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# Characterization of the *mgl* Operon of *Escherichia coli* by Transposon Mutagenesis and Molecular Cloning

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We used transposon insertion mutagenesis, molecular cloning, and a novel procedure for in vitro construction of polar and nonpolar insertion mutations to characterize the genetic organization and gene products of the β-methylgalactoside (Mgl) transport system, which utilizes the galactose-binding protein. The data indicate that the *mgl* operon contained three genes, which were transcribed in the order *mglB*, *mglA*, and *mglC*. The first gene coded for the 31,000 $M_r$ galactose-binding protein, which was synthesized as a 3,000-dalton-larger precursor form. The *mglA* product was a 50,000 $M_r$ protein which was tightly associated with the membrane, and the *mglC* product was a 38,000 $M_r$ protein which was apparently loosely associated with the membrane and was probably located on the internal face of the cytoplasmic membrane. Identification of gene products was facilitated by in vitro insertion of a fragment of Tn*5* containing the gene conferring kanamycin resistance into a restriction site in the operon. The fragment proved to have a polar effect on the expression of promoter-distal genes only when inserted in one of the two possible orientations. The three identified gene products were necessary and apparently sufficient for transport activity, but only the binding protein was required for chemotaxis towards galactose. The transport system appeared to contain the minimum number of components for a binding protein-related system: a periplasmic recognition component, a transmembrane protein, and a peripheral membrane protein that may be involved in energy linkage.

A large variety of water-soluble, ligand-binding proteins are found in the periplasm of gram-negative bacteria (17). It is likely that each of these proteins is a component of the transport system for the specific ligand (35), and thus binding protein-mediated uptake represents an important mechanism for transport into bacterial cells. In the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, three sugar-binding proteins are involved in a second process in addition to transport; each functions as a specific chemoreceptor, interacting with the chemotactic sensory system which controls motility (16).

Genetic analyses have revealed that binding protein-related transport systems are encoded by more than one gene. For the most extensively studied systems, those transporting histidine (3, 19a), maltose (5, 32, 33), and leucine (R. C. Landick, C. J. Daniels, P. Mauromara, and D. L. Oxender, Fed. Proc. **40**:1894, 1981), gene products in addition to the respective binding proteins have been detected and shown to be membrane associated.

The galactose-glucose-binding protein has been the focus of considerable interest since the initial observation of periplasmic proteins. It was one of the earliest binding proteins to be identified and purified (4), the first, along with the histidine protein (2), for which convincing evidence was presented for its essential role in transport (6), and the first chemoreceptor protein to be identified (14). Mutations causing defective functioning of the β-methylgalactoside (Mgl) transport system were divided by Ordal and Adler (25) into three complementation groups. Mutations in *mglB* define the gene coding for galactose-binding protein. The available data suggested that the *mglA* and *mglC* complementation group could represent either two distinct genes, the interpretation favored by Ordal and Adler (25), or intracistronic complementation of mutations within a single gene. Robbins (29) identified *mglD* mutations with the properties of operator mutations, rendering constitutive the expression of an operon consisting of *mglA*, *mglB*, and *mglC*.

Many *mglA*, *mglC*, and *mglAC* mutants exhibit normal galactose taxis but defective galac-

tose uptake (26), confirming an earlier conclusion that transport of the sugar is not required for chemoreception (1). However, a substantial number of *mglA*, *mglC*, and *mglAC* mutants are vastly defective in tactic response to galactose (26), raising the possibility that those gene products are required for normal chemoreception in a manner independent of the transport function.

We used transposon insertion mutagenesis, molecular cloning, and in vitro manipulation to define and characterize the *mgl* operon and its products.

## MATERIALS AND METHODS

**Materials.** [$^{35}$S]methionine (>1,000 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels) and D-[1-$^{14}$C]galactose (56.5 mCi/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Restriction endonucleases were from Takara Ltd., Kyoto, Japan. All sugars used were D-forms.

**Bacterial strains and plasmids.** The strains used in this work are all derivatives of *Escherichia coli* K-12 and are listed, along with the plasmids used, in Table 1.

**General methods.** Procedures for cell culture, assay of transport by filtration through 0.45-μm pore-size filters, assay of tactic response of tethered cells to temporal gradients, and preparation of periplasmic proteins by a "minishock" method have all been described previously (15). Techniques for conventional genetic manipulation, cloning, in vitro manipulation of plasmids, mapping of restriction endonuclease sites, preparation of minicells, analysis of proteins synthesized in minicells, limited proteolysis, gel electrophoresis, and autoradiography were all performed as previously outlined (12). Immune precipitation of trichloroacetic acid-precipitated proteins synthesized in minicells was performed as described in reference 21.

**Complementation and testing of Mgl phenotype.** Each transposon insertion was introduced into strain EJ502, which is His$^-$; the resulting strains were made *recA*, and the appropriate F-prime was introduced by mating and selection for His$^+$ Str$^r$. The Mgl phenotype was determined on chemotactic swarm plates containing 50 μM galactose. For transport tests or osmotic shock, cells were grown in minimal salts-glycerol plus 1 mM fucose. Initial rates of uptake were determined for 30 s at 30°C.

