of glucitol-resistant derivatives of an srlD mutant is most conveniently performed on glucitol MacConkey indicator plates, on which Srl+ strains form large, crimson colored colonies, SrlA- or SrlC- (i.e., glucitol transport deficient) strains form large, white colonies, and SrlD-A+C+ (i.e., glucitol-6-phosphate dehydrogenase deficient, glucitol transport proficient) strains form small, transparent colonies that, with time, give rise to faster growing, white (SrlA- or SrlC-) papillae (cf., SOLOMON and LIN 1972). When 10^8 cells of JC10279 or L163 were plated on glucitol MacConkey plates, faster growing, glucitol-resistant colonies were apparent on the lawn after 24 hr of incubation. To detect strains carrying deletions of recA, such glucitol-resistant derivatives were streaked out on glucitol MacConkey plates, and single colonies were tested for recombination proficiency, UV resistance and in the case of derivatives of JC10279, for tetracycline resistance. Any glucitol-resistant, recombination-deficient strain was examined further as a candidate for carrying a deletion of recA.

Method II: Resistance to the thermo-induction of λ in the tif-1 background: The rationale for the second method of enriching for recA deletion mutants was that recA mutations prevent the thermo-induction (e.g., at 42°) of λ in a tif-1 lysogen, thereby allowing survival under inducing conditions (CASTELLAZZI, GEORGE and BUTTIN 1972a,b). The srlR3Ol::TnlO allele was transduced from JC10275 into JC10269 (srl+ tif-1 A), and one transductant, JC10276, which was srlR301:: TnlO srlD+ srlA+ srlC+ tif-1 λ, was used for the enrichment of recA deletion mutants. This was accomplished by plating 10^8 cells of an overnight L broth culture of JC10276 on glucose minimal plates, supplemented with denine present at 75 µg per ml to enhance the efficiency of λ induction (cf., CASTELLAZZI, GEORGE and BUTTIN 1972b), and by incubating at 42° for 48 hr. Colonies from surviving cells were streaked out under selective conditions and then tested for tetracycline resistance, growth on glucitol, UV sensitivity and recombination proficiency. TetS Srl- UV® Rec-survivors were presumed to be strains with deletions of recA because only deletions could have inactivated in one step at least three (i.e., fet, s11 and red) genes.

For further characterization, the mutations obtained by both methods were transduced into JC10206 (alaS3), selecting growth at 42° on glucose minimal plates. In this manner, the presumed recA deletions have been transduced into the genetic background of AB1157 (isogenic with JC10206 except for being alaS+, cf., Table 1), which has been used for much of the characterization of other alleles in the recA cluster (CLARK 1973).

General genetic methods and media

The phage used in generalized transductions was P1 vir. The growth media have been described previously (WILLITTS, CLARK and Low 1969), with the one addition that tetracycline, when used, was present at 15 µg per ml in complex media and at 10 µg per ml in minimal media. All cultures were incubated with aeration at 37° unless stated otherwise. Most details of strain construction entailed standard techniques of E. coli genetics described in the text by MILLER (1972).

Determination of UV sensitivity

For a qualitative survey, colonies were patched to an L agar plate (complex medium) incubated overnight and replica plated to two L plates, one of which was irradiated with a UV dose of 90 Joules per m² produced by 2 GE 15W germicidal lamps. The plates were incubated, in the dark, overnight, and UV sensitivity was judged by comparison of growth on irradiated and non-irradiated plates. The UV dose used caused only a slight decrease in the growth of recA+ strains, while it completely prevented growth of recA mutants. For a quantitative test, strains were grown in liquid L broth culture to a density of 10^8 cells per ml, sedimented and resuspended at the original density in medium 56/2. A 5 ml portion was irradiated with UV light, with samples removed at various points corresponding to different UV doses. The viability after exposure to each UV dose was determined on L agar plates, incubated in the dark.
TN10 INDUCED srl recA DELETIONS

Determination of recombination proficiency

For qualitative testing, colonies were patched to L agar plates and after overnight incubation, they were replica plated to a lawn for 10^8 cells of an appropriate Hfr strain on plates that were selective for the inheritance of early markers donated by the Hfr. When derivatives of JC10279 (metB+) were tested, the Hfr was KL25, and the ability to inherit metB+ was scored. In the case of strains isolated from JC10276 (thr-1 leuB6) the Hfr was JC158, and inheritance of thr+ and leu+ was selected. In both crosses, streptomycin was used to kill donor cells.

For quantitative testing, strains, all carrying the genetic background of AB1157 (thr-1 leuB6), were grown in L broth to a density of 10^8 cells per ml, as was the Hfr strain JC158 and the F thr+ leu+ (pJC356) strain JC7221. A 4 ml volume of each F- strain to be tested was mixed with 0.8 ml of the culture of JC158 or JC7221 and incubated with slow shaking for 60 min. Then, the mating mixtures were diluted serially and 0.1 ml portions of the various dilutions were spread on plates selective for the inheritance of the thr+ and leu+ alleles by the F- strains, with streptomycin present to kill the donors. The results are expressed as the “Recombination Deficiency Index” which, for each recipient, is calculated as the frequency of Thr+ Leu+ StrR transconjugants obtained when the F’ strain JC7221 was the donor divided by the frequency of Thr+ Leu+ StrR transconjugants obtained when JC158 was the donor, with the ratio normalized to the value for the Rec+ strain AB1157 taken as 1.0.

