Proc. Nat. Acad. Sci. USA
Vol. 72, No. 2, pp. 423–427, February 1975

Evidence for Binding Protein-Independent Substrate Translocation by the Methylgalactoside Transport System of *Escherichia coli* K12

(active transport/galactose-binding protein/autoradiography/genetic complementation/galactose transport)

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Communicated by Saul Roseman, November 15, 1974

ABSTRACT **Three genes, *mgl A*, *B*, and *C*, are required for active transport of substrate by the methylgalactose permease of *E. coli* K12. We report here that only two of these genes are required for substrate translocation, as seen by the ability or inability of isogenic *mgl* mutants (referred to as Tra$^+$ and Tra$^-$, respectively) to grow on methyl-β-D-galactopyranoside, supplied as sole carbon source. Individual mutants of both the Tra$^+$ and Tra$^-$ classes exhibited no detectable intracellular accumulation of methyl-β-D-galactopyranoside; thus, the Tra$^+$ phenotype cannot be explained by the mutants' levels of residual active transport. The phosphotransferase (Pts), the β-galactoside (LacY), and the arabinose (Ara E and Ara F) transport systems are not required for substrate translocation by Tra$^+$ cells. The Tra$^+$ phenotype was identified with mutants defective in the *mgl B*, locus of the galactose-binding protein, by genetic complementation; the Tra$^-$ phenotype was observed with both *mgl A* and *mgl C* mutants. The conclusion that the galactose-binding protein is not required for substrate translocation was supported by direct assays of the *mgl* mutants' binding protein activity. Mutants capable of translocation all showed reduced galactose-binding protein activity; mutants incapable of translocation exhibited binding protein activity equal to that of the *mgl*$^+$ parent.**

The intracellular accumulation of D-galactose and β-D-galactosides by way of the methylgalactoside transport system (methylgalactoside permease, MeGalP) of *Escherichia coli* K12 is dependent on the presence of the galactose-binding protein (GBP). Mild osmotic shock effects the release of this binding protein from the cell and a simultaneous decrease in accumulation of substrates of MeGalP (1). Induction of transport activity and GBP synthesis is coordinate and effected by the same inducers (2). Boos has demonstrated that mutation in a locus closely linked or identical to *mgl*, previously established as coding for MeGalP (3), causes concomitantly a decrease in transport activity, a decrease in the ability of GBP to bind substrate *in vitro*, and an alteration in the peptide map of GBP (4).

While GBP is necessary for active transport by MeGalP, the isolation of MeGalP$^-$ mutants with functional GBP suggested that additional MeGalP components are also required for active transport (5, 6). Genetic evidence for this has recently been presented by Ordal and Adler (7, 8), who have defined three complementation groups within the *mgl* locus, one of which, *mgl B*, codes for GBP. Since each and all of the three *mgl* genes are required for intracellular accumulation of substrate, an assay of MeGalP activity independent of substrate accumulation is necessary if the activity of any given *mgl* gene is to be correlated with a discrete step in the process of active transport.

In this paper we present a method for measuring translocation of a MeGalP substrate in mutants unable to accumulate this substrate. Using this method we demonstrate that translocation does not require the galactose-binding protein, but is dependent on the other two genes, *mgl A* and *mgl C*.

Abbreviations: MeGal, methyl-β-D-galactopyranoside; MeGalP, methylgalactoside permease; GBP, galactose-binding protein; del, deleted.

* In partial fulfillment of the requirements for the degree of Doctor of Philosophy.

MATERIALS AND METHODS

Bacterial Strains. Genotypes of the bacteria used in this study are given in Table 1. All strains were derived from *E. coli* K12, with the exception of SB1094, a derivative of *E. coli* B/r.

