JOURNAL OF BACTERIOLOGY, July 1975, p. 69-74

Vol. 123, No. 1
Printed in U.S.A.

Regulation of the *Escherichia coli* Methylgalactoside Transport System by Gene *mglD*

APRIL R. ROBBINS[1]

Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

Received for publication 27 March 1975

Constitutive activity of the methylgalactoside transport system of *Escherichia coli* K-12 is shown to result from mutation of a genetic locus distinct from the two previously described regulatory loci for this permease. Employing an autoradiographic procedure whereby constitutive and inducible cells can be differentiated, it is demonstrated that this locus, termed *mglD*, is 20% cotransducible with *ptsF* by bacteriophage P1. Selection for constitutive mutants among an inducible population yielded cells whose mutations mapped in *mglD*. Cotransduction of *mglD* with *mglB*, *-C*, and *-A*, three genes required for activity of the methylgalactoside transport system, is 95, 88, and 81%, respectively. The results of recombination studies employing three and four factors indicate that the order of genes in this region is *ptsF*, *mglD,B,C,A*.

Transport via the methylgalactoside permease (MeGalP) of *Escherichia coli* K-12 has been shown to require three genes, *mglA*, *-B*, and *-C*, located at approximately 40 min on the chromosome (7, 8). Two regulatory loci have been described that affect MeGalP activity: the RMG (3), now called *mglR* (12), 100% cotransducible with mutations of *galK* at 17 min, and a second *mglR* (4), located between 56 min and 74 min.

In this report we demonstrate the existence of a third regulatory locus for the MeGalP which is closely linked to *mglA*, *-B*, and *-C*. I used this regulator gene, termed *mglD*, to determine the order of genes in the *mgl* region.

MATERIALS AND METHODS

Organisms. The bacterial strains used in this study are listed in Table 1. All are derived from *E. coli* K-12.

Media and growth conditions. Cells were cultured at 37 C. Two complete media were used: for experiments involving bacteriophage, L broth (9); for all other purposes, antibiotic medium 3 (Difco). The minimal medium used was DM (10). Amino acids, where required, were supplied at a final concentration of 10 μg/ml. Media were solidified with 0.9% (wt/vol) Oxoid agar agar no. 3. Liquid cultures were grown in tubes or flasks; the former were aerated in a tube roller and the latter in a rotatory shaker. For induction of the MeGalP, cells were grown overnight in the presence of 1 mM D-fucose.

Chemicals. Methyl-β-D-[1-^{14}C]galactopyranoside (4.7 mCi/mmol) was purchased from New England Nuclear Corp.; this carbohydrate was purified by paper chromatography (9). D-[6-^{3}H]fucose (200 mCi/mmol) was purchased from CalAtomic. Methyl-β-D-galactopyranoside (MeGal) and isopropyl-1-thio-β-D-galactopyranoside were purchased from Nortok Associates. D-Fucose was purchased from Schwarz/Mann.

Permease assays. Intracellular accumulation of both [1-^{14}C]MeGal and D-[6-^{3}H]fucose was measured using cells grown in DM sodium lactate (0.4%) medium to stationary phase (4×10^8 to 6×10^8 cells per ml). Cells grown in the presence of inducer were washed two times with inducer-free DM medium before testing. The assay mixtures (3 ml final volume) contained: for MeGal accumulation, 1×10^8 cells in DM-lactate (0.4%) medium, 100 μg of chloramphenicol, and 0.46 μM [1-^{14}C]MeGal (10^4 counts/min); for D-fucose accumulation, 1.6×10^9 cells in DM-lactate (0.4%), 100 μg of chloramphenicol, and 10 μM D-[6-^{3}H]fucose (10^4 counts/min). The assay mixtures were incubated with rotation for 15 min at room temperature (22 to 24 C) and then filtered through HA membrane filters (Millipore Corp.). The membranes were dried, and their radioactivity was determined by liquid scintillation (efficiency for ^{14}C, 68%; for ^{3}H, 23%).

