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Recombination Between Two IS*1*s Flanking the r-Determinant of R100-1: Involvement of *dor* and *recA* Gene Functions in *Salmonella typhimurium*

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Drug resistance genes of the r-determinant component of a composite R plasmid R100-1 were frequently lost in *Salmonella typhimurium*. Various deletion mutants were analyzed by restriction endonuclease cleavage, Southern blotting, and hybridization techniques. The loss of the r-determinant was found to be the result of a reciprocal recombination between the two IS*1*s flanking the r-determinant. This recombination depended upon both *dor* and *recA* gene functions.

The drug resistance plasmid R100-1 (7) is a transfer-derepressed mutant of R100 and a composite molecule like many of the FII incompatibility R plasmids, comprising a resistance transfer factor (RTF) component and an r-determinant (r-det) component. The RTF component harbors the tetracycline (Tc) resistance transposon Tn*10* (referred to herein as RTF-Tc). The r-det component confers on the host resistance to chloramphenicol (Cm), streptomycin (Sm), sulfonamides (Su), and mercuric ions (Mer). These two regions are separated by two directly repeated IS*1*s (9, 18).

In *Escherichia coli*, R100-1 exists almost exclusively in the form of a composite molecule. In *Salmonella typhimurium*, however, there is a frequent loss of the r-det component from the composite molecule (23, 29, 30).

We isolated a *S. typhimurium* mutant (29) in which the frequency of loss of the r-det was reduced about 100 times when compared with that in the wild strain. We designated the mutation *dor* (deletion of r-det). The *dor* mutation (29) was mapped to a position near 57 units in the linkage map of *S. typhimurium* (22). This mutation caused a slight increase in UV sensitivity, but did not affect generalized recombination.

In this study, we show that both *dor* and *recA* gene functions are essential for the frequent deletion of the r-det from R100-1 in *S. typhimurium*, and that the deletion occurs as the result of a reciprocal recombination between two IS*1* elements flanking the r-det.

MATERIALS AND METHODS

Bacterial strains, plasmids and phages. The bacterial strains and R plasmids used in this study are listed in Table 1. The *dor* phenotype was examined as previously described (29). The *recA1* allele was transduced into HW204 and HW205 by phage P22 *int*, grown on the *recA1 srl*::Tn*10* strain TT521, by selecting for tetracycline resistance and screening for UV sensitivity. Tetracycline-sensitive (Tc^s) and srl^+ strains were isolated by the fusaric acid (12 μg/ml) resistance-positive selection method for loss of tetracycline resistance (2). The existence of the *dor* gene in HW213 was confirmed by the fact that *dor* was cotransduced into purG303 strain at 22% linkage with $purG^+$ (57 units) by phage P22 *int*. Phages used were λ *c*I-71 (provided by H. Ikeda), λ *r*14 *c*I857 *c*II::IS*1* *N*53 *N*7 (3) (provided by S. Iida) as a source of IS*1*, and P22 *int-11* (provided by M. Levine).

Media and drugs. Penassay broth (Difco Laboratories) and agar (1.5%) were routinely used. Minimal agar medium consisted of medium A (5) with 1% glucose, 0.08% bromthymol blue and 1.5% agar (Difco). λ broth contains 1% tryptone (Difco) and 0.25% NaCl. L broth (13) was used for P22 phage transduction. Each medium was supplemented with streptomycin (200 μg/ml) for LD-1 streptomycin-resistant (Sm^r) and LD-1 streptomycin-dependent (Sm^d) strains or with thymine (25 μg/ml) for thymine-requiring strains. When required, amino acids were added to minimal medium to a final concentration of 25 μg/ml each.

λ dilution buffer contains 10 mM $MgSO_4$ and 10 mM Tris-hydrochloride (pH 7.4). SSC contains 0.15 M NaCl and 0.015 M sodium citrate. BSG buffer contains 8.5 g of NaCl, 300 mg of KH_2PO_4, 600 mg of Na_2HPO_4, and 100 mg of gelatin in 1,000 ml of distilled water.

The concentrations of drugs for selection of resistant bacteria were as follows: tetracycline hydrochloride and streptomycin sulfate, 6.25 μg/ml; chloramphenicol and mercuric chloride, 12.5 μg/ml; sulfonamide, 100 μg/ml. Sulfonamide resistance was examined on minimal medium.

Transconjugation and transduction. The methods for transconjugation and transduction were described previously (29).

