JOURNAL OF BACTERIOLOGY, Apr. 1980, p. 145-152
0021-9193/80/04-0145/08$02.00/0

Vol. 142, No. 1

Salmonella typhimurium LT2 Mutation Affecting the Deletion of Resistance Determinants on R Plasmids

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Plasmid Rms312, specifying resistance to tetracycline (Tc), chloramphenicol (Cm), streptomycin (Sm), sulfonamide (Su), and mercury chloride (Mer), deletes both Tc and Cm Sm Su Mer determinants at a high frequency in *Salmonella typhimurium* LT2. *S. typhimurium* mutants that were stable carriers of Rms312 were isolated by alternate culture of R-bearing cells in a medium containing either tetracycline or chloramphenicol. In one of these mutants the deletion frequency of drug resistance determinants was decreased by about 100-fold not only in Rms312, but also in R100, R1, and R6-5. This mutation caused a slight reduction of ultraviolet resistance but did not affect generalized genetic recombination, indicating that the mutation is different from *recA*. The mutation, designated *dor* (deletion of r-determinants), was mapped to a position near 57 units in the new linkage map of *S. typhimurium* LT2 (K. E. Sanderson and P. E. Hartman, Microbiol. Rev. **42**:471-519, 1978). The *dor* mutation had no effect on IS*1*-mediated illegitimate deletion, indicating that the *dor* mutation is different from the *del* mutation described by Nevers and Saedler (P. Nevers and H. Saedler, Mol. Gen. Genet. **160**:209-214, 1978).

It has been shown that insertion elements play significant roles in chromosomal rearrangement, such as deletion, transposition, and duplication, since insertion elements were first found in the genes of several bacterial operons (27). In particular, insertion sequence IS*1* was found to enhance the frequency of deletion of DNA sequences adjacent to IS*1* by as much as 1,000-fold in *Escherichia coli* strains harboring this element in the *galT* gene of the *gal* operon (20).

These insertion elements are present as repeated sequences in bacterial plasmids, such as the fertility factor F and R plasmids (10, 19). Electron microscope heteroduplex studies on plasmid DNA molecules showed that the ends of insertion elements which flanked the r-determinant of the FII incompatibility group R plasmids also act as hot spots in the formation of deletions. Insertion-specific deletion formation can result from either legitimate or illegitimate recombination. Legitimate recombination occurs between two IS*1* sequences and is apparently responsible for the dissociation of R plasmids occurring during (i) r-determinant amplification in *Proteus mirabilis* (21); (ii) the generation of an r-determinant molecule in an integratively suppressed strain of *E. coli* (2); and (iii) the growth of *Salmonella typhimurium* carrying R100 (22, 26). This dissociation is dependent on *recA*$^+$ gene function (22, 31). Illegitimate recombination occurs between one IS*1* and a nonhomologous second site, as in the generation of miniplasmids from R12 (13) and pKN102 (18).

Mutations affecting formation of insertion-mediated deletions were first studied by Nevers and Saedler (16) and designated *del*. A *del* mutation causes a reduction in frequency of deletion formation between IS*1* and a nonhomologous second site in the *galT*::IS*1* system in *E. coli*, indicating that *del* affects illegitimate recombination.

In *E. coli*, FII R plasmids R100, R1, and R6 are stable composite molecules consisting of two units, RTF and an r-determinant (3, 17). In *S. typhimurium*, however, there is a frequent loss of the drug resistance conferred by the r-determinant (30, 31), presumably due to R-plasmid dissociation involving a legitimate recombination between two IS*1* sequences and subsequent loss of the r-determinant (22). The isolation and characterization of *S. typhimurium* mutants affected by this deletion may be a way to explore the mechanisms of legitimate recombination involving insertion elements. We used Rms312 as an FII R plasmid, because its tetracycline (Tc) resistance determinant was lost at a very high frequency in *S. typhimurium* (7) and because this property is convenient for isolating host mutants by positive selection. Rms312 differs in this property from R100 and its relatives.

In this communication, we describe the isolation and characterization of a novel *S. typhimurium* mutant altered in the recombination-

promoted deletion of r-determinants from FII plasmids including Rms312. The mutation is different from both *recA* and *del*, and is designated *dor* (deletion of r-determinants).

