JOURNAL OF BACTERIOLOGY, Dec. 1977, p. 904-911
Vol. 132, No. 3
Copyright © 1977 American Society for Microbiology
Printed in U.S.A.

Genetic Analysis of the *Escherichia coli* K-12 *srl* Region

KEVIN McENTEE†

Departments of Biochemistry and of Biophysics and Theoretical Biology, University of Chicago, Chicago, Illinois 60637

Received for publication 26 July 1977

Specialized transducing λ derivatives, deletion mapping, and P1*kc* transductional crosses have been used to analyze the genetic organization and regulation of the *srl* genes. Transducing phages obtained from a secondary site λ insertion in *srlA* are of two types: λp*srlC*1 and λp*recA* are substituted in the *b*2 region of the λ chromosome (galtype) and carry the *srlC* gene but not *srlD*; λp*srlD* is substituted in the early region of the phage deoxyribonucleic acid (biotype) and carries the *srlD* gene but not *srlC*. The λp*srlC*1 phage, which lysogenizes at *att*λ, complements *srlC* mutants in *trans*, indicating that this gene codes for a diffusable positive regulatory element. The *srl* genes have been ordered relative to the *cysC, recA,* and *alaS* genes by two- and three-factor P1*kc* crosses. The order, *cysC* ... *srlD-srlA-srlC-recA-alaS*, has been obtained. The *srlA* and *srlD* genes comprise an operon with *srlD* operator distal. From the secondary site lysogen, it has been possible to obtain deletion mutants of this region that are sensitive to ultraviolet light and are recombination deficient. Genetic evidence suggests that these deletions extend from *srl* into the *recA* gene.

The *srl* locus of *Escherichia coli* is a group of genes that are necessary for metabolizing D-glucitol (sorbitol). At least three genes are involved specifically in D-glucitol utilization, and two of these genes, *srlA* and *srlD*, code for enzymes that participate in converting D-glucitol to fructose 6-phosphate. The *srlA* gene product (D-glucitol enzyme II) catalyzes the phosphorylation of D-glucitol to D-glucitol 6-phosphate via the phosphoenolpyruvate-dependent phosphotransferase system (6, 7). Nicotinamide adenine dinucleotide-linked D-glucitol 6-phosphate dehydrogenase (EC 1.1.1.140), the *srlD* gene product, converts D-glucitol 6-phosphate to fructose 6-phosphate. In this paper the *srlA* and *srlD* genes are viewed as being identical to the genes called *gutA* and *gutD*, respectively, in Lengeler's nomenclature. Lengeler and Lin (8) have demonstrated that the *srlA*- and *srlD*-coded activities are induced in cells by growth in the presence of D-glucitol. Mutations that constitutively express the *srlA* and *srlD* genes have been described by Lengeler (6) and are tightly linked to *srlA* and *srlD*. Reversion and complementation experiments have suggested that these constitutive mutations are *cis*-dominant regulatory mutations that could alter an operator or an initiator site in *srl*. Jones-Mortimer and Kornberg (5) have described a class of conditional regulatory mutations whose properties are consistent with negative regulation of the *srlA* and *srlD* genes. The relationship of these mutations to the constitutive class isolated by Lengeler was not elucidated, however.

I have recently demonstrated that the *srl* genes contain a site for *int*-promoted insertion of phage λ (11). This secondary site is revealed when the host *att*λ region is deleted. The secondary-site lysogens have been used to generate transducing phages for the nearby *recA* gene (12). The studies of the *recA* transducing phages indicated that at least one *srl* gene is incorporated into the phage chromosome. Furthermore, it is possible to isolate phages that carry this *srl* gene (or part of it) but do not transduce *recA* (13). In this paper I report the results of a genetic investigation of the organization and regulation of the *srl* region. The availability of transducing phages for the *srl* genes, as well as a lysogen with λ inserted into *srl*, has facilitated these studies. The results indicate that *srlA* and *srlD* are part of an operon which is under control of a positive regulatory element, *srlC*. The *srlC* gene acts in *trans* and therefore codes for a diffusable product. It has also been possible to study the *srl-recA* region by using deletions obtained from the secondary site lysogen. Evidence is presented that deletions extending from *srlA* into *recA* can be isolated. These deletions are helpful in further analyzing this region as well as studying the specificity of λ insertion into the *srl* operon.

† Present address: Department of Biochemistry, Stanford University Medical School, Stanford, CA 94305.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The bacterial strains and phages used in this study are listed in Table 1. Genetic abbreviations are those of Bachmann et al. (1).

Media. KML complex medium and K115 minimal medium have been described (4). Solid minimal media contain 10 g of the appropriate sugar per liter, except that D-glucitol minimal medium contains 3 g/liter. Minimal media are supplemented with amino acids at 25 mg/liter and with vitamins at 1 mg/liter as required. Nitrofurantoin plates contain 2 mg of the drug per liter of KML medium. Indicator media consist of 10 g of the appropriate sugar and 40 g of MacConkey agar base (Difco Laboratories, Detroit, Mich.) per liter.

