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Translocation of Capsular Polysaccharides in Pathogenic Strains of *Escherichia coli* Requires a 60-Kilodalton Periplasmic Protein

RICHARD P. SILVER,†* WENDY AARONSON, AND WILLIE F. VANN

Division of Bacterial Products, Office of Biologics Research and Review, Food and Drug Administration, Bethesda, Maryland 20892

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An 11.6-kilobase (kb) region of a 34-kb fragment of *Escherichia coli* DNA that encodes the K1 capsular polysaccharide genes is necessary for translocation of the K1 polysaccharide to the bacterial cell surface. This 11.6-kb region contains a gene, *kpsD*, encoding a 60-kilodalton protein. The *kpsD* gene was localized to a 2.4-kb *Pst*I-*Bam*HI fragment. Cells harboring a Tn*1000* insertion in *kpsD* did not synthesize the 60-kilodalton protein and did not express polysaccharide on the cell surface. Immunodiffusion and rocket immunoelectrophoresis of cell extracts, however, demonstrated that K1 polysaccharide was synthesized by these cells. We present evidence that the *kpsD* gene product is synthesized as a precursor and that the processed form is located in the periplasmic space. Analysis of alkaline phosphatase activity of a *kpsD-phoA* fusion demonstrated that *kpsD* expression was under positive regulation. A 260-base-pair *Alu*I fragment located within the *kpsD* coding sequence was used as a probe and was found to hybridize to chromosomal DNA from *E. coli* that synthesizes the K2, K5, K7, K12, and K13 capsular polysaccharides but not K3 and K100. These results suggest that the *kpsD* gene product may be required for export not only of K1 but for other K antigens as well.

The K1 capsular polysaccharide is an important virulence determinant of invasive *Escherichia coli* (35). K1-producing isolates account for 80% of *E. coli* neonatal meningitis cases and are common in cases of neonatal septicemia and childhood pyelonephritis (21, 33). The K1 polysaccharide is an α-2,8-linked linear homopolymer of sialic acid (*N*-acetylneuraminic acid [NeuAc]; 2, 9). The initial events in the synthesis of the K1 polysaccharide have been extensively investigated (40, 42). A membrane-associated sialyltransferase complex catalyzes the polymerization of NeuAc from CMP-NeuAc. These reactions involve a NeuAc–P-undecaprenol intermediate, and synthesis requires endogenous sialyl acceptors within the membrane (41, 43). In contrast, the mechanism of translocation of the polysaccharide from its site of synthesis on the cytoplasmic membrane to the bacterial cell surface is poorly understood. It has been postulated that transfer occurs at sites of adhesion between the inner and outer membranes (Bayer junctions; 3). There is, however, a paucity of information about these events at the genetic and molecular levels.

The genes encoding proteins required for the synthesis and expression of the *E. coli* K1 capsular polysaccharide have been cloned (11, 38) and analyzed by genetic techniques (34, 39). These genes are organized in three coordinately regulated clusters. Deletion of one of these clusters, an 11.6-kilobase (kb) region, inactivated the expression but not the synthesis of the K1 polysaccharide (39). We therefore proposed that this one cluster was responsible for transport of the polysaccharide to the cell surface. Five protein species with molecular masses of 80, 77, 60, 40, and 37 kilodaltons (kDa), encoded within the 11.6-kb region, were postulated to be associated with the translocation process (39).

In this communication we present evidence that one of these proteins, p60, is required for K1 translocation. We demonstrate that the protein is synthesized as a precursor and that the processed form is located in the periplasmic space. Furthermore, we show that a DNA probe specific for the gene encoding p60, which we designate *kpsD*, hybridized to chromosomal DNA from *E. coli* that synthesizes non-NeuAc-containing capsular polysaccharides. These results indicate that *kpsD* may be involved in a general process that results in the translocation of capsular polysaccharides in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage. The bacterial strains and plasmids used in this study are described in Table 1. Bacteriophage E is specific for *E. coli* that synthesizes the K1 polysaccharide (14). Bacterial cultures were grown at 37°C in L broth or on L agar (23) and were supplemented with appropriate antibiotics (39).