**Fractionation of minicells.** The procedure for fractionating minicells closely resembled that used previously (24). Proteins synthesized by 0.8 optical density units of minicells (equivalent to $4 \times 10^8$ whole cells) were labeled with [$^{35}$S]methionine (12) in the presence or absence of 1 mM fucose. Synthesis was halted by cooling the labeled minicells to 4°C. The minicells were removed from the incubation solution by centrifugation for 10 min in an Eppendorf centrifuge (8,000 × *g*) and then subjected to osmotic shock under the conditions previously described (15). Shocked minicells were suspended in 350 μl of 30 mM Tris-hydrochloride (pH 8.0)–5 mM EDTA–1 mg of lysozyme per ml, incubated at 35°C for 60 min, frozen at −20°C, and then thawed at room temperature. The suspension was cooled in an ice-salt bath and sonicated four times for 30 s each, with a 60-s pause between each 30-s sonication. Unbroken minicells were removed from the suspension by centrifugation for 2 min in the Eppendorf centrifuge. The suspension was separated into supernatant and pellet by centrifugation at 40,000 rpm in a 42.2 Beckman rotor (200,000 × *g*) for 2.5 h.

TABLE 1. Bacterial strains and plasmids[a]

| *E. coli* strain | Genotype and source (reference) |
|---|---|
| EJ502 | F$^-$ *his rpsL* (derived from W3110) |
| UH925 | F$^-$ *minA minB mglB109*::Tn*5 recA srl*::Tn*10 rpsL* |
| TH1344 | F131(*mglA22*) *his rpsL mglB107*::Tn*5 recA* (derivative of EJ502) |
| TH1345 | F131(*mglC31*) *his rpsL mglB107*::Tn*5 recA* (derivative of EJ502) |
| F131 | F′ carrying *his* and *mgl* from J. Adler (25) |
| F131(*mglA22*) | J. Adler (25) |
| F131(*mglB5*) | J. Adler (25) |
| F131(*mglC31*) | J. Adler (25) |

[a] For plasmids isolated or constructed in this work, see Fig. 1.

## RESULTS

**Insertion mutations in *mgl*.** From a collection described previously (15), we identified eight Tn*5* and seven Tn*10* insertion mutations which conferred an Mgl$^-$ phenotype, which is the inability to form a chemotactic ring on a semisolid agar swarm plate containing 50 μM galactose (25).

**Complementation of *mgl* insertion mutations.** We used strain F131, which carries the *mgl* region, as well as derivatives of that F-prime carrying representative *mglA*, *B*, and *C* mutations (25), to determine the complementation patterns of Mgl$^-$ transposon insertion mutations. One insertion, *mgl-109*::Tn*5*, conferred an MglB$^-$A$^-$C$^-$ phenotype (for clarity, we list the genes in the operon order determined below); another insertion, *mgl-114*::Tn*5*, conferred an MglC$^-$ phenotype; and the remaining thirteen insertions all conferred an MglB$^+$A$^-$C$^-$ phenotype. These results were consistent with a gene order in the operon of *mglB-A-C*.

**Phenotypes of *mgl* insertion mutants.** Ordal and Adler (26) showed that all classes of *mgl* mutants are defective in the transport of galactose present at micromolar concentrations. Mgl$^-$ strains are not Gal$^-$, since several other systems transport galactose at the concentration (10 mM) present in conventional growth media (31). Each of the *mgl* insertion mutants was vastly defective in the ability to take up galactose from a 1 μM solution, with initial uptake rates less than 5% of those in the wild-type parent.

Each mutant was examined for the presence

of galactose-binding protein in the periplasm by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of proteins released by cold osmotic shock (17). No band corresponding to authentic galactose-binding protein was observed among the proteins released from a strain carrying *mgl-109*::Tn*5*, whereas all other *mgl* insertions mutants exhibited essentially wild-type levels of galactose-binding protein. These results were consistent with the observed patterns of complementation.

Transposons block expression of the gene within which they are inserted as well as the expression of promoter-distal genes (23), and thus the tactic behavior of appropriate *mgl* insertion mutants should reflect the null phenotype of *mglAC* mutations. The response to temporal gradients of 10 mM galactose by cells carrying *mglA110*::Tn*5*, which complemented neither *mglA* nor *mglC* mutations, was indistinguishable from the response of the wild-type parent strain (data not shown), indicating that chemoreception occurred normally in the complete absence of the *mglA* and *mglC* products (see below). This observation eliminated the possibility that either the MglA or MglC protein performed any necessary role in the chemoreception of galactose.