Media and Growth Conditions. Cells were cultured at 37°. Three complete media were used: for experiments involving bacteriophage, L broth [1% (w/v) Bacto-tryptone, 86 mM NaCl, and 2.5 mM $CaCl_2$; the calcium was added after the medium was adjusted to pH 7.8 and autoclaved]; for scoring of intracellular MeGal accumulation, [*1*-^{14}C]MeGal agar (13) [0.89% (w/v) Bacto-tryptone, 94 mM NaCl, 1 mM KH_2PO_4, and 10^4 cpm/ml of [*1*-^{14}C]MeGal]; and, for all other purposes, Difco Antibiotic Medium 3. The minimal medium used was DM [Davis Medium (14)]. Amino acids, where required, were supplied at a final concentration of 10 μg/ml. Media were solidified with 0.9% Oxoid Agar Agar no. 3. Liquid cultures were grown in either tubes or flasks; the former were aerated in a tube roller and the latter in a rotatory shaker. For induction of the *lac* operon, cells were grown in the presence of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside.

Chemicals. Methyl-β-D-[*1*-^{14}C]galactopyranoside (4.7 mCi/mmol) was purchased from New England Nuclear Corp. and D-[*1*-^{3}H]galactose (5.7 Ci/mmol) from Amersham/Searle Corp. Both radioactive carbohydrates were purified by paper chromatography on 589 Orange Ribbon from Carl Schleicher and Schuell Co. in butanol–pyridine–water (63:35:48). The D-[*1*-^{3}H]galactose was chromatographed in two dimensions in order to separate it from glucose. Methyl-β-D-galactopyranoside and isopropyl-1-thio-β-D-galactopyranoside were purchased from Nortok Assoc.

Isolation of MeGalP$^-$ Mutants. After mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (15), the MeGalP$^+$ strain S181 was grown for 1 hr in broth, then stored at 4°. Samples from this mutagenized culture were spread on nutrient agar. Colonies on these plates were replicated onto [*1*-^{14}C]MeGal agar; then the colonies appearing on the latter

TABLE 1. *Bacterial strains*

Strain	Relevant genotype	Source
Che105	Hfr, *pts I*	W. Epstein (9)
RV20	*leu, str, lac(Z,Y,A)*$_{del}$	D. Perrin, Collection of Institute Pasteur
S181	*pts I, leu, str, lac(Z,Y,A)*$_{del}$	Che105 × RV20
S181-(1,2···135)	*mgl, pts I, leu, str, lac(Z,Y,A)*$_{del}$	Mutagenesis of S181
RV/F'MS1054	*lac(Z,Y,A)*$_{del}$/F' *lac* Z^+,Y_{del}	M. H. Mallamy (10)
KL16-21	Hfr, *pts F*	W. Boos (4) and H. L. Kornberg (11)
S182	*pts* I^+, *pts F, leu, str, lac(Z,Y,A)*$_{del}$	KL16-21 × S181
S183	*his, pts* I^+, *pts F, leu, str, lac(Z,Y,A)*$_{del}$	Penicillin selection of S182
OW4/F'4	*thr, leu, his, mgl B*/F'*his*$^+$, *mgl B*	G. W. Ordal and J. Adler (7)
OW18/F'18	*thr, leu, his, mgl A*/F'*his*$^+$, *mgl A*	G. W. Ordal and J. Adler (7)
OW31/F'31	*thr, leu, his, mgl C*/F'*his*$^+$, *mgl C*	G. W. Ordal and J. Adler (7)
SB1094	B/r, *ara(C,O)*$_{del}$	R. W. Hogg (12)

del, deleted.

were imprinted on filter paper, which was subsequently dried and exposed to x-ray film (Kodak BB-54) for 44–52 hr. After development of the film, the imprints of MeGalP$^+$ colonies could be distinguished from those of the MeGalP$^-$, because while the former produced black images, the latter caused no darkening of the film (13). All putative mutants were picked from the original plate and purified by two successive isolations on nutrient agar. Relevant parental markers were tested prior to use of the MeGalP$^-$ mutants.

