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P1 Transduction Mapping of the *trg* Locus in *rac*$^+$ and *rac* Strains of *Escherichia coli* K-12

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The *trg* locus, which had been located at min 31 in the cotransduction gap in the terminus region of the chromosome of *Escherichia coli*, has been mapped by transduction with bacteriophage P1. This locus exhibited no cotransduction with *fnr* when *rac*$^+$ strains were used. If *rac* strains were used, which removed approximately 27 kilobase pairs of DNA, *trg* and *fnr* exhibited 8.2% cotransduction. Although this mapping of *trg* at min 31.1 considerably reduces the size of the cotransduction gap, *trg* exhibited no cotransduction with a Tn*10* insertion located on the other side of the gap at min 34.2.

The termination of chromosome replication in *Escherichia coli* occurs between the *manA* (mannosephosphate isomerase, min 35.7) and *rac* (recombination activation) loci (13, 14, 17, 18). The *rac* locus is the site of a cryptic prophage (5, 10, 19), and we have recently mapped this site by means of Tn*10* insertions in the cryptic prophage (3). These Tn*10* insertions are located at min 29.7 and 30.0. Very few genetic loci have been mapped in the terminus region (1), and this has complicated defining with greater precision where termination occurs. Some of the other loci that have been mapped in this region are *pnt* (pyridine nucleotide transhydrogenase, min 35.4), *relB* (4) (relaxed regulation of RNA synthesis, min 34.4), and *sbcA* (min 29.7). Mutations at this last locus are mutations in the *rac* prophage (11), which affect expression of the *recE* gene and suppress *recB* and *recC* mutations (15). On the current map of the *E. coli* chromosome there is a cotransduction gap present in the terminus region which extends from the *relB* locus (min 34.4) to the *rac* locus and the loci associated with it (*sbcA* and *recE*).

The *trg* (taxis to ribose and galactose) locus has also been mapped in the terminus region. This locus was mapped at min 31 by interrupted-mating experiments (8), and it appears to be in the cotransduction gap. The *rac* prophage separates the *trg* locus from the cotransduction map that extends through the *trp* (min 27.5), *pyrF* (min 28.2), and *fnr* (fumarate, nitrate, nitrite reductase; min 29.3) loci. We have developed procedures that promote the excision of this prophage and which convert *rac*$^+$ strains to *rac* (3). The *rac* prophage contains approximately 27 kilobase pairs of DNA (10), and this is equivalent to 0.69 min on the genetic map (2). This suggests that the *trg* locus might show cotransduction with the *fnr* locus if *rac* strains are used, but not if *rac*$^+$ strains are used. The experiments reported here demonstrate this, and they consequently extend the cotransduction map to the *trg* locus, which is at min 31.1.

Fouts and Barbour (6) have very recently isolated mutations in the *ksgD* (kasugamycin resistance) locus, and they have mapped these mutations with respect to the *sbcA* locus. The *ksgD* locus maps at min 30.4. This locus should also show increased cotransduction with the *fnr* locus, if *rac* strains or *sbcA* mutations that are deletions (11) are used in the crosses.

MATERIALS AND METHODS

Strains. The bacterial strains listed in Table 1 are all derivatives of *E. coli* K-12. The λ reverse *c*I857 *S7* was obtained from M. Gellert, and the λ *b*221 *O*29 *c*I171::Tn*10* was obtained from N. Kleckner. The P1Cm and P1kc used for transduction were from this laboratory's collection.

Chemicals. [5-^{3}H]uracil, [*methyl*-^{3}H]thymine and [1-^{14}C]leucine were obtained from Amersham Corp. Tetracycline and kanamycin sulfate were purchased from Sigma Chemical Co.

