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Genetic and Physical Location of the *Escherichia coli rap* Locus, Which Is Essential for Growth of Bacteriophage Lambda

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The *Escherichia coli rap* mutant does not support the growth of bacteriophage λ (D. Henderson and J. Weil, Virology 71:546–559, 1976). We located the *rap* site at 26 min in the *E. coli* genetic map and determined the gene order *fadR-rap-supF-trp* from our transduction experiments. Plasmid pHO1 harbors a 5.6-kilobase-pair segment of the *E. coli* chromosome which contains the *pth* gene (B. Hove-Jensen, Mol. Gen. Genet. 201:269–276, 1985). This plasmid complemented *rap* bacteria, suggesting that it carries the dominant allele *rap*$^+$. Subcloning experiments reduced the *rap*-complementing segment to 1.5 kilobase pairs. This segment still contained *pth*; thus, both loci are tightly linked. The *lit* mutations that inhibit phage T4 growth in *E. coli* are located nearby at 25 min (W. Cooley, K. Sirotkin, R. Green, and L. Snyder, J. Bacteriol. 140:83–91, 1979). We showed that *rap* and *lit* mutations are phenotypically and genetically different.

The study of the interactions between bacteriophage λ and *Escherichia coli* has been fruitful in promoting the knowledge of both phage and host functions. We wished to pursue the study of a particularly interesting interaction reported initially by Henderson and Weil, that is, phage growth inhibition by the *E. coli rap* mutant (7). The *rap* mutation has been located near *trp* and *rac* loci in the genetic map of *E. coli* (7). Aside from its lambda phenotype, very little is known about the physiology of the *E. coli rap* mutant.

Plasmid pHO1 harbors a 5.6-kilobase-pair (kb) DNA segment of the *E. coli* chromosome from the region around 26 min, which contains the *pth* and *prs* genes that encode for the synthesis of peptidyl-tRNA hydrolase and phosphoribosylpyrophosphate synthetase, respectively (8, 9, 14). It has been proposed that the Pth enzyme participates in an editing mechanism to reduce errors during protein synthesis (13). The cotransduction frequency of *pth* and *trp*, located at 27 min, is 7.5%. A *pth* temperature-sensitive mutant stops protein synthesis abruptly at 43°C; thus, *pth* may be an essential gene for *E. coli* (14).

Phage mutations that overcome Rap inhibition, named *bar* (for blanco de acción de rap), have been mapped to four loci in the λ genome (7; P. Guzmán and G. Guarneros, manuscript in preparation). An analogous exclusion of phage T4 by *E. coli lit*(Con), a mutation that maps near the *rap* region in the *E. coli* chromosome, has been reported (6, 11). Phage T4 *gol* mutants overcome Lit inhibition (4).

In the present study, we further characterized the *rap* mutation through fine genetic mapping and plasmid complementation experiments. We show evidence that the *rap* locus is independent from the *lit* locus and that *rap* is closely linked to *pth*.

MATERIALS AND METHODS

Strains. The bacterial strains, bacteriophages, and plasmids used in this study, their relevant characteristics, and their construction or sources are listed in Table 1.

Media. LB broth contained 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter. The pH was adjusted to 7.2 by the addition of NaOH. Super broth was LB broth containing fivefold the normal amount of tryptone and yeast extract. T broth was LB broth without yeast extract. L-agar and T-agar plates were prepared from the corresponding broths with the addition of 12 g of agar per liter. Agar (7 g per liter of T broth) was added to the soft agar overlayer. MacConkey-maltose plates were prepared from MacConkey agar base supplemented with 1% of L-maltose. The tryptone, yeast extract, agar, maltose, and MacConkey agar base were obtained from Difco Laboratories, Detroit, Mich. Antibiotics were added to the following final concentrations: ampicillin, 25 to 50 μg/ml; chloramphenicol, 10 to 30 μg/ml; and tetracycline hydrochloride, 12.5 to 15 μg/ml.