DNA techniques. Purification of plasmid DNA by cesium chloride-ethidium bromide density gradient centrifugation, restriction enzyme analysis, ligation reactions, agarose gel electrophoresis, and plasmid transformation were done as previously described (39). Plasmid DNA was also prepared by the rapid alkaline extraction procedure of Ish-Horowicz and Burke (19). Chromosomal DNA was isolated essentially as described by Moxon et al. (27).

Transposon mutagenesis. Insertions of Tn*1000* (γδ) into plasmid DNA were generated by transforming plasmids into the F^+ $\gamma\delta^-$ strain MG1656 and mating the resultant strain with the F^- Sm^r recipient JC1569 as previously described (15). Transpositions of Tn*phoA* (Tn*5* IS*50*$_L$::*phoA*; kindly provided by J. Mekalanos) to pSR102 were obtained by first isolating a random insertion of Tn*phoA* into the chromosome of HB101 (24). The resultant strain, RS1602, was transformed with pSR102 and cells grown for 8 h in L broth containing 500 μg of ampicillin and 100 μg of kanamycin per ml and subcultured into L broth containing 500 μg of ampicillin and 300 μg of kanamycin per ml. Plasmid DNA was prepared from an overnight culture and used to transform HB101, with selection of blue alkaline phosphatase-

* Corresponding author.
† Present address: Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642.

TABLE 1. Bacterial strains and plasmids

E. coli strain or plasmid	Genotype or relevant characteristics[a]	Source or reference
K-12 strains		
C600	F⁻ *thr leu thi supE lacY tonA*	23
HB101	F⁻ *lacY galK ara mtl xyl pro hsdS supE rpsL recA*	23
RS1602	HB101::Tn*phoA*	This study
DS410	*minA minB ara xyl mtl azi thi*	10
EV80	*nanA4 zgj-791*::Tn*10 fadE mel supF*	E. Vimr (45)
JC1569	F⁻ *arg his met leu lacY gal xyl rpsL recA*	5
MG1656	F⁺/γδ⁻ derivative of W1485	M. Guyer (16)
Encapsulated strains		
RS218	O18:K1:H7, NeuAc	37
F1961	O86:K2:H2, galactose, glycerol-PO_4	31
U4-41	O4:K3:H5, ND[b]	31
Bi8337-41	O10:K5:H4, *N*-acetylglucosamine, glucuronic acid	31
RS448	ND:K7:ND, *N*-acetylmannosaminouronic acid, glucose	R. Meyerowitz
Su65-42	O4:K12:NM,[c] rhamnose, 2-keto-3-deoxyoctulosonic acid	31
Su4344-41	O6:K13:H1, ribose, 2-keto-3-deoxyoctulosonic acid	31
Bos-12	O16:K92:NM, NeuAc	36
Easter	O75:K100:H5, ribose, ribitol-PO_4	12
Plasmids		
pUC12	Ap[r]	26
pACYC184	Cm[r] Tc[r]	23
pSR23	34-kb *E. coli* fragment encoding *kps* genes cloned in pHC79	39
pSR25	5-kb *Bam*HI fragment of pSR23 in pHC79	39
pSR28	5- and 6.6-kb *Bam*HI fragments of pSR23 in pHC79	39
pSR46	pSR23::Tn*5 kps*⁺	39
pSR50	pSR23*kps4*::Tn*5*	39
pSR95	3.9-kb *Hind*III fragment derived from pSR46; includes 1.2 kb of Tn*5*	This study
pSR102	2.4-kb *Pst*I-*Bam*HI fragment of pSR28 cloned in pUC12	This study
pSR123	2.4-kb *Pst*I-*Bam*HI fragment from pSR102 cloned in pACYC184	This study
pSR127	pSR50*kpsD26*::Tn*1000*	This study
pSR128	pSR102*kpsD45*::Tn*phoA*	This study
pSR129	pSR102*kpsD25*::Tn*phoA*	This study