Genetic methods. Transduction with P1*kc* and isolation of λ^r strains were performed as described by Miller (14). Matings were performed by mixing 0.2 ml of a recipient culture in KML medium containing 4×10^8 cells per ml with an equal volume of donor culture at the same cell density, incubating without agitation

TABLE 1. *Bacterial strains and phages*

Strain	Relevant genotype[a]	Origin or reference[b]
Bacterium		
KL 16-99	Hfr KL16 *recA1 thiA*	K. B. Low
KL387	F^- *argA cysC alaS5 supD*	K. B. Low
KM601	F^- *recA1 supE thr leu rpsL*	
KM2136	F^- *lysA argA (gal-att λ-bio) Δ2134 srl(λcI857) mtl*	(12)
L144	F^- *argA metB hisl galT mtlA gutC^{c}A51D$^+$ lacY rpsL*	J. Lengeler
L146	As L144 except *galK gutC^{c}A50D$^+$*	J. Lengeler
L163	F^- *mtlA gutC^{c}D gatC^{c}A metB rpsL*	J. Lengeler
L174	As L144 except *lacI gutC^{c}A174*	J. Lengeler
L195	As L144 except *nalA lysA gutC^{c}A195*	J. Lengeler
L245	Hfr Cavalli *gutC$^+$D245*	J. Lengeler
SC1100	F^- *lysA argA cysC39 rpsL lac*	
WA5022	F^- (P2)	J. Zissler
KM100[c]	*srlC1 cysC39*	
KM500[c]	*srlC5 cysC39*	
KM600[c]	*cysC39 alaS5*	(KL387) × KM100 → *srl*$^+$
KM700[c]	*srlC5 alaS5*	
KM800[c]	*gutC^{c}D163 alaS5*	(L163) × KM600 → *cys*$^+$
KM900[c]	*cysC39*	
KM1612[c]	*gutC^{c}D163 recA12*	
KM2307[c]	*(srl-recA) Δ7*	This work
KM2321[c]	*(srl-recA) Δ21*	This work
KM2400	*srlC1 cysC39 pheA*	
KM2445	*pheA gutC$^+$D245*	(L245) × KM2400 *srl*$^+$ → *cys*$^+$
KM2473	*pheA gutC^{c}D163*	(L163) × KM2400 *srl*$^+$ → *cys*$^+$
KML144	See Results	(L144) × KM600 → *alaS*$^+$
KML146	See Results	(L146) × KM600 → *alaS*$^+$
KM174	See Results	(L174) × KM600 → *alaS*$^+$
KM195	See Results	(L195) × KM600 → *alaS*$^+$
Bacteriophage[d]		
λ	wild type	
λ*bio11nin5*	(*fec*$^-$)	R. Gayda
λ*proAB*	(*fec*$^-$)	R. Weisberg
λp*srlC1*		(13)
λp*recA*		(11)
λp*recA99*		(12)

[a] All strains obtained from J. Lengeler or transductants derived therefrom are described by the genetic symbol *gut* instead of the equivalent *srl* designation.

[b] Crosses show donor first, recipient second. The selected marker is indicated after the arrow. Transductional crosses with P1*kc* are listed with the donor in parentheses. Where no source is given, the strain is from the laboratory collection.

[c] Constructed from strain SC1100; all are F^- *lysA argA mtl rpsL lac* and nonsuppressing unless otherwise stated.

[d] All λ derivatives carry the temperature-sensitive repressor mutation *c*I857.

for a sufficient time (usually 30 to 60 min at 37°C), and plating volumes of serial dilutions on appropriate selective medium. Phage plating and titering were done as described (12). For plating of phage λ and λ transducing derivatives, all host strains were pregrown in KML medium containing 0.2% maltose.

Selection for *alaS*$^{+}$ transductants was done at 42°C on D-glucitol MacConkey plates, whereas that for *recA*$^{+}$ was done on nitrofurantoin plates. Strains carrying *recA* mutations are killed by 2 μg of this drug per ml, whereas *recA*$^{+}$ strains grow at this concentration. In both of these selections, the cells were grown under permissive conditions (30°C and absence of nitrofurantoin, respectively) for 2 h after P1*kc* adsorption and then were plated on selective medium. Reversion analysis of *srl* mutants was done by plating cells grown on KML medium onto D-glucitol minimal medium. Srl^{+} colonies were picked and tested on indicator plates.

Measurement of UV killing. Semiquantitative determination of ultraviolet (UV) sensitivity was performed by streaking cells from a log-phase culture onto KML plates. Streaks were irradiated in zones for 5, 10, 20, and 30 s of UV exposure (ca 50 to 300 ergs/mm^{2}). Strains carrying *recA* mutations were completely killed by a 10-s exposure and showed poor survival in the 5-s exposure zone. Wild-type strains showed confluent growth in all exposure zones. Quantitative UV survival measurements were performed by diluting logarithmic-phase cells grown in K115 minimal medium containing glucose and Casamino Acids each at 2 g/liter with K115 minimal medium to a cell density of 1×10^{8} to 2×10^{8} cells per ml. Cells were irradiated in sterile petri dishes on a shaking platform. Dilutions of the culture were made in K115 medium, and volumes were plated. Plates were incubated for 24 to 36 h at 30°C.

