JOURNAL OF BACTERIOLOGY, Mar. 1984, p. 984–986
0021-9193/84/030984-03$02.00/0

Vol. 157, No. 3

Clustering of Genes for L-Fucose Dissimilation by *Escherichia coli*

T. CHAKRABARTI,† YU-MEI CHEN, AND E. C. C. LIN*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Received 14 November 1983/Accepted 22 December 1983

Aerobic and anaerobic L-fucose utilization by *Escherichia coli* involves an inducible trunk pathway mediated by a permease, an isomerase, a kinase, and an aldolase. Tn*5* insertion mutants of a parental strain expressing this pathway constitutively were used to map the positions of the structural genes by transduction. Results from this and previous studies show that all of the structural genes of the L-fucose trunk pathway map between *eno* and *argA* at minute 60.2 of the chromosome.

L-Fucose is metabolized through an inducible pathway in *Escherichia coli* by the sequential action of L-fucose permease (8), L-fucose isomerase (6), L-fuculose kinase (10), and L-fuculose 1-phosphate aldolase (5). The last enzyme catalyzes the formation of dihydroxyacetone phosphate and L-lactaldehyde. Aerobically, an NAD-linked dehydrogenase is induced to oxidize L-lactaldehyde to L-lactate (7, 18) which in turn induces a flavin-linked dehydrogenase (3). Anaerobically, an NADH-linked oxidoreductase is induced to reduce L-lactaldehyde to L-1,2-propanediol which is excreted into the medium apparently via a facilitator protein of the inner cell membrane (3, 7).

The *fuc* locus within which mutations can prevent the utilization of L-fucose, first reported in 1963 (4), is presently placed at minute 60.2 of the chromosome (2). Analysis of the genetic organization of the L-fucose system, however, has been hindered by the difficulty in distinguishing loss of enzyme activities caused by mutations that affect an activator protein (9, 13, 17) from those that prevent the formation of the true inducer from the substrate (in *Klebsiella pneumoniae* L-fuculose 1-phosphate serves as the effector, but not L-fucose and L-fuculose [14]). Attempts to isolate one-step mutants that become constitutive in the pathway have been unsuccessful (13; T. Chakrabarti, unpublished data). In this study, we exploited a suppressor mutant that produces the permease, the isomerase, the kinase, and the aldolase constitutively to find out whether all of the genes in this trunk pathway map in the same locus.

The suppressor mutant was derived by the following procedure. By selecting for aerobic growth on L-1,2-propanediol as the sole source of carbon and energy, a mutant with constitutive aerobic expression of the NAD-linked oxidoreductase was first isolated (19). Selection for a mutant with improved growth rate on L-1,2-propanediol resulted in enhanced level of the oxidoreductase. Unexpectedly, this mutation was pleiotropic: the aldolase became constitutive but the permease, the isomerase, and the kinase became noninducible (8, 9). Consequently, the cells lost the ability to grow on L-fucose (3). Spontaneous L-fucose-positive derivatives were isolated, and these pseudorevertants expressed the entire trunk pathway constitutively (8). One such suppressed mutant, strain ECL56 (Table 1), was used as the parental strain for the isolation of L-fucose-negative mutants by Tn*5* mutagenesis (15). Loss of catalytic proteins of the pathway should therefore result directly from disruption of their structural genes rather than indirectly because of induction failure.

Tn*5* mutagenesis (15) of 10 separate clones of strain ECL56 was carried out by infecting the cells with λ::Tn*5* at a multiplicity of 0.5. The infected cells were incubated for 30 min at 37°C in LB broth containing 20 mM sodium citrate. Appropriately diluted samples were spread on MacConkey agar containing L-fucose (10 mg/ml) and kanamycin (20 μg/ml). Pale colonies were purified on the same agar and tested for failure to grow on L-fucose (2 mg/ml) as the sole source of carbon and energy. Seven independent kanamycin-resistant mutants of strain ECL56 that also lost the ability to grow on L-fucose were thus isolated and identified. (All seven mutants retained constitutive propanediol oxidoreductase activity and the ability to grow on L-1,2-propanediol.) Five of the mutants simultaneously lost the activities of the permease, the isomerase, and the kinase; one lost the activities of both the isomerase and the kinase; and one lost only the aldolase activity. Table 2 shows the activity profiles of the parent (strain ECL56), one triply affected mutant (strain ECL429), and one singly affected mutant (strain ECL475).

