The malX malY operon of Escherichia coli encodes a novel enzyme II of the phosphotransferase system recognizing glucose and maltose and an enzyme abolishing the endogenous induction of the maltose system.

J Reidl and W Boos
The malX malY Operon of Escherichia coli Encodes a Novel Enzyme II of the Phosphotransferase System Recognizing Glucose and Maltose and an Enzyme Abolishing the Endogenous Induction of the Maltose System

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Mutants lacking MalK, a subunit of the binding protein-dependent maltose-maltodextrin transport system, constitutively express the maltose genes. A second site mutation in malL abolishes the constitutive expression. The malL gene (at 36 min on the linkage map) codes for a typical repressor protein that is homologous to the Escherichia coli LacI, GalR, or CytR repressor (J. Reidl, K. Römisch, M. Ehrmann, and W. Boos, J. Bacteriol. 171:4888-4899, 1989). We now report that MalL regulates an adjacent and divergently oriented operon containing malI and malY. malX encodes a protein with a molecular weight of 56,654, and the deduced amino acid sequence of MalX exhibits 34.9% identity to the enzyme II of the phosphotransferase system for glucose (ptsG) and 32.1% identity to the enzyme II for N-acetylglucosamine (nagE). When constitutively expressed, malX can complement a ptsG ptsM double mutant for growth on glucose. Also, a ΔmalE malT(Con) strain that is unable to grow on maltose due to its maltose transport defect becomes Mal+ after introduction of malL::Tnl0 and the plasmid carrying malX. MalX-mediated transport of glucose and maltose is likely to occur by facilitated diffusion. We conclude that malX encodes a phosphotransferase system enzyme II that can recognize glucose and maltose as substrates even though these sugars may not represent the natural substrates of the system. The second gene in the operon, malY, encodes a protein of 43,500 daltons. Its deduced amino acid sequence exhibits weak homology to aminotransferase sequences. The presence of plasmid-encoded MalX alone was sufficient for complementing growth on glucose in a ptsM ptsG glk mutant, and the plasmid-encoded MalY alone was sufficient to abolish the constitutivity of the mal genes in a malK mutant. The overexpression of malY in a strain that is wild type with respect to the maltose genes strongly interferes with growth on maltose. This is not the case in a malT(Con) strain that expresses the mal genes constitutively. We conclude that malY encodes an enzyme that degrades the inducer of the maltose system or prevents its synthesis.

The maltose-maltodextrin system of Escherichia coli consists of a number of genes coding for proteins whose function is the uptake and metabolism of maltose and maltodextrins (23, 58, 65). The system is regulated by MalT, a transcriptional activator that is needed together with the inducer for the expression of all maltose-regulated genes (54). In vitro transcription experiments with purified MalT have shown that, of all dextrins tested, only maltotriose is effective in stimulating the action of MalT. This is in contrast to the in vivo situation, where the maltose system is induced by the presence of maltose or maltodextrins in the growth medium (53). Null mutations in malT no longer express the mal genes, whereas point mutations in malT [malT(Con)] have been isolated that express the maltose genes constitutively (18). In most of these mutants, MalT exhibits a higher affinity for maltotriose (15).

The transport system for maltose and maltodextrins is a multicomponent, binding protein-dependent system (16, 28, 69). One of its subunits, MalK (70), has several functions. (i) It contains an ATP binding site (13, 26) such as is found in all analyzed corresponding components of other binding protein-dependent transport systems (29) as well as in other transport-related proteins of prokaryotic and eucaryotic origin (29). The ATP binding site in MalK is thought to be responsible for the energization of active transport (44). (ii) MalK is the target for inducer exclusion, mediated by the unphosphorylated enzyme III (EII) of the phosphotransferase system (PTS) for glucose and resulting in the inhibition of maltose transport (17, 33). (iii) Mutants lacking MalK not only are negative in maltose transport but also express the mal genes constitutively (10, 19, 30). In line with this phenomenon is the observation that overproduction of MalK results in the repression of the remaining mal genes (57). The constitutive expression of the mal genes in the absence of MalK and the inability to express the maltose genes when MalK is overproduced are dependent on a wild-type malT gene, since malK::lacZ fusions are no longer expressed in strains that carry a malT::Tnl0 insertion (10) and mal gene expression is not reduced by overexpressed MalK in strains that carry a malT(Con) mutation (57). The three functions of MalK, in transport, inducer exclusion, and regulation, can be separated by malK mutation analysis, indicating a domain structure for MalK (33).

The effect of MalK on mal gene expression has been studied extensively in mutants that carry a malK::lacZ fusion (10, 19). The fusion mutant exhibits high β-galactosidase activity when grown on glycerol; this activity is repressed by the presence of overproduced MalK (57). A mutation, malL, was found that abolished the constitutive expression of Φ(malK::lacZ). malL was mapped at 36 min on the linkage map, a position not connected to any previously known mal genes (19). The cloning and sequencing of malL revealed that malL codes for a protein exhibiting high homology to the typical E. coli repressor proteins LacI, GalR, and
CytR. Next to malL, and oriented divergently to it, we observed the start of an open reading frame, called malX, whose control region was very similar to that of malL. We concluded that MalL was a repressor for malX as well as for malL itself. To explain the role of malX mutations in the repression of $\Phi$ (malK::lacZ), we proposed that the gene product of malX is an enzyme that eliminates an internal inducer for the maltose system. This implied that the function of elevated levels of MalK in the downregulation of mal gene expression is similar to that of MalX, that is, the elimination of an internal inducer (56).

