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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

JOACHIM REIDL AND WINFRIED BOOS*

Department of Biology, University of Konstanz, P.O. Box 5560, D-7750 Konstanz, Federal Republic of Germany

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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 *malI* abolishes the constitutive expression. The *malI* 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 MalI regulates an adjacent and divergently oriented operon containing *malX* 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 phosphototransferase 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 Δ*malE malT*(Con) strain that is unable to grow on maltose due to its maltose transport defect becomes Mal$^+$ after introduction of *malI*::Tn*10* 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 procaryotic 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 (EIII) 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*::Tn*10* 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, *malI*, was found that abolished the constitutive expression of Φ(*malK*::*lacZ*). *malI* 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 *malI* revealed that *malI* codes for a protein exhibiting high homology to the typical *E. coli* repressor proteins LacI, GalR, and

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant known genotype[a]	Origin of strain or allele
E. coli K-12 strains		
MC4100	F⁻ *araD* Δ(*araF-lac*)*U169 flbB5301 ptsF25 rbsR relA1 rpsL150*	11
HS3018	MC4100 Δ*malE444 malT*(Con)	68
JB3018-2	HS3018 *malE*⁺ *malT*(Con)	8
KM225	HS3018 *malI*::Tn*10*	This study
BRE1162	MC4100 Φ(*malK*::*lacZ*)	9
ME429	BRE1162 *malI*::Tn*10*	19
KR36	ME429 *malI* Tet^s	56
CC321	F′ *lacI*^{q} *pro zzf*::Tn*phoA*/CC114	39
CC114	Δ(*ara-leu*)*7697 galE galK hsr hms*⁺ *rpsL rpoB argE*(Am) *srl*::Tn*10 recA1 lacZ*	
REI7	ME429 *malI malX* Tet^s	56
DS410T	*minB ara lacY malA mtl xyl rpsL thi tonA azi gyrA* Δ(*glpT-glpA*)*593*	36
UE26 (ZCS112)	*glk-7 ptsG2 ptsM1 rpsL*	12
WK124	UE26 *glk*⁺ *zfc-765*::Tn*10*	W. Klein
WK126	UE26 *glk*⁺, Tet^s *zfc-765*::Tn*10* derivative	W. Klein
REI199	WK126 *malI*::Tn*10*	This study
REI215	UE26 *malI*::Tn*10*	This study
REI216	REI7 *malT*::Tn*10*	This study
PPA69	Hfr KL16 *thi* Δ(*ptsHI-crr*) *galR*	7
REI300	KR36 Δ(*ptsHI-crr*) *zfc-765*::Tn*10*	This study
LR2-167	F⁻ *argG6 galT his-1 manI metB nagE ptsM rpsL thi-1*	J. Lengeler
Plasmids		
pLG339	Kan^r Tet^r	74
pHSG575	Cm^r	76
pNM480-2	*lacZ lacY* Amp^r	45
ptrC99	Amp^r *lacI*^{q}	1
pTC156	*glk* from *Z. mobilis* Amp^r	3
pTSG5	*ptsG lacI*^{q} Amp^r	B. Erni
pJR1	pBR322 *malI* Ap^r	56
pJR101	pLG339 *malX malY* Kan^r	This study
pJR102	pLG339 *malX malY* Kan^r	This study
pJR103	pHSG575 *malX malY* Cm^r	This study
pJR105	pLG339 *malX* Kan^r	This study
pJR108	pLG339 Φ(*malI*::*lacZ*)(Hyb) Kan^r	This study
pJR110	pLG339 Φ(*malX*::*lacZ*)(Hyb) Kan^r	This study
pJR111	pLG339 *malX* Φ(*malY*::*lacZ*)(Hyb) Kan^r	This study
pJR115	ptrC99 *malY*	This study
pJR116	pJR103 *malX*::Tn*phoA malY*	This study

[a] The genotypes given for plasmids refer to the wild-type allele, except for fusions.

CytR. Next to *malI*, 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 *malI*. We concluded that MalI was a repressor for *malX* as well as for *malI* itself. To explain the role of *malI* mutations in the repression of Φ(*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^{Glc} (EII^{Glc}) and to enzyme II^{Nag} (EII^{Nag}) of the PTS. *ptsG ptsM* double mutants are unable to grown on glucose but were observed to grow on glucose when *malX* was expressed constitutively, as in *malI* 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 imidazolylacetolphosphate:L-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 μg/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-β-D-galactopyranoside (X-gal) at a final concentration of 40 μg/ml were used. P1 transductions were done by the method of Miller (43). Selection of Tet^s derivatives of Tn*10* insertion strains was done by the method of Bochner et al. (6). Techniques involving manipulation of

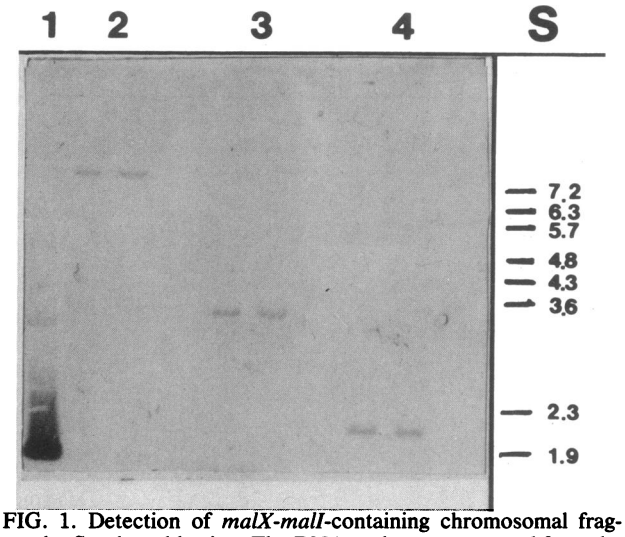

FIG. 1. Detection of *malX-malI*-containing chromosomal fragments by Southern blotting. The DNA probe was prepared from the 644-bp *Hin*cII fragment of pJR1 that overlaps with the *malX-malI* intergenic region (56). Lanes: 1, 1,824-bp *Pst*I fragment of pJR1 (control); 2, chromosomal DNA digested with *Hin*dIII; 3, chromosomal DNA digested with *Pvu*II; 4, chromosomal DNA digested with *Pst*I; S, size (kilobase) standards from λ DNA digested with *Bst*EII.