[a] For encapsulated strains, serotypes and the chemical composition of capsular polysaccharides (20, 22) are given.
[b] ND, Not determined.
[c] NM, Nonmotile.

producing transformants on L agar containing 300 μg of kanamycin, 100 μg of ampicillin, and 40 μg of 5-bromo-4-chloro-3-indoyl phosphate–*p*-toluidine salt per ml (24). The sites of Tn*phoA* insertions in pSR102 were determined by restriction mapping. The orientation of insertions was determined by dideoxy DNA sequencing of fusion junctions directly from plasmid DNA (7) with a *phoA*-specific primer (R. Taylor, personal communication). The primer was synthesized with a 380A DNA synthesizer (Applied Biosystems).

Minicell analysis. Minicells were prepared and labeled with [^{35}S]methionine as previously described (39). Labeled products were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and the labeled bands were visualized by autoradiography as previously described (39). Periplasmic fractions were prepared from labeled minicells by either (i) the cold osmotic-shock procedure of Hazelbauer and Harayama (17) or (ii) conversion to spheroplasts by Tris-lysozyme-EDTA treatment as described by Witholt et al. (46). Membrane fractions were prepared from spheroplasts lysed by 100-fold dilution in water. Unbroken cells were removed by centrifugation at 5,000 × *g*, and membrane fractions were recovered after centrifugation at 200,000 × *g* for 2 h.

Cell fractionation. Early-exponential-phase cells were washed twice with 0.1 M Tris (pH 8) and lysed by two passages through a French pressure cell (American Instruments) at 12,000 lb/in^2. After removal of unlysed cells and debris by centrifugation at 8,000 × *g* for 10 min, membrane and soluble fractions were separated by centrifugation in an airfuge (Beckman Instruments, Inc.) at 30 lb/in^2 for 20 min.

Hybridization. A single-strand specific hybridization probe was prepared with the M13 Universal Hybridization Probe Primer (Bethesda Research Laboratories Inc.) essentially as described by Messing (26). The probe was annealed to an M13mp18 clone that contained a 260-base-pair *kpsD Alu*I fragment by incubation at 65°C for 15 min followed by cooling to room temperature for 15 min. The primer was extended by the addition of deoxynucleotide triphosphates and DNA polymerase I (Klenow fragment; 26). Fragments generated by *Bam*HI-digested chromosomal DNA were separated on a 0.6% agarose gel in Tris-acetate-EDTA buffer, transferred to NYTRAN (Schleicher & Schuell, Inc.), and hybridized as previously described (39).

Immunological techniques. Immunodiffusion and rocket immunoelectrophoresis were performed as previously described (39).

In vitro transcription-translation. Purified plasmid DNA (1 to 2 μg) was used as a template with an in vitro transcription-translation kit (Amersham Corp.). The reactions were carried out according to the instructions of the manufacturer. Labeled

products were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as previously described (39).

Enzyme assay. Alkaline phosphatase (PhoA) activity was measured by production of *p*-nitrophenol essentially as previously described (24) and was expressed in enzyme units per unit of optical density at 600 nm. Cells were grown to mid-log phase in M63 medium (23) supplemented with glucose, thiamine, and 0.2% Casamino Acids and were permeabilized by the addition of 1 drop of toluene. The toluene was evaporated by incubation at 37°C for 45 min.