Complementation analysis of the glucitol-resistant mutants

Mutations causing resistance to glucitol in the srlD50 genetic background may restore the function of srlD and lead to restored ability to utilize glucitol as carbon and energy source. Alternatively, they may cause loss of glucitol transport activity, and the mutant, although resistant to glucitol, will not utilize the sugar for growth. The latter mutations could affect srlA, srlC or other genes involved in generalized sugar transport (such as ptsH, ptsI, cya, crp, etc.) (LENGELER 1975b). In order to verify that a given mutation leading to glucitol resistance, while retaining inability to ferment glucitol, was specifically in the srl operon, the SrlR mutants obtained from L163 (srlR+ srlD50) or JC10279 (srlR301::Tn10, srlD50) were mated with JC9915, an F+ strain that in addition harbored pLC18-42, a hybrid ColEl plasmid carrying the srlA+ srlC+ and recA+ alleles and possibly srlR+ (CLARKE and CARBON 1976). Srl+ Ilv+ Leu+ transconjugants were selected by spotting drops of overnight cultures of the glucitol-resistant derivatives of L163 or JC10279 on a lawn of 10^8 cells of a late log-phase culture of JC9915 on glucitol plates supplemented only with methionine and thiamine. Thick growth where donor was spotted was interpreted to mean that the mutation under examination was recessive to srl+ and hence was located in the srl operon.

Among mutations confined to the srl locus, the loss of either srlA or srlC could result in glucitol-transport deficiency. We determined the relative frequency of mutations that affected srlC by mating, in the manner described above, derivatives of L163 and JC10279 with JC9918, an F+ strain that carried the hybrid ColEl plasmid pJC597 containing the srlR+ srlD+ srlA+ srlC1 and recA+ alleles. On selection for Srl+ Ilv+ Leu+ transconjugants, three kinds of responses were observed: (1) thick growth where the recipient was spotted, which was interpreted as an indication of complementation between the mutation in question and srlC1, (2) variable numbers of isolated colonies where the recipient was spotted, which was taken to mean that the mutation could not complement srlC1 but could recombine it to generate an intact srl+ locus, and (3) no growth at all, which was taken to mean that the two mutations could neither complement nor recombine with each other to restore growth on glucitol, presumably because the srlC locus had been deleted.

Determination of the translocation frequency of genetic elements coding for tetracycline resistance

Among presumed recA deletion SrlR mutants were two strains, JC10283 and JC10284, which were resistant to similar concentrations of tetracycline as was the parental strain, JC10279 (Figure 1). These two were thought to retain all or most of Tn10 (Figure 2). As a bookkeeping device, not necessarily implying any difference from wild-type Tn10, we shall denote the drug-
resistance element present in JC10283 at Tn10-83, the one in JC10284 as Tn10-84 and the one in JC10279 as Tn10-79. Mapping results implied that the tetracycline-resistance character in these strains was still closely linked to \( \text{alaS} \). To find out whether the deletions might have removed parts of Tn10, we tested the translocation ability of the residual drug-resistance elements, in the following manner. First, Tn10-83, Tn10-84 and Tn10-79 were transduced into a similar genetic background, creating three Tet\(^R\) \( \text{thr-1 leuB6} \) strains, JC10288, JC10289 and JC10285, respectively (cf., Table 5). Then F \( \text{thr}^+ \text{ leu}^+ \) (pJC536) was transferred to these strains by conjugation with JC7221. The resultant F' transconjugants were in turn mated with KL386 (F- \( \text{leuB6 recA1} \)) electing for \( \text{Leu}^+ \) or Tet\(^R\) transconjugants with the donors being counterselected by their amino acid requirements. The transfer of tetracycline resistance under these conditions, when both donor and recipient strains were recA mutants, occurred presumably as a result of a transposition event of the drug-resistance element from the chromosome to the F'. The transposition efficiency is expressed as the ratio of the number of Tet\(^R\) transconjugants obtained divided by the number of \( \text{Leu}^+ \) transconjugants obtained.

RESULTS

Mapping \( sr1R301::Tn10 \)

The mutation \( sr1R301::Tn10 \) was originally detected as a suppressor of the mannitol transport defect in JC10206 (\( \text{mtl-1} \)), producing a Mtl\(^+\) phenotype while retaining a Srl\(^+\) phenotype. Initial mapping of the \( sr1R \) mutation was performed by transducing the Tet\(^R\) Srl\(^-\) Mtl\(^-\) \( \text{AlaS}^* \) strain KL385 (\( sr1R^+ \text{sr1C1 mtl-1 alaS}^* \)) to Tet\(^R\) by a P1 lysate grown on the Tet\(^R\) Srl\(^+\) Mtl\(^+\) \( \text{AlaS}^+ \) strain JC10275 (\( sr1R301::Tn10 \text{sr1C}^+ \text{mtl-1 alaS}^+ \)). The following classes were found among 191 Tet\(^R\) transductants: Srl\(^+\) Mtl\(^+\) Alas\(^+\) (80\%), Srl\(^+\) Mtl\(^+\) \( \text{AlaS}^* \) (13\%) and Srl\(^-\) Mtl\(^-\) \( \text{AlaS}^* \) (7%). The high co-inheritance (93\%) of Tet\(^R\) and Srl\(^+\) indicates the close linkage of \( sr1R301::Tn10 \) to the \( \text{sr1C}^+ \) allele. The fact that the Tet\(^R\) Srl\(^-\) transductants were also Mtl\(^-\) and that there were no (<0.6\%) transductants that were Srl\(^+\) Mtl\(^-\) or Srl\(^-\) Mtl\(^+\) indicates the failure of \( sr1R301::Tn10 \) to suppress \( \text{mtl-1} \) when the strain is \( \text{sr1C1} \). This is significant in our interpretation of the functions of \( sr1R \) and \( \text{sr1C} \), although it does not assist in mapping \( sr1R \).

