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Carbohydrate Accumulation and Metabolism in *Escherichia coli*: the Close Linkage and Chromosomal Location of *ctr* Mutations

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Six pleiotropic *ctr* mutations of *Escherichia coli*, affecting the ability to utilize 10 carbohydrates, were found to be closely linked to one another and to the mutation of strain MM6 causing lack of enzyme 1 of the phosphotransferase system. These mutations are located at 46 to 47 min on the *E. coli* map. Preliminary biochemical evidence indicates that the *ctr* mutants also lack enzyme 1, although they have a different phenotype from MM6.

Pleiotropic mutations affecting the utilization of at least 10 diffeerent carbohydrates by *Escherichia coli* were described in earlier communications (13; R. J. Wang and M. L. Morse, Bacteriol. Proc., p. 37, 1966; p. 105, 1967). Cells with these mutations were unable to grow on glucose, fructose, mannose, mannitol, galactose, maltose, lactose, melibiose, glycerol, and succinate. They had no defects in the glycolytic or pentose shunt pathways and they grew on glucose-6-phosphate. The mutants were found to be incapable of accumulating glucose, fructose, lactose, galactose, mannitol, glycerol, and maltose, even after prior exposure to these compounds during growth.

It is our purpose here to report the chromosomal location of these mutations and their close genetic linkage to one another. Some phenotypically similar mutants described by others are also considered in relation to the mutants described here.

MATERIALS AND METHODS

Cultures. The cultures employed are described in Table 1. All were derivatives of *E. coli* K-12.

Phage. The phage used in transduction experiments was P1(v), a virulent mutant of phage P1 received from A. L. Taylor.

Media. The media used were described previously (13). Calcium chloride was added to LB medium for transductions at a concentration of 2×10^{-3} M; adenosine was used (50 mg/liter) for growth of purine auxotrophs.

Bacterial matings. The procedure of DeHaan and Gross (3) was employed. The ratio of Hfr to F^- was usually 8×10^7 per ml to 4×10^8 per ml. Matings were interrupted by shaking the dilution tube vigorously on a Turbo-mixer (Technilab Instruments, Los Angeles, Calif.) for 1 min. Streptomycin, when used as a counterselective agent, was added in the soft-agar pour at a concentration of 200 μg/ml.

Transduction. Phage P1 stocks were prepared according to the method of Adams (1). Transductions were performed by the technique of Rothman (8). The selection medium was Davis minimal medium with appropriate supplements.

RESULTS

Approximately 80 phenotypically *ctr* mutants have been isolated for one purpose or another after mutagen exposure (ultraviolet light and nitrosoguanidine) and platings on indicator agar containing glucose. The primary selection has been for inability to utilize glucose, and subsequent testing has determined utilization or growth on other carbohydrates. Among mutants selected in this way, only 1 of about 80 has been affected only for glucose utilization. The remainder have the Ctr phenotype. The *ctr* mutation is a frequent occurrence in *E. coli* after such mutagen exposures. As a cautionary note, it should be mentioned that many *ctr* mutants are, however, unstable; qualitatively speaking, they return to the wild type or other partial wild types at a rate

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TABLE 1. *Stock cultures employed*[a]

Strain[b]	Genotype	*ctr* mutation
903C	F+ *his pro* λ^s	1
2092C	F− *his purF pheA argH thi lac malA mtl xyl tsx str* λ^s *mu*+	2
2570C	Hfr (O-*lysA-pheA-purF-glyA-his*) *thi*	3
911IVA	F− *ilv his str* λ^s	4
AB2547	Hfr (0–12, *str-mtl-met*) *ilv188 arg-1 xyl-4 pur-1 supN23*	
909A	F− *his pro* λ^s *str*	1
PA3306C	F− *thi arg-8 purC nicB str gal*	6
AT2570	Hfr (O-*lysA-pheA-purF-glyA-his*) thi	Wild type
MM6	Hfr (lacks enzyme I)	Wild type
MM6SF	F− *str* (derivative of MM6)	
1101	Hfr *thi* (deficient in HPr)	Wild type
2547A	(derivative of AB2547)	5

