JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 449–456
0021-9193/91/020449-08$02.00/0

Vol. 173, No. 2

Plasmid-Mediated Sucrose Metabolism in *Escherichia coli*: Characterization of *scrY*, the Structural Gene for a Phosphoenolpyruvate-Dependent Sucrose Phosphotransferase System Outer Membrane Porin

CYNTHIA HARDESTY,† CLAUDIA FERRAN, AND JOSEPH M. DiRIENZO*

Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received 15 June 1990/Accepted 22 October 1990

The *scrY* gene, part of the pUR400-borne sucrose regulon, appeared to be transcribed from its own promoter, with the transcriptional start site located 58 bp upstream from the initiation codon. An open reading frame encoding a polypeptide of 505 amino acid residues (M_r 55,408) was identified. The first 22 amino acid residues formed a leader sequence typical of those found in other procaryotic outer membrane and periplasmic proteins. A frameshift mutation in the *scrY* gene resulted in a dramatic decrease in sucrose transport with no effect on in vitro phosphorylation activity associated with enzyme II^{Scr}. The rate of diffusion of sucrose was 96 times greater than the rate of diffusion of lactose or maltose in liposomes containing the ScrY protein. This increase in sucrose permeability provided strong evidence that the ScrY protein functions as a sucrose porin. There was 23% amino acid sequence identity between the ScrY protein and LamB, a maltose porin from *Escherichia coli*.

One of the mechanisms by which a wide variety of bacterial species transport and phosphorylate sugars is the phosphoenolpyruvate (PEP)-dependent carbohydrate phosphotransferase system (PTS). The components of the PTS are the soluble cytoplasmic proteins enzyme I and HPr, which are general phosphoryl transfer proteins that participate in the phosphorylation of all PTS class sugars and a series of sugar group-specific integral cytoplasmic membrane proteins, enzymes II, which act as permeases. The enzymes II may function in a complex with another sugar group-specific class of membrane-associated phosphoproteins known as enzymes III. The characteristics of the various PTS components and the details of the phosphorylation cascade that drives the system have been thoroughly reviewed (9, 29, 31).

The *ptsI* (enzyme I), *ptsH* (HPr), and *crrA* (enzyme III^{Glc}) genes constitute an operon, at 52 and 49 min on the *Escherichia coli* (3) and *Salmonella typhimurium* (32) linkage maps, respectively. A number of the enzyme II genes that have been mapped in *E. coli* are widely dispersed on the chromosome (3, 29). However, many of these enzyme II genes are arranged in operons or regulons which usually contain a regulatory gene, the corresponding enzyme III gene, if required, and structural genes that code for substrate-metabolizing enzymes. (See reference 31 for a list of the genes which have been identified in some of these operons.)

A sucrose PTS regulon from *S. typhimurium* is located on a conjugative plasmid, pUR400, which has been transferred to *E. coli* (36, 40). Enteric bacteria other than *Klebsiella pneumoniae* cannot ferment sucrose unless they harbor this or a similar plasmid. The structure of the sucrose regulon of pUR400 has been reported (3, 16, 35, 36) and is similar to those of chromosomally located sucrose regulons in *Bacillus subtilis* (11) and *Streptococcus mutans* (34). In our studies, restriction fragments of pUR400 were cloned in *E. coli* K-12, and inducible sucrose fermentation was exhibited by clones that contained a 5.7-kb *Sal*I fragment (16). When sucrose-inducible gene products were expressed in maxicells, a previously unidentified protein having an apparent molecular weight of 68,000 was observed. This protein appeared to be localized in the outer membrane and did not appear to be either enzyme II^{Scr} (*scrA* gene) or β-D-fructofuranoside fructohydrolase (EC 3.2.1.26) (*scrB* gene). Analysis of a collection of plasmids with progressively larger deletions indicated that this protein was the product of a new gene, designated *scrC*. This gene was located immediately upstream from the *scrA* and *scrB* genes. Deletions resulting in the loss of this gene coincided with dramatically reduced sucrose uptake but had no effect on PEP-dependent sucrose phosphorylation associated with enzyme II^{Scr} (16). Schmid et al. (35) obtained similar results except that the apparent molecular weight of their gene product was 58,000. They designated the new gene *scrY*, and we will adopt that designation in this study for the sake of consistency.

Thus, an unusual feature of the plasmid-encoded sucrose PTS regulon was the existence of a new gene involved in sucrose transport. Here we present the complete nucleotide sequence of the *scrY* gene, demonstrate the porinlike properties of the gene product (ScrY), and show similarities between the gene product and LamB protein, a maltose porin found in the outer membrane of *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *E. coli* TB1 [Δ(*lac-pro*) *rpsL ara thi* φ80d*lacZ*ΔM15 *hsdR*] (Life Technologies, Inc., Gaithersburg, Md.) and RR1(λ *c*I857) (7) were used as plasmid hosts in this study. *E. coli* JM107

* Corresponding author.
† Present address: Gene Expression Sciences, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

TABLE 1. Plasmids used in this study

Plasmid	Relevant phenotype[a]	Source or reference
pUC18, pUC19	Amp^r LacZ	Cloning vector; Life Technologies[b]
pBR328	Amp^r Cml^r Tet^r	Cloning vector; ATCC[c]
pEV-vrf1	Amp^r λ p_L	Expression vector (7)
pJD2000	Scr^+ Amp^r Cml^r	5.7-kb *SalI* fragment containing the sucrose regulon cloned in pBR328 (16)
pCH183	Scr^+ Amp^r	5.7-kb *SalI* fragment from pJD2000 cloned in pUC18 (16)
pCH186	Scr^+ Amp^r	Insert in opposite orientation relative to pCH183 (16)
pCH186Δ104	$ScrY^-$ Amp^r	1.6-kb *SalI-PstI* deletion (16)
pCH186Δ470	Scr^- Amp^r	2.6-kb *EcoRI-SalI* deletion (16)
pCH183Δ52	EII^{Scr-} $ScrY^-$ Amp^r	2.9-kb *SalI-SmaI* deletion (16)
pCH183Δ21	EII^{Scr-} $ScrY^-$ Amp^r	3.1-kb *SalI-EcoRI* deletion (16)
pCH2	$ScrY^-$ Amp^r	Frameshift mutation in *scrY*; this study
pCV139	$ScrY^+$ Amp^r	*scrY* cloned in pEV-vrf1; this study