Isolation of mgl Transductants. Lysates of bacteriophage P1kc were prepared on plates with 0.45% Oxoid Agar Agar no. 3 overlays (16). Each lysate was initiated from a single plaque and cycled at least three times on the respective *mgl, pts* F^+ host to ensure genetic homogeneity. Transductions were performed at a multiplicity of infection of one, according to methods described by Lennox (17). After infection of the recipient S183 (*pts F, mgl*$^+$), the cells were washed and plated on DM agar containing 0.2% D-fructose as sole carbon source. Colonies from these plates were scored on [*1*-^{14}C]MeGal plates and purified as described in *Isolation of MeGalP$^-$ Mutants.*

Construction of Merodiploids. Two methods were used; spot-matings for the scoring of F-ductant phenotype, and liquid matings for determining the frequency of a given F-ductant phenotype.

(*a*) For spot-matings, the F$^-$ and F' strains were grown to exponential phase in broth; then 0.005 ml of the F$^-$ strain was spotted on the appropriate selection plate. After the spot had dried, 0.005 ml of the F' strain was applied onto the same spot.

(*b*) For liquid matings, equal volumes of the F$^-$ and F' strains, grown to exponential phase in broth, were mixed to give a final concentration of 1×10^8 and 2.5×10^7 cells per ml, respectively. The mating mixtures were incubated, standing, for 60 min at 37°, agitated on a Vortex Mixer for 30 sec, diluted, and plated on the appropriate selective plates.

The selective plates used in matings between F' *his*$^+$, *mgl* strains and the F$^-$ *his, mgl* transductants were DM–0.4% sodium lactate agar, containing [*1*-^{14}C]MeGal, without histidine or threonine (required by the F$^-$ and F' strains, respectively). The imprinting and scoring were conducted as described above. For matings between F' MS1054 (F'*lacZ*$^+$, Y_{del}) and either F$^-$ *mgl* mutants or transductants, cells were plated on DM agar, with streptomycin (20 μg/ml), containing as sole carbon source either MeGal or 0.2% lactose in the presence of 0.2 mM isopropyl-1-thio-galactopyranoside.

Galactose-Binding Protein Assays. Assays were performed with cell-free extracts from bacteria grown to stationary phase in DM–0.4% lactate. The extracts were prepared as described (18), except that the extracts were dialyzed for 36 hr with five changes of buffer. The ratio of buffer volume to extract volume was 20:1. The immunochemical assay for GBP activity (19) was carried out with the modifications described (18). The linearity of this assay was ascertained by measuring samples of the extracts at three protein concentrations. The radioactivity of the bound galactose was determined by liquid scintillation with an efficiency of 17% for ^{3}H.

Isolation of ara (C,O)$_{del}$ *Transductants.* A lysate of SB1094 was prepared with bacteriophage P1kc as described under *Isolation of mgl Transductants,* using a phage inoculum that had been previously cultured on *E. coli* B/r (this original phage inoculum was kindly provided by R. W. Hogg). Infection of the *leu, ara*$^+$ recipients was followed by selection for *leu*$^+$ recombinants. The *leu*$^+$ colonies were purified and tested for *ara (C,O)*$_{del}$ by plating on DM–0.2% arabinose.

Permease Assays. Permease activity was measured by the intracellular accumulation of [*1*-^{14}C]MeGal, using cells grown in DM–0.4% lactate to stationary phase (4 to 6×10^8 cells per ml). The assay mixture (3 ml final volume) contained 4×10^8 cells in DM–0.4% lactate, 100 μg of chloramphenicol, and 0.46 μM [*1*-^{14}C]MeGal (10^4 cpm). The assay mixture was incubated with rotation for 15 min at room temperature (22–24°) and filtered through HA Millipore membranes. Then the membranes were dried and their radioactivity was determined by liquid scintillation, with an efficiency of 62%.