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 modification described by Reeve (55). Labeling with 10 μCi of [^{35}S]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 *Hin*cII 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 immunoassaying 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 *Pvu*II chromosomal fragment hybridized with the probe (Fig. 1). Chromosomal DNA digested with *Pvu*II 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 Φ(*malK*::*lacZ*) in strain REI7.

Subcloning and construction of *lacZ* fusions. Nearly the entire chromosomal insert of pJR101 (Fig. 2) was transferred as the *Fsp*I 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 *Xmn*I fragment from pJR102, yielding pJR105. The removal of the *Xmn*I fragment deleted a gene located distal to *malX* that we subsequently named *malY*. pJR115 carries the *Sma*I-*Hin*dIII fragment of pJR102 ligated in the *Sma*I and *Hin*dIII sites of ptrC99 (1), placing *malY* under *trc* promoter control. The *Xmn*I site in pJR105 was used to introduce *lacZ* (*Sma*I-*Dra*I 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 *malX* promoter activity. The corresponding fragment of pNM482 (45) carrying *lacZ* in a different frame was cloned into the *Pst*I and *Xmn*I sites of pJR102, yielding *lacZ* fused in frame to *malX* (pJR110). For the construction of a *malI*::*lacZ* fusion, the '*malX malI*' fragment consisting of the 644-bp *Hin*cII fragment of pJR1 (56) (Fig. 2) was subcloned into the *lacZ*-carrying plasmid pNM480 (45), which was opened at the *Sma*I site within the multiple cloning site. This resulted in an in-frame fusion of *malI* with *lacZ*. Because of the multicopy state of this plasmid and the subsequent overproduction of the deleterious hybrid protein, this construct was not stable. Therefore, the *malI*:*lacZ* fusion was subcloned as a *Eco*RI-*Dra*I fragment into the low-copy-number plasmid pLG339 (74) by replacement of the *Eco*RI-*Hin*cII fragment of this plasmid. The resulting construct pJR108 is shown in Fig. 2. Plasmid pJR116 carries a Tn*phoA* insertion in *malX*. The insertion was done by the method of Manoil and Beckwith, using the transfer of Tn*phoA* from an F′ episome in strain CC321 (which was transformed with pJR103) and selecting for high Kan^r (40).

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

DNA sequencing. The dideoxy 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 *Fsp*I-*Pst*I fragment and the 1.26-kb *Pst*I-*Fsp*I 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 *Eco*RI site in M13mp19 and at the *Hin*dIII site in M13mp18 and digesting with *Eco*RI and *Hin*dIII, 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 *Pst*I-*Fps*I fragment was sequenced by using specific oligonucleotide primers (AR1 through AR4) purchased from Mycrosynth 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 *Pst*I, *Hin*dIII, and *Pvu*II were blotted against a DNA probe containing the intergenic region between *malI* and *malX* (56). We observed hybridization to fragments of 14 kb

