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recB and *recC* Genes of *Salmonella typhimurium*

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We have investigated the genetic organization of the *recB* (exonuclease V) and *recC* (exonuclease V) genes of *Salmonella typhimurium*. A detailed genetic map is constructed that includes the relative order in the chromosome, P22 cotransduction frequencies, and the orientation of transcription of the *recB* and *recC* genes. In addition, the isolation and characterization of Mu dJ insertion mutations in *recB* and *recC* are discussed.

The *recB* and *recC* genes of *Escherichia coli* and *Salmonella typhimurium* encode subunits of exonuclease V, a major enzyme involved in recombination (13, 14, 20, 21). Experiments in vitro have shown that several activities are associated with this enzyme. These include an ATP-dependent exonuclease, an ATP-stimulated endonuclease, a DNA-dependent ATPase, and a DNA helicase (15, 16, 20). Strains containing *recBC* deficiencies display multiple phenotypic defects: sensitivity to UV light (8, 9); reduction in cell viability (1); and reduction in transductional (5, 8), conjugal (5, 8), and intrachromosomal recombination (M. J. Mahan and J. R. Roth, Genetics, in press) (for reviews, see references 5 and 19). While these genes have been extensively characterized in *E. coli*, they are less well known in *S. typhimurium*. Since this enzyme plays a central role in recombination, it is important to have characterized mutations for the study of this process in *Salmonella* spp. We describe here genetic characterization of the *recBC* region in *S. typhimurium*.

Isolation of Mu dJ insertion elements in the *recB* and *recC* genes. The *recB* and *recC* genes are cotransducible with *thyA* at 61 min on the chromosome map of *S. typhimurium* (17). Derivatives of bacteriophage Mu, Mu d prophages, were used for insertional mutagenesis of this region. These prophages, referred to here as Mu dJ, are transposition-defective elements that form operon fusions; they were constructed by Casadaban and co-workers (2, 3). Localized insertional mutagenesis of the *recBC* region was achieved by growing P22 transducing phage on a pool of strains containing random chromosomal insertions of Mu dJ; this pool was generated as described by Hughes and Roth (12). The pooled P22 lysate was used to transduce TR5125 (*thyA383*) to

TABLE 1. Bacterial strains and relevant characteristics

Strain	Genotype and relevant characteristics	Source or reference
S. typhimurium		
LT2	Wild type	Lab collection
SL4213	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404 H1-b nml H2-enx* (Fels 2)$^-$	J. L. Ingraham
GW476	*recB503*::Tn*10 hisG46*	G. C. Walker
TR5124	*recBC10 hisD1447*	A. Eisenstark (8)
TR5125	*thyA383 hisD1447*	Lab collection
TT215	*lysA565*::Tn*10*	Lab collection
TT7689	*hisD9950*::Mu dA	Lab collection
TT7692	*hisD9953*::Mu dA	Hughes and Roth (11)
TT13229	*recB497*::Mu dJ *hisD1447*	This work
TT13230	*recC498*::Mu dJ *hisD1447*	This work
TT13231	*recC499*::Mu dJ *hisD1447*	This work
TT13232	*recC500*::Mu dJ *hisD1447*	This work
TT13233	*recC501*::Mu dJ *hisD1447*	This work
TT13234	*recC502*::Mu dJ *hisD1447*	This work
TT13855	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404 H1-b nml H2-enx* (Fels 2)$^-$; pCDK3; *recB*$^+$ *recC*$^+$ *thyA*$^+$	Recombinant plasmid obtained from S. R. Kushner (7)
TT13856	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404 H1-b nml H2-enx* (Fels 2)$^-$; pCDK25; *recC*$^+$ *thyA*$^+$	Recombinant plasmid obtained from S. R. Kushner (7)
TT13858	*hsdL6 hsdA29 galE496 metA22 metE551 ilv-452 rpsL120 xyl-404 H1-b nml H2-enx* (Fels 2)$^-$; pCDK30; *recB*$^+$ *recD*$^+$ *argA*$^+$	Recombinant plasmid obtained from S. R. Kushner (7)
TT13862	*recB503*::Tn*10 recC498*::Mu dJ *thyA383 hisD1447*	This work
TT13863	*recC498*::Mu dJ *thyA383 hisD1447*	This work
TT13864	*recC498*::Mu dA *hisD1447*	This work
TT13865	*recB497*::Mu dA *hisD1447*	This work
TT13866	*recC502*::Mu dA *hisD1447*	This work