Isolation of ptsF exconjugants. Equal volumes of the Hfr (KL16-21) and F$^-$ (S181) strains, grown to exponential phase in broth, were mixed to give a final concentration of 2.5×10^7 and 1×10^8 cells per ml, respectively. The mating mixtures were incubated, with slow rotation, for 30 min at 37 C, agitated on a Vortex mixer for 45 s, diluted, and plated on DM-mannitol (0.2%) agar containing streptomycin (20 μg/ml) to select *ptsI*$^+$ exconjugants. Colonies on these plates were replicated onto DM-fructose (0.2%) agar, with streptomycin, to identify *ptsF* recombinants among the *ptsI*$^+$ cells. Exconjugants with the desired phenotype were purified twice and then tested for relevant markers prior to use.

[1] Present address: Cancer Research Center, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

TABLE 1. *Bacterial strains*

Strain	Relevant genotype[a]	Reference or derivation
KL16-21	Hfr, *ptsF*	2, 7[b]
S181	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD*	9
S201, 202 . . . 220	*ptsF, leu, str, lac*$(Z,Y,A)_{del}$	KL16-21 × S181
S251, 252, 253, 254	*ptsF, leu, str, lac*$(Z,Y,A)_{del}$, *mglD*	KL16-21 × S181
S183	*ptsF, leu, str, lac*$(Z,Y,A)_{del}$, *his*	9
S184	*leu, str, lac*$(Z,Y,A)_{del}$, *his*	Spontaneous revertant of S183
S181-91	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,B*	9
S181-29	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,B*	9
S181-27	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,B*	9
S181-27T	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,B,C*	Mutagenesis of S181-27
S181-71	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,C*	9
S181-93	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,C*	9
S181-95	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,C*	9
S181-10	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,A*	9
S181-72	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,A*	9
S181-94	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,A*	9
S181-96	*ptsI, leu, str, lac*$(Z,Y,A)_{del}$, *mglD,A*	9
OW4/F'4	*thr, leu, his, mglB*/F' *his*$^+$, *mglB*	7[b]
OW18/F'18	*thr, leu, his, mglA*/F' *his*$^+$, *mglA*	7[b]
OW31/F'31	*thr, leu, his, mglC*/F' his$^+$, *mglC*	7[b]
RV20	*leu, str, lac*$(Z,Y,A)_{del}$	—[c]
RV/F'MS1054	*lac*$(Z,Y,A)_{del}$/F'*lacZ*$^+$, Y_{del}	6[d]
RV20/F'MS1054	*leu, str, lac*$(Z,Y,A)_{del}$/F'*lacZ*$^+$, Y_{del}	RV/F'MS1054 × RV20

[a] Genetic symbols are those of Taylor and Trotter (12).
[b] Obtained from J. Adler.
[c] Collection of Institute Pasteur, obtained from D. Perrin.
[d] Obtained from M. H. Malamy.

Transductions. Lysates of bacteriophage P1kc were prepared on plates with 0.45% Oxoid agar agar no. 3 overlays (11). Each lysate was initiated from a single plaque and cycled at least twice on the respective *ptsF*$^+$ host to ensure genetic homogeneity. Transductions were performed at a multiplicity of infection of one by methods described by Lennox (5). After infection of the recipient, the cells were washed and plated on DM agar containing 0.05% D-fructose as sole carbon source. Colonies from these plates were replicated onto DM-fructose (0.05%) agar containing [1-^{14}C]MeGal (10^4 counts/min per ml). The colonies on the latter plates were imprinted on filter paper which was subsequently dried and exposed to X-ray film as previously described (9, 13).