[a] Amp^r, ampicillin resistance; Cml^r, chloramphenicol resistance; Tet^r, tetracycline resistance; LacZ, β-galactosidase; λ p_L, lambda promoter; Scr^+, sucrose fermentation; EII^{Scr}, enzyme II^{Scr}; ScrY, 68-kDa protein.
[b] Life Technologies, Gaithersburg, Md.
[c] American Type Culture Collection, Rockville, Md.

[Δ(*lac-pro*) *thi gyrA96 endA1 relA1 supE44*(F′ *traD36 proAB lacI*^{q}ZΔM15 *hsdR17*) was used as the host for bacteriophage M13mp19 (26). The growth media used were L broth, K medium, and M9 minimal medium (24). Broth media were supplemented with either glycerol, glucose, fructose, or sucrose (0.4% final concentration unless otherwise noted) and ampicillin, tetracycline, or chloramphenicol (50 μg/ml). The M9 medium was also supplemented with 0.1 μg of thiamine per ml and the appropriate amino acids (40 μg/ml). *K. pneumoniae* CH, grown in brain-heart infusion broth, was used as a source of DNA for hybridization experiments. Solid media consisted of the media listed above or MacConkey agar base supplemented with 1% sucrose and ampicillin (50 μg/ml). The plasmids used in this study are described in Table 1.

DNA sequencing. Deletions were made with restriction enzymes or exonuclease III (18, 30) and were cloned in either pUC19 or M13mp19 (26). Plasmid DNA for sequencing of double strands was purified by a modification of the rapid boiling method (15). Sequencing was performed by the dideoxy chain termination method (33) with commercial and synthetic primers and the Sequenase kit (version 2; U.S. Biochemical Corp., Cleveland, Ohio). Both strands were sequenced a minimum of two times.

Primer extension. Total RNA was extracted (14) from late-log-phase cells of TB1(pCH186) or TB1(pCH183Δ52) grown in K medium supplemented with 0.4% sucrose. The RNA start site for the *scrY* gene was determined as described before (4) by using the synthetic primers 5′-GATAAGCATCGCAAGTGTGC-3′ and 5′-GCTTCAATG GTGCTTATATC-3′.

Construction of plasmids. A frameshift mutation in the *scrY* gene was made by treating pJD2000 DNA with *KpnI* and Klenow fragment. Blunt-end ligation was carried out as described previously (16). The 5.7-kb *SalI* fragment was then excised and cloned in pUC18 to create pCH2. The mutation was verified by DNA sequencing. To create the expression clone pCV139, pEV-vrf1 (7) was digested with *EcoRI*, and a 2-kb *Avi*II-*Eco*RI fragment was isolated from pCH186. The 5′ overhanging region of the plasmid and the fragment were filled in by using Klenow and deoxynucleotide triphosphates to create blunt ends. *E. coli* RR1(λ *c*I857) was transformed at 30°C following ligation. Transformants were selected by hybridization with a 1.1-kb *Sph*I DNA fragment, from pCH186, containing the 5′ end of the *scrY* gene.

Isolation of the ScrY protein. Three liters of a culture of TB1(pCH186) was lysed by sonication, and the membrane fraction was collected by centrifugation at 100,000 × *g* (Spinco 70Ti rotor) for 1 h. The membrane fraction was then extracted with 0.5% sodium Sarkosyl for 30 min at room temperature, followed by 1% sodium dodecyl sulfate (SDS)–0.4 M NaCl–5 mM EDTA–10 mM Tris hydrochloride (pH 8.0) for an additional 30 min at room temperature. The soluble fraction (2 mg of protein) from the SDS extraction was loaded on a Sephadex G-200 column (2 by 75 cm) equilibrated in the same SDS buffer. Fractions containing the ScrY protein were pooled and run on a Sepharose 6B column (2 by 75 cm). Fractions containing the ScrY protein were exhaustively dialyzed against deionized water for incorporation into liposomes. The ScrY protein constituted 61% of the total protein in the final fraction, as determined by scanning densitometry of gels stained with Coomassie blue.

Assays. The uptake of carbohydrate (36), PEP-dependent sugar phosphorylation activity (20), and sucrase activity (16) were measured as described previously except that cells were grown in 0.1 to 0.6% sucrose. The soluble PTS protein fraction was obtained from *E. coli* TB1 by published procedures (20). Phospholipids, used to form the liposomes, were extracted from *E. coli* TB1 by the method of Ames (1). Porin activity was measured by the liposome swelling method as described before (23). Briefly, 6 μmol of phospholipid was suspended in 200 μl of chloroform and dried under nitrogen. The sample was placed in a vacuum desiccator for 30 min, 400 μl of water was then added, and the suspension was sonicated for 1 min. Outer membrane protein (75 μg) or partially purified ScrY protein (10 μg) from cells grown in 0.1% sucrose was added, and the suspension was sonicated for an additional 15 s. The liposomes were then dried under nitrogen in a 45°C water bath. The liposomes were resuspended in 400 μl of 20 mM stachyose–2 mM Tris hydrochloride (pH 7.5). The liposomes (30 μl) were added to 600 μl of various isotonic sugar solutions, and the change in OD_{400} was determined. Isotonic solutions were prepared by using liposomes that did not contain protein. The rate of swelling was determined as $d(1/OD_{400})/dt$ between 7 and 14 s after the addition of liposomes to each of the test sugar solutions.