The \( sr1R301::Tn10 \) mutation was further localized by transducing JC10207 (\( sr1R^+ \text{sr1D50 gutC300 alaS}^* \text{mtl-1} \)) to Tet\(^R\) at 33\(^\circ\), to Srl\(^+\) at 33\(^\circ\) and to AlaS\(^+\) at 42\(^\circ\) by a P1 lysate grown on JC10275 (\( sr1R301::Tn10 \text{sr1D}^+ \text{sr1A}^+ \text{sr1C}^+ \text{gutC}^+ \text{alaS}^+ \text{mtl-1} \)). Since both the donor and recipient strains carried suppressors of \( \text{mtl-1} \) (both the \( sr1R301::Tn10 \text{and gutC300 alleles produce constitutivity of the sr1 genes} \), growth on mannitol could not be used to detect uniquely the inheritance of \( sr1R301::Tn10 \), and Tet\(^R\) was used instead. The results of the three-factor cross are presented in Table 2. When AlaS\(^+\) was selected, the frequency of Srl\(^+\) Tet\(^R\) transductants (3.6\%) was greater than that of the Srl\(^-\) Tet\(^R\) transductants (1.2\%). Making the assumptions that quadruple crossovers are less probable than double crossovers and that \( \text{alaS} \) does not lie between the two \( sr1 \) genes, the gene order consistent with these results is \( sr1R301::Tn10 \text{sr1D50 alaS}^3 \). When Tet\(^R\) was selected, the frequency of Srl\(^+\) AlaS\(^*\) transductants (18\% of the total) was greater than that of the Srl\(^-\) AlaS\(^+\) transductants (<0.5\%), a finding consistent with the gene order deduced.
**TABLE 2**

*Linkage of SrlR, srlD and alaS in P1 transduction*

<table>
<thead>
<tr>
<th>Selected phenotype</th>
<th>Number tested</th>
<th>AlaS+</th>
<th>Srl+</th>
<th>TetR</th>
<th>Srl+TetR</th>
<th>Srl+TetE</th>
<th>Srl+TetE</th>
<th>Srl-TetR</th>
<th>Srl-TetE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlaS+</td>
<td>167</td>
<td>85</td>
<td>82</td>
<td>81</td>
<td>14</td>
<td>3.6</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetA AlaS+</td>
<td>92</td>
<td>98</td>
<td>89</td>
<td>&lt;0.9</td>
<td>9.0</td>
<td>2.5</td>
<td>&lt;0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetE AlaS+</td>
<td>94</td>
<td>98</td>
<td>89</td>
<td>&lt;0.9</td>
<td>9.0</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetX AlaS+</td>
<td>100</td>
<td>96</td>
<td>90</td>
<td>86</td>
<td>5</td>
<td>1.5</td>
<td>&lt;0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Strain JC10207 (srlR+ srlD50 gutC300 alaS+ mtl-1) was transduced at 33° to growth on glucitol or to tetracycline resistance (on glucose) or to growth at 42° (on glucose) by a P1 lysate grown on JC10275 (srlR301::Tn10 srlD+ srlA+ gutC+ gutC+ alaS+ gutC+ mut-1). The Srl- phenotype, scored on glucitol MacConkey plates, was a combination of inability to ferment glucitol and glucitol sensitivity typical of srlD50 srlA+ srlC+ gutC+ strains. Every TetR transductant was Mtl+ because the donor carried the srlR301::Tn10 mutation, which caused srl constitutivity, hence growth on mannitol. There were no TetR Mtl+ transductants, which would have been indicative of the srlR+ gutC300 genotype. This may indicate that srlR and gutC are very close together.

Detection of Tn10 induced mutations to glucitol resistance

On glucitol MacConkey plates, glucitol-sensitive (Srl-) cells grow slowly making a thin flat colony against which faster growing glucitol resistant (Srl+) papillae can be seen. More such papillae appear in colonies of the srlR301::Tn10 srlD50 strain, JC10279, than in those of its srlR+ srlD50 parent, L163. When 10^8 cells of each strain were plated on glucitol MacConkey medium, faster growing Srl+ colonies appeared against a thin background lawn. Even though such colonies continued to appear on prolonged incubation, the initial mutant frequency was estimated by determining the number of the Srl+ colonies present 24 hours after plating. In this manner, it was estimated that a culture of L163 (SrlR+ srlD50 gutC300) contained glucitol resistant derivatives at a frequency of two per 10^8 cells plated, whereas a culture of JC10279, isogenic to L163 except for being srlR301::Tn10, contained glucitol resistant derivatives at a frequency of 8.5 × 10^3 per 10^8 cells plated. Thus, the presence of Tn10 in close proximity to genes required for the functioning of the glucitol transport system (srlA and srlC) resulted in an apparent 4 × 10^3-fold increase in the mutation frequency to glucitol-resistant mutants. This effect could not be mimicked by the presence of Tn10 elsewhere in the chromosome, because when it was in a cys gene, approximately one minute from the srl operon (mapping data not shown) in strain JC10301 (cys-300::Tn10 srlD50 gutC300), the frequency of glucitol-resistant derivatives was three per 10^8 cells, or very nearly the same as was obtained with the TetR parent, L163.

The increase in frequency of glucitol-resistant derivatives of JC10279, as compared to L163, is presumably due to the ability of Tn10 to induce chromosomal deletions or inversions near its site of insertion (Botstein and Kleckner 1977; Kleckner, Roth and Botstein 1977; Kleckner and Ross 1978), which in this...
instance inactivates srlA or srlC. The glucitol-resistant strains obtained from JC10279 were first screened for the loss of tetracycline resistance. Tetracycline-sensitive derivatives were readily obtained, though their frequency varied from experiment to experiment. In 13 independent isolations, screening approximately 200 glucitol resistant colonies in each, the frequency of Tet<sup>+</sup> mutants varied from a low of 0.5% to a high of 85%, with 15% the median figure.