Materials. The following commercially available materials were used: restriction enzymes, T4 DNA ligase, large fragment of DNA polymerase I (Klenow), and Universal Hybridization Probe Primer (Bethesda Research Laboratories); agarose (FMC Corp., Marine Colloids Div.); procaryotic in vitro-coupled transcription-translation system (Amersham); 5-bromo-4-chloro-3-indoyl phosphate–*p*-toluidine salt and NeuAc (Sigma Chemical Co.); deoxynucleotides and dideoxynucleotides (Pharmacia, Inc.); and L-[^{35}S]methionine (800 Ci/mmol) and [^{35}S]deoxyadenosine 5′-(thio)triphosphate (500 Ci/mmol) (New England Nuclear Corp.).

RESULTS

Localization and characterization of the *kpsD* gene. Plasmid pSR23 contained the K1 genes on a 34-kb insert (Fig. 1). Minicells harboring pSR28, a subclone derived from pSR23 (Fig. 1), expressed at least five proteins (p80, p77, p60, p40, and p37; Fig. 2, lane A). The *kpsD* gene, encoding p60, was localized to a 2.4-kb *Pst*I-*Bam*HI fragment of pSR28 by Tn*1000* mutagenesis (data not shown) and by further subcloning. Minicells harboring plasmid pSR102, constructed by inserting the 2.4-kb *Pst*I-*Bam*HI fragment into pUC12, expressed the 60-kDa *kpsD* gene product (Fig. 2, lane B).

To provide direct evidence for the involvement of p60 in the translocation process, a Tn*1000* insertion in the *kpsD* gene was isolated and characterized. Since we were concerned about the potential instability of mutants unable to transport polymer, we used pSR50, a Tn*5*-derived mutant of pSR23, for these experiments. Cells carrying pSR50 are defective in capsule synthesis and synthesize capsular polysaccharide only when exogenous NeuAc is provided (39). The site of the insertion of Tn*1000* within the 2.4-kb *Bam*HI-*Pst*I fragment of pSR50 is shown in Fig. 1. Minicells harboring the *kpsD26*::Tn*1000* mutant plasmid, designated pSR127, did not express the 60-kDa polypeptide (data not shown).

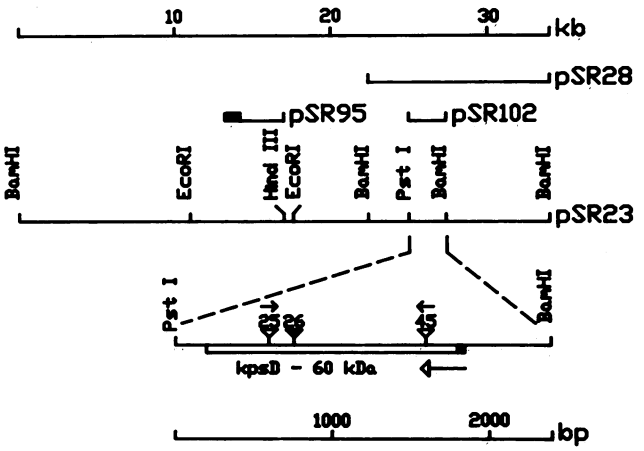

FIG. 1. Genetic and physical map of the K1 plasmid pSR23 and the *kpsD* gene. Plasmid pSR95 was derived from a K1$^+$ Tn*5* derivative of pSR23, pSR46 (39); the closed area in pSR95 represents Tn*5* sequences. Symbols above *kpsD* represent the site of Tn*1000* (▼) and Tn*phoA* (▽) insertions. The arrows above Tn*phoA* indicate the orientation of the *phoA* gene. The closed area in the box representing the *kpsD* gene product shows the signal peptide of the protein. The arrow below *kpsD* indicates the direction of transcription. bp, Base pairs.