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. The bacterial strains and R plasmids used in this study are listed in Table 1. Nal^r types were selected on plates of nutrient agar containing nalidixic acid (Nal; 25 μg/ml), which was used as a counterselection in conjugal transfer of R plasmids from *E. coli*. All *thyA* mutants were selected on plates of minimal medium containing trimethoprim (10 μg/ml) and thymine (50 μg/ml), and they were used for selection of recombinants with Hfr mating and transduction.

Rms312 is a conjugative plasmid that was derived from a *'Shigella* strain (7) and belongs to FII incompatibility group. The molecular weight of Rms312 is 6.4×10^7. R100-Tc is a mutant of R100 which is deleted for Cm Sm Su Mer determinants but maintains Tc resistance and transferability. P1 *vir* (11) and P22 (kindly provided by K. Mise) were used for transduction.

Media and drugs. L-broth and L-agar (12) were routinely used for liquid and plate cultures, respectively. AG-agar medium consisted of medium A (5) with 1% glucose, 0.08% BTB, and 1.5% agar. Each medium was supplemented with streptomycin (Sm; 200 μg/ml) in LD-1 Sm^r and LD-1 Sm^d, or thymine (25 μg/ml) in thymine-requiring derivatives. LB Ca (1) was L-broth containing 10^{-3} M $CaCl_2$. BSG buffer consisted of 8.5 g of NaCl, 300 mg of KH_2PO_4, 100 mg of gelatin, and 1,000 ml of distilled water. Antibiotics etc. used: streptomycin sulfate (Toyojozo Co., Japan), tetracycline hydrochloride (Japan Lederle Co.), chloramphenicol (Cm; Toyojozo Co., Japan), ampicillin (Ap; Toyama Chemical Industries, Japan), kanamycin sulfate (Km; Meiji Seika, Co., Japan), sulfisomidine (sulfonamide, Su; Dainihon Pharmaceutical Co., Japan), nalidixic acid (Dai-ichi Pharmaceutical Co., Japan), and mercury chloride (Mer; Wako Pure Chemical Industries, Ltd., Japan). Concentration of drugs in solid media for selection of resistant bacteria was: streptomycin, tetracycline, chloramphenicol, ampicillin, kanamycin, and nalidixic acid, 25 μg/ml; sulfonamide, 100 μg/ml; mercury chloride, 12.5 μg/ml.

Transconjugation of R plasmids. Exponential-

TABLE 1. *Bacterial strains and R plasmids used*

Strain, R plasmid	Genotype[a] or phenotype[b]	Source (reference)
S. typhimurium LT2 derivatives		
4526	F$^-$ *metA metE trpB val str hsdSA hsdL galE* P1 phage sensitive	C. Colson (4)
ML4910	As 4526, also Nal^r	Nal^r derivative of 4526
ML4912	As ML4910, also *dor*	This paper
ML4913	As ML4910, also *thyA*	Tp^r derivative of ML4910
ML4914	As ML4912, also *thyA*	Tp^r derivative of ML4912
SA540	Hfr *purE rfx*	K. E. Sanderson (23)
ML4915	As ML4914, but *thy*$^+$ *trp*$^+$ Hfr	SA540 × ML4914, *thy*$^+$ *pur*$^+$ selection
ML4916	As SA540, also *thyA*	Tp^r derivative of SA540
ML4918	As ML4916, but *thy*$^+$ *dor*	ML4915 × ML4916, *thy*$^+$ *met*$^+$ selection
ML4919	As ML4918, also *thyA*	Tp^r derivative of ML4918
TR2246	Hfr *metA recA str*	J. Ishizu
ML4920	F$^-$ *metA recA str*	Derived from TR2246
JB564	F$^-$ *serA glyA*	J. E. Brenchley
ML4922	As JB564, but *gly*$^+$ *dor*	ML4918 × JB564, *gly*$^+$ *pur*$^+$ selection
E. coli K-12 derivatives		
LD-1 Sm^r	Sm^r	T. Miki
LD-1 Sm^d	Sm^d	T. Miki
W3630	F$^-$ *mal*	J. Lederberg
R plasmids[c]		
R100	Tc^r Cm^r Sm^r Su^r Mer^r	Y. Hirota
R100-Tc	Tc^s Cm^s Sm^s Su^s Mer^s	This paper
Rms312	Tc^r Cm^r Sm^r Su^r Mer^r	H. Hashimoto (7)
R6-5	Cm^r Sm^r Su^r Mer^r Km^r	K. Timmis (29)
R1	Cm^r Sm^r Su^r Ap^r Km^r	N. Datta

[a] Abbreviation and nomenclature are essentially that of Demerec et al. (6) and Sanderson and Hartman (24).

