Physical Map Location of the New *Escherichia coli* Gene *eco*, Encoding the Serine Protease Inhibitor Ecotin

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Ecotin is a pancreatic serine protease inhibitor which is found in the periplasm of *Escherichia coli* and is remarkable in its ability to inhibit chymotrypsin, trypsin, and elastase equally well (3). We recently cloned and sequenced the 486-bp ecotin gene and determined its reactive site (7) and have overexpressed ecotin to facilitate determination of an X-ray crystal structure (6). The function of ecotin remains unknown, but since it resides in the periplasm and does not inhibit any *E. coli* proteases or esterases thus far tested (3, 10), it may be a defensive molecule (2). We propose the name *eco* for the ecotin gene.

The position of the *eco* gene was determined by using a gene-mapping membrane, kindly provided by Akihiro Noda, which contains the 476-clone minisat that includes the entire *E. coli* genome (4). A 448-bp DNA fragment was used as the probe; it consists of 273 bases 5' to the initiating ATG codon and 175 bases encoding the N-terminal region of the protein. The probe hybridized to clones 372 (19D1) and 373 (22A6), placing it between kb 2308 and 2318 on the physical map, which corresponds to approximately 48 min on the genetic map (1, 11). The *eco* gene contains sites for only two of the enzymes used to construct the map (*Pst*I and *Bgl*II), which appear to be coincident on the Kohara map; thus, the orientation is uncertain. The DNA sequence of the *spi* gene shows that the *Pst*I restriction site is 37 bp 5' to the *Bgl*II restriction site (7). According to the restriction map of Médigue et al. (8), the *Bgl*II restriction site closely precedes the *Pst*I restriction site, placing the *eco* gene counterclockwise on the *E. coli* map (Fig. 1). However, as noted by Rudd et al. (11), closely spaced sites can sometimes be mapped in inverted order, making this assignment provisional. ECOEOA does not overlap the neighboring *ada-alkB* operon at physical map location 2318 kb (9) or the *proL* gene at physical map location 2294 kb (5).

Nucleotide sequence accession number. The nucleotide sequence of the *eco* gene has been assigned GenBank accession number M60876.

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REFERENCES

1. Bachmann, B. J. 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. 54:130-197.
2. Brecher, A. S., and R. D. Pugatch. 1969. A non-dialyzable

FIG. 1. Restriction map, adapted from Kohara et al. (4) and Médigue et al. (7), showing the overlap region of the *E. coli* genome between clones 22A6 and 19D6. The black bar denotes the *eco* gene, and the larger box denotes the sequenced DNA used for restriction analysis. Map units are indicated in kilobase pairs.

inhibitor of proteolytic activity in soluble extracts of *Escherichia coli*. Experientia 25:251-252.

3. Chung, C. H., H. E. Ives, S. Almeda, and A. L. Goldberg. 1983. Purification from *Escherichia coli* of a periplasmic protein that is a potent inhibitor of pancreatic proteases. J. Biol. Chem. 258:11032-11038.
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. Komine, Y., T. Adachi, H. Inokuchi, and H. Ozeki. 1990. Genomic organization and physical mapping of the transfer RNA genes in *Escherichia coli* K12. J. Mol. Biol. 212:579-598.
6. McGrath, M. E., T. Erpel, M. F. Browner, and R. J. Fletterick. Expression of the protease inhibitor ecotin and its cocrystallization with trypsin. J. Mol. Biol., in press.
7. McGrath, M. E., W. M. Hines, J. Sakanari, R. J. Fletterick, and C. S. Craik. 1991. The sequence and reactive site of ecotin: a general inhibitor of pancreatic serine proteases from *E. coli*. J. Biol. Chem. 266:6620-6625.
8. Médigue, C., J. P. Bouché, A. Hénaut, and A. Danchin. 1990. Mapping of sequenced genes (700 kbp) in the restriction map of *Escherichia coli* chromosome. Mol. Microbiol. 4:169-187.
9. Médigue, C., A. Hénaut, and A. Danchin. 1990. *Escherichia coli* molecular genetic map (1000kbp): update 1. Mol. Microbiol. 4:1443-1454.
10. Palmer, S. M., and A. C. St. John. 1987. Characterization of a membrane-associated serine protease in *Escherichia coli*. J. Bacteriol. 169:1474-1479.
11. Rudd, K. E., W. Miller, J. Ostell, and D. A. Benson. 1990. Alignment of *Escherichia coli* K12 DNA sequence to a genomic restriction map. Nucleic Acids Res. 18:313-321.

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