Here we report the cloning and sequencing of malX as well as malY, a gene distal to malX in the same operon. We found that malX encoded a protein of 56,654 daltons with a deduced amino acid sequence that is homologous to that of enzyme II$^{\text{Sc}}$ (EII$^{\text{Sc}}$) and to enzyme II$^{\text{Sa}}$ (EII$^{\text{Sa}}$) of the PTS. ptsG ptsM double mutants are unable to grow on glucose but were observed to grow on glucose when malX was expressed constitutively, as in malL mutants, or when present on a multicopy plasmid. Thus, malX encodes a PTS EII that can recognize glucose as a substrate. The second gene, malY, encodes a protein of 43,500 daltons that shows homology to an apparently essential Bacillus subtilis protein of unknown function (49) that is homologous to the hisC gene product (27) of E. coli encoding imidazolylacetophosphate:1-glutamate aminotransferase. Sequences that are conserved in aminotransferases can be recognized in MalY. We demonstrate that only MalY is involved in the endogenous induction of the maltose system.

**MATERIALS AND METHODS**

**Bacterial strains and genetic methods.** Strains and plasmids are listed in Table 1. Strains were grown in Luria broth (LB) or minimal medium A (MMA) (43) with 0.2% carbon source. Amino acids as auxotrophic requirements were added by a 1:100 dilution from stock solutions containing 4 mg of amino acid per ml. Ampicillin, kanamycin, chloramphenicol, and tetracycline were used at 75, 50, 30, and 10 $\mu$g/ml, respectively. For qualitative screening of the expression of lacZ fusions, MMA plates with glycerol as a carbon source and 5-bromo-4-chloro-3-indolyl-p-D-galactopyranoside (X-gal) at a final concentration of 40 $\mu$g/ml were used. P1 transductions were done by the method of Miller (43). Selection of Tet$^+$ derivatives of Tn10 insertion strains was done by the method of Bochner et al. (6). Techniques involving manipulation of

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**TABLE 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant known genotype*</th>
<th>Origin of strain or allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12 strains</td>
<td></td>
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</tr>
<tr>
<td>MC4100</td>
<td>F$^-$ araD $\Delta$(araF-lac)U169 fbsB301 ptsF25 rbsR relA1 rpsL150</td>
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<tr>
<td>HS3018</td>
<td>MC4100 $\Delta$malE444 malT(Con)</td>
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<tr>
<td>JB3018-2</td>
<td>HS3018 malE+ malT(Con)</td>
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<tr>
<td>KM225</td>
<td>HS3018 mal::Tn10</td>
<td>This study</td>
</tr>
<tr>
<td>BRE1162</td>
<td>MC4100 $\Phi$(malK::lacZ)</td>
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</tr>
<tr>
<td>ME429</td>
<td>BRE1162 mal::Tn10</td>
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</tr>
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<td>ME429 malL Tet$^+$</td>
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<td>CC321</td>
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<td>$\Delta$(ara-leu)769 galE galK hsr hms$^+$ rpsL rpoB argE(Am) srn::Tn10 recA1 lacZ</td>
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<td>REI7</td>
<td>ME429 malL Tet$^+$</td>
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<td>DS410T</td>
<td>minB ara T7 malA metY xyl rpsL thi tonA azi gyrA $\Delta$(gltP-glpA)759</td>
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<td>gld-7 ptsG2 ptsM1 rpsL</td>
<td>W. Klein</td>
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<td>UE26 gld$^+$, Tetr$^+$ zfc-765::Tn10 derivative</td>
<td>This study</td>
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<td>WK126 mal::Tn10</td>
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<td>REI3600</td>
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<td>J. Lengeler</td>
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Plasmids

- pLG339: Kan$^+$ Tet$^+$
- pHSG575: Cm$^+$
- pNM480-2: lacZ lacY Amp$^+$
- ptrC99: Amp$^+$ lacP$^+$
- pTTC156: gld from Z. mobilis Amp$^+$
- pTSG5: ptsG lacP$^+$ Amp$^+$
- prIR1: pBR322 malL Ap$^+$
- pIR101: pLG339 malX malY Kan$^+$
- pIR102: pLG339 malX malY Kan$^+$
- pIR103: pHSG575 malX malY Cm$^+$
- pIR105: pLG339 malX Kan$^+$
- pIR106: pLG339 $\Phi$(malK::lacZ)Hyb Kan$^+$
- pIR110: pLG339 $\Phi$(malX::lacZ)Hyb Kan$^+$
- pIR111: pLG339 malX $\Phi$(malY::lacZ)Hyb Kan$^+$
- pIR115: ptrC99 malY
- pIR116: pIR103 malX::TnphoA malY

* The genotypes given for plasmids refer to the wild-type allele, except for fusions.
DNA, such as the analysis by restriction endonucleases, cloning, and transformation, were done as described by Maniatis et al. (38). Plasmid-directed protein synthesis in the minicell-producing strain DS410T (36) was done by the method of Meagher et al. (41) with the protocol described by Reeve (55). Labeling with 10 μCi of [35S]methionine (1,000 mCi/mmol; Amersham) was for 1 h at 37°C. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out on 12% polyacrylamide slab gels with the buffer system of Laemmli (35). The samples were routinely heated at the temperature of boiling water for 4 min before they were loaded onto the gel.

Probe labeling and selection of the chromosomal DNA fragment containing malX and malY. We used the commercial DIG DNA labeling and detection kits supplied by Boehringer (Mannheim, Federal Republic of Germany) to label the 644-bp HincII fragment of pJR1. It was labeled by random-primed incorporation (22) of digoxigenin-labeled dUTP. Chromosomal DNA was prepared as described by Silhavy et al. (71). Chromosomal restriction fragments were separated by 0.8% agarose gel electrophoresis and transferred onto a nylon filter (Nytran 13; Schleicher und Schuell, Federal Republic of Germany) by the Southern transfer method (72). Detection of specific hybridization was done by immunohasysaying with anti-digoxigenin--alkaline phosphatase conjugate and staining with 5-bromo-3-indolyl phosphate (25). Results with this technique indicated that a 3.3-kb PvuII chromosomal fragment hybridized with the probe (Fig. 1). Chromosomal DNA digested with PvuII was separated by agarose gel electrophoresis, and the fraction containing the 3.3-kb fragments was ligated in pLG339. pJR101 was identified as carrying malX and malY by its ability to repress the expression of Ph (malK::lacZ) in strain RE17.