[a] Abbreviations used (text and tables): *his*, histidine; *str*, streptomycin; *purF*, purine; *pheA*, phenylalanine; *cysC*, cysteine; *glyA*, glycine; *nicB*, nicotinic acid; *supN*, suppressor; *ilv*, isoleucine + valine; *lysA*, lysine; *mtl*, mannitol; *lac*, lactose; *gal*, galactose; *suc*, succinate; *glp*, glycerol; *pro*, proline; *argH*, arginine; *thi*, thiamine; *malA*, maltose; *xyl*, xylose; *tsx*, phage T6; *mu*, phage mu; *met*, methionine; *ade*, adenine; *rha*, rhamnose; *rbs*, ribose; *fuc*, fucose; *gua*, guanine; *man*, mannose; *ara*, arabinose; *s*, sensitive; *R*, regulator gene.

[b] We thank E. A. Adelberg, R. Lavalle, C. F. Fox, and A. L. Taylor for the gift of bacterial strains.

which complicates study. In the studies reported here, and subsequently, control assays were always made on cultures to insure that the cells in the cultures studied were *ctr*.

For the purpose of studies to be reported later, six independent *ctr* mutants have been selected for special study. The data reported here show that the mutations of these cultures have a closely linked chromosomal site, on the basis of time of entry in bacterial matings or on the basis of close linkage in transductional analysis.

Location of the ctr mutations by bacterial mating. In preliminary crosses to locate the site of the *ctr* mutations, *ctr-1* was located approximately 8 min from *his* (39 min) toward *str*. By use of this information, the *ctr-2* mutation was induced in the F− strain 2092C, which has the mutations *purF* (44 min) and *pheA* (50 min). Bacterial matings with this mutant, selecting for the wild-type alleles of *pheA*, *ctr-2* (on glucose), and *purF* are shown in Fig. 1. The wild-type allele of *ctr-2* enters approximately halfway between *pheA* and *purF* or at approximately 46 to 47 min, which is in good agreement with the map position of *ctr-1*. The *E. coli* map of Taylor and Trotter (12) is shown in abbreviated form in Fig. 2. On this map are shown the genes of *E. coli* for carbohydrate metabolism, including those that are affected by the *ctr* mutation. In addition, this map shows the genes *cysC* (53 min), *pheA* (50 min), *glyA* (49 min), *nicB* (46 min), and *supN* (45 min), which appear to be in the region adjacent to the preliminary location of the first two *ctr* mutations.

Location of ctr mutations by transduction. Transductional analysis of *ctr-1* and *ctr-2* was made by using phage P1 to test for linkage of these mutations to any of the adjacent mutations. Linkage of *ctr-1* or *ctr-2* to *pheA*, *purF*, *cysC*, or *glyA* was not detected (Table 2). This result might have been expected, if the preliminary location of *ctr-1* and *ctr-2* is correct, as Taylor and Trotter (12) reported cotransduction frequencies by P1 of less than 0.3% with gene pairs separated by 2 min.

To define further the location of the *ctr* mutations, the *ctr-4* mutation was induced in a strain (911IVA) requiring isoleucine and valine (*ilv*,

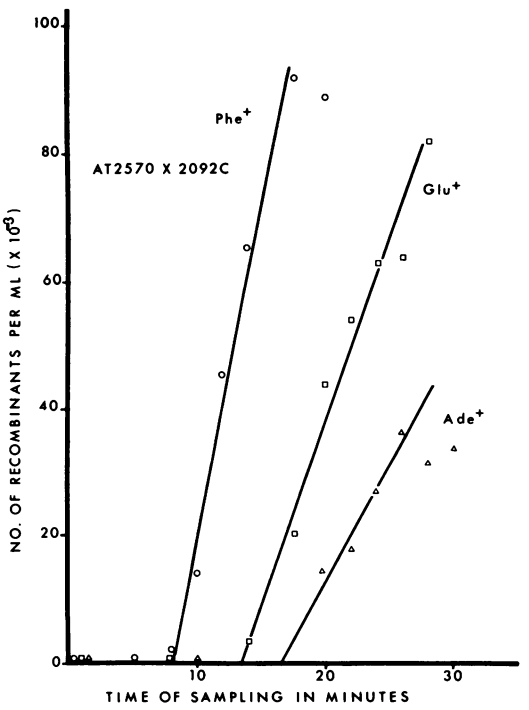

FIG. 1. *Kinetics of chromosome transfer by donor strain AT2570 to recipient 2092C. Strain AT2570 is phe+ ctr+ ade+, and 2092C is pheA ctr purF.*