General procedures. Plasmid and genomic DNA were isolated as described previously (16). Southern blotting and DNA-DNA hybridizations were performed as described before (16). Outer membrane was recovered by isopycnic sucrose density gradient centrifugation (28), and the proteins

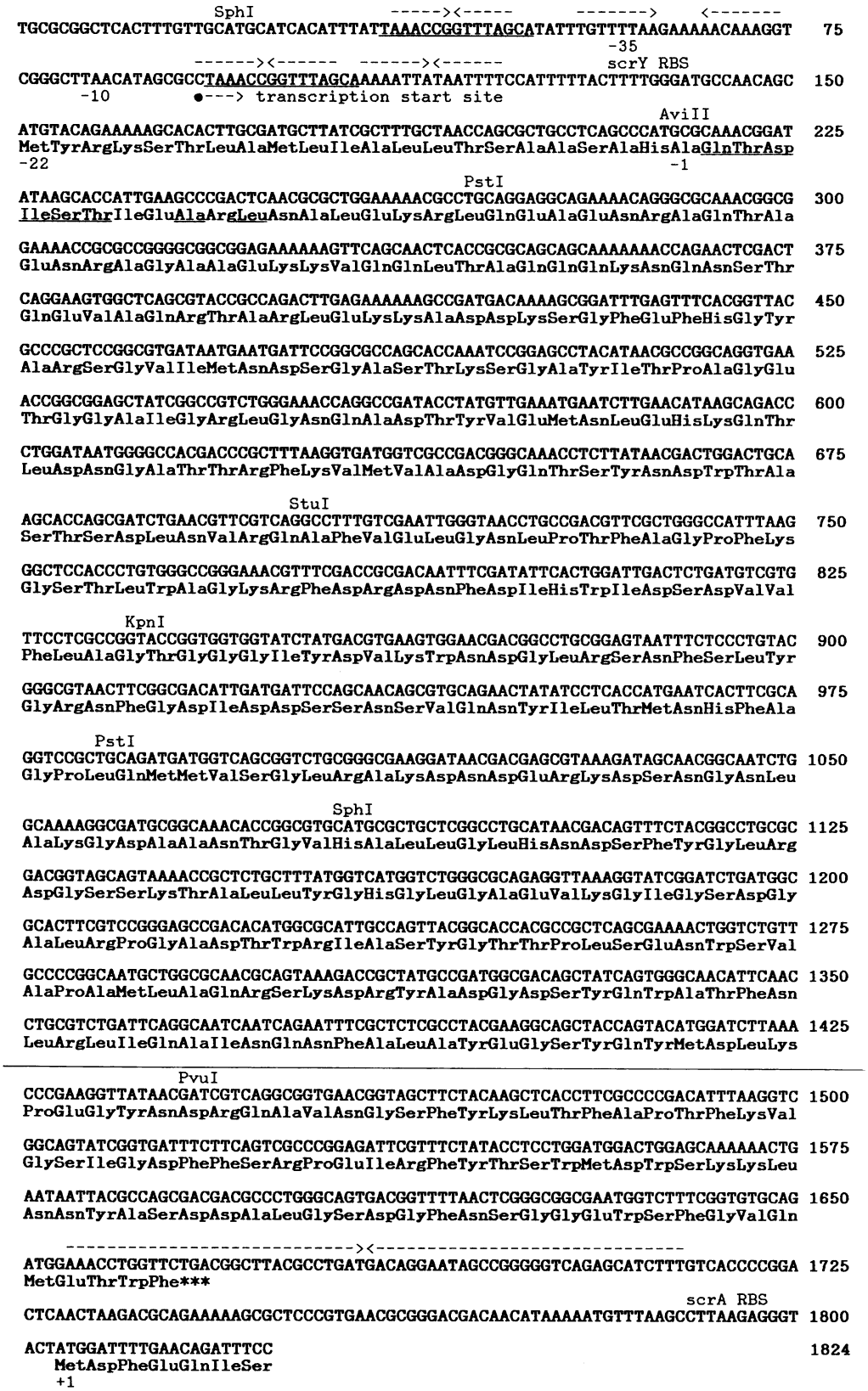

FIG. 1. Nucleotide sequence of the *scrY* gene and its upstream region. Selected restriction sites and the transcription start site are labeled. A direct repeat (bases 36 to 50 and 94 to 108) is underlined, and four inverted repeats, upstream from the *scrY* coding region, are identified by dashed arrows above the sequence. A putative ribosome-binding site (RBS) and promoter region (−10 and −35) are shown. A putative transcription termination loop (bases 1655 to 1714) is also labeled by dashed arrows. The deduced amino acid sequence of the gene product is written below the nucleotide sequence. Negative numbers designate the leader sequence of the ScrY protein. Amino acid residues determined by amino-terminal sequence analysis of the 68-kDa protein are underlined. The 5′ end of the *scrA* gene (10) is shown along with its putative ribosome-binding site. The deduced amino acid sequence of the amino-terminal end of the *scrA* gene product begins at residue +1.

