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Escherichia coli K-12 Mutants That Allow Transport of Maltose via the β-Galactoside Transport System

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We have isolated mutants of *Escherichia coli* that have an altered β-galactoside transport system. This altered transport system is able to transport a sugar, maltose, that the wild-type β-galactoside transport system is unable to transport. The mutation that alters the specificity of the transport system is in the *lacY* gene, and we refer to the allele as $lacY^{mal}$. The $lacY^{mal}$ allele was detected originally in strains in which the *lac* genes were fused to the *malF* gene. Thus, as a result of gene fusion and isolation of the $lacY^{mal}$ mutation, a new transport system was evolved with regulatory properties and specificity similar to those of the original maltose transport system. Maltose transport via the $lacY^{mal}$ gene product is independent of all of the normal maltose transport system components. The altered transport system shows a higher affinity than the wild-type transport system for two normal substrates of the β-galactoside transport system, thiomethyl-β-D-galactoside and *o*-nitrophenyl-β-D-galactoside.

The metabolism of the disaccharide lactose by *Escherichia coli* requires the products of two of the genes of the *lac* operon. The *lacZ* gene codes for the enzyme β-galactosidase, which catalyzes the intracellular hydrolysis of lactose to glucose and galactose. The *lacY* gene codes for a protein of the cytoplasmic membrane which is necessary and sufficient for the accumulation of lactose within energized cells. This β-galactoside transport system is the most thoroughly studied bacterial transport system (12, 19, 22). The *lacY* gene product, the M protein, has been identified by Fox, Kennedy, and co-workers as a protein with a molecular weight of 30,000 which is localized in the cytoplasmic membrane and binds various substrates of the transport system (5, 22). The substrate specificity of the β-galactoside transport system is very broad; many β-galactosides and α-galactosides are transported efficiently (13, 20).

In contrast, the accumulation of the disaccharide maltose and higher dextrins by *E. coli* is more complex. The products of the five genes in the *malB* region (Fig. 1) are involved in the normal function of this transport system. Two of these five components have been identified. The product of the *malE* gene is the periplasmic maltose-binding protein (11). The product of the *lamB* gene is an outer membrane protein that is the receptor for bacteriophage lambda and is required for the transport of maltose only at low concentrations and of maltodextrins at all concentrations (17, 24, 25). The products of the other genes *malF*, *malK*, and *malG* (7; T. J. Silhavy, unpublished data) have not yet been identified, although at least one of them must be associated with the cytoplasmic membrane and participate in the translocation of substrate across the membrane.

We wish to report a novel class of mutations in the *lacY* gene that extend the specificity of the β-galactoside transport system to include the sugar maltose, which is not a substrate for the wild-type β-galactoside transport system. As a result, strains which lack the four required components of the maltose transport system are able to utilize maltose as a carbon source if they contain a copy of this *lacY* mutation. We have named mutations of this class $lacY^{mal}$.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains are listed in Table 1.

Media and genetic techniques. Minimal medium 63, rich media, and indicator agar media have been described (6). F′ matings, P1 transductions, and phage induction and isolation were performed as described by Miller (16).

Isolation of transducing phages that carry the gene fusion. Lambda transducing phages carrying the *malF-lacZ* gene fusion were isolated from the strains containing the fusion by induction of the phage with UV light. Fresh colonies of the fusion strain were picked onto a lawn of indicator bacteria growing on a tryptone/yeast extract agar plate and irradiated with UV light. After incubation in the dark at 37°C for 18 h, zones of lysis could be seen where colonies of the

fusion strain had been deposited. Transducing phages were purified from this mixture of phage by streaking phage-containing material on a lawn of indicator bacteria that are deleted for the *lac* region and growing on minimal medium maltose agar plates which contain a chromogenic substrate of β-galactosidase, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XGal). The indicator bacteria grow and produce a white lawn because they contain a deletion of the structural gene, *lacZ*, for β-galactosidase. Phage that carry the gene fusion and the promoter of the *malEFG* operon (see Fig. 1) produce blue plaques because these phage allow synthesis of β-galactosidase which cleaves the XGal to

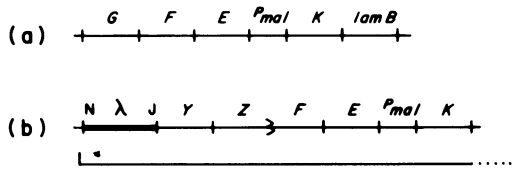

FIG. 1. *Structure of the malB region of E. coli K-12. (a) The wild-type region is composed of two operons that are transcribed in opposite directions (7). (b) The structure of the malE,F,G operon after genetic fusion of the lacZ and lacY genes to malF. The fusion joint is indicated by a wavy line. The lower line indicates genetic material carried by λpmalF-lacZ transducing phages (T. J. Silhavy et al., unpublished data). The gene fusion technique has been described (23). Pmal is the promoter for the two malB operons.*

produce the blue dye indigo. The blue plaques were purified twice and used to grow stocks of the transducing phages.