Growth media and conditions. Luria broth and M9 media have been previously described (20). Scoring for *fnr*$^+$ was done on GF plates (16) in anaerobic jars containing GasPaks (BBL Microbiology Systems) as H_2 generators. Tests for chemotaxis were done as described by Hazelbauer and Harayama (9). Chemotaxis buffer consisted of M9 medium containing 2 μg of uracil per ml and 20 μg each of methionine, leucine, histidine, threonine, and tryptophan per ml. Chemotaxis swarm plates contained 0.35% agar (Difco Laboratories) and 0.003% galactose or 0.02% ribose, which were used to test for *trg*$^+$, or 0.02% maltose, which was used to test *trg* mutants for chemotaxis to a different attractant. All growth of bacteria was at 37°C. Selection for tetracycline or kanamycin resistance was done on Luria broth medium containing 1.5% agar and

TABLE 1. List of bacterial strains

Strain	Genetic markers	Source
BD342	F⁻ *manA relB argA pheA trp supE rpsL*	B. Diderichsen
GMS343	F⁻ *thi aroD362 argE manA4 mtl gal lac rpsL*	G. Novel
HB234	F⁻ *thr leu his eda rpsL lac ara xyl tonA tsx trg-1*::Tn*5*	G. Hazelbauer (8)
HB235	As HB234 but contains *trg-2*::Tn*10* instead of *trg-1*::Tn*5*	G. Hazelbauer (8)
LS489	F⁻ *trpR trpA9605 his-29 ilv pro arg thyA deoB* or *deoC tsx rac*⁺	L. Soll
PLK831	F⁻ *fnr-1 gal-25 rpsL195 pyrF287 trpE5 rac*⁺	Binding et al. (3)
PLK983	As PLK831 but *rac*	this paper
PLK1091	As LS489 but *rac*	this paper
PLK1110	F⁻ *argA pheA trp supE rpsL zde-234*::Tn*10*	This paper; derived from BD342
PLK1165	As PLK1091 but contains *trg-2*::Tn*10*	P1(HB235) × PLK1091; Tc^r selection
PLK1184	As RP3342 but *trg-1*::Tn*5* replaces *trg-2*::Tn*10*	P1(HB234) × RP3342; Km^r selection
PLK1233	As LS489 but contains *trg-2*::Tn*10*	P1(HB235) × LS489; Tc^r selection
RP3342	F⁻ *thi thr leu his met eda rpsL lac ara xyl mtl trg-2*::Tn*10*	S. Parkinson

20 μg of tetracycline or 50 μg of kanamycin per ml. Screening of *manA* was done on MacConkey agar base medium (Difco) containing 1.5% mannose.

Genetic procedures. Bacterial conjugations and P1 transductions were performed as described by Miller (20). Recombinant colonies were restreaked once on the original type of selection plate and then subsequently scored on the appropriate plates for additional markers.

Determination of *relB* genotype. The *relB* genotype was determined as described by Friesen et al. (7). Exponentially growing cultures were labeled with [1-^{14}C]leucine (6.6 μCi/ml) and [5-^{3}H]uracil (22 μCi/ml), and 250 μg of valine per ml was added after 20 min. Samples were taken 10 and 120 min after the addition of valine, and the amount of radioactivity precipitable by trichloroacetic acid was determined. The ratio of [^{3}H]uracil/[^{14}C]leucine incorporated was used to distinguish the *relB*⁺ and *relB* genotypes.

DNA-DNA hybridization. The conditions and the λ bacteriophage used for the hybridizations have been described previously (13, 14).

RESULTS

***fnr* and *trg* are cotransducible in *rac* strains.** Harayama et al. (8) used interrupted matings with two different Hfr's to demonstrate that the *trg* locus was located at min 31. They also demonstrated that this locus was on the F506 episome and that it was not on F123 (Fig. 1). Both of these episomes were obtained from HfrB7, and they contain regions of the bacterial chromosome that are located on the clockwise and counterclockwise sides, respectively, of the F-DNA in HfrB7. As a preliminary step to mapping the *trg* locus further, we tested two similar, but smaller, episomes that we had isolated from HfrB7. Consistent with the results of Harayama et al., we found that *trg* mutants were complemented by episome F'621, which contains the region of the chromsome from the F-DNA through the *manA* gene (unpublished data; Figure 1). The *trg* mutants were not complemented by episome F'618, which contains the region of the chromosome from the F-DNA through the *trkE* locus.

