REVERTANT INSTABILITY IN *ESCHERICHIA COLI*. II. GENETIC STUDIES[1]

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FOUR unstable suppressors of the *lac* z_{1S} mutation are cotransduced with the *ile ilv metE* region of the *E. coli* K-12 chromosome by phage P1*kc* (SCHWARTZ 1967a,b). However, one of the unstable suppressors studied, USRev 1, showed no linkage to these markers or the bacterial chromosome in conjugation experiments (SCHWARTZ 1965). In part, the experiments reported here were undertaken in an attempt to resolve this difference in linkage observed in transduction and conjugation experiments. A brief summary of some of these experiments has appeared (SCHWARTZ 1967a).

MATERIALS AND METHODS

The bacterial strains employed are derivatives of *E. coli* K-12 (Table 1). Standard media and culture techniques and exceptions to these have been previously described (SCHWARTZ 1965, 1967b).

RESULTS AND DISCUSSION

ilv and *metE* (1) are located close to the origin of the Hfr R1 chromosome (HAYES 1964), (2) are transferred at high frequency by Hfr R1, 8 (*ilv*) and 9 (*metE*) minutes after mating (Figure 1), and (3) are cotransduceable by P1*kc* (EGGERTSSON and ADELBERG 1965). USRev 1, an unstable suppressor of *lac* z_{1S}, does not have a chromosomal location since it is transferred at low frequency soon after the start of mating by Hfr R1 (and by four other Hfr types) and it does not show linkage to unselected chromosomal markers (SCHWARTZ 1965). Therefore, the finding that USRev 1 was cotransduced with *ile*$^+$ (SCHWARTZ 1967a) was unexpected. USRev 1 is linked to *ile ilv* and *metE* since approximately 70% of the unstable suppressed *lac* z_{1S} recombinants selected in transductions of *lac* z_{1S}, *ile* or *ilv metE* recipients by P1 grown on *lac* z_{1S} USRev 1 (*ile*$^+$ *ilv*$^+$ *metE*$^+$) strains inherit unselected *ile*$^+$, *ilv*$^+$ or *metE*$^+$ (Table 2).

In view of this result, we decided to re-examine the frequency and kinetics of USRev 1 transfer employing as donor Hfr R1, now comparing it to that obtained for the cotransduceable *ilv*$^+$ and *metE*$^+$ markers. The Hfr R1 USRev 1 strain used was derived by selecting a lactose-utilizing recombinant from transduction to a *lac* z_{1S} (*ilv*$^+$ *metE*$^+$) Hfr R1 strain by P1 grown on the original unstable revertant, 3300 *lac* z_{1S} USRev 1 (SCHWARTZ 1965, 1967b). Preliminary broth

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TABLE 1

*Bacterial strains**

Strain number	Pertinent characteristics	Origin, source or derivation
Hfr R1	Reeves Hfr R1 prototroph	AB674, EAA Yale University
Hfr R1 *lac* z_{1S}	*lac* z_{1S} suppressible by USRev 1	SCHWARTZ 1965, 1967b
3300 *lac* z_{1S} USRev 1	Original unstable suppressed *lac* z_{1S} mutant	SCHWARTZ 1965
311S-14	Nitrosoguanidine induced *ile* of Hfr Hayes *lac* z_{1S}	SCHWARTZ 1965
Hfr R1 *lac* z_{1S} *ilv5*	Nitrosoguanidine induced *ilv* of Hfr R1 *lac* z_{1S}	Hfr R1 *lac* z_{1S}
AB2291 *lac* z_{1S}	*lac* z_{1S} (*gal*+) derivative of F- AB2291 *ilv188 metE46 his4 str*^{r}	AB2291, EAA Yale University

* The following gene symbols are employed: *metE*–mutation resulting in methionine or vitamin B_{12} requirement, *ilv*–isoleucine-valine, *ile*–isoleucine, *his*–histidine, *lac z*–beta-galactosidase structural gene within the lactose operon, *gal*–galactose, *str*–streptomycin, P1*kc*–transducing phage P1. Superscript + and – signs indicate ability and inability to utilize indicated carbon sources. Superscript *s* and *r* indicate sensitivity and resistance to streptomycin. USRev 1 is an extrachromosomal unstable suppressor of *lac* z_{1S} (SCHWARTZ 1965). An italicized gene symbol indicates genotype; the same symbol employed without italics is used to indicate phenotype only. Strains with AB numbers were either obtained or derived from the collection of DR. EDWARD A. ADELBERG (EAA), Yale University.