Enzyme assays. Cells were grown overnight in K115 medium containing Casamino Acids (1 g/liter) and glycerol (2 g/liter), with or without D-glucitol (2 g/liter). The cells were centrifuged, washed with a solution containing 50 mM tris(hydroxymethyl)aminomethane-chloride (pH 7.6), and disrupted with a Branson sonifier. The D-glucitol enzyme II assay was performed essentially as described by Curtis and Epstein (3). The assay contained (in a total volume of 0.1 ml): D-[*U*-^{14}C]glucitol (9.5 mCi/mmol), 50 μM; phosphoenolpyruvate, 12.5 mM; enzyme I-Hpr extract (50 mg/ml), 20 μl; $MgCl_2$, 50 μM; tris(hydroxymethyl)aminomethane-chloride (pH 7.6), 25 mM; and 5 μl of crude extract. D-glucitol 6-phosphate dehydrogenase was measured by the coupled reduction of dichlorophenolindophenol as described by Lengeler and Lin (8). Both assays were performed under conditions in which activity was proportional to the amount of extract and linear with time. In all extracts, including those from strains grown without inducer and the *srl* mutants, a background activity was detected that interfered with D-glucitol enzyme II measurements. Because this activity was most prominent during the first 8 min of the reaction, the D-glucitol enzyme II levels for all strains were calculated from the amount of product formed between 8 and 15 min of incubation, when the contribution from this second activity was low. Protein concentrations were determined by the method of Lowry et al. (10).

Chemicals. D-Glucitol was purchased from Pfanstiehl Laboratories, Waukegan, Ill. D-Glucitol 6-phosphate and nitrofurantoin were obtained from Sigma Chemical Co., St. Louis, Mo. D-[*U*-^{14}C]glucitol was purchased from Amersham/Searle, Arlington Heights, Ill., and was purified on Dowex-1 to remove contaminants.

RESULTS

Characterization of λp*srlC* and λp*srlD* transducing phages. We have previously reported that the λp*recA* and λp*srl*-1 transducing phages, which were obtained by thermal induction of strain KM2136, carry a portion of the *srl* region (12). These phages transduce strains KM100 and KM500 to growth on D-glucitol. To determine the *srl* gene(s) carried by λp*recA* and λp*srl*-1, these phages were tested for *srlD* transducing activity in strains KM2445 and KM2473. Neither phage transduced these *srlD* mutants to *srl*$^{+}$ ($<10^{-7}$), indicating that the *srlD* gene is not carried by these phages.

The λp*recA* and λp*srl*-1 phages have incorporated bacterial deoxyribonucleic acid into the nonessential *b2* region of the phage chromosome (12), i.e., galtype substitutions. A second class of transducing phage, substituted in the early region of λ (biotype), was selected by plating a low-frequency transducing lysate from strain KM2136 onto the P2 lysogenic host WA5022. Phages that form plaques on this strain (Spi^{-}) have lost the *gam* and *red* genes; wild-type λ (and λp*srl*-1) fail to grow (Spi^{+}). Three Spi^{-} phages were grown lytically on strain KM100, and the lysates were tested for *srlD* transducing activity. All three Spi^{-} phages transduced the *srlD* mutants to *srl*$^{+}$ in spot tests on minimal or MacConkey medium. However, none of these Spi^{-} derivatives transduced strains KM500 or KM100 to *srl*$^{+}$ ($<10^{-5}$).

Two independent *srlD*$^{+}$ lysogens of strain KM2445 were purified, grown, and thermally induced (see Materials and Methods). Each of the cultures produced low titer lysates (ca. 10^{6} to 10^{7} plaque-forming units per ml) that contained *srlD* transducing particles. Because the λp*srlD* phages are deleted for early phage genes, they lack the *int* gene and lysogenize by *recA*-dependent homologous recombination at the host *srl* region. This *recA* dependent lysogenization by λp*srlD* was confirmed by using strain KM1612, a *srlD recA12* double mutant. This strain could not be transduced to *srl*$^{+}$ with the *srlD* transducing phages.