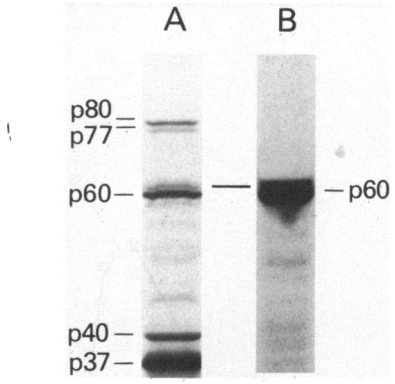

FIG. 2. Autoradiogram of [^{35}S]methionine-labeled proteins in minicells harboring pSR28 (lane A) and pSR102 (lane B). Samples were analyzed in an SDS-10% polyacrylamide gel. Only the relevant portions of the gels are shown. p80, p77, p60, p40, and p37 were previously designated P1, P2, P5, P9, and P10, respectively (39). Molecular weight standards were myosin, 200,000; phosphorylase *b*, 94,400; bovine serum albumin, 68,000; ovalbumin, 43,000; chymotrypsinogen, 25,700; β-lactoglobulin, 18,4000; and lysozyme, 14,300.

Sensitivity to the K1-specific bacteriophage E was used as an indicator of surface-exposed polymer. This phage binds specifically to the K1 capsule and degrades the polysaccharide by means of a specific endoneuraminidase (44). Cells harboring pSR50 were able to synthesize and express the K1 capsular polysaccharide following growth in medium containing 100 μg of NeuAc per ml. These cells showed marked sensitivity to K1 phage (Fig. 3). In contrast, cells carrying pSR127 grown under identical conditions were resistant to phage infection (Fig. 3).

The *kpsD26*::Tn*1000* mutation on pSR127 was complemented by a wild-type copy of this gene. The 2.4-kb *Pst*I-*Bam*HI fragment carrying the *kpsD* gene was inserted into pACYC184. This plasmid, designated pSR123, and pSR127 have compatible replicons and can coexist within the same cell. When grown in the presence of exogenous NeuAc, cells harboring pSR123 and pSR127 were as sensitive to the K1 phage as cells harboring pSR50 alone (data not shown). These results also suggest that the *kpsD* gene constitutes a single transcriptional unit.

Expression of K1 polysaccharide. The presence of K1 polysaccharide on intact cells as well as in cell extracts was examined by immunodiffusion against group B meningococcal antiserum. An immune precipitate was observed with intact cells harboring pSR50 but not pSR127 (Fig. 4A, wells 1 and 2). K1 polysaccharide was found, however, in soluble extracts of both cultures (Fig. 4A, wells 3 and 4). These results indicate that polysaccharide was produced in cells harboring pSR127 but was not transported to the cell surface. Preliminary studies suggest that in the mutant strain, the polysaccharide was localized to the periplasmic space (R. P. Silver and W. Aaronson, unpublished observation). K1 polysaccharide was also associated with a total mem-

