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Location of the *gltP* Gene on the Physical Map of *Escherichia coli* K-12

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Recently, a gene (*gltP*) that encodes the proton-dependent glutamate-aspartate carrier protein of *Escherichia coli* was cloned and sequenced (3, 5, 6). Evidence for the function of *gltP* is as follows. The open reading frame encodes a protein of 437 amino acids (molecular mass, 47.2 kDa) that is hydrophobic in nature and contains 12 possible membrane-spanning α-helical regions (5). A substantial proportion of GltP expressed from a multiple-copy-number plasmid was located in the membrane fraction of the cells (6). Further, a strain of *E. coli* (BK9MDG) that harbored *gltP* on a multiple-copy-number plasmid had 5.5-fold-higher uptake of L-glutamate and 4.5-fold-higher uptake of aspartate than those of BK9MDG without the plasmid (6). Uptake was insensitive to the concentration of Na^+ and was inhibited by L-cysteate and β-hydroxyaspartate but not by α-methylglutamate.

A 1.6-kb fragment of DNA containing *gltP* was removed from pBW1 (6) by digestion with *Mlu*I and *Nsi*I, purified in low-melting-point agarose gels and used to prepare a digoxigenin-labelled probe (digoxigenin labelling and detection kit; catalog no. 1175033; Boehringer-Mannheim). This probe was hybridized to the *E. coli* Gene Mapping Membrane (Takara Shuzo Co. Ltd., Kyoto, Japan) which contains a miniset of 476 recombinant lambda phage clones arranged in an ordered array on a nylon membrane (4). The conditions used for hybridization and for detection of clones to which the probe bound were those suggested by Boehringer-Mannheim. The *gltP* probe hybridized to lambda clones 10G7 and 1A11, indicating that *gltP* lies in the overlap region of the two phages, i.e., between kb 4369 and 4377 on the physical map of the *E. coli* chromosome (Fig. 1). The pattern of restriction enzyme sites between kb 4372 and 4378 on the physical map corresponds with that of the insert of pBW1 and confirms this as the correct position for the fragment used as the probe. The *gltP* gene must therefore lie between kb 4373.4 and 4375. It should be noted, however, that whereas only one *Eco*RV site is recorded at kb 4374 on the physical map, two *Eco*RV sites, 225 bp apart, occur at the same relative position on pBW1 (Fig. 1). Position 4374.6 on the physical map corresponds to approximately min 92.5 on the temporal map of the *E. coli* chromosome (1). Recently, a mutation responsible for resistance to L-cysteate in a mutant of *E. coli* was mapped at min 92.5 by Booth et al. (2), who suggested that the mutated gene was *gltP*. The results presented in this communication support their suggestion.

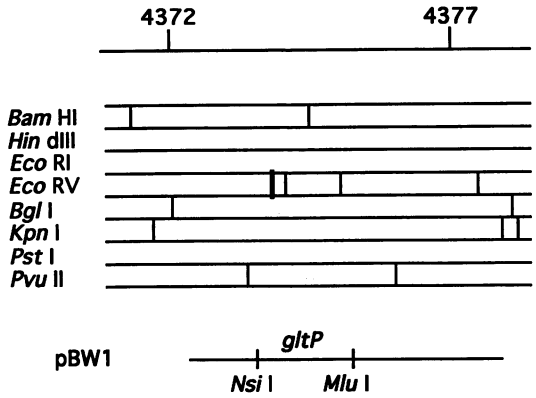

FIG. 1. Location of *gltP* on the physical map of the *E. coli* chromosome represented in the format of Kohara et al. (4). The *Nsi*I-to-*Mlu*I fragment was used to probe the *E. coli* Gene Mapping Membrane. All restriction enzyme sites (|) within the insert DNA of pBW1 corresponded to those on the physical map except for an additional *Eco*RV site (‖) located within the *gltP* fragment.

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