Subcloning and construction of lacZ fusions. Nearly the entire chromosomal insert of pJR101 (Fig. 2) was transferred as the FspI fragment into two different vectors. One was pLG339 (74), yielding pJR102, and the other was pHSG575 (76), yielding pJR103. Both hybrid plasmids exhibited the MalX+ phenotype. Further subclones were obtained by deleting the XmnI fragment from pJR102, yielding pJR105. The removal of the XmnI fragment deleted a gene located distal to malX that we subsequently named malY. pJR115 carries the Smal-HindIII fragment of pJR102 ligated in the Smal and HindIII sites of ptrC99 (1), placing malY under trc promoter control. The XmnI site in pJR105 was used to introduce lacZ (Smal-HindII fragment of pNM481 (45)) by blunt end ligation. This construct, pJR111, contained lacZ, the gene for β-galactosidase, fused in frame to the malY gene. It was used to test the malY promoter activity. The corresponding fragment of pNM482 (45) carrying lacZ in a different frame was cloned into the PstI and XmnI sites of pJR102, yielding lacZ fused in frame to malX (pJR110). For the construction of a malY::lacZ fusion, the 'malX::malY' fragment consisting of the 644-bp HincII fragment of pJR1 (Fig. 2) was subcloned into the lacZ-carrying plasmid pNM480 (45), which was opened at the Smal site within the multiple cloning site. This resulted in an in-frame fusion of malY with lacZ. Because of the multi-copy state of this plasmid and the subsequent overproduction of the deleterious hybrid protein, this construct was not stable. Therefore, the malY::lacZ fusion was subcloned as an EcoRI-DraI fragment into the low-copy-number plasmid pLG339 (74) by replacement of the EcoRI-HincII fragment of this plasmid. The resulting construct pJR108 is shown in Fig. 2. Plasmid pJR116 carries a TnphoA insertion in malX. The insertion was done by the method of Manoil and Beckwith, using the transfer of TnphoA from an F' episome in strain CC321 (which was transformed with pJR103) and selecting for high Kanamycin resistance (40).

We noticed that the presence of plasmids harboring malX and malY and more so plasmids harboring malX alone, particularly in malY mutants, was not very healthy for the cells. When kept on LB plates, they rapidly lost their glucose-complementing capability.

DNA sequencing. The dyeoxy nucleotide chain termination method of Sanger et al. (62), as modified by Biggin et al. (5), with the commercial Sequenase kit (U.S. Biochemical Corp.) (75) was used. The 1.84-kb FspI-PstI fragment and the 1.26-kb PstI-FspI fragment of pJR102 (Fig. 2) were cloned into M13mp18 and M13mp19 (79). Deletions were introduced into the single-stranded DNA with T4 DNA polymerase after annealing with special primers around the EcoRI site in M13mp19 and at the HindIII site in M13mp18 and digesting with EcoRI and HindIII, respectively. This was done by the protocol of Dale et al. (14) with the commercial IBI system for rapid deletion subcloning. The noncoding DNA strand of the PstI-FspI fragment was sequenced by using specific oligonucleotide primers (AR1 through AR4) purchased from Myrcysynth Corp. (Switzerland).

Enzymatic activity. β-Galactosidase activity in permeabilized whole cells was determined as described by Miller (43). Nucleotide sequence accession number. The sequence data shown in Fig. 5 have been assigned the GenBank accession number M60722.

RESULTS

Cloning of malX and malY. DNA fragments obtained by digestion of chromosomal DNA with the restriction endonucleases PstI, HindIII, and PvuII were blotted against a DNA probe containing the intergenic region between malX and malY (56). We observed hybridization to fragments of 14 kb.
FIG. 2. Plasmids used in this study and their corresponding segments in the physical map of *E. coli*. The sequenced region containing *malI-malX malY* was computer analyzed for the restriction sites of the restriction endonucleases used to construct the physical map of *E. coli* (31) and found to fit well to kb 1715 to 1720 of the map corresponding to 36 min on the genetic map of *E. coli* (2). The *HindIII* fragment of pJR1 was used as probe to isolate the chromosomal *PvuII* fragment containing the entire *malX* and *malY* genes. The black boxed area of pJR1 indicates the multiple cloning site on mini-Mu used to clone *malI* (56). Vector DNA (---) and the *malI, malX,* and *malY* genes (■■■) are indicated. In pJR105 the *malY* gene is interrupted by the deletion of an *XmnI* fragment. The *lacZ* gene (■■■) was fused in frame to *malY, malX,* and *malI* by ligating the appropriate fragments from pNM481, pNM482, and pNM480 (45), respectively, after digestion with the indicated restriction enzymes into JR102. pJR108 is described in Material and Methods. pJR115 carries the *Smal-HindIII* fragment of pJR102 in *ptrC99,* placing *malY* under *trc* promoter control. pJR116 is a pJR102 derivative carrying a *TnpmA* insertion. The *phoA* transcriptional direction is opposite to that of *malX* and had occurred between nucleotides 565 and 566 (see Fig. 5). Restriction enzymes: *H*, *HinClI*; *P*, *PstI*; *Pv*, *PvuII*; *X*, *XmnI*; *K*, *KpnI*; *Bg*, *BglI*; *E*, *EcoRI*; *B*, *BamHI*; *S*, *SmaI*; *D*, *DraI*; *F*, *FspI*. The numbers (1 to 4) indicate kilobases.
with HindIII, fragments of 3.3 kb with PvuII, and fragments of 2 kb with PsI (Fig. 1). PvuII chromosomal fragments of about 3.3 kb were eluted from an agarose gel and cloned into the HincII restriction site of the low-copy-number vector pLG339 (74). The pooled plasmids were transformed into RE17 or pJR101 and the resulting transformants were screened for the repression of the malK::lacZ fusion on X-gal-containing plates with glycerol as the carbon source. The rationale of this screening procedure was that overproduction of the MalX-encoded protein would result in the degradation of the endogenous inducer of the mal system and therefore the malK::lacZ fusion would no longer be strongly expressed (56). In this way pJR101 was identified and chosen for further studies. It hybridized to the malL-containing probe, and its presence in RE17 strongly reduced the expression of MalX::lacZ. Therefore, it carried MalX as well as part of malL. To express MalY without MalX, the Smal-HindIII fragment of pJR102 was cloned in front of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible trc promoter of ptrC99 (1). pJR116 carries a TnphoA insertion in malX with the direction of phoA transcription opposite to that of malX. The restriction analyses of pJR101 and of pJR1 (the previously isolated plasmid containing malL and the beginning of malX [56]), the different subcloned plasmids, and the constructions carrying the lacZ gene fused to malX malY and malL are shown in Fig. 2.

