JOURNAL OF BACTERIOLOGY, May, 1966
Vol. 91, No. 5

Chromosomal Location of the Structural Gene for Glycerol Kinase in *Escherichia coli*

N. R. COZZARELLI AND E. C. C. LIN
Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts

Received for publication 11 January 1966

ABSTRACT

COZZARELLI, N. R. (Harvard Medical School, Boston, Mass.), AND E. C. C. LIN. Chromosomal location of the structural gene for glycerol kinase in *Escherichia coli*. J. Bacteriol. **91**:1763–1766. 1966.—A glycerol kinase mutant site has been mapped by transduction and sexual conjugation. Three-factor crosses with the two procedures yielded the following gene order: arginine-1–methionine-1–glycerol kinase–isoleucine, valine-16. An additional 13 independent glycerol kinase mutant sites mapped in the same region. Since some of the mutants were able to produce a protein serologically indistinguishable from the wild-type enzyme, it is concluded that the region mapped represents the structural gene for the kinase.

Studies on inducible catabolic pathways in *Escherichia coli* have centered on those which are coordinately controlled. We have been investigating a converging pathway responsible for the dissimilation of glycerol and L-α-glycerophosphate (L-α-GP) which is under the influence of a single regulator gene but is noncoordinately controlled (10). As can be seen in Fig. 1, the first step in the metabolism of glycerol requires a kinase, and the initial process of utilization of L-α-GP involves an active transport system. In both cases, intracellular L-α-GP is the product, and its further metabolism depends upon a dehydrogenase (4, 5).

In a preliminary communication, it was reported that the genes for the kinase, the transport system, and the dehydrogenase are not linked (Cozzarelli, Hayashi, and Lin, Federation Proc. **24**:417, 1965). In this paper, a more detailed analysis of the position of the structural gene for glycerol kinase is presented.

MATERIALS AND METHODS

Bacteria. All mutants lacking glycerol kinase were derived from *E. coli* K-12 Hfr C (2). Three of these mutants, strains 4, 9, and 61, have been described (4, 6, 10). The remaining mutants, isolated in this laboratory by J. P. Koch and G. J. Doyle and kindly made available to us for this study, were obtained by the following procedure. After treatment with the mutagen ethyl methanesulfonate (12), the cell suspension was diluted 20 times with glucose medium, grown out, and plated on MacConkey Agar in which glycerol was substituted for lactose. This agar contains 10 g of glycerol, 17 g of peptone (Difco), 3 g of Proteose Peptone (Difco), 1.5 g of Bile Salts No. 3 (Difco), 5 g of sodium chloride, 0.03 g of Neutral Red (Difco), 0.001 g of Crystal Violet (Difco), 13.5 g of agar (Difco), and water to a total volume of 1 liter. Pale colonies on these plates were streaked upon simple agar containing either glycerol or L-α-GP as the sole source of carbon and energy. Those which grew only on L-α-GP were collected and grown out on casein hydrolysate in the presence ot the inducer, L-α-GP. Extracts of these cells were then examined for the absence of glycerol kinase activity (11).

P678 is an F^- strain (7) from the Pasteur Institute collection. A revertant of this strain selected for growth on galactose was used for the present experiment. F′ AB1206 and F^- AB1450 (15) were gifts of E. A. Adelberg. Strain 161 is a glycerol kinase-negative recombinant obtained from a cross between strain 61 and AB1450.

Conditions of mating. The mating procedure was essentially that described by Adelberg and Burns (1). Parental cells were grown in rich broth to a density of approximately 5×10^8 cells per milliliter. A 4.5-ml amount of the female culture was mixed with 0.5 ml of the male culture in a 125-ml Erlenmeyer flask, and mating was allowed to proceed for 2 hr at 37 C without shaking. The mixture was diluted into rich broth and plated on selective agars. Colonies arising from recombinants were recloned and scored for unselected traits by means of replica plating.

Procedure for transduction. Phage P1kc, from the laboratory of S. E. Luria, was used as the vector of transduction according to the method of Luria, Adams, and Ting (13). Transductants were selected on the solid mineral media described by Adelberg and Burns (1), supplemented with a carbon source at a concentration of 0.2% and the appropriate amino acids and vitamins.

Immunochemistry. Specific antiserum was isolated from rabbits immunized against crystalline preparations of glycerol kinase from *E. coli*. The Ouchterlony

double-diffusion technique (9) was used to detect the presence of cross-reacting material in extracts of mutants with little or no glycerol kinase activity (6).

