JOURNAL OF BACTERIOLOGY, Apr. 1983, p. 72–75
0021-9193/83/040072-04$02.00/0
Copyright © 1983, American Society for Microbiology

Vol. 154, No. 1

Mapping of the Gene for Cytidine Deaminase (*cdd*) in *Escherichia coli* K-12

JYTTE JOSEPHSEN,† KARIN HAMMER-JESPERSEN,* AND TONNY D. HANSEN

Enzyme Division, University Institute of Biological Chemistry B, DK-1307 Copenhagen K, Denmark

Received 16 November 1982/Accepted 17 January 1983

The structural gene encoding cytidine deaminase (*cdd*) has been mapped in *Escherichia coli* K-12. It is located counterclockwise to *ptsF* between 46 and 47 min. The gene order in this region of the *E. coli* chromosome was found to be *his-udk-gat-dld-cdd-ptsF*.

The enzyme cytidine deaminase or (deoxy)cytidine aminohydrolase (EC 3.5.4.5) is encoded by the *cdd* gene. The enzyme converts cytidine (deoxycytidine) to uridine (deoxyuridine). It belongs to a group of enzymes and transport proteins which are involved in the catabolism of nucleosides and which are regulated by the repressor encoded by the *cytR* gene (8, 13). The other genes encoding enzymes in this group have previously been located precisely on the *Escherichia coli* chromosome (14, 15). There has been some disagreement about the precise location of the *cdd* gene in *E. coli* K-12 (6, 19). Since we have initiated a study of the regulation of the *cdd* gene (9), we found it relevant to map the gene. Earlier we found that the *cdd* gene is transcribed clockwise on the chromosome (9). In the present study we have used P1 transduction to determine the location of the *cdd* gene relative to the *his*, *gat*, *udk*, *dld*, and *ptsF* genes.

MATERIALS AND METHODS

The bacterial strains used in this study are all derivatives of *E. coli* K-12 (Table 1). The bacteria were grown in phosphate-buffered minimal medium (5) supplemented with a carbon source as indicated and with the nutrient requirements of the strains being tested. Solid medium contained 1.5% agar. Hfr crosses and P1 transductions were performed as described by Miller (12) and Rosner (18), respectively.

Selection and testing of genotypes were based on the following phenotypic traits: Cdd$^+$ strains can utilize 0.1% cytidine as the sole carbon source (7); Dld$^+$ strains can grow on 0.1% D-lactate as the carbon source; PtsF$^+$ can grow on 0.054% fructose (3 mM) as the sole carbon source (17); and Gat$^+$ strains can utilize 0.2% galactitol (dulcitol) as the sole carbon source at 30°C (11). Strains which contain a *udk* mutation are resistant to 5 μg of 5-fluorouridine per ml on glucose minimal medium containing uracil (10 μg/ml) (2).

RESULTS

The chromosomal location of the *cdd* gene encoding for cytidine deaminase was initially determined by conjugation with Hfr strain KL 16-21-23 as the donor. In the first cross, with S01519 as the recipient, *glpT*$^+$ recombinants were selected. The unselected markers were inherited with the following frequencies: *gyrA* (96%), *cdd* (53%), *udk* (37%), and *his* (29%), indicating the clockwise order *his-udk-cdd-gyrA*. In the second cross, with S0423 as the recipient, *his*$^+$ recombinants were selected. The unselected markers were inherited with the following frequencies: *gat*$^+$ (68%), *cdd* (47%), and *ptsF* (24%), indicating the clockwise order *his-gat-cdd-ptsF*.

To obtain a more precise location of the *cdd*, gene P1 transductions were performed. The cotransduction frequencies found between the markers spanning the *his-ptsF* region are collected in Table 2. The segregation of the unselected markers in some of the crosses are given in Table 3.

Cross 1 (Table 2) established the order *his-udk-gat*. Neither the *cdd* gene nor the *ptsF* gene cotransduces with the *his* gene.

In cross 2, *gat*$^+$ transductants were selected. Cotransduction with all the other markers was observed. The order *his-udk-gat* was also indicated here. Of the nine *his*$^+$ transductants (Table 3), none had received *cdd* or *ptsF*. Of the eight *cdd*$^+$ transductants, none had received *his*, showing that *his* and *cdd* are located on opposite sites of the *gat* gene. The two *ptsF* recombinants found had also inherited *cdd*$^+$, but not *udk*$^+$ or *his*$^+$, from the donor. This indicates the order *his-udk-gat-cdd-ptsF*.

Cross 3 (Table 2) gave the cotransduction frequency between *ptsF* and *cdd* of 26%. No *gat*, *udk*, or *his* recombinants were found. This is in accordance with the results from cross 2.