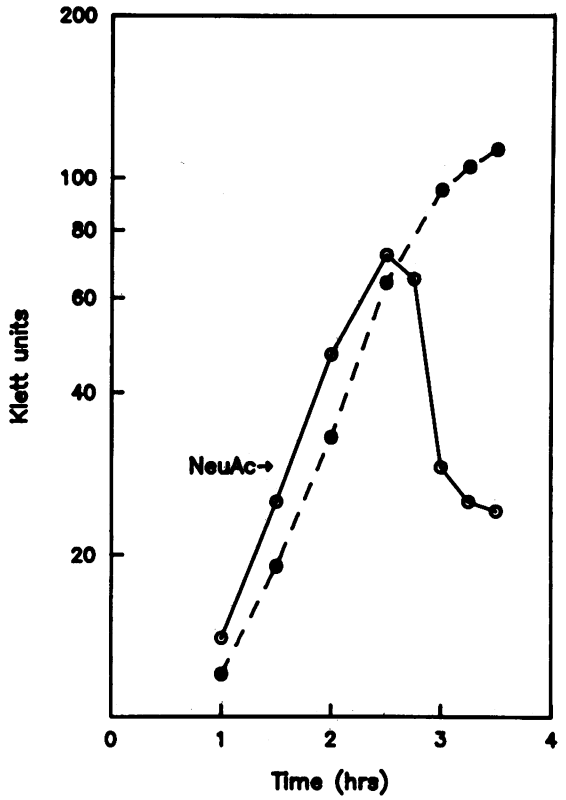

FIG. 3. Effect of K1-specific bacteriophage on growth of pSR50 (○) and pSR127 (●). EV80, the host organism, is unable to use NeuAc as a carbon source (45). Cells were grown at 37°C in L broth, and NeuAc (100 μg/ml) was added at a cell density indicated by the arrow (~2 × 10^8 to 4 × 10^8 cells/ml). Growth was monitored with a Klett-Summerson photoelectric colorimeter with a green filter. Fifteen minutes after the addition of NeuAc, K1-specific bacteriophage E was added (multiplicity of infection, 1).

brane fraction from both cultures (Fig. 4A, wells 5 and 6). The amount of capsular polysaccharide present in the extracts was assayed by rocket immunoelectrophoresis. Similar amounts of K1 polysaccharide were observed in the soluble extracts (Fig. 4B, wells 1 and 2), but reduced levels of membrane-associated polysaccharide were seen in cells harboring the mutant plasmid (Fig. 4B, wells 3 and 4).

Identification of the *kpsD* gene product as a periplasmic protein. The subcellular location of the KpsD protein was determined. The 60-kDa polypeptide was released in high levels from ^{35}S-labeled minicells during formation of spheroplasts (Fig. 5, lane b) as well as from osmotically shocked minicells (data not shown). Both procedures selectively release periplasmic proteins (29). Additional evidence for the periplasmic location of the *kpsD* gene product was provided by the observation that the 60-kDa protein is synthesized as a precursor protein of about 62 kDa. Treatment of minicells with 9.5% ethanol, a procedure that inhibits processing of signal peptides (32), inhibited synthesis of p60 and enriched for the precursor (Fig. 6A, lane b). In addition, when pSR102 DNA was added to a coupled transcription-translation system, only the 62-kDa precursor was expressed (Fig. 6B, lane b). In these experiments, β-lactamase, a periplasmic protein expressed by the vector, served as a useful internal control (data not shown).

Fusion of alkaline phosphatase to the KpsD protein. To be enzymatically active, the periplasmic protein alkaline phosphatase (PhoA) must be exported from the cytoplasm (24, 25). Since the signal sequence of alkaline phosphatase encoded by the transposon Tn*phoA* has been deleted, fusion of Tn*phoA* to a gene that encodes an exported protein is required to yield an active enzyme (24, 25). We isolated a derivative of pSR102, pSR128, in which insertion of Tn*phoA* in the *kpsD* gene yielded active alkaline phosphatase. From the orientation of *phoA* in pSR128, we concluded that transcription of *kpsD* was from right to left on the physical map (Fig. 1). In contrast to the situation with pSR128, insertion of Tn*phoA* in *kpsD* did not give an active hybrid in pSR129. The observation that the orientation of *phoA* in pSR129 was opposite to the orientation in pSR128 is consistent with the predicted direction of transcription of *kpsD* (Fig. 1). These results also show that the signal sequence of the KpsD protein could promote export of alkaline phosphatase and provide additional support for the concept that the *kpsD* product is a periplasmic protein.

