Chromosomal Transformation of *Escherichia coli* recD Strains with Linearized Plasmids†

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Received 10 March 1988/Accepted 9 February 1989

Wild-type *Escherichia coli* are resistant to genetic transformation by purified linear DNA, probably in part because of exonuclease activity. We demonstrate that *E. coli* containing a recD mutation could be easily transformed by linearized plasmids containing a selectable marker. The marker was transferred to the chromosome by homologous recombination, whereas plasmid markers not in the region of homology were lost.

In contrast to some other bacteria, *Escherichia coli* is not readily transformable by linear pieces of DNA (34). This is due in part to degradation of the incoming DNA by intracellular exonucleases (13, 43). Oishi and Cosley (8, 9, 26) showed that *E. coli* strains lacking exonuclease V (ExoV) by virtue of recB or recC mutations can be transformed by linear DNA if they carry as well the sbcB (and sbcC) mutations that restore recombination proficiency to the recB recC mutant. Similarly, recD mutants also are lacking in exonuclease activity, but unlike the recB recC mutants are robust and recombination proficient without the requirement of additional mutations (5).

Jasin and Schimmel (14) and Winans et al. (44) used recB recC sbcB strains to transform with linearized plasmids and homologously replace a chromosomal allele with an allele that had been modified. Yamaguchi and Tomizawa (46) and Gutterson and Koshland (12) used strains defective in polA to transfer genes from plasmids to the chromosome. Strains carrying recB, recC, and sbcB grow poorly and generally carry a mutation in sbbc (22). In addition, strains carrying polA are not robust (15). These considerations make the general use of such strains unattractive.

Using the *E. coli* chemotaxis system genes as a chromosomal target, we show here that recD strains can be transformed with linear DNA. The availability of recD alleles that are mutant by virtue of a mini-Tet insertion facilitates the construction of transformable derivatives of strains of interest by one-step bacteriophage-mediated transduction.

**MATERIALS AND METHODS**

**Strains, media, and chemicals.** The bacterial strains, plasmids, and phages used are listed in Tables 1 and 2. LB medium and Trypoinre broth were as described previously (2). The lactose phenotypes of the bacterial strains were determined on agar plates containing 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal) at 20 μg/liter and isoprpyl-β-D-thiogalactopyranoside (IPTG) at 1 mM.

**Swarm assay for chemotaxis.** Colonies were screened for chemotaxis ability on semisolid agar plates. Che- strains form large circular swarms within a few h at 30°C; strains that are Che- but still motile form fuzzy diffuse colonies; and nonmotile strains form sharp colonies. Che+ revertants form swarms that bulge out from one side of the smaller colonies, analogous to sectored colonies on colorimetric indicator plates.

**Methyl esterase assays.** The activity of the CheB protein, a methyl esterase whose activity is modulated in response to attractants and repellents, can be measured in vivo by the amount of radioactive methanol released from cells that have been incubated with methyl-labeled radioactive methionine (16, 18, 19, 37, 41, 42). Steady-state cumulative evolution of methanol was measured by the method of Toews and Adler (41) as described by Kehry et al. (16). Stimulus-dependent esterase activity was measured in the flow apparatus as described by Kehry et al. (6, 16, 17).

**Transfer of chromosomal DNA to plasmids.** The method for transferring chromosomal DNA to plasmids is described in detail in the accompanying paper (30). Briefly, plasmid pCR176 was introduced into a strain, resulting in streptomycin sensitivity (11). Revertants to streptomycin resistance that still carried ampicillin resistance were selected. Plasmids were harvested from these revertants, and their restriction maps were examined to determine the structure of the DNA in the region of interest.

**Screen for RecD phenotype.** Our screen for RecD relies on the inability of Red- Gam- λ phage lacking Chi sequences to form large plaques on wild-type bacterial lawns (36). We used phage carrying the deletion b1453 and the substitution imm21 to provide the Red- Gam- phenotype and allow plating on lambda lysogens, respectively. On rec+ strains, Chi- versions of our phage make smaller plaques than do Chi+ versions. Chi- and Chi+ Red- Gam- λ form indistinguishable large plaques on recD strains (5, 33). Methods for plating λ were as described by Arber et al. (2).