Detection of R plasmid mutants sensitive to streptomycin. Detection of mutants was performed by the

TABLE 1. Bacterial strains and R plasmids used

Strain or R plasmid	Properties[a]	Source or reference
S. typhimurium LT2 derivatives		
JB564	*serA glyA*	J. E. Brenchley
HW201	*serA glyA thy*	Tp^{rb} derivative of JB564
ML4922	Hfr *dor*	(29)
HW204	*serA thy dor*	P22(ML4922) × HW201
HW205	*serA thy*	P22(ML4922) × HW201
TT521	*srl*::Tn*10 recA1*	J. Ishizu
HW207	*serA thy srl*::Tn*10 recA1*	P22(TT521) × HW205
HW209	*serA thy dor srl*::Tn*10 recA1*	P22(TT521) × HW204
HW211	*serA thy recA1*	Tc^s and *srl*$^+$ derivative of HW207
HW213	*serA thy dor recA1*	Tc^s and *srl*$^+$ derivative of HW209
purG303	*purG303 thi*	K. E. Sanderson
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		
R100-1	Tc^r Cm^r Sm^r Su^r Mer^r	(7)
R100-11	Tc^r Cm^s Sm^s Su^s Mer^s	This paper
R100-22	Tc^r Cm^r Sm^s Su^s Mer^s	This paper
R100-42	Tc^r Cm^r Sm^s Su^s Mer^s	This paper
R100-25	Tc^r Cm^s Sm^s Su^s Mer^s	This paper
R100-45	Tc^r Cm^s Sm^s Su^s Mer^s	This paper

[a] Abbreviations and nomenclature are essentially as in Demerec et al. (6) and Sanderson and Hartman (22).
[b] Tp, Trimethoprim.

transfer of the plasmid to a Sm^d strain as described previously (29). This technique (21) was based on the observation that only Sm^s R plasmids can survive in an Sm^d strain because Sm^r R plasmids encode an enzyme that inactivates streptomycin, which is required for the growth of Sm^d cells.

Donor cells harboring R100-1 were cultured to a density of 2×10^8 cells per ml in Penassay broth containing a low concentration of chloramphenicol (3 μg/ml). The culture was then diluted 10^6-fold with fresh drug-free Penassay broth and grown at 37°C with gentle shaking to a density of 2×10^8 cells per ml after about 20 cell doublings. The ratio of r-det loss of R100-1 per total cells at this time in HW205, HW204, HW211, and HW213 was about 12, 0.1, <0.1 and <0.1%, respectively. The donor culture and a recipient culture (LD-1 Sm^d or LD-1 Sm^r, about 2×10^8 cells per ml) were mixed at a volume ratio of 1:4. After 2 h of incubation at 37°C, the cells were washed twice in BSG buffer and plated on minimal agar medium containing Casamino Acids (2 mg/ml), streptomycin (200 μg/ml), and either chloramphenicol (12.5 μg/ml) or tetracycline (6.25 μg/ml). After 48 h incubation, transconjugants were scored and examined for their resistance markers by streaking on drug-containing plates.

Preparation of phage and phage DNA. The phage was prepared by temperature induction of a lysogen or infection of bacteria in broth, concentrated by polyethylene glycol 6000, and purified by high-speed centrifugation and then banding in a CsCl density gradient. Mature DNA was prepared by extracting purified phage particles in SSC with SSC-saturated phenol in the presence of 0.2% sodium dodecyl sulfate. DNA thus obtained was dialyzed against SSC.

Preparation of plasmid DNA. DNA of all R plasmids was prepared from W3630 (R$^+$). Stationary phase cells were harvested, suspended in cold 25% (wt/vol) sucrose–0.05 M Tris–0.001 M EDTA, (pH 8.0), and added with RNase (20 μg/ml). Cells were lysed by adding cold Triton X-100 to a final concentration of 0.05% (vol/vol) after treatment with lysozyme (1 mg/ml) for 10 min at 0°C and 0.5 M EDTA (pH 8.0) for an additional 10 min at 0°C. DNA was concentrated by 10% (wt/vol) polyethylene glycol (Carbowax 6000) and further purified by preparative centrifugation in a neutral CsCl gradient. Plasmid DNA from CsCl gradients was dialyzed against 0.1 × SSC and stored at 4°C.

Restriction endonuclease digestion and agarose gel electrophoresis. DNA was completely digested with *Eco*RI (New England Biolabs) or *Pst*I (Boehringer Mannheim Corp.) in the appropriate buffer. In most reactions 2 to 4 U of enzyme per μg of DNA was used, and digestion was carried out in 30- to 50-μl volumes for 2 h at 37°C. The reactions were terminated by the addition of a mixture of sodium dodecyl sulfate sucrose, bromophenol blue, and EDTA to final concentrations of 1%, 10%, 0.005%, and 0.04 M, respectively. Restriction fragments were resolved by electrophoresis through 0.8 or 1.2% agarose (Wako Pure Chemical Industries, Ltd.) vertical slab gels (12 by 13 by 0.3 cm) with TEAS electrophoresis buffer (0.05 M Tris, 0.02 M sodium acetate, 0.002 M EDTA, 0.018 M sodium chloride, pH 8.05). Gels were run at 32 V/cm, stained with 0.5 μg of ethidium bromide per ml, and photographed under UV illumination.