The relevant restriction sites of the chromosomal portion of the plasmids correlated well with the physical map of E. coli at around kb 1715 to 1720 (31), corresponding to 36 min on the linkage map (2) and in agreement with previous mapping data (19). There were a few minor deviations between our restriction analysis and the physical map. The BamHI site reported at kb 1716 consisted of a doublet of two BamHI sites. Also, the EcoRV site that should be present within the cloned chromosomal fragment of pJR101 could not be found. From the orientation of malX and malY on the various plasmids and the comparison with the physical map, one can conclude that malL is transcribed counterclockwise and that malX and malY are transcribed clockwise.

We had previously reported (56) that plasmid pJR1 harboring malL, contained within the 5' end of the malX gene (to the right of the second HincII site of pJR1 in Fig. 2) the restriction sites StyI, BamHI, Aval, Smal, and EcoRI. As will be explained below (sequence of malX), these sites originate from the multiple cloning site of mini-Mu that had been used to clone malL. Therefore they do not belong to restriction sites of the chromosomal insert.

Expression of malX and malY in minicells. Plasmids to be tested for their encoded proteins were transformed into the minicell-producing strain DS410T (36). After the plasmid-encoded proteins were labeled with [35S]methionine, they were analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography (Fig. 3). The plasmids pJR101, pJR102, and pJR103 (Fig. 2), which were able to repress the constitutive phenotype of MalX::lacZ, synthesized two proteins that were not present when the vector plasmids pLG339 and pHSG575 were used as templates. These proteins exhibited apparent molecular weights of 52,000 and 42,000. pJR105, a derivative of pJR102 in which an XmnI fragment was deleted, synthesized only the 52,000-Da protein. Therefore, this protein must be the MalX protein, whereas the protein with the apparent molecular weight 42,000 must be the product of malY, located distal to malX. Both proteins disappeared when the lacZ gene was fused with malX (pJR110). Instead, a fusion protein with a molecular weight of over 120,000 was synthesized, presumably representing a MalX-β-galactosidase hybrid protein.

Sequencing of malX and malY. The two FspI-PsiI fragments of pJR101 were subcloned into M13mp18 and M13mp19 for sequencing by the dideoxy termination method (62), and deletion fragments were obtained by partial T4 polymerase treatment (14). The lengths of the sequenced portions and their individual start points are shown in Fig. 4. To sequence one strand of the 1.26-kb fragment, we used synthetic primers (AR1 through AR4). The DNA sequence and the deduced amino acid sequence of the three coding regions of the entire FspI fragment are shown in Fig. 5. The sequence begins with the FspI site in malL, followed by the intergenic control region (bases 158 to 331), the malX gene (bases 332 to 1921), and finally the malY gene (bases 1934 to 3103). In the intergenic control region upstream of malX, we found putative -10 and -35 regions, one CAP binding site, and the binding sites for the Mal protein (O1 and O2) (56). malX and malY are preceded by sequences representing good ribosomal binding sites (67). According to the above sequence, the malX gene encodes a protein of 530 amino acids with a molecular weight of 56,654 and malY encodes a protein of 390 amino acids with a molecular weight of 43,500. From its position, its induction pattern (Table 2), and the polar effect on malY of an insertion in malX (Table 3), it is clear that malY is the second gene in an operon with malX, but it is not clear whether malY is the last gene in this operon.

We noticed that the 5' portion of the malY gene contains
stretches of sequence that would allow the formation of two stable stem-and-loop structures exhibiting AGs of $-25.2$ and $-21.4$ kcal (ca. $-105$ and $-89.5$ kJ), respectively (calculated by the method of Tinoco et al. [77]). The possible significance of these structures is unknown and was not further pursued.

We had previously sequenced the 5' portion of malX (56) encoding the first 171 amino acids. The present sequence (Fig. 5) was identical only up to amino acid 119. From amino acids 120 to 171, both the amino acid sequence and the DNA sequence were entirely different. Closer analysis revealed that pJR1, the plasmid used in the previous study, still carried the MuS end with the multiple cloning site from mini-Mu fused to the 3' end of malX. Its deduced amino acid sequence was in frame with malX.

**Homology of MalX to EII^Glc and EII^Nag of the PTS.** A computer-aided search for protein sequence homology with the Fasta program of Pearson and Lipman (48) revealed that MalX was homologous to EII^Glc (20) and EII^Nag (50, 59) of the PTS. When small gaps were introduced for optimal alignment (Fig. 6), the MalX sequence was identical at 175 positions out of 530 amino acids of MalX and out of 477 amino acids of EII^Glc. By using the analysis of Pearson and Lipman (48), 34.9% identity was determined for a continuous stretch of 430 amino acids. The comparison of MalX with EII^Nag (Fig. 6) showed that 161 amino acids were identical, resulting in 32.1% identity in a stretch of 461 amino acids. EII^Nag is much larger than MalX or EII^Glc. The entire C-terminal sequence of EII^Nag that corresponds to the sequence of EII^Glc (50, 59) is missing in MalX.