[b] Phenotype abbreviations: Nal, nalidixic acid; Tp, trimethoprim; Tc, tetracycline; Cm, chloramphenicol; Sm, streptomycin; Su, sulfonamide; Mer, mercury chloride; Ap, ampicillin; Km, kanamycin; r, resistance; s, sensitivity; d, dependence.

[c] All of the R plasmids used belonged to the FII incompatibility group.

phase L-broth cultures (about 2×10^8 cells per ml) of donor and recipient were mixed at a volume ratio of 1:4. The mixture was incubated for 2 h at 37°C without aeration, diluted appropriately, and then plated on selective media.

Hfr mating. Donor and recipient cultures (about 2×10^8 cells per ml) in exponential growth phase in L-broth were mixed at a volume ratio of 1:4. After 150 min of static incubation at 37°C, the culture was mechanically agitated to separate mating pairs, washed two times in BSG buffer, and plated on selective media. Each 100 recombinant colonies were purified by three successive single-colony isolations and scored on supplemented minimal agar for unselected markers.

Population analysis of resistant cells. Cells from a colony were suspended in 1 ml of BSG buffer, and one loopful of suspension was spread on a drug-free plate for purification. After overnight culture, 20 colonies were streaked on plates containing either chloramphenicol (25 μg/ml) or tetracycline (25 μg/ml), and the population of resistant cells was scored.

Curing of R plasmids. The method for curing R plasmids followed that described by Hirota (8). About 10^6 cells were inoculated into 10 ml of L-broth containing 25 μg of acridine orange per ml and incubated overnight at 37°C. The culture was diluted appropriately and spread on drug-free plates. After overnight culture, colonies were replicated onto plates individually containing each of the drugs for which the R plasmid confers resistance, and a colony without any of the resistance markers was selected.

Estimation of spontaneous loss frequency of resistance markers. A transconjugant colony with all the markers of the R plasmid was picked from a drug-free plate, suspended in 10 ml of L-broth (about 10^5 cells per ml), and grown with gentle shaking to late log phase (about 4×10^8 cells per ml), at 37°C. Appropriate dilutions of the culture at the start and end of the experiment were spread on L-agar plates, and the number of colonies was calculated after overnight culture. About 1,000 colonies in total were replicated onto plates, each containing one of the drugs for which the R plasmid confers resistance. After overnight culture, loss of each marker was scored. Loss frequency was estimated by use of the following equation described by Stent (28); M (loss of frequency per cell per generation) $= (\pi - \pi_0)/\ln(p/p_0)$, where π_0 and p_0 are, respectively, the proportion of mutants without resistance marker(s) and the total number of cells in the culture at the start of the experiment, and π is the proportion of mutants without resistance marker(s) after the number of cells has risen to p.

Detection of R-plasmid mutants sensitive to streptomycin. Detection of the mutants was performed by the transfer of the R plasmid to a streptomycin-dependent (Sm^d) strain. This technique was developed by T. Miki (22), based on the observation that only streptomycin-sensitive (Sm^s) R plasmids could survive in an Sm^d strain because an Sm^r R plasmid encodes an enzyme that inactivates streptomycin, and this drug is required for the growth of Sm^d cells (9). An Sm^d strain was cultured in a medium containing 200 μg of streptomycin per ml. Transconjugation was performed under the conditions described above. Transconjugants were selected on L-agar plates containing streptomycin (200 μg/ml) and an appropriate drug and examined by replica plating for their resistance markers.

Preparation of phage and transduction. Donor bacteria were grown at 37°C in 5 ml of L-broth to a concentration of about 2×10^8 cells per ml. The culture was centrifuged, and the precipitate was suspended in the same volume of LB Ca. A 0.1-ml sample of P1 phage (10^8 plaque-forming units per ml) was added to the bacterial suspension and vigorously shaken for 3 h at 37°C, followed by addition of $CHCl_3$, and the debris was removed by centrifugation. Recipient bacteria grown in L-broth to a concentration of 5×10^8 cells per ml were centrifuged, suspended in the same volume of LB Ca, and infected at a multiplicity of 0.2 with P1 phage prepared as described above. After adsorption of phage for 20 min at 37°C, the recipient bacteria were washed three times with BSG buffer and plated on selective media. The plates were incubated at 37°C for 48 h. P22 phage transduction was performed by the same method as P1 transduction except that there was no use of $CaCl_2$ and cells were infected with phage at a multiplicity of 5.