Construction of *lacI*$^+$ derivatives of *lacI*$^-$ *lacY*^{mal} strains. To isolate *lacI*$^+$ derivatives of *lacI*$^-$ *lacY*^{mal} strains, we transferred F plasmids carrying the *lacI*$^-$ *lacY*^{mal} genes into a strain which is *lacI*$^+$ and deleted for *lacZ* and *lacY* (X8504). After overnight growth of the merodiploid strains to allow recombination, the F′ plasmids were transferred into a strain with a deletion of the entire *lac* operon (X7131). Episomes which had recombined with the *lacI*$^+$ gene in strain X8504 were recognized as being inducible for β-galactosidase expression in strain X7131. That is, the *lacI*$^+$ bacteria were pale blue on glucose minimal agar plates containing XGal, whereas *lacI*$^-$ bacteria are very dark blue on these plates because they synthesize β-galactosidase constitutively.

Assays. β-Galactosidase activity in whole cells was assayed by the method of Miller (16). Entry of *o*-nitrophenyl-β-D-galactoside (ONPG) was measured to 30°C by incubating washed exponential cells (0.8 ml) with various concentrations of ONPG (0.2 ml). The ONPG that enters the cells is hydrolyzed by the intracellular β-galactosidase to galactose and *o*-nitrophenol, which is released into the medium. The reaction was terminated by the addition of 1 ml of ice-cold 1 M Na_2CO_3. Whole cells were removed by centrifugation, and the amount of *o*-nitrophenol in the supernatant fluid was determined spectrophotometrically at 420 nm. Samples assayed in the presence of thiodigalactoside, a specific inhibitor of the β-galactoside trans-

TABLE 1. *Bacterial strains*[a]

Strain	Genotype	Origin
MC4100	F$^-$ Δ*lacU169 araD139 rpsL thi*	Casadaban (4)
MC4417	F′ *metB*$^+$ *malE*$^+$*F*$^+$*G*$^+$/Δ*lacU169 araD139 rpsL thi* Φ(*malF-lacZ* hyb 11-1)	Silhavy (23)
HS6501	F′ *metB*$^+$ Φ(*malF-lacZ* hyb 11-1)/Δ*lacU169 araD139 rpsL thi* Φ(*malF-lacZ* hyb 11-1)	Homogenote of MC4417/KLF10
HS6503	F′ *metB*$^+$ Φ(*malF-lacZ* hyb 11-1 *lacY*^{mal})/Δ*lacU169 araD139 rpsL thi* Φ(*malF-lacZ* hyb 11-1)	This study
HS4600	F′ *lacI3 lacY*^{mal} *proAB*$^+$/Δ(*lac-pro*)*X111* Δ*malB101 rpsL thi rpoB*	This study
HS4511	F′ *lacI3 lacZ*$^+$ *lacY*$^+$ *proAB*$^+$/Δ(*lac-pro*)*X111* Δ*malB101 rpsL thi rpoB*	This study
HS4701	F′ *lacI3 lac*$^+$ *proAB*$^+$/Δ(*lac-pro*)*X111 malT rpsL thi*	This study
HS4702	F′ *lacI3 lacZ*$^+$ *lacY*^{mal} *proAB*$^+$/Δ(*lac-pro*)*X111 malT rpsL thi*	This study
HS2511	F′ *lacI3 lacZ*$^+$ *lacY*^{mal} *proAB*$^+$/*malF49* Δ*lacU169 araD139 rpsL thi rpoB*	This study
MGB75	F′ *lacY*::Tn*9*/*thi rha trkA trkD lacY*::Tn*9*	From J. A. Shapiro and L. MacHattie
MC4331	F$^-$ Δ*lacU169 araD139 malF*::Muc(Ts) *rpsL thi*	This study
HS6101	F$^-$ Δ(*lac-pro*)*X111 rpsL thi* Φ(*malF-lacZ* hyb 11-1 *lacY*^{mal})	This study
HS2503	F′ *lacI3 lac*$^+$ *proAB*$^+$/Δ*lacU169 araD139 rpsL rpoB thi* Δ*malB101*	This study
X8504	F$^-$ Δ(*lac-pro*)*X111* φ80d*lacI*$^+$Δ(*lacZ lacY*)	This study
X7131	F$^-$ Δ(*lac-pro*)*X111 rpsE thi*	This study
HS4005	F$^-$ Δ(*lac-pro*)*X111 rpsL malF8 thi*	This study