The λp*recA* phage lysogenizes by integrating at or near the *att*λ site (12). Therefore, the ability of λp*recA* to transduce strains KM100

and KM500 to *srl*⁺ is due to complementation of the *srl* mutations in these strains rather than recombination at *srl*. The λp*srl*-1 phage was obtained from the same secondary site lysogen as λp*recA* and contains the same hybrid attachment site (ΔOP′). It is expected, therefore, that λp*srl*-1 also lysogenizes at or near the normal attachment site. Two experiments were performed to confirm that λp*srl*-1 integrates in the *att*λ region. (i) An *att*λ deletion derivative of strain KM100 was infected with λp*srl*-1. The lysogenization frequency of this strain is reduced more than 100-fold, compared to the *att*λ⁺ parental strain. (ii) Strain KM5123 (*srl5 recA123*) is efficiently transduced to *srl*⁺ with λp*srl*-1 or λp*recA*99 (13), which lack a functional *recA* gene. I conclude from these experiments that λp*srl*-1 carries an intact *srl* gene that complements in *trans* the *srl* mutations of strains KM100 and KM500.

Regulation of the *srl* genes. The nature of the defect of D-glucitol metabolism in strains KM100 and KM500 was investigated by measuring D-glucitol enzyme II and D-glucitol 6-phosphate dehydrogenase in extracts of these strains (see Materials and Methods). The results of these experiments are shown in Table 2. Neither strain KM100 nor KM500 shows significant induction of either enzymatic activity after growth in the presence of D-glucitol. In the *srl*⁺ control strain, the levels of these activities increased 10- to 20-fold after induction (Table 2). Lysogenization of strain KM500 by λp*srl*-1 restores the ability of this mutant to induce high levels of both of the D-glucitol enzymes. Similar results were obtained with strain KM100 (data not shown). Although the *srl*-1 and *srl-5* mutants lack both activities, reversion of these mutations to *srl*⁺ occurs at frequencies typical of single mutations, 3×10^{-7} to 5×10^{-7}. I conclude that the pleiotropic effect of the *srl-1* and *srl-5* mutations is due to inactivation of a control gene, *srlC*. (Henceforth, the *srl-1* and *srl-5* mutations will be called *srlC1* and *srlC5* mutant alleles, respectively.) The λp*recA* and λp*srl*-1 phages carry an intact *srlC* gene that complements these mutations in *trans*. (The λp*srl*-1 phage will be designated λp*srlC*1 for the remainder of this paper.) The results of these complementation studies indicate that the *srl* genes are positively regulated by *srlC* and that this gene codes for a *trans*-acting (diffusable) product.

A different mode of regulation for the *srl* genes has been suggested by the genetic results of Lengeler (6). He has described the isolation of *srl* mutants starting with a strain constitutive for *srl* expression (designated *gutC*ᶜ). By using several selection or enrichment techniques, he was able to isolate mutants that could not utilize D-glucitol. The characterization of these *srl* mutants suggested that the genotype of these strains was *gutC*ᶜ*A*. In complementation tests these "*gutC*ᶜ*A*" strains behaved as if the original *gutC*ᶜ mutation was *cis* dominant. Another interesting feature of these strains is that little or no D-glucitol 6-phosphate dehydrogenase activity (gutD) is detectable in cell extracts, suggesting that this group of genes is an operon and that the *gutA* mutations are polar on *gutD*.

This model for the organization and regulation of *srl* can be tested by using the λp*srlC*1 and λp*srlD* transducing phages. Polar *gutA* mutations should not be complemented by λp*srlC*1 since this phage does not carry the *srlD* gene. Recombination of λp*srlC*1 at *srl* would relieve the polarity if the phage inserted in the promoter proximal section of the operon and reconstituted a wild-type *srl* operon. However, as demonstrated earlier, λp*srlC*1 efficiently lysogenizes at

TABLE 2. *D-Glucitol enzyme II and D-glucitol 6-phosphate dehydrogenase activities*[a]

Strain	*srl* Genotype	Enzyme II (nmol/min per mg of protein)		D-Glucitol 6-phosphate dehydrogenase (μmol/min per mg of protein)	
		Basal	Induced	Basal	Induced
KM900	*srl*⁺ *mtl*	0.22	5.9	0.01	0.113
KM100	*srlC1 mtl*	0.67	0.73	0.01	0.02
KM500	*srlC5 mtl*	0.01	0.12	0.01	0.013
KM500 (λp*srlC*1)	*srlC5 mtl* (λp*srlC*1)	0.20	2.1	0.028	0.103
KM2136	*srl*(λ) *mtl*	0.26	0.38	0.01	0.02
KM2307	deletion Δ 7	0.11	0.46	0.01	0.01
KM2307 (λp*srlC*1)[b]	deletion Δ 7 (λp*srlC*1)	0.39	0.39	0.016	0.016

[a] Cells were grown, sonic extracts were prepared, and assays were performed as described in the text. Basal activities were measured in extracts from cells grown in Casamino Acids and glycerol-containing medium; induced activities were from cells grown in medium that also contained D-glucitol.

[b] An Srl⁻ lysogen obtained after λp*srlC*1 infection (see text).