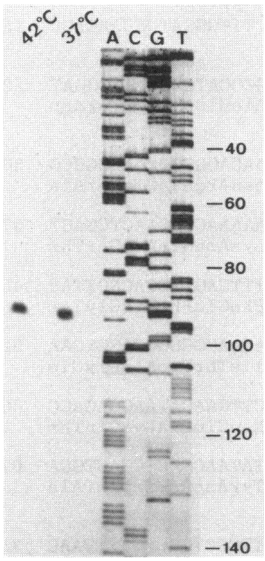

FIG. 2. Primer extension experiment showing the *scrY* transcription start site. The synthetic primer 5'-GCTTCAATGGTGCT TATATC-3' was end labeled and hybridized to total RNA from TB1(pCH186). Hybridization was performed at 48°C for 3.5 h. Separate reaction mixtures were incubated at 37 and 42°C for 1 h, and the samples were loaded on a 6% sequencing gel (lanes designated 37°C and 42°C). The corresponding nucleotide sequence of the primer-binding region, of the noncoding strand, was determined on the same gel (lanes A, C, G, and T). The numbers refer to the base positions shown in Fig. 1. The autoradiogram is shown. Some curvature in the running gel is evident in the first lane.

were analyzed by SDS-polyacrylamide gel electrophoresis on 17.5% slab gels. Samples were treated for electrophoresis as described before (16). Gels were stained with Coomassie brilliant blue and scanned with an LKB laser densitometer (Pharmacia-LKB Instruments, Paramus, N.J.). Total protein was determined by the method of Lowry et al. (22) with bovine serum albumin as a standard.

Amino acid sequence analysis was performed by D. Speicher, Wistar Institute, Philadelphia, Pa., for samples electroblotted on polyvinylidene difluoride membranes (Immobilon; Millipore Corp., Bedford, Mass.) as described before (25). Primers for DNA sequencing and primer extension experiments were made by M. Mitchell of the DNA Synthesis Service, University of Pennsylvania. Nucleic acid and protein data base searches were performed with software developed by the University of Wisconsin Genetics Computer Group (8).

Nucleotide sequence accession number. The sequence shown in Fig. 1 has been assigned GenBank accession no. M38416.

RESULTS AND DISCUSSION

Nucleotide sequence and amino acid sequence of the plasmid-encoded sucrose transport protein. A 1.1-kb *Sph*I fragment of DNA containing part of the *scrY* gene hybridized to RNA isolated from TB1(pCH183), TB1(pCH186), TB1(pCH 186Δ470), and TB1(pCH2) but not to RNA from TB1(pCH 186Δ104), TB1(pCH183Δ52), or TB1(pCH183Δ21) (data not shown), supporting the general location of the *scrY* gene. Since the exact boundaries of the *scrY* gene were not known, the nucleotide sequence of a 1,850-bp region upstream of and including the 5' end of the *scrA* gene was determined. The complete nucleotide sequence of this region is presented in Fig. 1. A single open reading frame was found beginning at base 151 and extending to base 1665. The open reading frame specified as protein containing 505 amino acids having a molecular weight of 55,408. However, the deduced gene product was smaller than the 68-kDa (16) and 58-kDa (35) proteins made by maxicells and minicells, respectively. To establish that the open reading frame identified corresponded to the previously assigned *scrC*/*scrY* gene, the 68-kDa protein was extracted from TB1(pCH186) and transferred to a polyvinylidene difluoride membrane for amino-terminal sequence analysis. The resulting sequence, Gln-Thr-Asp-Ile-Ser-Thr-X-X-Ala-X-Leu, was identical to the deduced amino acid sequence beginning at base 217 (Fig. 1, underlined residues). Since two Met residues were present, upstream and in frame, at positions −14 and −22, translation could be initiated at either of these two sites. From comparisons with other procaryotic signal sequences, the ScrY protein is most likely made with a 22-amino-acid leader sequence. A 14-amino-acid leader sequence would lack the positively charged amino terminus thought to be required for interaction of the elongating nascent peptide during protein translocation with the negatively charged inner surface of the cytoplasmic membrane (13). Procaryotic leader sequences generally range from 18 to 26 amino acids, with some as long as 31 residues (13). It is unlikely that the open reading frame began further upstream than at the start codon specifying the Met residue at position −22, since there was a termination codon (bases 94 to 96) in frame to the two putative initiation codons (bases 151 to 153 and 175 to 177).

A transcription initiation site was identified by primer extension experiments. Two synthetic primers, corresponding to nucleotides 164 to 183 and 223 to 242, hybridized to RNA isolated from TB1(pCH186). Identical results were obtained with both of these primers; however, only the experiment with the latter primer is shown (Fig. 2). The 5' end of the reverse transcript mapped at the C at base 93 (Fig. 1), 58 bases upstream from the first Met in the open reading frame. No hybridization was obtained with RNA from the deletion mutant TB1(pCH183Δ52). The −10 and −35 promoter regions were inferred from their predicted locations, since there was not good agreement to *E. coli* consensus promoter sequences (17) or to promoter sequences deposited in GenBank. A putative Shine-Dalgarno sequence (GGA) may be present 11 bases upstream from the first Met (residue −22) in the open reading frame. A 15-base direct repeat was found at positions 36 to 50 and 94 to 108 in the noncoding region (underlined bases in Fig. 1). An inverted repeat was found within each direct repeat, and two additional short inverted repeats were present at positions 53 to 72 and 109 to 122 (dashed arrows in Fig. 1). The significance of the direct repeat and inverted repeats is not known at present. Although these short inverted repeats at the 5' end of the mRNA did not appear to form stable secondary structures, a relatively stable hairpin-loop structure (ΔG, −20.8 kcal; calculated as in reference 38) may be formed at the 3' end of the molecule (bases 1655 to 1714 in Fig. 1).