**Cloning the *mgl* region.** We isolated several ColE1 hybrid plasmids that complemented *mglB109*::Tn*5*, which complemented none of the three classes of *mgl* mutations, from a pooled collection of strains constructed by Clarke and Carbon (7). Since those plasmids were able to provide the Mgl function for a mutant lacking all Mgl complementation activity, the cloned DNA was likely to contain the entire *mgl* operon. Analysis by restriction endonucleases and agarose gel electrophoresis revealed that two $Mgl^+$ hybrid plasmids, pUH19 and pUH21, contained 15 and 18 kilobase pairs (kb), respectively, of chromosomal DNA and that 13 kb of that DNA was common to both. The mutations *mglA107*::Tn*5* and *mglA110*::*Tn5* were each introduced by recombination into pUH19, creating plasmids pTH39 and pTH42, respectively. Determination of the location of restriction endonuclease sites on those three plasmids (Fig. 1) allowed identification of the *mgl* region along the cloned DNA. Two pieces of DNA from pUH19, both of which contained the sites of the *mglA* insertion mutations, were cloned into pBR322 (Fig. 1). pTH76, which carried the 11-kb *Pst*I fragment of pUH19, complemented *mglB109*::Tn*5* in a *recA* strain, and thus should carry the complete *mgl* operon. PTH62, which carried the 11-kb *Eco*RI-*Hin*dIII fragment of pUH19, did not complement either *mglB109*::Tn*5* or *mglB* point mutations (25), but did complement *mglA* and *mglC* mutations (25) in appropriate *recA* hosts. Thus, all of *mglA* and *mglC*, but not *mglB*, should be to the right of the *Eco*RI site (Fig. 1).

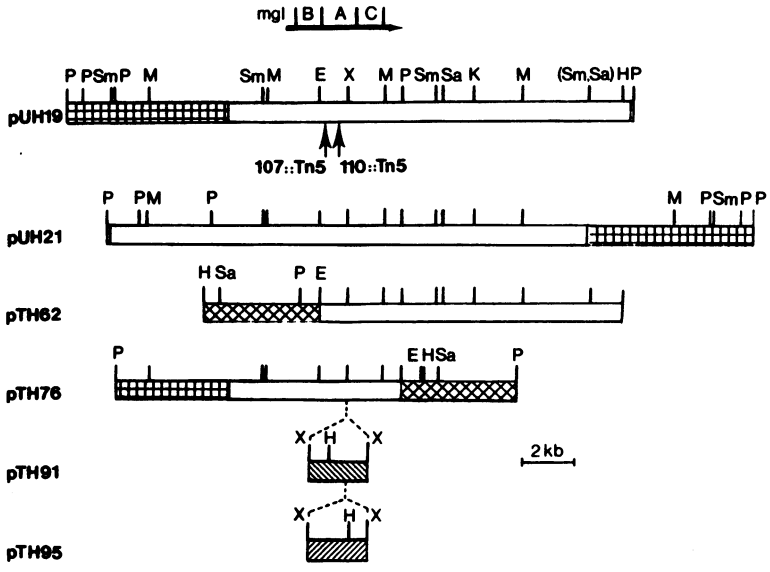


FIG. 1. Physical maps of plasmids carrying the *mgl* operon. Symbols: checkerboard areas, ColE1 DNA; crosshatched areas, pBR322 DNA; diagonally lined areas, Tn*5* DNA; blank areas, cloned chromosomal DNA. Restriction endonuclease sites are labeled as follows: P, *Pst*I; Sm, *Sma*I; M, *Mlu*I; E, *Eco*RI; X, *Xho*I; Sa, *Sal*I; K, *Kpn*I; H, *Hin*dIII. The deduced placement of the *mgl* operon is indicated at the top, with the length of the genes corresponding to the sizes of the products.

We presume that expression of *mglA* and *mglC* in pTH62 was from a promotor carried on the vector (see below).

**Expression of *mgl* products from cloned genes.** Cells carrying *mglB109*::Tn*5* on the chromosome but also containing pUH19, pUH21, pTH76, or pTH42 exhibited a significantly higher content of galactose-binding protein than did normal strains, as revealed by a predominant band corresponding to that protein seen in patterns of total cell protein on Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gels (data not shown). These observations imply that an increased dosage of *mglB* resulted in an increased cellular content of galactose-binding protein.

To identify the other *mgl* products, we introduced appropriate plasmids into minicell-producing strains and examined the [$^{35}$S]methionine-labeled products synthesized in minicells. As expected, *mglB*$^+$ plasmids directed the synthesis of galactose-binding protein, identified by comigration with authentic protein (Fig. 2) through characteristic release from minicells by cold osmotic shock (see below) and immune precipitation (data not shown). It was previously noted that in minicells containing plasmids carrying a gene for an exported protein, a significant proportion of the protein synthesized is present in its precursor form, 2,000 to 3,000 daltons larger than the mature protein (20). This is probably the result of defective processing of precursor forms in minicells (28). A polypeptide approximately 3,000 daltons larger than mature galactose-binding protein was synthesized in minicells containing *mglB*$^+$ plasmids (Fig. 2) and was present in increased amounts relative to galactose-binding protein when synthesis occurred in the presence of 50 μM carbonyl cyanide-*m*-chlorophenyl hydrazone, an uncoupler known to inhibit proteolytic processing (10) of precursor forms (data not shown). The polypeptide was specifically precipitated by anti-galactose-binding protein serum and thus is related to the galactose-binding protein and is probably the precursor form.