[a] Genetic nomenclature is from Bachmann et al. (2). The symbol Φ designates a gene fusion which is described in parentheses. For example, Φ(*malF-lacZ* hyb 11-1) is a gene fusion that produces a hybrid protein which has an amino-terminal region coded for by part of the *malF* gene and a carboxyl-terminal region coded for by part of the *lacZ* gene. The hyb designation refers to the fact that a hybrid protein is produced; 11-1 is the number assigned to the particular fusion.

port system, provided control values for entry of ONPG not mediated by the β-galactoside transport system.

Transport of thiomethyl-β-D-galactoside (TMG) by whole cells was measured by uptake of ^{14}C-labeled sugar. Washed exponential cells were incubated at room temperature with radioactive substrate, and samples were taken at various times, filtered on membrane filters (45-μm pore size; Millipore Corp.), and washed with 5 ml of medium 63 salts. The filters were dried and counted with Liquifluor counting fluid (New England Nuclear Corp.) at 89% efficiency in a Packard scintillation counter.

Chemicals. Medium components were obtained from Difco. All chemicals were of the highest grade. ONPG, thiodigalactoside, and TMG were obtained from Sigma Chemical Co. [^{14}C]TMG was purchased from New England Nuclear Corp.

RESULTS

The *lacY*^{mal} mutations were initially detected in strain HS6501 in which the *lacZ* and *lacY* genes were genetically fused to one of the genes of the *malB* region, *malF* (Fig. 1). In strains which carry *mal-lac* fusions, the expression of the *lac* genes is determined by the promoter and regulatory components of the maltose system. As a result, the expression of the *lac* genes is maximal in the presence of maltose, the inducer of the maltose system. In this particular strain, the *lacZ* and *malF* genes are fused such that a hybrid protein is produced which is comprised of an amino-terminal portion of the *malF* gene product and all but a small portion of the carboxy-terminal portion of β-galactosidase. This hybrid retains β-galactosidase activity (23). This strain is unable to utilize maltose as a carbon source since the *malF* and *malG* genes have been inactivated. At the same time, elimination of the maltose transport system results in a considerably higher level of expression of the *mal* operon compared to the uninduced level of wild-type Mal$^+$ bacteria (8). The mechanism for this constitutive expression of *mal* genes in mutants deficient in maltose transport is not understood. The resulting constitutive expression of the *lacZ* and *lacY* genes in this strain is sufficient to allow growth on lactose. This is a crucial property of these strains, since, as we show below, the detection of the *lacY*^{mal} mutations requires constitutive synthesis of the *lacY*^{mal} gene product.

Isolation of the lacY^{mal} mutant. The experiments which led to the isolation of the *lacY*^{mal} mutants were initiated because of our interest in the purification of the hybrid protein product of the *malF-lacZ* gene fusion. To increase the amount of this protein in the cell, we had constructed a strain, HS6501, which is diploid for the fusion, having copies on both the chromosome and an F plasmid. The synthesis of the hybrid protein, although somewhat constitutive, was still not maximal since the absence of the maltose transport system does not allow entry of maltose and induction of the operon. For this reason, we sought mutants of strain HS6501 which could utilize maltose and thus permit maximal induction in the fusion strains.

For selection of Mal$^+$ mutants, approximately 10^9 bacteria of strain HS6501 were spread on minimal agar plates containing 0.4% maltose. After 1 week of incubation at 37°C, one to three colonies appeared on plates from six independent cultures. One Mal$^+$ isolate was chosen for further study. This strain, HS6503, grows on maltose minimal agar plates and has a doubling time of 1.5 h in liquid minimal media containing maltose.

The effect of maltose in the growth media on the level of β-galactosidase activity is shown in Table 2. The level of hybrid β-galactosidase activity present in strain HS6503 is 10-fold higher than that found in strain MC4417. This 10-fold increase in specific activity of the hybrid protein has greatly facilitated its purification (H. A. Shuman, unpublished data).