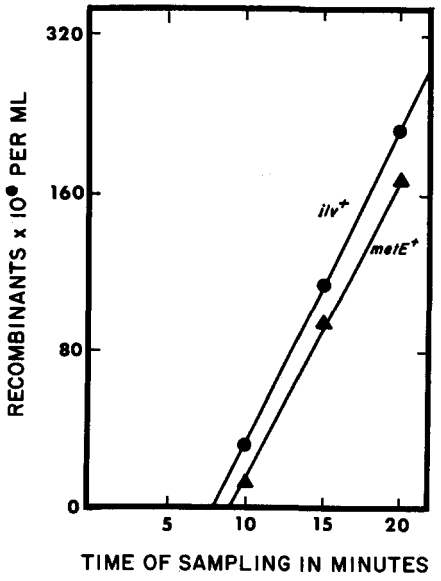

FIGURE 1.—Kinetics of recombinant formation for *ilv188*+ and *metE46*+ markers in an interrupted mating experiment. Donor Hfr R1; recipient AB2291 *lac* z_{1S}.

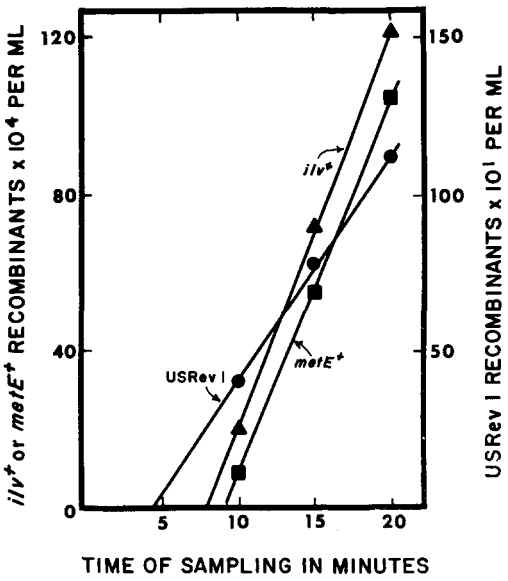

FIGURE 2.—Kinetics of recombinant formation for *ilv188*+, *metE46*+ and USRev 1 markers in an interrupted mating experiment. Donor Hfr R1 *lac* z_{1S} USRev 1; recipient AB2291 *lac* z_{1S}.

TABLE 2

Cotransduction of ilv, ile *and* metE *with USRev 1**

P1 donor strain	Selected marker(s)	Unselected marker	Number transductants per 0.1ml	Number of transductants tested	Percent of transductants containing unselected marker
3300 *lac* z_{1S} USRev 1 (*ile*+ *ilv*+ *metE*+)	USRev 1	*ile*+	265	44	70
	USRev 1	*ilv*+	127	44	68
	USRev 1	*metE*+	127	44	73
	USRev 1 and *ilv*+	*metE*+	106	44	100
	USRev 1 *ilv*+ and *metE*+	....	95	..	..
Hfr R1 *lac* z_{1S} USRev 1 (*ile*+ *ilv*+ *metE*+)	USRev 1	*ilv*+	181	40	70
	USRev 1	*metE*+	181	44	73
	USRev 1 *ilv*+ and *metE*+	....	129	..	..

* Strains AB2291 *lac* z_{1S} *ilv metE* and 311S-14 *lac* z_{1S} *ile* were treated with the indicated phage lysates at a multiplicity of infection=2.

matings (45 minutes at 37°C) of Hfr R1 *str*^{s} *lac* z_{1S} USRev 1 × F$^-$ AB2291 *str*^{r} *lac* z_{1S} *ilv188 metE46* yielded the following numbers of recombinants (per ml of mating mixture): 1.8×10^7 *ilv*+ *str*^{r}, 1.7×10^7 *metE*+ *str*^{r} and 1.2×10^3 USRev 1 *str*^{r}. Transfer curves for these markers were then obtained in interrupted mating experiments employing these strains. Curves for *ilv*+ and *metE*+, shown to be transferred at high frequency, were obtained by interrupting mating in samples of a diluted mating mixture (DE HAAN and GROSS 1962); USRev 1 transferred at low frequency was determined from the same but undiluted mating mixture by the T6 interruption method (HAYES 1957). The results obtained (Figure 2) confirm previous data and the conclusion that USRev 1 is extrachromosomal (SCHWARTZ 1965). Yet, in a transduction experiment employing P1 grown on the Hfr R1 *lac* z_{1S} USRev 1 culture, a portion of which was used in the conjugation experiment (Figure 2), 70% cotransduction of USRev 1 and *ilv*+ was obtained (Table 2). In order to account for this difference, we propose that the *ilv*+ marker linked to USRev 1 in transduction differs from the *ilv*+ marker transferred independent of USRev 1 in conjugation. On the basis of this model, both original unstable revertant and the Hfr R1 USRev 1 transductant derivative are partially diploid carrying two *ilv*+ regions, one linked to extrachromosomal USRev 1 and the second carried on the chromosome.