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TABLE 2. Complementation of chromosomal *recBC*::Mu dJ insertions with recombinant plasmids

Recipient strain	Relevant genotype	Color on X-gal[a]	Presence of donor phage[b]:			
			No plasmid (*recBC*)	TT13858 (*recB*+*C*)	TT13856 (*recBC*+)	TT13855 (*recB*+*C*+)
TT13229	*recB497*::Mu dJ	White	−	+	−	+
TT13230	*recC498*::Mu dJ	Blue	−	−	+	+
TT13231	*recC499*::Mu dJ	Blue	−	−	+	+
TT13232	*recC500*::Mu dJ	Blue	−	−	+	+
TT13233	*recC501*::Mu dJ	Blue	−	−	+	+
TT13234	*recC502*::Mu dJ	White	−	−	+	+
TR5124	*recB10*	NA[c]	−	+	−	+
GW476	*recB503*::Tn*10*	NA[c]	−	+	−	+
LT2	*recB*+*C*+	NA[c]	+	+	+	+

[a] The color of colonies (blue or white) is an indicator of the transcriptional orientation of the *lacZYA* genes contained in the Mu dJ insertion element.
[b] P22 phage grown on plasmid-containing strains was used to transduce recipient strains harboring *recBC*::Mu dJ insertions to chloramphenicol resistance. A + indicates restoration to wild type for the five phenotypes tested; a − indicates no restoration.
[c] NA, Not applicable; indicated strain does not contain a Mu dJ insertion element.

kanamycin resistance. (All strains used in this study are listed in Table 1; all nutritional supplements were as described previously [6].) Km^r transductants were scored for prototrophy (Thy$^+$) by replica printing to minimal medium containing kanamycin. The Thy$^+$ Km^r transductants have Mu dJ insertions near the *thyA* gene. These transductants were screened for sensitivity to UV light on solid nutrient broth medium. Six UV-sensitive Thy$^+$ Km^r recombinants were isolated from 1,032 Thy$^+$ Km^r transductants tested. Each of six isolates containing the putative *recBC*::Mu dJ insertions displayed the following characteristics: (i) an 8- to 20-fold reduction in recombination as judged by the ability to serve as a recipient in a transduction cross; (ii) sensitivity to UV light; (iii) a 50 to 70% reduction in cell viability; (iv) formation of dark-green colonies on green indicator medium of Chan et al. (4) characteristic of strains containing *recA*, *recB*, or *recC* mutations in *S. typhimurium* (M. J. Mahan, unpublished results); and (v) slow growth on nutrient broth medium.

Complementation of *recB* and *recC* insertion mutations with recombinant plasmids. P22 phage grown on strains containing cloned *E. coli recB* (pCDK30), *recC* (pCDK25), or *recBC* (pCDK3) genes (obtained from Sydney Kushner [7]) was used to transduce strains containing the putative *recBC*::Mu dJ insertions to chloramphenicol resistance. The Cam^r transductants, which inherit the plasmids of the donor, were scored for restoration to wild type of the following phenotypes: resistance to UV light, normal cell viability, normal transduction ability, formation of light-green colonies on green indicator medium, and fast growth on nutrient broth medium. Table 2 shows the complementation profile of strains containing the six *recBC*::Mu dJ insertion mutations. In addition to causing mutations, Mu dJ insertions form operon fusions (2, 3). Table 2 shows the color of colonies (blue or white) that results when strains containing *recBC*::Mu dJ insertions were single-colony isolated on minimal medium containing the chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). A summary of the data indicates that the sole *recB* insertion did not form an operon fusion but four of the five *recC* insertions did form operon fusions. Also characterized is a *recB10* mutant (obtained from A. Eisenstark [8]).