There appeared to be an increased use of rare codons except for those used for Phe and Pro. One codon each for Leu (UUA), Val (GUA), and Pro (CCU) were not used. Although these three codons end in A or U, this did not appear to be the result of a distinct preference for G and C over A and T, since the G+C content of the *scrY* gene was 49%. In addition, the two codons for Cys (UGU and UGC) were not used. Codon usage for translation of the *scrA* gene

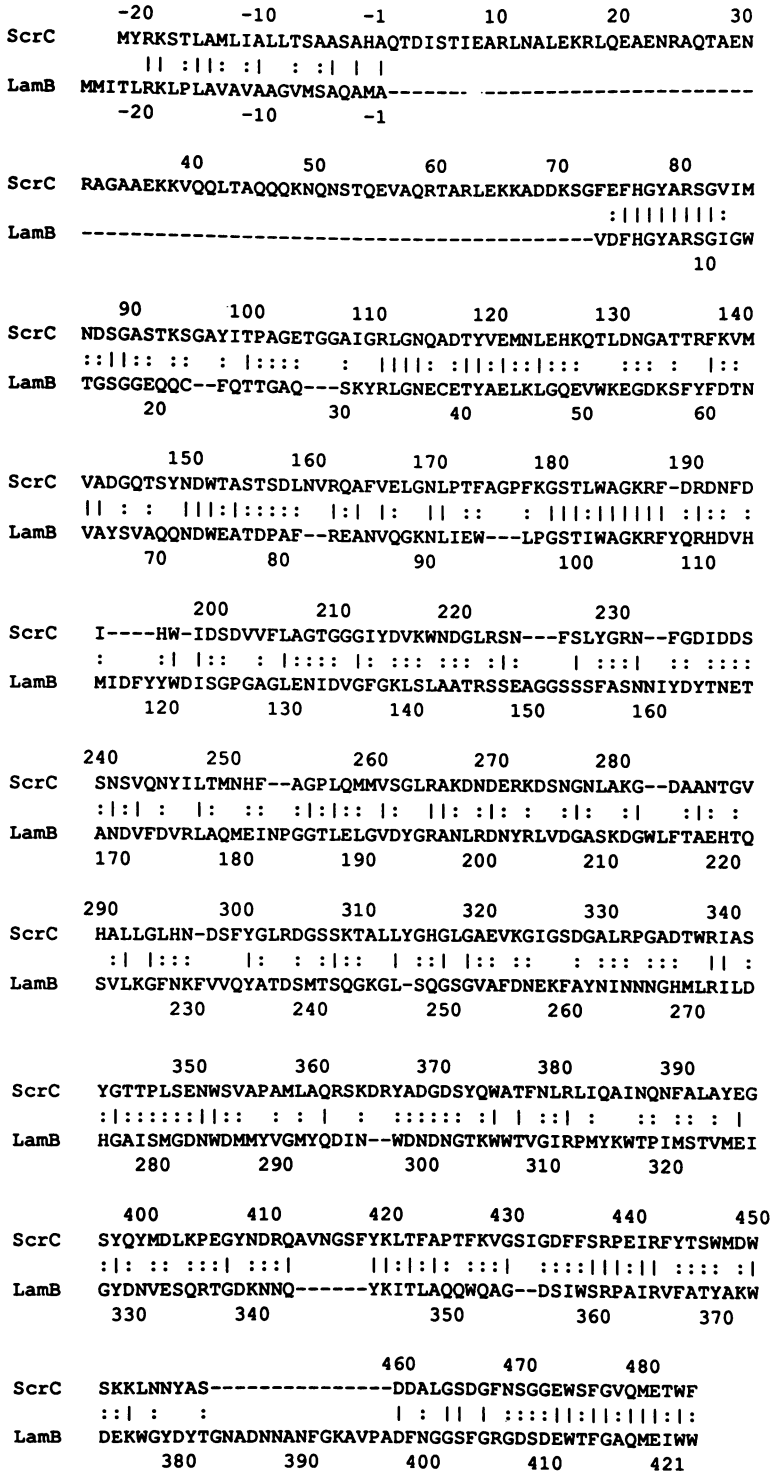

FIG. 3. Aligned amino acid sequences of ScrY (ScrC) and LamB. Identical matches are connected by vertical lines, and conserved residues are connected by dots. Gaps introduced in each sequence to improve the fit are shown by dashed lines.

from pUR400 was also biased towards rare codons (11). The use of rare codons for the translation of at least two genes in the *scr* regulon is consistent with that of genes within the plasmid-encoded *raf* operon of *E. coli* (2).

Sequence similarities. A search of the National Biomedical Research Foundation (NBRF) protein data bank revealed 23% sequence identity between the ScrY protein and LamB, an outer membrane maltose porin and bacteriophage lambda receptor from *E. coli* K-12 (19, 23). The computer-aligned sequences of the two proteins are shown in Fig. 3. The ScrY

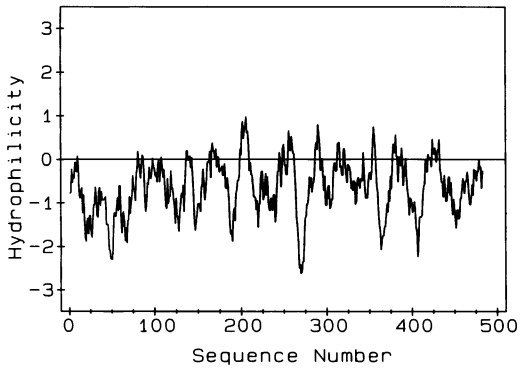

FIG. 4. Hydropathy plot of ScrY. The method used was that of Kyte and Doolittle (21), with a window size of nine amino acids. Hydrophilic values are negative.