**Characterization of the glucitol-resistant mutants**

Based on the observations of Kleckner and Ross (1978), we have sketched in Figure 2A the structure of Tn10-induced deletions that would be expected to result in glucitol resistance in JC10279. Most deletions extending rightward from Tn10 would be expected to terminate at the right-hand boundary of either of the inverted repeat sequences flanking the tet genes of Tn10 (Kleckner and Ross 1978). Thus, we expect some deletions to have removed the tet genes and others not. All deletions producing glucitol resistance are expected to have extended into or through srlA, so that entry of glucitol and accumulation of glucitol-6-phosphate would be prevented. Four classes of deletions can be recognized under our conditions: (1) Those that do not extend into srlC, (2) those that extend into srlC but do not remove the wild-type site corresponding to srlCI (3) those that do remove the wild-type site corresponding to srlCI but do not extend as far as recA or its promoter, and (4) those that extend into or beyond recA but do not affect the vital gene alaS. The first class would both complement and recombine with srlCI. The second class would not complement with srlCI but would recombine with it. The third and fourth classes would neither complement nor recombine with srlCI. They would differ from each other in that the third class would be Rec<sup>+</sup> UV<sup>+</sup>, while the fourth class would be Rec<sup>-</sup> UV<sup>+</sup>. Point mutants in srlA or srlC would behave like deletion mutants of classes 1 and 2.

One hundred glucitol transport-deficient strains were obtained in a single selection from each of the Srl<sup>+</sup> strains, L163 and JC10279. They were tested for complementation with the srlID+ srlA+ srlC+ and srlID+ srlA+ srlCI alleles, present on hybrid CoE1 plasmids pLC18-42 and pJC597, respectively. All were restored to growth on glucitol upon the inheritance of pLC18-42, indicating that the mutations were in the srl operon and recessive to their wild-type alleles. The results of the complementation tests with the srlCI plasmid pJC597 are presented in Table 3. The majority of mutants from both strains fell into classes 1 and 2, which are indistinguishable from point mutants by the tests employed. Relatively more of the mutants (30% vs. 10%) from the Tn10-carrying strain JC10279 fell into classes 3 and 4. Since these mutants had apparently lost the ability to produce Srl<sup>+</sup> recombinants with srlCI, they were most probably deletion mutants. To determine whether any of the Srl<sup>+</sup> mutants were in class 4, they were tested for UV<sup>+</sup> and Rec<sup>+</sup>. None of the 200 mutants were in Class 4.

Even though the first experiment had not yielded any Rec<sup>-</sup> UV<sup>+</sup> mutants, the large difference in numbers of Srl<sup>+</sup> mutants associated with the presence of Tn10 and the relatively large percentage falling in class 3 led us to test more Srl<sup>+</sup> mutants derived from JC10279. From each of 13 independent cultures of
Patterns of complementation between $srlD^+$ $srlA^+$ $srlC^+$ and the mutations conferring glucitol-transport deficiency present in glucitol-resistant derivatives of L163 ($srlD^{50}$) and JC10279 ($srlR^{301}:Tnl^O srlD^{50}$)*

<table>
<thead>
<tr>
<th>Response upon inheritance of pJC597 (ColE1 $srlD^+$ $srlA^+$ $srlC^+$ recA*)</th>
<th>Percent of glucitol-resistant derivatives with the specified response†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Srl phenotype</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
</tr>
<tr>
<td>3 &amp; 4</td>
<td>−</td>
</tr>
</tbody>
</table>

* One hundred derivatives of L163 or JC10279 were tested for ability to grow on glucitol upon mating with JC9918, an F+ strain carrying pJC597 (ColE1 $srlD^+$ $srlA^+$ $srlC^+$ recA*). The details of the experimental procedure and the interpretation of the results are explained in MATERIALS AND METHODS.

† JC10279 can give rise to two kinds of glucitol-resistant derivatives: tetracycline-sensitive ones and tetracycline-resistant ones, which are tabulated in separate columns according to the response given upon the inheritance of pJC597; L163 can give rise only to tetracycline-sensitive derivatives.

JC10279, approximately 200 colonies were screened and five UV-sensitive, recombination-deficient strains were found. Three of the five originated in the same culture, and all of these were tetracycline sensitive. Since these three might have been siblings, only one, which was called JC10282, was characterized further. The other two isolates were obtained from different cultures. Both were tetracycline resistant; they were named JC10283 and JC10284.

Derivatives of JC10276 ($srlR^{301}:Tn10 tif-1 \lambda$) resistant to the thermoinduction of $\lambda$

As was stated earlier, the fact that a functional recA product is required for the 42° induction of $\lambda$ in the tif-1 background was used as the basis for an alternate method of detection of recA deletions. We anticipated finding deletions that removed the entire srl operon and part or all of recA, with or without affecting the tetracycline-resistance genes (Figure 1B). We also anticipated finding inversions (not shown in Figure 1B) that had one endpoint in recA and the other in or adjacent to Tn10. These inversions would be expected not to interfere with the expression of the srl structural genes and to fall into two classes depending on whether or not the functioning of the Tet$^a$ genes was affected.