Since the *fuc* locus is situated between *eno* (minute 59.6) and *argA* (minute 60.5), these two genes were used as reference points in transductional mapping. For this purpose, an *eno argA*::Tn*10* strain was selected on glycerol (2 mg/ml)–succinate (2 mg/ml) (11, 12) agar supplemented with arginine (20 μg/ml) and tetracycline (20 μg/ml) as a transductant of strain ECL248 exposed to a P1 lysate of strain ECL357. The transductant, strain ECL289, was tested for its *arg* marker on glycerol-succinate agar (*eno* strains will grow on these two carbon sources together but not on glucose) with or without the amino acid and for the *eno* marker by growth failure on glucose (2 mg/ml) agar supplemented with arginine. Strain ECL289 was used as the recipient in every transductional cross with the Tn*5* insertion mutants. Minimal agar with the following supplements was used for selection of the nutritional markers: glycerol and succinate for arg^+; glucose and arginine for eno^+; and glucose alone for both arg^+ and eno^+. The arg^+ transductants were tested for the unselected marker kan^r on kanamycin-glycerol-succinate agar and the unselected marker *fuc* on fucose-succinate agar. The eno^+ transductants and the arg^+ eno^+ double transductants were tested for the unselected marker kan^r on LB-kanamycin agar and the unselected marker *fuc* on MacConkey-fucose agar. All kanamycin-resistant transductants were unable to utilize L-fucose and vice versa. The results in Table 3 show that the Tn*5* insertion in strain ECL429 disrupting the synthesis of the permease, the isomerase, and

* Corresponding author.
† Present address: Biochemistry Division, National Chemical Laboratory, Pune 411 008 India.

TABLE 1. *E. coli* and bacteriophage strains

Strain	Derived from strain:	Genotype or phenotype	Source or reference
Bacteria			
ECL56	ECL3	HfrC *phoA8 relA1 tonA22* T_2^r (λ) (Fuc$^+$ and constitutive)	8
ECL248	K10	HfrC *eno relA1 ton22* T_2^r (λ)	D. Fraenkel
ECL289	ECL248	HfrC *eno argA*::Tn*10 relA1 tonA22* T_2^r (λ)	This study
ECL357	W3110(SY660)	F$^-$ *argA*::Tn*10 recB21 thyA36*	M. Syvanen (see also reference 1)
ECL429	ECL56	HfrC *fucP*::Tn*5 phoA8 relA1 tonA22* T_2^r (λ)	This study
ECL475	ECL56	HfrC *fucA*::Tn*5 phoA8 relA1 tonA22* T_2^r (λ)	This study
Bacteriophages			
P1(vir)			S. E. Luria
λ::Tn*5*		*c*I857 *b*221 *O*am80 cIII::Tn*5*	M. Syvanen

the kinase and the Tn*5* insertion in strain ECL475 disrupting the synthesis of only the aldolase both map between *argA* and *eno*. Like the *fuc* locus, the mutations mapped closer to *argA* than to *eno* (2).

Previous findings on mutational effects on regulatory pattern of the catalytic L-fucose proteins suggested that the permease, the isomerase, and the kinase belong to a single operon (8) but that the aldolase, L-1,2-propanediol oxidoreductase, and L-lactaldehyde dehydrogenase each belong to a separate operon (8, 9). The phenotypes of the Tn*5* insertion mutants analyzed in the present study are consistent with this view. Moreover, since insertion of the transposon has a strong polar effect, the isolation of mutants triply defective in the permease, the isomerase, and the kinase and doubly defective in the isomerase and the kinase suggests that the permease gene is proximal to the promoter.

Results from this and other studies also indicate that all of the known genes associated with L-fucose utilization are clustered in a single region. Specialized transduction with λ showed that mutations causing sensitivity to L-fucose (presumptive aldolase defect) or expanding the inducer specificity of the L-fucose system (alteration of the activator protein) are closely linked to *argA* (16, 17). Also localized in the *fuc* region are mutations that cause the constitutive synthesis of L-1,2-propanediol oxidoreductase (3), constitutive synthesis of L-lactaldehyde dehydrogenase (7), or loss of L-lactaldehyde dehydrogenase (Y.-M. Chen, unpublished data). Finally, three independent fusions of the *lac* genes to the promoter of the L-1,2-propanediol oxidoreductase gene are 50% linked to *argA* (Y.-M. Chen, E. C. C. Lin, J. Ros, and J. Aguilar, J. Gen. Microbiol, in press). Deletion mapping is in progress to order the seven known genes of the L-fucose system.

TABLE 2. Activities of L-fucose trunk pathway enzymes in various mutants[a]

Strain	Activity			
	Permease	Isomerase	Kinase	Aldolase
ECL56	12	500	10	100
ECL429	0.03	5	0.4	180
ECL475	11	450	9	3

[a] The cells were grown aerobically at 37°C to midexponential phase in mineral medium (20) with 0.5% casein acid hydrolysate as carbon and energy source. Preparation of cells and extracts, as well as the methods of assays, were as previously described (8) except for the following. L-Fucose permease activity was determined at 37°C by the initial rate of cellular uptake using L-[1-^{3}H]fucose at a concentration of 20 μM. Fuculose kinase activity was determined at 30°C from the rate of L-[1-^{3}H] fuculose 1-phosphate formation. A mixture (80 μl) containing labeled L-fucose (1 μmol, 0.25 μCi), partially purified fuculose isomerase (0.24 μmol/min with no detectable fuculose kinase and fuculose 1-phosphate aldolase activities), $MgCl_2$ (0.5 μmol), and Tris-hydrochloride (pH 7.8; 5 μmol) was preincubated for 15 min to allow adequate equilibration of the isomerization reaction. ATP (1 μmol) and cell extract (20 μl) were then added to initiate the reaction. After 15 min, 50 μl of the mixture was withdrawn and spotted on a Whatman DE81 filter paper disk (diameter, 2.5 cm) which was then dropped into 80% ethanol, washed with water, dried, and determined for its radioactivity by scintillation counting. Under the conditions used, the rate of fuculose 1-phosphate formation remained linear up to 20 min and the activity was proportional to the concentration of cellular protein.

