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Molecular Analysis of *treB* Encoding the *Escherichia coli* Enzyme II Specific for Trehalose

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A gene bank of partially *Sau*3A-digested *Escherichia coli* DNA ligated in plasmid pBR322 was screened for the ability to complement a mutant unable to metabolize trehalose at low osmolarity. The resulting plasmid was shown to contain the genes encoding transport (*treB*) and metabolic (*treC*) functions. The complementing DNA region was sequenced and shown to contain an operon of two genes, with *treB* as the promoter proximal gene and with *treC* as the promoter distal gene. The transcriptional start point was determined, and one major transcript was detected. The control region of the operon was found to contain consensus binding motifs for the cyclic AMP-catabolite activator protein complex and for a specific repressor protein whose gene, *treR*, is located immediately upstream of *treB*, being transcribed in the same direction as *treB treC*. The products of both genes could be expressed in minicells in which TreB revealed itself as a protein with an apparent molecular weight of 42,000. The gene product of *treB* consists of 485 amino acids with a calculated molecular weight of 52,308. It showed high homology to enzymes II^{Scr} of enteric bacteria specific for the uptake of sucrose and encoded by plasmid pUR400 of enteric bacteria. Like enzyme II^{Scr}, enzyme II^{Tre} belongs to the EIIBC domain type and lacks a covalently bound EIIA domain. Instead, enzyme II^{Tre}-mediated phosphorylation of trehalose requires the activity of enzyme IIA^{Glc}, a component of the major glucose transport system.

The disaccharide trehalose serves as an osmoprotectant in many different organisms. Its two glucose molecules are linked 1-1 α-glycosidically. Thus, the sugar is nonreducing and has the unique quality of maintaining the fluidity of membranes under conditions of dryness and desiccation (13). In *Escherichia coli*, trehalose is synthesized internally in response to osmotic stress (20, 57, 59). Among other more prominent osmoprotectants such as glycine-betaine or proline (37), trehalose can contribute up to 20% of the entire capacity of the osmotic protection of the cell (16, 29). To synthesize the osmoprotectant trehalose at high osmolarity, UDP-glucose and glucose 6-phosphate are used to form trehalose-6-phosphate, regardless of the carbon source. Trehalose-6-phosphate is subsequently dephosphorylated to give free trehalose. Trehalose-6-phosphate synthase is encoded by *otsA* (osmotic trehalose synthesis), and trehalose-6-phosphate phosphatase is encoded by *otsB*. The two genes are localized at 42 min on the chromosome, *otsB* being the promoter proximal gene of the operon (16, 23, 59). The operon is induced by high osmolarity, and at least the synthase is activated by potassium (59) which is accumulated as a response to osmotic stress (7). *E. coli* can grow on trehalose as the sole source of carbon at low osmolarity and high osmolarity, when the sugar is synthesized and accumulated internally. This seemingly paradoxical situation of degrading trehalose as a carbon source and synthesizing it as an osmoprotectant at the same time is solved by *E. coli* in an intriguing way. The bacterium has developed two different systems of trehalose metabolism, one for high-osmolarity and one for low-osmolarity growth conditions.

Under conditions of high osmolarity, trehalose is hydrolyzed to glucose by a periplasmic trehalase, encoded by *treA*, located at 26 min on the *E. coli* chromosome (6, 21). Glucose is subsequently taken up by the phosphotransferase system (PTS) as glucose 6-phosphate and enters glycolysis. The periplasmic trehalase, TreA, is induced by 250 mM NaCl in the medium but not by trehalose. Mutants in *treA* can no longer grow on trehalose as the single source of carbon at high osmolarity (6), but they do at low osmolarity (<170 mosM NaCl). Therefore, it is clear that, at low osmolarity, a second system for trehalose degradation exists.

As described previously (6, 36), trehalose is transported at low osmolarity via a PTS for trehalose, with a specific $EIICB^{Tre}$ (EII^{Tre}) and the $EIIA^{Glc}$ ($EIII^{Glc}$) of the glucose-PTS, delivering trehalose-6-phosphate to the cytoplasm. The resulting trehalose-6-phosphate is then hydrolyzed to glucose and glucose 6-phosphate by trehalose-6-phosphate hydrolase, the gene product of *treC* (44). Glucose 6-phosphate and glucose (after being phosphorylated by glucokinase) then enter glycolysis.

TreB (the EII^{Tre} protein) and TreC are both induced by trehalose-6-phosphate (26), and their genes have been mapped at 96.5 min on the *E. coli* chromosome (6).

In the present publication, we describe the molecular characterization of the *treB* gene. We present its sequence and the product of its expression. We demonstrate that *treB* and *treC* form an operon with *treB* as the promoter proximal gene. Upstream of *treB* and transcribed in the same direction, but not part of the operon, we found an open reading frame, *treR*, encoding the repressor of the system.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used are listed in Table 1. They were grown under aeration in tryptone broth (TB) or minimal medium A (MMA) with 0.2% carbon source (38). High-osmolarity minimal medium contained in addition 250 mM NaCl. Strain constructions were done by P1 *vir*-mediated transduction by the method of Miller (38). Selection for Tet^r was done without phenotypic expression by plating on DYT (double yeast tryptone) (38) containing 5 μg of tetracycline per ml. Selection for loss of Tet^r was by the method of Bochner et al. (3). In order to construct a deletion of the *tre* genes, a Tn*10* insertion was placed next to the *treC*::*lacZ* fusion. After selection for loss of Tet^r, the candidates were tested for loss of the *treC*::*lacZ* fusion (Lac^- Tre^- and kanamycin sensitivity). From the resulting strain, WK208, chromosomal DNA was prepared by the method of Silhavy et al. (55). The deletion of all *tre*

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Known genotype	Source or reference
Strains		
CC118	*araD139* Δ(*ara-leu*)*7697* Δ(*lacX*)*74* Δ(*phoA*)*-20 argE*(Am) *galE galK recA1 rpoB rpsE thi*	34
CC311	Δ(*ara-leu*)*7697* Δ(*lacX*)*74 galE galK recA1 rpsL thi* pO*xgen*::Tn*lacZ*	32
DS410T	*ara azi lacY malA minB mtl rpsL thi tonA xyl*	17
ECL116	F$^-$ *endA hsdR* Δ(*argF-lac*)*U169 thi*	12
HSK42	MC4100 *polA*	46
KRIM4	UE15 *treB*	6
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U169 deoC1 flbB5301 ptsF25 rbsR relA1 rpsL150*	10
RHo14	MC4100 *treA*:Ω-sp	This study
RHo16	RHo14 *treR*::Tn*10*	This study
RHo17	RHo14 Φ(*treB*::*lacZ*)*hyb2*	This study
RHo18	RHo16 Φ(*treB*::*lacZ*)*hyb2*	This study
RHo19	RHo14 Φ[(*treC*::*lacZ*$^+$)*1*]λp*lac*Mu55	This study
RHo20	RHo16 Φ[(*treC*::*lacZ*$^+$)*1*]λp*lac*Mu55	This study
RHo21	RHo19 *rpoS*::Tn*10*	This study
RHo22	RHo20 *rpoS*::Tn*10*	This study
TG1	F′[*lacI*^{q} *lacZM15 proA*$^+$*B*$^+$ *traD36*] *hsdD5* Δ(*lac-pro*) Δ(*pro-lac*) *supE thi*	
UE14	MC4100 *treA*::Tn*10*	6
UE15	UE14 Tet^s derivative	6
UE26	F$^-$ *glk7 ptsG2 ptsM1 rpsL150*	15
UE49	UE14 Φ[(*treC*::*lacZ*$^+$)*1*]λp*lac*Mu55	6
WK208	UE15 Δ(*treR treB treC*)	This study
WK211	UE14 Φ(*treC*::*lacZ*)*hyb31*	This study
WK213	UE14 Φ(*treB*::*lacZ*)*hyb105*	This study
M13mp18/19	M13 sequencing vector	39
Plasmids		
pACYC184	Amp^r Cam^r	11
pBR322	Amp^r Tet^r	4
pHP45Ω	Amp^r Sp^r	
pHSG575	Cam^r	61
pKM100	pBR322 *treB*$^+$ *treC*$^+$ *treR*$^+$ Amp^r	This study
pRHo14	pTRE11 *treA*:Ω-Sp Amp^r	This study
pRHo400	pACYC184 *treB*$^+$ *treC*$^+$ Cam^r	This study
pRHo500	pACYC184 *treR*$^+$ Tet^r	This study
pRIM11[a]	pHSG575 *treB*$^+$ *treC*$^+$ Cam^r	44
pTRE11	pBR322 *treA*$^+$ Amp^r	21
pUE1	pBR322 *treB*$^+$ *treC*$^+$ *treR*$^+$ Amp^r	6
pWK11[a]	pHSG575 *treB*$^+$ *treC*$^+$ Cam^r	This study
pWK11F1	pWK11 Φ(*treB*::*lacZ*)*hyb31*	This study
pWK11F2	pWK11 Φ(*treB*::*lacZ*)*hyb2*	This study
pWK12	pHSG575 *treB*$^+$ Cam^r	This study
pWK14	pACYC184 *treB*$^+$ Cam^r	This study