Genetic manipulations. Phage P1*clr*-100::Tn*9* was used to lysogenize strains at 30°C, and the lysogens were induced at 42 to 37°C to prepare lysates. The lysates were used in transduction experiments as described elsewhere (17). Tc^r transductants were selected on L-agar with tetracycline, purified, and assayed for the unselected markers *supF*, *rap*, and *lit*. The assay for *supF* relied on two facts, i.e., that *supF* transductants are able to plate λ Sam7 phage and that *supF* transductants, when in combination with the *malB*(Am) marker (receptor strain MBM7014), form red colonies on MacConkey-maltose plates. Phages λ and λ *bar* were used to check Rap phenotypes, and phages T4 and T4 *gol* were used to check Lit phenotypes. Phage suspensions (10^2 to 10^7 PFU/10 μl) were spotted on bacterial lawns on T-agar plates; *rap* bacteria are unable to grow λ at 37°C, and *lit*(Con) cells prevent the growth of T4 at 30°C at the appropriate phage concentration after overnight incubation. Phages λ *bar* and T4 *gol* grow on the respective bacterial mutants under the conditions described above. Lawns of transformants for plasmids carrying Ap^r or Tc^r markers were cultured in L medium supplemented with the appropriate antibiotic. Plasmids used in complementation assays were grown on strain C600, checked by restriction analysis, and transformed into the appropriate strain, i.e., C600 *rap*, DH173 *rap*, or AA7852 *pth*. The *pth* bacteria transformed with *pth*$^+$ plasmids were prevented from dying at 42°C. The lysogens of strain C600 *rap* that were used for complementation assays were con-

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TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain, phage, or plasmid	Relevant characteristics	Source, derivation, or reference
E. coli		
C600	*thr-1 leu-6 thi-1 supE44 tonA lacY1*	Our collection
C600 *rap*	C600 *rap zch*::Tn*10*	C600 with P1 (DH173 *zch*::Tn*10*), Tc^r; this work
DH173	C600 *rap lac Y14*(Am)	7
DH173 *zch*::Tn*10*	DH173 *rap zch*::Tn*10*	DH173 with P1 (MEM7030), Tc^r; this work
MBM7014	F$^-$ *araC*(Am) *araD* Δ(*argF-lac*) *U169 trp*(Am) *malB*(Am) *rpsL relA thi tyrT* (*supF*)	17
MBM7014 *rap*	MBM7014 *rap zch*::Tn*10*	MBM7014 with P1 (DH173 *zch*::Tn*10*), Tc^r; this work
MBM7030	MBM7014 *sup*^{0} *zch*::Tn*10*	M. Berman; transposon inserted between *tyrT* (*supF*) and *trp*
SA2140	SA500 *his ilv tyrT* (*supF*) *trp*::Tn*10 galL3*::IS2	S. Adhya
GG283	*lit-6*(Con) *fadR*::Tn*10*	L. Snyder
AA7852	*arg his leu thr thi pth*(Ts)	14
Bacteriophages		
λ *lac trpW205 red*114 *imm*434	Excluded by *E. coli rap*, Bar$^+$ phenotype	15
λ *lac trpW205 bar*101 *red*114 *imm*434	Grows on *E. coli rap*	P. Guzmán
λ *b2 imm*21 *S*am7	Bar phenotype phage harboring a *supF* suppressible mutation	Our collection
λ HO1	λ D69 *imm*21 *prs*$^+$ *pth*$^+$ (*Bam*HI segment, 5.6 kb)	9
λ *imm*21c *int-2 red-3*	Clear-plaque phage	
T4	Wild-type, unable to grow on *E. coli lit*(Con)	L. Snyder
T4 *gol* 6B	Grows on *E. coli lit*(Con)	L. Snyder
P1 Tn*9 clr*100	Thermoinducible phage, transduces chloramphenicol resistance	17
Plasmid pHO1	pBR322 *prs*$^+$ *pth*$^+$(*Bam*HI segment, 5.6 kb) Ap^r	B. Hove-Jensen

structed by infection with λ HO1 and selection of colonies immune to λ *imm*21c phage.