We also observed cotransduction between

---

† Present address: Department of Microbiology, The Technical University of Denmark, DK-2800 Lyngby-Copenhagen, Denmark.

TABLE 1. Strains used

Strain	Known genotype
S0423	F$^-$ *cdd-5 his metB udk upp gat* (7)
S01515	F$^-$ *metB rpsL his udk cdd gat*$^+$ *ptsF3 relA1* λ^{r}[a]
S01519	F$^-$ *metB rpsL his udk cdd gat upp glpT gyrA relA1 thi* λ^{r}[b]
C312	F$^-$ *lacZ his gyrA dld gat*[c]
KL16-23	Hfr *thi-1 relA1 ptsF5* λ^-[d]
KL16-21-23	Hfr *thi-1 relA1 ptsF3 ptsM4* λ^-(P045)[d]

[a] From S01519 by mating with KL16-21 (Hfr KL16 *ptsF3 ptsM4*) selected for *glpT*$^+$.

[b] From S0423 by mating with KK406 (HfrC *glpT nalA*) (Olle Karlström) selected for Nal^r.

[c] From Steven Short.

[d] From *E. coli* Genetic Stock Center through B. Bachmann.

dld, encoding D-lactate dehydrogenase, and *cdd* (cross 6). In crosses 4 and 5, the same donor and recipient strains were used. In cross 4, *dld*$^+$ transductants were selected, and these were five to seven times more frequent than the *gat*$^+$ transductants selected in cross 5. When *dld*$^+$ was selected, no inheritance of other markers was found. When *gat*$^+$ transductants were isolated, we found 7 to 8% cotransduction with *cdd* and *dld*$^+$.

The inheritance of nonselected markers from crosses 5 and 6 is given in Table 3. The results in cross 5 indicate the order *gat-dld-cdd-ptsF*. This is confirmed in cross 6, in which the order *udk-gat-dld-cdd-ptsF* was found.

The genetic map constructed from these data is shown in Fig. 1. The map distances can be estimated from the cotransduction frequencies by the formula of Wu (22). By assuming the position of *his* at 44 min (1), we calculated *udk* to be placed at 44.6 min, *gat* at 45.1 to 45.4 min, *dld* at 46.2 to 46.5 min, *cdd* at 46 to 47 min, and *ptsF* at 46.5 to 47 min.

DISCUSSION

For the first time it has been possible to span the cotransductional gap on the *E. coli* map between *his* and *ptsF* by P1 transduction. We find that the *cdd* gene cotransduces with *udk*, *gat*, *dld*, and *ptsF*, indicating a location between *ptsF* and *his* in agreement with the results of other laboratories (4, 6, 21). Our data indicate the following gene order in this region: *his-udk-gat-dld-cdd-ptsF*.

This gene order differs, however, from that obtained by Boos et al. (4), who postulated the gene order *his-cdd-fpk-ptsF-mglB-gatA*. They located *gat* relative to the insertion element zef-700::Tn*10*, which they mapped clockwise of *ptsF*. From their data it cannot be ruled out that zef-700::Tn*10* is located counterclockwise to *ptsF*, resulting in the gene order *his-gatA-mglB-cdd-ptsF-fpk*. This would also be in agreement with the results of Lengeler (10), who found that *gat* cotransduced 1 to 2% with *his*, suggesting the same position as the one we found. It would also locate the *mglB* gene between *ptsF* and *his*, in agreement with the results of Sunshine and Kelly (20), who found that chromosome deletions caused by P2-mediated eduction included *his* and ended near or in *mgl*. Neuhard and Thomassen (16) found that these eductants were *udk*. Furthermore, Fuchs and Karlstrøm (6) have shown that P2-mediated education does not include *cdd*.

From the cotransduction frequencies between markers in the *his-ptsF* region, we believe that the distance between *his* and *ptsF* is larger than depicted on the latest linkage map of *E. coli* (1). This would indicate that *ptsF* should cotransduce with *gyrA* and *glpT*, provided that the distance between *his* and *gyrA* is 4 min.