Isolation of triple mutants. An *mglD,B* mutant in the merodiploid configuration S181-27/F'MS1054 was mutagenized with *N'*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1), grown for 1 h in broth, and then stored at 4 C. Samples from the mutagenized culture were spread on nutrient agar, and then colonies on the latter plate were replicated onto both DM-MeGal (0.1%) agar (9) and DM-lactose (0.2%) agar with isopropyl-1-thio-β-D-galactopyranoside (200 μM). Those colonies whose replicas grew on the latter, but not the former, medium were isolated and purified. To ascertain the genotype of the mutant derivative, a P1 lysate of this strain was prepared and used to infect S183. The resulting *ptsF*$^+$ transductants were screened by autoradiography to identify the *mgl* recombinants. These *ptsF*$^+$, *mgl* recombinants were then purified and tested by complementation analyses (9). One of these transductants was *mglB* and 29 were *mglB,C*.

RESULTS

Evidence for a third regulatory site for the MeGalP, distinct from RMG and *mglR*, was obtained from the results of interrupted matings between strains KL16-21, an Hfr inducible for this permease, and S181, constitutive (Table 2). Following a 30-min mating, recombinants which had received both the PtsI$^+$ and PtsF$^-$ characters from the Hfr were isolated and tested for inducibility of the permease. As shown in Table 2, 20 of these recombinants were inducible and 4 were constitutive for the MeGalP. The origin, 56 min, and counterclockwise direction of transfer of strain KL16-21 make it unlikely that either the RMG or *mglR* was transferred in this cross.

To proceed with the mapping of this regulatory locus (hereafter referred to as *mglD*), a method suitable for differentiating constitutive and inducible cells on a large scale was de-

TABLE 2. *Inducibility of MeGalP activity in ptsF exconjugants of KL16-21 × S181*

Strain	Intracellular accumulation (nmol/mg of dry cells)			
	[1-^{14}C]MeGal[a]		D-[6-^{3}H]fucose[b]	
	Induced	Noninduced	Induced	Noninduced
Exconjugants[c]				
S201, 202 . . . 220	9.0–10.8	0.66–0.78	3.8–4.9	0.51–0.68
S251, 252, 253, 254	10.5–12.6	11.1–12.0	4.1–5.6	3.6–4.9
Parents[d]				
S108	10.2	9.6	3.6	3.6
KL16-21	ND[e]	ND[e]	2.4	0

[a] A value of 10 nmol/mg of dry cells is equivalent to 2,360 counts/min per 10^8 cells. Results are corrected for a blank value obtained with formaldehyde-treated cells (175 to 200 counts/min).

[b] A value of 5 nmol/mg of dry cells is equivalent to 890 counts/min per 1.6×10^8 cells. Results are corrected for a blank value obtained with formaldehyde-treated cells (84 to 109 counts/min).

[c] The values presented are the minimum and maximum of the range obtained with the respective group of exconjugants. Each exconjugant was assayed twice in independent cultures.

[d] The results presented are the average of three independent determinations.

[e] Due to the presence of the *lacZ*$^+$ gene, accumulation of [1-^{14}C]MeGal cannot be determined in this strain.

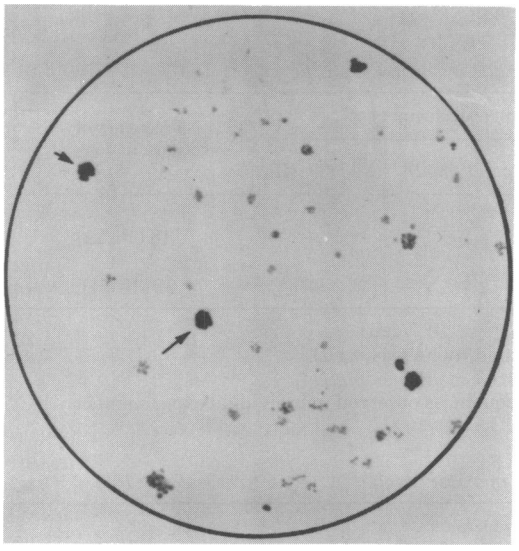


FIG. 1. *Autoradiograph from a [1-^{14}C]MeGal agar plate with colonies of both inducible and constitutive cells. Arrows indicate two of the five constitutive colonies present on this plate.*