DNA manipulations. Plasmid DNA was isolated essentially as previously described (2). When necessary, plasmid DNA was concentrated from bacterial super-broth cultures by CsCl-ethidium bromide equilibrium gradients (16). Bacteria were transformed with plasmid DNA by the $CaCl_2$ procedure (5). pGM plasmids were generated by restriction of pHO1 DNA with single enzymes, religation of the fragments resolved by gel electrophoresis, and selection of transformants on ampicillin medium. The restriction enzymes used are indicated in Fig. 2. Plasmid pEG-1 was constructed by cloning a *Pvu*I-*Eco*RI fragment from pHO1 DNA into pBR322 restricted with the same enzymes. This procedure was followed by ligation and selection of transformants on tetracycline medium. Plasmid pEG-2 was generated by an *Nco*I deletion of pEG-1. Restriction enzyme digestion and ligation of DNA were done by the procedures recommended by the suppliers (New England BioLabs, Inc., Beverly, Mass., and Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Gel electrophoresis was done as described by Maniatis et al. (12) with Tris-acetate buffer. DNA fragments for ligation were recovered after gel electrophoresis in low-melting-point agarose (Bethesda Research Laboratories).

RESULTS

Transductional mapping of *rap*. Earlier conjugational mapping located the *rap* mutation near the *trp* operon and the *rac* locus in the *E. coli* chromosome (7). To locate *rap* more precisely and to determine if the λ exclusion phenotype involved a single mutation, a P1 lysate grown on SA2140 *trp*::Tn*10 tyrT* (*supF*) was used to transduce strain DH173 *rap*. Our results showed that *supF* cotransduces 25% with *trp*::Tn*10*, in agreement with the 21% cotransduction frequency previously observed (S. Adhya, personal communication). However, in the same cross, the *rap*$^+$ allele cotransduced with *trp*::Tn*10* with a frequency of only 2% (1 of 50 Tc^r isolates). This transductant also inherited the unselected marker *supF* from the donor strain, suggesting that the marker order was *rap-supF-trp*::Tn*10*. A P1 lysate grown on MBM7030 *sup*^{0} *malB*(Am) *zch*::Tn*10*, which harbors Tn*10* inserted between *tyrT* (*supF*) and *trp*, cotransduced Tn*10* (Tc^r) and the unselected marker *rap*$^+$ with a frequency of 17%. These results confirmed our supposition that *rap* was linked to *supF* and was therefore located counterclockwise from *trp*.

A P1 lysate made on DH173 *rap zch*::Tn*10* (Tc^r) from the previous experiment was used to transduce strain MBM7014 *malB*(Am) *supF*. Among the Tc^r transductants, 13% had also inherited *rap*, which confirmed the previous experimental results. All the *rap* transductants analyzed had also inherited *sup*^{0}; *supF* and Tn*10* cotransduced with a frequency of 60%. From these results, the gene order *rap-supF-zch*::Tn*10* was inferred. A summary of these data and the resulting linkage map is shown in Fig. 1.

The Rap phenotype of transductants C600 *rap* and MBM7014 *rap* was not as stringent as that of the parental strain DH173 *rap*; phage λ *imm*434 formed minute plaques on the *rap* transductants but was totally excluded on DH173. Additionally, DH173 and *rap*$^+$ isolates of DH173 grew slowly in liquid medium and formed small colonies on L-agar plates, as compared to the C600 and MBM7014 isolates. This suggested that the growth defect and the Rap exclusion in DH173 were due to independent mutations whose combination caused a more stringent phenotype.

Complementation with *rap* cloned on phage and plasmids.