74 min) but whose requirement for amino acids is suppressible by *supN* (4). As the data in Table 2 indicate, the wild-type allele of *ctr-4* is cotransducible with *supN* at 1.9%, which places *ctr-4* about 1.5 min from *supN*, or at about 46.5 min. In a similar experiment, selection for *supN* again was used, but testing was for the cotransduction of *ctr-5* with *supN*. This experiment was unsuccessful because the presence of the *ctr* mutation and the suppressor affected the growth characteristics of P1, its plaque morphology, and possibly its usefulness in transduction analysis.

The data thus far place *ctr-1*, *ctr-2*, and *ctr-4* at about 46 to 47 min on the *E. coli* map, very close to the position given for *nicB* (46 min). In a direct test of the site of the *ctr* mutations in relation to *nicB*, an *nicB* strain with a *ctr* mutation (*ctr-6*) was used. P1 grown on a wild-type strain was the donor. Whereas about 50% cotransduction was expected, none of 97 transductants examined received the wild-type allele of *ctr-6*. Since it will be shown in a later section that *ctr-6* is linked to the other *ctr* mutations, we conclude that the position of *nicB* is not correctly given.

Linkage of ctr-1, ctr-2, and ctr-3 in bacterial crosses. To test for the linkage of the first three *ctr* mutants, recombination was measured between *ctr-3*, which is in an Hfr strain, and *ctr-2* in an F⁻ *purF* (44 min) strain, between *ctr-3* and *ctr-1* in an F⁻ *his* (39 min) strain. Selection in the *ctr-3* × *ctr-2* cross was for adenine independence, and in the *ctr-3* × *ctr-1* cross was for histidine independence. In each case, the selected recombinants were examined for ability to utilize glucose. As the data in Table 3 indicate, there was a small amount of recombination between *ctr-3* and *ctr-2*, and between *ctr-3* and *ctr-1*, which positions these three mutations closely linked at 46 to 47 min on the *E. coli* map.

Close linkage of ctr-3-ctr-6 by P1 transduction. To place the first six *ctr* mutations into a cluster at about 46.5 min on the *E. coli* genetic map, recombination between *ctr-3* and the *ctr-4*, *ctr-5* and *ctr-6* mutations was measured by P1 transduction. Phage grown on a wild-type strain was used as a reference, and selection for a nutritional independence was also made as an internal measure of transduction efficiency. Selection for recombination between *ctr* mutants was on minimal mannitol medium (with necessary supplements) except in one case when glucose was used. The unadjusted data are presented in Table 4. Although there was considerable variation in transduction efficiency from recipient to recipient, the data clearly indicate that the yield of wild-type between the three *ctr* mutants and *ctr-3* is small and that the four mutations are all closely linked. Coupled with the bacterial cross data and the cotransduction with *supN*, the six mutations form a cluster at about 46.5 min.

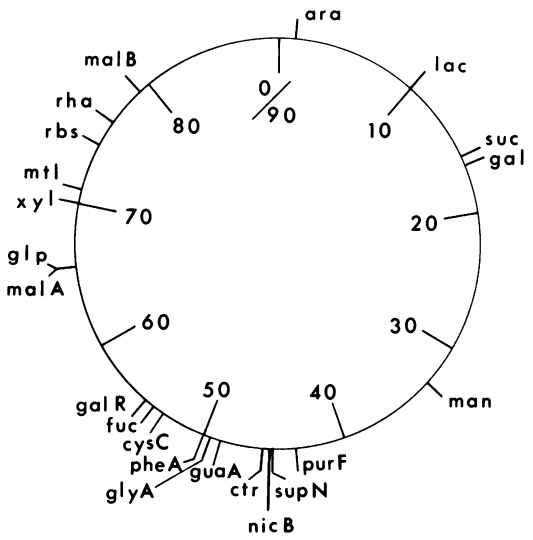

FIG. 2. *Linkage map of E. coli chromosome, redrawn from Taylor and Trotter (12).*