Determination of UV sensitivity. The culture in the exponential growth phase was pelleted by centrifugation and suspended in BSG buffer to give a concentration of 1×10^5 to 2×10^5 cells per ml. A 5-ml bacterial suspension in a 90-mm petri dish was irradiated using a National GL-15 lamp from a distance of 30 cm. Irradiated bacteria were diluted with BSG buffer and plated on L-agar. Colonies were scored. The relation between irradiation time and the number of viable cells was examined.

Determination of UV sensitivity and *dor* marker in recombinants. Cells from a recombinant colony were suspended in 0.5 ml of BSG buffer, and a 0.05-ml sample was spotted on an L-agar plate and irradiated with a UV lamp as described above. The *dor* marker was examined as follows. Rms312 was transferred from an appropriate donor into a recombinant by selection for Tc^r. A colony of the R^+ recombinant was spread on a drug-free plate to be free from selective force of tetracycline. Spreading was performed by the same procedure described above under "Population analysis of resistant cells." After overnight incubation, one colony on the plate was examined for the population of Tc^s progeny: the colony was spread on a drug-free plate and incubated overnight, and 10 progeny colonies were streaked on plates containing tetracycline (25 μg/ml). The *dor* recombinant maintained Tc^r in 9 to 10 of 10 progeny colonies, but the dor^+ recombinant lost Tc^r in all 10 colonies.

RESULTS

Isolation of the *dor* mutant. Rms312 specifies, on its host, resistance to tetracycline, chloramphenicol, streptomycin, sulfonamide, and mercury chloride. In *S. typhimurium*, Rms312 loses independently not only the Cm Sm Su Mer determinants but also the Tc determinant at a high frequency (7). To isolate *S. typhimurium* mutants that stably maintain all of the resistance markers of Rms312, we performed alternate

culturing of Rms312$^+$ strain in a broth containing tetracycline or chloramphenicol.

Rms312 was transferred from *E. coli* K-12 W3630 to *S. typhimurium* LT2 ML4910 by selection for Tc^r. Eight transconjugants that retained all of the R markers were independently suspended into eight test tubes with 10 ml each of L-broth. After an overnight incubation, each culture was found to contain about 80% tetracycline-sensitive cells and 20% chloramphenicol-sensitive cells. A portion of the culture was diluted 10-fold with fresh L-broth containing tetracycline (25 μg/ml) and incubated overnight. Then similar dilution and incubation was made with L-broth containing chloramphenicol (25 μg/ml). These alternate cultivations were repeated 10 times. One loopful of the final culture was spread on a drug-free plate for purification, and 10 colonies from each culture were examined for their population of resistant cells as described in Materials and Methods. Nine or 10 out of 10 colonies from each of the eight cultures were found to consist of chloramphenicol- and tetracycline-resistant cells. Then, one progeny clone that stably maintained the resistance determinants was selected from each of the eight final cultures.

The eight clones thus obtained were then examined to see whether the mutation resides on the R plasmid or on the host chromosome. The R plasmids in the eight clones were conjugally transferred to ML4916 by selection for Tc^r. Three of eight R plasmids in ML4916 were found to have lost the Tc determinant at a frequency of more than 80% after overnight culture, indicating the mutation did not affect the R plasmid. The three R plasmids were then cured from the original clones by the acridine dye method, and the resultant R$^-$ clones were termed ML4911, ML4912, and ML4917. Rms312 was newly transferred from W3630 to these three hosts, and the stability of tetracycline resistance was examined. After an overnight culture of Rms312-bearing ML4911, ML4912, or ML4917, tetracycline-sensitive cells appeared at a frequency of less than 1%. On the other hand, when Rms312 was transferred to ML4910, tetracycline resistance was lost at a frequency of more than 80%. The site of the mutation governing loss of Tc in ML4910 was considered to be on the host chromosome, and the gene it defines was designated *dor*.

