JOURNAL OF BACTERIOLOGY, Dec. 1993, p. 7745–7746
0021-9193/93/237745-02$02.00/0

Vol. 175, No. 23

Physical Mapping of the *Escherichia coli pepT* and *potABCD* Genes

MARY-JANE LOMBARDO,[1] CHARLES G. MILLER,[1]* AND KENNETH E. RUDD[2]

Department of Microbiology, University of Illinois at Urbana-Champaign, 131 Burrill Hall, 407 S. Goodwin, Urbana, Illinois 61801,[1] and National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland 20894[2]

The anaerobically regulated *pepT* gene of *Salmonella typhimurium* encodes an aminotripeptidase (8–10). *pepT* has been mapped to 25 map units on the *Salmonella* chromosome (9), and the locus has been cloned and its nucleotide sequence has been determined (6) (GenBank accession number M62725). A recent GenBank search identified an *Escherichia coli* sequence, ECOPOTABCD (accession number M64519), with 87% identity in a 569-bp overlap with a region of the *pepT* sequence. This overlap includes the 5′ translated regions of both *pepT* and the *potABCD* operon, indicating that *pepT* and *potABCD* are divergently transcribed. The translation start sites are separated by only 250 bp, and the −35 sequence for one of the two *pepT* promoters (the "aerobic" promoter [6]) is located only 5 bp from the suggested −35 sequence for *potABCD* (2). In many cases, such an arrangement implies coordinate regulation of the two divergently transcribed genes (1). Nothing is known about the regulation of *potABCD*. *pepT* is controlled by the *oxrA*(*fnr*) gene product, the transcriptional activator of a family of anaerobically expressed genes (9). The *E. coli potABCD* operon encodes a putrescine/spermidine transport system (2, 3). This locus has not been previously identified in *S. typhimurium*. The presence of a *pepT* gene in *E. coli* had been suggested previously on the basis of the ability of a multiply peptidase-deficient *E. coli* strain to use tripeptides as amino acid sources when grown anaerobically (9). The partial amino acid sequence of *E. coli* peptidase T deduced from ECOPOTABCD has been deposited with the SwissProt protein sequence database as PEPT_ECOLI (accession number P29745). This sequence of 43 amino acids (MDKLLERFL NYVSLDTQSKAGVRQVPSTEGQWKLLHLLKEQLE) differs from the corresponding *S. typhimurium* peptidase T sequence only at the four underlined positions.

The *potABCD* operon has been located at 15 map units on the *E. coli* chromosome (3). This map position differs from the 25-map-unit position of *Salmonella pepT*. Alignment of the restriction map predicted by the ECOPOTABCD sequence with the physical map of Kohara et al. (4) using the MapSearch program (7) initially failed to find a significant match to any region of the genomic restriction map. Identification of the partial *pepT* sequence in the ECOPOTABCD led us to examine the 25-centisome (percent physical map units) region of the *E. coli* genomic restriction map in more detail. The region of the genomic restriction map covered by miniset clone E4C2[238] appeared to have an unusual paucity of restriction sites. An inspection of the individual restriction map for clone E4C2[238] provided with the miniset clones (2a) revealed that

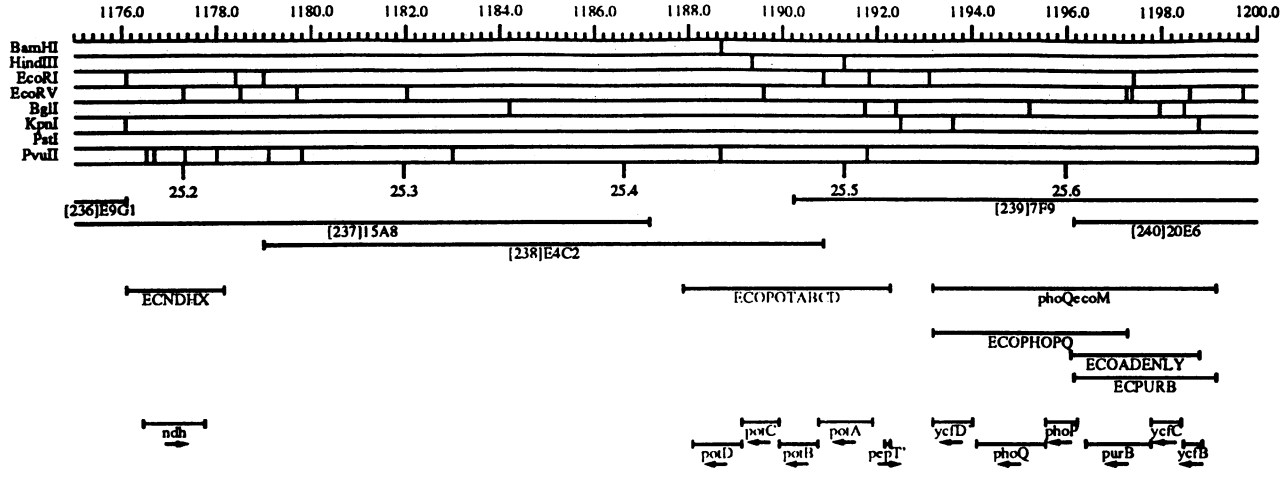

FIG. 1. The 1,175- to 1,200-kb region of the integrated *E. coli* genomic restriction map. DNA sequence information is aligned and integrated with the genomic restriction map as previously described (6). The accession numbers for the aligned GenBank sequences are as follows: ECNDHX, V00306; ECOPOTABCD, M64519; ECOPHOPQ, D90393; ECOADENLY, M74924; ECPURB, X59307. *ycfB*, *ycfC*, and *ycfD′* are names assigned to open reading frames of unknown function. phoQecoM is a melded EcoSeq entry. The numbers directly below the restriction map are physical map units in centisomes. pepT′ indicates that only a part of the *E. coli pepT* gene has been sequenced.