RESULTS

In a previous report we described means for selecting, among a population of permease-negative bacteria, individual cells that possess the MeGal permease. The basis for selection is the ability of the MeGalP$^+$ cells to utilize methyl-β-D-galactopyranoside (MeGal) as sole carbon source (20). Subsequently, we have observed that by varying the concen-

TABLE 2. *Intracellular accumulation of [1-^{14}C]MeGal in MeGalP$^-$ mutants*

Strain	Intracellular accumulation (%)*	
S181 (parent)	100	(5850 cpm)†
S181-10	0.6	
-27	0	
-29	0	
-71	0.1	
-72	0	
-81	0.4	
-91	0.2	
-92	0	
-93	1.4	
-94	0.3	
-95	1.4	
-96	0.8	
-106	0.2	
-121	0	

* Results are corrected for a blank of 180–200 cpm; this was obtained when formaldehyde-treated cells were used in the permease assay (14). Each value is the average of three determinations.

† Equivalent to an intracellular concentration of 3.4 nmol of MeGal per mg of dry cells, indicating a ratio between intracellular and extracellular MeGal of about 4400.

tration of MeGal in the plates we can distinguish not only MeGalP$^+$ from MeGalP$^-$ strains, but also differentiate two groups among the MeGalP$^-$ strains.

For this investigation we used a MeGalP$^+$ derivative of *E. coli* K-12 (S181) containing a deletion of the three structural genes of the *lac* operon. Such a deletion is essential since MeGal, the substrate used in our assays, is also transported by the Y permease and is hydrolyzed by β-D-galactosidase. MeGalP$^-$ mutants of S181 were obtained by mutagenesis, followed by screening for clones unable to accumulate radioactive MeGal against a concentration gradient (see *Materials and Methods*). After purification of putative MeGalP$^-$ mutants, these cells were tested quantitatively for their levels of intracellular accumulation. Into those mutants with little or no accumulation (Table 2), we introduced the gene for β-D-galactosidase by means of an F'*lacZ*$^+$,Y_{del} episome. The resulting merodiploids have all the enzymes necessary for the metabolism of intracellular MeGal and were tested for their abilities to grow at varying concentrations of MeGal, supplied as sole carbon source.

While merodiploids constructed from the MeGalP$^+$ parent grew at or above 50 μM MeGal, none of the MeGalP$^-$ merodiploids grew at 500 μM MeGal or less. At substrate concentrations greater than 500 μM, the MeGalP$^-$ mutants were observed to fall into two categories, those which formed colonies at or above 1 mM MeGal, and those which were unable to grow at any of the MeGal concentrations tested (up to and including 5 mM). Of 105 independent MeGalP$^-$ mutants tested, 17 belong to the former and 88 to the latter category.

These two phenotypes observed among the mutants could result from differences in either the translocation of MeGal or its metabolism. Accordingly, we examined the various steps involved in MeGal metabolism. No differences were observed between the mutants and the MeGalP$^+$ parent with respect to their levels of β-galactosidase activity in cultures either induced by isopropyl-1-thio-galactopyranoside or not induced, as

TABLE 3. *Cotransduction of Tra with mgl*

Donor				
Strain	Tra phenotype*	*mgl* among *pts* F$^+$ transductants	Frequency of cotransduction (%)	Tra phenotype† of *mgl* transductants
S181-27	+	52/208	25	+
-29	+	50/201	25	+
-81	+	51/196	26	+
-91	+	45/181	25	+
-121	+	40/174	23	+
-10	−	48/190	25	−
-71	−	48/190	25	−
-72	−	60/206	29	−
-92	−	39/157	25	−
-93	−	48/192	25	−
-94	−	36/176	20	−
-95	−	48/200	24	−
-96	−	56/222	25	−
-106	−	56/256	22	−
S181		0/250	0	
S183		0/250	0	
None		0/500	0	

* Measured with the respective F'*lacZ*$^+$,Y_{del} merodiploids.

† Scored by spot-mating transductants with the F'*lacZ*$^+$,Y_{del} strain. At least 30 independent transductants from each donor were tested.

measured by *o*-nitrophenyl-β-D-galactopyranoside hydrolysis *in vivo* and *in vitro* (21). Also, no differences were observed regarding growth on galactose as sole carbon source over the range 0.05–5 mM. From these results we proceeded on the hypothesis that the two phenotypes, hereafter referred to as Tra$^+$ and Tra$^-$, reflect the different abilities of the mutants to translocate MeGal. (Translocation is defined here as the movement of substrate across the cellular membrane regardless of the mechanism involved.)