Mid-log-phase cultures of each candidate, grown in Trypontine broth supplemented with thiamine, MgSO4, and 0.2% maltose, were mixed for 10 min with about 100 PFU of either MMS1443 or its Chi+ derivative, SMR46. They were then plated on Trypticase agar (BBL Microbiology Systems, Cockeysville, Md.) (2). Plates were incubated overnight at 37°C and examined for plaque size.

**Construction of bacterial strains.** Transduction with phage P1 was performed as described by Miller (24). RP487 is a standard chemotaxis wild-type strain (28). D63 is a λ ind lysogen of RP487. CR101 was made by transduction of D63 to Tet+ from NK5992. D308 resulted from transduction of CR101 to Arg- and screening for the RecD phenotype. D300 was constructed by transducing D63 to Tet+ with P1 grown on SG1039 and screening for white colonies on plates containing IPTG and X-Gal. D310 was made by transducing...
TABLE 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype or phenotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>D63</td>
<td>RPs47 (Che lacY eda)</td>
<td>J. S. Parkinson (28)</td>
</tr>
<tr>
<td>DPB271</td>
<td>rec::mini-Tet</td>
<td>S. Cohen (4)</td>
</tr>
<tr>
<td>SG1039</td>
<td>Δ(lacIZYA) U169 zai::Tn10 proC</td>
<td>S. Gottesman</td>
</tr>
<tr>
<td>NK992</td>
<td>argA::Tn10</td>
<td>N. Kleckner</td>
</tr>
<tr>
<td>A535</td>
<td>recD1009</td>
<td>A. Chaudhury (5)</td>
</tr>
<tr>
<td>YK4136</td>
<td>flal eda*</td>
<td>Y. Komeda</td>
</tr>
<tr>
<td>D300</td>
<td>D63 Δ(lacIZYA) U169 Tet Pro*</td>
<td>P1 transduction from SG1039</td>
</tr>
<tr>
<td>D310</td>
<td>D63 Δ(lacIZYA) U169</td>
<td>P1 transduction of D300 from D63</td>
</tr>
<tr>
<td>D301</td>
<td>D310 recD1003::mini-Tet</td>
<td>P1 transduction from DPB271</td>
</tr>
<tr>
<td>CR101</td>
<td>D63 argA::Tn10</td>
<td>P1 transduction from NK992</td>
</tr>
<tr>
<td>D308</td>
<td>CR101 recD1009</td>
<td>P1 transduction from A535</td>
</tr>
</tbody>
</table>

D300 back to Pro* and screening for Tet*. D301 was the result of transducing D63 to Tet* and screening for RecD. Construction of the other strains is described below.

Construction of plasmids. DNA manipulations were performed as described by Maniatis et al. (23). The plasmids contain DNA from various stretches of the E. coli meche operon (Fig. 1). Plasmid pRSW220 carries the cheB gene. Plasmid pCR67 carries the tar, tap, cheR, and cheB genes. Plasmid pCR70 carries the tar, tap, cheR, cheB, and cheY genes and a small part of the cheZ gene. Plasmid pCR73 carries the entire operon. Plasmids pCR174 and pCR175 were constructed from pCR73 by inserting the lacZ gene from pMC1871 between the HindIII site in cheB and the SalI site in cheY and inserting a 1.3-kilobase fragment from pUC4-KSAC containing the kanamycin resistance gene into the BamHI site adjacent to the SalI site. Plasmid pCR173 was constructed in the same process but lost the lacZ gene. Plasmid pCR176, made from pCR173, contains the streptomycin sensitivity gene from pN01523 in place of the kanamycin resistance gene.

Transformation with linear DNA. The E. coli chemotaxis gene cluster was used as the chromosomal target for transformation experiments. Figure 1 shows the genetic map of that region.

The transforming DNA was a linearized plasmid containing the kanamycin resistance gene from Tn9O3 replacing parts of the cheB and cheY genes (Fig. 1). Plasmid pCR173 contains unique XbaI and EcoRI sites on the E. coli DNA flanking the chemotaxis genes. However, there is an additional EcoRI site in lacZ in plasmids pCR174 and pCR175. The plasmid was cut with XbaI, EcoRI, or both, and the extent of digestion was determined by gel electrophoresis. Strains were made competent by treatment with cold CaCl2 as described by Maniatis et al. (23) and exposed to the transforming DNA, exactly as if introducing a circular plasmid. Kanamycin-resistant transformants were selected and screened for ampicillin resistance. Ampicillin-resistant transformants were generally discarded as being the probable result of transformation by uncut plasmid or religation of