[a] These plasmids express *treB* and *treC* constitutively.

genes was verified by Southern hybridization according to the protocol of Sambrook et al. (48) with different digoxigenin-labeled DNA fragments (manual from Boehringer Mannheim) of the *tre* genes from plasmid pUE1. The plasmid pTRE11 (Ap^r) harboring *treA* (21) was digested with *Hpa*I, and the 920-bp fragment of *treA* was replaced by a *Sma*I fragment of pHP45Ω containing the Ω interposon mediating resistance to spectinomycin (Sp^r) (19). The resulting plasmid, pRHo14 (pTRE11 *treA*:Ω-sp), was inserted into the chromosome by linear transformation into strain MC4100 *recD*::Tn*10*. The resulting strain, RHo14, was selected for Sp^r and screened for Amp^s as well as the inability for growth on trehalose under high osmotic conditions. *treA*:Ω-sp showed 100% cotransducibility with *treA*::Tn*10*.

Molecular biological techniques. Plasmid preparation and transformation were done according to the method described by Sambrook et al. (48). For restriction and ligation of DNA, the buffer systems of the suppliers were used. DNA fragments were separated in 0.7% agarose gels, and the DNA of the appropriate bands was eluted with the Jetsorb kit (Genomed Inc.). DNA sequencing was done by the dideoxy chain termination technique of Sanger et al. (49), as modified by Biggin et al. (2). After subcloning of the appropriate fragments in the M13mp18/19 vector system, deletions were generated according to the method of Dale et al. (14). The sequence reactions were done with digoxigenin-labeled primers and resolved by direct blotting electrophoresis on a GATC sequencer with chemiluminescence detection according to the manufacturer's manual (GATC Inc., Konstanz, Germany). Overlapping fragments were sequenced on double-stranded DNA of pUE1. The mRNA start was mapped by the reverse transcriptase method of primer extension according to the work of Belfort et al. (1). Twenty micrograms of total cellular RNA and 5 pmol of digoxigenin-labeled primer were annealed; this was followed by extension with 10 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) for 90 min. Detection was the same as described for sequencing.

Sequence homologies were found by queries to the latest release of all available databases, with the BLAST server at the National Center for Biotechnology, Bethesda, Md. Protein homology comparisons were done with the ClustalV program.

Cloning of *treB* and *treR* and construction of plasmids (see Fig. 1 and Table 2). Chromosomal DNA of strain ECL116 (12), partially digested with *Sau*3A and ligated into the *Bam*HI site of pBR322 (4), was used to transform strain UE49. Selection was for growth on trehalose. Plasmid pUE1 was isolated in this way. A *Bam*HI deletion of pUE1 yielded plasmid pKM100. Subcloning of a 6.5-kb *Pst*I fragment of pUE1 into pHSG575, a *tac* promoter-carrying plasmid (61), yielded plasmid pRIM11. By cutting with *Cla*I and *Asu*II followed by religation of the plasmid, pRIM11 was shortened to pWK11. Digestion of pWK11 with *Acc*I and *Asu*II followed by religation yielded plasmid pWK12. To subclone *treB* and *treC* with their own promoter, a 4.4-kb *Bsp*EI-*Cla*I fragment of pUE1 was ligated into a *Ngo*MI-*Cla*I-opened pACYC184 vector (11). The resulting plasmid, pRHo400, was isolated by its ability to complement both *treB* and *treC* mutations. A deletion of pRHo400 with *Sph*I resulted in plasmid pWK14. *treR* was cloned by ligating a 1.9-kb *Sna*BI-*Nco*I fragment from pKM100 into *Nco*I-*Xmn*I of pACYC184, yielding plasmid pRHo500. The screen was for repression of a *treB*::*lacZ* fusion.

In vivo selection of plasmid-encoded *treB*::*lacZ* fusions. To generate *lacZ* fusions in *treB*, the Tn*lacZ* transposable element (35) was used. For this purpose, plasmid pWK11 was transformed into strain CC311 (32). Cells (10^8) were spread on agar plates containing 250 μg of kanamycin per ml. The colonies of each plate were harvested, and the plasmid DNA was prepared, yielding several independent pools of mutagenized pWK11. Transformation into strain CC118 allowed screening for active *lacZ* fusions on 5-bromo-4-chloro-3-indolylgalactopyranoside (XG) indicator plates. The fusion points between the *tre* gene and *lacZ* were determined by double strand sequencing. Of 25 independent pools of mutagenized plasmid DNA, 10 active *lacZ* fusions were isolated. Two of these 10 were *treB*::*lacZ* fusions; the other eight were *treC*::*lacZ* fusions. Active *lacZ* fusions were moved into the chromosome by the recombination technique of Saarilahti and Palva (46) by selection for Kan^r Lac$^+$ Tre$^-$ Cam$^-$ phenotype. Strains WK211 and WK213 were isolated in this way (Table 1).

Expression of TreB in the minicell system. The plasmids pWK11, pWK12, and, as vector control, pHSG575 were transformed into strain DS410T (17). Minicells were prepared, and newly synthesized proteins were labeled with [^{35}S]methionine as described previously (43). Minicells containing 25 μg of protein were resuspended in 500 μl of MMA containing 0.4% glycerol and all amino acids except methionine (5 mM each). Ten microcuries of [^{35}S]methionine (Amersham International, Little Chalfont, Buckinghamshire, United Kingdom) was added, and the suspension was incubated for 30 min at 37°C. The incorporation of labeled methionine was stopped by adding 50 μl of 3 mM unlabeled methionine, and the incubation was continued for 5 min. The minicells were harvested, washed once in 1 ml of TNE (50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 1 mM EDTA), and resuspended in 25 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. After incubation for 4 h at 50°C or at room temperature overnight, the samples were heated for 2 min at 95°C and separated by SDS-PAGE (12.5% polyacrylamide gel) (28) and autoradiographed.

Enzymatic assays. β-Galactosidase was assayed according to the method described by Miller (38). Specific activity is given in micromoles of *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein at room temperature. The protein concentration was taken from the optical density at 578 nm of the bacterial culture, with the correlation that the optical density at 578 nm of 1.0 is equivalent to 107 μg of protein per ml. The activity of the trehalose-6-phosphate hydrolase was measured according to the method described by Rimmele and Boos (44). The specific activity is given in micromoles of *para*-nitrophenyl-β-D-glucopyranoside hydrolyzed per minute per milligram of protein at room temperature.

Transport activity of trehalose and other sugars. Transport activity was assayed as described previously (6).

Nucleotide sequence accession number. The nucleotide sequence of *treB* reported in this paper has been submitted to GenBank under accession number U06195. The amino acid sequence of TreB has been submitted to the Swissprot database under accession number P36672.