The linkage between *mgl*, the locus of the MeGal permease, and the locus of the Tra phenotype was determined as follows. The *mgl* mutations of the tested strains were introduced into S183 (*pts F, mgl P*$^+$, *his*) by transduction, selection for *pts F*$^+$, and screening for *mgl*. The frequencies of *pts F*$^+$-*mgl* cotransduction ranged from 20 to 30% (Table 3). The F'*lacZ*$^+$,Y_{del} was then introduced into the *pts F*$^+$, *mgl* transductants in order to test the resulting merodiploids for their ability to grow on MeGal. We found that all *mgl* transductants from Tra$^+$ mutants were Tra$^+$, while all from Tra$^-$ mutants were Tra$^-$ (Table 3). Thus, the Tra locus lies close to or within the *mgl*.

The isogenic Tra$^+$ and *mgl*$^+$ transductants in the F'*lac Z*$^+$,Y_{del} merodiploid configuration were compared in liquid cultures with respect to their growth rates on varying concentrations of MeGal. The doubling time of the *mgl*$^+$ strain was the same at 50, 5, and 2.5 mM MeGal, 126 ± 6 min. The doubling times of the Tra$^+$ mutants at these same MeGal concentrations were 212 ± 20, 336 ± 36, and 718 ± 30 min, respectively. The Tra$^-$ mutants did not grow under these conditions.

Having shown this relationship between Tra and *mgl*, we next determined whether the Tra phenotype could be assigned by complementation analysis to any of the three *mgl* genes, *A*, *B*, or *C* (7, 8). F' *his*$^+$ episomes with mutations in *mgl A*,

TABLE 4. *Complementation analysis of mgl transductants*

Transductant	Tra phenotype*	Complementation to MeGalP+		
		F'*mgl A*†	F'*mgl B*†	F'*mgl C*†
S183-27	+	+ (229/233)	− (0/246)	+ (188/191)
-29	+	+ (248/254)	− (0/254)	+ (203/211)
-81	+	+	−	+
-91	+	+	−	+
-121	+	+	−	+
-71	−	+ (254/258)	+ (326/332)	− (0/180)
-93	−	+ (273/280)	+ (257/262)	− (0/203)
-95	−	+	+	−
-10	−	− (0/206)	+ (206/218)	+ (164/184)
-72	−	− (0/284)	+ (283/290)	+ (195/201)
-94	−	−	+	+
-96	−	−	+	+
-106	−	−	+	+
-92	−	−	−	−

* From Table 3.

† Mutants were assigned to their respective complementation groups by spot-mating transductants with the respective F'*his*+, *mgl* strains. At least 30 independent transductants from each donor strain were tested. Numbers in parentheses denote the ratio of MeGalP+, *his*+ merodiploids to the total *his*+ merodiploids obtained on mating the indicated strains and scoring the *his*+ progeny *individually* for MeGalP activity.

B, or *C* were introduced into the *pts* F^+, *mgl*, *his* transductants, obtained in the previous experiments, and the resulting *his*+ F-ductants were scored for their ability to accumulate MeGal against a concentration gradient (see *Materials and Methods*). These experiments showed that all the Tra+ transductants were defective in the *mgl B* locus. The Tra− phenotype was observed in both *mgl A* and *mgl C* mutants (Table 4).

We repeated several of the crosses described above in order to test the resulting *his*+ F-ductants individually for their ability to accumulate MeGal. Scoring about 200 independent merodiploids per mating, we found no MeGalP+ colonies on crossing mutants defective in the same *mgl* locus, as defined by the spot crosses described above, while crosses between mutants defective in different *mgl* loci yielded more than 90% MeGalP+ F-ductants (Table 4). When these MeGalP+ merodiploids were cured of the F' *his*+ episome with acridine orange (22), the resulting *his* derivatives were all MeGalP−. Furthermore, complementation of these *his*, *mgl* derivatives revealed no differences between them and the respective original *mgl* transductants.