The mutation is located in the malB region of the fusion strain. We considered several possible explanations for the mutation which yielded the Mal$^+$ phenotype. (i) The mutation altered a gene for another transport system, extending its specificity to maltose. (ii) The mutation resulted in constitutive expression of another hitherto undetected maltose transport system which is normally not expressed. (iii) The mutation altered one of the remaining components of the normal maltose transport system so as to obviate the need for the *malF* and *malG* products. (iv) The mutation altered the *malF-lacZ* hybrid protein so as to generate a protein active in transport. (v) The mutation altered the *lacY* gene to extend the specificity of its protein product to maltose. With explanations i and ii, the mutation would, in all probability, be unlinked to the *malB* region.

To determine whether the Mal$^+$ phenotype was due to a mutation linked to the *lacZ-malF* fusion, we transduced the fusion into a different strain background. Since the original fusion strain was constructed in such a way that the bacteriophage genome is inserted adjacent to the *lacZ-malF* fusion, it is possible to isolate λ transducing phages which carry the fusion and a portion of the *malB* region. Strain HS6503 was irradiated with UV light to induce the λ phage. Transducing phages which carried the fusion were detected as described in Materials and Methods.

TABLE 2. *β-Galactosidase activity in strains containing the malF-lacZ gene fusion*

Strain	Mal phenotype	Medium	Activity[a]
MC4417	Mal$^+$ (F′ *malB*$^+$/F$^-$ *malF-lacZ*)	M63 gly	4
		M63 mal	111
HS6501	Mal$^-$ (fusion/fusion) (F′ *malF-lacZ*/F$^-$ *malF-lacZ*)	M63 gly	112
		M64 gly mal	59[b]
HS6503	Mal$^+$ (revertant) (F′ *malF-lacZ*/F$^-$ *malF-lacZ*)	M63 gly	46
		M63 gly mal	370
		M63 mal	600
		M63 gly	134[c]
		M63 mal	1,298[c]

[a] One unit of β-galactosidase is that amount of enzyme which hydrolyzes 1 nmol of ONPG per min per mg of protein.

[b] The lower amount of constitutive expression in *malB* strains when grown in the presence of maltose is not understood.

[c] These values were obtained with cultures that were in early stationary phase. This increase in activity is not understood.

Since strain HS6503 is diploid for the fusion, induction of lambda could lead to production of phages from both copies of the *malB* region in each cell. Therefore, even if the Mal$^+$ phenotype was due to alteration of a gene that could be transduced by the phage, only some fraction of the transducing particles could be expected to transduce a *malF* recipient to Mal$^+$. When eight independent λ*malF-lacZ* transducing phages were isolated from both HS6501 and HS6503, it was found that none of the eight phages isolated from HS6501 was able to transduce a *malF* strain (MC4331) to Mal$^+$, whereas three of the eight phages isolated from HS6503 transduced this recipient to Mal$^+$. The *malF* strain that was used as a recipient was *malF*::Muc(Ts). Because of the location of the Mu insertion, the *malF* region in this strain cannot recombine with the *malF* region carried by the λ*malF-lacZ* transducing phages to give an intact *malF* gene. This result indicated that the Mal$^+$ phenotype was due to alteration of a gene that is carried by the λ*malF-lacZ* transducing phage, thus making explanations i and ii highly unlikely.

A second mutation in the *lacY* gene eliminates the Mal$^+$ phenotype. We next proceeded directly to determine whether the Mal$^+$ phenotype was due to a mutation in the *lacY* gene. If this were the case, then inactivation by further mutation of the altered *lacY* gene should result in a Mal$^-$ phenotype. We have chosen to use an insertion of a translocon carrying chloramphenicol resistance, Tn*9*, in the *lacY* gene (J. A. Shapiro, personal communication) to test this possibility.

To facilitate selection of Tn*9* insertions in the *lacY* gene in such fusions, we first transferred the altered *mal-lac* fusion region from the strain diploid for the fusion using phage P1. The P1 transduction was done into a recipient which is haploid for the fusion and which carries a large deletion of the chromosome at the normal site of the *lac* genes, selecting for the Mal$^+$ phenotype. The *lac* deletion is large enough so that insertion of the *lac* genes in that region by homologous recombination is not possible. The resulting strain, HS6101, is Mal$^+$ but has the *malF-lacZ* fusion region and has only that one copy of *lacY*. When strain HS6101 was transduced to chloramphenicol resistance by use of P1 grown on a strain with Tn*9* inserted into *lacY*, all of the chloramphenicol-resistant, Lac$^-$ transductants were Mal$^-$ (12 of 12). This experiment demonstrates that the integrity of the *lacY* gene is necessary for the Mal$^+$ phenotype.