Removal of *Eco*RI DNA fragments from agarose. DNA was removed from agarose by the glass-binding technique (28).

Labeling λ DNA by nick translation. Purified *Eco*RI fragment B of λ *r*14, in which the fragment IS*1* element was present (27), and λ *c*I-71 were labeled with [^{32}P]dCTP to high specific radioactivities (10^8 cpm/μg) in vitro by the procedure of Rigby et al. (19). ^{32}P-labeled *Eco*RI fragment B of λ *r*14 was used as a probe for IS*1*.

Blotting and hybridization procedures. Fragments of DNA were transferred from agarose gels to nitrocellulose filters (Shleicher & Schüll Co., BA85) and hybridized to a radioactive probe by a slight modification of the procedure of Southern (25). Before transfer, the DNA fragments were treated with 0.1 N HCl for 10 min, denatured by incubating the gel for 30 min in 0.5 M NaOH–1.5 M NaCl, and neutralized by a further 30-min incubation in 3 M NaCl–0.5 M Tris-hydrochloride (pH 7.4). After transfer the filters were rinsed with 2× SSC, air dried, and then baked at 80°C under vacuum for 2 h. Before hybridization, the nitrocellulose filter was incubated at 68°C for 2 h in 200 ml of 3× SSC–0.1% sodium dodecyl sulfate–10× Denhardt solution (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), yeast RNA (10 μg/ml), and heat-denatured calf thymus DNA (50 μg/ml). After pretreatment, the filter was air dried and placed in a plastic bag, and 5 ml of hybridization solution was added. The hybridization solution included 3× SSC, 10× Denhardt solution, yeast RNA (10 μg/ml), heat-denatured calf thymus DNA (50 μg/ml), and labeled probe DNA containing between 4×10^5 and 1×10^6 cpm. The plastic bag was sealed and immersed at 68°C for 24 h. Hybridization was terminated by cutting open the bag and washing the nitrocellulose filter four times (30 min each) in a solution with both 0.1× SSC and 0.1% sodium dodecyl sulfate at 68°C and finally washed twice in 0.1× SSC. After air drying the filter was exposed to preflashed Fuji X-ray film with a Du Pont Lightning Plus intensifying screen at −80°C for 1 to 6 days.

RESULTS

Dependence of r-det deletions on both *dor* and *recA* functions. The r-det of R100-1 is flanked by the two IS*1*s and specifies resistance to mercuric ion, sulfonamides, streptomycin, and chloramphenicol (Fig. 1). It is possible to detect deletions of the Sm gene or of all of the r-det including the Sm gene by the transfer of R100-1 into a streptomycin-dependent strain as previously described (21, 29). By using different drugs for the selection of transconjugants, different types of deletions can be obtained (21, 29). By selecting for Tc^r, deletions of the entire r-det were mainly detected. By selecting for Cm^r, the deletions of a part of the r-det (sensitive to mercuric ion, sulfonamides, and streptomycin; designated Sm^s Cm^r) were mainly detected. Using this system, we previously reported that the loss of the entire r-det of R100 depended upon a *dor* gene function (29). In this paper, the effect of *recA* mutation was examined.