The results of these complementation studies indicated that mutation of either the *mgl A* or *mgl C* locus results in the loss of both accumulation and translocation of MeGal. If a single point mutation is sufficient to impair both these transport functions, then reversion of the *mgl A* and *mgl C* mutants to Tra+ should concomitantly restore the MeGalP+ phenotype. Accordingly, Tra+ revertants were obtained by plating Tra− in the F'*lacZ*+,Y_{del} merodiploid configuration on 5 mM MeGal. The Tra+ revertants were purified and tested for restoration of the MeGalP+ character by assaying their ability to grow on 0.5 mM MeGal as well as their ability to accumulate radioactive D-fucose, a nonmetabolizable substrate of the MeGalP. The Tra+ revertants all formed colonies at 0.5 mM MeGal; their intracellular accumulation of D-fucose was observed to be comparable to that of the MeGalP+ parent. One of the *mgl C* (S183-71) and two of the *mgl A* (S183-10 and S183-72) transductants were observed to revert to MeGalP+ at frequencies greater than 1×10^{-7} per cell.

TABLE 5. *Transport activity and galactose-binding protein of mgl transductants*

Transductant (gene assignment)	Transport: Intracellular MeGal accumulation (%)	Binding protein Galactose bound*: (cpm)	(pmol/mg of total protein)
S183-181 (*mgl*+)	100 (6660 cpm)†	2013	17.7
-91 (*B*)	0.1	6	0.1
-27 (*B*)	0	33	0.3
-81 (*B*)	0.2	55	0.5
-121 (*B*)	0	78	0.7
-92 (*A,B,C*)	0	135	1.2
-29 (*B*)	0	584	5.1
-94 (*A*)	0.1	1947	17.1
-72 (*A*)	0.1	2077	18.2
-106 (*A*)	0	2084	18.3
-10 (*A*)	0.2	2098	18.4
-95 (*C*)	1.1	2161	19.0
-93 (*C*)	2.2	2173	19.1
-96 (*A*)	0.8	2174	19.1
-71 (*C*)	0	2407	21.1

* Results are corrected for a blank of 33–35 cpm; this was obtained when buffer was substituted for cell extract in the assay mixture. The values given are the average of the results obtained with two independent preparations.

† Equivalent to an intracellular accumulation of 3.9 nmol of MeGal per mg of dry cells, indicating a ratio between intracellular and extracellular MeGal of 5000. The values given are the average of three independent determinations, and are corrected for a blank value of 180–200 cpm obtained when formaldehyde-treated cells were used in the permease assay (14).

From the complementation analysis we concluded that mutation in the *mgl B*, locus of GBP, does not prevent translocation. Support for this conclusion was obtained from determinations of GBP activity in cell-free preparations of isogenic Tra+ and Tra− mutants. Extracts of the Tra+ mutants evinced as little as 0.1% of the galactose-binding specific activity of the wild-type preparation (Table 5). Extracts of Tra− cells (*mgl A* and *mgl C*) contained GBP with specific activity equal to that found in the extracts of the MeGalP+ parent (Table 5).

We examined the possibility that the arabinose-binding protein, product of the *ara F* (23), which exhibits a substrate specificity similar to that of GBP and crossreacts immunologically with GBP (24), is required for MeGal translocation. We tested the effect on the Tra phenotype of an *ara C,O* deletion, which prevents synthesis of both arabinose transport systems, Ara E and Ara F (25, 12). It was observed that the *mgl B*, *ara* $(C,O)_{del}$ transductants remained Tra+, and the *mgl A*, *ara* $(C,O)_{del}$ and *mgl C*, *ara* $(C,O)_{del}$ transductants remained Tra−.