Recombination of the *lacY* mutation onto an F′ *lac* plasmid. The original isolate of the *lacY*^{mal} allele was recombined onto F′ *lac pro* by introduction of F′ *lacI*$^-$ *lacZ*$^+$ *lacY*$^+$ *pro*$^+$ into a strain that carried a copy of the *malF-lacZ* fusion region and the *lacY*^{mal} allele at the *malB* region of the chromosome. A double crossover event, pictured in Fig. 2, results in the formation of F′ *lacI*$^-$ *lacZ*$^+$ *lacY*^{mal}. Recombinant episomes carrying the *lacY*^{mal} allele were detected by their ability to transfer the Mal$^+$ Pro$^+$ phenotype to a strain that is *malF* and deleted for the *lac-pro* region. The frequency of the recombinant episomes, that is, Mal$^+$ Pro$^+$ F-ductants, among the Pro$^+$ F-ductants was 0.4%. This frequency is much higher than the frequency of spontaneous reversion of the *malF* mutation to Mal$^+$ and presumably represents the recombinational event described.

Are any components of the maltose transport system required in addition to the *lacY*^{mal} allele? The original isolate of the *lacY*^{mal} mutation was obtained in a strain that

was *malF malG*. We wanted to test the possibility that the remaining components of the maltose transport system are required, in addition to the *lacY*^{mal} gene product, for growth on maltose.

A series of strains was constructed which contained a deletion of the *lac* genes and various mutations in the *malB* region. F′ *lac* episomes were transferred into each of these strains. The F′ *lac* episomes contained a mutation in *lacI*, which results in constitutive expression of the *lac* genes, and either a *lacY*$^{+}$ or a *lacY*^{mal} allele (see below). The ability of these strains to utilize maltose was examined by streaking on maltose minimal agar. We found that all of the strains containing the *lacY*^{mal} allele are able to grow on maltose, including a strain in which the entire *malB* region is deleted.

These results indicate that the transport of maltose by the *lacY*^{mal} gene product is independent of all of the normal components of the maltose transport system. Apparently the function of the normal maltose transport system which requires four gene products can be replaced by a transport system that requires only a single component.

The normal maltose transport system can also permit accumulation of maltodextrins. To determine whether maltodextrins can be transported by the *lacY*^{mal} gene product, we streaked strain HS2511, which is *malF lacY*^{mal} *lacI lamB*$^{+}$, on minimal agar plates containing maltodextrins as carbon source. The *lamB* gene product is necessary for the diffusion of maltodextrins across the outer membrane. This strain failed to grow on maltodextrin as carbon source even with the partially constitutive level of *lamB* gene product (see above) and constitutive levels of the *lacY*^{mal} gene product sufficient to allow growth on maltose. Even though the β-galactoside transport system can transport the disaccharide maltose, it is unable to transport maltodextrin polymers.

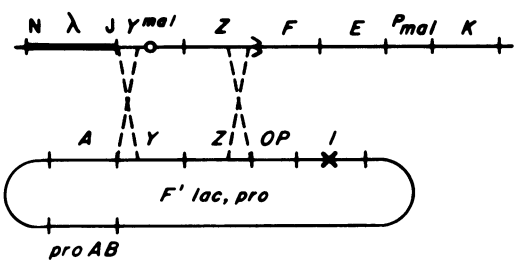

FIG. 2. *Recombination of the lacY*^{mal} *allele from the gene fusion onto F′ lac pro. The symbol ○ indicates the lacY*^{mal} *allele. The symbol × indicates the lacI3 mutation present on the episome. The dashed lines represent the double crossover event.*

Isolation of the *lacY*^{mal} mutation in strains with an unfused, normal *lac* operon. The results so far suggest that two conditions are necessary to allow entry of maltose through the β-galactoside transport system. First, it seems likely that there must be at least partially constitutive expression of the *lacY* gene, since maltose does not ordinarily induce the expression of the *lac* operon. This condition is met by the fused *lac* operon as described above. Second, there must be a *lacY* mutation that alters the specificity of the transport system. Thus, it should be possible to obtain *lacY*^{mal} mutations in a normally functioning *lac* operon, not fused to the *mal* genes, as long as the operon is induced or rendered constitutive by a *lacI* mutation.

We have sought Mal^{+} derivatives of a strain which is deleted for the *malB* region and which carries an F′ *lac* episome with a *lacI* mutation. When 2-ml amounts of overnight cultures of strain HS2503 were spread on maltose minimal agar, between 10 and 100 colonies appeared on each plate after 3 to 4 days. If, however, the parent strain was *lacI*$^{+}$, no colonies appeared, even after 1 week. Ten independent Mal^{+} colonies were obtained by use of this selection. To confirm that the Mal^{+} phenotype was under control of the *lac* repressor, we constructed *lacI*$^{+}$ derivatives of the Mal^{+} mutants (see Materials and Methods), and these did not grow on maltose. However, they were able to grow on maltose (5.5 mM) in the presence of 0.5 mM isopropyl-β-D-thiogalactoside, a gratuitous inducer of the *lac* operon. All 10 of the *lacY*^{mal} strains that were isolated appeared to have similar growth rates on maltose minimal agar plates.