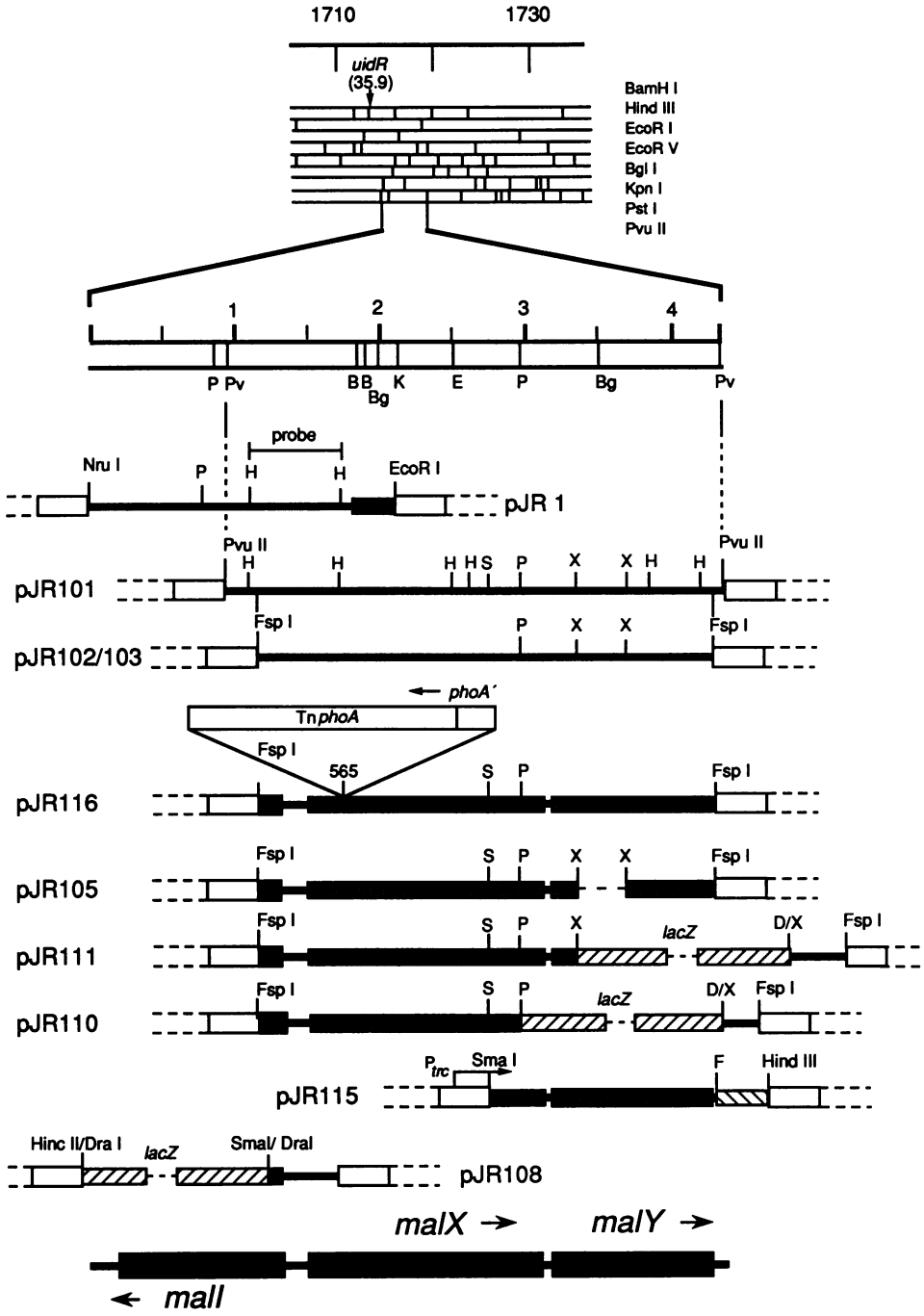

FIG. 2. Plasmids used in this study and their corresponding segments in the physical map of *E. coli*. The sequenced region containing *malI-malX malY* was computer analyzed for the restriction sites of the restriction endonucleases used to construct the physical map of *E. coli* (31) and found to fit well to kb 1715 to 1720 of the map corresponding to 36 min on the genetic map of *E. coli* (2). The *Hin*cII fragment of pJR1 was used as probe to isolate the chromosomal *Pvu*II 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 *Xmn*I fragment. The *lacZ* gene ( ) was fused in frame to *malY*, *malX*, and *malI* by ligating the appropriate fragments form 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 *Sma*I-*Hin*dIII fragment of pJR102 in ptrC99, placing *malY* under *trc* promoter control. pJR116 is a pJR102 derivative carrying a Tn*phoA* 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, *Hin*cII; P, *Pst*I; Pv, *Pvu*II; X, *Xmn*I; K, *Kpn*I; Bg, *Bgl*I; E, *Eco*RI; B, *Bam*HI; S, *Sma*I; D, *Dra*I; F, *Fsp*I. The numbers (1 to 4) indicate kilobases.

with *Hin*dIII, fragments of 3.3 kb with *Pvu*II, and fragments of 2 kb with *Pst*I (Fig. 1). *Pvu*II chromosomal fragments of about 3.3 kb were eluted from an agarose gel and cloned into the *Hin*cII restriction site of the low-copy-number vector pLG339 (74). The pooled plasmids were transformed into REI7 [Φ(*malK*::*lacZ*) *malI malX*], 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 *malI'*-containing probe, and its presence in REI7 strongly reduced the expression of Φ(*malK*::*lacZ*). Therefore, it carried *malX* as well as part of *malI*. To express *malY* without *malX*, the *Sma*I-*Hin*dIII fragment of pJR102 was cloned in front of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *trc* promoter of ptrC99 (1). pJR116 carries a Tn*phoA* 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 *malI* and the beginning of *malX* [56]), the different subcloned plasmids, and the constructions carrying the *lacZ* gene fused to *malX* *malY* and *malI* 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 *Bam*HI site reported at kb 1716 consisted of a doublet of two *Bam*HI sites. Also, the *Eco*RV site that should be present within the cloned chromosomal fragment of pJR101 could not be found. From the orientation of *malX* and *malI* on the various plasmids and the comparison with the physical map, one can conclude that *malI* is transcribed counterclockwise and that *malX* and *malY* are transcribed clockwise.

We had previously reported (56) that plasmid pJR1, harboring *malI*, contained within the 5' end of the *malX* gene (to the right of the second *Hin*cII site of pJR1 in Fig. 2) the restriction sites *Sst*II, *Bam*HI, *Ava*I, *Sma*I, and *Eco*RI. 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 *malI*. 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 [^{35}S]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 Φ(*malK*::*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 *Xmn*I 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.

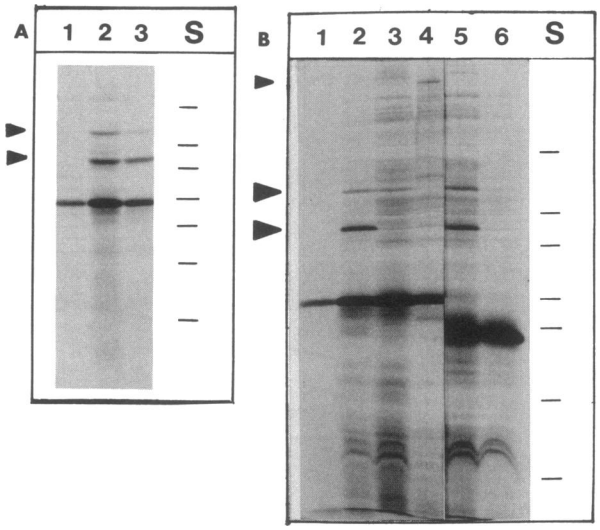

FIG. 3. Identification of plasmid-encoded proteins MalX and MalY by the minicell technique. Shown is the autoradiogram of the SDS-polyacrylamide gel electrophoretic analysis of ^{35}S-labeled proteins synthesized in minicells (strain DS40T [36]) that were programmed with the following different plasmids. (A) Lanes: 1, pLG339 (vector plasmid for pJR101 and pJR102); 2, pJR101 (MalX$^+$ MalY$^+$); 3, pJR102 (MalX$^+$ MalY$^+$). (B) Lanes: 1, pLG339; 2, pJR102 (MalX$^+$ MalY$^+$); 3, pJR105 (MalX$^+$); 4, pJR110 [Φ*malX*::*lacZ*(Hyb)]; 5, pJR103 (MalX$^+$ MalY$^+$); 6, pHSG575 (vector plasmid for pJR103). The large arrowheads on the left indicate the position of MalX, with an apparent molecular weight of about 52,000, and of MalY, with an apparent molecular weight of about 42,000. The small arrowhead indicates the position of the MalX-LacZ hybrid protein. The molecular weights of the standard proteins are as follows (from top to bottom): 66,000, 45,000, 36,000, 29,000, 24,000, 20,000 and 14,000.

Sequencing of *malX* and *malY*. The two *Fsp*I-*Pst*I 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 *Fsp*I fragment are shown in Fig. 5. The sequence begins with the *Fsp*I site in *malI*, 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 MalI protein (O_1 and O_2) (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