*att*λ far from *srl*, and recombination of the phage into the *srl* region is rare. These properties of the λp*srlC*1 phage predict that it should not transduce *gutC*^{c}*A* strains to *srl*$^+$ at high frequency. Several "*gutC*^{c}*A*" mutants (Table 1), generously provided by J. Lengeler, were tested for complementation by λp*srlC*1 and λp*srlD*. Because most of the original strains were resistant to λ, the *srl* regions of these strains were transduced by P1*kc* into strain KM600 by linkage to *alaS* (see below). As expected, no *srl*$^+$ transductants ($<10^{-5}$) were obtained after infection with the λp*srlD* lysate. When λp*srlC*1 or λp*recA* lysogens of strains KML144 (*gutC*^{c} *gutA51*), KML146 (*gutC*^{c} *gutA50*), KML174 (*gutC*^{c} *gutA*), and KML195 (*gutC*^{c} *gutA*) were selected and tested for *srl*, all 160 (40 lysogens of each strain) were *srl*$^+$ on indicator medium. Curing of the λp*srlC*1 prophage by heat pulse restored the Srl$^-$ phenotype of the parent. One *srl*$^+$ lysogen of each mutant strain was grown in liquid and thermally induced. Each culture produced a high frequency transducing lysate for λp*srlC*1 with a titer between 3×10^8 and 3×10^9 phages per ml. These results are not compatible with the suggestion that revertants of the *gutC*^{c} mutation are polar extracistronic *gutA* (*srlA*) mutations. An alternative model for the regulation of the *srl* genes suggested by my results is that the *gutC* gene is identical to the *srlC* gene described in this paper. The polar revertants obtained by Lengeler, i.e., "*gutCA*," are actually *gutC* mutations that inactivate the positive regulatory protein and are similar to the *srlC1* and *srlC5* mutations. The loss of *gutD* (*srlD*) activity in these revertant strains is not due to polarity of *gutA* mutations (which is ruled out by the transducing phage results), but to the loss of *srlC* function. The complementation data of Lengeler are also easily explained by this model (see Discussion).

Transductional mapping of the *srl* region. Our laboratory (12) and others (6) have reported a clustering of the *srl* and *recA* genes on the *E. coli* genetic map. The relative order of these genes with respect to outside markers has not been established. Furthermore, the literature reports considerable variation in the linkage of *srl* to *alaS*, the structural gene for alanyl-transfer ribonucleic acid synthetase (15). For these reasons, a more complete genetic analysis of this region was performed. Two- and three-factor transductional crosses were performed by using several of the strains listed in Table 1. The results of two-factor P1*kc* crosses are shown as linkage values in Fig. 1. The order of the genes represented in Fig. 1 is derived from the two-factor linkage values, previously published data (12), the results of three-factor P1*kc* crosses shown in Table 3, and the properties of the λ transducing phages for this region (13). The crosses listed in Table 3, section (a) establish the gene order *srlD-srlC-alaS*. Previously we have shown by three-factor analysis that the *srlC* gene lies between *recA* and *cysC* (12). These data together are consistent with

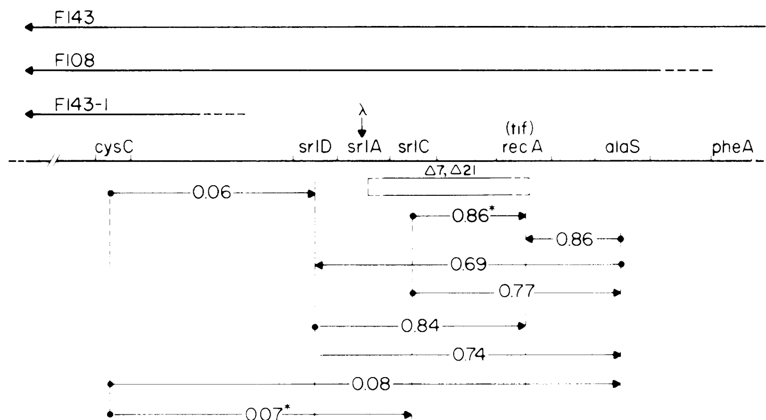

FIG. 1. *A genetic map of the srl-recA region of E. coli showing co-transduction frequencies for markers connected by arrows. The arrow points to the unselected marker in P1kc transductions, and the order of the genes is determined from the data of Table 3 and from the complementation properties of specialized transducing phages for this region. Insertion of λ between the srlD and srlC genes (presumably in srlA) is described in the text. The open box represents the recA deletions obtained in strain KM2136. Co-transduction values with an asterisk have been reported previously (12). The location of the tif-1 mutation is from Castellazzi et al. (2) and from three-factor transductional data of this laboratory (K. McEntee, unpublished results). Episomes for this region, F108, F143, and F143-1, are represented above the line (9).*