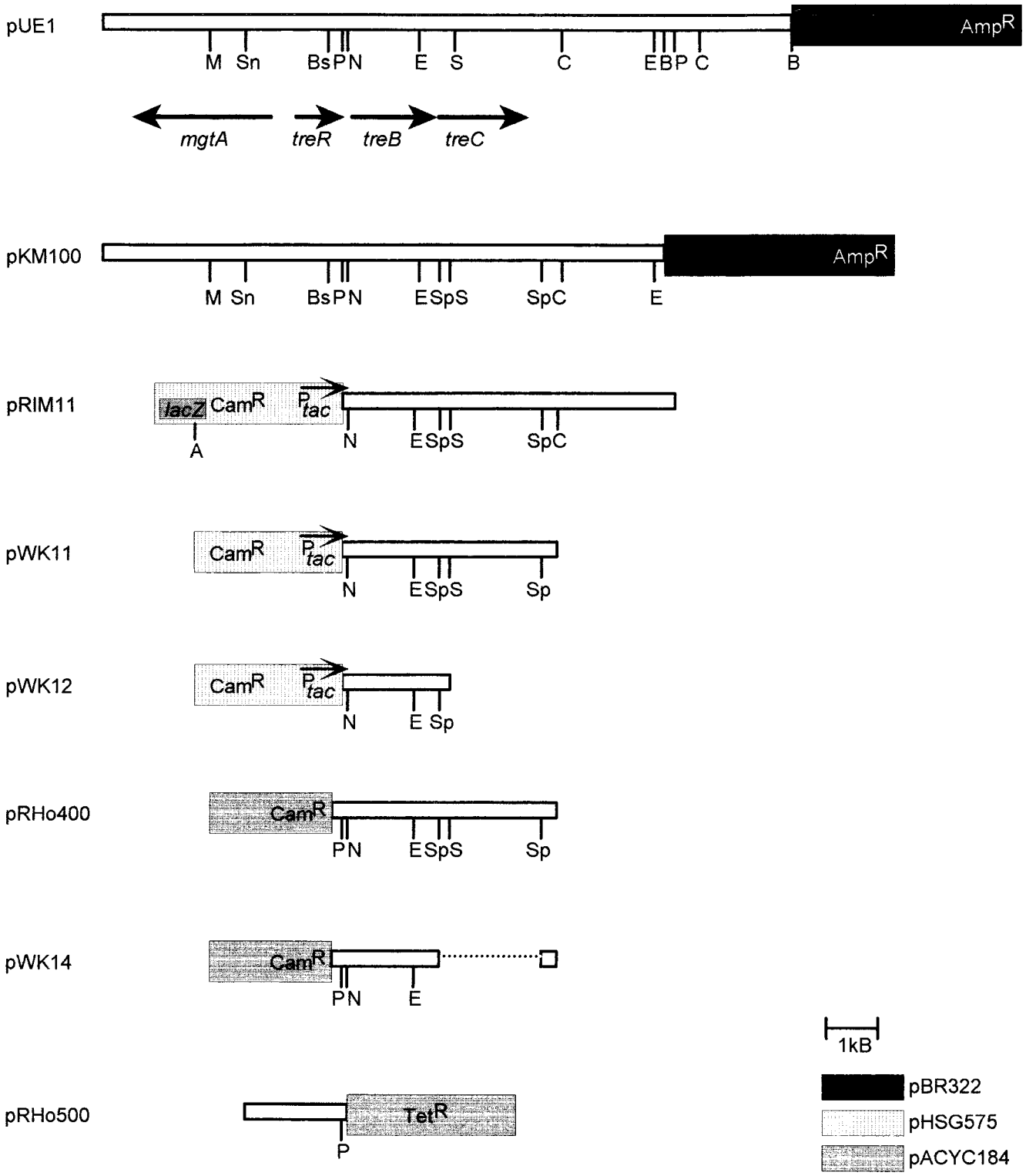


FIG. 1. Plasmids used in this study. The DNA represents an area of the 96-min region of the genetic map of the *E. coli* chromosome that is depicted in the clockwise orientation from left to right. All plasmids are derived from pUE1, which is a clone from a DNA library obtained by a limited *Sau*3A digestion of chromosomal DNA (strain ECL116 [12]) ligated in pBR322. The cleavage sites of the following endonuclease restriction enzymes are shown: A, *Asu*II; B, *Bam*HI; Bs, *Bsp*EI; C, *Cla*I; E, *Eco*RI; M, *Mlu*I; N, *Nco*I; P, *Pst*I; S, *Sal*I and *Acc*I; Sn, *Sna*BI; Sp, *Sph*I.

TABLE 2. Complementational analysis of the different plasmid subclones for growth on trehalose

Plasmid	Growth on MMA trehalose when transformed into strain[a]:			
	UE15 (*treB*$^+$ *treC*$^+$)	UE49 (*treB*$^+$ *treC*::*lacZ*)	KRIM4 (*treB treC*$^+$)	WK208 Δ(*treB-treC*)
pBR322	+	−	−	−
pHSG575	+	−	−	−
pACYC184	+	−	−	−
pUE1	+	+	+	+
pKM100	+	+	+	+
pRIM11	+	+	+	+
pWK11	+	+	+	+
pWK12	+	−	+	−
pRHo400	+	+	+	+
pWK14	+	−	+	−

[a] Several independent selected transformants have been tested for their ability to restore growth on trehalose. The appropriate antibiotics were added to the media for maintenance of the plasmids. Growth was scored after 2 days at 37°C.

RESULTS

Cloning of the *treB treC* region. Strain UE49 was used as a recipient strain for transformation with a gene bank of partially *Sau*3A-digested DNA fragments (6 to 20 kb) of strain ECL116 (12) into the *Bam*HI site of pBR322. UE49 is defective in *treA* and carries a *treC-lacZ* fusion. It cannot grow on trehalose as the sole source of carbon either at low or at high medium osmolarity (6). A plasmid, pUE1, complementing UE49 for growth on trehalose at low but not high osmolarity was isolated. Therefore, it was likely to carry the *treB treC* region but not *treA*. The plasmid not only complemented UE49, being defective in *treC*, but also complemented KRIM4, a strain that is defective in trehalose transport (6). Subcloning into pHSG575, a *tac* promoter-carrying plasmid (61), yielded the plasmids pRIM11, pWK11, and pWK12. In Fig. 1, the size of these plasmids is shown, and in Table 2, their ability to complement strains UE49 and KRIM4 is shown. pWK12, the plasmid with the smallest chromosomal insert, 2 kb, was able to complement the transport function in KRIM4 but not the trehalose-6-phosphate hydrolase activity lacking in strain UE49. The presence of pWK11 and pWK12 in strain UE26, wild type in respect to the *tre* gene cluster, after growth on glycerol increased the transport activity only for trehalose. The uptake of sucrose, maltose, glucose, and galactose was not affected (Table 3).

Thus, the 2-kb fragment of pKW12 must contain the entire *treB* gene encoding the capacity to take up trehalose.

Expression of *treB* and *treC* in minicells. The minicell-producing strain DS410T was transformed with plasmids pWK11 (*treB*$^+$ *treC*$^+$) and pWK12 (*treB*$^+$) as well as the vector plasmid pHSG575 (Fig. 1). Minicells were isolated, and newly synthesized proteins were labeled with [^{35}S]methionine. The proteins were analyzed by SDS-PAGE and autoradiographed. The results are shown in Fig. 2. Both pWK11 and pWK12 (but not the vector plasmid) direct the synthesis of a 42-kDa protein and can complement KRIM4 for growth on trehalose. Plasmid pWK12, which is unable to complement strain UE49 for growth on trehalose, lacks the 62-kDa TreC protein (44). Thus, it is clear that the 42-kDa protein represents the *treB* gene product.

TABLE 3. Transport of several sugars in UE26 (*treB*$^+$ *treC*$^+$) carrying plasmid-encoded *treB*$^+$[a]

Plasmid	Initial rate of transport of sugar				
	Trehalose	Sucrose	Maltose	Glucose	Galactose
pHG575	0.65	0.12	2.2	25	5.22
pWK11	8.6	0.13	2.9	48	6.3
pWK12	4.38	0.09	2.3	35	4.25

[a] The strain was grown on glycerol. Initial rate of transport of radioactive sugars was measured in cells resuspended in minimal medium. Substrate concentration was 30 nM. The rate is given in picomoles per minute per 10^9 cells.

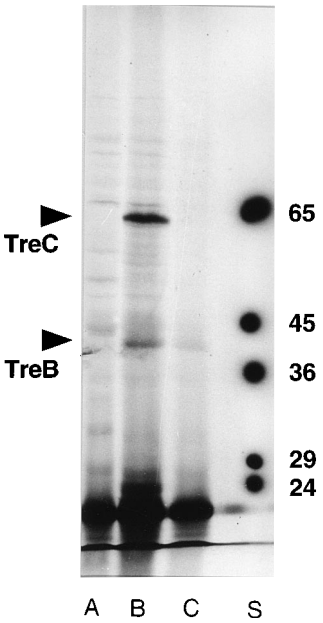


FIG. 2. Expression of plasmid-encoded *treB* and *treC* in minicells. Plasmid-encoded proteins were labeled with [^{35}S]methionine and analyzed by SDS-PAGE (12.5% polyacrylamide gel). The autoradiogram shows the proteins encoded by the following plasmids: lane A, vector pHG575; lane B, pWK11 (*treB*$^+$ *treC*$^+$); lane C, pWK12 (*treB*$^+$); lane S, molecular weight standards (numbers are in thousands). The positions of TreB and TreC are indicated by arrowheads.

