NOTES

Complementation of the \textit{lytD} Mutation of \textit{Escherichia coli} by Either the \textit{cI} or \textit{cro} Gene of Bacteriophage \textit{\lambda}

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The \textit{lytD} mutant of \textit{Escherichia coli} exhibits temperature-sensitive growth which is attributed to cellular autolysis at the restrictive temperature. Either of two cloned phage \textit{\lambda} genes, identified as \textit{cI} and \textit{cro}, suppressed the \textit{lytD}(Ts) lysis phenotype, suggesting that \textit{lytD} encodes a DNA-binding protein with a DNA-binding specificity similar to that of \textit{CI} and \textit{Cro}. LytD may be a repressor of a gene(s) involved in cellular autolysis.

\textit{Escherichia coli} possesses at least eight enzymes which hydrolyze cell wall peptidoglycan (9). The activities of some of these peptidoglycan hydrolases may cause bacteriolysis under certain circumstances, e.g., during treatment with inhibitors of peptidoglycan synthesis. We (5) have recently described a temperature-sensitive mutation in a new \textit{E. coli} gene, designated \textit{lytD}, which is associated with peptidoglycan hydrolase-mediated lysis at the restrictive temperature.

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FIG. 1. Restriction map of the 7-kb \textit{BamHI} fragment from plasmid pDD52, a derivative of pUC19, and the strategies for subcloning the \textit{\lambda cro} and \textit{cI} genes. Abbreviations for restriction enzyme cleavage sites: \textit{B}, \textit{BamHI}; \textit{Bg}, \textit{BglII}; \textit{H}, \textit{HindIII}; \textit{S}, \textit{SmaI}; \textit{T}, \textit{TaqI}. The boxes indicate the locations of \textit{cI} and \textit{cro}, and the arrows within the boxes indicate the transcriptional orientations of the genes. The \textit{cI} clone pDD65 is a derivative of pUC19. The 2.5-kb \textit{HindIII-SmaI} fragment from pDD52 was cloned in both orientations in plasmids pAA-pZ718 and pAA-pZ719 (Gold Biotechnology, Inc., St. Louis, Mo.) to yield plasmids pDD54 and pDD55, respectively. Sets of nested deletions derived from pDD54 (pDD56, pDD57, and pDD58) and pDD55 (pDD59, pDD60, and pDD61), prepared as described by Ahmed (1), were used to document the \textit{lytD1}-complementing activity of \textit{cro}. Each of the deletions was confirmed by DNA sequencing. Their \textit{lytD1}-complementing activities are indicated to the right.
carrying fragment was e.g., pDD60, and genome sequencing. DNA gene BamHI site pUC19 unexpectedly indicated strain VC4014 to ren clones including partial (prototrophic, elsewhere (3). genes, identified (42°C).

Four clones which complemented the lytD1 mutation were selected from a gene library consisting of fragments from a partial Sau3A1 digest of chromosomal DNA from E. coli W3110 (prototrophic, λ−) which were ligated into the unique BamHI site of the single-copy phasmid vector λSE6 (7). All four clones contained identical 7-kb BamHI fragments on which resided the lytD1-complementing activities. The fragment from one of these clones, pDD51, was subcloned into pUC19 to yield plasmid pDD52 (Fig. 1). Several criteria, including a partial nucleotide sequence and a physical map, unexpectedly indicated that the fragment represented the N to ren gene segment (35.3 to 42.3 kb [8]) of the phage λ genome and was clearly not derived from the E. coli chromosome (3). In initial experiments, cro was identified as a lytD1-complementing gene by deletion analysis (Fig. 1) and DNA sequencing. Plasmids carrying the intact cro gene, e.g., pDD61, conferred lysis tolerance to the lytD1 mutant, strain VC4014 (5) at 42°C, whereas cro deletion derivatives, e.g., pDD60, did not (Fig. 2).

The presence of a wild-type cl gene on the 7-kb BamHI fragment was readily verified by demonstrating that bacteria carrying plasmid pDD52 were immune to phages λ cI857 (at 42°C). We report here that either of two cloned phage λ genes, identified as cI and cro, complemented the lytD1 mutation. Details of these experiments have been presented elsewhere (3).

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FIG. 4. Southern blot analysis of BamHI-digested E. coli and phage λ genomes with a cro probe derived from pDD61 (Fig. 1). DNA samples were transferred to Zeta-Probe membranes (Bio-Rad Canada) by the method of Southern (13). The procedures for labeling DNA probes with digoxigenin-11-dUTP, for hybridization, and for detection of hybridized DNA were provided by Boehringer-Mannheim Canada. Lanes: a, no DNA; b, λ DNA; c, phasmid pDD51, a derivative of λSE6 (7) and the source of the 7-kb BamHI fragment in pDD52 (Fig. 1); d, W3104 (λ lysogen) chromosomal DNA; e, W3110 (λ-) chromosomal DNA. DNA standard size markers are indicated on the left (in kilobases).

Figure 4 shows a Southern blot analysis of the phage λ and E. coli genomes with the 1.1-kb cro-carrying fragment from pDD61 (Fig. 1) as a probe. This experiment was designed primarily to determine the origin of the 7-kb BamHI λ DNA fragment in the gene library. The DNAs to be probed were digested with BamHI. As expected, the probe hybridized to a single 7-kb fragment derived from λ DNA (lane b). Lane c represents pDD51, one of the four original λD1-complementing phasmid clones isolated from the gene library. In this case, the probe hybridized to both the 33-kb phasmid vector (λSE6 contains cro but not cI [7]) and the 7-kb insert. As already noted, neither copy of cro in pDD51 is expressed because of the cI gene on the insert. Finally, the BamHI-digested chromosomal DNAs of strains W3104 (λ lysogen) and W3110 (nonlysogenic) were compared. The strongly hybridizing 7-kb fragment from W3104 DNA (lane d) was undoubtedly derived from the λ prophage in this strain. This component was not observed in W3110 (lane e). These results confirm that the 7-kb BamHI fragment did not come from strain W3110. We are uncertain about the origin of this λ DNA fragment, but we suggest that the λSE6 vector preparation was apparently contaminated with DNA derived from a λ helper phage. If so, the 7-kb fragments were probably generated when the vector preparation was digested with BamHI during the construction of the gene library.

Interestingly, Fig. 4 shows that both W3104 and W3110 exhibited at least six common fragments (31, 26, 13, 6.8, 5.5, and 5 kb) which hybridized weakly with the probe, suggesting that E. coli K-12 DNA contains several sequences related to the cI-cro region. It remains to be determined whether any of these λ-related sequences represents the chromosomal λD locus. Cryptic λ-related sequences have been demonstrated previously in several regions of the chromosomes of E. coli and other enterobacteria by DNA hybridization with λ inserts DNA probe (2, 12). The location of λD (12.7 min on the linkage map) actually coincides with that of the cryptic lambdoid prophage called Qsr' (10). In view of the λD lysis phenotype, this is especially interesting because the Qsr' module is composed of analogs of the λ lysis genes S and R and their positive regulator, Q. It is possible that λD lies within the Qsr' module. However, this seems unlikely since the SR module is positively regulated by Q (8), and it is difficult to reconcile this with the observed λD-complementing activities of Cro and CI. Alternatively, λD may regulate a nonlambdoid gene(s) involved in a cellular function and may fortuitously possess structure-function features common to Cro and CI. The identification of the λD locus and its putative target gene may resolve these issues.

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