ML4912, one of the *dor* strains, and its parent strain, ML4910, were used for further study of the *dor* character.

Loss of resistance specified by various R plasmids in a *dor* mutant. We examined loss of resistance specified by various R plasmids in the *dor* mutant ML4912. R plasmids were transferred from W3630 to ML4910 and ML4912, using nalidixic acid and an appropriate drug for selection. Frequency of loss of resistance determinants was estimated as described in Materials and Methods (Table 2). Tested markers of FII R plasmids were lost about 100-fold less frequently in a *dor* host than in a *dor*$^+$ host. Loss frequency of the tetracycline resistance of R100, however, was less than 1.3×10^{-4} in both hosts.

Resistance markers other than Tc in FII R plasmids were lost jointly, indicating that the loss of resistance was caused by deletion. Ten deletion mutants from each of the FII R plasmids were selected, they were transferred from the Sm^r host ML4910 to the streptomycin-sensitive host W3630, and the presence of the Sm^r allele was examined. All of the deletion mutants tested were streptomycin sensitive, indicating that the Sm allele was deleted jointly with Cm in these R plasmids.

Effect of the *dor* mutation on deletion of the resistance determinant of R100. We examined the effect of the *dor* mutation on various deletions of the r-determinants. For this purpose, we used a streptomycin-dependent strain (LD-1, Sm^d) as described in Materials and Methods, because in this host it was possible to detect deletions of some or all of the r-determinant including the Sm gene (22). We transferred R100 from a *dor* or *dor*$^+$ donor to a streptomycin-dependent or streptomycin-resistant recipient. Since no Sm^r plasmids survive in Sm^d hosts, the ratio of Tc or Cm transconjugants in the Sm^d/Sm^r hosts is taken as a measure of the frequency of preexisting Sm deletions in the donor. R100-Tc and LD-1 Sm^r were used for controls concerning ability to transfer.

R100-Tc was transferred to both recipients at about the same frequency, and transfer frequency to LD-1 Sm^r was about 10^{-4} in all cases (Table 3). These results indicate that ability to transfer is not involved in the measurement of the frequency of Sm deletions. When a *dor*$^+$ R100 donor was used, the ratio of Tc^r transconjugants in the Sm^d/Sm^r hosts was 100-fold higher than the ratio of Cm^r transconjugants. When a *dor* R100 donor was used, the ratio of Tc^r transconjugants was similar to that of Cm^r transconjugants. These results suggest that the type of streptomycin-sensitive R plasmids differs between Tc and Cm selection. The *dor* mutation affects only the number of streptomycin-sensitive mutants obtained by Tc selection.

We then analyzed the resistance pattern of transconjugants in the Sm^d recipient (Table 4). After the selection for Tc, the majority of transconjugants had lost resistance to chloramphenicol, streptomycin, sulfonamide, and mercury chloride, indicating that the entire r-determinant was lost. After the selection for Cm, most

TABLE 2. *Loss of resistance marker(s) specified by various FII R plasmids in a dor mutant*

R plasmid	Markers lost[a]	Dor[b] of host	Total cell no. at start (p_0) (10^8)	Proportion of mutants losing resistance at start (π_0) (%)	Total cell no. at end (p) (10^8)	Proportion of mutants losing resistance at end (π) (%)	Loss frequency[c] (M) (10^{-2})
Rms312	Tc	+	1.6	24.1	3.6	88.1	8.3
		−	1.1	<0.1	3.8	0.4	0.036–0.049
	Cm Sm Su Mer	+	1.6	0.8	3.6	16.2	1.9
		−	1.1	<0.1	3.8	<0.1	<0.012
R100	Cm Sm Su Mer	+	1.0	0.6	3.1	15.1	1.8
		−	2.0	<0.1	3.8	<0.1	<0.013
	Tc	+	1.0	<0.1	3.1	<0.1	<0.012
		−	2.0	<0.1	3.8	<0.1	<0.013
R6-5	Cm Sm Su Mer Km	+	1.0	0.8	4.0	12.5	1.4
		−	1.2	<0.1	3.0	<0.1	<0.013
R1	Cm Sm Su Ap Km	+	1.2	0.7	4.1	11.4	1.3
		−	2.1	<0.1	3.7	<0.1	<0.013

[a] For phenotype abbreviations, see Table 1, footnote *b*.
[b] Dor$^+$, ML4910; Dor$^-$, ML4912.
[c] Loss frequency was estimated by the method described in the text. $M = (\pi - \pi_0)/\ln(p/p_0)$.