An *ilv* derivative of Hfr R1 *lac* z_{1S} was isolated after nitrosoguanidine treatment (ADELBERG, MANDEL and CHEN 1965). This *ilv* derivative was then used as a recipient with P1 grown on 3300 *lac* z_{1S} USRev 1. From this an unstable lactose-utilizing transductant which inherited unselected *ilv*+ was isolated. On

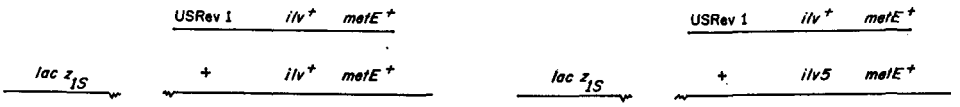

FIGURE 3.—Genetic constitution of unstable suppressed mutants and transductant derivatives. Left *lac* z_{1S} *ilv*+ *metE*+/*metE*+ *ilv*+ USRev 1 homogenote; right *lac* z_{1S} *ilv*/*ilv*+ USRev 1 heterogenote.

the basis of our model this strain, Hfr R1 *lac* z_{1S} *ilv5*/USRev 1 *ilv5*+, should have only one functional *ilv* locus. Figure 3 shows the genomes of strains described. The following experiments were performed in order to test our hypothesis.

Conjugation experiments: Hfr R1 *str*^{s} *lac* z_{1S} *ilv5*/USRev 1 *ilv5*+ was mated with F$^-$ AB2291 *str*^{r} *lac* z_{1S} *ilv188 metE46*. A preliminary broth cross yielded the result anticipated if the only *ilv*+ locus of the male parent were linked to extrachromosomal USRev 1. After 45 minutes of mating at 37°C, the following recombinants (per ml of mating mixture) were obtained: 1.6×10^3 *ilv*+ *str*^{r}, 1.9×10^3 USRev 1 *str*^{r} and 2.1×10^7 *metE*+ *str*^{r}. In contrast to the previous results presented for the donor strain, Hfr R1 *str*^{s} *lac* z_{1S} *ilv*+ *metE*+/USRev 1 *ilv*+, both the frequencies of USRev 1 *str*^{r} and *ilv*+ *str*^{r} recombinants are greatly depressed (to about the same level) in comparison to the frequency of *metE*+ *str*^{r} recombinants. Figure 4 shows the results of a mating experiment interrupted to time the entry of *ilv*+, USRev 1 and *metE*+ from Hfr R1 *lac* z_{1S} *ilv5*/USRev 1 *ilv5*+. The time of entry curves obtained for extrachromosomal USRev 1 and *ilv5*+ are indistinguishable from each other, and different from the *metE*+ curve. The results obtained for chromosomal *metE*+ transfer are identical to those obtained using the wild-type Hfr R1 donor (Figure 1).

Transduction experiments: The validity of the genetic models proposed in Figure 3 can also be tested by transduction analyses. If strains which carry the USRev 1 mutation are diploid for the *ilv metE* region, then as discussed below,

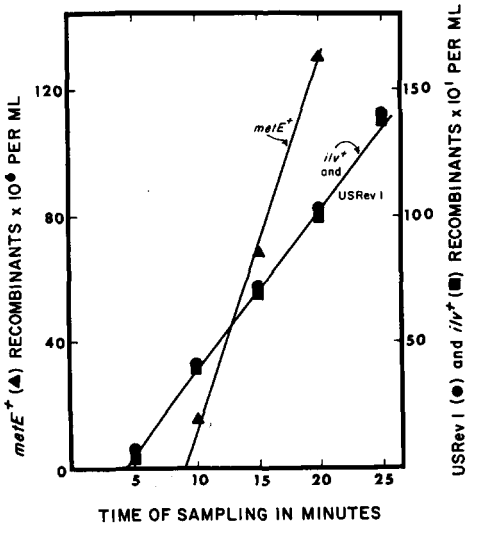

FIGURE 4.—Kinetics of recombinant formation for *ilv5*+, USRev 1 and *metE46*+ markers in an interrupted mating experiment. Donor Hfr R1 *lac* z_{1S} *ilv5 metE*+/*metE*+ *ilv5*+ USRev 1; recipient AB2291 *lac* z_{1S}.