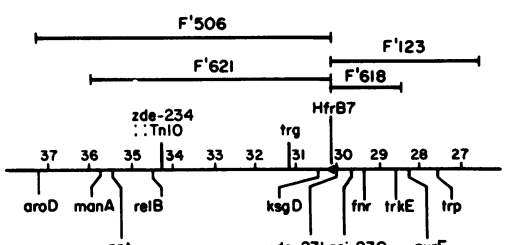

FIG. 1. Segment of the *E. coli* genetic map from min 27 to 38 (1) and F' episomes derived from Hfr B7. Markers listed above the horizontal line are mapped by data in this paper.

The Tn*10* insertion in the *trg* gene, *trg-2*::Tn*10* (8), was used to map the *trg* locus by transduction. This insertion did not show cotransduction with *pyrF*, *fnr*, or *manA* (Table 2, lines 1a, 1b, 1c, and 2) when *rac*⁺ strains were used. These loci flank the terminus region (Fig. 1), and the results are consistent with the location of *trg* in the cotransduction gap, at approximately min 31.

It occurred to us that the removal of the *rac* prophage from between *trg* and *fnr* might permit the cotransduction of these loci. To test this idea, we constructed *rac* strains by using the procedures that we have recently described (3). Briefly, strains LS489 and PLK831 were transduced with bacteriophage P1 to obtain derivatives that contained the *zcj-230*::Tn*10* insertion in *rac*, and these were then lysogenized with λ reverse *c*I857 *S*7 (λ*rev*). Lysogens that had lost the Tn*10* insertion were selected, and these were then cured of the λ*rev* by selecting cells that grew at 42°C. These procedures produced strains PLK1091, which was a *rac* derivative of LS489, and PLK983, which was a *rac* derivative

TABLE 2. P1 transductions

Transduction no.		Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency (%)
1	a	PLK1233	PLK831	*trg-2*::Tn*10*	*fnr*$^+$	0/240
	b	*rac*$^+$ *fnr*$^+$ *trg-2*::Tn*10*	*rac*$^+$ *fnr*	*trg-2*::Tn*10*	*pyrF*$^+$	0/240
	c			*pyrF*$^+$	*trg-2*::Tn*10*	0/200
2		PLK1233 *manA*$^+$ *trg-2*::Tn*10*	BD342 *manA*	*trg-2*::Tn*10*	*manA*$^+$	0/400
3	a	PLK1165	PLK983	*trg-2*::Tn*10*	*fnr*$^+$	18/248 (7.3)
	b	*rac fnr*$^+$ *trg-2*::Tn*10*	*rac fnr*	*trg-2*::Tn*10*	*pyrF*$^+$	0/248
	c			*pyrF*$^+$	*trg-2*::Tn*10*	0/400
4	a	PLK1110	GMS343	*zde-234*::Tn*10*	*aroD*$^+$	0/100
	b	*aroD*$^+$ *manA*$^+$	*aroD manA*	*aroD*$^+$	*zde-234*::Tn*10*	0/100
	c	*zde-234*::Tn*10*		*zde-234*::Tn*10*	*manA*$^+$	66/2,335 (2.8)
5	a	PLK1110	BD342	*zde-234*::Tn*10*	*manA*$^+$	17/690 (2.5)
	b	*manA*$^+$ *relB*$^+$	*manA relB*	*manA*$^+$	*zde-234*::Tn*10*	2/108 (1.9)
	c	*zde-234*::Tn*10*		*zde-234*::Tn*10*	*relB*$^+$	36/50 (72)
	d			*manA*$^+$	*relB*$^+$	8/70 (8.8)
6		PLK1110 *zde-234*::Tn*10*	PLK1184 *trg-1*::Tn*5*	*zde-234*::Tn*10*	Km^s	0/240
7		HB234 *trg-2*::Tn*10*	PLK1184 *trg-1*::Tn*5*	*trg-2*::Tn*10*	Km^s	95/100 (95)

of PLK831. Finally, PLK1091 was transduced with P1 grown on HB235 to obtain PLK1165, which contained the *trg-2*::Tn*10* insertion.