TABLE 3. Transduction mapping of *fuc*::Tn*5* mutants[a]

Donor	Selected marker(s)	Unselected marker	% Linkage with *fuc*::Tn*5*
ECL429	arg$^+$	*fucP*::Tn*5*	57
	eno$^+$	*fucP*::Tn*5*	40
	arg$^+$ *eno*$^+$	*fucP*::Tn*5*	96
ECL475	*arg*$^+$	*fucA*::Tn*5*	50
	eno$^+$	*fucA*::Tn*5*	34
	arg$^+$ *eno*$^+$	*fucA*::Tn*5*	94

[a] A total of 96 clones were analyzed for each combination of selected and unselected markers.

We thank Sarah Monosson for editorial assistance.

This work was supported by grant PCM79-24046 from the National Science Foundation and Public Health Service grant 5-R01-GM11983 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12 (edition 7). Microbiol. Rev. **47**:180–230.
3. **Cocks, G. T., J. Aguilar, and E. C. C. Lin.** 1974. Evolution of L-1,2-propanediol catabolism in *Escherichia coli* by recruitment of enzymes for L-fucose and L-lactate metabolism. J. Bacteriol. **118**:83–88.
4. **Falkow, S., H. Schneider, L. S. Baron, and S. B. Formal.** 1963. Virulence of *Escherichia-Shigella* genetic hybrids for the guinea pig. J. Bacteriol. **86**:1251–1258.
5. **Ghalambor, M. A., and E. C. Heath.** 1962. The metabolism of L-fucose. II. The enzymatic cleavage of L-fuculose-1-phosphate. J. Biol. Chem. **237**:2427–2433.
6. **Green, M., and S. S. Cohen.** 1956. The enzymatic conversion of L-fucose to L-fuculose. J. Biol. Chem. **219**:557–568.
7. **Hacking, A. J., J. Aguilar, and E. C. C. Lin.** 1978. Evolution of

propanediol utilization in *Escherichia coli*: mutant with improved substrate-scavenging power. J. Bacteriol. **136**:522–530.
8. **Hacking, A. J., and E. C. C. Lin.** 1976. Disruption of the fucose pathway as a consequence of genetic adaptation to propanediol as a carbon source in *Escherichia coli*. J. Bacteriol. **126**:1166–1172.
9. **Hacking, A. J., and E. C. C. Lin.** 1977. Regulatory changes in the fucose system associated with the evolution of a catabolic pathway for propanediol in *Escherichia coli*. J. Bacteriol. **130**:832–838.
10. **Heath, E. C., and M. A. Ghalambor.** 1962. The metabolism of L-fucose. I. The purification and properties of L-fuculose kinase. J. Biol. Chem. **237**:2423–2426.
11. **Hillman, J. D., and D. G. Fraenkel.** 1975. Glyceraldehyde 3-phosphate dehydrogenase mutants of *Escherichia coli*. J. Bacteriol. **122**:1175–1179.
12. **Irani, M., and P. K. Maitra.** 1974. Isolation and characterization of *Escherichia coli* mutants defective in enzymes in glycolysis. Biochem. Biophys. Res. Commun. **56**:127–133.
13. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106**:90–96.
14. **St. Martin, E. J., and R. P. Mortlock.** 1976. Natural and altered induction of the L-fucose catabolic enzymes in *Klebsiella aerogenes*. J. Bacteriol. **127**:91–97.
15. **Shaw, K. J., and C. M. Berg.** 1979. *Escherichia coli* auxotrophs induced by insertion of the transposable element Tn*5*. Genetics **92**:742–747.
16. **Skjold, A. C., and D. H. Ezekiel.** 1982. Analysis of lambda insertions in the fucose utilization region of *Escherichia coli* K-12: use of λ*fuc* and λ*argA* transducing bacteriophages to partially order the fucose utilization genes. J. Bacteriol. **152**:120–125.
17. **Skjold, A. C., and D. H. Ezekiel.** 1982. Regulation of D-arabinose utilization in *Escherichia coli* K-12. J. Bacteriol. **152**:521–523.
18. **Sridhara, S., and T. T. Wu.** 1969. Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J. Biol. Chem. **244**:5233–5238.
19. **Sridhara, S., T. T. Wu, T. M. Chused, and E. C. C. Lin.** 1969. Ferrous-activated nicotinamide adenine dinucleotide-linked dehydrogenase from a mutant of *Escherichia coli* capable of growth on 1,2-propanediol. J. Bacteriol. **98**:87–95.
20. **Tanaka, S., S. A. Lerner, and E. C. C. Lin.** 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. **93**:642–648.