When plated at 42° on adenine-supplemented media, strain JC10276 gave rise to survivors at a frequency of eight per 10$^9$ cells plated, which did not differ substantially from the survival frequency (four per 10$^5$) of the isogenic strain JC10269 not harboring Tn10. Survivors obtained from JC10276 were checked for their ability to grow on glucitol, for being tetracycline sensitive, UV sensitive and recombination deficient. From one typical selection, the most prevalent class (237 out of 300 survivors) consisted of Tet$^a$ Srl$^+$ UV$^a$ Rec$^+$ strains, which could be back-mutants of tif-1 to tif$^+$ or mutants with intragenic or extragenic suppressors of tif-1 that did not cause UV sensitivity or recombination deficiency (CASTELAZZI, GEORGE and BUTTIN 1972b). A considerable number (58 out of 300) were Tet$^a$ Srl$^+$ UV$^a$ Rec$^+$, with various gradations of UV sensitivity and
recombination deficiency. These could be strains with point mutations of the recA, lexB or zab variety (CASTELLAZZI, GEORGE and BUTTIN 1972b), or they could be strains carrying inversions with one endpoint in recA and the other in Tn10 that does not interfere with the TetR genes. For our purposes, the interesting mutants were those that became TetR, and these fell into three categories: TetR Srl- UVs Rec- (two out of 300), TetR Srl+ UVs Rec- (two out of 300) and TetR Srl+ UVr Rec+ (one out of 300). The TetR Srl- UVs Rec- strains, having suffered the loss of three unrelated functions were presumed to carry deletions. One was called JC10277. Another strain, JC10278, with identical phenotype was isolated in an independent selection that yielded similar frequencies of mutants with the different phenotypes. Both were still lambda lysogens. The TetR Srl+ UVs Rec- strains could have suffered deletion of the tet genes of Tn10, coupled with an inversion with one endpoint in recA such as described by KLECKNER and Ross (1978). The TetR Srl+ UVr Rec+ strain may have been a double mutant. It and the presumed inversions were not characterized further.

Characterization of the putative recA deletion mutants

With two alternate selection procedures, we isolated five independent UV-sensitive and recombination-deficient mutants, three of which (JC10277, JC10278 and JC10282) also lost both the ability to express tetracycline resistance, conferred originally by Tn10 and the function of at least one of the srl genes. Since these lost in one step three unrelated functions, we hypothesized that they carried deletions that extended from a point within Tn10, past srlD, srlA and srlC, into or beyond recA. The other two mutants being TetR might have been produced by deletions or they might have been produced by inversions associated or not with Tn10. Characterization of the TetR mutants will be taken up in the next section.

In general, the most rigorous genetic criterion that a given mutation is a deletion is that there exists a set of mutations of different map positions within the gene in question that are unable to recombine with the presumed deletion. Since we are dealing with mutations that confer extreme deficiency in recombination (see below), we could not carry out the usual genetic mapping to prove that the mutations isolated are deletions of recA. Two less definitive tests that we could perform were to see whether, in the presumed deletion-bearing strains, the srl and recA mutations were completely linked and whether the linkage between srl and alaS had been increased.

To perform these tests, the rec mutations obtained by both selections were transduced into strain JC10206 (alaS3), selecting growth at 42°C on glucose minimal media. For the sake of comparison of the linkages of srl and recA point mutations, a transduction was also carried out using strain JC10285 (srl301::Tn10 srlD50 recA35) as the donor. At the level of resolution of the scoring allowed by 100 recombinants in each cross, there was complete linkage of the srl and rec defects from the putative deletion-bearing strains (see Table 4), whereas with the double point mutant JC10285 as the donor, 10% of the transductants that inherited recA35 did not inherit srlD50. Furthermore, when the presumed dele-
TABLE 4

The linkage, in P1 transduction, of the srl recA mutations to alas*

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Genotype</th>
<th>Number of 100 Alas+ transductants with phenotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Srl-Rec+</td>
</tr>
<tr>
<td>JC10277</td>
<td>del(srlR-recA)302</td>
<td>100</td>
</tr>
<tr>
<td>JC10278</td>
<td>del(srlR-recA)303</td>
<td>100</td>
</tr>
<tr>
<td>JC10282</td>
<td>del(srlR-recA)304</td>
<td>91</td>
</tr>
<tr>
<td>JC10285</td>
<td>srlID50 recA35</td>
<td>87</td>
</tr>
</tbody>
</table>

* In each cross, the recipient was JC10206 (srl+aZaS3) and growth on glucose minimal medium at 42° was selected.
+ The derivation of the first five strains is indicated in Figure 1; the derivation of the sixth is in Table 1.

Some of the phenotypic consequences of the three recA deletions so defined were measured after the deletions had been transduced into the genetic background of AB1157. The transductants were compared with the isogenic strains JC2926 and JC2922 carrying the point mutations recA13 and recA35, respectively, and also with those of the recA+ strain AB1157. These two point mutations were chosen because the recA13 allele is dominant to recA+ in ColEl recA13/recA+ heterozygotes, whereas recA35 is essentially recessive when carried by the plasmid (L. Csonka, A. Templin and A. J. Clark, manuscript in preparation). As can be seen from the data in Table 5, the reduction in recombinational proficiency caused by the recA deletions was similar to that conferred by the two point mutations. The growth rates and UV sensitivities of the strains

TABLE 5

The loss in recombinational proficiency conferred by the srl recA mutations*

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Genotype</th>
<th>Recombinational deficiency index</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157</td>
<td>recA+</td>
<td>1.0</td>
</tr>
<tr>
<td>JC10290</td>
<td>del(srlR-recA)302</td>
<td>3.6 \times 10^4</td>
</tr>
<tr>
<td>JC10291</td>
<td>del(srlR-recA)303</td>
<td>5.1 \times 10^4</td>
</tr>
<tr>
<td>JC10287</td>
<td>del(srlR-recA)304</td>
<td>5.6 \times 10^4</td>
</tr>
<tr>
<td>JC10288</td>
<td>crg(srl-recA)305</td>
<td>1.8 \times 10^4</td>
</tr>
<tr>
<td>JC10289</td>
<td>del(srlR-recA)306</td>
<td>3.6 \times 10^4</td>
</tr>
<tr>
<td>JC2922</td>
<td>recA35</td>
<td>6.1 \times 10^4</td>
</tr>
<tr>
<td>JC2926</td>
<td>recA13</td>
<td>5.1 \times 10^4</td>
</tr>
</tbody>
</table>