TABLE 2. *Cotransduction test of the ctr mutations*

Donor	Recipient	Selection	Transductants	Cotransductants
903C *ctr-1*	W2664 *cysC*	Cysteine-independent	107	0
911 (wild type)	2092C *pheA ctr-2*	Phenylalanine-independent	112	0
911	2092C *purF ctr-2*	Adenine-independent	96	0
903C *ctr-1*	AT2457 *glyA*	Glycine-independent	241	0
2547A *ctr-5*	911IVA *ilv*	Isoleucine-valine-independent	546	0
AB2547 *supN*	911IVA *ilv ctr-4*	Isoleucine-valine independent	1,454	28 (1.9%)
AB312 (wild type)	PA3306 *nicB ctr-6*	Nicotinic acid-independent	97	0

TABLE 3. *Linkage between ctr-1, ctr-2, ctr-3 in bacterial crosses*

Hfr	F⁻	Selection	No. of selected recombinants	*glu*⁺[a]
2570C *ctr3*	2092C *purF ctr-2*	Adenine-independent	216	4
2570C *ctr3*	909A *his ctr-1*	Histidine-independent	144	3

[a] Recombination between the *ctr* mutations was measured by selecting for a (nearby) gene and testing the selected recombinants for the ability to use glucose.

TABLE 4. *Linkage of ctr-3 to ctr-4, ctr-5, and ctr-6 as detected by P1 transduction*

Recipient	Donors					
	2570C *ctr-3*			2570		
	Growth on mannitol	Selection	No. of transductants[a]	Growth on mannitol	Selection	No. of transductants
911IVA *ctr4*	0	Histidine-independent	270	3,000	Histidine-independent	1,800
2547A *ctr5*	2	Arginine-independent	572	153	Arginine-independent	618
PA3306A *ctr6*	0[b]	Arginine-independent	183	95	Arginine-independent	147

[a] Number of transductants per 10^8 recipient cells. Multiplicity of infection, 5 phage per bacterium. The lysates applied to 911 *ctr-4* were different from those applied to *ctr-5* and *ctr-6*.

[b] Since PA3306A is unable to grow on mannitol because of a second mutation, selection for *ctr* recombinants in this experiment was made on minimal medium containing glucose.

Location of the mutant site of MM6. MM6, a mutant of *E. coli* unable to grow on mannitol, fructose, and mannose, has been found to lack the enzyme (enzyme I of the phosphotransferase system) that transfers phosphate from phosphoenolpyruvate (PEP) to a heat-stable protein (HPr) that is used in subsequent carbohydrate phosphorylations (7, 11). The approximate chromosomal site of the mutation in MM6 was sought by time of entry in bacterial crosses. Since MM6 is an Hfr strain, an F⁻ streptomycin-resistant derivative was selected from it for use in crosses. Crosses were made between this strain and AT2570 (O-*lysA*, 55 min), and selection for the wild-type allele of the MM6 mutation was made on minimal mannitol-streptomycin-agar. The data from two crosses are given in Fig. 3, which indicates the site of the MM6 mutation at about 8 to 9 min from the origin (*lysA*, 55 min) of AT2570, or at about 46 to 47 min on the *E. coli* genetic map.