In the course of our studies, we have occasionally observed that from *cdd*$^+$ (*his*$^+$, *udk*$^+$) transductants *cdd* (*his*, *udk*) segregants could be obtained after several rounds of single-colony purification. This was also noticed for other

TABLE 2. P1 cotransduction frequencies

Cross	Donor	Recipient	Selected marker[a]	% Cotransduction with unselected markers
1	KL16-23	S0423	*his*$^+$ (200)	34% *udk*$^+$, 2% *gat*$^+$, 0% *cdd*$^+$, 0% *ptsF*
2	KL16-23	S0423	*gat*$^+$ (200)	45% *udk*$^+$, 5% *his*$^+$, 4% *cdd*$^+$, 1% *ptsF*
3	S0423	KL16-23	*ptsF*$^+$ (98)[b]	26% *cdd*, 0% *gat*, 0% *udk*, 0% *his*
4	S01515	C312	*dld*$^+$ (167)	0% *ptsF*, 0% *cdd*, 0% *gat*$^+$, 0% Nal^s
5	S01515	C312	*gat*$^+$ (176)	8% *dld*$^+$, 7% *cdd*, 1% *ptsF*
6	C312	S01515	*cdd*$^+$ (46)	26% *dld*, 22% *ptsF*$^+$, 15% *gat*, 4% *udk*$^+$

[a] Numbers in parentheses give numbers of transductants tested.

[b] A total of 196 *ptsF*$^+$ colonies were purified and tested, but since half of these were revertants, 98 were used to calculate the frequency of cotransduction. A reversion frequency of 5.0×10^{-6} was found for *ptsF5* when uninfected cells of KL16-23 were plated on fructose as the sole carbon source.

TABLE 3. Position of *cdd* with respect to *ptsF*, *gat*, *udk*, and *his* as determined by P1 transduction

Cross	Strains and relevant genotype		Selected marker	Genotypes of transductants				% of total[a]
	Donor	Recipient						
				ptsF	*cdd*	*gat*	*udk*	
1	KL16-23 *ptsF*	S0423 *cdd gat udk his*	*his*$^+$	+	−	−	−	67 (133)
				+	−	−	+[b]	32 (63)
				+	−	+[b]	+[b]	2 (4)
				ptsF	*cdd*	*udk*	*his*	
2	KL16-23 *ptsF*	S0423 *cdd gat udk his*	*gat*$^+$	+	−	−	−	51 (102)
				+	−	+[b]	−	41 (81)
				+	−	+[b]	+[b]	4 (8)
				+	−	−	+[b]	1 (1)
				−[b]	+[b]	−	−	1 (2)
				+	+[b]	−	−	2 (4)
				+	+[b]	+[b]	−	1 (2)
				ptsF	*cdd*	*dld*		
5	S01515 *cdd ptsF3*	C312 *dld gat*	*gat*$^+$	+	+	−		91 (161)
				+	+	+[b]		2 (3)
				+	−[b]	−		1 (1)
				+	−[b]	+[b]		5 (9)
				−[b]	−[b]	+[b]		1 (2)
				ptsF	*dld*	*gat*	*udk*	
6	C312 *dld gat*	S01515 *cdd ptsF udk*	*cdd*$^+$	−	+	+	−	59 (27)
				+[b]	+	+	−	15 (7)
				+[b]	−[b]	+	−	7 (3)
				−	−[b]	−[b]	−	11 (5)
				−	−[b]	+	−	4 (2)
				−	−[b]	−[b]	+[b]	4 (2)

[a] Numbers are shown in parentheses. Totals were 200 for crosses 1 and 2, 176 for cross 5, 46 for cross 6.
[b] Marker inherited from the donor.

transductants involving markers in this region (3, 4). These phenomena may be due to gene duplications, explaining why the region between *his* and *ptsF* has been so difficult to map by cotransduction (1).

The map position of *dld* is not completely

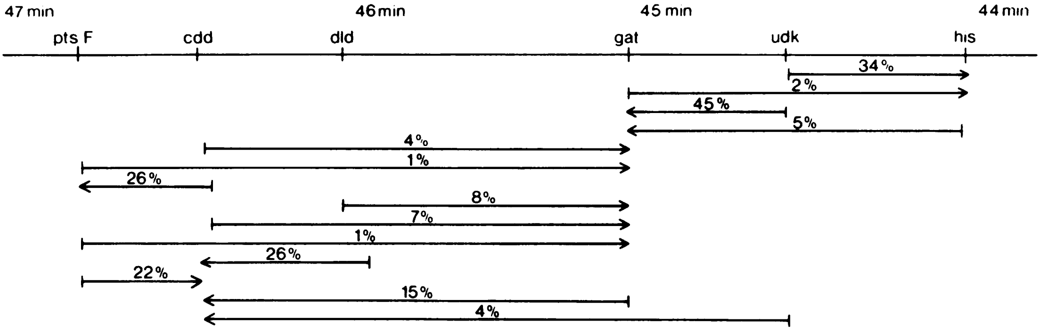

FIG. 1. Linkage of markers in the *his-ptsF* region. The numbers below the map indicate cotransductional frequencies, with the head of the arrow representing the selected marker. Distances are not drawn to scale.

resolved by the data in this publication. At present we cannot explain the lack of linkage of *dld* to other markers when Dld$^+$ recombinants were selected (Table 2, cross 4). They were, however, obtained five to seven times more frequently than the Gat$^+$ recombinants in the same experiment (Table 2, cross 5). The phenomenon might, therefore, be due to hot spots of recombination and may be related to chromosomal aberrations in this region of the chromosome.