cotransduction frequencies in this region should differ being dependent upon the origin of the transducing phage. Cotransduction of USRev 1 amongst selected *ilv*$^+$ recombinants employing a P1-USRev 1 *ilv*$^+$/*ilv* heterogenotic donor should be greater than that obtained using a USRev 1 *ilv*$^+$/*ilv*$^+$ homogenotic donor. This should be the case since according to our model only *ilv*$^+$ transducing fragments derived from the exogenote should contain USRev 1; *ilv*$^+$ recombinants issuing from transductions of endogenotic *ilv*$^+$ are not expected to contain USRev 1.

It must be recognized that the transduction frequency for a given marker (e.g. *ilv*$^+$) is greater when located on the endogenote of the P1-donor (Table 3). This is expected since exogenote markers are unstable being rapidly lost during growth in nonselective complex nutrient media required for obtaining high titer P1 lysates. However, the observed (individual) frequencies of either exogenotic USRev 1 or exogenotic *ilv*$^+$ transduction, employing a P1-USRev 1 *ilv*$^+$/*ilv* heterogenote donor are similar (Table 3).

ilv$^+$ transductants of strain AB2291 *lac* z_{1s} *ilv* were selected in three different crosses employing phage derived from (1) original unstable strain 3300 *lac* z_{1s} *ilv*$^+$/*ilv*$^+$ USRev 1, (2) unstable transductant derivative Hfr R1 *lac* z_{1s} *ilv*$^+$/*ilv*$^+$ USRev 1 and (3) unstable transductant derivative Hfr R1 *lac* z_{1s} *ilv5*/*ilv5*$^+$ USRev 1. In agreement with predictions made on the basis of our model (Figure 3), approximately 7% of the *ilv*$^+$ transductants inherited unselected USRev 1 in the cross employing the *ilv*/*ilv*$^+$ heterogenote, and only about 0.5% of the *ilv*$^+$ inherited USRev 1 when the transducing phage was derived from either of the two *ilv*$^+$/*ilv*$^+$ homogenotes (Table 3). In all three crosses, when initial selection was made for lactose-utilizing (USRev 1) recombinants, about 70% inherited unselected *ilv*$^+$ (Table 4). This result can be explained on the basis

TABLE 3

USRev 1 ilv metE *linkage in transduction**

P1 donor strain	Selected marker	Number of transductants per 0.1ml	Unselected marker	Number of transductants tested for unselected marker	Percent transductants inheriting unselected marker
3300 *lac* z_{1s} *ilv*$^+$ *metE*$^+$/ *metE*$^+$ *ilv*$^+$ USRev 1	*ilv*$^+$	3230	USRev 1	880	0.5
	USRev 1	171	*ilv*$^+$	44	70.
	metE$^+$	625	*ilv*$^+$	88	60.
Hfr R1 *lac* z_{1s} *ilv*$^+$ *metE*$^+$/ *metE*$^+$ *ilv*$^+$ USRev 1	*ilv*$^+$	3120	USRev 1	396	0.5
	USRev 1	183	*ilv*$^+$	44	68.
	metE$^+$	645	*ilv*$^+$	44	59.
Hfr R1 *lac* z_{1s} *ilv5* *metE*$^+$/ *metE*$^+$ *ilv5*$^+$ USRev 1	*ilv*$^+$	223	USRev 1	176	6.8
	USRev 1	216	*ilv*$^+$	44	70.
	metE$^+$	490	*ilv*$^+$	176	9.1

* Recipient strain AB2291 *lac* z_{1s} *ilv metE* was crossed with phage grown on each donor strain. Transductants were scored and tested for unselected markers by replica plating.

that when initial selection is made for USRev 1 (which must be carried on exogenote) a constant fraction of these recombinants inherit exogenotic *ilv*$^+$ regardless of the state of the *ilv* allele located on the endogenote.