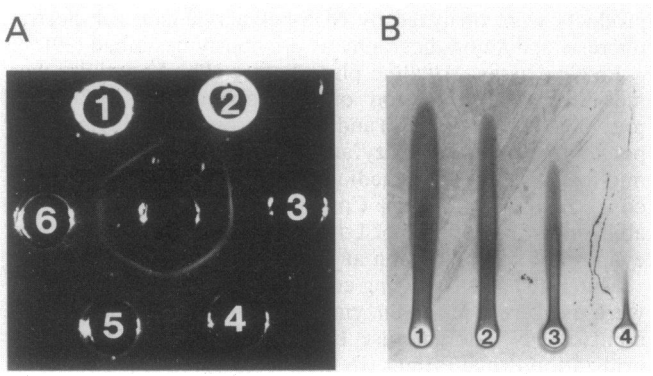

FIG. 4. (A) Immunodiffusion with group B meningococcal antiserum (center well) and extracts (5 μl) from cells harboring pSR50 and pSR127. Exogenous NeuAc (100 μg/ml) was added to the cultures prior to cell fractionation. Wells: 1, pSR50 cells; 2, pSR127 cells; 3, pSR50 soluble extract; 4, pSR127 soluble extract; 5, pSR50 membranes; 6, pSR127 membranes. (B) Rocket immunoelectrophoresis in 10% agarose containing 6.25% group B meningococcal antiserum. 1, pSR50 soluble extract; 2, pSR127 soluble extract; 3, pSR50 membranes; 4, pSR127 membranes.

Cells harboring pSR128 expressed moderate levels of alkaline phosphatase (~40 U/U of optical density at 600 nm). Introduction of pSR95 into these cells consistently yielded a two- to threefold increase in enzymatic activity. pSR95 was derived from pSR23 and includes the 2-kb region of pSR23 (Fig. 1) that we previously postulated to be involved in the

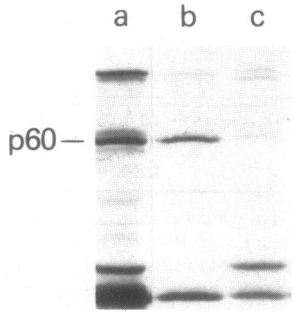

FIG. 5. Autoradiogram of [^{35}S]methionine-labeled proteins in minicells harboring pSR28. Proteins were analyzed in SDS-10% polyacrylamide gel. Lane a, Whole minicells; lane b, periplasmic fraction; lane c, total membranes.

regulation of K1 synthesis (39). These results are consistent with that hypothesis.

Presence of *kpsD* sequences in other *E. coli* capsular types. *E. coli* synthesizes more than 70 distinct capsular (K) antigens, and it has been shown that some of the steps in the biosynthesis of distinct K antigens are shared (34; R. P. Silver and W. Aaronson, in press). To determine whether *kpsD* gene sequences were present in *E. coli* strains synthesizing non-NeuAc-containing capsular polysaccharides (Table 1), we performed Southern blot hybridization with a probe derived from the *kpsD* gene. The coding sequence of the *kpsD* gene has been identified (R. P. Silver and W. Aaronson, unpublished data), and a 260-base-pair *Alu*I fragment located within this *kpsD* coding sequence was used as the hybridization probe. This probe hybridized to a 5-kb *Bam*HI chromosomal DNA fragment from *E. coli* K1, K2, K5, K7, K12, K13, and K92 but not to K100 and K3 (Fig. 7).

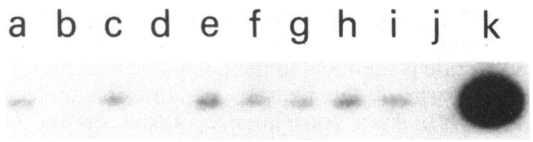

FIG. 7. Hybridization of a *kpsD*-specific probe with DNA from *E. coli* expressing different K antigens. *Bam*HI digests of chromosomal DNA (10 μg) and pSR25 (200 ng) were separated on a 0.6% agarose gel and hybridized as described in Materials and Methods. The 5.0-kb *Bam*HI fragment of pSR25 includes the gene encoding p60. Lanes: a, K1; b, K100; c, K2; d, K3; e, K5; f, K7; g, K12; h, K13; i, K92; j, C600; k, pSR25.