* The recombinational ability of the strains was quantitated by recombinational deficiency index, as explained in MATERIALS AND METHODS.
† Five of these strains are not included in Table 1. Each is an Alas+ transductant derived by P1 transduction of JC10206. Each transductant had also inherited Rec-Srl+ properties characteristic of their donor parents. Pairs of donor and transductant progeny are, respectively, as follows: JC10277 and JC10290, JC10278 and JC10291, JC10282 and JC10287, JC10283 and JC10288, JC10284 and JC10289. Derivation of the donor strains is indicated in Figure 1.
with the *recA* deletions were similar to those of the strains with the point mutations (Figure 3). Strains carrying *recA* deletions exhibited similar sensitivity to nitrofurantoin as did the point mutants (data not shown) and were unable to plaque *red gam* double-mutant derivatives of phage λ (data not shown). In addition, neither spontaneous nor UV-stimulated induction of λ occurred in the deletion-bearing strains (data not shown). Qualitative tests showed that the

![Figure 3](image_url)

**Figure 3.**—Survival of *recA* deletion strains following UV irradiation. Cells were irradiated and plated as described in MATERIALS AND METHODS. The strains tested were as follows: *recA*+, AB1157; *recA35*, JC2922; *recA13*, JC2926; del(*srR-recA*)302, JC10290; del(*srR-recA*)303, JC10291; del(*srR-recA*)304, JC10287; crg(*srR-recA*)305, JC10288; del(*srR-recA*)306, JC10289. For derivation of the latter five strains, see footnote to Table 5. Because results for the deletion and possible deletion mutant strains fell in a narrow range, we have averaged them. The range at each point is shown by the vertical line.
deletions were recessive to the wild-type allele when recA+ was carried on a ColE1 plasmid. As yet, we have not isolated plasmids carrying the deletions, so that their expected recessiveness as plasmid markers was not tested.

**Characterization of the TetR UV8 Rec- mutants**

Two TetR UV8 Rec- strains had been isolated from the selection that had yielded glucitol-resistant mutants. Three types of mutations might account for this phenotype: (1) deletions stemming from the right-hand terminus of Tn10 (see Figure 2), (2) inversions in which one end point was in srl and the other in recA, and (3) double point mutations. Some evidence that the strains do not carry point mutations in both srl and recA is shown in Tables 4 and 6. No separation of the Srl- and Rec- properties was observed in the crosses of the putative deletion mutants, while 3.2% of the transductants from a bona fide double point mutant were Srl+ Rec-. Without knowing which type of rearrangement had occurred, we provisionally called the mutations crg(srl-recA)305 and crg(srl-recA)306 in JC10283 and JC10284, respectively.

The cross results shown in Table 6 also allowed us to test the closeness of these mutations to Tn10, since the frequency of TetR Srl+ recombinants indicates the frequency of recombinational separation of Tn10 and the mutation causing Srl-. From the srlR301::Tn10 srlD50 recA35 donor, one of the AlaS+ transductants (0.5% of the total) was TetR Srl+ and two (1.1%) were TetS Srl-, indicating that recombination could take place between Tn10 and srlD50. No such transductants were found when either of rearrangement mutants was the donor. In addition with the double point mutant donor, seven (3.8%) of the AlaS+ transductants were TetR Rec-, indicating the amount of recombination

**TABLE 6**

<table>
<thead>
<tr>
<th>Donor strain and genotype</th>
<th>Number of transductants screened</th>
<th>Percent of AlaS+ transductants with phenotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Srl-Rec-</td>
</tr>
<tr>
<td>JC10283 (srlR301)</td>
<td>188</td>
<td>TetR 94.1</td>
</tr>
<tr>
<td>::Tn10-83 crg(srl-rec)305</td>
<td></td>
<td>TetS &lt;0.6</td>
</tr>
<tr>
<td>JC10284 (srlR301)</td>
<td>189</td>
<td>TetR 89.9</td>
</tr>
<tr>
<td>::Tn10-84 del(srl-recA)306</td>
<td></td>
<td>TetS &lt;0.6</td>
</tr>
<tr>
<td>JC10285 (srlR301)</td>
<td>185</td>
<td>TetR 86.6</td>
</tr>
<tr>
<td>::Tn10-79 srlD50 recA35</td>
<td></td>
<td>TetS 1.1</td>
</tr>
</tbody>
</table>

* The linkage of the tetracycline resistance element to alaS was determined by the transducing JC10206 (alaS3) to growth at 42° by a P1 lysate grown on the indicated donor strains.

† Derivation is indicated in Figure 1 and Table 1.
between srlR301::Tn10 and recA35. No such recombinants were found with the strains carrying the rearrangements.

Although this test showed that the rearrangements were very closely linked to Tn10, we wanted to increase the sensitivity of the test. Thus, the transductions were repeated with JC10206 (srl+ mtl-l alaS3) as recipient, selecting this time for TetR at 33°C on glucitol MacConkey plates supplemented with tetracycline. On these indicator plates, it was possible to spot easily by color any tetracycline-resistant transductants that were Srl+. With JC10285 (the srlD50 strain) as the donor, from 2154 TetR transductants, 144 (6.7%) were Srl+. This gives us the frequency of recombination we can expect between an srl point mutation and Tn10. Additionally we tested these TetR Srl+ derivatives to determine what fraction retained the srlR301::Tn10 mutation capable of suppressing mtl-l. Of the 144 such derivatives, 141 (97.9%) were Mtl+ and thus carry srlR301::Tn10. Three (2.1%) of the TetR Srl+ transductants were formed by a process that did not retain the srlR mutation. One possibility is that transposition of Tn10 occurred from a transducing fragment to the chromosome outside the srl region. Such an event, although at higher frequencies, has been described by Kleckner et al. (1978).