In addition to galactose-binding protein and its precursor form, there were two bands, of 38,000 and 50,000 apparent $M_r$, respectively, which exhibited the properties of Mgl proteins. All four bands were synthesized in minicells containing the plasmids pUH19 (Fig. 2, lanes 1 and 2), pUH21 (data not shown), and pTH76 (Fig. 2, lanes 9 and 10), which complementation studies indicated should contain a complete *mgl* operon. Furthermore, the amount of each of the four polypeptides synthesized was increased in the presence of 1 mM fucose, a characteristic inducer (31) of the *mgl* operon (Fig. 2). In minicells containing pTH42, in which *mglA* and *C* were

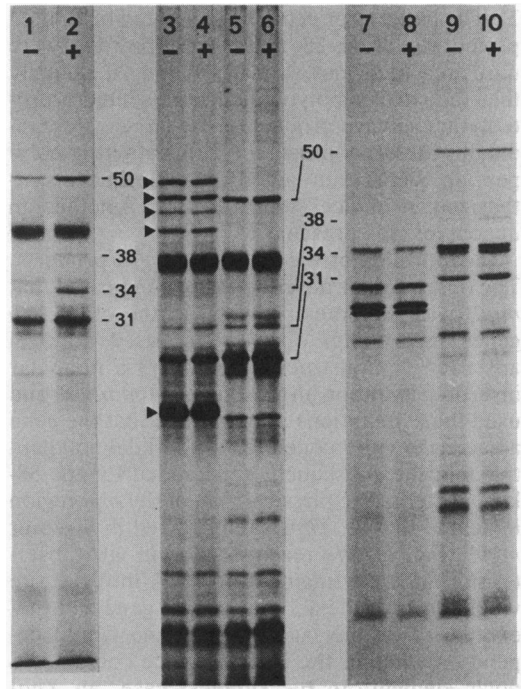


FIG. 2. Proteins synthesized in minicells containing plasmids carrying the *mgl* operon. The three panels are autoradiograms of sodium dodecyl sulfate-polyacrylamide (12, 10, and 14%, respectively) gels containing [$^{35}$S]methionine-labeled proteins. Products coded for by the *mgl* operon are indicated by numbers (in kdal). The band at 31-kdal corresponds precisely to the position of authentic galactose-binding protein. Products coded for by Tn*5* are indicated by arrowheads. For each plasmid, radioactive labeling of products was done in the absence (−) or presence (+) of inducer (1 mM fucose). Minicells from strain UH925 contained the following plasmids: (lanes 1, 2, 5, and 6) pUH19, a ColE1-*mgl* hybrid plasmid; (lanes 3 and 4) pTH42, which is pUH19 carrying *mglA10*::Tn*5*; (lanes 7 and 8) pBR322; and (lanes 9 and 10) pUH76, a pBR322-*mgl* hybrid plasmid.

inactivated by the insertion of *mglA110*::Tn*5*, neither the 38- nor the 50-kilodalton (kdal) protein was synthesized, although both galactose-binding protein and its precursor form were produced in normal amounts (Fig. 2, lanes 3 and 4). In minicells containing pTH62, which complemented *mglA* and *mglC* but not *mglB* mutations, the 50-kdal protein but not the 31-kdal galactose-binding protein was synthesized, although the level of synthesis was not influenced by fucose (data not shown). This was consistent with transcription from a promoter other than the usual *mgl* promoter (see above). Synthesis of the 38-kdal polypeptide was not detected, probably because the amount of that protein synthesized in uninduced conditions is quite low (Fig.

2). The patterns of peptides generated by limited proteolysis of the 38- and 50-kdal proteins were unrelated (data not shown), making it unlikely that the smaller polypeptide represented a proteolytic cleavage product of the larger. No candidates for larger precursor forms of either polypeptide were seen among the products synthesized in minicells regardless of whether an uncoupler was present.

**Identification of the *mglA* and *mglC* products.** The observations described above indicated that *mglB* was promoter proximal to the other two genes in the *mgl* operon. We used a novel approach to construct a polar and a nonpolar insertion mutation in the gene after *mglB* and used those mutations to determine that the gene adjacent to *mglB* coded for the 50-kdal polypeptide and the subsequent gene coded for the 38-kdal protein. The physical map of the *mgl* region indicated that an *Xho*I site occurred in *mglA* or *mglC* (Fig. 1). We reasoned that in vitro insertion of an *Xho*I fragment of DNA into the site would destroy the activity of that gene and that expression of any subsequent gene would depend on whether the inserted piece contained a polar element. In the simplest case, an *Xho*I fragment would exert a polar effect inserted in one orientation but not in the other. We found precisely this feature for the 2.4-kb *Xho*I fragment which contains the gene for neomycin phosphotransferase II from Tn*5* (30). Two derivatives of pTH76, pTH91 and pTH95, which each carried the *Xho*I fragment inserted in a different orientation, were identified by restriction endonuclease analysis of plasmids constructed in vitro (Fig. 1). Both insertion orientations eliminated production of the 50-kdal protein in minicells containing the plasmids (Fig. 3). In contrast, a substantial amount of the 38-kdal polypeptide was synthesized in minicells containing pTH91, whereas only a very small amount was synthesized in minicells containing pTH95 (Fig. 3). Synthesis of the 38-kdal protein directed by pTH91 was increased by the inducer fucose. We concluded that the *Xho*I site was located within the gene coding for the 50-kdal polypeptide and that the subsequent gene in the *mgl* operon coded for the 38-kdal polypeptide.