The sequence of *treB*. The DNA region of the *Pst*I-*Acc*I fragment contained in pWK12 was sequenced on both strands with the dideoxy termination technique on single-stranded DNA after subcloning deletion fragments into M13. Overlapping fragments on both ends were sequenced on double-stranded DNA of pUE1. The result is shown in Fig. 3. An open reading frame was found starting at the ATG codon at position 398 and ending with the TAA stop codon at position 1853. This open reading frame encodes 485 amino acids and represents *treB*. Four nucleotides downstream of the stop codon of *treB*, the ATG start codon of *treC* was recognized, continuing with the known sequence of *treC* (44). Upstream of the ATG start codon of *treB*, sequences were recognized that are likely to function as −10 and −35 regions of a σ^{70}-dependent promoter. Indeed, primer extension analysis with reverse transcriptase (Fig. 4) revealed the start point of *treB* transcription 7 nucleotides downstream of the proposed −10 region. In addition to the promoter, we recognized two sequences, indicated in Fig. 3, that could act as recognition sites for the cyclic AMP (cAMP)-catabolite activator protein complex (potential half-sites are underlined). This is consistent with a previous observation that *treA* Δ*crp* or *treA* Δ*cya* strains are not able to grow on trehalose as the sole carbon source (6). One of these potential cAMP-catabolite activator protein sites is framed by two nearly palindromic sequences that are likely to represent

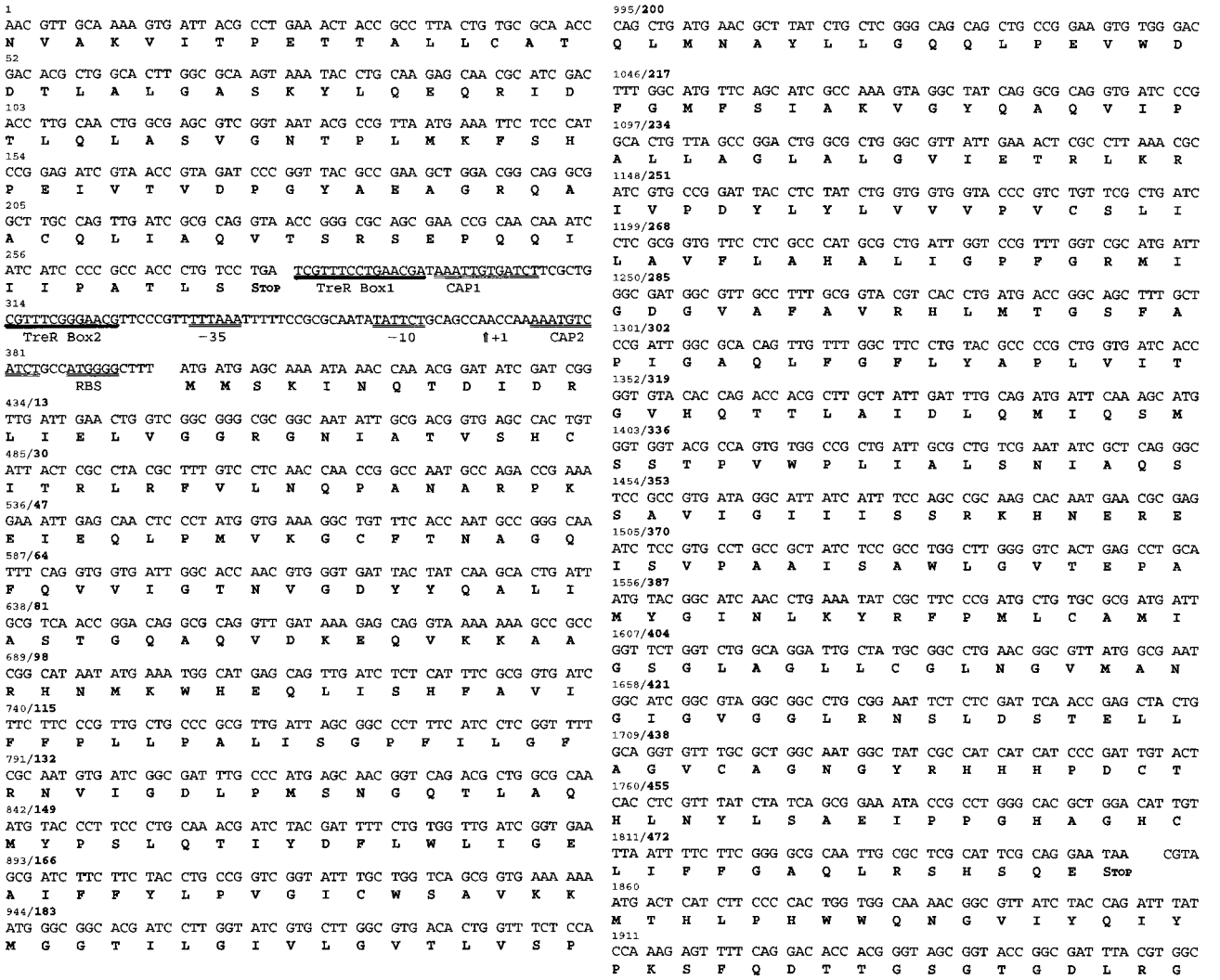


FIG. 3. DNA sequence of *treB* and flanking regions. The open reading frame representing *treB* starts with the first ATG codon at nucleotide 398 of the sequence and ends with the TAA stop codon at nucleotide 1856 of the sequence. The deduced amino acids are numbered (1 to 485) in boldface type. Potential −35 and −10 regions of the promotor are doubly underlined. Potential binding sites for the TreR repressor are underlined and indicated as TreR Box1 and Box2 (solid lines). Similarly, potential catabolite activator protein binding sites and the potential ribosome binding site are indicated by CAP1 and CAP2 and RBS, respectively (double lines). The start of *treB* transcription as determined by reverse transcriptase (Fig. 4) begins with nucleotide 368 and is indicated by an arrow. The open reading frame upstream of *treB* represents the 3′ portion of the *treR* gene encoding the trehalose repressor. The open reading frame downstream of *treB* represents *treC* encoding trehalose-6-phosphate hydrolase (44).

binding sites for TreR, the negative regulator of the system. The primer extension experiment shown in Fig. 4 revealed the presence of a second transcript which apparently is not under the control of induction by trehalose and is seen only with plasmid as template. This transcript has its origin 20 nucleotides downstream of the trehalose-inducible promoter. Its significance for the transcription of the *tre* operon in vivo is unclear at present.

We recognized the existence of another open reading frame preceding the *treB* control region whose C-terminal portion is also shown in Fig. 3. This open reading frame is part of *treR* encoding the repressor of the system. The sequence, the purification, and the properties of this protein will be the subject of another publication.

TreB is homologous to the enzymes II^{Sucrose} of different bacterial origin. The search for homology to the *treB*-encoded protein revealed homology (36.5% identity) to another enzyme II of the PTS, specific for sucrose, encoded by *srcA* (18) that is carried on the plasmid pUR400, derived from enteric bacteria (52). The *treB*-encoded enzyme is also homologous (33 to 37% identity) to enzymes II^{Src} of other organisms listed in the review of Lengeler et al. (31). The comparison of the deduced amino acid sequence of TreB with that of the pUR400-encoded SrcA protein is shown in Fig. 5. The figure indicates the conserved regions (R1 to R6) recognized in many enzymes II analyzed so far (41). Conserved at the amino terminus is the CATRLR amino acid sequence motif, characteristic for the EIIB domain (nomenclature of Saier and Reizer [47]), thought to be involved in the phosphoryl transfer from the phosphohistidine of EIIA^{Glc} to the incoming sugar substrate (40, 42, 64). Characteristic for the enzyme IIC domain aside from its hydrophobic nature is the GITE motif, which in the case of TreB is GVTE at position 384. The function of this sequence motif is also thought to be important for the phosphoryl transfer to the incoming substrate, since mutations in this motif result in the uncoupling of transport and phosphorylation (30,

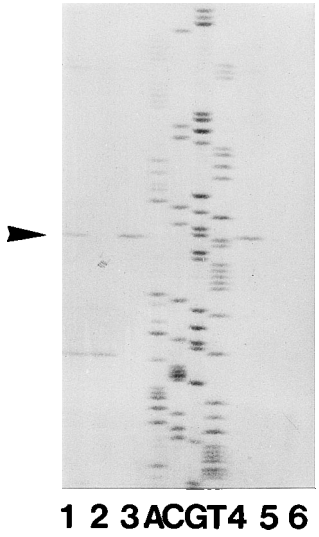


FIG. 4. Determination of the start of transcription of the *treB treC* operon. The method of primer extension by reverse transcriptase was used. The primer consisted of the oligonucleotide representing nucleotides 450 to 432 of the sequence shown in Fig. 3. The primer extension reaction was done with RNA isolated from the following strains: lane 1, UE14/pUE1, grown on trehalose; lane 2, UE14/pUE1, grown on glycerol; lane 3, UE14, grown on trehalose; lane 4, UE15, grown in TB plus trehalose; lane 5, WK139 (Δ*cya*) (6), grown in TB plus trehalose; lane 6, WK140 (Δ*crp*) (6), grown in TB plus trehalose. Lanes A, C, G, and T represent the sequencing reactions with the same primer.