Genetic mapping of the *recB* and *recC* genes. The relative order *recB-recC-thyA* was determined by three-factor crosses (Table 3, cross 1). Furthermore, two-factor crosses showed that both *argA* and *lysA* are linked to *recB*, *recC*, and *thyA* but are unlinked to each other, indicating that *argA*

TABLE 3. Three-factor crosses: relative order of *recB-recC-thyA-lysA* in the *Salmonella typhimurium* chromosome

Cross[a]	Selected marker (no. scored)	Presence of resulting characters:				No. with indicated genotype	Relative frequency (% total)
		recB	*recC*	*thyA*	*lysA*		
1	Thy$^+$ (200)	+	+	+	NA[b]	90	45
		+	−	+	NA	0	0
		−	+	+	NA	91	46
		−	−	+	NA	19	9
2	Thy$^+$ (200)	NA	+	+	+	146	73
		NA	+	+	−	37	19
		NA	−	+	+	13	6
		NA	−	+	−	4	2
2	Tc^r (300)	NA	+	+	−	147	49
		NA	+	−	−	6	2
		NA	−	+	−	30	10
		NA	−	−	−	117	39

[a] Cross 1 determines the relative order *recB-recC-thyA*. Cross 2 determines the relative order *recC-thyA-lysA*. For cross 1, donor was strain LT2 and recipient was strain TT13862 (*thyA383 recB503*::Tn*10 recC498*::Mu dJ). For cross 2, donor was strain TT215 (*lysA565*::Tn*10*) and recipient was strain TT13863 (*thyA383 recC498*::Mu dJ).
[b] NA, Not applicable.

TABLE 4. Transcription orientation of the *recB* and *recC* genes

P22 donors (color)[a]	No. of:			
recBC::Mu dA, *hisD*::Mu dA	Ap^r transductants	Histidine auxotrophs	UV-sensitive transductants	UV-resistant Ap^r His^+ transductants
recB497::Mu dA (white), *hisD9953*::Mu dA (blue)	116	107	6	3
recC502::Mu dA (white), *hisD9953*::Mu dA	100	3	69	28
recC498::Mu dA (blue), *hisD9953*::Mu dA	77	8	69	0
recB497::Mu dA (white), *hisD9950*::Mu dA (white)	75	15	60	0
recC502::Mu dA (white), *hisD9950*::Mu dA	71	5	66	0
recC498::Mu dA (blue), *hisD9950*::Mu dA	83	2	78	3

[a] The recipient in all crosses was LT2. The donors were TT13865 (*recB497*::Mu dA), TT13866 (*recC502*::Mu dA), TT13864 (*recC498*::Mu dA), TT7692 (*hisD9953*::Mu dA), and TT7689 (*hisD9950*::Mu dA). Mu dA insertions form operon fusions (2, 3, 10). Strains containing the Mu dA insertions were single-colony isolated on minimum medium containing X-gal. The color of colonies (blue or white) is an indicator of the transcriptional orientation of the *lacZYA* genes contained in the Mu dA insertion element.

and *lysA* are outside markers (Fig. 1). Table 3 (cross 2) shows the relative order *recC-thyA-lysA*, thus allowing the inference that the relative order of all five genes is *argA-recB-recC-thyA-lysA*. This is identical to the relative order described previously for the five genes in *E. coli* (22). Figure 1 illustrates a detailed genetic map of the *recB-recC* region in *S. typhimurium*; the map includes P22-mediated transduction frequencies between relevant genetic markers.