We determined that the 50- and 38-kdal proteins were the products of *mgl*A and *mgl*C, respectively, by complementation studies with F-primes carrying a representative *mglA* or *mglC* missense mutation. Derivatives of strain F131 carrying *mglA22* or *mglC31* (25) were introduced into a *recA* host containing *mglB109*::Tn*5*, creating strains with mutations in only *mglA* or *mglC*, respectively. The plasmids pTH91 and pTH95 were subsequently introduced into these mutants, and the activity of the Mgl transport system in the resulting strains was

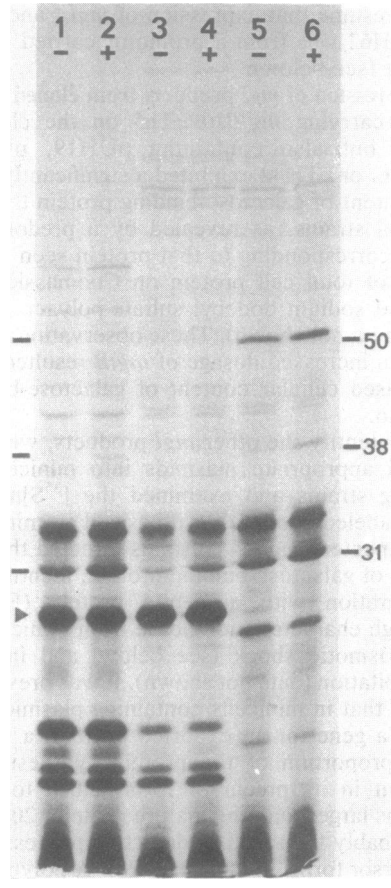


FIG. 3. Proteins synthesized in minicells containing *mgl* plasmids carrying an inserted *Xho*I fragment from Tn*5*. Procedures and symbols are described in the legend to Fig. 2. Only one protein, the neomycin phosphotransferase II (arrowhead), was synthesized from the insert. In this particular gel, the band of precursor galactose-binding protein was obscured by comigration with an outer membrane protein. On the original autoradiogram, a very faint band at 38 kdal was visible in lanes 3 and 4. The plasmids were pTH76 ($MglB^+A^+C^+$) in lanes 5 and 6; pTH91 ($MglB^+A^-C^+$) in lanes 1 and 2; and pTH95 ($MglB^+A^-C^-$) in lanes 3 and 4.

determined (Table 2). The absence of complementation of *mglA22* by either plasmid, strong complementation of *mglC31* by pTH91, and weak complementation of *mglC31* by pTH95 implied that the 50-kdal polypeptide, synthesized by neither plasmid, corresponded to the *mglA* product and that the 38-kdal polypeptide, synthesized extensively by pTH91 and weakly by pTH95, corresponded to the *mglC* product. The induction of transport activity by fucose corresponded to induction of synthesis of the 38-kdal polypeptide (Fig. 3).

**Localization of the Mgl proteins.** We fraction-

TABLE 2. Complementation of transport defects by Mgl plasmids[a]

| Host strain (phenotype) | Induction (1 mM fucose) | Initial rate of galactose uptake (pmol/min per μg of protein) | | |
|---|---|---|---|---|
| | | No plasmid | pTH91 | pTH95 |
| W3110 (Mgl$^+$) | − | 3.4 | ND[b] | ND |
| | + | 10 | ND | ND |
| TH1344 (MglA) | − | ND | 0.4 | 0.4 |
| | + | 0.4 | 0.4 | 0.4 |
| TH1345 (MglC) | − | ND | 3.6 | 1.0 |
| | + | 0.4 | 6.7 | 1.1 |

[a] Cells were grown in minimal medium containing glycerol with or without 1 mM fucose. Uptake was determined at 25°C with 2 μM galactose.

[b] ND, Not determined.

ated minicells containing [$^{35}$S]methionine-labeled Mgl proteins to determine in which compartment each of the three gene products was located (Fig. 4). The majority of the galactose-binding protein was released by osmotic shock, as was expected for a protein located in the periplasm. Both the 38- and 50-kdal proteins were present in the particulate fractions, which indicated that the gene products were probably membrane proteins. However, most of the 38-kdal protein was found in the soluble fraction, which implied that the protein was only loosely attached to the membrane.