FIG. 1. Structures of plasmids. The top line shows the structure of the chromosome in the chemotaxis region; the next four lines show the portions of the chemotaxis genes on plasmids used in complementation experiments. pCR173, pCR174, and pCR175 are the plasmids used in linear transformations of the chromosome; all three have the general features shown for pCR173.
TABLE 3. Results of transformation with linearized plasmids

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plasmid</th>
<th>Enzyme(s)</th>
<th>Enzyme Sites</th>
<th>Kan&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Amp&lt;sup&gt;-&lt;/sup&gt;/Kan&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Che&lt;sup&gt;-&lt;/sup&gt;/Amp&lt;sup&gt;-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pCR173</td>
<td>XbaI, EcoRI</td>
<td>1,050</td>
<td>490</td>
<td>428/490</td>
<td>10/10</td>
</tr>
<tr>
<td>2</td>
<td>pCR173</td>
<td>XbaI</td>
<td>980</td>
<td>1,050</td>
<td>13/122</td>
<td>10/10</td>
</tr>
<tr>
<td>3</td>
<td>pCR173</td>
<td>EcoRI</td>
<td>1,050</td>
<td>1,050</td>
<td>5/135</td>
<td>4/5</td>
</tr>
<tr>
<td>4</td>
<td>pCR174</td>
<td>XbaI, partial EcoRI</td>
<td>4</td>
<td>4</td>
<td>2/4</td>
<td>1/2</td>
</tr>
<tr>
<td>5</td>
<td>pCR174</td>
<td>XbaI</td>
<td>6</td>
<td>3/6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>pCR175</td>
<td>XbaI, partial EcoRI</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>pCR175</td>
<td>XbaI</td>
<td>26</td>
<td>9/26</td>
<td>8/9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All experiments were performed with approximately 10<sup>8</sup> E. coli D308 (recD1009). 120 ng of DNA was used in experiments 1 to 3, and 10 ng of DNA was used in experiments 4 to 7.

the plasmid in the bacterial cell. Ampicillin-sensitive transformants were screened for the ability to swarm on soft agar plates as a test for chemotaxis. Isolates that lost the ability to swarm were defined as transformed at the chemotaxis locus.

RESULTS

Selection of transformants. The results of one series of transformations are shown in Table 3. Two different sorts of transformations were attempted. In the first type, the plasmid was cut on both sides of selected kan DNA (Table 3, experiment 1), liberating a fragment that had only DNA that was homologous to the target (in this case, the chromosomal che locus) on both sides of kan and no plasmid replication origin. In the second type (experiments 2 and 3), the plasmid was cut on just one side of kan, resulting in DNA with the kanamycin gene sandwiched between two regions of homology with the chromosome and a nonhomologous piece of DNA at one end that contained the ampicillin resistance gene and origin of replication. Transformation was found to be efficient in both cases, with mildly increased efficiency when both ends of the homologous DNA were cleaved. Over several experiments, the efficiency of transformation by the single-cut DNA was 10 to 80% that of the double-cut DNA. Transformation was also possible when a cleavage site outside the region of homology was chosen. These results are consistent with the model of RecBC as a traveling recombinase entering double-strand ends, as discussed below, and somewhat different from the case in organisms in which the transforming DNA enters the cell as a single strand (33, 34).

Chromosomal transformation to kanamycin resistance is less easily demonstrated when the linear donor DNA piece contains the origin of replication. Under these conditions, the probability is increased for transformation by a recircularized plasmid or by one that was never linearized, and plasmids were always recoverable from kanamycin-resistant, Che<sup>+</sup> transformants. None of several Che<sup>-</sup> isolates appeared to contain plasmids.

The ability to transform with plasmids cut at only one site is useful when one does not have convenient restriction sites on both sides of the marker. An example is the pair of plasmids pCR174 and pCR175. These plasmids contain a convenient XbaI site to one side of the cheB-lacZ fusion, but the second EcoRI site, in lacZ, makes use of EcoRI more difficult for providing a DNA fragment with bacterial DNA on both sides of the dominant selectable marker. Transformations were performed with plasmids cut only at the XbaI site and also with plasmids cut at XbaI and partially cut at EcoRI (Table 3, experiments 4 to 7).