Similar transductions were carried out using AlaS+ Rec- Srl- TetR transductant derivatives purified from the crosses described in Table 6. The results for the two strains differed and will be discussed separately. In the transduction with JC10288 (carrying crg (srl-recA)305 from JC10283) as donor, 103 out of 1418 TetR transductants (7.3%) had retained Srl+, a result similar to the 6.7% that had retained Srl+ when JC10285 (srlD.50) had been used as donor. Of the 103 TetR Srl+ transductants, 101 (97.1%) had retained the srlR301::Tn10 mutation suppressing mtl-l, a result similar to that obtained with the srlD point mutant. This implies that part of the srl locus remains between Tn10 and the mutation causing Srl-; hence, the left-hand boundary of crg (srl-recA)305 can not be the right-hand boundary of Tn10. This separation of the mutation from Tn10 means that we can not be certain that the mutation is a deletion rather than an inversion. We think a deletion is more likely, because all of the 1315 TetR Srl- transductants in this cross were glucitol resistant, indicating no recombinational separation between srlD50 and crg(srl-recA)305.

With the donor JC10289 (a derivative of JC10284), only seven (0.13%) of 5091 TetR transductants retained the Srl+ phenotype of the recipient and all seven were Mtl-, indicating that they did not carry srlR301::Tn10. Presumably these resulted from transposition of Tn10 since the frequencies of such transductants in the previous cases were similar: three of 2154 (0.13%) and two of 1418 (0.14%). Thus, it appears that recombinational separation between Tn10 and crg(srl-recA)306 can not be obtained, and the left-hand end point of the deletion must either be at the right-hand end point of Tn10 or between the tet genes and this right-hand end point. (Figure 2). Since the deletion thus removes part of srlR and inactivates either or both srlA and srlC, as well as recA, we can change the name of the mutation to del(srlR-recA)306.
Results in Table 5 and Figure 3 indicate that the Rec- and UV-sensitive phenotypes associated with crg(srl-recA)305 and del(srl-recA)306 are those of standard recA point mutations.

**Characterization of the Tn10 transposons associated with crg(srl-recA)305 and del(srl-recA)306**

Consideration of Figure 2 shows that del(srl-recA)306 may have its left-hand end point within Tn10 and still leave the tet genes intact. If this were the case, part or all of the 1.4 kb inverted repeat sequence would be lost. Since intact inverted repeat sequences are essential for the transposition of Tn3 (Heffron et al. 1977), it seems likely that they would also be required for transposition of Tn10. To test the possibility that Tn10–84 (the transposon in the del(srl-recA)306 mutant) may be transposition deficient, we compared its transposition ability with those of Tn10–83 and Tn10–79, the transposons in the crg(srl-recA)305 strain and the original srlR300::Tn10 strain, respectively. The results (Table 7) show that all three transposons transpose with about the same frequency. Thus, it seems likely that the left-hand endpoint of del(srl-recA)306 is the right-hand endpoint of Tn10–84.

**DISCUSSION**

**Phenotype associated with deleting recA**

Many point mutations in recA drastically reduce homologous recombination and resistance to UV irradiation in E. coli. In addition, they show varying degrees of dominance to recA+ (Clark 1967, 1973; L. Csonka, A. Templin and A. J. Clark, in preparation), implying an interference by the mutant recA product with the functioning of the wild-type recA product or the products of other genes. These we call standard recA mutations. Other mutations apparently in recA have intermediate and differential effects on recombination and UV resistance (cf., Castellazzi, George and Buttin 1972a,b; Glickman, Gulit and Morand 1977). These mutations may owe part or all of their phenotypic defects to inter-

**TABLE 7**

*Translocation ability of the tetracycline resistance elements Tn10–83 and Tn10–84*

<table>
<thead>
<tr>
<th>Donor strain†</th>
<th>Genotype‡</th>
<th>Translocation frequency of the tetracycline resistance element</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC10304</td>
<td>srlR301::Tn10–83 crg(srl-recA)305</td>
<td>4.4 × 10⁻⁶</td>
</tr>
<tr>
<td>JC10305</td>
<td>srlR301::Tn10–84 del(srl-recA)306</td>
<td>5.6 × 10⁻⁶</td>
</tr>
<tr>
<td>JC10302</td>
<td>srlR301::Tn10–79 srlD50 recA35</td>
<td>3.7 × 10⁻⁶</td>
</tr>
</tbody>
</table>

*The translocation frequency of the drug-resistance element is quantitated as the ability of the tetracycline-resistance characteristic to be mobilized by an F factor (cf., MATERIALS AND METHODS).† These strains were made by crossing each of three srlR301::Tn10 strains with JC7221 as donor. Selection was made for Thr+ Leu+ [Ura+] transconjugants inheriting pJC536. Recipients and derived transconjugants are, respectively, as follows: JC10288 and JC10304, JC10289 and JC10305, JC10285 and JC10302. The derivation of the three recipient strains is explained in Table 1 and a footnote to Table 5.‡ The tetracycline-resistance element in JC10304 is denoted Tn10–83 and the one in JC10305 is denoted Tn10–84, even though they are probably identical to Tn10–79 in JC10302.
action of mutant recA protein with other gene products; e.g., lexA protein. To identify the phenotype of a mutant devoid of recA product, we isolated a strain carrying srlR3O::Tn10 to use the Tn10 element to generate deletion mutations affecting nearby recA.