ACKNOWLEDGMENTS

We thank Steven Short for suggesting the use of the *dld* marker and for supplying us with a *dld* strain. We are grateful to Jan Neuhard for critical reading of the manuscript.

This work was supported by a fellowship to J.J. from the Danish National Science Research Council and the Carlsberg Foundation.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Beck, C. F., J. L. Ingraham, J. Neuhard, and E. Thomassen.** 1972. Metabolism of pyrimidines and pyrimidine nucleosides by *Salmonella typhimurium*. J. Bacteriol. **110**:219–228.
3. **Blumenthal, T.** 1972. P1 transduction: formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. Virology **47**:76–93.
4. **Boos, W., I. Steinacher, and D. Engelhardt-Altendorf.** 1981. Mapping of *mglB*, the structural gene of the galactose-binding protein of *Escherichia coli*. Mol. Gen. Genet. **184**:508–518.
5. **Clark, D. J., and O. Maaløe.** 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. **23**:99–112.
6. **Fuchs, J. A., and H. O. Karlstrøm.** 1976. Mapping of *nrdA* and *nrdB* in *Escherichia coli* K-12. J. Bacteriol. **128**:810–814.
7. **Hammer-Jespersen, K., and A. Munch-Petersen.** 1973. Mutants of *Escherichia coli* unable to metabolize cytidine: isolation and characterization. Mol. Gen. Genet. **126**:177–186.
8. **Hammer-Jespersen, K., and A. Munch-Petersen.** 1975. Multiple regulation of nucleoside catabolizing enzymes: regulation of the *deo* operon by the *cytR* and *deoR* gene products. Mol. Gen. Genet. **137**:327–335.
9. **Josephsen, J., and K. Hammer-Jespersen.** 1981. Fusion of the *lac* genes to the promoter for the cytidine deaminase gene of *Escherichia coli* K-12. Mol. Gen. Genet. **182**:154–158.
10. **Lengeler, J.** 1975. Mutations affecting transport of the hexitols D-mannitol, D-glucitol, and galactitol in *Escherichia coli* K-12: isolation and mapping. J. Bacteriol. **124**:26–38.
11. **Lengeler, J.** 1977. Analysis of mutations affecting the dissimilation of galactitol (dulcitol) in *Escherichia coli* K-12. Mol. Gen. Genet. **152**:83–91.
12. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Munch-Petersen, A., and B. Mygind.** 1976. Nucleoside transport systems in *Escherichia coli* K-12: specificity and regulation. J. Cell. Physiol. **89**:551–559.
14. **Munch-Petersen, A., B. Mygind, A. Nicolaisen, and N. J. Pihl.** 1979. Nucleoside transport in cells and membranes vesicles from *Escherichia coli* K-12. J. Biol. Chem. **254**:3730–3737.
15. **Munch-Petersen, A., P. Nygaard, K. Hammer-Jespersen, and N. Fiil.** 1972. Mutants constitutive for nucleoside-catabolizing enzymes in *Escherichia coli* K-12. Eur. J. Biochem. **27**:208–215.
16. **Neuhard, J., and E. Thomassen.** 1976. Altered deoxyribonucleotide pools in P2 eductants of *Escherichia coli* K-12 due to deletion of the *dcd* gene. J. Bacteriol. **126**:999–1001.
17. **Reiner, A. M.** 1977. Xylitol and D-arabitol toxicities due to derepressed fructose, galactitol, and sorbitol phosphotransferases of *E. coli*. J. Bacteriol. **132**:166–173.
18. **Rosner, J. L.** 1972. Formation, induction and curing of bacteriophage P1 lysogene. Virology **48**:679–689.
19. **Schnier, J., and K. Isono.** 1979. The gene for ribosomal protein L25 (*rplY*) maps at 47.3 min near *nalA* in *Escherichia coli* K-12. Mol. Gen. Genet. **176**:313–318.
20. **Sunshine, M. G., and B. Kelly.** 1971. Extent of host deletions associated with bacteriophage P2-mediated eduction. J. Bacteriol. **108**:695–704.
21. **Tabor, H., E. W. Hafner, and C. W. Tabor.** 1980. Construction of an *Escherichia coli* strain unable to synthesize putrescine, spermidine, or cadaverine: characterization of two genes controlling lysine decarboxylase. J. Bacteriol. **144**:952–956.
22. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54**:405–410.