Genetic nomenclature. The following abbreviations for genetic markers are used. They are listed in the order of their injection by Hfr C: *lac* for lactose fermentation; *pro* for proline dependence; *thr-leu* for threonine and leucine dependence; *thi-2* for thiamine depencence; *arg-1* for arginine dependence; *met-1* for methionine dependence; *ilv-16* for isoleucine and valine dependence; *mtl* for mannitol fermentation; *str* for response to streptomycin; and *his* for histidine dependence. For an explanation of the allele numbers, see Pittard et al. (15).

Since glycerol kinase, the L-α-GP transport system, and the L-α-GP dehydrogenase share the same specific repressor gene and the inducer, L-α-GP, the abbreviation glp (for glycerophosphate) will be used to designate genes belonging to this regulon (14). Accordingly, *glp-K* denotes the gene for glycerol kinase.

RESULTS AND DISCUSSION

In view of the fact that the linear order of the genes in an Hfr strain can be determined from the gradient of their transmission frequency to an F^- strain (8), this technique was used to discover the general region containing the kinase locus. Hfr C (*lac*$^+$, *thr-leu*$^+$, *mtl*$^+$, *glp-K*$^+$, *str-s*) was mated with a *glp-K*$^-$ derivative of P678 (*lac*$^-$, *thr-leu*$^-$, *mtl*$^-$, *str-r*). Streptomycin-resistant recombinants for each of the donor markers were selected. The relative recombination frequencies placed the kinase gene between *thr-leu* and *mtl* (in the neighborhood of the *thi* locus).

More precise location of the kinase gene required an F^- strain with several markers in this region. AB1450 (*arg-1*$^-$, *met-1*$^-$, *glp-K*$^+$, *str-r*) was therefore chosen as the recipient in a cross with the Hfr, strain 61 (*arg-1*$^+$, *met-1*$^+$, *glp-K*$^-$, *str*-s). From this cross, *arg-1*$^+$, *str-r* and *met-1*$^+$, *str-r* recombinants were selected which were then scored for the unselected traits. The results are given in Fig. 2. For the *arg-1* recombinants, the least frequent class is *arg-1*$^+$, *met-1*$^-$, *glp-K*$^-$, and, for the *met-1* recombinants, it is *arg-1*$^-$, *met-1*$^+$, *glp-K*$^+$. Both of these findings are consistent with the order: *arg-1—met-1—glp-K*.

This genetic order was confirmed by three-point transduction tests. The recipient was *arg-1*$^-$, *met-1*$^-$, *glp-K*$^-$ (strain 161), and the donor was positive for all three. Transductants selected for a single marker, *arg-1*$^+$, *met-1*$^+$, or *glp-K*$^+$, were each scored for the other two (Fig. 3). The least frequent genotype among all the recombinants is *arg-1*$^+$, *met-1*$^-$, *glp-K*$^+$ as predicted by the order *arg-1—met-1—glp-K*. The map distances in the kinase region based on these transduction experiments are shown in Fig. 4. The orientation of *arg-1* and *met-1* relative to *thi-2* and *ilv-16* is drawn according to published results (3, 8, 15) which were confirmed for the strains used in this study.

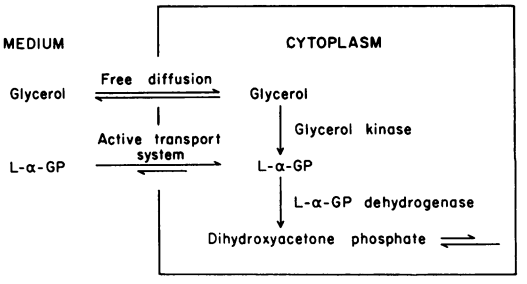

FIG. 1. *Metabolism of glycerol and* L-α-*glycerophosphate.*

Thirteen additional independent glycerol kinase-negative mutants of Hfr C were mated with AB1450. The recombinants for *met-1*$^+$, *str-r* or *arg-1*$^+$, *str-r* were scored for *glp-K*. In all cases, about 80% of the *met-1* recombinants and 65% of the *arg-1* recombinants acquired the kinase marker of the male parent. The similarity in recombination pattern suggests that all these glycerol kinase mutant sites are closely linked. This was tested by measuring the map distances between several of these loci. Hfr strains with genotype *glp-K*^-_x, *ilv-16*$^+$, *str-s* were mated with F^- strains with genotype *glp-K*^-_y, *ilv-16*$^-$, *str-r*. *Glp-K*$^+$, *ilv-16*$^+$, *str-r* recombinants and *ilv-16*$^+$, *str-r* recombinants were selected. The ratio of the two classes gives the crossover frequency between the two sites of mutation. The selection of the distal *ilv-16* marker as a control for variations in mating efficiency does not bias the incorporation of the *glp-K* gene of the male. The minimal crossover frequency detectable by this method was 10^{-6}. For the four crosses performed with six different kinase-negative mutants, including strain 161 used in the above three-factor analysis, the crossover frequency varied from 2×10^{-4} to 5×10^{-3}. Thus, we conclude that mutations affecting glycerol kinase occur in one small region of the chromosome.