Segregation and linkage of exogenote markers: Some evidence for segregation and crossing over involving the exogenote, *ilv*$^+$ USRev 1, of a *lac* z_{1S} *ilv/ilv*$^+$ USRev 1 strain would be obtained by finding phenotypic Lac$^-$, stable-Ilv$^+$ segregants from this phenotypically unstable-Lac$^+$ unstable-Ilv$^+$ partial diploid. Cultures of partial diploid strains, *lac* z_{1S} *ilv/ilv*$^+$ USRev 1 and *lac* z_{1S} *ile/ile*$^+$ USRev 1 were grown in minimal-lactose media (lacking isoleucine-valine or isoleucine, respectively) and plated on EMB lactose agar. Lac$^-$ sectors each derived from different lactose-variegated colonies were picked and purified from single colony isolates on EMB lactose agar. The Ilv (or Ile) phenotype of these Lac$^-$ clones was then tested by replica plating onto minimal glucose media with and without isoleucine-valine (or isoleucine) supplementation. Approximately 10% of these *lac* segregants had stable Ilv$^+$ (or Ile$^+$) phenotypes. (About 90% of the *lac* were stable *ilv* or *ile*.)

Transduction and conjugation experiments were performed to demonstrate that Lac$^-$ segregants from *lac* z_{1S} *ilv/ilv*$^+$ USRev 1 were genetically haploid *lac* *ilv*$^+$, and not either *lac* (1) suppressed *ilv* mutants or (2) *ilv/ilv*$^+$ partial diploid heterogenotes. To distinguish *ilv*$^+$ from possible *ilv su*, phage P1 grown on the *metE*$^+$ Ilv$^+$ strain in question was used as donor in transduction of strain AB2291 *metE46 ilv188*. *metE*$^+$ transductants were selected and the colonies obtained picked and patched with a toothpick onto media of the same composition as used for their initial selection. After overnight growth, the patches were replica plated onto media lacking both methionine and isoleucine-valine in order to determine the percent inheritance of unselected *ilv*$^+$ amongst selected *metE*$^+$. Control crosses employing (1) a *metE*$^+$ *ilv*$^+$ donor yielded 66% cotransduction of *ilv*$^+$ with *metE*$^+$, (2) a *metE*$^+$ *ilv5* donor yielded 0 *ilv*$^+$ recombinants amongst the 132 *metE*$^+$ transductants that were tested (Table 4). P1 grown on Lac$^-$ Ilv$^+$ segregant (No. 1) from strain Hfr R1 *lac* z_{1S} *ilv5/ilv5*$^+$ USRev 1 yielded 67% cotransduction of *ilv*$^+$ amongst 132 selected *metE*$^+$ (Table 4). This result demonstrates that the genetic alteration responsible for the *ilv*$^+$ phenotype of segregant No. 1 maps in the *ilv metE* region.

TABLE 4

Genetic nature of lac ilv$^+$ *segregants from* lac z_{1S} ilv/ilv$^+$ *USRev 1**

P1 donor strain	Number of selected metE$^+$ transductants tested	Percent of *metE*$^+$ transductants inheriting unselected *ilv*$^+$
Hfr R1 wild type (*metE*$^+$ *ilv*$^+$)	88	66
Hfr R1 *lac* z_{1S} *ilv5*	132	0
Hfr R1 *lac* z_{1S} *ilv*$^+$ segregant No. 1	88	67
Hfr R1 *lac* z_{1S} *ilv5 metE*$^+$/ *metE*$^+$ *ilv5*$^+$ USRev 1	132	9.1

* Recipient strain AB2291 *ilv188 metE* was crossed with each of the indicated phage donors and plated for *metE*$^+$ transductants. The percent of *metE*$^+$ recombinants which inherited unselected *ilv*$^+$ was determined by replica plating.

Phage P1 derived from the unstable partial diploid strain, Hfr R1 *lac* z_{1s} *ilv5/ilv5*$^+$ USRev 1, yielded 9% cotransductants *metE*$^+$ *ilv*$^+$, amongst 132 selected *metE*$^+$ recombinants (Table 4). This finding indicates that this partial diploid is actually *lac* z_{1s} *metE*$^+$ *ilv5/ilv5*$^+$ *metE*$^+$ USRev 1, being diploid for the *ilv metE* region. Comparison of the *metE*$^+$ *ilv*$^+$ cotransduction frequencies 67% (segregant No. 1) and 9% (known heterogenote) rules out the possibility that segregant No. 1 is also a heterogenote, (*ilv5/ilv5*$^+$). Finally, interrupted mating experiments employing Hfr R1 *lac ilv*$^+$ segregant No. 1 and F$^-$ strain AB2291 *metE ilv* results in transfer kinetics and entry times for *ilv*$^+$ and *metE*$^+$ which are indistinguishable from the results obtained with the wild-type Hfr R1 donor.