TABLE 3. *Ordering the srl region by three-factor transductional crosses*

Trans-duction	Donor	Recipient	Selected marker	No. of transduc-tants	Unselected markers	No. of transduc-tants
a	KM500 (*srlC5*)	KM800 (*srlD163 alaS5*)	*alaS*+	200	*srl*+	0
	KM2463 (*srlD163*)	KM700 (*srlC5 alaS5*)	*alaS*+	520	*srl*+	46
b	FEJ5 (*srlC5*)	KM600 (*cysC alaS5*)	*cysC*+	154	*srl*+ *alaS*+	0
					srl+ *alaS*	141
					srl alaS+	12
					srl alaS	1
	L163 (*srlD163*)	KM600 (*cysC alaS5*)	*cysC*+	146	*srl*+ *alaS*+	1
					srl+ *alaS*	131
					srl alaS+	12
					srl alaS	2
c	KM2412 (*recA12*)	KM800 (*srlD163 alaS5*)	*alaS*+	52	*srl*+ *recA*+	3
					srl+ *recA*	33
					srl recA+	4
					srl recA	12

two possible gene orders: *srlD-srlC-alaS-recA* or *srlD-srlC-recA-alaS*. However, only the latter gene order is compatible with the genetic properties of the λp*recA* transducing phage. This phage carries a *srlC* gene and the *recA* gene, but does not carry the *alaS* gene (13). Therefore, I conclude that *alaS* does not lie between the *srlC* and *recA* genes, but is distal to *recA* with respect to *srlC*. This order is confirmed by the results of transductional crosses in sections (b) and (c) of Table 3, which indicate a gene order *srlD-srlC-recA-alaS*.

Characterization of λ insertion in the *srl* region. Strain KM2136 is Srl⁻ due to the insertion of λ into one of the *srl* genes. Although this strain cannot utilize D-glucitol, it is not similar to *srlD* mutant strains since it is not inhibited for growth by this hexitol. As shown in Table 2, neither D-glucitol enzyme II nor D-glucitol 6-phosphate dehydrogenase is inducible in strain KM2136. Since λp*srlC1* and λp*recA* transducing phages that are obtained from this lysogen carry an intact *srlC* gene, the prophage must be located between the *srlD* and *srlC* genes. The properties of the transducing phages and the secondary-site lysogen can be explained most easily by assuming that the *srlA* and *srlD* genes are part of an operon with *srlD* operator distal. Integration of λ into the promoter proximal *srlA* cistron would inactivate *srlA* expression and would be polar on expression of the distal *srlD* cistron. Polarity due to λ insertion has been observed in the *trp* operon (16).

Although Lengeler has concluded that *srl* is an operon with *srlA* operator proximal (6), his conclusion is based upon presumed polarity effects of his *gutA* revertants. As described previously, complementation properties of these *gutA* strains argue against polarity effects of these mutations.

Deletions of the *srl-recA* region. The inefficient excision of λ from the secondary attachment site after temperature shift of strain KM2136 provides a powerful selection method for isolating deletions in this region. Survival of strain KM2136 is extremely low (ca 10^{-8}) at 42°C, and most survivors are *srl* mutants. These mutants are frequently deletions of the prophage that extend into adjacent host deoxyribonucleic acid. Among 1,600 *srl* survivors, six colonies were isolated that are sensitive to UV irradiation in semiquantitative survival tests (see Materials and Methods). These strains are also recombination deficient in spot matings with Hfr strains that do not transfer the *recA* gene.

Two putative *srl-recA* deletions, designated Δ*7* and Δ*21*, were chosen for further study. Transductional crosses confirmed the linkage of these mutations with *cysC* (5% [4/80] for Δ*7*, and 12.5% [10/80] for Δ*21*) and demonstrated that Δ*21* was linked to *alaS* at high frequency (95% [39/41]). The Srl and UV-sensitive properties were 100% linked in the *cysC*+ or *alaS*+ recombinants selected in these crosses. One *cysC*+ Δ*7* transductant, designated KM2307, was assayed for D-glucitol enzyme II and D-glucitol 6-phosphate dehydrogenase activity after growth in the presence of D-glucitol. Neither activity was induced in this strain (Table 2). This result is consistent with a deletion of part of the *srlA* gene and the *srlC* genes.

Lysogenization of strain KM2307 with λp*recA* or λp*srlC1* produces both *srl*+ and *srl* strains at about equal frequency. It was unexpected that

λp*srlC*1 or λp*recA* could complement a deletion of part of the *srl* operon. For this reason, the nature of the *srl*⁺ lysogens was investigated further. Upon temperature shift, these strains produced low titer lysates in which less than 0.5% (0/200) of the phages released had transducing activity for *srlC*. Strain KM2307 was lysogenized with λ*c*I or with heteroimmune λ*imm*434 *c*I⁺, and the corresponding lysogen was superinfected with λp*srlC*1 or λp*recA*. Both *srl* and *srl*⁺ transductants were obtained after infection of the heteroimmune lysogen, but only *srl* transductants were obtained in the homoimmune strain. I conclude that transduction to *srl*⁺ by λp*srlC*1 in strain KM2307 is independent of *recA*, but is dependent upon phage gene expression. These results can be explained if strain KM2307 contains a deletion that does not remove the right prophage attachment site, Δ′*OP*. Transduction to *srl*⁺ would occur by site specific recombination between this site and the Δ*OP*′ hybrid site on the phages. This reciprocal exchange would regenerate a normal phage attachment site (*POP*′) and the Δ*O*Δ′ secondary site, restoring an intact *srlA* gene. Consistent with this interpretation is the observation that deleting *att*λ from strain KM2307 reduces the frequency of *srl* lysogens formed after λp*srlC*1 infection, but does not affect the number of *srl*⁺ transductants.