45). The positioning of the CATRLR amino acid sequence motif at the N terminus and the GVTE motif at the C terminus as well as the lack of a covalently attached EIIA domain characterizes the TreB protein as an EIIBC-type enzyme II of the PTS.

The formation of an amphipathic helix with the amino acids 90 to 106 at the N-terminal portion of the protein can be envisioned as shown in Fig. 6. The function of such a potential amphipathic helix is unclear. However, a role as a topological signal for membrane assembly has been discussed for a similar helix with the mannitol enzyme II of *E. coli* (65) and of the chemotactic signal transducer, Tsr (54).

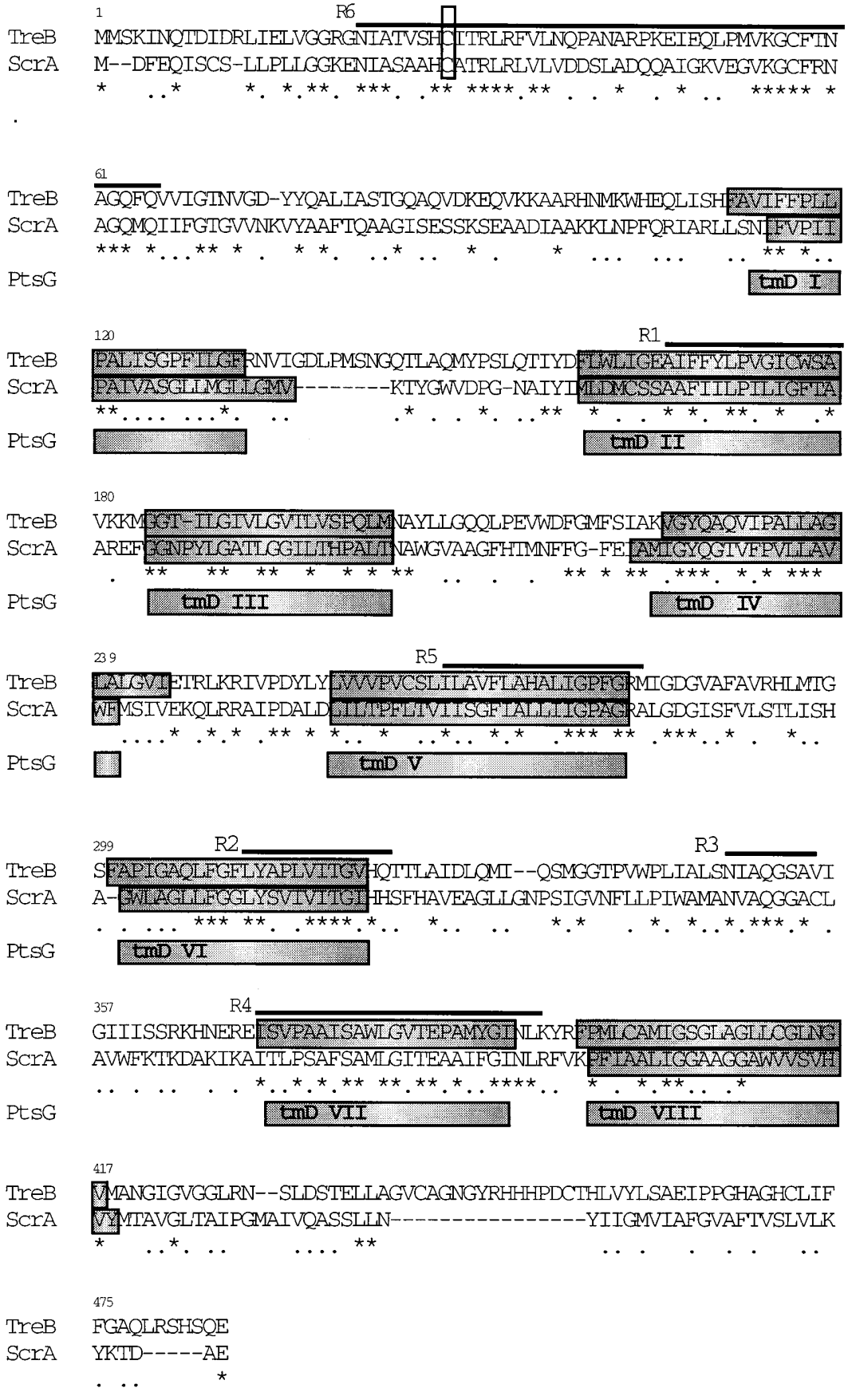
***treB* and *treC* form an operon.** The positioning of *treB* and *treC* only four nucleotides apart (Fig. 3) suggested that they form an operon. This was supported by the finding that *lacZ* fusions in *treB* exhibit a strong polar effect on the expression of the *treC* gene. This is shown in Table 4. Plasmid pWK11 expresses *treC*-encoded trehalose-6-phosphate hydrolase constitutively. The introduction of a *lacZ* fusion into pWK11, early in the *treB* gene, abolishes the synthesis of trehalose-6-phosphate hydrolase. After transfer of the *treB-lacZ* fusion into the chromosome, the same phenomenon can be observed.

Northern (RNA) analysis of total RNA, with a 2.4-kb *Eco*RI-*Eco*RV DNA fragment containing *treC* as probe, revealed one band with the length of about 3,100 nucleotides, consistent with an mRNA harboring both genes (data not shown).

The *treB treC* operon is negatively regulated by TreR, whose gene *treR* is located upstream of *treB* but is not part of the operon. Sequencing of the DNA contained in pUE1 carrying a large chromosomal insert of more than 12 kb including the *treB treC* cluster (Fig. 1) revealed a sequence that appeared highly homologous to a sequence found in *Salmonella typhimurium* encoding MgtA, a transport system for magnesium (62). Next to the *mgtA* sequence in *S. typhimurium*, an open reading frame had been found exhibiting homology to typical repressor proteins even though this sequence was apparently not involved in the regulation of *mgtA* (62). Thus, it appeared likely that the open reading frame would also be present in *E. coli* and could be relevant to the regulation of the *treB treC* operon instead of the *mgtA* gene. The region upstream of *treB* was therefore cloned separately and tested for its ability to down regulate the expression of *treB-lacZ* and *treC*. pRHo500, the smallest plasmid, contains a 1.9-kb chromosomal DNA fragment that begins with a *Sna*BI site within *mgtA* and ends with a *Nco*I site early in *treB* (Fig. 1). In addition, a mini-Tn*10* insertion carrying a chloramphenicol resistance marker (24) (strain RHo18) was isolated in a *treB-lacZ* strain that was cotransducible by P1 transduction with the *treB-lacZ* fusion. As shown in Table 5, this strain exhibits a high and constitutive β-galactosidase activity in contrast to its parent, strain RHo17, not containing the chloramphenicol insertion. Therefore, the insertion had occurred in a gene whose function is that of a repressor. We called the repressor-encoding gene *treR*. After transformation of strain RHo18 with pRHo500, the constitutive expression of *treB-lacZ* was abolished. Similarly, when the insertion in *treR* was combined with a *treC-lacZ* fusion (strain RHo20), the expression of *treC-lacZ* became constitutive but was repressed after transformation of RHo20 with the *treR*$^+$-carrying plasmid pRHo500. Transforming strain RHo16 carrying the insertion in *treR* and otherwise *treB*$^+$ *treC*$^+$ wild type with pRHo500 abolished the constitutive expression of the trehalose-6-phosphate hydrolase activity of strain RHo16 (Table 6). Plasmid pWK14 harboring the *tre* promoter but not *treR* was able to titrate the repressor function. Strain RHo17 (*treB-lacZ treR*$^+$) when grown on glycerol strongly increased the expression of *treB-lacZ* after transformation with pWK14 (Table 5). These experiments demonstrated that the open reading frame upstream of *treB* encodes a repressor for the *treB* as well as the *treC* expression.

