

Locations of Genes in the *nar-adhE* Region of the *Escherichia coli* K-12 Chromosome

MICHAEL R. LEONARDO AND DAVID P. CLARK*

Department of Microbiology, Southern Illinois University, Carbondale, Illinois 62901

The known genes in the *nar-adhE* region of the *Escherichia coli* K-12 chromosome (27 min) have been located on the physical map of Kohara et al. (7) (Table 1). We used the Kohara phage λ D8 to clone the *adhE* gene. A restriction map of the cloned DNA was compared with restriction maps of other cloned genes in the region for correspondence to the physical map.

The restriction maps of the cloned genes were mostly in agreement with the physical map, except that one *Hind*III site centrally located and a *Pvu*II site located at the 5' end in the *tyrT* clone (11) are absent from the physical map (7), whereas in comparison to *narL* clones (6, 12) an extra *Pvu*II

some (Table 1 and Fig. 1). The correlation of restriction data for individual clones to the physical map of Kohara strongly suggests the gene order *galU-drc-drs-irk-bglY-adhE* (Fig. 1). This conflicts with earlier cotransductional mapping by our laboratory which suggested the gene order *bglY-galU-adhE* (2). This disparity could result from the difficulty of screening for *bglY* (3). The gene for thymidine kinase (*tdk*) has been cotransductionally mapped between *tyrT* and *galU* (1). Because *tdk* has not been cloned or sequenced, precise placement is impossible and *tdk* has been omitted from Fig. 1.

Another point to note is the lack, in the clone of Goodlove et al. (4), of two *Hind*III sites 5' of the *adhE* gene (i.e.,

TABLE 1. Physical locations of genes in the *nar-adhE* region of the *E. coli* K-12 chromosome

Gene	Genetic map location (min) ^a	Physical map location ^b		Reference(s)	Comments
		min	kb		
<i>narL</i>	27.18	27.18	1291	6, 12	<i>nar</i> regulatory gene
<i>narX</i>	27.18	27.21	1292	12	<i>nar</i> regulatory gene
<i>narK</i>	27.18	27.22	1293	12	<i>nar</i> regulatory gene
<i>narGHJI</i>	27.26	27.27	1295	10, 13, 14	Nitrate reductase structural genes
<i>tyrT</i>	27.36	27.37	1300	11	tRNA ^{Tyr} , <i>supF</i> , <i>supE</i> , <i>tyrV</i>
<i>tdk</i>	27.50	— ^c	— ^c	1	Thymidine kinase
<i>galU</i>	27.56	[27.43]	[1303]	8	Glucose-1-phosphate uridylyltransferase
<i>drc, drs, irk</i>	— ^d	[27.47]	[1305]	8	Decrease in chloramphenicol and L-serine resistance; increase in kanamycin resistance
<i>bglY</i>	27.43	27.49	1306	5, 8	H1 proteins
<i>adhE</i>	27.66	27.62	1312	4	Coenzyme A-linked acetaldehyde and alcohol dehydrogenases
IS2C	— ^d	27.68	1315	15	Insertion sequence 2C

^a From reference 1.

^b Positions in minutes are derived from the exact physical position in kilobase pairs by dividing by 47.5 (1). Brackets indicate positions deduced for genes strongly suspected to lie close to physically mapped genes.

^c Not noted on linkage map in reference 1.

^d No physical data available to place gene.

site was noted (Fig. 1). These discrepancies may be due to imprecisions caused by the construction of the physical map (7), but they could also be the result of strain differences, as duplications in the *tyrT* region could promote genetic rearrangement.

A report of the cloning of the *bglY-galU* region has been published (8). *bglY* mutants with several alternate phenotypes have been isolated under different names. Recently, the gene *bglY*, referred to as *drdX* by Göransson et al. (5), has been cloned and sequenced. Because of incomplete restriction data, *galU* and the *drc, drs, and irk* sequences of Lejeune et al. (8) cannot be precisely placed, but they are located between 27.40 and 27.49 min on the *E. coli* chromo-

some (Table 1 and Fig. 1). The clone lacking these sites did not exhibit anaerobic regulation (4). However, when cloned from λ D8, the *adhE* gene retained the *Hind*III sites and was subject to anaerobic regulation (unpublished data). Umeda and Ohtsubo (15) identified the positions of insertion sequences on the physical map, and alignment of their data with our own places the insertion sequence IS2C 1.4 kb upstream (i.e., counterclockwise) of *adhE*. It is possible that an IS2-mediated deletion event led to the loss of both *adhE* regulation and the two *Hind*III sites in the previous clones (4).

The *ana* mutation has also been cotransductionally mapped to this region (1). Three-point crosses showed a greater than 95% cotransduction frequency between *ana* and *adhC* and confirmed the gene order *zch::Tn10-(ana, adhC)-galU* (unpublished results). This, coupled with the data that *adhE* clones complement the *ana* mutation (4), implies that

* Corresponding author.