The ratio of ampicillin-sensitive transformants to the total number of transformants varied from 20 to 100% for XbaI-EcoRI (two scissions)-cut pCR173 and from 2 to 80% for XbaI- or EcoRI (single scission)-cut pCR173 or pCR174. The same DNA samples gave similar results in transformations into either D308 (recD1009) or D301 (recD1003), which implied that the variability was in the DNA preparation rather than the cell preparation. The frequency of chromosomal transformation to kan at the che locus with XbaI-EcoRI-cut pCR173 was approximately 10<sup>-4</sup> relative to normal transformation with the same quantity of uncut circular plasmid.

The frequency of linear transformation decreased with decreasing lengths of homologous DNA flanking the kanamycin resistance marker. The frequency of transformation with pCR173 cut with EagI (1,900-base-pair homology) (25) was less than 20% of the frequency with XbaI-cut plasmid (4,700-base-pair homology). The frequency of transformation with BsaHI-cut pCR173 (300-base-pair homology on either side) was less than 2% that of XbaI-EcoRI-cut plasmid.

Analysis of chemotaxis functions in the transformants. Eight of the transformants from Table 3 were examined in more detail to determine the structure of the chromosome in the region around cheB and cheY. The eight chosen were four each from transformations with pCR174 and with pCR175. Each of four set had three isolates from transformation with XbaI-cut plasmid and one from plasmid cut with XbaI and partially cut with EcoRI. If the linear transformation event is conversion of the bacterial chromosome to the allele carried on the linearized plasmid, the inserted lac and kan sequences should partially delete the cheB and cheY genes while leaving intact the remainder of the meche operon containing the tar, tap, cheR, and cheZ genes (Fig. 1). The candidates were examined for swimming behavior, ability to be complemented by a plasmid carrying the entire meche operon, ability to be rescued to Che<sup>+</sup> by recombination with a plasmid carrying DNA that spans the insert, presence of a functional cheR gene, and absence of a functional cheB gene.

The cheY gene codes for a protein responsible for causing clockwise rotation of the bacterial flagella (7, 20, 29, 45). Disruption of the cheY gene results in bacteria whose flagella turn only counterclockwise; therefore, the bacteria always swim without tumbling (20, 27, 45). All 8 candidates, as well as 16 other presumed transformants, exhibited no tumbling behavior, which supported the idea that the cheY deletion had been transferred to the chromosome.

The eight candidates were transformed to ampicillin resistance with intact plasmids pRSW220 (which carries just the cheB gene), pCR67 (which carries the cheR and cheB genes and part of the cheY gene but does not overlap the right-hand end of the insert), pCR70 (which carries cheR, cheB, and cheY and does overlap both ends of the insert), and pCR73 (which expresses cheR, cheB, cheY, and cheZ). The results for all eight candidates were the same. Plasmid pCR73 complemented the chromosomal transformants to approximately wild-type levels on swarm plates, whereas the other three did not. The small swarms formed by the strains carrying pCR70 resembled those of a cheRERY strain carrying pCR70. In this case, however, the small swarms produced fast-swarming flares, which then swarmed as wild type. When these fast-swarming variants were tested on
kanamycin plates, 37 of 40 had lost kanamycin resistance. These results suggest that the insertion in the chromosome is polar, eliminating expression of the cheZ gene, but that recombination from pCR70 into the chromosome restores the original structure. Presumably, the 10% that retained kanamycin resistance had transferred the resistance gene to a plasmid, either by reciprocal recombination or by prior transfer to another of the multiple copies of the plasmid. No reversion to Che+ was observed in the presence of plasmid pCR67 or pRSW220, which indicated that no homology exists for recombination downstream from the insert, as one would predict.

Expression of the cheR and cheB genes can be assayed biochemically. The products of these genes are a methyl transferase and a methyl esterase, respectively (32, 35, 37), which are responsible for transferring methyl groups from $S$-adenosylmethionine to the chemotaxis signal transducer-receptor proteins and from the transducers-receptors into methanol (41). The continuous evolution of methanol requires that both gene products be present, and radioactive methanol is released as a result of incubation with [methyl-$^{3}H$]methionine. The lesion introduced into the chromosome disrupts cheB but not cheR. Therefore, a plasmid complementing cheB should be required to restore methanol production without any necessity to complement cheR. All tested candidates showed increased release of volatile radioactivity and stimulus-dependent esterase activity when carrying pRS220, which provides just the CheB function. The enhancement of methanol evolution as a result of adding CheB demonstrates both the absence of a functional cheB gene and the presence of a functional cheR gene.