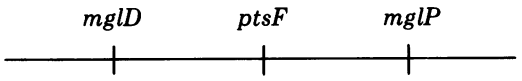
signed. This method is based on a modification of the Wilson and Kashket (13) autoradiographic procedure whereby colonies of MeGalP$^+$ and MeGalP$^-$ cells, grown on solid medium containing [1-^{14}C]MeGal, can be differentiated (9). Figure 1 shows the appearance of constitutive and inducible colonies on autoradiographs. The darker appearance of the constitutive is explained by its 15-fold greater intracellular concentration of [1-^{14}C]MeGal in the absence of induction (Table 2). The radioactive plates contain no inducer of the MeGalP (10).

The reliability of this assay was assessed by plating a mixture of inducible and constitutive cells in a 10:1 ratio. Fifty colonies were isolated by their dark appearance on the autoradiographs, cultured, and tested quantitatively for inducibility of the MeGalP. All 50 colonies were constitutive. No exceptions have been observed with respect to the correlation between dark colonies and the constitutive phenotype. Using this method we repeated the mating between strains KL16-21 and S181. Of 126 *ptsI*$^+$, *ptsF* recombinants, 101 were inducible and 25 were constitutive.

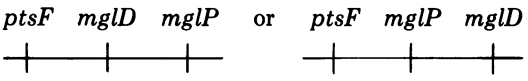
The cotransducibility of *mglD* with *ptsF* was tested since three *mgl* genes, *A*, *B* and *C*, have been shown to cotransduce with *ptsF* (7). A *ptsF*, *mglD*$^+$ recipient, S183, was infected with P1 lysates of a *ptsF*$^+$, *mglD* donor (S181); *ptsF*$^+$ transductants were selected and then screened by autoradiography to determine the frequency of *ptsF*$^+$, *mglD* recombinants among the transductants. Cotransduction between *mglD* and *ptsF* was found to be 18% (Table 3). In a similar manner, the transduction of *mglD*$^+$ into an *mglD* recipient was measured by infecting the *ptsF*, *mglD* strain, S252, with lysates prepared on a *ptsF*$^+$, *mglD*$^+$ donor (S184), selecting, and screening as above. In these experiments cotransduction of the *mglD* and *ptsF* loci was 21% (Table 3).

The results indicate that the distance between *ptsF* and *mglD* is similar to that between *ptsF* and the other *mglP* genes (7, 9). (The term *mglP* is used here to encompass the three genes

A, *B* and *C*.) If the order of genes were:



then little, if any, cotransduction of *mglD* and *mglP* would be expected since the distance separating *mglD* and *mglP* would be larger (1.6×) than the maximum length of DNA carried by P1. On the other hand, if the order were:



one would predict a high frequency of cotransduction between *mglD* and *mglP*.

I measured the cotransduction of *mglD* and *mglP* by comparing the frequencies of *mglD*,P^+ recombinants obtained using *mglD*,P^+ and *mglD*,*P* donors. For these experiments it was necessary to employ a set of *mglA*, *-B*, *and -C* mutants derived from the constitutive strain S181. These mutants have been described in a previous report (9). S183 (*ptsF*, *mglD*$^+$,P^+) was infected with lysates of these double mutants, *ptsF*$^+$ transductants were selected, and the transductants were screened by autoradiography to determine the number of recombinants in each of the following categories: *mglD*$^+$,P^+; *mglD*,P^+; and *mglP*. (Since *mglP* cells do not cause darkening of the autoradiographs, it is not possible to distinguish by this method those *mglP* recombinants that are *mglD*$^+$ from those that are *mglD*.)