The presence of a right prophage attachment site in strain KM2307 is useful for determining whether there is more than one site for λ insertion in *srl*. Transduction of strain KM2307 to *srl*⁺ by λp*srlC*1 is due to *int*-promoted recombination between the right prophage site on the chromosome and the left prophage site on the phage. Transducing phages arising from a distinct secondary site in *srl* will contain a left prophage attachment site different from that of λp*srlC*1. Such phages should not be able to transduce strain KM2307 to *srl*⁺ at high frequency since restoration of an intact *srlA* gene will not occur. Fourteen independent secondary-site *srl* insertions have been obtained in this laboratory (K. McEntee, unpublished results). Transducing phages for *srlC* were isolated from these strains and were used to infect strain KM2307. In spot transductions, *srl*⁺ recombinants were obtained at high frequency with 13 of the 14 lysates. All 14 of the lysates could efficiently complement strains KM100 and KM500 for growth on D-glucitol, suggesting that the insertion site of λ in one of the lysogens was distinct from the secondary site in strain KM2136. These results indicate that there are at least two sites for integration into *srl*, a major site in *srlA* and a minor site that has not been characterized.

The RecA⁻ phenotype of strain KM2321 was analyzed further by genetic tests. Quantitative

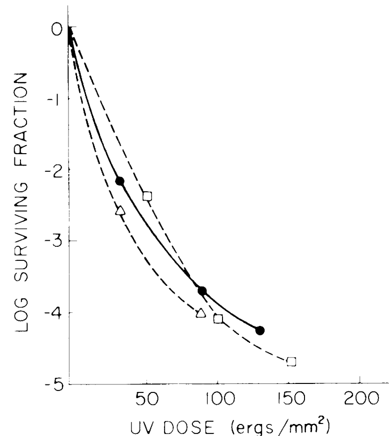

FIG. 2. *UV survival of strains carrying recA deletions Δ7 (KM2307) and Δ21 (KM2321). Cells were irradiated, and survival was measured after 36 h of incubation at 30°C as described in the text. The UV survival curve of a recA1 mutant is shown for comparison. Symbols:* ●, *recA1 strain KM601;* □, *recA Δ7 strain KM2307;* Δ, *recA Δ21 strain KM2321.*

UV survival curves (Fig. 2) indicate that the putative deletion strains are as sensitive to killing as the *recA1* missense mutant, KM601. No *arg*⁺ recombinants were formed in strain KM2321 in crosses with P1*kc* grown on strain FEJ1 or with *recA1* Hfr strain KL16-99. This recombination defect was complemented by λp*recA* (246 *arg*⁺ recombinants per 10^7 P1*kc*), but not by λp*recA*99. The λp*recA* lysogen of strain KM2321 was as UV resistant as strain KM100 in semiquantitative tests. Another characteristic of *recA* mutants is their inability to support growth of λ mutants lacking recombination functions (λ*fec*). Two λ*fec* mutants (λ*bio11* and λp*roAB*) form plaques on strain KM2321 at an extremely low efficiency, about 10^{-3}, compared with plating on the *recA*⁺ strain KM100. The efficiency of plating of λ*fec*⁺ is normal on strain KM2321 (0.68 compared with the efficiency on strain KM100).

Revertants of the *srl* mutation of strain KM2321 were sought by plating this strain on D-glucitol minimal medium. After 3 days of incubation at 37°C, slow-growing colonies were detected at a frequency of about 10^{-8}. These colonies gave a weak indicator reaction on D-glucitol MacConkey medium. Twenty recloned colonies were tested for mannitol utilization on mannitol MacConkey plates. Each gave a positive *mtl*⁺ reaction, suggesting that all of the Srl⁺ colonies are *mtl*⁺ revertants. P1*kc* grown on two of these revertants were used to transduce strain KM600 to *alaS*⁺. An *srl* mutation linked to *alaS* at high frequency was recoverable from these strains. These results suggest that reversion of strain KM2321 to D-glucitol utilization is due to

forward mutation at the *mtl* locus rather than the *srl* locus.

DISCUSSION

A genetic analysis of the *srl* locus has been performed by using specialized transducing phages for this region, P1*kc* transductional crosses, and deletion isolation. The available evidence is consistent with the *srlA* and *srlD* genes forming an operon that is under positive control by *srlC*. The *srlC* gene codes for a diffusable product, which is required for induction of the *srl* operon in the presence of D-glucitol. The genetic and enzymatic properties of *srlC* mutants are identical to the "*gutC*^{c}*A*" mutants isolated by Lengeler. These mutations result in the loss of both *srlA* and *srlD* gene expression, and this defect is complemented in *trans* by λp*srlC*1. This latter result argues against polarity effects in the "*gutC*^{c}*A*" strains and strongly suggests that these mutants lack *srlC* (*gutC*) function.