We employed two different selections to detect mutants among which we expected to find recA deletions. In the first case, we asked for loss of function of the glucitol-transport genes, srlA or srlC. In this case, we found a 10-fold greater number of SrlR mutants in the presence of Tn10 than in its absence, implying that only about 0.1% of mutants from the Tn10 strain would have been generated spontaneously rather than because of Tn10. An average of 15% of the 2600 Tn10 generated SrlR mutants were tetracycline sensitive and, of these, three from one culture were Rec- UVs. Suspecting that the three might represent a clone from a single mutation, we examined only one. Several pieces of evidence indicate that this TetR mutant is not an inversion mutant like those studied by Kleckner and Ross (1978), but rather is a deletion mutant. First, glucitol-transport function determined by srlA and srlC was affected along with recA. Second, the mutant did not have the wild-type functional srlC gene nor did it have the wild-type site corresponding to srlC1, as shown by absence of complementation and SrlR recombinant formation. Third, no recombinational separation of mutations leading to the Srl- and Rec- defects occurred.

The second selection did not show an increase in the number of 42CR mutants correlated with the presence of Tn10 near the recA allele, tif-1, probably due both to the high background frequency of spontaneous back-mutations and intragenic suppressor mutations in recA and to a low frequency of deletions extending as far as recA from Tn10. Nonetheless, two Rec- UVs mutants were found that had become Srl-. These also failed to complement and recombine with srlC1. In addition, recombinational separation of mutations determining Srl- and Rec- was not observed. Taken together, these results indicate that these two TetR Rec- strains had suffered a deletion and not an inversion coupled with loss of the tet genes and a second mutation producing SrlR.

The two TetR Rec- strains detected in the screening for SrlR mutants also probably carry deletions affecting recA. Neither show complementation or recombination with srlC1, nor allow recombinational separation of mutations leading to Srl- and Rec- . In one case, the mutation seemed to terminate at one of the outside ends of Tn10, which fits the hypothesis of Kleckner and Ross (1978) for the nature of Tn10-generated deletions. In the other case, however, the mutation terminated at a distance from Tn10, and we could not decide whether it is a deletion or an inversion. Since neither possibility would fit the types of inversions and deletions generated by Tn10 and studied by Kleckner and Ross (1978), we have had to ask whether or not this mutation was generated by the proximity of Tn10. The alternative that it was a spontaneous mutation can not be ruled out from our evidence on mutant frequencies. Should it happen to have been Tn10 generated, it would be important because neither end point of the deletion would be adjacent to any part of the transposon.
The phenotype of five deletion mutants mimics that of standard recA point mutants with regard to both recombination deficiency and UV sensitivity. The only discrepancy appears at higher doses of UV irradiation, where the numbers of survivors of the standard point mutants are greater than those of the deletion mutants. Discounting the possible significance of these survivors, we conclude that it seems unlikely that standard recA mutations owe their recombination deficiency and UV sensitivity to an interaction of mutant recA protein with other gene products.

At present, however, we do not know which deletion mutations have removed all and which only part of the recA gene. In the latter, the promoter of recA would be deleted since it is thought to be on srl side of the gene (K. McEntee, personal communication; D. Rupp, personal communication) whence stem all five deletion mutations. Whether any remaining portion of recA would be sense-wise expressed from another promoter to which it had been fused is a matter for conjecture. With two of the deletion mutants, we have been unable to detect by one-dimension acrylamide gels any UV induction of recA protein (M. Volkert and A. J. Clark, unpublished results). Since there is a slight amount of protein at the position of recA protein in nonirradiated cells, we are unable to say at this time that the deletion mutants produce absolutely no recA protein. Two-dimensional gels or experiments with antiserum to recA protein may settle this matter.

Regulation of the srl operon

On the basis of the observation that srlC mutations abolish the expression of both srlA and srlD, and that this effect can be corrected by lysogenization with a λ phage carrying srlC+, but not srlA+ and srlD+, McEntee (1977a) proposed that srlC+ is a positive regulatory gene for the other two srl genes. Negative control had, however, already been suggested by Jones-Mortimer and Kornberg (1976). Our isolation of a Tn10-induced mutation (srlR301::Tn10), which confers constitutivity to the srl structural genes and which Joseph Lengeler (personal communication) finds is recessive to srlR+, supports the hypothesis of negative control. While it is possible that srlA and srlD are under both positive and negative control, we would like to suggest the alternative hypothesis that srlC is not a positive regulatory gene. The product of the srlC+ gene might instead be a component of the glucitol uptake and phosphorylation system, perhaps the enzyme III of glucitol transport hypothesized by Saier (1977). In this case, srlC mutations would have a pleiotropic negative effect because induction of srlA and srlD depends on a low constitutive functioning of the uptake system for the accumulation of the inducer (Lengeler and Steinberger 1978). Such pleiotropic negative effects of transport-deficiency mutations have been found in experiments with the put, mal and lac operons (Ratzkin, Grabnar and Roth 1978; Silhavy et al. 1976; and Beckwith and Rossow 1974, respectively).

There is some evidence, that supports our hypothesis. Glucitol can also be taken up and converted to glucitol-6-phosphate by the transport system normally induced by a third hexitol, galactitol, provided the relevant gene, gatA, is expressed constitutively (Lengeler 1975b, 1977). Strains constitutive for gatA
can grow on glucitol even if they carry the \(sr\)l\(C\) mutation (A. Templin and A. J. Clark, unpublished results; J. Lengeler, personal communication). The implication is that the product of the \(sr\)l\(D\) gene, which is absolutely required for the metabolism of glucitol-6-phosphate (Lengeler 1975a, 1977), can be expressed without a functional \(sr\)l\(C\)+ gene product. This would not be expected if \(sr\)l\(C\) were an obligatory positive control gene.

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LITERATURE CITED


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