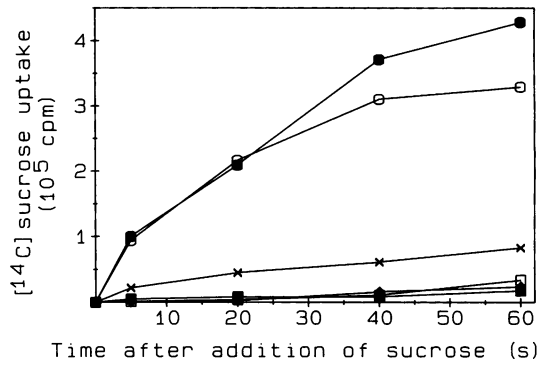

FIG. 5. Effect of a frameshift mutation in the *scrY* gene on the uptake of [^{14}C]sucrose. *E. coli* TB1, TB1(pCH186), and TB1(pCH2) were grown in minimal medium containing 0.1, 0.2, 0.4, or 0.6% sucrose to induce expression of *scrY*. The cells were washed extensively to remove sucrose and adjusted to an A_{490} of 1.2 (0.5 mg of cell protein per ml) in phosphate buffer. [^{14}C]sucrose (10 μCi [540 mCi/mmol]), was added to 1 ml of each cell suspension. Uptake was determined at 20 nM sucrose as described in Materials and Methods. Symbols: ●, TB1(pCH186), 0.1% sucrose; ○, TB1(pCH186), 0.2% sucrose; ×, TB1(pCH186), 0.4% sucrose; ■, TB1(pCH2), 0.1% sucrose; □, TB1(pCH2), 0.2% sucrose; ◆, TB1(pCH2), 0.4% sucrose. Uptake values for *E. coli* TB1, TB1(pCH186), and TB1 (pCH2) induced in 0.6% sucrose are not shown. These values were the same or lower than those shown for TB1(pCH2) grown in 0.4% sucrose (◆).

and LamB proteins contain leader sequences of 22 and 25 amino acids, respectively. Eight of these amino acids were perfect matches, while five additional amino acids showed conservative exchanges. Four regions, corresponding to amino acids 75 to 91, 110 to 126, 179 to 195, and 459 to 483 in the ScrY sequence (Fig. 3), exhibited strong identity. These regions did not correspond to the starch and phage lambda-binding domains of the LamB protein (6) and may therefore represent more common sequences related to the general structure of sugar-specific porins.

Figure 4 shows the hydropathy profile of the ScrY protein. Weakly hydrophobic regions alternated with moderate to strong hydrophilic domains. Similarities in structure to the LamB protein were also found (data not shown). The ScrY protein appears to be more hydrophilic than typical membrane proteins. For example, the polarity index (5) of the ScrY protein is 47.9. This value is closer to the polarities of soluble proteins and membrane proteins that can be solubilized in aqueous media. Ionic detergents were required to solubilize the ScrY protein (16). The reduced hydrophobicity of the ScrY protein may account for its anomalous migration on SDS gels due to the decreased binding of SDS.

Function of the ScrY gene product. Deletions at the 5′ end of the 5.7-kb *Sal*I fragment in pCH183 that extended to each of the *Pst*I sites within the *scrY* gene correlated with a significant reduction in sucrose uptake but did not affect the phosphorylation activity associated with enzyme II^{Scr} (16). However, approximately 500 bp of DNA immediately upstream from the *scrY* gene was also deleted from those plasmids, and this may have influenced sucrose transport. Therefore, a frameshift mutation was made in pJD2000 by taking advantage of the unique *Kpn*I site. The mutation in TB1(pCH2) altered the amino acids at positions 208 to 211 in the wild-type protein from Thr-Gly-Gly-Gly to Trp-Trp-Tyr-Leu and introduced a termination codon (bases 850 to 852) resulting in a truncated gene product (molecular weight, 25,610 before processing). The mutant exhibited a 24-fold reduction in sucrose uptake measured 60 s after the addition of radiolabeled sucrose when uptake was induced by growing the cells in 0.1% sucrose (Fig. 5). The in vitro sucrose phosphorylation activity of the mutant was not affected compared with that of TB1(pCH186). Membrane preparations from TB1(pCH2) and TB1(pCH186) produced 7.6 and 7.3 pmol of sucrose phosphate per min per mg of membrane protein, respectively. These results demonstrated that the decrease in sucrose uptake in TB1(pCH2) was not due to a polar effect on *scrA* gene expression. Growth of TB1 (pCH186) in increasing concentrations of sucrose, ranging from 0.1 to 0.6%, resulted in increased repression of sucrose transport (Fig. 5). This decrease in sucrose transport appeared to be correlated with decreasing amounts of the ScrY protein, as measured on SDS-polyacrylamide gels (data not shown).

Several attempts to segregate the ScrY uptake activity from that of enzyme II^{Scr} by subcloning the *scrY* gene in pUC18 and in the low-copy-number plasmid pSC101 were unsuccessful. Although an *scrY* probe (1.1-kb *Sph*I fragment) hybridized to mRNA from TB1(pCH186Δ470), neither the gene product nor sucrose uptake could be detected. The *scrY* gene was successfully subcloned and expressed, without the leader sequence, in pEM-vrf1, where it was transcribed from a temperature-sensitive lambda promoter. The ScrY protein constituted 39% of the total cell protein in this clone, as determined by scanning densitometry. However, the gene product was rapidly lost during efforts to isolate the protein from cell fractions. The addition of protease inhibitors failed to significantly reduce the loss of the protein. These results precluded efforts to measure sucrose uptake in clones containing the segregated gene and appear to reflect the instability of the ScrY protein in the absence of enzyme II^{Scr} and sucrose hydrolase.

Since the ScrY protein facilitated the transport of sucrose, was located in the outer membrane, and showed identity to LamB, it was tested for porin activity. Outer membrane was prepared from TB1(pCH186) by isopycnic sucrose gradient centrifugation, and the proteins were analyzed by SDS-polyacrylamide gel electrophoresis. The ScrY protein represented 11% of the total protein contained in this fraction. The porin activity of this outer membrane fraction was determined by the liposome swelling method. Comparisons of the relative diffusion rates of various sugars in liposomes made with outer membranes from TB1, TB1(pUC18), and

TABLE 2. Diffusion rates of various sugars in liposomes containing the ScrY protein

Sugar	$d(1/OD_{400})/dt$ (min^{-1})			Relative rate of permeation (%)		
	Outer membrane[a]		Protein[b] (ScrY)	Outer membrane		Protein (ScrY)
	TB1	TB1 (pCH186)		TB1	TB1 (pCH186)	
DL-Arabinose	1.16	0.73	1.18	1,055	109	122
D-Glucose	0.71	0.61	1.12	645	91	117
D-Fructose	1.08	1.00	0.63	982	149	66
Sucrose	0.11	0.67	0.96	100	100	100
Lactose	0.22	0.22	<0.01	200	33	—[c]
Maltose	0.27	0.22	<0.01	245	33	—
Raffinose	0.26	0.13	<0.01	236	19	—
Melezitose	<0.01	<0.01	0.18	—	—	19
Maltoheptaose	<0.01	0.18	<0.01	—	27	—

[a] Liposomes contained 75 μg of outer membrane protein.
[b] Liposomes contained 10 μg of partially purified ScrY protein. OmpC plus OmpF and ScrY represented 4% and 61%, respectively, of the total protein in this sample.
[c] —, Below limit of detection.