If this region codes for the structure of glycerol kinase, then some of the *glp-K* mutants should produce an enzymatically inactive but serologically related protein. Cross-reacting material was sought in cell-free extracts of 10 mutants grown in the presence of the inducer. By use of the Ouchterlony double-diffusion technique, positive results were obtained with six of them. In each of these cases, a continuous band without spurs was formed in the agar between the central well charged with antiserum and peripheral wells inoculated alternately with extracts of mutant

Recombinants selected for	Genotype of recombinants					
	$\overline{+}\,\underline{-}\,\overline{-}$	$\underline{-}\,\overline{+}\,\underline{+}$	$\overline{+}\,\underline{-}\,\overline{+}$	$\underline{-}\,\overline{+}\,\underline{-}$	$\overline{+}\,\overline{+}\,\underline{+}$	$\overline{+}\,\overline{+}\,\underline{-}$
	Per cent distribution					
arg I^+	2	--	19	--	13	66
met I^+	--	3	--	8	12	77

FIG. 2. *Three-factor cross analysis of arg-1, met-1, and glp-K. Hfr C (arg-1^+, met-1^+, glp-K^-, str-s) was crossed with AB1450 (arg-1^-, met-1^-, glp-K^+, str-r). A total of 164 arg-1^+, str-r and 136 met-1^+, str-r recombinants were analyzed for the unselected markers. The "+" and "−" symbols in triplets refer to the arg-1, met-1, and glp-K genes, respectively. A bar above the symbol denotes that the gene is contributed by the donor and a bar below denotes that the gene is contributed by the recipient. The local crossovers between the donor and recipient required by the order arg-1—met-1—glp-K are indicated by dotted lines.*

Transductants selected for	Genotype of recombinants						
	$\underline{+}\,\overline{+}\,\underline{-}\,\overline{+}\,\underline{+}$	$\underline{-}\,\overline{+}\,\underline{-}\,\overline{+}\,\underline{+}$	$\underline{+}\,\overline{+}\,\underline{+}\,\overline{+}\,\underline{+}$	$\underline{-}\,\overline{+}\,\underline{+}\,\overline{+}\,\underline{+}$	$\underline{-}\,\overline{-}\,\underline{+}\,\overline{+}\,\underline{+}$	$\underline{+}\,\overline{+}\,\underline{-}\,\overline{-}\,\underline{-}$	$\underline{+}\,\overline{+}\,\underline{+}\,\overline{+}\,\underline{-}$
	Per cent distribution						
arg I^+	0	--	21	--	--	64	15
met I^+	--	19	19	41	--	--	22
glp K^+	0	--	19	48	33	--	--

FIG. 3. *Three-point transduction of arg-1, met-1, and glp-K. The donor was arg-1^+, met-1^+, glp-K^+, and the recipient was arg-1^-, met-1^-, glp-K^-. A total of 149 arg-1^+, 128 met-1^+, and 144 glp-K^+ transductants were analyzed for the unselected markers. The "+" and "−" symbols in triplets refer to the arg-1, met-1, and glp-K genes, respectively. A bar above the symbol denotes that the gene is contributed by the donor and a bar below denotes that the gene is contributed by the recipient. The crossovers between the donor and recipient required by the order arg-1—met-1—glp-K are indicated by dotted lines.*

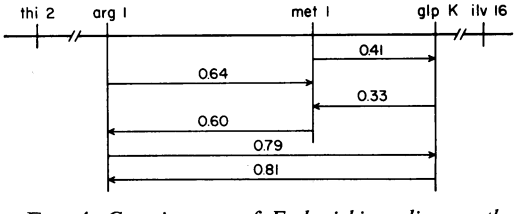

FIG. 4. *Genetic map of Escherichia coli near the structural gene for glycerol kinase as determined by transduction. The numbers are the crossover frequencies as decimal fractions between the selected marker (at the tail of the arrow) and the unselected marker (at the head of the arrow).*

and wild-type cells. The production of cross-reacting material has been quantitated for one of these mutants (strain 61), and the antigen titer has been found to be the same as for wild-type cells (6). These mutations which lower the enzymatic activity without lowering the amount of protein produced established the nature of the region mapped as a structural and not a regulator gene (16).