* Corresponding author. Electronic mail address: charles_miller@qms1.life.uiuc.edu.

it included restriction sites that were not indicated on the consensus genomic restriction map. Strikingly, the five restriction sites at the right end of the insert in clone E4C2[238] matched very well to the sites determined from the last 1.4 kb of the ECOPOTABCD DNA sequence. This allowed us to align and integrate the ECOPOTABCD DNA sequence into the genomic restriction map as indicated in Fig. 1. This alignment predicts that part of *potABCD* and all of *pepT* should be located on clone 7F9[239]. The remainder of *potABCD* should be on clone E4C2[238]. These clones carry sequences located at approximately 25 centisomes on the *E. coli* chromosome. To test this prediction, we have probed a blot of the Kohara miniset (4) (purchased from Takara Shuzo Co., Ltd.) with a 2.4-kb *Eco*RI fragment from pJG17 (bp 55 to 2475 [6]). This fragment includes the entire coding region of *Salmonella pepT*, 135 bp of the coding region of *potABCD*, and the intergenic region. Only one clone in the miniset, 7F9[239], was found to hybridize to this fragment. This result places *pepT* and *potABCD* at 25.5 centisomes (approximately 1,191 kb) on the *E. coli* chromosome, a position corresponding almost exactly to that determined by genetic methods for *S. typhimurium*.

The alignment of ECOPOTABCD with the genomic restriction map shown in Fig. 1 also predicts a counterclockwise orientation of *potABCD* and a gap of about 1 kb between ECOPOTABCD and ECOPHOPQ. To test these predictions, we have used primers specific to ECOPOTABCD and ECOPHOPQ to amplify the region between these two sequences using the polymerase chain reaction (PCR). The ECOPOTABCD-specific primer (pot1-5′CGCGGTTGATTA TTCAATTTCTTACTCTGTCCCA3′) was derived from the *Salmonella pepT* sequence (bp 155 to 188), and the ECOPHO PQ-specific primer (phoQ1-5′AACACTTTAAGCAATGG TTT3′) was derived from the ECOPHOPQ sequence (bp 4042 to 4061). If *potABCD* is oriented counterclockwise, then these two primers should amplify a sequence of approximately 1,500 bp. If the orientation is opposite to that shown or if the map assignment is incorrect, no PCR product is expected. These primers were used in a PCR with a single-colony suspension of *E. coli* MG1655 providing the template DNA as described previously (5), except that the annealing temperature was 45°C. A single product of approximately 1,850 bp was observed, indicating that the counterclockwise orientation of *potABCD* is correct and that the gap between the ECOPOT ABCD and ECOPHOPQ sequences is approximately 1,380 bp, consistent with the alignment shown in Fig. 1.

This work was supported by grant AI10333 from the National Institute for Allergy and Infectious Diseases.

REFERENCES

1. **Beck, C. F., and R. A. Warren.** 1988. Divergent promoters, a common form of gene organization. Microbiol. Rev. **52:**318–326.
2. **Furuchi, T., K. Kashiwagi, H. Kobayashi, and K. Igarashi.** 1991. Characteristics of the gene for a spermidine and putrescine transport system that maps at 15 min on the *Escherichia coli* chromosome. J. Biol. Chem. **266:**20928–20933.

2a. **Isono, K., and Y. Kohara.** Personal communication.

3. **Kashiwagi, K., N. Hosokawa, T. Furuchi, H. Kobayashi, C. Sasakawa, M. Yoshikawa, and K. Igarashi.** 1990. Isolation of polyamine transport-deficient mutants of *Escherichia coli* and cloning of the genes for polyamine transport proteins. J. Biol. Chem. **265:**20893–20897.
4. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
5. **Lombardo, M. J., D. Bagga, and C. G. Miller.** 1991. Mutations in *rpoA* affect expression of anaerobically regulated genes in *Salmonella typhimurium*. J. Bacteriol. **173:**7511–7518.
6. **Miller, C. G., J. L. Miller, and D. A. Bagga.** 1991. Cloning and nucleotide sequence of the anaerobically regulated *pepT* gene of *Salmonella typhimurium*. J. Bacteriol. **173:**3554–3558.
7. **Rudd, K. E., W. Miller, C. Werner, J. Ostell, C. Tolstoshev, and S. G. Satterfield.** 1991. Mapping sequenced *E. coli* genes by computer: software, strategies and examples. Nucleic Acids Res. **19:**637–647.
8. **Strauch, K. L., T. H. Carter, and C. G. Miller.** 1983. Overproduction of *Salmonella typhimurium* peptidase T. J. Bacteriol. **156:**743–751.
9. **Strauch, K. L., J. B. Lenk, B. L. Gamble, and C. G. Miller.** 1985. Oxygen regulation in *Salmonella typhimurium*. J. Bacteriol. **161:** 673–680.
10. **Strauch, K. L., and C. G. Miller.** 1983. Isolation and characterization of *Salmonella typhimurium* mutants lacking a tripeptidase (peptidase T). J. Bacteriol. **154:**763–771.