Linkage of ctr-1 and ctr-5 to MM6 by P1 transduction. Transductions were made between MM6 as donor to *ctr-1* and *ctr-5* as recipients, selecting for wild-type recombinants on minimal mannitol medium. The data (Table 5) show clearly that the yield of wild-type recombinants between MM6 and the *ctr* mutants is small (<10%) when compared with the formation of *mtl*⁺ from wild-type. From this, we conclude that

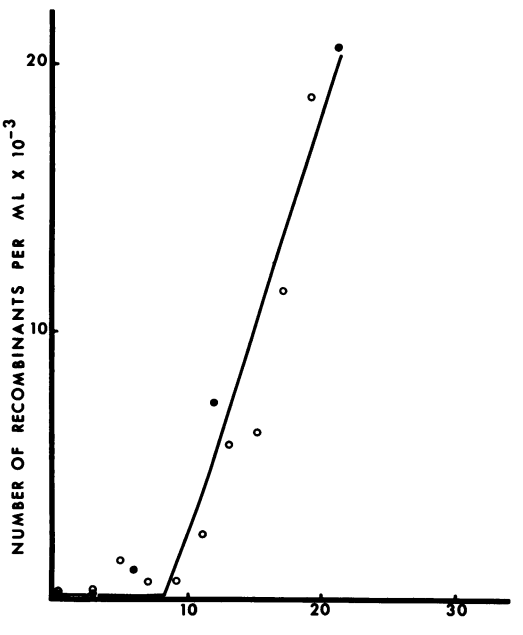

FIG. 3. *Kinetics of chromosome transfer by donor strain AT2570 to an F⁻ str derivative of MM6, a culture lacking enzyme I of the phosphotransferase system. The origin of AT2570 is at lysA (55 min), and the data indicate the site of the enzyme I gene at 46 to 47 min.*

TABLE 5. *Linkage of ctr-1 and ctr-5 to the mutation in mutant MM6 causing enzyme I deficiency*

Recipient	Transduction donors					
	Wild type			MM6		
	Growth on mannitol	Selection	No. of transductants[a]	Growth on mannitol	Selection	No. of transductants
ctr-1	1,570[a]	Histidine-independent	700	80	Histidine-independent	480
ctr-5	5,095	Arginine-independent	8,855	5	Arginine-independent	2,870

[a] Number of transductants per 10^8 recipient cells. Multiplicity of infection, 5 phage per bacterium. The lysates applied to *ctr-1* were different from those applied to *ctr-5*.

the site of mutation affecting mannitol utilization in MM6 is closely linked to the *ctr* mutations.

Preliminary biochemical characterization of a ctr mutant. β-Glucosides are phosphorylated by the phosphotransferase system (7, 10, and see below) and the phosphorylated β-glucosides are cleaved by a phosphoglucosidase (5). A test of whether the *ctr* mutations affected the phosphotransferase system was made by inducing a *ctr* mutation in a strain which contains phosphoglucosidase and the enzyme II for β-glucosides. The *ctr* mutation caused the loss of ability to utilize salicin as well as to hydroylze *o*-nitrophenyl-β-D-glucoside. The site of the *ctr* mutation's effect was sought further by employing extracts of known mutants. With this technique (the synthesis of *o*-nitrophenyl-β-D-glucoside phosphate, its hydrolysis to yield *o*-nitrophenol by the β-glucosidase), it was possible to show the following: (i) an extract of strain 1101, a mutant deficient in HPr, contained a factor which permitted *o*-nitrophenol formation by extracts of the *ctr* mutant; (ii) an extract of strain MM6, which lacks enzyme I of the phosphotransferase system (11), does not restore the formation of *o*-nitrophenol by the *ctr* extract. From these results, we conclude that the *ctr* mutant is lacking enzyme I of the phosphotransferase system.

DISCUSSION

Six *ctr* mutations have been found to be closely linked and located at 46 to 47 min on the *E. coli* chromosomal map. In addition, the mutant MM6, which is lacking enzyme I of the PEP-dependent phosphotransferase system (7), has been located at the same place and found to be closely linked to the *ctr* mutants. In preliminary biochemical experiments, a *ctr* mutant was found not to complement MM6 in the synthesis of *o*-nitrophenyl-β-D-glucoside-6-phosphate, and it can be concluded that the *ctr* mutants also lack enzyme I.