The homologies of MalX to EII^Glc and EII^Nag are clustered, and they coincide with domains that are conserved in several EII proteins (50, 61). Around position 471, corresponding to the essential cysteiny1 residue 421 in EII^Glc (46), the sequence is highly conserved (Fig. 6). The corresponding sequence has also been found in several different EIIIs (78). Peri and Waygood (50) reported that there are three conserved histidiny1 residues in several EII proteins that might be involved in phosphorylation. Two of these conserved histidiny1 residues can also be found in MalX at positions 240 and 356. The third, at position 264, is exchanged for serine.

Two regions that do not contain cysteiny1 or histidiny1 residues but that are highly conserved between MalX, EII^Glc, and EII^Nag are from isoleucine 168 to isoleucine 185 of MalX and from glycine 301 to alanine 308 of MalX. All of these conserved sequences include or overlap regions that have been defined by Peri and Waygood (50) as being conserved in many different EIIIs (no. 1 to 6 in Fig. 6).

A Kyte and Doolittle hydrophathy plot (34) of MalX and EII^Glc is shown in Fig. 7. The structures of the two proteins are very similar. The MalX protein appears to be different from EII^Glc in only three stretches: one is between amino acids 110 and 150, the second is around amino acids 250 to 300, and the third is at the carboxyl terminus. The predicted topological similarity of the two proteins can be observed even in regions of little sequence homology. The same comparison of MalX with EII^Nag reveals very little similarity (data not shown).

**Homology of MalY to aminotransferases.** Comparison of the deduced amino acid sequence of MalY with the most recent EMBL protein sequence data base (24) revealed 21.3% identity in a 357-amino-acid overlap with protein OrfY from *B. subtilis*; the function of OrfY is unknown but is apparently essential (49). In turn, OrfY exhibits homology to the *E. coli* imidazolylacetolphosphate:gamma-glutamate aminotransferase, the hisC gene product (27). The alignment of the three proteins is shown in Fig. 8. The comparison also indicates the 12 amino acid residues that have been found invariant in most aminotransferases (42). Seven of these conserved amino acids can be found at the corresponding position in MalY.

*MalX* regulates the expression of *malX* and *malY* as well as its own expression. We had previously reported that the intergenic region between *malX* and *malY*, cloned on a multicopy plasmid, reduced the expression of $\Phi$(*malk::lacZ*) in a *mal*- strain, supposedly by binding and therefore eliminating the repressor protein for *malX*. Since the identical palindromic operatorlike sequences O$_1$ and O$_2$ were found within the nontranslated regions of both *malX* and *malY*, it was likely that *Mal* controlled not only the expression of *malX* but also that of *malY* itself (56). To further study the regulatory features of this system, we constructed plasmid-
FIG. 5. Complete DNA sequence of the FspI fragment containing malX and malY. The DNA sequence of the 3,119-bp FspI fragment is shown in the 5′ to 3′ orientation with respect to the transcription of malX and malY as shown in Fig. 2. The deduced amino acid sequence of MalX and MalY (and Mal') is given in the one-letter code. malX starts at nucleotide 332 and ends at nucleotide 1921, whereas malY starts at nucleotide 1933 and ends at nucleotide 3104. Potential ribosomal binding sites (67) at positions 320 and 1922 are indicated by underlining. Presumptive promoter elements are indicated at positions 196 (−35) and 225 (−10). Two direct palindromic repeats at positions 190 through 201 and 268 through 279, marked with O₁ and O₂, respectively, are likely to represent the binding sites for the Mal repressor protein (56). A potential CAP box is indicated by underlining at positions 238 through 258. Toward the 5′ end of the malY gene (at nucleotides 2109 and 2172) are two pairs of sequences (dashed arrows) that would be able to form two stable stem-and-loop structures. The beginning of themalgene at nucleotide 157, transcribed divergently to malX, is shown.
encoded lacZ fusions to maltX, malY, and malf and analyzed their expression in a malf+ strain and a malf:-Tn10 strain (Table 2). F(malt::lacZ)(Hyb) and F(malY::lacZ)(Hyb) expression were reduced 8- and 10-fold, respectively, in the presence of an intact malf+ gene. The expression of F(malX::lacZ)(Hyb) could not be measured reproducibly in a malf::Tn10 strain, since the overproduction of the corresponding fusion protein appeared to be deleterious for the cells.

MalfX allows growth on glucose in a ptsG ptsM mutant and growth on maltose in a mutant lacking the maltose binding protein-dependent maltose transport system. Double mutants defective in ptsG and ptsM (manX) are unable to grow on glucose as the only source of carbon (52). Into such a strain

FIG. 5—Continued.
**TABLE 2.** mall-dependent expression of Φ(mall::lacZ)(Hyb) and Φ(malY::lacZ)(Hyb)

<table>
<thead>
<tr>
<th>Chromosomal state of mall</th>
<th>Plasmid</th>
<th>β-Galactosidase activity (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mall*</td>
<td>pLG339</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>mall::Tn10</td>
<td>pLG339</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>mall*</td>
<td>pJR108 [Φ(mall::lacZ)(Hyb)]</td>
<td>0.1</td>
</tr>
<tr>
<td>mall::Tn10</td>
<td>pJR108 [Φ(mall::lacZ)(Hyb)]</td>
<td>0.8</td>
</tr>
<tr>
<td>mall*</td>
<td>pJR111 [malX+ Φ(malY::lacZ)(Hyb)]</td>
<td>0.0058</td>
</tr>
<tr>
<td>mall::Tn10</td>
<td>pJR111 [malX+ Φ(malY::lacZ)(Hyb)]</td>
<td>0.056</td>
</tr>
<tr>
<td>mall*</td>
<td>pJR110 [Φ(mall::lacZ)(Hyb)]</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* One unit of β-galactosidase activity hydrolyzes 1 μmol of o-nitrophenyl-β-D-galactopyranoside per min at room temperature.