TABLE 3. *Frequency of preexisting Sm deletions of R100 in a dor strain*[a]

Dor phenotype[b] of donor	R plasmid in donor	Selective drug	Frequency of transfer to:		Ratio Sm^d/Sm^r[c]
			LD-1 Sm^d	LD-1 Sm^r	
+	R100-Tc	Tc	7.1×10^{-5}	8.3×10^{-5}	8.5×10^{-1}
+	R100	Tc	3.7×10^{-5}	1.0×10^{-4}	3.7×10^{-1}
+	R100	Cm	3.3×10^{-5}	8.5×10^{-5}	3.9×10^{-3}
−	R100	Tc	6.4×10^{-7}	1.2×10^{-4}	5.3×10^{-3}
−	R100	Cm	3.2×10^{-7}	9.8×10^{-5}	3.3×10^{-3}

[a] Measured by transfer of the R plasmid to an Sm^d recipient. Transfer of R plasmid was performed under the conditions described in the text. Selection medium was L-agar containing streptomycin (200 μg/ml) and either chloramphenicol (25 μg/ml) or tetracycline (25 μg/ml). Transfer frequency was expressed as the number of transconjugants per input donor cell.
[b] Dor$^+$, ML4916; Dor$^-$, ML4919.
[c] The frequency of transfer to LD-1 Sm^d divided by that to LD-1 Sm^r. The ratio is taken as a measure of the frequency of preexisting Sm deletions in a donor.

TABLE 4. *Progeny analysis of transconjugants in matings using a streptomycin-dependent recipient*[a]

Donor	Selective drug in matings	No. of colonies tested	Phenotype of transconjugant				No. of colonies obtained
			Mer^r[b]	Su^r	Cm^r	Tc^r	
ML4916(R100)	Tetracycline	100	−	−	−	+	100
	Chloramphenicol	100	−	−	+	+	91
			+	+	+	+	9
ML4919(R100)	Tetracycline	100	−	−	−	+	95
			−	−	+	+	4
			+	+	−	+	1
	Chloramphenicol	100	−	−	+	+	92
			+	+	+	+	8

[a] Methods are described in the text.
[b] Map of the resistance markers of R100: -IS*1*-Mer-Su-Sm-Cm-IS*1*-Tc- (15).

of the transconjugants had lost resistance to streptomycin, sulfonamide, and mercury chloride (designated Sm^s Cm^r in this paper).

From the above results, it is concluded that the *dor* mutation did affect formation of the entire r-determinant deletion, but did not affect formation of Sm^s Cm^r deletions.

Effect of the *dor* mutation on UV sensitivity and generalized recombination. It is known that a *recA* mutation reduces the deletion formation of the r-determinant of R100 in *S. typhimurium* (31). The *dor* mutation was compared with a *recA* mutation for their UV-light sensitivity and effect on generalized recombination. The *dor* mutant ML4912 was more sensitive to UV light than its parent strain ML4910, but less sensitive than a *recA* strain ML4920 (Fig. 1).

We then examined the frequency of generalized recombination after conjugation with an Hfr and after transduction. The Hfr strain SA540 was crossed with a *dor*$^+$, *dor*, or *recA* strain, and *met*$^+$ *pur*$^+$ recombinants were selected (Table 5). The frequency of recombination was the same in both *dor*$^+$ and *dor* strains, whereas the recombination frequency in *recA* strain was greatly decreased. Table 5 also shows that the frequency of the *thy*$^+$ transductants was the same in both *dor*$^+$ and *dor* strains. These results indicate that the *dor* mutation had no effect on the frequency of generalized recombination and that *dor* was distinct from *recA*.

