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 pH01 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 pH01 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 pyridoxal-5′-triphosphate 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. Guarnéros, 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. L agar (7 g per liter of T broth) was added to the soft agar overlay. 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 P1cr100::Tn9 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' 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 bar were used to check Rap phenotypes, and phages T4 and T4 gol were used to check Lit phenotypes. Phage suspensions (102 to 105 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' or Tc' 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-
structured by infection with λ HO1 and selection of colonies immune to λ imm21c 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 CaCl₂ 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 PvuI-EcoRI 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 Ncol 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 conjugal 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::Tn10 tyrT (supF) was used to transduce strain DH173 rap. Our results showed that supF cotransduces 25% with trp::Tn10, in agreement with the 21% cotransduction frequency previously observed (S. Adhya, personal communication). However, in the same cross, the rap⁺ allele cotransduced with trp::Tn10 with a frequency of only 2% (1 of 50 Tc⁺ isolates). This transductant also inherited the unselected marker supF from the donor strain, suggesting that the marker order was rap-supF-trp::Tn10. A P1 lysate grown on MBM7030 supF malB(Am) zch::Tn10, which harbors Tn10 inserted between tyrT (supF) and trp, cotransduced Tn10 (Tc⁺) 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::Tn10 (Tc⁺) from the previous experiment was used to transduce strain MBM7014 malB(Am) supF. Among the Tc⁺ transductants, 13% had also inherited rap, which confirmed the previous experimental results. All the rap transductants analyzed had also inherited supF, supF and Tn10 cotransduced with a frequency of 60%. From these results, the gene order rap-supF-zch::Tn10 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 imm434 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.

<table>
<thead>
<tr>
<th>Strain, phage, or plasmid</th>
<th>Relevant characteristics</th>
<th>Source, derivation, or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli C600</td>
<td>thr-1 leu-6 thi-1 supE44 tonA lacY1</td>
<td>Our collection C600 with P1 (DH173 zch::Tn10), Tc⁺; this work</td>
</tr>
<tr>
<td>C600 rap</td>
<td>C600 rap zch::Tn10</td>
<td>7 DH173 with P1 (MEM7030), Tc⁺; this work</td>
</tr>
<tr>
<td>DH173</td>
<td>C600 rap lac Y4(Am)</td>
<td>MBM7014 with P1 (DH173 zch::Tn10), Tc⁺; this work</td>
</tr>
<tr>
<td>DH173 zch::Tn10</td>
<td>17</td>
<td>M. Berman; transposon inserted between tyrT (supF) and trp</td>
</tr>
<tr>
<td>MBM7014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBM7014 rap</td>
<td>MBM7014 rap zch::Tn10</td>
<td></td>
</tr>
<tr>
<td>MBM7030</td>
<td>MBM7014 supF zch::Tn10</td>
<td></td>
</tr>
<tr>
<td>SA2140</td>
<td>SA500 his ilv tyrT (supF) trp::Tn10 galL3::1S2</td>
<td>S. Adhya</td>
</tr>
<tr>
<td>GG283</td>
<td></td>
<td>L. Snyder</td>
</tr>
<tr>
<td>AA7852</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Bacteriophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>λ lac trpW205 red14</td>
<td>Excluded by E. coli rap, Bar⁺ phenotype</td>
<td>15</td>
</tr>
<tr>
<td>imm434</td>
<td>Grows on E. coli rap</td>
<td>P. Guzmán</td>
</tr>
<tr>
<td>λ b2 imm21 Sam7</td>
<td>Bar phenotype phage harboring a supF suppressible mutation</td>
<td>Our collection</td>
</tr>
<tr>
<td>λ HO1</td>
<td>λ D69 imm21 prs⁺ pth⁺ (BamHI segment, 5.6 kb)</td>
<td>9 B. Hove-Jensen</td>
</tr>
<tr>
<td>λ imm21c int-2 red-3</td>
<td>Clear-plaque phage</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Wild-type, unable to grow on E. coli lit(Con)</td>
<td>L. Snyder</td>
</tr>
<tr>
<td>T4 gol 6B</td>
<td>Grows on E. coli lit(Con)</td>
<td>L. Snyder</td>
</tr>
<tr>
<td>P1 Tn9 clt100</td>
<td>Thermolabile phage, transduces chloramphenicol resistance</td>
<td>17</td>
</tr>
<tr>
<td>Plasmid pH01</td>
<td>pBR322 prs⁺ pth⁺(BamHI segment, 5.6 kb) Ap⁺</td>
<td>B. Hove-Jensen</td>
</tr>
</tbody>
</table>
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 imm434 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 *Avai* 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 *Avai* 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 *EcoRV* and *Avai* 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 *EcoRV* and *Avai* 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 *gor*, 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 *Tn10* 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 map counts clockwise to *fadR* (11). A P1 lysate prepared on strain GG283 *lit* *fadR* was used to transduce strain MBM7014 *malB* (Am) *supF*. Table 2 shows the results of such experiments. Among the Tc' transductants, 5% also inherited

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**FIG. 2.** Restriction map of plasmid pH01 and the structures and phenotypes of subclones. The upper line represents pHO1 linearized at the *EcoRI* site in pBR322. The thick lines indicate *E. coli* and the thin portions indicate pBR322-derived DNAAs. The indicated restriction enzyme sites are: EI, *EcoRI*; H, *HindIII*; B, *BamHI*; P, *PvuI*; EV, *EcoRV*; Nc, *NcoI*; Nr, *NruI*; Sa, *Sall*; A, *Avai*; Sp, *SphI*. 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

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Selected marker (no. scored)</th>
<th>Unselected markers</th>
<th>Genotype</th>
<th>Recombinants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C600</td>
<td>Tn10 (92)</td>
<td>lit</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lit+</td>
<td></td>
<td>190</td>
</tr>
<tr>
<td>MBM7014 supF</td>
<td>Tn10 (37)</td>
<td>lit supF</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lit+ supF</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>DH173 rap</td>
<td>Tn10 (84)</td>
<td>lit</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lit+ rap</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lit+ supF</td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

* Transductions were performed as described in Materials and Methods. The donor strain was GG283 lit(Con)/fadR::Tn10 sup^B.

lit(Con) and 11% inherited sup^B from the donor strain, but none of them received both lit(Con) and sup^B. From these results we concluded that lit and sup^F lie on opposite sides of fadR::Tn10. Since sup^F 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::Tn10 were used to transduce strain DH173 rap. Transductants resistant to tetracycline were tested for the Lit and Rap phenotype; 54% received rap^+ only, but 5% inherited lit(Con) from the donor strain. An even smaller number of Tc^+ 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 conjugal 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::Tn10 (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 pth and pth genes (9). The pth 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 pth (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.

**ACKNOWLEDGMENTS**

We thank L. Snyder, J. Menninger, and B. Hove-Jensen for sharing their unpublished results and providing strains, plasmids, D. Court for suggesting the use of plasmid pHO1; R. Barrera and K. Cannon for typing the manuscript; and F. Bastarrachea, C. Montañez, and D. Court for critical reading of the manuscript.

This work was supported partly by grants from CONACYT and the Foundation Zedada, Mexico. Gabriel Guarneros was the recipient of a fellowship from the John Simon Guggenheim Foundation during the development of this research.

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