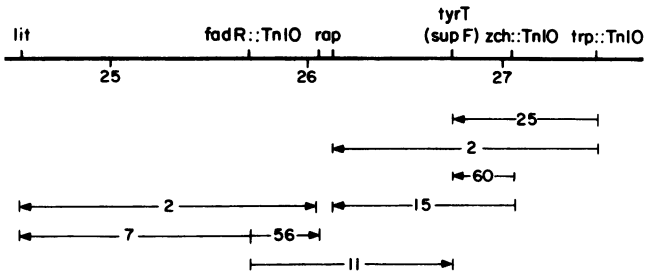

FIG. 1. Map of the *fadR-trp* region of the *E. coli* chromosome. The scale below the thick line represents minutes in the genetic map. The *fadR*::Tn*10* marker, which was used as a reference, was fixed at 25.7 min (1). Values in the arrows are the cotransduction percentages of the respective markers, which are based on data from Table 2 and data cited in the text. Arrowheads point to the unselected markers. The relative distances between markers were calculated by the equation of Wu (19), with the assumption that the effective length of the phage P1-transducing fragment is 2 min (1). A slight discrepancy in the position of *rap* is shown, depending on the location of the Tn*10*, which was used as a selected marker, either in *fadR*, *zch*, or *trp*.

Plasmid pHO1 and phage λ HO1 harbor an *E. coli* DNA segment of 5.6 kb which contains the *pth* gene (9). Plasmid or phage complementation experiments were carried out to determine whether *rap*$^+$ was present in the cloned segment. Strain C600 *rap* was transformed with pHO1 or lysogenized with λ HO1. Unlike the C600 *rap* strain, both the transformants and the lysogens grew phage λ *imm*434 normally (data not shown), indicating that the *rap* gene was harbored in the cloned bacterial DNA. In addition, this result showed that *rap*, in both high- and low-copy numbers, was dominant over the mutant allele.

To delimit the *rap* locus on the 5.6 kb of the bacterial DNA insert in pHO1, a set of in vitro deletions was generated by using the appropriate restriction enzymes (Fig. 2). The plasmid deletion isolates were used to complement *rap* or *pth* bacteria. The deletion plasmids pGM-3, pGM-4, and pGM-5, which eliminate up to 3.3 kb of chromosomal DNA to the right of the leftmost *Ava*I site, complemented the C600 *rap* bacteria by transformation (Fig. 2). This result suggested that the remaining 2.3-kb segment of insert DNA in pGM-5 contained *rap*. Deletions of insert DNA to the left of the same *Ava*I site, such as those in pGM-1, pEG-2, and pGM-6 but not in pGM-2, lost the *rap*-complementing activity (Fig. 2). These results indicated that the 1.5-kb segment defined by deletions *Eco*RV and *Ava*I in the chromosomal insert of pHO1 contained *rap*.

The *prs* gene has been located in a 1,785-base-pair DNA segment on the right side of the bacterial insert (Fig. 2) (9, 10). Therefore, plasmids carrying *rap* that have lost variable lengths on the right end of the bacterial insert, such as pGM-3, pGM-4, pGM-5, and pEG-1, must not carry a complete *prs* gene (Fig. 2). The *pth* gene must be present in the *rap*-complementing plasmids pGM-2, pGM-5, and pEG-1 because they complement *pth* too. Thus, *rap* and *pth* are closely linked loci on the leftmost part of the insert between the *Eco*RV and *Ava*I restriction sites. The *rap* phenotype of a *pth* mutant was assayed at the permissive temperature (37°C). Phage λ$^+$ was excluded more effectively than λ *bar* mutants (data not shown). This result suggested that *pth* and *rap* affect the same locus.