The frequency of appearance of *lacY*^{mal} mutants is difficult to quantitate since colonies continued to appear on the selection plates after many days. We believe that contamination of the maltose by another sugar may allow sufficient growth of the parental strain so that mutants continue to accumulate in the population during the selection.

The frequency of appearance of the *lacY*^{mal} mutants seemed to be much lower in the original selection in which the *malF-lacZ* fusion strain was used as the parent than the frequency obtained when the strain with the unfused *lac* genes was used. This difference may be related in some way to the fact that the strain containing the wild-type *lac* operon genes and the *lacI* mutation probably has a higher constitutive level of *lacY* expression than does the strain containing the *mal-lac* fusion.

Inhibition of maltose transport by thiodigalactoside. If the ability of the bacteria without the normal maltose transport system to

grow on maltose is dependent on a functional β-galactoside transport system, then inhibition of this system should prevent growth on maltose. Thiodigalactoside, a specific inhibitor of the β-galactoside transport system (12), was added to cultures, and the amount of growth, after a 16-h incubation, at 37°C was measured. From the results presented in Table 3, it can be seen that growth on maltose is dependent on the *lacY*^{mal} allele, since the isogenic *lacY*$^{+}$ strain fails to grow. In addition, the presence of 4 mM thiodigalactoside in the media prevents the utilization of the (5.5 mM) maltose by the bacteria that have the *lacY*^{mal} allele. This indicates that a functioning β-galactoside transport system is necessary for the transport of maltose by bacteria with the *lacY*^{mal} allele.

Effect of the *lacY*^{mal} allele on the transport of normal substrates of the β-galactoside transport system. Since the *lacY*^{mal} mutation results in alteration of the specificity of the β-galactoside transport system, this change may have altered the transport of normal substrates. Initially, we examined the growth of strains carrying either the *lacY*$^{+}$ or *lacY*^{mal} allele on minimal agar plates containing either lactose or melibiose. Both strains grew very well on lactose minimal agar plates. The *lacY*^{mal} strain, however, grew very poorly on melibiose minimal agar plates. Since melibiose is a very efficient inducer of the *lac* operon (10), the fact that the *lacI*$^{+}$ *lacY*^{mal} strain grows poorly on melibiose probably reflects decreased ability of the *lacY*^{mal} transport system to transport melibiose.

TABLE 3. *Effect of thiodigalactoside (TDG) on the growth of ΔmalB strains on maltose*[a]

Strain	*lac* genotype	TDG	Growth
HS4511	*lacI lacY*$^{+}$	−	0.088
	lacI lacY^{+}	+	0.086
HS4600	*lacI lacY*^{mal}	−	2.90
	lacI lacY^{mal}	+	0.075

[a] Amounts of 5 ml of M63 containing 0.2% maltose were inoculated with 10^{7} bacteria of the indicated strain, either in the presence or absence of 10 mM TDG. Growth is expressed as the optical density of the cultures at 600 nm determined after 16 h at 37°C.

To determine whether the *lacY*^{mal} allele affected the kinetic parameters of the β-galactoside transport system for other substrates, we measured the initial velocity of transport as a function of external substrate concentration for TMG and ONPG in both the *lacY*$^{+}$ strain and the *lacY*^{mal} mutant.

A double-reciprocal plot of the initial velocity of [^{14}C]TMG uptake versus the external concentration of TMG is shown in Fig. 3a. The data indicate that the maximal velocity of TMG transport is the same in strains containing either the wild-type β-galactoside transport system or the *lacY*^{mal} transport system. The concentration which results in half-maximal velocity is 0.5 mM TMG in the *lacY*$^{+}$ strain, whereas it is 0.11 mM TMG in the *lacY*^{mal} strain.

Figure 3b shows similar data for ONPG transport. Again, the maximal velocity of transport appears to be the same in both strains, but the concentration of ONPG which results in half maximal rate of transport is lower in the *lacY*^{mal} strain (0.21 mM) than in the *lacY*$^{+}$ strain (0.9 mM).