## DISCUSSION

**Genetic organization.** The polarity of the sequence promoter-*mglB-mglA-mglC* deduced from our results was consistent with the identification by Ordal and Adler (25) of mutations with complementation patterns *mglBAC* and *mglAC* but not any other combinations, as well as with the sequence operator-*mglB*-(*mglAC*) suggested by the results of three-point crosses (29). The lack of complementation between any *mglAC-mglA* pairs and moderate degrees of complementation between several *mglAC-mglC* pairs observed by Ordal and Adler (25) could be explained if *mglAC* mutations were located in *mglA* and had varying degrees of polarity on *mglC*. We do not understand why the results of a previous cotransduction study (29) imply the order *mglC-mglA*, the opposite of that indicated by our results. However, since the only available phenotypic distinction between *mglA* and *mglC* mutations is the product identification documented here, we suggest that the order of *mgl* genes is best defined by the present studies.

**How many components in Mgl?** There was coding capacity for an additional 40-kdal protein after *mglC* on pTH76 (Fig. 1); however, there was no indication of an additional *mgl*-related gene from complementation studies or from characterization of products expressed from *mgl* hybrid plasmids. The most conservative conclusion is that only three polypeptides are involved in the transport of galactose across the cytoplasmic membrane via the Mgl system. The three other binding protein-related transport systems that have been characterized in sufficient detail all comprise more components than does Mgl. The transport of maltose across the cytoplasmic membrane requires the activity of one gene for the binding protein (22), two genes for membrane proteins (5, 32, 33), and one gene for an unidentified product (34). Recent observations raised the possibility that there may be yet

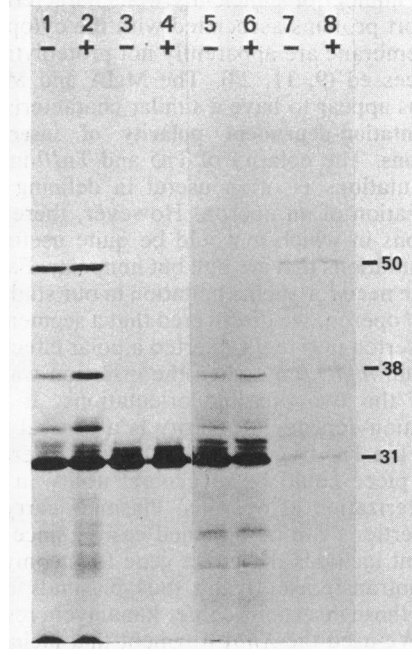


FIG. 4. Fractionation of Mgl polypeptides into different cellular compartments. After proteins were synthesized in the presence of [$^{35}$S]methionine, minicells containing pTH76 were fractionated. Numbers and symbols are described in the legend to Fig. 2. Lanes 1 and 2, Total minicells; lanes 3 and 4, periplasmic fractions released by osmotic shock; lanes 5 and 6, soluble fractions; lanes 7 and 8, membrane fractions pelleted by high-speed centrifugation. Equivalent amounts of material were applied to each lane. A significant proportion of minicells were not broken by the lysis procedure, and those cells were removed by a clarification step (see the text). For this reason, the intensity of the bands in lanes 5–8 was lower than in the initial sample. However, the amount of material from the clarified suspensions in lanes 5 and 6 was equivalent to the amount in lanes 7 and 8, allowing conclusions to be drawn about the distribution of polypeptides between the soluble and pelleted fractions.

another component in the maltose system (8). Genes coding for the histidine transport system include two for histidine- and lysine-arginine-orithine-binding proteins (18), respectively, two genes coding for membrane proteins (3, 19), and an additional gene whose product is unknown (19a). The leucine transport system involves a leucine-specific and a leucine-isoleucine-valine-binding protein (27) and at least two other gene products, one of which has been identified as a membrane protein (R. C. Landick et al., Fed. Proc. 40:1894, 1981).

**Biosynthetic precursor forms.** The identification of a larger precursor form of galactose-binding protein added another example to the long list of exported proteins which are synthesized as longer precursors (20). In contrast, the limited number of sufficiently well characterized transport proteins associated with the cytoplasmic membrane are apparently not proteolytically processed (9, 11, 24). The MglA and MglC proteins appear to have a similar characteristic.

**Orientation-dependent polarity of insertion mutations.** The polarity of Tn*5* and Tn*10* insertion mutations is often useful in defining the organization of an operon. However, there are situations in which it would be quite useful to have mutations that are null but nonpolar. Faced with the need for such a mutation in our study of the *mgl* operon, we discovered that a segment of Tn*5* inserted into *mglA* exerted a polar effect on the distal *mglC* only when the insertion was in one of the two possible orientations. If this orientation-dependent polarity is a general feature of the Tn*5* fragment, then in vitro insertion of the piece could be of general utility in the characterization of operons. Plasmids carrying the insertions can be obtained easily, since the fragment includes the intact gene for neomycin phosphotransferase II and thus plasmids containing these insertions confer kanamycin resistance. We used the *Xho*I fragment that included the resistance gene, but the region is also bracketed by *Bgl*I, *Pst*I, and *Hin*dII sites, and thus those fragments are also good candidates for possible orientation-dependent polarity.