(WK126) we introduced the mall::Tn10 insertion (REI199) and observed growth on glucose. In contrast, the triple mutant ptsG ptsM glk (UE26), lacking glucokinase in addition to the two major transport systems for glucose, remained unable to grow on glucose after the introduction of mall::Tn10 (strain REI215). Since growth on glucose was dependent on glucokinase, it follows that MalX, when expressed constitutively from the chromosomal malX gene, can mediate glucose transport without concomitant phosphorylation. Only when MalX was overexpressed from the plasmid-encoded malX gene, in a strain that is mall::Tn10, was growth on glucose independent of glucokinase (Table 3). Apparently, MalX is also able to vectorally phosphorylate glucose, although with low efficiency.

To test the possibility that MalX also recognizes maltose and maltdextrins, we introduced plasmids carrying mall, malY, or both mall and malY in a malE mall mutant strain mall::Tn10 strain and tested for growth on agar plates containing maltose as the only carbon source (Table 4). We observed growth after 3 days in strains carrying mall alone and weaker growth with strains carrying mall and malY but no growth in strains carrying malY alone or the vector plasmid. Growth on maltose was clearly dependent on the presence of the maltose enzymes, since the introduction of malT::Tn10 abolished growth. This had to be tested in a different set of strains, since the above ΔmalE mutant did not allow the easy introduction of mall::Tn10. Strain RE17 does not transport maltose and does not grow on maltose because of its malK::lacZ fusion; it lacks mall and malX on the chromosome and expresses the maltose genes constitutively (56). After the introduction of plasmid-encoded malX, the strain grows after 3 days on maltose-containing plates, but its derivative carrying mall::Tn10 does not. Surprisingly, with overexpression of ptsG the same set of strains also showed EII\textsuperscript{Glc} mediated growth on maltose. Since the maltose degradative enzymes amylomaltase and maltdextrin phosphorylase are geared for the utilization of unphosphorylated maltdextrins and no MalT-dependent maltdextrin phosphorylase has been found yet, it follows that MalX and EII\textsuperscript{Glc} are likely to transport maltose by facilitated diffusion without phosphorylation.

MalX exhibited homology not only to EII\textsuperscript{Glc} but also to EII\textsuperscript{Nag}. However, the introduction of mall::Tn10 into a nagE mutant (strain LR2-167) that was unable to transport N-acetylglucosamine did not allow growth on this amino sugar.

**Role of MalX and MalY in downregulating the maltose system.** We had previously proposed that malX codes for an enzyme that eliminates an as yet undefined endogenous inducer of the maltose system and that the expression of malX is controlled by mall, the product of which functions as a repressor (56). With the present knowledge of two genes in the operon and the conclusion that MalX is a PTS EII, it was of interest to test whether mall alone or both mall and malY were necessary for the repression of the malK::lacZ fusion in a strain lacking mall and malX. In particular, it seemed plausible that it was the removal by PTS-mediated phosphorylation of internal glucose that abolished endogenous induction of the maltose system. The data shown in Table 3 demonstrate that this is not the case. Plasmid pJR102 (containing mall and malY) reduced malK::lacZ expression, whereas pJR105 (carrying mall alone) did not, even though it was sufficient to complement a ptsG ptsM glk mutant for growth on glucose. Similarly, the expression of

**TABLE 3.** Effect of multiple copies of mall and malY on Φ(malK::lacZ) and on complementation of the growth of a ptsM ptsG glk mutant on glucose

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>β-Galactosidase activity (U/mg of protein)</th>
<th>Growth on glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE17 [mall malX Φ(malK::lacZ)]</td>
<td>pLG339 (vector)</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pTRC99B (vector)</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pHSG575 (vector)</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR103 (malX+ malY+*)</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR102 (malX+ malY+*)</td>
<td>0.0017</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR105 (malX+*)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p115 (malY+*)</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p115 (malY+*) + IPTG</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR116 (malX::TaphoA malY+*)</td>
<td>1.017</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>β-Galactosidase activity (U/mg of protein)</th>
<th>Growth on glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>REI215 (ptsG ptsM glk mall::Tn10)</td>
<td>pLG339 (vector)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR102 (malX+ malY+*)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR105 (malX+*)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p115 (malY+*) + IPTG</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR110 [Φ(malX::lacZ)(Hyb)]</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR111 [malX+ Φ(malY::lacZ)(Hyb)]</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pJR116 (malX::TaphoA malY+*)</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* One unit of β-galactosidase activity hydrolyzes 1 μmol of o-nitrophenyl-β-D-galactopyranoside per min at room temperature. The cells were grown with glycerol as the carbon source.
MalX  MTKATPKVTLEWFFQQQLGKTFLMPVALLSFLCGIMLGSSLSDHHVTLIPVLGNPVVLQ
E2Glc  MFKNAFAN------LQKVGKSLMLPVSPLIAIGILLGVGA------NFSLPAVSVHV--
E2Nag  MNILGF------FQRLGRALQLP1AVLPAVARLRFQPQ--DL------LNVAFA--

MalX  AIFTWSMKGSFASFSLFVMEFCIAPIGLARENKGVAFAFGITYAMVNLAVNFVLTKNG
E2Glc  ------MAAAGGSVFNAMPLIFAIQAGFTTND--GVSALAVVAYGIMVTKM-------
E2Nag  ------IAQAGGAIADNLLAIQAGVAASWISDSAGAALAGAVGFFLTKAM-------

MalX  ILPTTDAVLKANNQISQILQDSYTGILGAVIAVIWMLHERFHNLRPDAAILAFFG
E2Glc  ------AVAPFLVHLPAEIEASKHLADTVLGLGGISGAIAAMNFRYIKLPCIYLFAGK
E2Nag  ------TQPEIM----------GLAVGIGTVLQAGAYAANRSDIKLPLFGSFGK

MalX  RFVPIIISLVMGVLGLVPLVPFWIFAMIGSGLGMINSAGDFGPM-LFGTGERLLFLPG
E2Glc  RFVPIIISLGLALIAITFVGVLSPFVPPIGSAIQTSFSQMAAYQVPFAISFIERCLVPFG
E2Nag  RFVPIATQFGCLVLAAIFGVYVPVQHAIAGGEWVSGALGS---GIFGPFRNLPLIPGL