The results of these experiments (Table 4) indicate that the order of genes is *ptsF*, *mglD*, *mglP*. We observed that *mglD*,P^+ recombinants are obtained with the *mglD*,P^+ donor 3 to 20 times more frequently than with the double mutant *mglD*,*P* donors (Tables 3 and 4). These frequencies vary with the location of the *mglP*

TABLE 3. *Cotransduction of mglD with ptsF by Plkc*

Donor (genotype)	Recipient (genotype)	No. of transductants			Frequency of cotransduction (%)
		Total *ptsF*$^+$[a]	*ptsF*$^+$, *mglD*[b]	*ptsF*$^+$, *mglD*$^+$[c]	
S181 (*ptsF*$^+$ *mglD*)	S183 (*ptsF*, *mglD*$^+$)	589	106		18 (16–25)[d]
S184 (*ptsF*$^+$, *mglD*$^+$)	S252 (*ptsF*, *mglD*)	467		98	21 (19–31)

[a] Corrected for the reversion to *ptsF*$^+$ of uninfected cells (5×10^{-7}/cell).
[b] Among the 500 *ptsF*$^+$ revertants of uninfected S183 tested, none was *mglD*.
[c] Among the 500 *ptsF*$^+$ revertants of uninfected S252 tested, none was *mglD*$^+$.
[d] Values in parentheses are the range of cotransduction frequencies observed in individual experiments.

TABLE 4. *Cotransduction of mglD with mglA, -B, and -C in a ptsF, mglD*$^+$,A^+,B^+,C^+ *recipient (S183)*

Donor	Type[a]	No. of transductants			Frequency (%)		
		Total *ptsF*$^+$[b]	*ptsF*$^+$, *mglD*,P^+[c]	*ptsF*$^+$, *mglP*[d]	*mglD*, P^+/*ptsF*	*mglP*/*ptsF*$^+$	*mglD*, P^+/*mglP*
S181-29	*D*,*B*	767	7	138	0.9	18	5 (0–6)[e]
S181-27	*D*,*B*	540	6	113	1.1	21	5 (0–7)
S181-91	*D*,*B*	691	7	124	1.0	18	6 (0–6)
S181-71	*D*,*C*	484	16	102	3.3	21	16 (14–17)
S181-93	*D*,*C*	865	13	165	1.5	19	8 (7–10)
S181-95	*D*,*C*	481	12	101	2.5	21	12 (11–16)
S181-10	*D*,*A*	733	26	132	3.5	18	20 (18–23)
S181-72	*D*,*A*	453	25	110	5.5	24	23 (22–27)
S181-94	*D*,*A*	725	24	144	3.3	20	17 (14–22)
S181-96	*D*,*A*	614	18	104	2.9	17	17 (17–25)

[a] The classification of these mutant donors has been previously described (9).
[b] Corrected for the number of *ptsF*$^+$ revertants obtained with uninfected cells (5×10^{-7} to 1×10^{-6}/cell).
[c] Of 12,576 *ptsF*$^+$ revertants of uninfected cells tested, 2 were *mglD*,P^+.
[d] Of 12,576 *ptsF*$^+$ revertants of uninfected cells tested, 1 was *mglP*.
[e] The values in parentheses represent the range of frequencies observed in individual experiments.

mutation. With *mglD,B* donors 0.9 to 1.1% of the *ptsF*$^+$ recombinants were *mglD,P*$^+$; with *mglD,C*, 1.5 to 3.3%; and with *mglD,A*, 2.9 to 5.5% (Table 4). The frequencies of *ptsF*$^+$,*mglP* recombinants (Table 4) are similar to those previously reported (9). These results are consistent with the order *mglD,B,C,A*.

To further test this order, I repeated the experiments described above substituting a triple mutant *mglD,B,C* for the double mutant donors. The *ptsF*$^+$ transductants were classified as *mglD*$^+$,*P*$^+$, *mglD,P*$^+$, or *mglP*, and the *mglP* recombinants were assayed by complementation analyses (9) to determine the number of recombinants in the following categories: *mglB*; *mglC*; and *mglB,C*. The results shown in Table 5 corroborate the order *ptsF, mglD,B,C,A*. The *ptsF*$^+$, *mglD,P*$^+$ recombinants were obtained with the triple mutant donor at a frequency of 1.0%. This is similar to the frequency observed (1.1%) using as donor the double mutant *mglD,B* from which the triple mutant was derived. Complementation analyses of the *mglP* recombinants showed that 95% were *mglB,C* and 5% were *mglB,C*$^+$; no *mglB*$^+$,*C* recombinants were observed.