These data indicate that the *lacY*^{mal} mutation

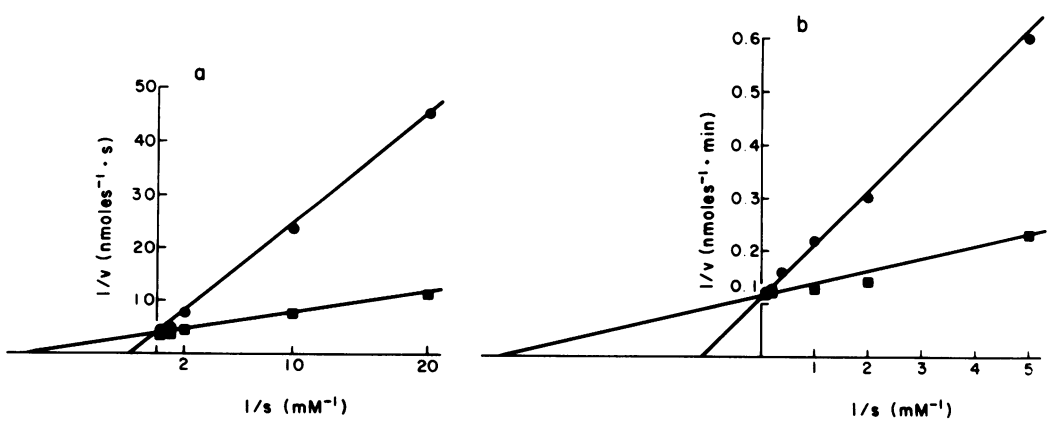

FIG. 3. *Double reciprocal plots of the initial rates of TMG and ONPG transport. (A) The initial rate of TMG transport was measured at different TMG concentration per 1.5 × 10*^{8} *cells. (●) HS4511, lacY*$^{+}$*; (■) HS4600, lacY*^{mal}*. (B) The initial rate of ONPG hydrolysis in intact cells was measured at different ONPG concentrations per 7 × 10*^{7} *cells. (●) HS4511, lacY*$^{+}$*; (■) HS4600, lacY*^{mal}*.*

has altered the β-galactoside transport system such that the apparent affinity of the altered transport system for ONPG and TMG is higher than that of the wild-type transport system for these substrates.

DISCUSSION

We have described the isolation and characterization of a class of mutations in the *lacY* gene of *E. coli* K-12 that alter the specificity of the β-galactoside transport system, so that a novel substrate, maltose, can be transported. Such mutations eliminate the need for the four protein components of the maltose transport system necessary specifically for growth on maltose. The evidence indicating that these are indeed mutations in the *lacY* gene comes from genetic studies, specific inhibitor studies, and studies of the effect of the mutation on the transport of normal substrates of the β-galactoside transport system. We believe that the *lacY*^{mal} mutation represents the first instance of a mutation in the structural gene of a transport system that allows recognition and transport of a novel substrate.

The genetic conversion of the wild-type *lac* operon to one which can permit maltose transport requires two mutational events. First, a regulatory mutation is necessary to permit constitutive expression of the *lacY* gene, and, second, the *lacY*^{mal} mutation must occur. A number of examples have been reported of laboratory-induced mutational evolution of new functions in bacteria. In some of these cases, both regulatory and specificity mutations appear essential (14).

The strain in which we originally detected the *lacY*^{mal} mutation was one in which the *lacY* gene was fused to the *malE,F,G* operon. In this strain, not only is the specificity of the β-galactoside transport system extended to include maltose, but, in addition, the synthesis of the transport protein is induced by maltose. Thus, as a result of gene fusion and the subsequent isolation of the *lacY*^{mal} mutation, we have unexpectedly evolved a new transport system with both the regulatory and specificity properties of naturally occurring systems.

There are other cases in which a regulatory mutation alone has uncovered a secondary transport system for a given compound. For example, Messer isolated mutants that were able to grow on lactose in the presence of thiodigalactoside, a specific inhibitor of the β-galactoside transport system (15). It was shown that lactose entry in these mutants occurred via the high-affinity arabinose transport system (3, 21). Furthermore, the mutations were mapped in the *araC* gene, which codes for the regulatory protein of the arabinose regulon. These mutations also resulted in constitutive expression of the *araBAD* operon. In addition, it was shown that other strains which were *lacY* could grow on lactose if they contained an allele of the *araC* gene, *araC*^{c}, which resulted in constitutive expression of the arabinose regulon (15).