MalX  HHILVALIRITFDAQGTQUMCQGTQUSGALTIFOALSCPTTHGESATRSAFLPGK
E2Glc  HHINWPFQMQCQGETNAA--GQVFHGDIPRYMA--------GDPQAVKLSGHGFL-FKM
E2Nag  HQLVNTIAFQWQIGETNAAAGTVFHDGINRFA--------GDDQAGMFGFFPIMM

MalX  GGLPGAAALMYHACAREPHNHIKGLLLISGLACVVGCSLFEFLFLFVAPVYLVIH
E2Glc  YGLPAAAIAIWHASKPENRACKVGGMISALTSISITGITEPEIFEMFEVPAILYIHAL
E2Nag  FGLGPAALMYAPKERRMMGVGGMLLSAVTALFTGVEFLPLFMALPLLYLH

MalX  TGLGFQTVSGLVGTIQT-DGNIDFVFGGILHGLSTKWMYPVVAAIFVVFYVYIFRFA
E2Glc  AGLAFPICIIILGMDRTSFSGHLIDFIV--LSGNSKLLWFLPISGITYAIYYTIFRFL
E2Nag  TGISLFVATLLGIIACFSFSAGAIDYALMYNLPAASQVWMMLVNVGVIFFAIFYVFSLV

MalX  ITRFNKLTPGRDQSRVASSIEKAVAGPAPSICYNV-PAILAEGLGADNIVSDNCITIRL
E2Glc  IKALDLKTPGRDATED--AKATGSEPAPALVAAFAGKENGITNLDACITRLV
E2Nag  IRMNFKLTPGRKDEDEIVETEEANSNTEEGTLQATSNYIAYVGGTDNLKDAICITRL

MalX  SVKDOMLSVNVQLKDKNRAIGVQVLOQHNVQVFQVSVKDEMAAGLMHMTQVA
E2Glc  SVADDVSKVDAGGLKLLGAAGVQ-VAGSGVQAIFGKSDNLKEMDEYI--RNH
E2Nag  TVAASARVNTDMCKRLGAGGSKGKVQNIQIVQGVAKESGDAKMKV--ARGP

FIG. 6. Comparison of the amino acid sequence of MalX with that of EII\(^{Glc}\) and EII\(^{Man}\) of the PTS. For optimal alignment, small gaps (dashed lines) were introduced. Identical amino acids are indicated by asterisks and conserved amino acids are exchanged (according to Schwartz and Dayhoff [66]) are indicated by dots. Stretches of sequences that are highly homologous in all three proteins are underlined. The numbers 1 through 6 correspond to sequences defined by Peri and Waygood (50) as conserved in many different PTS EII\(\)s. The cysteine residue at position 471 of MalX corresponds to the cysteine residue 421 in EII\(^{Glc}\) (46) that is essential for function.

\(ptsG\) from \(E. coli\) or of \(glk\) from \(Zymomonas mobilis\) (3) did not reduce the expression of the \(maltX::lacZ\) fusion when tested on \(X\)-gal-containing indicator plates. On the other hand, plasmid pJR115, expressing only \(maly\) by the IPTG-inducible \(trc\) promoter, strongly reduced the expression of the \(maltX::lacZ\) fusion, even in the absence of IPTG. Thus, it is clear that only the second gene in the \(maltX : maly\) operon is responsible for controlling the endogenous induction of the maltose system. The plasmid-derived overproduction of \(Maly\) in the wild-type strain MC4100 strongly interfered with the ability of the strain to grow on maltose. This was not the case with strain JB3018-2 carrying a \(malt(\text{Con})\) mutation.

Cell extracts of strains carrying pJR115 that had been grown in the presence of IPTG contained the \(Maly\) protein as a prominent Coomassie blue-stained band of 42,000 molecular weight when analyzed by SDS-polyacrylamide gel electrophoresis (data not shown). In contrast to the expression of \(maltX\), the overexpression of \(maly\) was well tolerated by the cell.
malX and malY form an operon. To demonstrate that malX and malY form an operon, we isolated a TnphoA insertion in malX and tested its effect on the expression of malY, located distal to malX. Plasmid pJR116 carries a TnphoA inserted early in malX (between nucleotides 565 and 566 in Fig. 5) in which phoA is oriented in the opposite transcriptional direction from malX (Fig. 2). pJR116 was unable to complement a ptsG ptsM glk mutant for growth on glucose and no longer reduced the expression of Φ(malK::lacZ) (Table 3). Since malY is affected in its expression by the polar insertion of TnphoA in malX, it is clear that both genes form an operon with malX as the promoter-proximal gene and malY as the promoter-distal gene.

DISCUSSION

Sequence analysis of malX combined with mutant analysis allowed us to identify a novel PTS EII in E. coli that is able to recognize glucose and maltodextrins. Three lines of evidence support this conclusion: (i) the sequence of malX is highly homologous to ptsG coding for the major PTS EII for glucose (20); (ii) when MalX was overproduced in a ptsG ptsM glk malY mutant, growth on glucose was restored; (iii) when MalX was overproduced in a strain lacking the binding protein-dependent transport system for maltose but containing the maltose degradative enzymes constitutively, the strain regained the ability to grow on maltose.

Uptake and metabolism of glucose in E. coli can be achieved in several ways. The major route is EII^Glc (ptsG)-mediated uptake and phosphorylation. Similarly, EII^Man (ptsM) is able to recognize, transport, and phosphorylate glucose (21, 73). Glucose can also be taken up without chemical alteration by at least two active transport systems: one is the proton motive force (PMF)-dependent galactose transport system (galP) (37). Glucose is not an inducer of the GalP transport system. Thus, for growth on glucose the system has to be induced by the nonmetabolizable D-fucose, or galR mutants, which express galP constitutively, have to be used (7). The other transport system capable of recognizing glucose is the galactose-binding protein-dependent transport system for galactose and β-methyl galactoside, encoded by mgl (60, 64). This system is highly sensitive to catabolite repression (4) and can be expressed only when glucose cannot enter via a PTS-dependent route. Glucose transported by either the PMF-dependent GalP system or the binding protein-dependent Mgl system must be phosphorylated internally by glucokinase (glk) (12). Since ptsG ptsM mutants cannot grow on glucose, it follows that the chromosomally encoded malX system is not sufficiently expressed to allow growth. A strain that is in addition mal::TnJO and constitutively expresses malX-malY can grow on glucose, provided that glucokinase is present. This strongly indicates that MalX mediates glucose transport by facilitated diffusion. Only overexpression from a plasmid-encoded malX gene (in a malY mutant) allows growth on glucose in a ptsG ptsM glk mutant, demonstrating that MalX is also able to mediate vectorial phosphorylation of glucose, possibly in combination with EII^Glc.