Recently, Bourd et al. (2) reported for the mutant P-34 which they have been studying, and which resembles the *ctr* mutants, a genetic site of 48 min. This is very close to the genetic site of the *ctr* mutation and, within experimental error, is probably identical to it. It appears quite likely that the two laboratories are dealing with the same mutations, although a direct test must be performed. In addition, Bourd et al. reported that the P-34 mutant is lacking enzyme I and the thermostable protein, HPr, of the phosphotransferase system. This makes the P-34 mutant a double mutant, and compatible with this finding is their earlier observation (6) that P-34 does not revert, even after mutagen treatment.

The PEP-dependent phosphotransferase system phosphorylates many carbohydrates in *E. coli* according to the following scheme (7):

$$\text{PEP} + \text{HPr} \underset{\text{I}}{\overset{\text{enzyme}}{\rightleftharpoons}} \text{HPr} - \text{P} + \text{pyruvate}$$

$$\text{HPr} - \text{P} + \text{CHO} \underset{\text{II}}{\overset{\text{enzyme}}{\rightleftharpoons}} \text{CHO} - \text{P} + \text{HPr}$$

In this scheme, HPr is a histidine-containing heat-stable protein which is phosphorylated by enzyme I. The phosphate is transferred to a variety of carbohydrates by carbohydrate-specific enzymes of type II, which are membrane-bound.

The phenotype of *ctr* mutations includes changes in ability to utilize lactose, galactose, succinate, and glycerol. No connection between the utilization of these carbohydrates and the phosphotransferase system has been established. Indeed, the uptake of galactosides by *E. coli* has been shown to be dependent upon the hydrolysis of adenosine triphosphate (9). We have indicated earlier (13) that the *ctr* mutants have a complex reversion behavior, reverting for only portions of the phenotype, with some of the reversions not occurring at the *ctr* site. The loss of enzyme I cannot explain the *ctr* phenotype and its reversion behavior in a simple way. The *ctr* mutations appear to affect a more fundamental step in cell

metabolism than the phosphorylation of carbohydrates.

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LITERATURE CITED

1. Adams, M. H. 1950. Methods of study of bacterial viruses. Methods Med. Res. 2:1–73.
2. Bourd, I. V., I. Andreeva, V. P. Shabolenko, and V. N. Gershanovitch. 1968. The absence of the phosphotransferase system in the mutant of *E. coli* K-12 with damaged system of the carbohydrate transport. Mol. Biol. 2:89–94.
3. DeHaan, P. G., and J. D. Gross. 1962. Transfer delay and chromosome withdrawal during conjugation in *Escherichia coli*. Genet. Res. 3:251–272.
4. Eggertsson, G., and E. A. Adelberg. 1965. Map positions and specificities of suppressor mutation in *Escherichia coli* K-12. Genetics 52:319–340.
5. Fox, C. F., and G. Wilson. 1968. The role of a phosphoenolpyruvate-dependent kinase system in β-glucoside catabolism in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. 59:988–995.
6. Gershanovitch, V. N., G. I. Bourd, N. V. Jurovitzkaya, A. G. Skavronskaya, V. V. Klyutchova, and V. P. Shabolenko. 1967. *Escherichia coli* mutant K-12 with impaired systems of glucose utilization. Mol. Biol. 1:88–96.
7. Kundig, W., S. Ghosh, and S. Roseman. 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phosphotransferase system. Proc. Natl. Acad. Sci. U.S. 52:1067–1071.
8. Rothman, J. L. 1965. Transduction studies on the relation between prophage and host chromosome. J. Mol. Biol. 12: 892–912.
9. Scarborough, G. A., M. K. Rumley, and E. P. Kennedy. 1968. The function of adenosine-5′ triphosphate in the lactose transport system in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. 60: 951–958.
10. Schaefler, S., and W. K. Maas. 1967. Inducible system for the utilization of β-glucosides in *Escherichia coli*. II. Description of mutant types and genetic analysis. J. Bacteriol. 93:264–272.
11. Tanaka, S., D. G. Fraenkel, and E. C. C. Lin. 1967. The enzymatic lesion of MM6, a pleiotropic carbohydrate mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. 27:63–67.
12. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
13. Wang, R. J., and M. L. Morse. 1968. Carbohydrate accumulation and metabolism in *Escherichia coli*. I. Description of pleiotropic mutants. J. Mol. Biol. 32:59–66.