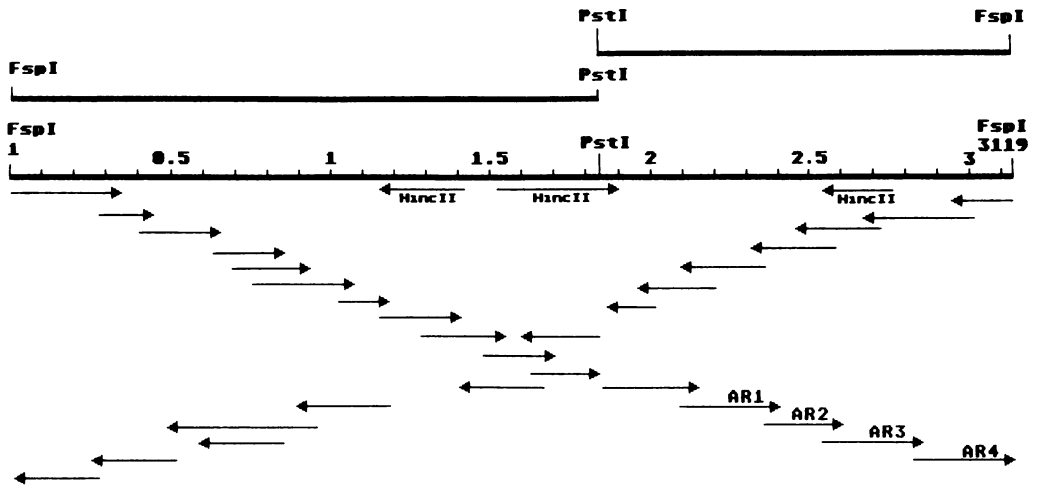

FIG. 4. Strategy for DNA sequencing. The *Fsp*I fragment of pJR102 was sequenced in two parts, a 1.84-kb *Fsp*I-*Pst*I fragment and a 1.26-kb *Pst*I-*Fsp*I fragment. The two fragments were cloned into M13mp18 and M13mp19, and overlapping deletions extending in both directions were created by digestion with T4 polymerase (14). The 1.26-kb *Pst*I-*Fsp*I fragment was sequenced in one direction by using oligonucleotide primers (17-mers, AR1 to AR4). In addition, three *Hinc*II fragments (Fig. 2) were cloned into M13 and sequenced as indicated.

stretches of sequence that would allow the formation of two stable stem-and-loop structures exhibiting ΔGs 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 EIII^{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 cysteinyl residue 421 in EII^{Glc} (46), the sequence is highly conserved (Fig. 6). The corresponding sequence has also been found in several different EIIs (78). Peri and Waygood (50) reported that there are three conserved histidinyl residues in several EII proteins that might be involved in phosphorylation. Two of these conserved histidinyl 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 cysteinyl or histidinyl 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 EIIs (no. 1 to 6 in Fig. 6).

A Kyte and Doolittle hydropathy 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 carboxy 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:L-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.

***MalI* regulates the expression of *malX* and *malY* as well as its own expression.** We had previously reported that the intergenic region between *malI* and *malX*, cloned on a multicopy plasmid, reduced the expression of Φ(*malK*::*lacZ*) in a *malI*$^+$ 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 *malI* and *malX*, it was likely that MalI controlled not only the expression of *malX* but also that of *malI* itself (56). To further study the regulatory features of this system, we constructed plasmid-

```
 FspI
TGCGCACAAATCCCAGCTCTTCAATGGCGGCGTTAACGCGTTCTCCTGTGGCGGTAGAGATTCGCCCTTTGCCAC 75
  R  V  F  G  L  E  E  I  A  A  N  V  R  E  G  T  A  T  S  I  R  G  K  G  S

TAAGCACCAGCGAAACGGTACTTACCGACACGCCCGCAGCCAGCGCAACATCATGAATGGTTATTTTTTTGGCGG 150
  L  V  L  S  V  T  S  V  S  V  G  A  A  L  A  V  D  H  I  T  I  K  K  A  T

                                                        -35->
TAGCCATGCAGATGACCTACTCCCTGATTATGTGACAGATAAAACGTTTTACCTTTTATTTATCTTATACCCGCT 225
  A  M  <-malI                                  O1                           

-10->
ATTATCGTTGCGTAATGTGATTTATGCCTCACTAAAATTTGATAAAACGTTTTATCTTCTCGCGCAATTTACTGA 300
          CAP                                   O2

                    S.D.
ATCCAGATTGTTCTCTACGAGGAGTCGTTTTATGACGGCGAAAACAGCACCGAAAGTCACGCTGTGGGAGTTCTT 375
                      malX -> M  T  A  K  T  A  P  K  V  T  L  W  E  F  F

CCAGCAGTTAGGCAAAACCTTCATGTTACCCGTGGCATTATTGTCGTTCTGCGGCATTATGCTCGGCATTGGTAG 450
  Q  Q  L  G  K  T  F  M  L  P  V  A  L  L  S  F  C  G  I  M  L  G  I  G  S

TTCTCTTAGCAGCCATGATGTCATAACCCTGATCCCGGTCCTGGGCAACCCCGTGTTGCAGGCTATCTTTACCTG 525
  S  L  S  S  H  D  V  I  T  L  I  P  V  L  G  N  P  V  L  Q  A  I  F  T  W

GATGAGTAAGATTGGCTCGTTTGCTTTTAGTTTCCTGCCTGTCATGTTCTGTATCGCCATCCCGCTGGGCCTGGC 600
  M  S  K  I  G  S  F  A  F  S  F  L  P  V  M  F  C  I  A  I  P  L  G  L  A

ACGCGAAAATAAAGGCGTAGCGGCATTCGCTGGCTTCATCGGTTATGCGGTAATGAACCTCGCGGTAAACTTCTG 675
  R  E  N  K  G  V  A  A  F  A  G  F  I  G  Y  A  V  M  N  L  A  V  N  F  W

GTTGACCAATAAAGGCATTCTGCCAACCACGGATGCCGCGGTTCTGAAAGCCAATAACATCCAGAGCATTCTTGG 750
  L  T  N  K  G  I  L  P  T  T  D  A  A  V  L  K  A  N  N  I  Q  S  I  L  G

GATCCAGTCGTACGATACCGGGATCCTCGGTGCGGTGATCGCCGGTATTATCGTCTGGATGCTGCATGAGCGTTT 825
  I  Q  S  Y  D  T  G  I  L  G  A  V  I  A  G  I  I  V  W  M  L  H  E  R  F

CCATAATATCCGCCTGCCGGATGCGCTGGCATTCTTCGGCGGTACGCGCTTCGTACCAATTATCTCCTCGCTGGT 900
  H  N  I  R  L  P  D  A  L  A  F  F  G  G  T  R  F  V  P  I  I  S  S  L  V

GATGGGCCTTGTCGGCCTGGTGATTCCATTAGTCTGGCCGATTTTCGCCATGGGTATTAGCGGCTTGGGCCATAT 975
  M  G  L  V  G  L  V  I  P  L  V  W  P  I  F  A  M  G  I  S  G  L  G  H  M

GATAAACAGCGCGGGTGATTTCGGACCGATGCTGTTTGGTACCGGTGAACGTCTGCTGTTGCCGTTTGGTCTGCA 1050
  I  N  S  A  G  D  F  G  P  M  L  F  G  T  G  E  R  L  L  L  P  F  G  L  H

TCACATTCTGGTGGCATTAATTCGCTTTACCGACGCAGGCGGCACGCAGGAAGTCTGCGGTCAAACCGTCAGCGG 1125
  H  I  L  V  A  L  I  R  F  T  D  A  G  G  T  Q  E  V  C  G  Q  T  V  S  G

CGCACTGACCATCTTCCAGGCGCAATTGAGTTGCCCGACCACTCACGGTTTTTCTGAAAGCGCCACGCGTTTCCT 1200
  A  L  T  I  F  Q  A  Q  L  S  C  P  T  T  H  G  F  S  E  S  A  T  R  F  L

TTCGCAAGGTAAAATGAATGCGTTTCTCGGCGGTCTGCCAGGTGCAGCGTTAGCTATGTATCACTGCGCGCGCCC 1275
  S  Q  G  K  M  N  A  F  L  G  G  L  P  G  A  A  L  A  M  Y  H  C  A  R  P

GGAAAATCGCCATAAAATTAAAGGTCTGCTGATTTCTGGCCTGATCGCCTGCGTCGTTGGCGGCACTACCGAACC 1350
  E  N  R  H  K  I  K  G  L  L  I  S  G  L  I  A  C  V  V  G  G  T  T  E  P

GCTGGAATTCCTGTTCCTGTTCGTAGCGCCAGTTCTGTATGTCATCCACGCGCTGTTAACCGGCCTCGGCTTCAC 1425
  L  E  F  L  F  L  F  V  A  P  V  L  Y  V  I  H  A  L  L  T  G  L  G  F  T

CGTCATGTCTGTGCTCGGCGTCACCATCGGTAATACCGACGGCAATATCATCGACTTCGTGGTGTTCGGTATTTT 1500
  V  M  S  V  L  G  V  T  I  G  N  T  D  G  N  I  I  D  F  V  V  F  G  I  L

GCATGGTCTGTCAACCAAGTGGTACATGGTGCCAGTGGTGGCGGCAATCTGGTTTGTCGTTTACTACGTCATCTT 1575
  H  G  L  S  T  K  W  Y  M  V  P  V  V  A  A  I  W  F  V  V  Y  Y  V  I  F

CCGTTTCGCTATCACCCGCTTCAATCTGAAAACCCCGGGGCGCGATAGCAGAGTTGCCAGCTCAATCGAAAAAGC 1650
  R  F  A  I  T  R  F  N  L  K  T  P  G  R  D  S  R  V  A  S  S  I  E  K  A
```