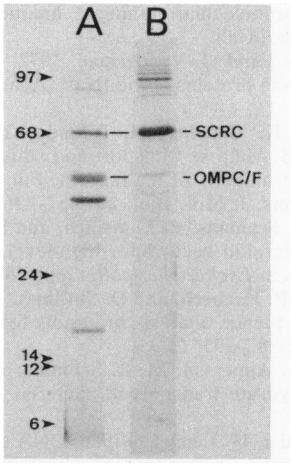

FIG. 6. Isolation of the ScrY (ScrC) protein for liposome swelling experiments. Details of the isolation scheme are provided in Materials and Methods. Protein samples were solubilized at 100°C for 10 min and examined by SDS-polyacrylamide gel electrophoresis. Lane A, Sarkosyl-insoluble membrane protein; lane B, ScrY-enriched fraction eluted from the Sepharose 6B column. Samples A and B contained 18 and 15 μg of protein, respectively. Gels were stained with Coomassie blue. Molecular size standards are indicated in kilodaltons. Positions of ScrY (ScrC) and OmpC plus OmpF proteins are shown by SCRC and OMPC/F, respectively.

TB1(pCH186) suggested that the presence of the ScrY protein markedly enhanced the rate of diffusion of sucrose (Table 2). Since the outer membrane fractions from TB1 and TB1(pCH186) contained the general diffusion pore proteins OmpC and OmpF, the disaccharides lactose and maltose and the trisaccharide raffinose were able to slowly diffuse through liposomes containing these proteins. It has been reported that small amounts of sucrose can traverse the outer membrane of *E. coli* through OmpC and OmpF, as evidenced by liposome swelling experiments (27).

The ScrY protein was then isolated from outer membrane by detergent extractions and gel filtration chromatography. We were not able to selectively solubilize the ScrY protein as in the case of LamB (23). The final ScrY protein preparation was relatively free of the major porins OmpC and OmpF; these proteins represented only 4% of total protein (Fig. 6, lane B). Membrane fractions containing considerably higher levels of the major porin proteins did not increase the permeability of the liposomes for sucrose relative to other disaccharides (Table 2 and Fig. 6, lane A). Several higher-molecular-weight polypeptides also appeared as contaminants in the ScrY protein preparation. Several of these polypeptides behaved anomalously during the protein separations, and studies are in progress to determine whether they represent homologous or heterologous aggregates containing the ScrY protein. These polypeptides were not labeled in maxicells containing pCH186 (16). The addition of this ScrY protein preparation to liposomes significantly increased the rate of diffusion of sucrose relative to the diffusion rates of the other disaccharides (Table 2). The diffusion rate of sucrose approached the diffusion rates of lower-molecular-weight sugars, such as arabinose and several hexoses. The results demonstrated that the ScrY protein imparted a structural specificity to the diffusion pores created in the liposomes. Thus, ScrY in the sucrose operon may function similarly to the LamB protein in the maltose regulon (19).

Origin of the *scrY* gene. We have previously shown that a probe containing the 5.7-kb *Sal*I fragment from pCH186 hybridized to chromosomal DNA from *K. pneumoniae* (16). A 1.1-kb *Sph*I fragment from pCH183, confined to the 5′ end of the *scrY* gene, hybridized to *K. pneumoniae* DNA digested separately with *Eco*RI, *Sal*I, and *Bam*HI (data not shown). The probe hybridized to a single restriction fragment in each digest. These data indicate that a related gene may reside in *K. pneumoniae*. Similarities between sucrose metabolism in *K. pneumoniae* and that directed by the *Salmonella* plasmid have led others to propose that the sucrose plasmid genes may have originated from the *Klebsiella* chromosome (35, 36). Recently, Wehmeiers et al. (39) used *lacZ* fusions to map a sucrose gene cluster on the *Klebsiella* chromosome. Although studies (37, 39) provided evidence, based on biochemical analyses, that an invertase, enzyme II^{Scr}, and an ATP-dependent fructokinase was present in the *Klebsiella* sucrose gene cluster, no data were presented to suggest the presence of a sucrose porin.

ACKNOWLEDGMENTS

We thank Robert Crowl (Hoffmann-La Roche) for making strains and plasmids available, Mary Brawner (SmithKline Beecham Pharmaceuticals) for performing the computer searches, and Jan Westpheling (University of Pennsylvania) for helpful discussions concerning the primer extension experiments.

This work was supported by Public Health Service grants DE08239 and DE06555 from the National Institute of Dental Research.