When the frequency of various deletions in a

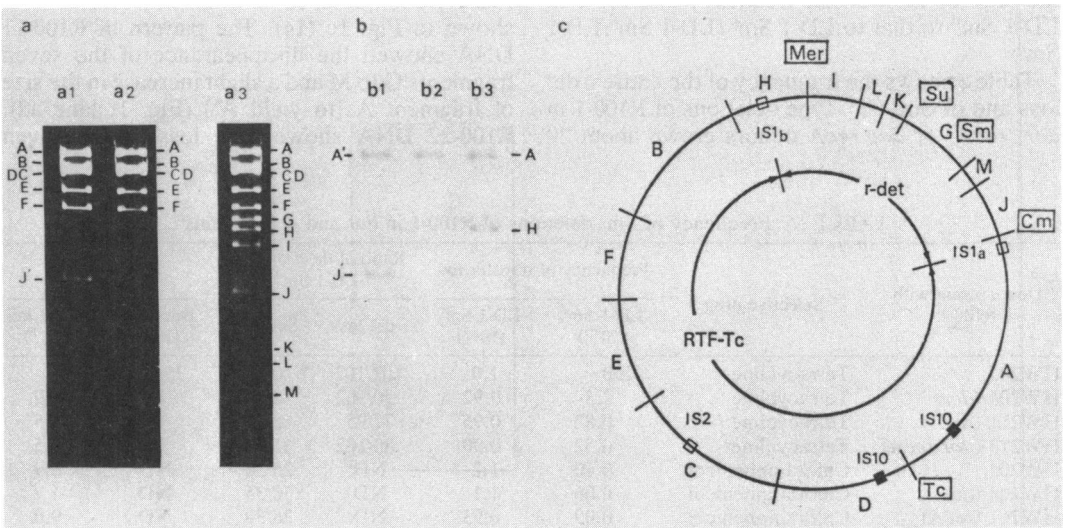

FIG. 1. Agarose gel electrophoresis of *Eco*RI fragments of deletion mutant DNAs derived from R100-1 and location of the IS*1* sequences by hybridization. Channels a1 through a3 show the patterns of *Eco*RI fragments of R100-22, R100-11, and R100-1, respectively, after electrophoresis on a 0.8% agarose gel. These fragments were denatured, transferred to a nitrocellulose membrane filter, and hybridized with ^{32}P-labeled λ *r*14 *Eco*RI B fragment. The results obtained from an autoradiograph of the filter are shown in channels b1 through b3 (R100-22, R100-11, and R100-1, respectively). DNA of R100-1 and its derivatives did not hybridize with ^{32}P-labeled λ *c*I-71 without the IS*1* sequences (data not shown). c, Summarized *Eco*RI restriction endonuclease map of R100-1 and the location of the resistance genes based on published data (14, 20, 26).

donor is compared with that in other donor by this transfer system, it is necessary to consider some factors which may influence the frequency. One is the effect of differences in growth rate between the cells with R100-1 and those with its deletion derivatives. We determined independently the generation time of HW205(R100-1), HW205(R100-11), and HW205(R100-22) from a plot of the logarithm of the optical density at 560 nm versus the time; the generation time was 28 ± 1 min in these three strains. In the case of other hosts, there was also no significant difference in the generation time between the cells with R100-1 and those with its deletion derivatives. The generation time of the strains with a *recA* mutation was about 1.5 times longer than that of the rec^+ strain. Another factor to be considered is the effect of differences in transfer frequency between R100-1 and its derivatives. R100-1, R100-11, and R100-22 were independently transferred from various hosts to LD-1 Sm^r. The average transfer frequency of R100-11 and R100-22 did not vary by more than 20% from that of R100-1 in each host (data not shown). The ability of LD-1 Sm^d and LD-1 Sm^r to serve as recipient strains was also examined and was found to be almost the same when HW205(R100-11) was used as a donor. These results indicate that these factors do not significantly influence the frequency of Sm^s deletions determined by the transfer system. Thus, the frequency of Sm^s deletions in donor cells could be estimated from the ratio of the transfer frequency of R100-1 to LD-1 Sm^d to that to LD-1 Sm^r (LD-1 Sm^d/LD-1 Sm^r).

Table 2 shows the frequency of the entire r-det loss and of Sm^s Cm^r-type deletions of R100-1 in *dor*, *recA*, or *dor recA* donors grown about 20 cell doublings in the nonselective medium before mating. Since some types of Sm^s mutants other than the r-det loss or Sm^s Cm^r types were detected among transconjugants in LD-1 Sm^d, the frequency of r-det loss or Sm^s Cm^r-type deletions was therefore adjusted by taking into account the frequency of these other types of deletions. In *dor*, *recA*, or *dor recA* mutants, the frequency of loss of the entire r-det was apparently lower than that in its wild-type parent. The function of $recA^+$ and dor^+ gene products was not multiplicative, but rather additive. The frequency observed in a *dor recA* double mutant was much higher than the frequency calculated on the assumption that a $recA^+$ and a dor^+ gene product functioned fully independently ($4.5 \times 10^{-4} > 1.5 \times 10^{-6} = 2.2 \times 10^{-3} \times 7.0 \times 10^{-4}$). The frequency of Sm^s Cm^r-type deletions was almost the same in all mutants as that in the wild-type parent.

***Eco*RI digestion and IS1 location of deletion mutants from R100-1.** To determine the sites of deletion hot spots of the entire r-det and Sm^s Cm^r-type deletion mutants, we chose R100-11 and R100-22 as the representative mutants, respectively, and analyzed their DNA by restriction cleavage analysis, Southern blotting, and hybridization.

The patterns of *Eco*RI fragment obtained from R100-1, R100-11, and R100-22 are shown in Fig. 1a. R100-1 DNA consisted of 13 major fragments (Fig. 1, lane a3, bands A through M). The relative position of these fragments on R100-1 is shown in Fig. 1c (14). The pattern of R100-11 DNA showed the disappearance of the seven fragments G to M and a slight increase in the size of fragment A (to yield A') (Fig. 1, lane a2). R100-22 DNA showed the loss of the seven

TABLE 2. Frequency of Sm^s deletions of R100-1 in *dor* and *recA* strains[a]