The data in line 3a of Table 2 demonstrate that the *trg-2*::Tn*10* and *fnr* loci exhibited 7.3% cotransduction when the donor and recipient strains were *rac* strains. This indicates that the *fnr* locus is 1.2 min from the *trg-2*::Tn*10* insertion in *rac* strains (21). Since the excision of the *rac* prophage caused the loss of approximately 27 kilobase pairs of DNA (10), which is equivalent to 0.69 min on the genetic map (1), the distance from *trg-2*::Tn*10* to *fnr* would be expected to be 1.9 min in *rac*$^+$ strains. This places the *trg* locus at min 31.1 on the genetic map of *rac*$^+$ strains. It should be mentioned that *pyrF* and *trg* continued to show no cotransduction, even when *rac* strains were used (Table 2, lines 3b and 3c).

The Tc^r *fnr*$^+$ cotransductants obtained as shown in line 3a of Table 2 were tested further to determine whether or not the Tn*10* insertion had remained at the *trg* locus and whether or not the cotransduction frequency of *trg-2*::Tn*10* and *fnr* was unchanged in these strains. Ten of these Tc^r recombinants were tested by a chemotaxis assay (9) and were Trg$^-$. P1 was grown on these strains and used to transduce PLK983. Again, all of the Tc^r recombinants that were tested were Trg$^-$. The Tc^r recombinants from three of the P1 stocks were tested for the cotransduction of *fnr* with *trg-2*::Tn*10*. The cotransduction frequencies observed were 6 of 72, 6 of 72, and 8 of 72, consistent with the data shown in line 3a. If all the cotransduction data are pooled; the cotransduction frequency is 8.2%, which places *trg-2*::Tn*10* at min 31.1 in *rac*$^+$ strains.

The cross shown in line 1a of Table 2 was performed with PLK1233 and PLK831, which are *rac*$^+$ strains, and the cross in line 3a was performed with PLK1165 and PLK983, which are *rac* strains. To test whether these strains were indeed *rac*$^+$ and *rac*, DNA-DNA hybridizations were conducted. Strains PLK1233 and PLK1165 were *thyA*, and they could be readily labeled with [^{3}H]-thymine. Derivatives of PLK831 and PLK983 that were *thyA* were isolated to facilitate labeling the DNA in these strains. The data presented in Table 3 demonstrate that strains PLK1233 and PLK831 *thyA* are *rac*$^+$, since they contain DNA homologous to the sequences from the *rac* locus that are present in λ*rev* (3). These sequences are not present in strains PLK1165 and PLK983 *thyA*, and these strains are consequently *rac*.

***trg* is not cotransducible with *relB*.** The *trg*

TABLE 3. Identification of *rac*$^+$ and *rac* strains by DNA-DNA hybridization

Strain	Input cpm	cpm hybridized				
		DNA on filter			Normalized data[a]	
		λ	λ*trp*	λ*rev*	λ*trp*	λ*rev*
PLK831 *thyA*	770,000	386	1,709	1,510	1,340	1,138
PLK983 *thyA*	780,000	534	1,522	552	988	18
PLK1233	605,000	356	1,222	990	1,130	817
PLK1165	610,000	328	1,336	360	1,290	41

[a] The data in these columns have been corrected for the hybridization to λDNA, and the data have been normalized to an input of 780,000 cpm.