Mapping of the *dor* mutation. Determination of the location of *dor* on the host chromosome is shown in Table 6. First, the Hfr × F$^-$ *dor* cross shown in Table 6 (experiment A) was performed, and the recombinants were selected for *trp*$^+$ *pur*$^+$ or *met*$^+$ *pur*$^+$. The linkages between *dor* and *trp*, and *dor* and *met*, were similarly low. Since *thy* is between *trp* and *met* in the linkage map of *S. typhimurium* (25), we isolated a *thy* mutant of ML4912, which was then crossed with the same Hfr. *dor* was found to show 54% linkage with *thy*. A reciprocal cross confirmed that the linkage of *dor* with *thy*$^+$ was similar to that of *dor*$^+$ with *thy*$^+$ (Table 6, experiment B). In this cross the map position of *dor* is either between *thy* and *trp* or between *thy* and *str*. To detail the site of *dor* in relation to *thy*, we used another F$^-$ strain JB564 to cross with the *dor* Hfr. As shown in experiment C (Table 6), *dor* was located between *thy* and *trpB*, showing 82% linkage with *glyA*.

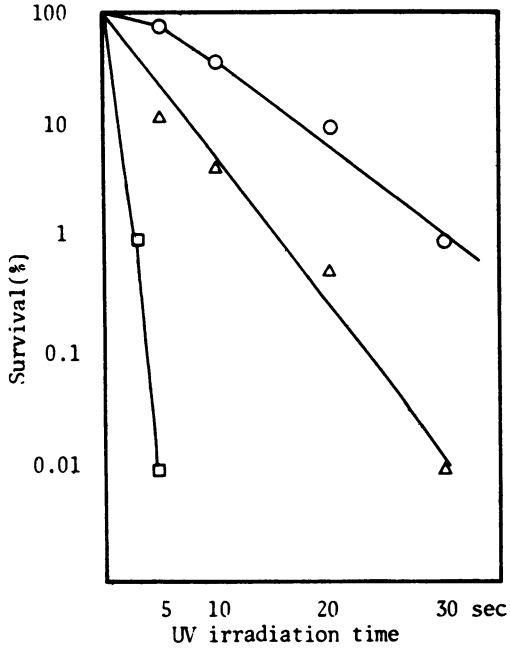

FIG. 1. *UV sensitivity. S. typhimurium ML4910* (○), *ML4912 (dor)* (△), *and ML4920 (rec A)* (□).

A transduction experiment was performed to determine more precisely the location of *dor*. P22 was propagated on a *glyA*$^+$ *dor* strain (ML4922), and the *glyA*$^+$ marker was transduced to a *glyA dor*$^+$ recipient (JB564). One hundred *glyA*$^+$ transductants were examined for both *dor* and UV sensitivity as unselected markers. *dor* was cotransduced at 11% linkage with *glyA* and did not segregate from UV sensitivity. This result indicated that *dor* is located within 1 unit of *glyA* because P22 covers a length of 1 unit (25). *glyA* is located at 57 units on the *S. typhimurium* map (25), so *dor* is between 56 and 58 units on the map.

DISCUSSION

In this paper we have described the isolation and characterization of a mutation (*dor*) in *S. typhimurium* which affects the deletion of r-determinants of R plasmids. The *dor* mutation is located close to *glyA* at about 57 units on the *S. typhimurium* chromosome, a region near which no genes responsible for recombination or repair have been observed.

TABLE 5. *Effect of the dor mutation on generalized recombination*

Mode of genetic transfer	Donor	Recipient	Frequency of recombinants	
			metA$^+$ *purE*$^+$	*thyA*$^+$
Hfr conjugation[a]	SA540 (*purE*)	ML4910 (*metA*)	1.7×10^{-5}	
		ML4912 (*metA dor*)	1.7×10^{-5}	
		ML4920 (*metA recA*)	$<10^{-8}$	
P1 transduction[b]	ML4910	ML4913 (*thyA*)		3.0×10^{-6}
		ML4914 (*thyA dor*)		2.0×10^{-6}

[a] Hfr mating was performed under the conditions described in the text. The *metA*$^+$ recombinants were selected on AG-agar containing 25 μg each of valine and tryptophan per ml and 4 pmol of cyanocobalamin per ml. Frequency of recombination was expressed as the number of recombinants per donor cell.

[b] Transduction was performed by the method described in the text. The *thyA*$^+$ recombinants were selected on AG-agar containing 25 μg each of valine, tryptophan, and methionine. Frequency of recombination was expressed as the number of recombinants per plaque-forming unit of input phages.