Donor strain with R100-1	Selective drug	Frequency of transfer to:		Ratio of deletions[b] in LD-1 Sm^d		Frequency[c] of	
		LD-1 Sm^d (10^{-5})	LD-1 Sm^r (10^{-2})	r-det loss	Sm^s Cm^r	r-det loss (10^{-3})	Sm^s Cm^r (10^{-5})
HW205	Tetracycline	250	1.0	102/102	0/102	250	
HW204 (*dor*)	Tetracycline	2.3	0.92	90/102	2/102	2.2	4.9
HW211 (*recA*)	Tetracycline	0.82	0.95	77/95	5/95	0.70	4.5
HW213 (*dor recA*)	Tetracycline	0.52	0.90	80/102	15/102	0.45	8.5
HW205	Chloramphenicol	0.10	1.2	ND[d]	25/30	ND	6.9
HW204 (*dor*)	Chloramphenicol	0.06	1.1	ND	30/35	ND	4.7
HW211 (*recA*)	Chloramphenicol	0.09	0.93	ND	28/30	ND	9.0
HW213 (*dor recA*)	Chloramphenicol	0.09	0.96	ND	28/30	ND	8.7

[a] See text. The frequencies in the table are the means of three determinations. The individual values usually did not vary by more than 30% from the average.

[b] Ratio of r-det loss or Sm^s Cm^r shows the number of Mer^s, Su^s, Sm^s, Cm^s, and Tc^r strains or the number of Mer^s, Su^s, Sm^s, Cm^r, and Tc^r strains among the tested transconjugants in LD-1 Sm^d.

[c] Determined by (transfer frequency to LD-1 Sm^d)/(transfer frequency to LD-1 Sm^r) × ratio of r-det loss or Sm^s Cm^r.

[d] ND, Not detected.

fragments G to M, but differed from R100-11 DNA in the formation of one new fragment (J') and an intact fragment A (Fig. 1, lane a1).

We then determined the location of IS1-specific sequences among the *Eco*RI fragments of R100-1, R100-11, and R100-22. The DNA fragments in the agarose gel shown in Fig. 1 were denatured and transferred to a nitrocellulose membrane filter by the method of Southern (25). The transferred fragments were hybridized with a ^{32}P-labeled preparation of the *Eco*RI fragment B of bacteriophage λ *r*14, which carries one copy of IS1 inserted into the *cII* gene (27), as described above. The results are shown in Fig. 1 (lanes b1 through b3). Both the R100-1 fragments A and H hybridized with IS1-specific DNA, and the result is consistent with those of Rownd et al. (21, 26) and Chandler et al. (4). IS1-specific DNA was also found in fragment A' of R100-11 (Fig. 1, lane b2) and fragment A and J' of R100-22 (Fig. 1, lane b1).

We analyzed eight plasmids in total; four plasmids Sm^s Cm^r-type deletions and the remainder deletions of the entire r-det. The results of the latter class showed that they were identical to R100-11 (data not shown). However, the sizes of J' fragments from the four Sm^s Cm^r-type plasmids were not the same, suggesting that this class of deletion is not formed in the same manner. Two of them are shown in Fig. 5.

From the above results, R100-11 without the entire r-det was considered to be identical to an RTF plasmid described by Tanaka et al. (26) and an RTF-Tc plasmid described by Chandler et al. (4). These suggested that R100-11 may be formed by a reciprocal recombination between two IS1s (which exist in fragment A and H) flanking the r-det and by the subsequent loss of the r-det. The *Eco*RI A' fragment should be a fusion product of part of fragments A and H. To confirm the prediction, we carried out further experiments as described below.

Only one copy of IS1 in R100-11. Although IS1-specific DNA was found in the A' fragment of R100-11 (Fig. 1), it is not clear whether only one IS1 element exists in the A' fragment. We examined how many IS1 elements are present in the A' fragment by using the fact that an IS1 element has only one cutting site for *Pst*I (17).

The A' fragment, extracted from an agarose gel, was digested with *Pst*I. The *Pst*I-digested *Eco*RI A' fragment on 1.2% agarose was transferred to a nitrocellulose filter, which was hybridized to a ^{32}P-labeled IS1 probe (Fig. 2, lanes a3 and b3). Two fragments bound IS1 specific DNA under conditions of agarose gel electrophoresis which would detect more than 200 base pairs of DNA. This result indicates that the A' fragment has one IS1 element. This was also confirmed by the result that two fragments of *Pst*I-digested R100-11 bound IS1 specific DNA, whereas *Pst*I-digested R100-1, which is known to have two IS1 elements, hybridized with IS1 specific DNA at four fragments (Fig. 3).