If the frequency of recombination between exogenote and endogenote markers is a function of map distance between the markers involved, then it should be possible to determine whether USRev 1 maps closer to *ilv* or *metE*. A derivative of strain AB2291, *lac* z_{1s} *ilv188 metE46* was used as recipient for transduction of USRev 1 by phage grown on the original unstable revertant strain 3300 *lac* z_{1s} *metE*$^+$ *ilv*$^+$/*ilv*$^+$ *metE*$^+$ USRev 1. A P1-sensitive lactose-utilizing transductant which had also inherited *ilv*$^+$ and *metE*$^+$ was obtained and tested for stability of the recombinant markers. In a preliminary experiment all three markers were found to be unstable, most of the Lac$^-$ segregants were also Ilv$^-$ and Met$^-$. The genotype of the recombinant is therefore *lac* z_{1s} *ilv metE/ilv*$^+$ *metE*$^+$ USRev 1.

Lac$^-$ segregants (244) from this strain each purified from different lactose-variegated colonies were replica plated to minimal-glucose agar lacking (1) methionine, and (2) isoleucine and valine. Out of 244 Lac$^-$ tested, 69 were Met$^+$ and 18 were Ilv$^+$. Ten Lac$^-$ prototrophic (5 Ilv$^+$ and 5 Met$^+$) strains tested were stable (haploids) since they did not yield auxotrophic segregants. The 87 prototrophic Lac$^-$ strains are probably similar in origin and genetic constitution to *lac ilv*$^+$ segregant No. 1 (Table 4). USRev 1 appears to map closer to *ilv* than to *metE* since out of 244 Lac$^-$, 226 were Ilv$^-$ and 175 Met$^-$, i.e., crossover and segregational events resulting in USRev 1 loss favor the loss of *ilv*$^+$ more than *metE*$^+$.

Partial diploid strain *lac* z_{1s} *ilv metE/metE*$^+$ *ilv*$^+$ USRev 1 was grown to saturation in minimal-lactose broth lacking isoleucine, valine and methionine, thereby forcing retention of the exogenote. The culture was then diluted and plated on minimal-lactose agar containing isoleucine, valine and methionine. Colonies obtained were directly replica plated to minimal-lactose media lacking (1) isoleucine and valine, (2) methionine, and (3) isoleucine, valine and methionine. Out of 8,661 lactose-utilizing clones tested, 28 Met$^-$, 11 Ilv$^-$ and 0 Ilv$^-$ Met$^-$ were found. Since these auxotrophs retained their USRev 1 phenotype (i.e. produced lactose-variegated colonies on EMB lactose agar), it is likely that they are homogenotic segregants, *ilv metE/metE ilv*$^+$ USRev 1 and *ilv metE/metE*$^+$ *ilv* USRev 1, respectively.

Although we do not know the mechanisms involved, USRev 1 is separated from *metE*$^+$ more frequently than from *ilv*$^+$. Therefore it follows from this experiment, as from the previous, USRev 1 maps closer to *ilv* than to *metE*.

I am indebted to Dr. Joan Stadler for providing many bacterial strains.

SUMMARY

An unstable suppressor of *lac* z_{1S}, USRev 1, is not linked to chromosomal markers *ilv* or *metE* in conjugation experiments. However USRev 1 is cotransduced with *ilv* and/or *metE* by phage P1*kc*. This apparant contradiction has been resolved by finding that the USRev 1 strain is partially diploid: *lac* z_{1S} *ilv metE/metE ilv* USRev 1. Recombinational events in a *lac* z_{1S} *ilv metE/metE*$^+$ *ilv*$^+$ USRev 1 strain results in (1) stable *lac ilv*$^+$ and *lac metE*$^+$ haploid segregants and (2) unstable homogenotic *ilv/ilv* USRev 1 and *metE/metE* USRev 1 segregants. The frequency of these segregants indicates that the order of exogenote markers is *metE ilv* USRev 1.

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