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

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*Escherichia coli* contains two proton motive force-driven, high-affinity uptake systems for nucleosides and deoxynucleosides. These are the products of genes termed *nupG* and *nupC* and differ in both substrate specificity and transcriptional regulation (5, 6, 8, 9). Both transport systems show wide substrate specificity for ribonucleosides and deoxyribonucleosides. However, these permeases can be distinguished by the inability of the *nupC* product to transport guanosine and deoxyguanosine. *nupG* maps between *metK* and *speC*, near 64 min on the genetic map of *E. coli*, and its sequence is known (11). We have recently confirmed the presence of the *nupG* gene on plasmid pLC20-5 from the Clarke-Carbon library (2).

*E. coli* cells are capable of producing, de novo, sufficient quantities of nucleosides for DNA and RNA synthesis. However, externally supplied nucleosides can also be utilized, following degradation via the "salvage pathway,"and may even serve as the sole source of carbon or nitrogen for growth (3). We have exploited this phenomenon to locate *nupC* on the overlapping series of physically mapped lambda clones, constructed by Kohara et al. (4). *nupC* has been reported to lie between 50 and 52 min on the chromosome (7) but has not been precisely mapped. We report here a more precise location.

Using P1 transduction from strains carrying defined transposon Tn*10*(Tc$^r$) and Tn*10*(Km$^r$) insertions in *nupC* and *nupG*, respectively (10), we have constructed a mutant (MPG100) of *E. coli* K-12 in which both transport systems have been inactivated, as evidenced by its nucleoside analog insensitivity and by the ability of plasmids carrying *nupG* to restore nucleoside transport (2). MPG100 was used as a host to screen the Kohara library for restoration of the ability of the cells to grow on nucleosides as the sole carbon source.

Strain MPG100 was transformed with plasmid pUN121, which encodes the lambda *cI* repressor and increases the number of homologous recombinants which arise from interaction between the chromosome and infecting lambda clones, by preventing the lytic cycle. MPG100(pUN121) was then infected with miniset clones of the Kohara library, spanning the 50.7-to-52.3 min region of the genetic map of *E. coli* (lambda clones 410 to 417). Recipients infected with λ415 or λ416 but not the others gave rise to recombinants able to use cytidine as sole source of carbon (3). Putative *nupC*$^+$ colonies proved to have a kanamycin-resistant, tetracycline-sensitive phenotype, indicating that the transposon in the *nupC* gene had been excised as a result of reciprocal recombination. As expected, these strains had also regained sensitivity to toxic nucleoside analogs.

The inserts in λ415 and λ416 overlap by about 11 kb (physical coordinates 2520 to 2531), as shown in Fig. 1. Subsequent subcloning, complementation, and sequencing studies have more accurately localized the *nupC* gene between the *Pvu*II site of λ415 and the more distal *Pst*I site (Fig. 1) and show that transcription occurs in a clockwise direction in relation to the chromosomal origin of replication.

Thus, we have located *nupC* at position 2526 on the physical map (4) between *amiA* and *glk*, near min 51.8 on the genetic map (1).

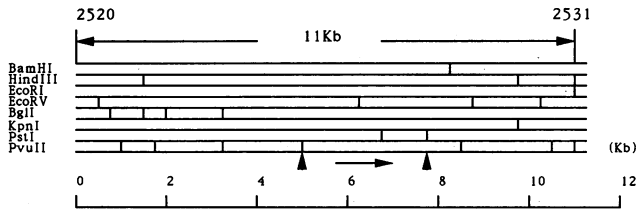


FIG. 1. Overlapping region of lambda clones 415 and 416 and published restriction analysis (4). *Pvu*II and *Pst*I sites which flank the *nupC* gene are indicated by arrowheads. The direction of transcription of *nupC* is shown by the horizontal arrow.

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