Lengeler's conclusion that the *gutC*^{c} mutation is *cis* dominant is derived from complementation tests performed with diploids: *mtlA gutC*^{c}*AD*$^+$/F143 *gutC*$^+$*A*$^+$*D*$^+$ and *mtlA gutC*^{c}-*A*$^+$*D*$^+$/F143 *gutC*$^+$*A*$^+$*D*$^+$. The former strains are Mtl^-, whereas the latter strains are Mtl^+, since strains with a high constitutive level of *gutA* activity (enzyme II) can phosphorylate mannitol and suppress the *mtlA* defect. However, the alternative explanation that the *gutC*^{c}*AD*$^+$ endogenote is actually *gutCA*$^+$*D*$^+$ predicts the properties of the diploids equally well, since reversion of the *gutC*^{c} mutation to *gutC* abolishes expression of the D-glucitol enzyme II. The *gutCA*$^+$ *mtlA* strains are phenotypically Mtl^-. Although the data presented here suggest that the *srl* operon is positively controlled by the *srlC* gene product, they cannot conclusively rule out a negative regulatory scheme similar to that proposed by Jones-Mortimer and Kornberg (5).

The order of markers in the *srl* region of the genetic map has been shown to be *cysC . . . srlD-srlA-srlC-recA-alaS* . . . In the secondary site *srl* lysogenic strain KM2136, the prophage is inserted into the *srlA* gene and is polar on *srlD* expression. A survey of transducing phages from 14 independent *srl* secondary site lysogens suggests that this region contains at least two sites for λ insertion. One site, defined by the Δ7 deletion, appears to be the most frequent site for integration in *srl*. Lambda insertion at one minor site has been detected and occurs less than 1/10 as frequently as λ integration at the major secondary site.

Deletions extending into the *recA* gene have been derived from one secondary site lysogen. By several genetic criteria, these mutants are similar to *recA* missense mutants in their UV sensitivity and recombination deficiency. Although these deletions have not been fully characterized, they suggest that the *recA* product may not be essential for cell viability. Further characterization of these deletions should be useful for understanding the regulation of the *recA* gene in vivo.

ACKNOWLEDGMENTS

I thank J. Lengeler and K. B. Low for providing strains used in this work. I am indebted to J. Hesse, W. Paxton, and A. Luncsford for valuable technical assistance and to W. Epstein for helpful discussion.

This work was supported by Public Health Service training grant GM780, by a National Research Service Award to K.M. from training grant GM7190, and by research grant GM23016 to W. Epstein, all of which are from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40:**116–167.
2. **Castellazzi, M., J. George, and G. Buttin.** 1972. Prophage induction and cell division in *E. coli*. I. Further characterization of the thermosensitive mutation *tif-1* whose expression mimics the effect of UV irradiation. Mol. Gen. Genet. **119:**139–152.
3. **Curtis, S. J., and W. Epstein.** 1975. Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. J. Bacteriol. **122:**1189–1199.
4. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108:**639–644.
5. **Jones-Mortimer, M. C., and H. L. Kornberg.** 1976. Uptake of fructose by the sorbitol phosphotransferase of *Escherichia coli* K-12. J. Gen. Microbiol. **96:**383–391.
6. **Lengeler, J.** 1975. Mutations affecting transport of the hexitols D-mannitol, D-glucitol, and galactitol in *Escherichia coli* K-12: isolation and mapping. J. Bacteriol. **124:**26–38.
7. **Lengeler, J.** 1975. Nature and properties of hexitol transport systems in *Escherichia coli*. J. Bacteriol. **124:**39–47.
8. **Lengeler, J., and E. C. C. Lin.** 1972. Reversal of the mannitol-sorbitol diauxie in *Escherichia coli*. J. Bacteriol. **112:**840–848.
9. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36:**587–607.
10. **Lowry, O. H., N. S. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
11. **McEntee, K.** 1976. Specialized transduction of *recA* by bacteriophage lambda. Virology **70:**221–222.
12. **McEntee, K., and W. Epstein.** 1977. Isolation and characterization of specialized transducing bacteriophages for the *recA* gene of *Escherichia coli*. Virology **77:**306–318.
13. **McEntee, K., J. E. Hesse, and W. Epstein.** 1976. Identification and radiochemical purification of the *recA* protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **73:**3979–3983.
14. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, New York.
15. **Ruffler, D., P. Buckel, W. Piepersberg, and A. Böck.** 1974. Alanyl-tRNA synthetase of *Escherichia coli*: genetic analysis of the structural gene and of suppressor mutations. Mol. Gen. Genet. **134:**313–323.
16. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal sites. I. Location of the secondary attachment sites and properties of the lysogens. J. Mol. Biol. **63:**483–503.