MalX is also able to recognize and transport maltose, again most likely by facilitated diffusion. Strains that lack the high-affinity and binding protein-dependent transport system for maltose and that express the maltodextrin degradative enzymes constitutively are able to grow slowly on maltose after introduction of the malX-containing plasmid in a background that is lacking malY (constitutive expression of malX-malY). Since all known maltodextrin-utilizing enzymes of E. coli, in particular amyloamylase and maltodextrin phosphorylase, recognize the free (nonphosphorylated) sugars, it appears very likely that MalX-mediated transport of maltodextrins occurs as a free sugar without concomitant phosphorylation. Transport of maltose via MalX cannot be very effective, since the usual transport assays with low concen-
trations of radioactive maltose have not given any significant rates of uptake (data not shown). EII-mediated facilitated diffusion is not without precedent. Supposedly EII^{Glc}-mediated facilitated diffusion of galactose in the absence of the general PTS components has been reported (32). Although EII^{Glc}-mediated uptake of glucose in the wild type always occurs by vectorial phosphorylation, mutations in EII^{Glc} have been isolated that uncouple transport from phosphorylation (51). Apparently these mutations are not rare events; they result in a dramatic increase of the apparent \( K_m \) (>10 mM) without affecting the \( V_{\text{max}} \) of glucose transport (60).

From all of these considerations it appears that glucose and maltodextrins may not be the natural substrates of the MalX system. From its glucose-recognizing capabilities one could argue that MalX might effectively transport a glucose-containing di- or polysaccharide. So far, we have excluded trehalose (7), cellobiose (47), and \( \beta \)-glucosides (63) as possible major substrates (data not shown). On the other hand, one might argue that the \( \text{malX} \) system represents a former glucose-maltodextrin transport system, outdated by evolution, that has lost its specific EII for phosphorylation. The system was replaced by the more efficient high-affinity and binding protein-dependent maltose transport system. Possibly, the \( \text{ptsG} \) gene has evolved by duplication of the ancient \( \text{malX} \) gene and has become specialized for the utilization of glucose, the smallest member of the maltodextrin family, which is no longer recognized by the modern maltose transport system.
TABLE 4. MalX-mediated growth on maltose of strains lacking the maltose binding protein-dependent transport system*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Growth on maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM225 [ΔmalE malT(Con)]</td>
<td>pLG339 (vector)</td>
<td>–</td>
</tr>
<tr>
<td>mali::Tn10</td>
<td>pJR102 (malX+ malY+)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>pJR105 (malX*)</td>
<td>+ +</td>
</tr>
<tr>
<td></td>
<td>pJR115 (malY+)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>IPTG</td>
<td></td>
</tr>
<tr>
<td>REI7 [Φ(malK::lacZ)] Δ(mal-malX)]</td>
<td>pLG339 (vector)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>pJR102 (malX+ malY+)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>pJR105 (malX*)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>pJR115 (malY+)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>IPTG</td>
<td></td>
</tr>
<tr>
<td>REI216 (malT::Tn10) ΦmalK::lacZ Δmali-malX</td>
<td>pLG339 (vector)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>pJR102 (malX+ malY*)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>pJR105 (malX*)</td>
<td>–</td>
</tr>
</tbody>
</table>

* Growth was scored after 3 days on plates containing minimal medium plus 0.4% maltose as the only carbon source.

The reason for analyzing the mali-malX malY gene cluster was its relation to the endogenous induction of the maltose system: the mali mutation had been discovered because of the loss of high expression of a malK::lacZ fusion (19). The subsequent finding that mali encodes a repressor protein led to the conclusion that the genes (malX malY) that are repressed by MalE include enzymes that would eliminate an endogenous inducer of the mal system (56). The discovery reported herein that MalX is homologous to EIIα (36) and that it can complement a glucose transport defect seemed at first relevant to the endogenous induction of the maltose system. Could not glucose itself be the endogenous inducer? The function of the MalX-MalY system would then be to eliminate internal free glucose by phosphorylation. This is clearly not the case. We could show that the expression of maliX alone did not cause the reduction in the expression of Φ(malK::lacZ), even though it complemented a glucose-negative growth phenotype. Also, the expression of ptsG (coding for EIIα of the PTS) (20) on a multicopy plasmid that is thought to also phosphorylate internal glucose had no effect on the expression of Φ(malK::lacZ). Similarly, the overexpression of the glucokinase gene from Z. mobilis (3) did not result in the reduction of Φ(malK::lacZ) expression.

As shown herein, the product of the mali gene alone was responsible for reducing Φ(malK::lacZ) expression. We found that maliX overexpression had such a dramatic effect in downregulating the maltose system that even wild-type strains were strongly affected in their ability to grow on maltose. The phenomenon was specific, since growth on glycerol or glucose was not affected. The situation is reminiscent of the overexpression of MalK (57). As with MalK, the mal gene-repressing activity was observed only in a malY+ strain, not in a malT(Con) strain that is independent of an inducer. This indicates that MalY eliminates the endogenous inducer or prevents its synthesis. At present the enzymatic activity of MalY, if it is indeed an enzyme, is not clear. From the deduced amino acid sequence of MalY, the activity of an aminotransferase is indicated. The purification of the easily available protein will hopefully provide us with the answer.

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