Composition of the A' fragment of R100-11. The *Eco*RI A' fragment of R100-11 and *Eco*RI A and H fragments of R100-1 were compared after digestion with *Pst*I and hybridization with ^{32}P-labeled IS1 probe (Fig. 2, lanes a2 through a4 and b2 through b4). The *Pst*I fragments A'-4 and

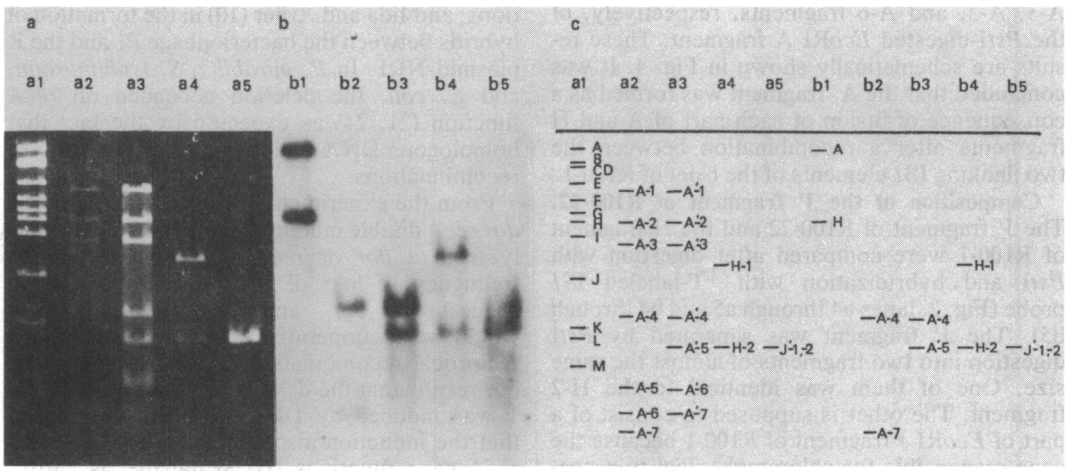

FIG. 2. Agarose (1.2%) gel electrophoresis of *Pst*I fragments of various *Eco*RI fragment DNAs from R100-1 and its deletion mutants, and location of the IS1 sequences by hybridization. a1, *Eco*RI-digested R100-1 DNA; a2, *Pst*I-digested *Eco*RI A fragment DNA from R100-1; a3, *Pst*I-digested *Eco*RI A' fragment DNA from R100-11; a4, *Pst*I-digested *Eco*RI H fragment DNA from R100-1; a5, *Pst*I-digested *Eco*RI J' fragment DNA from R100-22. Lanes b1 through b5 were a1 through a5 DNAs hybridized with ^{32}P-labeled λ *r*14 *Eco*RI B fragment, respectively. c, Diagram of a and b.

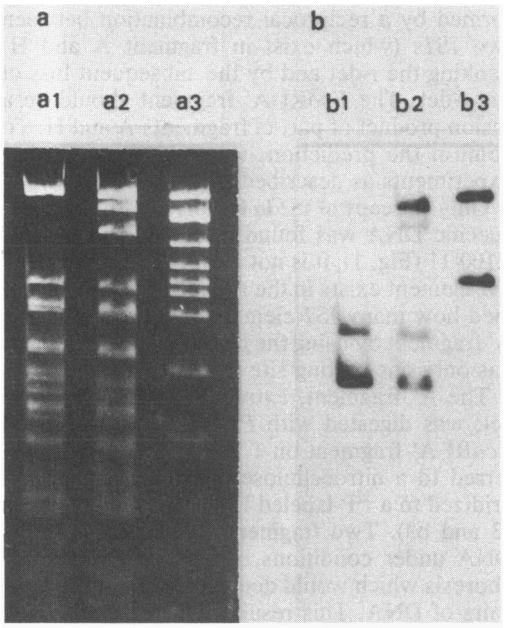

FIG. 3. Agarose (0.8%) gel electrophoresis of *Pst*I-digested R100-1 and its derivative DNA, and location of the IS*1* sequences by hybridization. a1, *Pst*I-digested R100-11 DNA; a2, *Pst*I-digested R100-1 DNA; a3, *Eco*RI-digested R100-1 DNA. Lanes b1 through b3 show the a1 through a3 DNAs transferred to filter hybridized with ^{32}P-labeled λ *r*14 *Eco*RI B fragment.

A′-5 on R100-11, which hybridized with IS*1* specific DNA, were identical to the A-4 and H-2 fragments of R100-1. The other *Pst*I fragments not binding IS*1* probe, A′-1, A′-2, A′-3, A′-6, and A′-7 on R100-11, were identical to A-1, A-2, A-3, A-5, and A-6 fragments, respectively, of the *Pst*I-digested *Eco*RI A fragment. These results are schematically shown in Fig. 4. It was concluded that the A′ fragment was formed as a consequence of fusion of each part of A and H fragments after a recombination between the two flanking IS*1* elements of the r-det of R100-1.