The constitutive expression of the *treB treC* operon in the absence of TreR is partially sensitive to high osmolarity. Induction of *treB treC* in a wild-type strain by trehalose in the growth medium is no longer possible in the presence of high osmolarity (6) (Table 7). On the basis of several findings, we had concluded that the reason for the inability to induce the *tre* operon at high osmolarity was degradation of the effective cytoplasmic inducer, trehalose-6-phosphate (26). Therefore, constitutive expression of the *tre* operon that is caused by the lack of repressor should be insensitive to high osmolarity. The availability of a *treR* mutation allowed us to test this prediction. Tables 5 and 6 show the results. Whereas the expression of *treB* and *treC* remained high in the constitutive genetic background (*treR*), the effect of high osmolarity was nevertheless still noticeable. On average, constitutive expression was reduced by a factor of 2 to 3. Thus, in addition to the degradation of inducer at high osmolarity, the expression of the *tre* operon may still be

FIG. 5. Amino acid sequence homology of TreB to ScrA, the enzyme II^{Scr} of enteric bacteria. An asterisk indicates sequence identity; a dot indicates homologous exchange. Black bars numbered R1 to R6 indicate regions conserved in PTS enzymes II as defined by Peri and Waygood (41). The boxed cysteine residue at position 29 of the TreB sequence represents the highly conserved cysteine of PTS enzymes II that is phosphorylated during transport. Shaded boxes indicate potential transmembrane domains as determined by hydrophobicity plots. These potential transmembrane domains are compared with the corresponding transmembrane domains (tmD I to VIII) as determined in the PtsG protein by *phoA* and *lacZ* fusions (8). The sequence data for ScrA are taken from references 18 and 31.


1
R6
TreB MMSKINQTDIDRLIELVGGRGNIATVSHCITRLRFVLNQPANARPKEIEQLPMVKGCFTN
ScrA M--DFEQISCS-LLPLLGGKENIASAAHCATRLRLVLVDDSLADQQAIGKVEGVKGCFRN
61
TreB AGQFQVVIGINVGD-YYQALIASTGQAQVDKEQVKKAARHNMKWHEQLISHFAVIFFPLL
ScrA AGQMQIIFGTGVVNKVYAAFTQAAGISESSKSEAADIAAKKLNPFQRIARLLSNIFVPII
PtsG tmD I
120
R1
TreB PALISGPFILGFRNVIGDLPMSNGQTLAQMYPSLQTIYDFLWLIGEAIFFYLPVGICWSA
ScrA PAIVASGLLMGLLGMV--------KTYGWVDPG-NAIYIMLDMCSSAAFIILPILIGFTA
PtsG tmD II
180
TreB VKKMGGT-ILGIVLGVILVSPQLMNAYLLGQQLPEVWDFGMFSIAKVGYQAQVIPALLAG
ScrA AREFGGNPYLGATLGGILTHPALTNAWGVAAGFHIMNFFG-FELAMIGYQGIVFPVLLAV
PtsG tmD III tmD IV
239
R5
TreB LALGVIETRLKRIVPDYLYLVVVPVCSLILAVFLAHALIGPFGRMIGDGVAFAVRHLMTG
ScrA WFMSIVEKQLRRAIPDALDLLLTPFLTVLISGFTALLIIGPAGRALGDGISFVLSTLISH
PtsG tmD V
299
R2
R3
TreB SFAPIGAQLFGFLYAPLVITGVHQTTLAIDLQMI--QSMGGTPVWPLIALSNIAQGSAVI
ScrA A-GWLAGLLFGGLYSVIVITGIHHSFHAVEAGLLGNPSIGVNFLLPIWAMANVAQGGACL
PtsG tmD VI
357
R4
TreB GIIISSRKHNERELSVPAAISAWLGVTEPAMYGINLKYRFPMLCAMIGSGLAGLLCGLNG
ScrA AVWFKTKDAKIKAITLPSAFSAMLGITEAAIFGINLRFVKPFIAALIGGAAGGAWVVSVH
PtsG tmD VII tmD VIII
417
TreB VMANGIGVGGLRN--SLDSTELLAGVCAGNGYRHHHPDCTHLVYLSAEIPPGHAGHCLIF
ScrA VYMTAVGLTAIPGMAIVQASSLLN----------YIIGMVIAFGVAFTVSLVLK
475
TreB FGAQLRSHSQE
ScrA YKTD-----AE

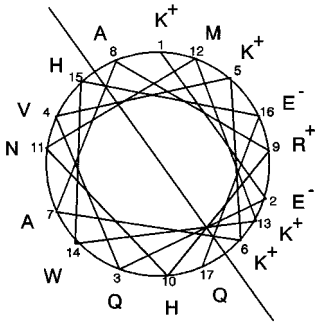


FIG. 6. Potential amphipathic helix at the N terminus of TreB. When amino acids 90 to 106 of TreB are drawn as an α-helix, the formation of an amphipathic helical structure with a predominantly positive charge distribution on one side can be visualized.

subjected to additional regulatory controls that are dependent on high osmolarity. We considered the possibility that the remaining reduction at high osmolarity was due to the effect of sigma factor RpoS, a central regulator of starvation and osmolarity (22). However, the introduction of an *rpoS*::Tn*10* mutation did not alter the twofold reduction in the constitutive expression of *treC-lacZ* at high osmolarity (Table 5).

TABLE 5. The expression of *treB* and *treC* is under the control of the TreR repressor and the constitutive expression of *treB* and *treC* in a *treR* strain is partially resistant against the repression by high osmolarity

Strain[a] and plasmids	Relevant genotype on:		β-Galactosidase activity[b]	
	Chromosome	Plasmid	No salt in the medium	250 mM NaCl in the medium
RHo17	*treB*::*lacZ treC*$^+$		0.0511	0.0157
pACYC184		Vector	0.0597	0.0211
pRHo500		*treR*$^+$	0.0067	0.0038
pWK14		*ptre treB*$^+$	1.295	ND[c]
RHo18	*treR treB*::*lacZ treC*$^+$		11.578	4.481
pACYC184		Vector	11.809	3.168
pRHo500		*treR*$^+$	0.0065	0.0024
RHo19	*treC*::*lacZ treB*$^+$		0.0061	0.0036
pACYC184		Vector	0.0072	0.0031
pRHo500		*treR*$^+$	0.0025	0.0022
RHo20	*treR treC*::*lacZ treB*$^+$		1.255	0.877
pACYC184		Vector	1.279	0.648
pRHo500		*treR*$^+$	0.0026	0.0020
RHo21	*treC*::*lacZ rpoS*		0.0054	0.0017
RHo22	*treR treC*::*lacZ rpoS*		1.326	0.696

[a] Strains were grown in MMA and glycerol as carbon source.
[b] β-Galactosidase activity is given in micromoles of ONPG hydrolyzed per minute per milligram of protein.
[c] ND, not determined.

DISCUSSION

In this paper, we describe the molecular characterization of TreB, the enzyme II^{Tre}, the PTS-dependent transport protein, specific for trehalose. Our analysis has shown that there is clear similarity of TreB to another enzyme II of the PTS, specific for the nonreducing disaccharide sucrose, found in many different bacteria (18, 31). This similarity is observed not only in the protein sequence homology but also in the sequence of the different domains that had been recognized in the PTS proteins. Thus, the TreB protein, like the ScrA protein, carries its B domain, the phosphoryl transfer domain, at the N terminus and the C domain proposed to be the permease proper at the C terminus. In addition, both proteins do not harbor the covalently bound phosphoryl donor domain EIIA but rely for this function on EIIA^{Glc}, the soluble EIIA domain (53) of the glucose transport system (9).

Very little is known about the membrane topology of EII proteins. The most thoroughly studied system is that of the *E. coli* glucose transporter (8), which is based on the generation of a nested set of *lacZ* and *phoA* fusions (33, 35). In this model, the EIIC domain spans the membrane eight times with both N- and C-terminal portions extending into the cytoplasm. So far, we were unable to isolate stable *phoA* or *lacZ* fusions to *treB*. Using the analysis for hydrophobicity according to the work of Kyte and Doolittle (27) and applying the positive inside rule for membrane protein topology (63) and the most sensible positioning of the sequence-derived amphipathic helix (Fig. 6), we propose a model that closely resembles that for the *E. coli* glucose transporter with eight membrane-spanning segments (8).

The *treB treC* operon is inducible by the presence of trehalose in the growth medium, but it becomes uninducible at high osmolarity (6). We had concluded that the inducer of the system is internal trehalose-6-phosphate which is formed by the TreB-mediated uptake of trehalose as trehalose-6-phosphate (26). From this study, we had also concluded that the major factor for the repression of the *treB treC* operon at high osmolarity was the inducer degradation by the cytoplasmic

TABLE 4. Polar effect of *treB*::*lacZ* fusions on the expression of *treC*[a]

Strain	Plasmid	β-Galactosidase activity	TreC activity
WK208 Δ(*treR treB treC*)	pHSG575 (vector)	<0.001	<0.0001
	pWK11 (*treB*$^+$ *treC*$^+$)	<0.001	0.136
	pWK11F1 (*treB-lacZ treC*$^+$)	2.405	<0.0001
	pWK11F2 (*treB-lacZ treC*$^+$)	4.875	<0.0001
UE14 (*treB*$^+$ *treC*$^+$)		<0.001	0.0600
WK211 *treB*$^+$ Φ(*treC*::*lacZ*)		0.30	<0.0001
WK213 *treC*$^+$ Φ(*treB*::*lacZ*)		0.10	<0.0001

[a] The specific activities are given in micromoles of hydrolyzed substrate per minute per milligram of protein. The strains were grown overnight in MMA with 0.2% both glycerol and trehalose. The cultures of plasmid-carrying strains contained 30 μg of chloramphenicol per ml.

