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Map Position of the *glnE* Gene from *Escherichia coli*

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Glutamine synthetase activity in *Escherichia coli* is controlled by a complex set of regulatory functions which affect the initiation of transcription as well as the covalent modification of the enzyme. Except for the *glnE* gene, the genes that encode these regulatory functions have all been located on the genetic map of *E. coli* chromosome. The *glnE* gene encodes the adenylyltransferase responsible for the reversible inactivation of glutamine synthetase by adenylylation. It has been known that *glnE* is not linked to other genes involved in the regulation of glutamine synthetase activity (namely, *glnA*, *glnB*, *glnD*, *glnG*, *glnL*, *gltB*, and *rpoN*). We determined the location of a Tn*5* insertion in *glnE* (generously provided by B. Magasanik), showing that it is located in the *dnaG* region of the genetic map, at about min 66.75 (Fig. 1). We then chose three lambda phage clones from the Kohara miniset bank (5) which defined this region, modified them so that lysogens could be selected directly (4), and lysogenized the *glnE* mutant with each of these clones. To aid in the selection and to stabilize the *glnE* insertion, we converted the original Tn*5* insertion to the tetracycline-resistant, transposase-negative derivative Tn*5-131* (2). Clone 9f9 (miniset no. 508) complemented the *glnE* mutation fully, whereas the adjacent clones 6B12 (miniset no. 507) and 19f2 (miniset no. 509) failed to complement *glnE* (Table 1). Southern blot analysis of wild-type and mutant chromosomal DNAs with miniset no. 508 as a probe (data not shown) indicated that the mobility of a 3.9-kb *Pvu*II fragment and not that of any other fragment was altered in the *glnE* mutant compared with the wild type. Thus, the *glnE* gene is located at map coordinate 3254 on the Kohara map scale, or min 66.75 on the genetic map of *E. coli*.

TABLE 1. Complementation of *glnE*::Tn*5* by phage miniset lysogens

Strain	Glutamine synthetase activity[a]		
	N starved[b] (nmol/min/mg)	N shocked[c] (nmol/min/mg)	Ratio (N starved/N shocked)[d]
Wild type (W3110)	920	100	9.2
glnE::Tn*5-131* (λ*c*I857)	1,010	960	1.0
glnE::Tn*5-131*(λ no. 507)	920	980	0.9
glnE::Tn*5-131*(λ no. 508)	840	140	6.0
glnE::Tn*5-131*(λ no. 509)	1,170	1,480	0.8

[a] Cells were grown to mid-log phase, and the unadenylylated glutamine synthetase activity was measured by using the γ-glutamyl transferase assay in the presence of 60 mM $MgCl_2$ as described previously (7).

[b] N starved, growth in glucose minimal medium with 0.1% (wt/vol) glutamine as the sole growth-rate-limiting nitrogen source.

[c] N shocked, same as N starved with ammonium sulfate added to 0.2% (wt/vol) for 2 min immediately before harvesting. Hexadecyltrimethylammonium bromide was added at harvesting to prevent change in adenylylation state (7).

[d] A high ratio indicates the ability to adenylylate.

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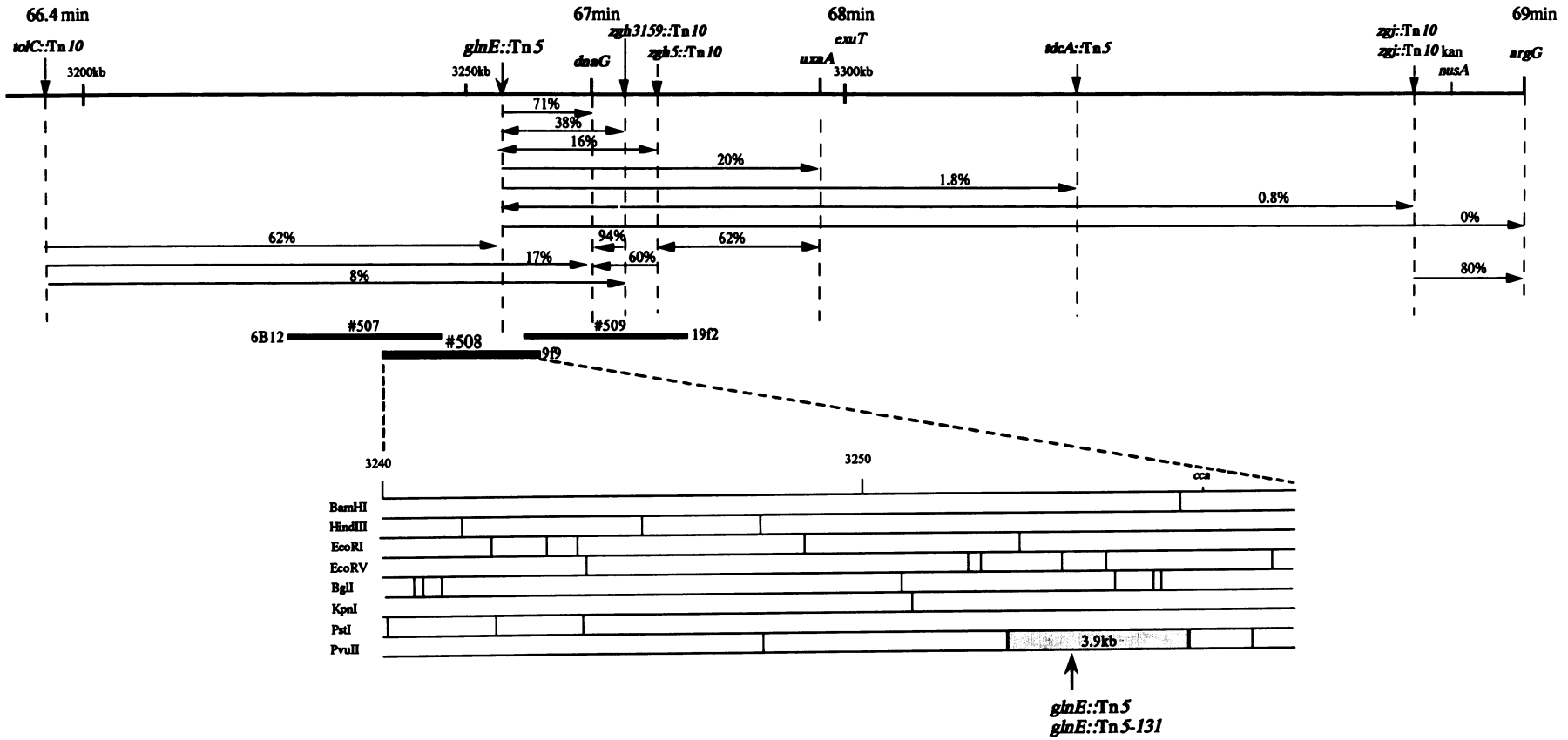

FIG. 1. Correlation of the physical map location of *glnE*::Tn*5* with the genetic map location. Arrows represent P1-mediated cotransduction frequencies. Positions of the indicated markers are redrawn from reference 1. The three thick black lines indicate Kohara phage miniset clones. The physical map is redrawn from reference 5. The shaded box indicates the 3.9-kb *Pvu*II fragment whose mobility was altered by the *glnE*::Tn*5* mutation. The *tolC*::Tn*10*, *zgh-3159*::Tn*10*, and *zgj*::Tn*10* mutations have been described previously (6), as have the *zgh*::Tn*10* and *tdc*::Tn*5* mutations (3).