DISCUSSION

Our results indicate that while all three *mgl* genes are necessary for the intracellular accumulation of MeGal, only two of these genes, *A* and *C*, are required for the translocation of this substrate. Using an assay of translocation based on the

ability of metabolically competent cells to grow on MeGal, we classified a series of isogenic MeGalP$^-$ mutants into two groups, Tra$^+$ and Tra$^-$. The *mgl* genes responsible for the Tra$^+$ phenotype were then determined by complementation analysis. The results of our genetic studies were supported by measurements *in vitro* of the activity of the galactose-binding protein, the *mgl B* product. The GBP obtained from each of the Tra$^+$ mutants exhibited significantly less specific activity than that from the *mgl*$^+$ parent. GBP from the Tra$^-$ mutants (both *mgl A* and *mgl C*) had activity equal to that from *mgl*$^+$ cells.

We conclude that a single point mutation in either *mgl A* or *mgl C* is sufficient to prevent both the intracellular accumulation and translocation of MeGal, since the Tra$^-$ mutants reverted at significant frequencies to MeGalP$^+$ on selection for the Tra$^+$ phenotype. We find no evidence for the participation of transport systems other than MeGalP in MeGal translocation. The Tra$^+$ phenotype of the *mgl B* mutants was observed irrespective of their loss, through mutation, of the Lac Y, Ara E, and Ara F permeases, as well as of the phosphotransferase system. Thus, it appears from the results of our investigation that the *mgl A* and *mgl C* genes are both necessary and sufficient for MeGal translocation.

For this investigation we have used a qualitative assay of MeGal translocation. Measuring translocation by a procedure independent of cell division (the details of which will be presented separately), we find that the translocation of MeGal by *mgl B* mutants is saturable and is competitively inhibited in the presence of other MeGalP substrates. Results of studies now in progress indicate that the K_m of substrate translocation in these mutants is about 1000 times higher than the K_m measured in MeGalP$^+$ cells, while the V_{max} of translocation observed in the *mgl B* mutants is the same as that in the MeGalP$^+$ parent.

A number of "periplasmic" binding proteins, including GBP, have been isolated from gram-negative bacteria after osmotic shock (26). It has frequently been proposed that the transport systems that depend on these binding proteins are multicomponent systems, requiring the activity of some additional protein(s) as well as of the respective binding protein (27). This hypothesis is consistent with the fact that active transport is a multistep process involving substrate recognition, translocation, and intracellular retention. In two systems, the high-affinity histidine transport system of *Salmonella typhimurium* (28) and MeGalP of *E. coli* K12 (7, 8), genetic evidence for the existence of additional components has been forthcoming. It is thus appropriate to ask whether the members of a multicomponent system are mutually dependent, in that inactivation of any individual component effects loss of all the transport functions. Our results indicate that in MeGalP, discrete steps in the process of active transport are catalyzed by individual components of the system; the activity of the complete system is required for neither substrate recognition nor translocation.

The finding that the *mgl A* and/or *mgl C* product is capable of recognizing substrate should prove useful in future attempts to identify and isolate these gene products. Moreover, comparison of the transport activities of *mgl B* and *mgl*$^+$ cells has significance for the question of the physiological role of GBP. We find that GBP is not required for translocation, yet we also observe that in the absence of active GBP the apparent affinity of the transport system for substrate decreases by several orders of magnitude. There is as yet insufficient information from which to derive the mechanism by which GBP mediates this increased affinity of the MeGalP. However, the results with an *mgl B* mutant S183-29 (Table 5), which exhibits no detectable substrate accumulation *in vivo* but whose binding protein shows significant activity *in vitro*, indicates that the binding of substrate by GBP is not sufficient to stimulate substrate accumulation. This mutant demonstrates that measurements of substrate binding *in vitro* provide only an incomplete assessment of activity of GBP *in vivo*. Indeed, it seems unlikely that the role of GBP will be fully understood until that protein can be studied *in vitro* in the presence of the other MeGalP components.

We thank J. Adler, W. Boos, W. Epstein, R. W. Hogg, A. Landy, M. H. Mallamy, G. W. Ordal, and D. Perrin for bacterial and bacteriophage strains. This work was supported by Grant AM-11194 from the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases; A.R.R. was supported by Training Grant AI-00418 from the National Institutes of Health.

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