Chromosomal structure of the transformants. Phage P1 transduction experiments (24) gave similar results for each of the eight candidates examined above. The 100% cotransduction of LacZ+ and Che− with kanamycin resistance (2,000 of 2,000 and 200 of 200, respectively) demonstrated that the plasmid structure remained basically intact when transferred to the chromosome. The 40% cotransduction of Eda− (85 of 205) confirmed that integration took place at the homologous che region of the chromosome, which is approximately 1 min away from eda on the E. coli chromosome.

The local physical structure of the chromosome was determined by selecting plasmids that had acquired the transformed segment of the chromosome and showing that their restriction maps were identical to the original structure of the plasmids used for the linear transformation (data not shown). Successful acquisitions were found by selecting for loss of the streptomyacin sensitivity conferred by a wild-type rpsL gene in pCR176 replacing the lacZ and kanamycin resistance inserts in the plasmids used for the original transformations. Homologous recombination with the chemotaxis region can replace the rpsL gene with the corresponding DNA from the chromosome. In this case, that DNA includes the kanamycin resistance gene and the lacZ gene from plasmid pCR174 or pCR175. In every candidate, loss of the rpsL gene resulted in plasmids acquiring the lacZ and kanamycin resistance genes and never reconstructed the original chemotaxis region. The apparent identity of the restriction maps of these plasmids with those of the plasmids used for the initial linear transformation demonstrated that the chromosome had not suffered any gross deletions in this region as a result of allele transfer to the chromosome.

**DISCUSSION**

The recB, recC, and recD genes of *E. coli* code for three polypeptides that make up a single complex protein (1, 21; reviewed in reference 10) containing several enzymatic activities, including a helicase (39) and ExoV (21). In wild-type *E. coli*, the RecBCD complex promotes homologous recombination, and mutations in *recB* or *recC* result in decreased recombination activity and increased sensitivity to UV light. ExoV activity is missing in extracts from either *recB* or *recC* mutants. However, one subunit required for ExoV activity, named α by Lieberman and Oishi, is not eliminated by mutations in either gene (21). Chaudhury and Smith (20) found nucleases that act on mutations in the vicinity of the *recBC* genes, and it was later shown that the ExoV activity can be eliminated by mutations in *recD* while leaving the bacteria proficient for recombination (1, 4, 5). This creates a situation similar to that found for the *recBC sbcB sbeC* background, also ExoV− and Rec+, which Oishi and Cosloy used for transformation of *E. coli* with linear DNA (8, 9, 26). Our results show that strains carrying just the *recD* mutation can also be transformed by linear DNA. Speculations on the mechanisms of recombination in these two kinds of strains have been offered elsewhere (39a, 40).

On a pragmatic level, the results shown here demonstrate that *E. coli* carrying mutations in *recD* can be used for strain construction with transformation by linear pieces of DNA, in this case linearized plasmids. The *recD* mutation does not noticeably interfere with bacterial growth and can easily be transduced into working strains either through linkage with *argA* (1) or by using a *recD::mini-Tet* (4). If the presence of the *recD* mutation is undesirable, the gene altered by transformation can be PI transduced to the original, rec+ strain.

For practical use, the transferred information should include a selectable marker such as the *kan* insert in the experiments described here. Another marker, which can be easily screened for (such as the *amp* gene in our plasmid), should be located adjacent to the plasmid origin of replication in order to score those transformation events that do not involve gene conversion in the chromosome. When scoring chromosomal events is difficult, it is also preferable that the plasmid be cleaved between the desired marker and the plasmid origin of replication. This places the cleavage on the opposite side of ori from the marker that is screened against, e.g., *amp*. Under these conditions, genetic information residing in a plasmid can be transferred to the chromosome and then stably maintained as a high-efficiency copy of *E. coli* genetics convenient access to some of the techniques possible with yeasts and some other bacterial species.

**ACKNOWLEDGMENTS**

We thank Frank Stahl for useful discussions, for initiating this collaboration, and for redaction of the manuscript.

This work was supported by Public Health Service grant AI-17808 from the National Institute of Allergy and Infectious Diseases to F. W. Dahlquist and by Public Health Service grant GM 33677 and National Science Foundation grant PCM 8408943 to F. W. Stahl.

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