**Localization of Mgl proteins.** The MglA protein was found almost exclusively in the membrane fraction of broken minicells, indicating that the protein was strongly associated with a membrane and a good candidate for an integral protein of the cytoplasmic membrane. In contrast, only some of the MglC protein was associated with the membrane fraction, whereas a greater amount was found in the soluble fraction. We interpreted these observations to mean that the MglC protein is weakly associated with the membrane, so that the fractionation procedure resulted in the release of much of it from the membrane. It is tempting to draw a parallel to the MalK protein, a component of the maltose transport system which is about the same size (40 kdal) as MglC. MalK associates with the cytoplasmic membrane only if the *malG* gene is active (32). Thus, the polypeptide appears to be a peripheral membrane protein, adhering to the membrane only as a result of interaction with MalG.

**Mgl system in transport and taxis.** The observations documented here lead us to suggest that the transport of sugars by the high-affinity Mgl system requires the functioning of only three proteins. The galactose-binding protein is a hydrophilic recognition component which binds the substrates glucose and galactose with high affinity. The MglA protein is firmly membrane bound and probably spans the cytoplasmic membrane, providing a pathway for the passage of the hydrophilic substrate through the hydrophobic permeability barrier by direct interaction with occupied binding protein on the periplasmic face of the membrane. The MglC protein may adhere to the inner face of the cytoplasmic membrane as a result of its direct interaction with a domain of the MglA protein that is exposed to the cytoplasm. In analogy to the functional role suggested for the MalK protein (32), MglA may serve as the link to the energy supply for this active transport system.

In contrast to transport, chemotaxis toward galactose requires only one of the three *mgl* products, the binding protein. The presence of the gene for the binding protein in an operon of transport system components, rather than in an operon of chemotaxis system components, classifies the protein as primarily a transport recognition component. This suggests that recruitment of the transport protein as a chemoreceptor was an event which occurred after the initial development of the bacterial sensory system.

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## ADDENDUM IN PROOF

In a recent publication, Rotman and Guzman (J. Biol. Chem. 257:9030–9034, 1982) concluded that the order of the *mgl* genes was the same as that established here and, consistent with our findings, identified the *mglA* product as a 52-kdal, membrane-associated protein.