Composition of the J′ fragment of R100-22. The J′ fragment of R100-22 and the H fragment of R100-1 were compared after digestion with *Pst*I and hybridization with ^{32}P-labeled IS*1* probe (Fig. 2, lanes a4 through a5 and b4 through b5). The J′ fragment was separated by *Pst*I digestion into two fragments of almost the same size. One of them was identical to the H-2 fragment. The other is supposed to consist of a part of *Eco*RI J fragment of R100-1 because the gene responsible for chloramphenicol transacetylase extends into the J fragment (Fig. 1c) (1, 14). These results are shown schematically in Fig. 4. The J′ fragment seems to be formed as a result of illegitimate recombination between one end of IS*1*b and a part of the J fragment.

***dor*-Dependent deletion of the chloramphenicol marker among Sm^s Cm^r-type mutants.** Sm^s Cm^r-type deletion mutants still had two IS*1* elements flanking a chloramphenicol resistance gene. We compared the frequency of loss of the chloramphenicol marker between *dor*$^+$ and *dor* strains (Table 3). The frequency of loss of the chloramphenicol marker of Sm^s Cm^r-type mutants was almost the same as that of the r-det of R100-1, and the loss depended upon *dor* gene function. The *Eco*RI fragment patterns of Cm^s clones (R100-25 and R100-45) from Sm^s Cm^r-type deletion mutants (R100-22 and R100-42, respectively) were identical to that of R100-11 (Fig. 5). These results also show that there is a frequent recombination between two IS*1*s of a composite R plasmid in *S. typhimurium* and that its recombination depends upon a *dor* gene function.

DISCUSSION

The results reported above indicated that both *dor* and *recA* genes mediated the frequent deletion of the entire r-det of a composite plasmid R100-1 in *S. typhimurium*, and that the sites of the reciprocal recombination were the two IS*1*s flanking the r-det. These genes, however, did not affect the frequency of illegitimate recombination.

The recombination between two IS*1*s has been reported by several workers: Tanaka et al. (26) in an analysis of a transitioned preparation of NR1(R100) isolated from *Proteus mirabilis*; Rownd et al. (21) in the loss of the re-det of R100 in *S. typhimurium*; Chandler et al. (4) in the formation of circular r-det elements in *Escherichia coli* under particular experimental conditions; and Iida and Arber (10) in the formation of hybrids between the bacteriophage P1 and the R plasmid NR1. In *P. mirabilis*, *S. typhimurium*, and *E. coli*, the deletion depended on *recA* function (21, 24) as expected by the fact that homologous DNA regions participated in these recombinations.

From the experimental results showing that a *dor recA* double mutant did not differ significantly from a *dor* or *recA* single mutant in the frequency of loss of the r-det (Table 2), we suggest that *dor*$^+$ and *recA*$^+$ gene products would work cooperatively on the IS*1*-mediated reciprocal recombination. This is supported by our results that the deletion of the r-det of R100-1 was induced by DNA-damaging agents and that the induction also depended upon *dor* and *recA* gene functions (H. Watanabe, K. Mise, and H. Hashimoto, submitted for publication). However, it is also possible that other gene functions might be involved in this recombination and affect the frequency of loss of the r-det in a *dor recA* mutant.