TABLE 6. *Genetic constitution of the recombinants*[a]

Expt	Cross		Selection	Presence of Hfr allele among 100 recombinants									
	Donor	Recipient		*metA* (90)[b]	*metE* (84)	*str* (72)	*serA* (63)	*thyA* (62)	*glyA* (57)	*dor*[c]	UV[c]	*trpB* (34)	*purE* (12)
A	SA540 (Hfr[d] *dor*$^+$)	ML4912 (F$^-$ *dor*)	*trpB*$^+$ *purE*$^+$	0	0	10				17	17	100	0
			metE$^+$ *purE*$^+$	97	100	72				6	6	0	0
B	SA540 (Hfr *dor*$^+$)	ML4914 (F$^-$ *dor*	*thyA*$^+$ *purE*$^+$	0	2	20		100		54	54	20	0
	ML4918 (Hfr *dor*)	ML4913 (F$^-$ *dor*$^+$)	*thyA*$^+$ *purE*$^+$	0	0	42		100		50	50	29	0
C	ML4918 (Hfr *dor*)	JB564 (F$^-$ *dor*$^+$)	*glyA*$^+$ *purE*$^+$				42		100	82	82		0
			serA$^+$ *purE*$^+$				100		37	34	34		0

[a] Mating was performed under the conditions described in the text. The growth of *metA*, *serA*, *thyA*, *glyA*, *trpB*, *purE*, or *metE* recombinants was supported by 25 μg of homocysteine, serine, thymine, glycine, tryptophan, or adenine per ml or 4 pmol of cyanocobalamin per ml, respectively.

[b] The figure in parentheses shows the map position quoted from Sanderson and Hartman (24).

[c] See the text.

[d] Genes transferred are o-*cysE-metE-metA-purE-trpB-...-str* (25).

Recently, Nevers and Saedler (16) reported an *E. coli* mutant defective in IS*1*-mediated deletion formation (designated *del*). The *del* mutation reduced the frequency of deletion caused by the *galT*::IS*1* system by as much as 100-fold (16). The *dor* mutation is different from the *del* mutation in the following three ways. (i) The deletion frequency of the Tc determinant of Rms312 was the same in both the *del* mutant and its parent strain (data not shown). However, the deletion frequency of the Tc determinant in the *dor* mutant was reduced in comparison with its parent strain. (ii) As shown in Table 3, the *dor* mutation did not affect the generation of Sm^s Cm^r deletions in R100. Rownd et al. obtained Sm^s Cm^r type deletions of R100 by using the LD-1 Sm^d strain, and they suggested that one of the two IS*1* elements that flank the r-determinant component served as a hot spot for this deletion formation (22). Thus, the *dor* mutation does not affect the formation of deletions mediated by a single IS*1*, although the *del* mutation affects the formation of deletions mediated by a single IS*1* as demonstrated in the *galT*::IS*1* system (16). (iii) The *del* mutation has been mapped to a position close to *lysA* at about 6 min distant from *glyA* on the *E. coli* chromosome, whereas *dor* is closely linked to *glyA* in *S. typhimurium*. Thus, the site of *del* is different from that of *dor*. This comparison appears warranted since it is clear that the genetic maps of *Salmonella* and *E. coli* bear a great degree of homology in overall organization (14, 23).

The r-determinants of R100, R1, and R6-5 are flanked by two directly repeated IS*1* sequences (10, 19). Chandler et al. (2) indicated that reciprocal recombination between the two IS*1* sites caused the separation of the RTF plasmid and the r-determinant element from R100.1. The *dor* mutation reduced the frequency of deletion formation of the r-determinant in R100, R1, and R6-5. The *dor* mutation is presumed to affect two IS*1*-mediated reciprocal recombinations, that is, legitimate recombinations. The *dor* mutation also affects the deletion formation of the Tc determinant and the Cm Sm Su Mer determinants of Rms312, suggesting that the Tc determinant and the Cm Sm Su Mer determinants of Rms312 are flanked by two repeated sequences or recombinational hot spots. This assumption is not inconsistent with our unpublished data that tetracycline-sensitive mutants spontaneously isolated from Rms312 always lost the same size of DNA fragment (12 megadaltons).

ACKNOWLEDGMENTS

We are grateful to Kenneth E. Sanderson, Jean E. Brenchley, Patricia Nevers, Kenneth Timmis, and Takeyoshi Miki for providing some strains used in this work.

This work was supported by the grants provided by the Ministry of Education, the Japanese Government (grants no. 15123, 311201, and 410701).

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