***rap* and *lit* are independent loci.** The *lit*(Con) mutations of *E. coli* inhibit the growth of phage T4. The locus *lit* has been located at 25 min in the bacterial genetic map between *purB* and *fadR* (6, 11). Since Lit and Rap phenotypes are similar and since the map locations of the respective sites are close to each other, we investigated whether the *lit* and *rap* mutations were allelic. The results showed that phage λ grew normally on *E. coli lit*; conversely, phage T4 grew well on *E. coli rap*. Phage λ *bar*, which grows on *rap* bacteria, and phage T4 *gol*, which plates on *lit*(Con) hosts, did not show any alteration in plating efficiencies on the heterologous hosts (data not shown). These results showed that the *lit* and *rap* mutations were not identical. The results also strongly suggested that the mutations belonged to independent loci. To critically test the validity of this assertion, transductional mapping experiments involving *rap* and *lit* markers were carried out.

A Tn*10* insertion in *fadR* was used as a reference marker to determine the relative positions of *rap* and *lit* loci. The gene *fadR* has been located around 25.5 min in the genetic map of *E. coli* (1, 8, 18). The *lit* locus maps counterclockwise to *fadR* (11). A P1 lysate prepared on strain GG283 *lit fadR*::Tn*10* was used to transduce strain MBM7014 *malB*(Am) *supF*. Table 2 shows the results of such experiments. Among the Tc^r transductants, 5% also inherited

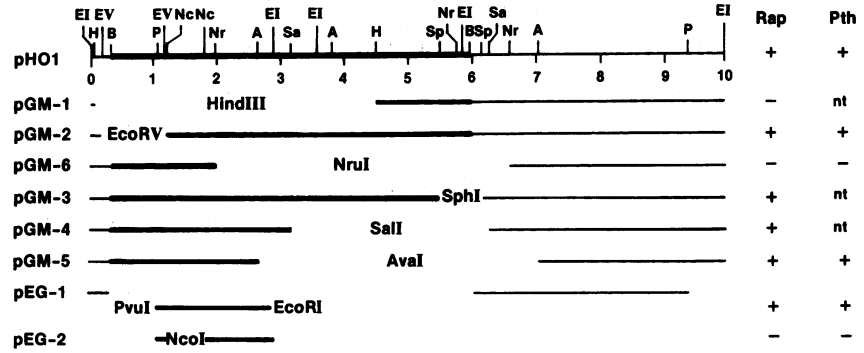

FIG. 2. Restriction map of plasmid pHO1 and the structures and phenotypes of subclones. The upper line represents pHO1 linearized at the *Eco*RI site in pBR322. The thick lines indicate *E. coli*, and the thin portions indicate pBR322-derived DNAs. The indicated restriction enzyme sites are: EI, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pvu*I; EV, *Eco*RV; Nc, *Nco*I; Nr, *Nru*I; Sa, *Sal*I; A, *Ava*I; Sp, *Sph*I. The extent of the DNA present in the subclones is indicated by the lines below the restriction map. The actual order of DNA segments in pEG-1 and pEG-2 is not the one in the diagram; this fact is represented by different levels for the insert and vehicle segments. The Rap and Pth phenotypes (column on the right) were determined on mutant bacteria transformed with the respective plasmids. nt, Not tested. An essentially similar restriction map has been generated by B. Hove-Jensen.

TABLE 2. Transductional mapping of *rap* and *lit* in the *fadR-trp* region[a]

Recipient	Selected marker (no. scored)	Unselected markers	
		Genotype	Recombinants (%)
C600	Tn*10* (92)	*lit*	10
		lit$^+$	90
MBM7014 *supF*	Tn*10* (37)	*lit supF*	5
		lit$^+$ *sup*^{0}	11
		lit$^+$ *supF*	84
DH173 *rap*	Tn*10* (84)	*lit rap*	5
		lit$^+$ *rap*$^+$	54
		lit rap$^+$	2
		lit$^+$ *rap*	39

[a] Transductions were performed as described in Materials and Methods. The donor strain was GG283 *lit*(Con) *fadR*::Tn*10* *sup*^{0}.

lit(Con) and 11% inherited *sup*^{0} from the donor strain, but none of them received both *lit*(Con) and *sup*^{0}. From these results we concluded that *lit* and *supF* lie on opposite sides of *fadR*::Tn*10*. Since *supF* maps clockwise from *fadR* (1), *lit* should lie counterclockwise from *fadR*.