REFERENCES

1. **Ames, G. F.-L.** 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. **95:** 833–843.
2. **Aslanidis, C., K. Schmid, and R. Schmitt.** 1989. Nucleotide sequences and operon structure of plasmid-borne genes mediating uptake and utilization of raffinose in *Escherichia coli*. J. Bacteriol. **171:**6753–6763.
3. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
4. **Calzone, F. J., R. J. Britten, and E. H. Davidson.** 1987. Mapping of gene transcripts by nuclease protection assays and cDNA primer extension, p. 611–632. *In* S. L. Berger and A. R. Kimmel

(ed.), Guide to molecular cloning techniques. Academic Press, Inc., San Diego, Calif.
5. **Capaldi, R. A., and G. Vanderkooi.** 1972. The low polarity of many membrane proteins. Proc. Natl. Acad. Sci. USA **69**:930–932.
6. **Charbit, A., K. Gehring, H. Nikaido, T. Ferenci, and M. Hofnung.** 1988. Maltose transport and starch binding in phage-resistant point mutants of maltoporin. Functional and topological implications. J. Mol. Biol. **201**:487–496.
7. **Crowl, R., C. Seamans, P. Lomedico, and S. McAndrew.** 1985. Versatile expression vectors for high-level synthesis of cloned gene products in *Escherichia coli*. Gene **38**:31–38.
8. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
9. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**: 385–418.
10. **Ebner, R., and J. W. Lengeler.** 1988. DNA sequence of the gene *scrA* encoding the sucrose transport protein enzyme II^{Scr} of the phosphotransferase system from enteric bacteria: homology of the enzymeII^{Scr} and enzymeII^{Bgl} proteins. Mol. Microbiol. **2**:9–17.
11. **Fouet, A., M. Arnaud, A. Klier, and G. Rapoport.** 1987. *Bacillus subtilis* sucrose-specific enzyme II of the phosphotransferase system: expression in *Escherichia coli* and homology to enzymes II from enteric bacteria. Proc. Natl. Acad. Sci. USA **84**:8773–8777.
12. **Garcia, J. L.** 1985. Cloning in *Escherichia coli* and molecular analysis of the sucrose system of the *Salmonella* plasmid SCR-53. Mol. Gen. Genet. **201**:575–577.
13. **Green, P., J. M. DiRienzo, H. Yamagata, and M. Inouye.** 1985. The biosynthesis of bacterial outer membrane proteins, p. 45–104. *In* B. K. Ghosh (ed.), Organization of prokaryotic cell membranes, vol. III. CRC Press, Boca Raton, Fla.
14. **Hagen, S. H., and E. T. Young.** 1978. Effect of RNase III on efficiency of translation of bacteriophage T7 lysozyme mRNA. J. Virol. **26**:793–804.
15. **Haltiner, M., T. Kempe, and R. Tjian.** 1985. A novel strategy for constructing clustered point mutations. Nucleic Acids Res. **13**:1015–1025.
16. **Hardesty, C., G. Colon, C. Ferran, and J. M. DiRienzo.** 1987. Deletion analysis of sucrose metabolic genes from a *Salmonella* plasmid cloned in *Escherichia coli* K12. Plasmid **18**:142–155.
17. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15**:2343–2361.
18. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
19. **Hofnung, M.** 1974. Divergent operons and the genetic structure of the maltose B region in *Escherichia coli* K12. Genetics **76**:169–184.
20. **Jacobson, G. R., L. A. Lee, and M. H. Saier, Jr.** 1979. Purification of the mannitol-specific enzyme II of the *Escherichia coli* phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **254**:249–252.
21. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
22. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
23. **Luckey, M., and H. Nikaido.** 1980. Specificity of diffusion channels produced by λ phage receptor protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:167–171.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. **262**:10035–10038.
26. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
27. **Nikaido, H., and E. Y. Rosenberg.** 1983. Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. J. Bacteriol. **153**:241–252.
28. **Osborn, M. J., and R. Munson.** 1974. Separation of the inner (cytoplasmic) and outer membranes of gram-negative bacteria. Methods Enzymol. **31**:642–653.
29. **Postma, P. W., and J. W. Lengeler.** 1985. Phosphoenolpyruvate: carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **49**:232–269.
30. **Putney, S. D., S. J. Benkovic, and P. R. Schimmel.** 1981. A DNA fragment with an α-phosphorothioate nucleotide at one end is asymmetrically blocked from digestion by exonuclease III and can be replicated in vivo. Proc. Natl. Acad. Sci. USA **78**:7350–7354.
31. **Saier, M. H., Jr.** 1985. Mechanisms and regulation of carbohydrate transport in bacteria, p. 50–56. Academic Press, Inc., Orlando, Fla.
32. **Sanderson, K. E., and J. R. Roth.** 1983. Linkage map of *Salmonella typhimurium*, edition VI. Microbiol. Rev. **47**:410–453.
33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
34. **Sato, Y., F. Poy, G. R. Jacobson, and H. K. Kuramitsu.** 1989. Characterization and sequence analysis of the *scrA* gene encoding enzyme II^{Scr} of the *Streptococcus mutans* phosphoenolpyruvate-dependent sucrose phosphotransferase system. J. Bacteriol. **171**:263–271.
35. **Schmid, K., R. Ebner, J. Altenbuchner, R. Schmitt, and J. W. Lengeler.** 1988. Plasmid-mediated sucrose metabolism in *Escherichia coli* K12: mapping of the *scr* genes of pUR400. Mol. Microbiol. **2**:1–8.
36. **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.
37. **Sprenger, G. A., and J. W. Lengeler.** 1988. Analysis of sucrose catabolism in *Klebsiella pneumoniae* and in Scr^+ derivatives in *Escherichia coli* K12. J. Gen. Microbiol. **134**:1635–1644.
38. **Tinoco, I., Jr., P. N. Borer, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothers, and J. Gralla.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) New Biol. **246**:40–41.
39. **Wehmeiers, U., G. A. Sprenger, and J. W. Lengeler.** 1989. The use of λ*plac*-Mu hybrid phages in *Klebsiella pneumoniae* and the isolation of stable Hfr strains. Mol. Gen. Genet. **215**:529–536.
40. **Wohlhieter, J. A., J. R. Lazere, N. J. Snellings, E. M. Johnson, R. M. Synenki, and L. S. Baron.** 1975. Characterization of transmissible genetic elements from sucrose-fermenting *Salmonella* strains. J. Bacteriol. **122**:401–4069.