locus is at min 31.1, and we wished to determine whether or not it exhibited cotransduction with the *relB* locus (min 34.4), which was the closest locus on the other side of the cotransduction gap. To test for cotransduction, we first isolated a Tn*10* insertion that was near *relB*. Approximately 10,000 random transpositions of Tn*10* were obtained by the procedure of Kleckner et al. (12), which allowed the isolation of transpositions from λ *b*221 *O*29 *c*I171::Tn*10* into the bacterial chromosome (3). These insertions were then transduced into BD342, and Tc^r *manA*$^+$ transductants were selected. The Tn*10* insertions were then screened further to obtain insertions that exhibited low cotransduction with *manA* (min 35.7) and no cotransduction with *aroD* (min 37.1). Strain PLK1110 contained *zde-234*::Tn*10*, which exhibited no cotransduction with *aroD* (Table 2, lines 4a and 4b), 2.8% cotransduction with *manA* (lines 4c, 5a, and 5b), and 72% cotransduction with *relB* (line 5c). These data indicate that the insertion is 0.2 min from *relB*. To determine on which side of *relB* the insertion was located, a three-factor analysis was conducted of the recombinants obtained from the cross in lines 5a and 5d. Twelve Tc^r *manA*$^+$ recombinants from line 5a were tested, and all were also recombinant for *relB*. Eight *manA*$^+$ *relB*$^+$ recombinants from line 5d were tested, and only one was also recombinant for Tc^r. These data place *relB* between *manA* and *zde-234*::Tn*10*. Therefore, *zde-234*::Tn*10* is located at min 34.2.

An insertion of the kanamycin resistance transposon Tn*5* in the *trg* gene, *trg-1*::Tn*5* (8), was used to test whether or not *zde-234*::Tn*10* exhibits cotransduction with the *trg* locus. Line 6 of Table 2 demonstrates that when *zde-234*::Tn*10* was crossed into a strain that contained *trg-1*::Tn*5*, none of the transductants became Km^s. To establish that the Tn*5* insertion in the recipient strain, PLK1184, was indeed in the *trg* gene and that no other Tn*5* insertions were present in the chromosome, these cells were also transduced with P1 grown on a strain that contained the *trg-2*::Tn*10* insertion. Tc^r recombinants were selected, and 95% of these recombinants were Km^s (Table 2, line 7). This demonstrates that a maximum of 5% of the PLK1184 cells had secondary insertions of Tn*5*. Consequently, the data in line 6 demonstrate that the *trg* locus is not cotransducible with *zde-234*::Tn*10*.

DISCUSSION

Our primary interest in mapping the *trg* locus by P1 transduction was because it is one of the closest loci to the replication terminus, and it appeared to be in the cotransduction gap. We are interested in extending the genetic map across this region, and the data presented here demonstrate that the *trg* locus exhibits cotransduction with the *fnr* locus, if *rac* strains are used. If the appropriate correction is made for the size of the *rac* cryptic prophage, the *trg* locus is positioned at min 31.1. This extends considerably the cotransduction map on the side of the terminus region that contains the *trp*, *pyrF*, and *fnr* loci.

The *trg* locus does not exhibit cotransduction with *zde-234*::Tn*10*, which is the closest locus that we presently have on the other side of the terminus. The genetic distance between these loci can presently only be determined by bacterial conjugation. The best estimate of this distance is 3.1 min based on the distance between *manA* and *trg* in the time of entry experiments of Harayama et al. (8) and the distance between *manA* and *zde-234*::Tn*10*, as determined by P1 cotransduction frequencies. This cotransduction gap between *zde-234*::Tn*10* and *trg* might be present merely because the distance is greater than the amount of DNA that can be packaged by P1-transducing particles. We tested for cotransduction, however, since it was possible that the distance between these loci might be overestimated by conjugation. It is interesting to note that the size of the cotransduction gap has decreased considerably since the genetic map of 1976 (2), in which the gap was 7.1 min. This indicates that the ultimate conjugation and P1 transduction maps of the terminus region will

probably not be substantially different from each other.

We are presently isolating a number of different transposon insertions in the interval between *zde-234*::Tn*10* and *trg*. Further mapping of these insertions will demonstrate whether or not it is possible to construct a complete cotransduction map of the terminus region. These insertions also provide landmarks that can be used to determine more precisely where the replication terminus is located, and they will facilitate the genetic manipulation of this region.

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