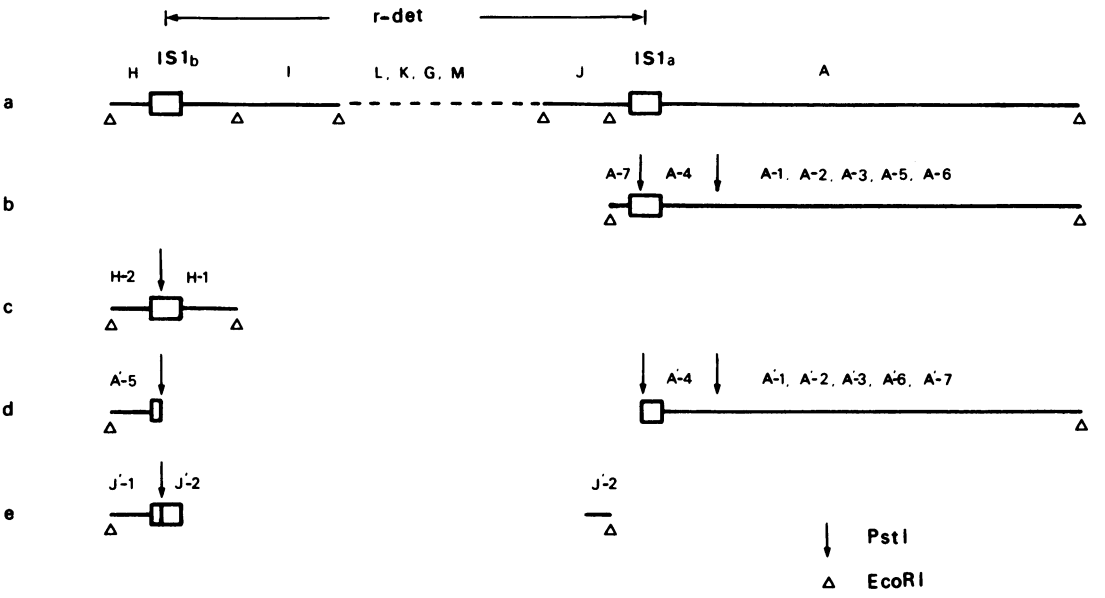

FIG. 4. Composition of *Eco*RI A' fragment DNA of R100-11 and *Eco*RI J' fragment DNA of R100-22. These figures show schematically the results obtained from Fig. 1 through 3. a, *Eco*RI restriction fragment map of the r-det of R100-1 (24); b, *Eco*RI A fragment from R100-1; c, *Eco*RI H fragment from R100-1; d, *Eco*RI A' fragment from R100-11; e, *Eco*RI J' fragment from R100-22. The order of *Pst*I fragments A-1, A-2, A-3, A-5, and A-6 was not determined.

The *dor* mutation affects IS*1*-mediated reciprocal recombination. IS*1* DNA sequences, however, do not seem to be the only site of *dor* function. We previously reported that the *dor* mutation affected the frequency of loss of the region encoding tetracycline resistance as well as the region encoding resistance to chloramphenicol, streptomycin, and sulfonamides in an FII group R plasmid, Rms312, and suggested that the region encoding tetracycline resistance is also flanked by hot spots for recombination (29). Using restriction endonuclease digestion and hybridization with IS*1* probe, we have now obtained experimental evidence that the region encoding tetracycline resistance of Rms312 has no IS*1*-specific DNA sequences (H. Watanabe, unpublished data). The *dor* gene product thus

TABLE 3. Dependence of Cm^r loss of Sm^s Cm^r-type deletion mutants upon a *dor* gene function

R plasmid	*dor* function[a] of host	% of Cm^s cells[b]
R100-1	Dor^+	7.0
	Dor^-	<0.2
R100-22	Dor^+	6.8
	Dor^-	<0.2
R100-42	Dor^+	6.0
	Dor^-	<0.2

[a] Dor^+, HW205; Dor^-, HW204.

[b] Cells harboring plasmids were cultured overnight at 37°C in Penassay broth containing a low concentration of chloramphenicol (3 μg/ml). The culture was then diluted 10^4-fold with fresh drug-free Penassay broth and incubated overnight at 37°C. Appropriate dilutions of the cells were spread on drug-free plates, and the drug resistance to chloramphenicol and tetracycline of individual colonies was tested by replica plating method. The percentage of Tc^s cells was less than 0.2 in all cases.

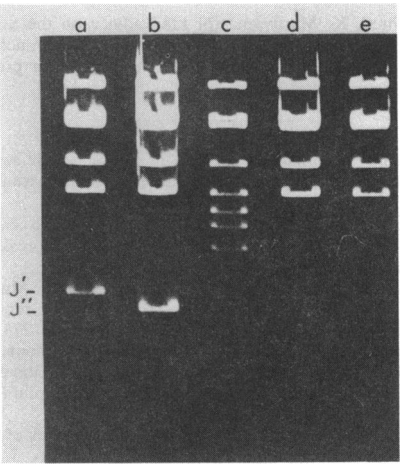

FIG. 5. Agarose (0.8%) gel electrophoresis of *Eco*RI fragments of R100-1 and its deletion mutants: a, R100-22; b, R100-42; c, R100-1; d, R100-25; e, R100-45.

also affects reciprocal recombination between repeated DNA sequences other than IS*1*. It is not certain whether IS*1* and such hot spots have short common sequences.

Sm^s Cm^r-type deletions resulted in loss of resistance to mercuric ion, sulfonamides, and streptomycin, and the *Eco*RI J′ fragments which we examined had different sizes. The production of these derivatives seems to be a result of IS*1*b-mediated illegitimate recombination. Some of them, however, may be caused by the involvement of the Tn*21* transposon, which specifies resistance to mercuric ion, sulfonamides, and streptomycin (11).

The integration and excision between λ and the host chromosome are known to result from a reciprocal recombination between homologous 15-base-pair regions within the attachment sites on both chromosomes (12). This recombination depends upon phage-encoded gene functions such as *int* and *xis* (16) and host-encoded gene functions such as hip (31) and him (15). The *dor* gene product may have a similar function. We are now examining whether the *dor* mutation affects cointegration by reciprocal recombination between two IS*1*s.

Hopkins and co-workers (8) have identified two genes (*ferA* and *ferB*) on the sex factor F which influence *recA* dependent recombination between two directly repeated IS*3*s. They also affect the *rec*-independent precise excision of the transposons Tn*5* and Tn*10*. A *dor* mutation did not affect the frequency of reversion to prototrophy of a Tn*10*-induced *his* auxotroph in *S. typhimurium* (unpublished data). Whether the *dor* and *fer* genes have related functions will require further analyses.

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