TABLE 6. *treC* expression and high osmolarity

Strain[a] and vector	Relevant genotype on:		TreC activity[b]	
	Chromosome	Plasmid	No salt in the medium	250 mM NaCl in the medium
RHo14	*treB*$^+$ *treC*$^+$		0.00015	0.00011
pACYC184		Vector	0.00047	0.00010
pRHo500		*treR*$^+$	<0.0001	<0.0001
RHo16	*treR treB*$^+$ *treC*$^+$		0.0364	0.0112
pACYC184		Vector	0.0281	0.0145
pRHo500		*treR*$^+$	<0.0001	<0.0001

[a] Strains were grown in MMA and glycerol as carbon source.
[b] TreC activity is given in micromoles of *para*-nitrophenyl-β-D-glucopyranoside hydrolyzed per minute per milligram of protein.

TABLE 7. *treC* expression is induced by trehalose in the medium but not at high osmolarity

Strain[a] and plasmids	Relevant genotype on:		TreC activity[b]	
	Chromosome	Plasmid	No salt in the medium	250 mM NaCl in the medium
RHo14 (uninduced)	*treB*$^+$ *treC*$^+$		0.0008	ND[c]
RHo14	*treB*$^+$ *treC*$^+$		0.0337	0.0001
pACYC184		Vector	0.0431	0.0002
pRHo500		*treR*$^+$	0.0044	0.0003

[a] Strains were grown in MMA and glycerol (0.4%)-trehalose (0.2%) as carbon source.
[b] TreC activity is given in micromoles of *para*-nitrophenyl-β-D-glucopyranoside hydrolyzed per minute per milligram of protein.
[c] ND, not determined.

trehalose-6-phosphate phosphatase that is induced at high osmolarity and is part of the biosynthetic pathway of trehalose synthesis at high osmolarity (58). The availability of a mutant defective in *treR* encoding the repressor of the *treB treC* operon allowed us to test whether or not the osmotic repression of the operon is due only to inducer degradation. The experiments shown in Tables 5 and 6 demonstrate that the constitutivity of the *treB treC* operon in the *treR* strains RHo16, RHo18, and RHo20 is partially repressed by osmolarity. Thus, inducer degradation is not the only but the major factor in the osmotic repression of the *treB treC* operon. The mechanism by which the remaining repression due to high osmolarity occurs remains unclear; RpoS, the central regulator of starvation and osmotic control (22), seems not to be participating.

High-affinity uptake systems for disaccharides exhibiting high rates of flux necessitate the presence of a specific porin in the outer membrane particularly at low substrate concentration. This can be seen in the case of the high-affinity and binding protein-dependent maltose transport system (60) as well as the plasmid-encoded PTS-dependent sucrose system (51). Since trehalose can be used as a carbon source and since its uptake system exhibits a reasonably high maximal rate of transport at the micromolar level of substrate, one would expect the presence in *E. coli* of a trehalose-inducible porin. The *treB treC* operon did not contain an additional gene encoding a specific outer membrane porin. Instead, trehalose metabolism induces the λ receptor that is very effective in facilitating the diffusion of trehalose through the outer membrane at low substrate concentration (25).

With investigation of the function of the periplasmic trehalase (encoded by *treA*) (5), the enzymes involved in the internal synthesis of trehalose (encoded by the *ots* genes) (23), and the proteins TreB and TreC, involved in the uptake and degradation of trehalose (encoded by the *tre* operon) as described in this publication, the complete set of proteins necessary to understand the metabolism of trehalose in *E. coli* appears to be known. Yet, there are still some questions. One of these questions concerns the switch from growth at high osmolarity, at which large amounts of trehalose accumulate, to low osmolarity at which high internal trehalose is actually growth inhibitory (6). There are reports of trehalose exit from the cell (50, 59), even though it is unclear by what pathway this may be achieved. Facilitated diffusion via TreB as a mode of exit seems to be excluded since this protein is not induced under these conditions. There are some experimental results indicating the presence of an as yet uncharacterized cytoplasmic trehalase that is induced under growth conditions of high osmolarity (6, 44). This enzyme may act as an emergency valve to prevent overproduction of internal trehalose and may be crucial for the transition of high to low medium osmolarity.

The pathway of trehalose metabolism is interwoven with that of maltose and maltodextrins. Thus, growth on trehalose induces the maltose system (25). The end products of trehalose degradation are glucose and glucose 6-phosphate. The compounds needed for the synthesis of maltotriose, the internal inducer of the maltose system, are glucose and glucose 1-phosphate (15). Therefore, it would seem that the maltose system in *pgm* mutants, lacking phosphoglucomutase, could no longer be induced by trehalose. However, this is not the case (our unpublished observations). This indicates either the presence of an alternative enzyme forming glucose 1-phosphate from glucose 6-phosphate in the presence of glucose and in the absence of phosphoglucomutase (56) or the existence of an alternate way of forming maltotriose from glucose and glucose 6-phosphate.

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REFERENCES

1. **Belfort, M., J. Pederson-Lane, D. West, K. Ehrenman, G. Maley, F. Chu, and F. Maley.** 1985. Processing of the intron-containing thymidylate synthase (td) gene of phage T4 is at the RNA level. Cell **41:**375–382.
2. **Biggin, M. D., T. J. Gibson, and G. F. Houg.** 1983. Buffer gradient gels and ^{35}S-label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:**3963–3965.
3. **Bochner, B. R., H. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.
5. **Boos, W., U. Ehmann, E. Bremer, A. Middendorf, and P. Postma.** 1987. Trehalase of *Escherichia coli*. Mapping and cloning of its structural gene and identification of the enzyme as a periplasmic protein induced under high osmolarity growth conditions. J. Biol. Chem. **262:**13212–13218.
6. **Boos, W., U. Ehmann, H. Forkl, W. Klein, M. Rimmele, and P. Postma.** 1990. Trehalose transport and metabolism in *Escherichia coli*. J. Bacteriol. **172:** 3450–3461.
7. **Booth, I. R., J. Cairney, L. Sutherland, and C. F. Higgins.** 1988. Enteric bacteria and osmotic stress: an integrated homeostatic system. J. Appl. Bacteriol. Symp. Suppl. 35S–49S.
8. **Buhr, A., and B. Erni.** 1993. Membrane topology of the glucose transporter of *Escherichia coli*. J. Biol. Chem. **268:**11599–11603.
9. **Buhr, A., K. Flukiger, and B. Erni.** 1994. The glucose transporter of *Escherichia coli*; overexpression, purification, and characterization of functional domains. J. Biol. Chem. **269:**23437–23443.
10. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:**541–555.
11. **Chang, A. C., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141–1156.
12. **Chen, Y.-M., Y. Zhu, and E. C. C. Lin.** 1987. The organization of the *fuc* regulon specifying L-fucose dissimilation in *Escherichia coli* K-12 as determined by gene cloning. Mol. Gen. Genet. **210:**331–337.
13. **Crowe, J. H., L. M. Crowe, J. F. Carpenter, A. S. Rudolph, C. Aurell Wistrom, B. J. Spargo, and T. J. Anchordoguy.** 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta **947:**367–384.
14. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid **13:**31–40.
15. **Decker, K., R. Peist, J. Reidl, M. Kossmann, B. Brand, and W. Boos.** 1993. Maltose and maltotriose can be formed endogenously in *Escherichia coli*