This conclusion is also consistent with the results of a dominance test. AB1206 (*pro*$^-$, *his*$^-$) was used as a donor strain because it harbors the F'_{14}, episome (*arg-*1^+, *met-*1^+, *ilv-16*$^+$) which was expected to contain the glycerol kinase structural gene. This donor strain was crossed with an F^- strain (*pro*$^+$, *arg-*1^-, *met-*1^-, *glp-*K^-, *ilv-16*$^-$, *his*$^-$), and conjugants were selected on agar containing glucose and histidine but not the other amino acids. Among 16 of the colonies examined, 15 were glycerol kinase-positive and were also genetic donors. After growth for a number of generations on rich broth, all but 2 of the 15 gave rise to glycerol kinase-negative segregants which had also lost the other three episomal markers. Therefore, conjugants heterozygous for *glp-K* were indeed formed, and in these merodiploids *glp-*K^+ is dominant to *glp-*K^-, as expected for a structural gene.

ACKNOWLEDGMENTS

We are grateful to J. P. Koch for his help and interest.

This investigation was supported by National Science Foundation grant GB-3527, by Public Health Service grant GM-11983, and by the American Cancer Society. N. R. Cozzarelli was supported by a predoctoral fellowship from the National Science Foun-

dation and E. C. C. Lin by a Research Career Development Award from the Public Health Service.

LITERATURE CITED

1. ADELBERG, E. A., AND S. N. BURNS. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321–330.
2. CAVALLI-SFORZA, L. L. 1950. La sessualità nei batteri. Boll. Ist. Sieroterap Milan. **29**:281–289.
3. GLANSDORFF, N. 1965. Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics **51**:167–179.
4. HAYASHI, S., J. P. KOCH, AND E. C. C. LIN. 1964. Active transport of L-α-glycerophosphate in *Escherichia coli*. J. Biol. Chem. **239**:3098–3105.
5. HAYASHI, S., AND E. C. C. LIN. 1965. Capture of glycerol by cells of *Escherichia coli*. Biochim. Biophys. Acta **94**:479–487.
6. HAYASHI, S., AND E. C. C. LIN. 1965. Product induction of glycerol kinase in *Escherichia coli*. J. Mol. Biol. **14**:515–521.
7. JACOB, F., AND E. L. WOLLMAN. 1956. Sur les processus de conjugaison et de recombinaison génétique chez *E. coli*. I. L'induction par conjugaison ou induction zygotique. Ann. Inst. Pasteur **91**:486–510.
8. JACOB, F., AND E. L. WOLLMAN, 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.
9. KABAT, E. A., AND M. M. MAYER. 1961. Experimental immunochemistry. Charles C Thomas, Publisher, Springfield, Ill.
10. KOCH, J. P., S. HAYASHI, AND E. C. C. LIN. 1964. The control of the dissimilation of glycerol and L-α-glycerophosphate in *Escherichia coli*. J. Biol. Chem. **239**:3106–3108.
11. LIN, E. C. C., J. P. KOCH, T. M. CHUSED, AND S. E. JORGENSEN. 1962. Utilization of L-α-glycerophosphate by *Escherichia coli* without hydrolysis. Proc. Natl. Acad. Sci. U.S. **48**: 2145–2150.
12. LIN, E. C. C., S. A. LERNER, AND S. E. JORGENSEN. 1962. A method for isolating constitutive mutants for carbohydrate-catabolizing enzymes. Biochim. Biophys. Acta **60**:422–424.
13. LURIA, S. E., J. N. ADAMS, AND R. C. TING. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
14. MAAS, W. K., AND A. J. CLARK. 1964. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. II. Dominance of repressibility in diploids. J. Mol. Biol. **8**:365–370.
15. PITTARD, J., J. S. LOUTIT, AND E. A. ADELBERG. 1963. Gene transfer by F′ strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. J. Bacteriol. **85**:1394–1401.
16. WILLSON, C., D. PERRIN, M. COHN, F. JACOB, AND J. MONOD. 1964. Non-inducible mutants of the regulator gene in the "lactose" system of *Escherichia coli*. J. Mol. Biol. **8**:582–592.