FIG. 5. Complete DNA sequence of the *Fsp*I fragment containing *malX* and *malY*. The DNA sequence of the 3,119-bp *Fsp*I 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 MalI′) 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 S.D. and underlining. Presumptive promoter elements are indicated at positions 198 (−35) and 225 (−10). Two direct palindromic repeats at positions 190 through 201 and 268 through 279, marked with O_1 and O_2, respectively, are likely to represent the binding sites for the MalI 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 the *malI* gene at nucleotide 157, transcribed divergently to *malX*, is shown.

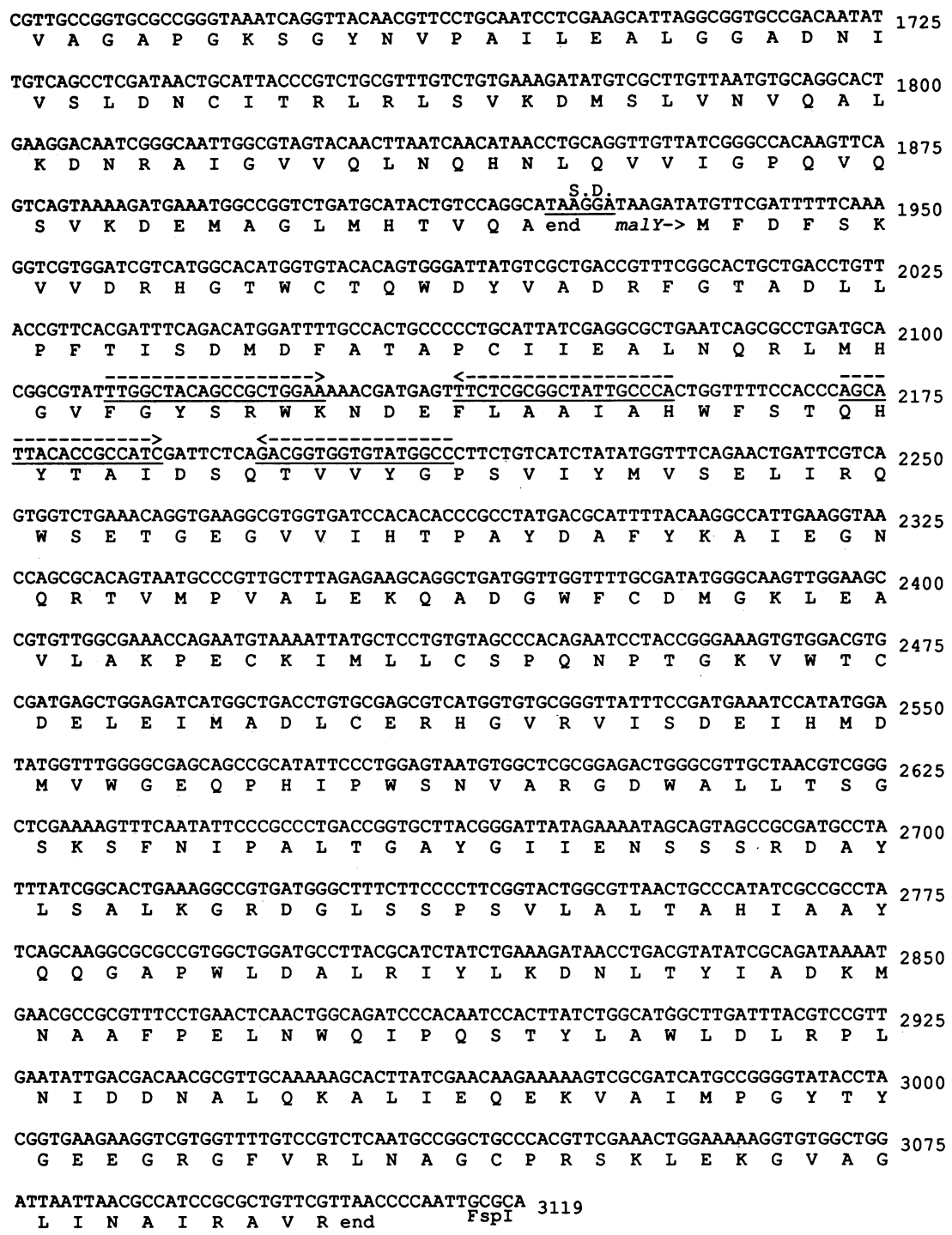

FIG. 5—*Continued.*

encoded *lacZ* fusions to *malX*, *malY*, and *malI* and analyzed their expression in a *malI*$^+$ strain and a *malI*::Tn*10* strain (Table 2). Φ(*malI*::*lacZ*)(Hyb) and Φ(*malY*::*lacZ*)(Hyb) expression were reduced 8- and 10-fold, respectively, in the presence of an intact *malI*$^+$ gene. The expression of Φ(*malX*::*lacZ*)(Hyb) could not be measured reproducibly in a *malI*::Tn*10* strain, since the overproduction of the corresponding fusion protein appeared to be deleterious for the cells.