## LITERATURE CITED


1. **Adler, J.** 1969. Chemoreceptors in bacteria. Science **166**:1588–1597.
2. **Ames, G. F.-L., and J. E. Lever.** 1972. The histidine-binding protein J is a component of a histidine transport. J. Biol. Chem. **247**:4309–4316.
3. **Ames, G. F.-L., and K. Nikaido.** 1978. Identification of a membrane protein as a histidine transport component in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. **75**:5447–5451.
4. **Anraku, Y.** 1968. Transport of sugars and amino acids in bacteria. I. Purification and specificity of the galactose- and leucine-binding proteins. J. Biol. Chem. **243**:3116–3122.
5. **Bavoil, P., M. Hofnung, and H. Nikaido.** 1980. Identification of a cytoplasmic membrane-associated component of the maltose transport system in *Escherichia coli*. J. Biol. Chem. **255**:8366–8369.
6. **Boos, W.** 1972. Structurally defective galactose-binding protein isolated from a mutant negative in the β-methylgalactoside transport system of *Escherichia coli*. J. Biol. Chem. **247**:5414–5424.
7. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *Escherichia coli* genome. Cell **9**:91–99.
8. **Clément, J.-M., C. Braun-Breton, E. Lepouce, C. Marchal, D. Perrin, H. Villarroya, and M. Hofnung.** 1982. A system for genetic analysis in gene *lamB*: first results with λ-resistant *tight* mutants. Ann. Microbiol. (Paris) **133**(Suppl. A):9–20.
9. **Ehring, R., K. Beyreuther, J. K. Wright, and P. Overath.** 1980. *In vitro* and *in vivo* products of *E. coli* lactose permease gene are identical. Nature (London) **283**:537–540.
10. **Eneqvist, H. G., T. R. Hirst, S. Harayama, S. J. S. Hardy, and L. L. Randall.** 1981. Energy is required for maturation of exported proteins in *Escherichia coli*. Eur. J. Biochem. **116**:227–233.
11. **Gunsalus, R. P., W. S. A. Brusilow, and R. D. Simoni.** 1982. Gene order and gene-polypeptide relationships of the proton-translocating ATPase operon (*unc*) of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **79**:320–324.
12. **Harayama, S., P. Engström, H. Wolf-Watz, T. Iino, and G. L. Hazelbauer.** 1982. Cloning of *trg*, a gene for a sensory transducer in *Escherichia coli*. J. Bacteriol. **152**:372–383.
13. **Harayama, S., and G. L. Hazelbauer.** 1982. A gene coding for a periplasmic protein is located near the locus for termination of chromosome replication in *Escherichia coli*. J. Bacteriol. **151**:1391–1396.
14. **Hazelbauer, G. L., and J. Adler.** 1971. Role of the galactose-binding protein in chemotaxis of *Escherichia coli* toward galactose. Nat. New Biol. **230**:101–104.
15. **Hazelbauer, G. L., and S. Harayama.** 1979. Mutants in transmission of chemotactic signals from two independent receptors of *E. coli*. Cell **16**:617–625.
16. **Hazelbauer, G. L., and J. S. Parkinson.** 1977. Bacterial chemotaxis, p. 61–98. *In* J. L. Reissig (ed.), Microbial interactions. Chapman & Hall, London.
17. **Heppel, L. A.** 1971. The concept of periplasmic enzymes, p. 223–247. *In* L. I. Rothfield (ed.), Structure and function of biological membranes. Academic Press, Inc., New York.
18. **Higgins, C. F., and G. F.-L. Ames.** 1981. Two periplasmic transport proteins which interact with a common membrane receptor show extensive homology: complete nucleotide sequences. Proc. Natl. Acad. Sci. U.S.A. **78**:6038–6042.
19. **Higgins, C. F., and G. F.-L. Ames.** 1982. Regulatory regions of two transport operons under nitrogen control:
19a. **Higgens, C. F., P. D. Haag, K. Nikaido, F. Ardeshur, G. Gareia, and G. Ferro-Luzzi Ames.** 1982. Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium*. Nature (London) **298**:723–727.
nucleotide sequences. Proc. Natl. Acad. Sci. U.S.A. **79**:1083–1087.
20. **Inouye, M., and S. Halegoua.** 1980. Secretion and membrane localization of proteins in *Escherichia coli*. Crit. Rev. Biochem. **7**:339–371.
21. **Josefsson, L.-G., and L. L. Randall.** 1981. Processing *in vivo* of precursor maltose-binding protein in *Escherichia coli* occurs post-translationally as well as co-translationally. J. Biol. Chem. **256**:2504–2507.
22. **Kellermann, O., and S. Szmelcman.** 1974. Active transport of maltose in *Escherichia coli* K12. Involvement of a "periplasmic" maltose binding protein. Eur. J. Biochem. **47**:139–149.
23. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. J. Mol. Biol. **116**:125–159.
24. **Lee, C. A., G. R. Jacobson, and M. H. Saier, Jr.** 1981. Plasmid-directed synthesis of enzymes required for D-mannitol transport and utilization in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:7336–7340.
25. **Ordal, G. W., and J. Adler.** 1974. Isolation and complementation of mutants in galactose taxis and transport. J. Bacteriol. **117**:509–516.
26. **Ordal, G. W., and J. Adler.** 1974. Properties of mutants in galactose taxis and transport. J. Bacteriol. **117**:517–526.
27. **Oxender, D. L., J. J. Anderson, C. J. Daniels, R. Landick, R. P. Gunsalus, G. Zurawski, E. Selker, and C. Yanofsky.** 1980. Structural and functional analysis of cloned DNA containing genes responsible for branched-chain amino acid transport in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:1412–1416.
28. **Palva, E. T., T. R. Hirst, S. J. S. Hardy, J. Holmgren, and L. L. Randall.** 1981. Synthesis of a precursor to the B subunit of heat-labile enterotoxin in *Escherichia coli*. J. Bacteriol. **146**:325–330.
29. **Robbins, A. R.** 1975. Regulation of the *Escherichia coli* methylgalactoside transport system by gene *mglD*. J. Bacteriol. **123**:69–74.
30. **Rothstein, S. J., R. A. Jorgensen, K. Postle, and W. S. Reznikoff.** 1980. The inverted repeats of Tn*5* are functionally different. Cell **19**:795–805.
31. **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. **16**:247–260.
32. **Shuman, H. A., and T. J. Silhavy.** 1981. Identification of the *malK* gene product, a peripheral membrane component of the *Escherichia coli* maltose transport system. J. Biol. Chem. **256**:560–562.
33. **Shuman, H. A., T. J. Silhavy, and J. R. Beckwith.** 1980. Labeling of proteins with β-galactosidase by gene fusion. Identification of a cytoplasmic membrane component of the *Escherichia coli* maltose transport system. J. Biol. Chem. **255**:168–174.
34. **Silhavy, T. J., E. Brickman, P. J. Bassford, M. J. Casadaban, H. A. Shuman, V. Schwartz, L. Guarente, M. Schwartz, and J. R. Beckwith.** 1979. Structure of the *malB* region in *Escherichia coli*. II. Genetic map of the *malE, F, G* operon. Mol. Gen. Genet. **174**:249–259.
35. **Wilson, D. B., and J. A. Smith.** 1978. Bacterial transport proteins, p. 495–557. *In* B. P. Rosen (ed.), Bacterial Transport. Marcel Dekker, Inc., New York.