To determine the relative positions of *lit* and *rap*, P1 phage grown on strain GG283 *lit fadR*::Tn*10* were used to transduce strain DH173 *rap*. Transductants resistant to tetracycline were tested for the Lit and Rap phenotype; 54% received *rap*$^+$, but only 5% inherited *lit*(Con) from the donor strain. An even smaller number of Tc^r transductants (2%) inherited both *rap*$^+$ and *lit*(Con) markers (Table 2). These results suggested that the gene order was *lit-fadR-rap* and were consistent with those of other authors who have located *lit* counterclockwise from *fadR* (6, 11). The relative distances between these markers are shown in Fig. 1.

DISCUSSION

This report describes the transductional and physical mapping of the *rap* gene at 26 min. Plasmid pHO1 (9) complemented for *rap*, and subclones located the complementing activity to a 1.5-kb DNA segment. In addition, *rap* was shown to be closely linked to *pth* and distinct from the previously identified *lit* gene.

The gene order near *rap* is *lit-fadR-(rap,pth)-supF-trp* (Fig. 1). The data in the present study confirm and refine previous conjugational mapping of *rap* (7) and are consistent with the mapping of nearby genes (1, 8, 18). The results of our experiments on the cotransduction between *lit* and *fadR*::Tn*10* (indicating a frequency of 2%) do not fully agree with the higher frequencies expected from the data obtained by Kao et al. (11). This apparent discrepancy may be explained by the fact that the *lit* donor strain carries the cryptic prophage e14 (L. Snyder, personal communication). This element has been mapped at 25 min on the *E. coli* chromosome (3). Thus, transduction frequencies of markers in this region to a nonimmune strain could be reduced by prophage induction. The results presented in this study show that Rap exclusion of phage λ differs from the previously described Lit exclusion of phage T4 (6) both phenotypically and genetically (Table 2).

Plasmid pHO1 and phage λ HO1, which bear *E. coli* DNA from the region around 26 min, complemented *rap* bacteria. This observation is consistent with the hypothesis of a *rap* gene harbored in the cloned segment whose product is dominant over that of the chromosomal *rap* mutant allele. Earlier complementation experiments had shown that the 5.6-kb DNA segment contained the *prs* and *pth* genes (9). The *prs* gene has been located in a 1.7-kb DNA segment near one end of the insert, and its nucleotide sequence has been determined (9, 10). The evidence in the present study indicates that *rap* and *pth* reside in a 1.5-kb DNA segment near the opposite end of the insert (Fig. 2). We were unable to separate *rap*- from *pth*-complementing activities with in vitro-generated deletions. Although *rap* and *pth* mutations determine somewhat different phenotypes, these results could be explained by a unique gene product with separate functional domains, independent but overlapping genes, or a single transcriptional unit for both *pth* and *rap*. The organization of *pth* and *rap* in the *E. coli* DNA awaits sequence analysis to be fully understood.

The chromosomal insert in pHO1 directs synthesis of two proteins with molecular masses of 33 and 13 kilodaltons (9). The 33-kilodalton polypeptide has been assigned to phosphoribosylpyrophosphate synthetase, the product of *prs* (10). The assignment of the 13-kilodalton polypeptide to a specific gene requires further investigation.

The function of *rap* in *E. coli* is not clear; *rap* transductants are indistinguishable from wild-type bacteria except for their inability to grow phage λ and to maintain plasmids harboring a functional λ *bar* site (P. Guzmán and G. Guarneros, unpublished results). The mechanism of Rap exclusion remains unclear. Our unpublished results show that *rap* inhibits plasmid protein synthesis and DNA replication when transcription occurs through a functional *bar* site. A molecular approach will be necessary to unravel the mechanism of this puzzling phenomenon.

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