MalX 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

TABLE 2. *malI*-dependent expression of Φ(*malI*::*lacZ*)(Hyb) and Φ(*malY*::*lacZ*)(Hyb)

Chromosomal state of *malI*	Plasmid	β-Galactosidase activity (U[a]/mg of protein)
malI$^+$	pLG339	<0.0002
malI::Tn*10*	pLG339	<0.0002
malI$^+$	pJR108 [Φ(*malI*::*lacZ*)(Hyb)]	0.1
malI::Tn*10*	pJR108 [Φ(*malI*::*lacZ*)(Hyb)]	0.8
malI$^+$	pJR111 [*malX*$^+$ Φ(*malY*::*lacZ*)(Hyb)]	0.0058
malI::Tn*10*	pJR111 [*malX*$^+$ Φ(*malY*::*lacZ*)(Hyb)]	0.056
malI$^+$	pJR110 [Φ(*malX*::*lacZ*)(Hyb)]	0.57

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

(WK126) we introduced the *malI*::Tn*10* 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 *malI*::Tn*10* (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 *malI*::Tn*10*, 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 maltodextrins, we introduced plasmids carrying *malX*, *malY*, or both *malX* and *malY* in a Δ*malE malT*(Con) *malI*::Tn*10* 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 *malX* alone and weaker growth with strains carrying *malX* 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*::Tn*10* abolished growth. This had to be tested in a different set of strains, since the above Δ*malE* mutant did not allow the easy introduction of *malT*::Tn*10*. Strain REI7 does not transport maltose and does not grow on maltose because of its *malK*::*lacZ* fusion; it lacks *malI* 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 *malT*::Tn*10* does not. Surprisingly, with overexpression of *ptsG* the same set of strains also showed EII^{Glc}-mediated growth on maltose. Since the maltose degradative enzymes amylomaltase and maltodextrin phosphorylase are geared for the utilization of unphosphorylated maltodextrins and no MalT-dependent maltodextrin phosphate-degradative enzyme has been found yet, it follows that MalX and EII^{Glc} are likely to transport maltose by facilitated diffusion without phosphorylation.

MalX exhibited homology not only to EII^{Glc} but also to EII^{Nag}. However, the introduction of *malI*::Tn*10* 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 *malI*, 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 *malX* alone or both *malX* and *malY* were necessary for the repression of the *malK*::*lacZ* fusion in a strain lacking *malI* 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 *malX* and *malY*) reduced *malK*::*lacZ* expression, whereas pJR105 (carrying *malX* 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 *malX* and *malY* on Φ(*malK*::*lacZ*) and on complementation of the growth of a *ptsM ptsG glk* mutant on glucose

Strain	Plasmid	β-Galactosidase activity (U[a]/mg of protein)	Growth on glucose
REI7 [*malI malX* Φ(*malK*::*lacZ*)]	pLG339 (vector)	1.2	
	ptrC99B (vector)	1.66	
	pHSG575 (vector)	1.64	
	pJR103 (*malX*$^+$ *malY*$^+$)	0.013	
	pJR102 (*malX*$^+$ *malY*$^+$)	0.0107	
	pJR105 (*malX*$^+$)	0.95	
	p115 (*malY*$^+$)	0.023	
	p115 (*malY*$^+$) + IPTG	0.012	
	pJR116 (*malX*::Tn*phoA malY*$^+$)	1.017	
REI215 (*ptsG ptsM glk malI*::Tn*10*)	pLG339 (vector)		−
	pJR102 (*malX*$^+$ *malY*$^+$)		+
	pJR105 (*malX*$^+$)		+
	p115 (*malY*$^+$) + IPTG		−
	pJR110 [Φ(*malX*::*lacZ*)(Hyb)]		−
	pJR111 [*malX*$^+$ Φ(*malY*::*lacZ*)(Hyb)]		+
	pJR116 (*malX*::Tn*phoA malY*$^+$)		−

[a] 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.