Since MeGal is a substrate, but not an inducer of the MeGalP (10), one would predict that constitutive cells would have a selective advantage when MeGal is supplied as sole carbon source. Accordingly, I attempted to isolate *mglD* mutants by plating the *mglD*$^+$ strain RV20/F'MS1054 on minimal medium containing 5×10^{-4} M MeGal; at this concentration, growth is dependent on transport by the MeGalP (9). From these plates eight colonies that appeared during the first 48 h were isolated, purified, and tested for inducibility of the MeGalP. All were found to be constitutive. I prepared P1 lysates of two of these constitutive mutants and used them to infect strain S183. Subsequent selection and screening were performed as described above. Among the *ptsF*$^+$ transductants obtained, 19 and 22% were constitutive for MeGalP activity. These values indicate that this procedure can be used to select cells mutant in the *mglD*.

DISCUSSION

I conclude that the order of genes in the region of *mgl* is *ptsF, mglD,B,C,A*. The rationale for this conclusion is as follows. (i) All four genes, *mglA*, *-B*, *-C*, and *-D*, are approximately 20% cotransducible with *ptsF*. If the order were *mglD, ptsF, mglP* (the term *mglD* encompasses the three genes *mglA*, *mglB*, and *mglC*), co-transduction of *mglD* and the *mglP* genes would occur infrequently, if at all, since the distance separating these loci would be approximately 1.6 times the length of the P1-transducing fragment. Instead, I observe between 77 and 95% cotransduction between *mglD* and the other *mgl* genes (Table 4). (ii) If the order were *ptsF, mglP, mglD*, a quadruple crossover would be required to generate *ptsF*$^+$, *mglD,P*$^+$ recombinants in those transductions in which the double *mglD,P* mutants were employed as donors. But, with the double mutant donors, we obtained these recombinants at 5 to 30% the frequency observed using the *mglD* single mutant donor (Tables 3 and 4). I consider these values to be higher than those expected for quadruple crossovers. (iii) The frequency of *mglD,P*$^+$ recombinants varies with the location of the *mglP* defect in the *mglD,P* double mutants (Table 4). The results indicate that *mglB* is most closely linked to *mglD*, followed by *mglC*, and then *mglA*. (iv) I obtained *ptsF*$^+$, *mglB,C*$^+$, but not *ptsF*$^+$, *mglB*$^+$*C*, recombinants using the triple mutant *mglD,B,C* donor. This corroborates the postulated order *ptsF*$^+$, *mglD,B,C*, since according to this order formation of *ptsF*$^+$, *mglB,C*$^+$ by transduction with the triple mutant donor would require a double crossover. Formation of *ptsF*$^+$, *mglB*$^+$,*C* would require a quadruple crossover.

I have found that *mglD,A* and *mglD,C* mu-

TABLE 5. *Transduction of mgl genes from an mglD,B,C mutant into a ptsF,mglD*$^+$,*A*$^+$,*B*$^+$,*C*$^+$ *recipient (S183)*

Donor	Type	No. of transductants						Frequency (%)	
		Total *ptsF*$^+$ [a]	*ptsF*$^+$, *mglD,P*$^+$ [b]	*ptsF*$^+$, *mglP* [c]	Total *mglB* [d]	*mglC* [d]	*mglB,C* [d]	*mglD, P*$^+$/ *ptsF*$^+$	*mglD/mglP*
S181-27T	*D,B,C*	1,265	13	208	12	0	196	1.0	6 (2-6) [e]
S181-27	*D,B*	540	6	113				1.1	5 (0-7)