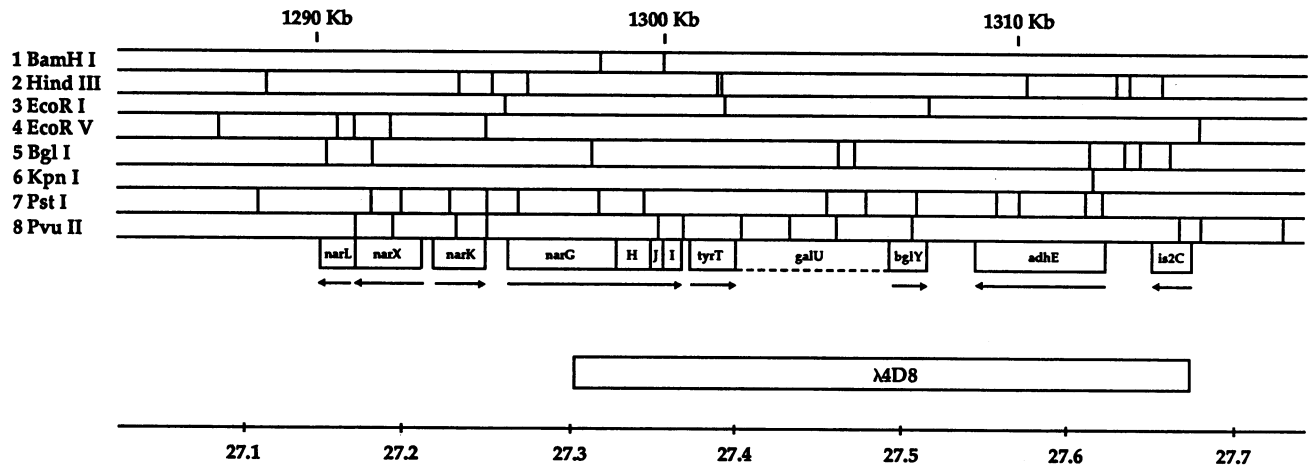


FIG. 1. Alignment of the genetic map of Bachmann (1) and the physical map of Kohara et al. (7). Data for gene alignment are from Leonardo and Clark (9) and references cited in Table 1. Arrows show transcriptional direction. The *PvuII* site at 1300 kb and the first *HindIII* site at 1302 kb were absent from the Kohara map. The *PvuII* site at 1291 kb is absent from the sequence of *narL* (12).

ana mutations are in the regulatory region of *adhE*, and therefore *ana* was omitted from Fig. 1.

Thus, the order of the genes in this region is *narL-narX-narK-narG-H-J-I-tyrT-(tdk-galU-drc-drs-irk)-bglY-adhE-IS2C* in a clockwise direction. The direction of transcription is as indicated by arrows in Fig. 1.

REFERENCES

- Bachmann, B. J. 1990. Linkage map of *Escherichia coli* K-12, edition 8. *Microbiol. Rev.* **54**:130-197.
- Cunningham, P. R., and D. P. Clark. 1986. The use of suicide substrates to select mutants of *Escherichia coli* lacking enzymes of alcohol fermentation. *Mol. Gen. Genet.* **205**:487-493.
- Defez, R., and M. De Felice. 1981. Cryptic operon for β -glucoside metabolism in *Escherichia coli* K12: genetic evidence for a regulatory protein. *Genetics* **97**:11-25.
- Goodlove, P. E., P. R. Cunningham, J. Parker, and D. P. Clark. 1989. Cloning and sequencing of the fermentative alcohol-dehydrogenase-encoding gene of *Escherichia coli*. *Gene* **85**:209-214.
- Göransson, M., B. Sondén, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin. 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. *Nature (London)* **344**:682-685.
- Gunsalus, R. P., L. V. Kalman, and R. R. Stewart. 1989. Nucleotide sequence of the *narL* gene that is involved in global regulation of nitrate controlled respiratory genes of *Escherichia coli*. *Nucleic Acids Res.* **17**:1965-1975.
- 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.
- Lejeune, P., P. Bertin, C. Walon, K. Willemot, C. Colson, and A. Danchin. 1989. A locus involved in kanamycin, chloramphenicol, and L-serine resistance is located in the *bglY-galU* region of the *Escherichia coli* chromosome. *Mol. Gen. Genet.* **218**:361-363.
- Leonardo, M. R., and D. P. Clark. Unpublished data.
- Li, S. F., and J. A. DeMoss. 1987. Promoter region of the *nar* operon of *Escherichia coli*: nucleotide sequence and transcription initiation signals. *J. Bacteriol.* **169**:4614-4620.
- Michelson, U., M. Bösl, T. Dingermann, and H. Kersten. 1989. The *tyrT* locus of *Escherichia coli* exhibits a regulatory function for glycine metabolism. *J. Bacteriol.* **171**:5987-5994.
- Nohono, T., S. Noji, S. Taniguchi, and S. Saito. 1989. The *narX* and *narL* genes encoding the nitrate-sensing regulators of *Escherichia coli* are homologous to a family of prokaryotic two-component regulatory genes. *Nucleic Acids Res.* **17**:2947-2957.
- Sodergren, E. J., and J. A. DeMoss. 1988. *narI* region of the *Escherichia coli* nitrate reductase (*nar*) operon contains two genes. *J. Bacteriol.* **170**:1721-1729.
- Sodergren, E. J., P.-Y. Hsu, and J. A. DeMoss. 1988. Roles of the *narJ* and *narI* gene products in the expression of nitrate reductase in *Escherichia coli*. *J. Biol. Chem.* **263**:16156-16162.
- Umeda, M., and E. Ohtsubo. 1989. Mapping of the insertion elements IS1, IS2, and IS3 on the *Escherichia coli* chromosome: role of the insertion elements in the formation of Hfrs and F' factors and in the arrangement of the bacterial chromosome. *J. Mol. Biol.* **208**:601-614.