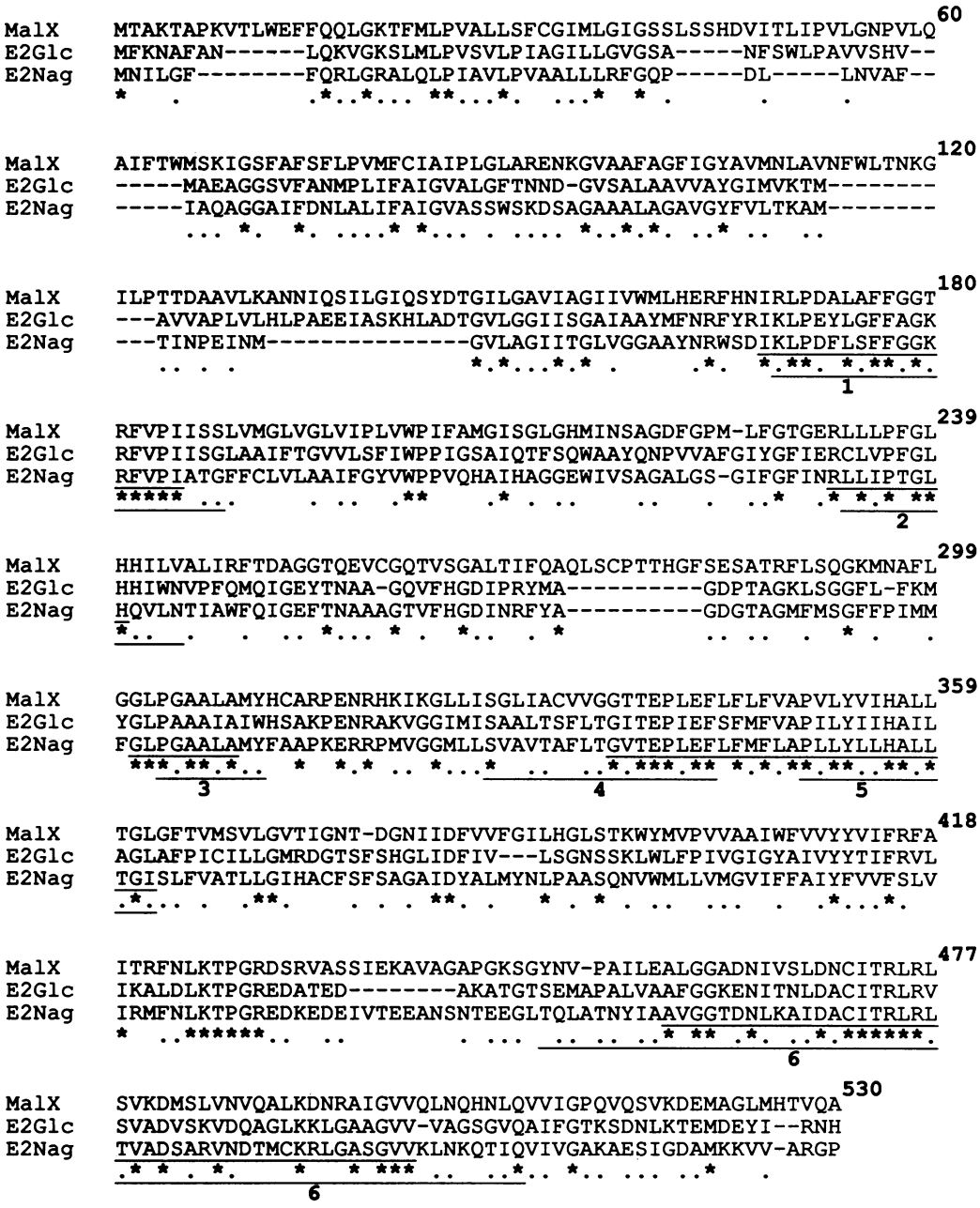

FIG. 6. Comparison of the amino acid sequence of MalX with that of EII^{Glc} and EII^{Nag} of the PTS. For optimal alignment, small gaps (dashed lines) were introduced. Identical amino acids are indicated by asterisks and conserved amino acid exchanges (according to Schwartz and Dayhoff [66]) are indicated by dots. Stretches of sequence that are highly homologous in all three proteins are underlined. The lines numbered 1 through 6 correspond to sequences defined by Peri and Waygood (50) as conserved in many different PTS EIIs. The cysteinyl residue at position 471 of MalX corresponds to the cysteinyl 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 *malK*::*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 *malK*::*lacZ* fusion, even in the absence of IPTG. Thus, it is clear that only the second gene in the *malX 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*(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 *malX*, the overexpression of *malY* was well tolerated by the cell.

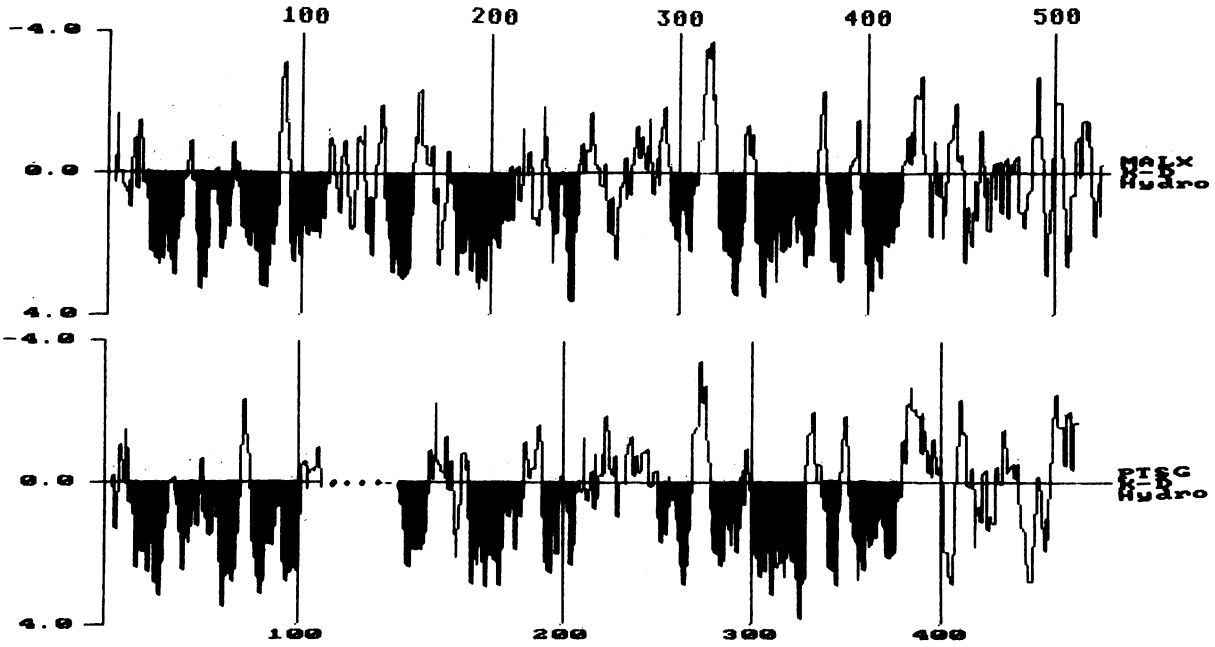

FIG. 7. Hydropathy plot of MalX in comparison to that of EII^{Glc}. The analysis was done by the method of Kyte and Doolittle (34) with a window of seven amino acids. For better alignment, a gap was introduced into EII^{Glc} at a position corresponding to amino acid 110 of MalX. Hydrophobic stretches are indicated by filled-in troughs. The numbers indicate the amino acids of MalX and PtsG.

***malX* and *malY* form an operon.** To demonstrate that *malX* and *malY* form an operon, we isolated a Tn*phoA* insertion in *malX* and tested its effect on the expression of *malY*, located distal to *malX*. Plasmid pJR116 carries a Tn*phoA* 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 Tn*phoA* 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 malI* mutant, growth on glucose was restored; (iii) when MalX was overproduced in a strain lacking the binding protein-dependent transport system for maltose but contained 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 *malI*::Tn*10* 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 *malI* 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 $EIII^{Glc}$.