[a] Corrected for the number of *ptsF*$^+$ revertants obtained with uninfected cells (5×10^{-7}/cell).
[b] Of 1431 *ptsF*$^+$ revertants of uninfected cells tested, none was *mglD,P*$^+$.
[c] Of 1431 *ptsF*$^+$ revertants of uninfected cells tested, none was *mglP*.
[d] Classified by complementation analyses with /F'4, /F'18, and /F'31 as previously described (9).
[e] The values in parentheses represent the range of frequencies observed in individual experiments.

tants are constitutive with respect to galactose binding protein synthesis. In heteromerodiploids of genotype *mglD,C/F'mglD*$^+$*,B* and *mglD,A/F'mglD*$^+$*B* I found that synthesis of galactose binding protein remains constitutive, whereas MeGalP activity is inducible (unpublished observations). This *cis*-dominant, *trans*-recessive effect is consistent with the *mglD* being an operator locus in a system of negative control. Moreover, these results indicate that synthesis of the products of *mglA* and *mglC* is coordinately induced with MeGalP activity. Neither of these gene products has yet been identified.

Both the screening and selection procedures described in this report were designed to identify *mglD* mutants that are constitutive for MeGalP activity. From the example of other regulatory systems it is predicted that a second category of *mglD* mutants will be found, i.e., *mglD* mutants in which transport activity of the MeGalP is abolished. My results indicate that this latter class of mutants would appear to be *mglA,B,C* on complementation and would exhibit MeGalP activity upon introduction of the F'*mglD*$^+$*,P*$^+$ episome. MeGalP$^-$ mutants exhibiting these characteristics have been described (7).

ACKNOWLEDGMENTS

I wish to thank Boris Rotman for his helpful consideration of this manuscript, Laneta Dorflinger for the isolation of strain S181-27T, and C. L. Howitt for her able technical assistance.

This investigation was supported by Public Health Service grant AM-11194 from the National Institute of Arthritis, Metabolism, and Digestive Diseases; I was supported by Public Health Service training grant AI-000418 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by *N'*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *E. coli* K12. Biochem. Biophys. Res. Commun. **18**:788-795.
2. Ferenci, T., and H. L. Kornberg. 1971. Role of fructose-1,6-diphosphatase in fructose utilization by *Escherichia coli*. FEBS Lett. **14**:360-363.
3. Ganesan, A. K., and B. Rotman. 1965. Transport systems for galactose and galactosides in *Escherichia coli*. I. Genetic determination and regulation of the methylgalactoside permease. J. Mol. Biol. **16**:42-50.
4. Lengeler, J., K. O. Hermann, H. J. Unsöld, and W. Boos. 1971. The regulation of the β-methylgalactoside transport system and of the galactose binding protein of *Escherichia coli* K12. Eur. J. Biochem. **19**:457-470.
5. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190-206.
6. Malamy, M. H. 1966. Frameshift mutations in the lactose operon of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **31**:189-201.
7. Ordal, G. W., and J. Adler. 1974. Isolation and complementation of mutants in galactose taxis and transport. J. Bacteriol. **117**:509-516.
8. Ordal, G. W., and J. Adler. 1974. Properties of mutants in galactose taxis and transport. J. Bacteriol. **117**:517-526.
9. Robbins, A. R., and B. Rotman. 1975. Evidence for binding protein independent substrate translocation by the methylgalactoside transport system of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. **72**:423-427.
10. Rotman, B., A. K. Ganesan, and R. Guzman. 1968. Transport systems for galactose and galactosides in *Escherichia coli*. II. Substrate and inducer specificities. J. Mol. Biol. **36**:247-260.
11. Swanstrom, M., and M. H. Adams. 1951. Agar layer method for production of high titer phage stocks. Proc. Soc. Exp. Biol. Med. **78**:372-375.
12. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:502-524.
13. Wilson, T. H., and E. R. Kashket. 1969. Isolation and properties of thiogalactoside transacetylase-negative mutants of *Escherichia coli*. Biochim. Biophys. Acta **173**:501-508.