from glucose and glucose-1-phosphate independently of enzymes of the maltose system. J. Bacteriol. **175:**5655–5665.
16. **Dinnbier, U., E. Limpinsel, R. Schmid, and E. P. Bakker.** 1988. Transient accumulation of potassium glutamate and its replacement by trehalose during adaptation of growing cells of *Escherichia coli* K12 to elevated sodium chloride concentrations. Arch. Microbiol. **150:**348–357.
17. **Dougan, G., and D. Sherratt.** 1977. The transposon Tn*1* as a probe for studying ColE1 structure and function. Mol. Gen. Genet. **151:**151–160.
18. **Ebner, R., and J. W. Lengeler.** 1988. DNA sequence of the gene *scrA* encoding the sucrose transport protein enzymeIISrc of the phosphotransferase system from enteric bacteria: homology of the enzymeIIScr and enzyme IIBgl proteins. Mol. Microbiol. **2:**9–17.
19. **Fellay, R., J. Frey, and H. Krisch.** 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for *in vitro* insertional mutagenesis of Gram-negative bacteria. Gene **52:**147–154.
20. **Giæver, H. M., O. B. Styrvold, I. Kaasen, and A. R. Strøm.** 1988. Biochemical and genetic characterization of osmoregulatory trehalose synthesis in *Escherichia coli*. J. Bacteriol. **170:**2841–2849.
21. **Gutierrez, C., M. Ardourel, E. Bremer, A. Middendorf, W. Boos, and U. Ehmann.** 1989. Analysis and DNA sequence of the osmoregulated *treA* gene encoding the periplasmic trehalase of *Escherichia coli* K12. Mol. Gen. Genet. **217:**347–354.
22. **Hengge-Aronis, R., R. Lange, N. Henneberg, and D. Fischer.** 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175:**259–265.
23. **Kaasen, I., J. Mcdougall, and A. R. Strom.** 1994. Analysis of the otsBA operon for osmoregulatory trehalose synthesis in *Escherichia coli* and homology of the OtsA and OtsB proteins to the yeast trehalose-6-phosphate synthase/phosphatase complex. Gene **145:**9–15.
24. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn10. Methods Enzymol. **204:**139–180.
25. **Klein, W., and W. Boos.** 1993. Induction of the λ receptor is essential for effective uptake of trehalose in *Escherichia coli*. J. Bacteriol. **175:**1682–1686.
26. **Klein, W., U. Ehmann, and W. Boos.** 1991. The repression of trehalose transport and metabolism in *Escherichia coli* by high osmolarity is mediated by trehalose-6-phosphate phosphatase. Res. Microbiol. **142:**359–371.
27. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
28. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
29. **Larsen, P. I., L. K. Sydnes, B. Landfald, and A. R. Strøm.** 1987. Osmoregulation in *Escherichia coli* by accumulation of organic osmolytes: betaines, glutamic acid, and trehalose. Arch. Microbiol. **147:**1–7.
30. **Lengeler, J. W.** 1990. Molecular analysis of the enzyme II-complexes of the bacterial phosphotransferase system (PTS) as carbohydrate transport systems. Biochim. Biophys. Acta **1018:**155–159.
31. **Lengeler, J. W., K. Jahreis, and U. F. Wehmeier.** 1994. Enzymes II of the phosphoenolpyruvate-dependent phosphotransferase systems: their structure and function in carbohydrate transport. Biochim. Biophys. Acta **1188:** 1–28.
32. **Manoil, C.** 1990. Analysis of protein localization by use of gene fusions with complementary properties. J. Bacteriol. **172:**1035–1042.
33. **Manoil, C.** 1991. Analysis of membrane protein topology using alkaline phosphatase and β-galactosidase gene fusions. Methods Cell Biol. **34:**61–75.
34. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA **82:**8129–8133.
35. **Manoil, C., and J. Beckwith.** 1986. A genetic approach to analyzing membrane protein topology. Science **233:**1403–1408.
36. **Maréchal, L. R.** 1984. Transport and metabolism of trehalose in *Escherichia coli* and *Salmonella typhimurium*. Arch. Microbiol. **137:**70–73.
37. **May, G., E. Faatz, M. Villarejo, and E. Bremer.** 1986. Binding protein-dependent transport of glycine betaine and its osmotic regulation in *Escherichia coli* K12. Mol. Gen. Genet. **205:**225–233.
38. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
39. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26:**101–106.
40. **Nuoffer, C., B. Zanolari, and B. Erni.** 1988. Glucose permease of *Escherichia coli*: the effect of cysteine to serine mutations on the function, stability, and regulation of transport and phosphorylation. J. Biol. Chem. **263:**6647–6655.
41. **Peri, K. G., and E. B. Waygood.** 1988. Sequence of cloned enzyme $II^{N\text{-acetylglucosamine}}$ of the phosphoenolpyruvate:*N*-acetylglucosamine phosphotransferase system of *Escherichia coli*. Biochemistry **27:**6054–6061.
42. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1993. Phosphoenolpyruvate-carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev. **57:**543–594.
43. **Reeve, J.** 1979. Use of minicells for bacteriophage-directed polypeptide synthesis. Methods Enzymol. **68:**493–503.
44. **Rimmele, M., and W. Boos.** 1994. Trehalose-6-phosphate hydrolase of *Escherichia coli*. J. Bacteriol. **176:**5654–5664.
45. **Ruijter, G. J. G., G. Van Meurs, M. A. Verwey, P. W. Postma, and K. Van Dam.** 1992. Analysis of mutations that uncouple transport from phosphorylation in enzyme II^{Glc} of the *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. J. Bacteriol. **174:**2843–2850.
46. **Saarilahti, H. T., and E. T. Palva.** 1985. In vivo transfer of chromosomal mutations onto multicopy plasmids utilizing *polA* strains: cloning of an *ompR* mutation in *Escherichia coli* K-12. FEMS Microbiol. Lett. **26:**27–33.
47. **Saier, M. H., and J. Reizer.** 1992. Proposed uniform nomenclature for the proteins and protein domains of the bacterial phosphoenolpyruvate-sugar phosphotransferase system. J. Bacteriol. **174:**1433–1438.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
50. **Schleyer, M., R. Schmid, and E. P. Bakker.** 1993. Transient, specific and extremely rapid release of osmolytes from growing cells of *Escherichia coli* K-12 exposed to hypoosmotic shock. Arch. Microbiol. **160:**424–431.
51. **Schmid, K., R. Ebner, K. Jahreis, J. W. Lengeler, and F. Titgemeyer.** 1991. A sugar-specific porin, *scrY*, is involved in sucrose uptake in enteric bacteria. Mol. Microbiol. **5:**941–950.
52. **Schmid, K., M. Schupfner, and R. Schmitt.** 1982. Plasmid-mediated uptake and metabolism of sucrose by *Escherichia coli* K-12. J. Bacteriol. **151:**68–76.
53. **Scholte, B. J., A. R. Schuitema, and P. Postma.** 1981. Isolation of III^{Glc} of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Salmonella typhimurium*. J. Bacteriol. **148:**257–264.
54. **Seligman, L., and C. Manoil.** 1994. An amphipathic sequence determinant of membrane protein topology. J. Biol. Chem. **269:**19888–19896.
55. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
56. **Stevens-Clark, J. R., M. C. Theisen, K. A. Conklin, and R. Smith.** 1968. Phosphoimidates. IV. Purification and characterization of a phosphoryl transfer enzyme from *Escherichia coli*. J. Biol. Chem. **243:**4468–4473.
57. **Strøm, A. R., P. Falkenberg, and B. Landfald.** 1986. Genetics of osmoregulation in *Escherichia coli*: uptake and biosynthesis of organic osmolytes. FEMS Microbiol. Rev. **39:**79–86.
58. **Strøm, A. R., and I. Kaasen.** 1993. Trehalose metabolism in *Escherichia coli*, stress protection and stress regulation of gene expression. Mol. Microbiol. **8:**205–210.
59. **Styrvold, O. B., and A. R. Strøm.** 1991. Synthesis, accumulation, and excretion of trehalose in osmotically stressed *Escherichia coli* K-12 strains: influence of amber suppressors and function of the periplasmic trehalase. J. Bacteriol. **173:**1187–1192.
60. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124:** 112–118.
61. **Takeshita, S., M. Sato, M. Toba, W. Masahashi, and T. Hashimoto-Gotoh.** 1987. High-copy-number and low-copy-number plasmid vectors for *lacZ* α-complementation and chloramphenicol- or kanamycin-resistance selection. Gene **61:**63–74.
62. **Tao, T., M. D. Snavely, S. G. Farr, and M. E. Maguire.** 1995. Magnesium transport in *Salmonella typhimurium*: *mgtA* encodes a P-type ATPase and is regulated by Mg2+ in a manner similar to that of the MgtB P-type ATPase. J. Bacteriol. **177:**2663–2672.
63. **von Heijne, G.** 1994. Membrane proteins: from sequence to structure. Annu. Rev. Biophys. Biomol. Struct. **23:**167–192.
64. **Wu, L. F., and M. H. Saier.** 1990. On the evolutionary origins of the bacterial phosphoenolpyruvate-sugar phosphotransferase system. Mol. Microbiol. **4:**1219–1222.
65. **Yamada, Y., Y. Y. Chang, G. A. Daniels, L. F. Wu, J. M. Tomich, M. Yamada, and M. H. Saier.** 1991. Insertion of the mannitol permease into the membrane of *Escherichia coli*—possible involvement of an N-terminal amphiphilic sequence. J. Biol. Chem. **266:**17863–17871.