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 *malI* (constitutive expression of *malX-malY*). Since all known maltodextrin-utilizing enzymes of *E. coli*, in particular amylomaltase 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-

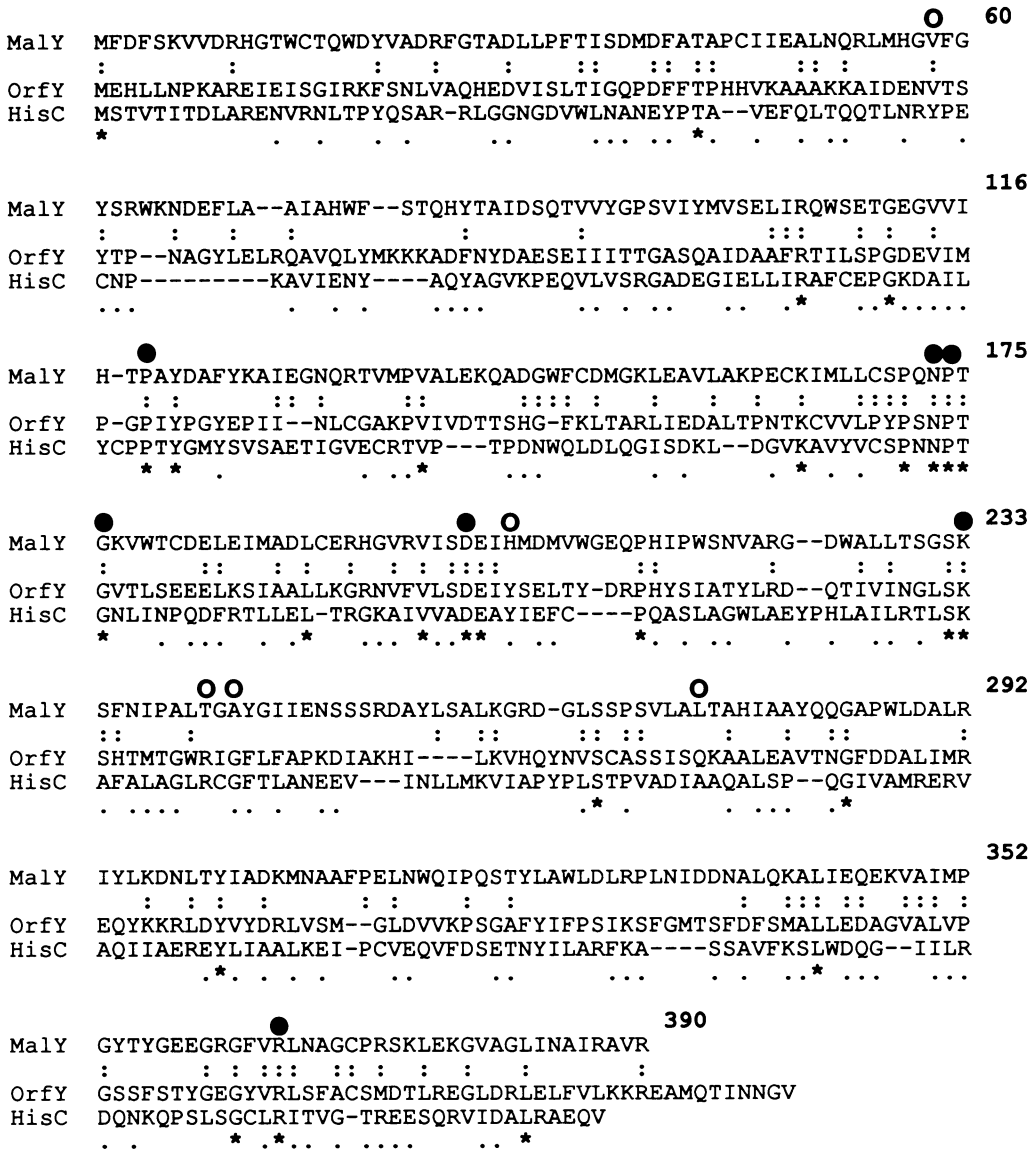

FIG. 8. Comparison of the amino acid sequence of MalY with that of OrfY from *B. subtilis* and imidazolylacetolphosphate:L-glutamate aminotransferase (HisC) from *E. coli*. For optimal alignment, small gaps (dashed lines) were introduced. Identical amino acids in all three proteins are indicated below the HisC sequence (27) by asterisks, and conserved amino acid exchanges (according to Schwartz and Dayhoff [66]) are indicated by dots. Identical amino acids between MalY and only OrfY (49) or HisC are indicated by a colon below the MalY sequence. Circles above the MalY sequence indicate 12 amino acids that are strictly conserved in 16 aminotransferases (42). Seven of these amino acids can be found in MalY and are indicated by filled circles.

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_{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 β-glucosides (63) as possible major substrates (data not shown). On the other hand, one might argue that the *malX* system represents a former glucose-maltodextrin transport system, outdated by evolution, that has lost its specific EIII for phosphorylation. The system was replaced by the more efficient high-affinity and binding protein-dependent maltose transport system. Possibly, the *ptsG* gene has evolved by duplication of the ancient *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[a]

Strain	Plasmid	Growth on maltose
KM225 [Δ*malE malT*(Con) *malI*::Tn*10*]	pLG339 (vector)	−
	pJR102 (*malX*$^+$ *malY*$^+$)	+
	pJR105 (*malX*$^+$)	++
	pJR115 (*malY*$^+$) + IPTG	−
	pTSG5 (*ptsG*$^+$) + IPTG	++
REI7 [Φ(*malK*::*lacZ*) Δ(*malI-malX*)]	pLG339 (vector)	−
	pJR102 (*malX*$^+$ *malY*$^+$)	+
	pJR105 (*malX*$^+$)	++
	pJR115 (*malY*$^+$) + IPTG	−
REI216 (*malT*::Tn*10* Φ*malK*::*lacZ* Δ*malI-malX*)	pLG339 (vector)	−
	pJR102 (*malX*$^+$ *malY*$^+$)	−
	pJR105 (*malX*$^+$)	−

[a] 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 MalI encode enzymes that would eliminate an endogenous inducer of the *mal* system (56). The discovery reported herein that MalX is homologous to EII^{Glc} 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 *malX* 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 EII^{Glc} 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 *malY* gene alone was responsible for reducing Φ(*malK*::lacZ) expression. We found that *malY* 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 *malT*$^+$ 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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