Orientation of transcription of the *recB* and *recC* genes. The orientation of transcription of the *recB* and *recC* genes was determined by the method of Hughes and Roth (11). In this procedure, duplication recombinants are formed when recipient cells are infected with a mixture of P22 lysates grown on two strains, each containing a Mu dA element at a different point in the chromosome; duplication formation only occurs if the Mu dA prophages are in the same orientation. Mu dA refers to a conditional transposition-defective derivative of the Mu d1(Lac Ap^r) phage described by Casadaban et al. (3) which forms operon fusions (10). To perform these crosses, Mu dJ insertions in *recB* or *recC* were first converted to the

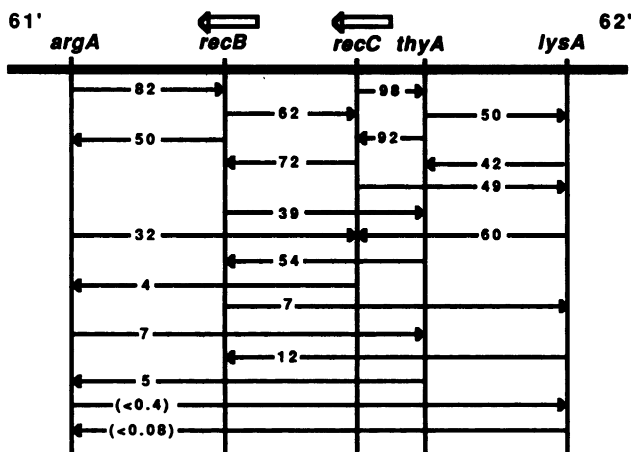

FIG. 1. Genetic map of the *recB* and *recC* genes. The numbers indicate P22-mediated cotransduction frequencies between genetic markers (at least 200 transductants were scored from each cross). Solid arrows placed with cotransduction frequencies point to the unselected marker in each cross. Insertion mutations were used in all crosses involving *recB*, *recC*, *argA*, or *lysA* (*recB497*::Mu dJ or *recB503*::Tn*10*, *recC498*::Mu dJ, *argA1832*::Tn*10*, and *lysA565*::Tn*10*, respectively). In crosses between *argA* and *lysA*, the recipient strain contained *argA69* instead of *argA1832*::Tn*10*. Open arrows represent the direction of transcription inferred (see text).

larger Mu dA insertions by homologous recombination. The conversion of Mu dJ insertions to allelic Mu dA insertions occurred by homologous recombination at the ends of the Mu, replacing a kanamycin resistance determinant with an ampicillin determinant. P22 phage was grown on strains containing *recBC*::Mu dA insertions and on strains carrying well-characterized *his*::Mu dA insertions; these lysates were mixed and used to transduce LT2 (wild type) to ampicillin resistance. Putative duplications were scored as UV-resistant Ap^r His^+ recombinants. The duplication structure of UV-resistant Ap^r His^+ recombinants from each cross was confirmed by their ability to segregate Ap^s recombinants at high frequency (25 to 40%) after 8 to 10 generations of nonselective growth (haploid strains containing *recBC*::Mu dA insertions segregate Ap^s colonies at low frequency [<0.09%]). Furthermore, no Ap^r UV-sensitive or Ap^r His^- segregants were observed (<0.067%), suggesting that the UV-resistant Ap^r His^+ recombinants are not the result of spontaneous duplications in which the Mu dA prophage inserted into either the *recB* or *hisD* genes. Duplications were obtained only if one donor strain contained a Mu dA element with *lac* genes that were being transcribed (indicated as blue on minimal plates containing X-gal) and one strain with *lac* genes that were not (indicated as white on minimal plates containing X-gal) (Table 4). These results indicate that the *recB* and *recC* genes are transcribed counterclockwise, opposite to that of the *his* operon. The orientation of transcription is the same as was determined previously for the *recB* and *recC* genes of *E. coli* (18).

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