Other mutations have been described which allow *malB* mutants to grow on maltose. Ricard et al. isolated strains that allowed *malB* mutants to grow on maltose and *lacY* mutants to grow on lactose (18). These strains also were hypersensitive to deoxycholate and probably represent a class of mutants with drastically altered membranes. Hofnung and Schwartz reported the isolation of a class of mutations that allowed *malB* strains to grow on maltose (9). They referred to these mutations as *bymA* (bypass for maltose permease) and were able to map the *bymA* locus at min 92 on the *E. coli* chromosome (2). They suggested that the *bymA* mutation unmasks a new system capable of transporting maltose.

The frequency of the *lacY*^{mal} mutation is low (10^{-7} to 10^{-8}), but probably too high to be due to double events. The properties of the mutants isolated so far appear to be similar, so it may be that only one or a few changes can result in the *lacY*^{mal} allele. Why is the normal maltose transport system, composed of four gene products, so complex, when it is possible for a single-component transport system (*lacY*^{mal}) to suffice? We can conceive of at least two explanations for the difference in complexity of the two systems. First, this maltose system transports dextrins in addition to maltose. In fact, the transport of maltodextrins requires the product of a fifth gene in the *malB* region, the *lamB* gene (17, 24, 25). The *lacY*^{mal} mutation, while allowing maltose transport, does not permit the transport of dextrins by the β-galactoside transport system.

Another property of the maltose transport system which might explain its complexity is the ability to transport substrate efficiently even at very low external substrate concentrations. The K_m of the maltose transport system for maltose is 10^{-6} M; at this concentration, bacteria are able to maintain a ratio of internal to external concentrations of maltose of approximately 8×10^4 (25). The chemoreceptor of the maltose transport system is the periplasmic maltose-binding protein which has an affinity for maltose that is very similar to the K_m of the transport system for maltose ($K_d = 10^{-6}$ M) (25). In contrast, the β-galactoside transport system has a K_m around 1 mM for lactose (although reported values vary widely; see [12] for summary) and is able to

concentrate substrates only on the order of 10^2 to 10^3 over the concentration present in the medium (12).

Andrews and Lin have compared different strategies for sugar accumulation in bacteria, with respect to versatility and scavenging power (1). Clearly, the maltose system is a much better scavenging system than the β-galactoside transport system. The β-galactoside transport system, however, is much more versatile than the maltose system since it can transport a wide variety of substrates including β-galactosides and even some α-galactosides such as melibiose. The maltose transport system on the other hand, seems to be specific for maltose and longer (1 → 4) glucose oligosaccharides (25). Not even glucose can be transported by this system.

Considering the versatility of the β-galactoside transport system, it may not be surprising that the capability to transport as additional substrate can be acquired as the result of a single mutation. One could suppose that the wild-type β-galactoside transport system has a very low affinity for maltose that is not sufficient to permit significant transport of maltose. If it were possible to increase the affinity of the β-galactoside transport system for a group of substrates, including maltose, then the altered transport system might gain the ability to transport maltose.

The data presented here concerning the effect of the $lacY^{mal}$ mutation on the affinity of the β-galactoside transport system for TMG and ONPG are consistent with this interpretation. The affinity of the $lacY^{mal}$ transport system for both TMG and ONPG is approximately fivefold higher than the affinity of the wild-type transport system. This indicates that the alteration of the β-galactoside transport system that allowed transport of a novel substrate simultaneously increased the affinity of the transport system for certain of the "normal" substrates. In contrast to these results, the transport of melibiose by the β-galactoside transport system is less efficient in the $lacY^{mal}$ strain than in the wild type. This suggests that the $lacY^{mal}$ allele has increased the efficiency of the transport system for some substrates (ONPG, TMG) and decreased it for others (melibiose). In order to test this idea further, it would be interesting to compare the kinetic parameters of the altered transport system with those of the wild-type system, for a wide variety of compounds including those that are recognized by the wild-type β-galactoside transport system and other compounds which might be novel substrates of the altered transport system.

Since the $lacY^{mal}$ mutation appears to affect only the affinity of the transport system for TMG and ONPG, without significantly altering the maximum velocity of transport, it might be reasonable to suppose that the site of the alteration in the $lacY^{mal}$ product is part of the substrate-binding site. If this is the case, then comparison of the primary structures of the $lacY^{mal}$ gene product and the wild-type M protein should provide some information about the location of the substrate-binding site of the β-galactoside transport system.

The rate of [^{14}C]maltose uptake mediated by the altered β-galactoside transport system is measurable but quite low (data not shown). From these data, active transport cannot be distinguished from facilitated diffusion coupled to metabolism of the internal maltose. A complete study of the physiological aspects of the $lacY^{mal}$ transport system is in progress.

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