DISCUSSION

In this study we have shown that the KpsD protein is required for translocation of the K1 capsular polysaccharide to the bacterial cell surface. The KpsD protein may be part of a multicomponent translocation system. Our preliminary results suggest that in the absence of the *kpsD* product, polysaccharide accumulates in the periplasmic space. Although the precise role of KpsD in the transport process is not yet known, we can conclude from our studies that synthesis of polysaccharide is not coupled to export. Our working model for biosynthesis of the K1 capsule includes polymerization of sialic acid by the sialyltransferase complex at the periplasmic face of the cytoplasmic membrane. We predict that this process involves a vectorial transfer of NeuAc from CMP-NeuAc to polymer by a mechanism analogous to that described for lipopolysaccharide and chitin biosynthesis (6, 28). Polysaccharide is then transferred to the exterior of the cell by the translocation machinery which includes the KpsD protein.

Previous studies indicate that different capsular polysaccharides are transported to the bacterial surface by a common mechanism (34; Silver and Aaronson, in press). Our results support this view. A probe specific for *kpsD* was found to hybridize to chromosomal DNA from *E. coli* synthesizing non-NeuAc-containing polymers. It is perhaps significant that these include capsular types commonly associated with invasive *E. coli* disease; the K1, K2, K5, K12, and K13 antigens are found in almost all *E. coli* isolates from the upper urinary tract.

The subcellular location of a protein can often provide information on the biological function of the protein. Our data indicate that the KpsD protein is itself located in the periplasmic space. Gram-negative bacteria generally contain two classes of periplasmic proteins: hydrolytic enzymes and substrate-binding proteins involved in transport. The role of KpsD as a binding protein is an intriguing possibility and could involve protein-carbohydrate or protein-protein interactions.

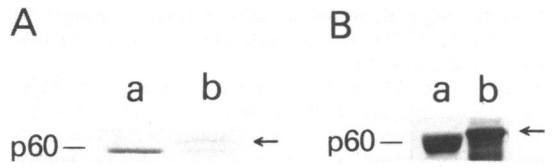

FIG. 6. Inhibition of processing of p60. (A) Minicells harboring pSR23 were labeled with [^{35}S]methionine (a) without ethanol and (b) with ethanol. (B) pSR102 labeled with [^{35}S]methionine (a) in minicells and (b) in vitro. The proteins were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

The concept of a periplasmic gel proposed by Hobot et al. (18) predicts that molecules located in the periplasmic space would have a relatively low mobility. Indeed, Brass et al. (4) recently showed that the rate of lateral diffusion of maltose-binding protein was very slow in the periplasm, about 1,000-fold lower than that observed in aqueous solution. These studies may imply a need for binding proteins to facilitate transport of polysaccharides across the periplasm or to specifically direct the polymer to an appropriate transport protein in the outer membrane. A functional porin in the outer membrane of *E. coli* is required for capsule expression (J. Foulds and W. Aaronson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, D21, p. 54) and is an attractive target for such an interaction. Analogous helper proteins have been implicated in the biogenesis of other supramolecular structures such as *E. coli* pili (8, 30).

In addition to *kpsD*, other genes within the 11.6-kb region are implicated in the transport process (39). Bacterial transport systems for the uptake of sugars, amino acids, and oligopeptides have been extensively characterized. These systems are typically composed of periplasmic substrate-binding proteins as well as membrane-associated components (1). It is therefore not surprising that several gene products may also be required for the export of polysaccharides in *E. coli*. How the components of this system are regulated and how they interact may provide information on protein-protein and on protein-carbohydrate interactions.

The molecular and genetic events involved in the regulation of K1 capsule synthesis have not been elucidated. We previously proposed that a 2-kb region, distinct from the transport cluster, was involved in the regulatory process (39). The *kpsD-phoA* fusion data suggest that the regulatory region encodes a *trans*-active factor that stimulates *kpsD* expression. We conclude from these results that at one level, expression of capsule synthesis is positively regulated. Complementation experiments have also shown a positive effect of the regulatory cluster encoded by pSR95 on the gene encoding CMP-NeuAc synthetase, the enzyme that catalyzes the activation of NeuAc to CMP-NeuAc (C. W. Finn and R. P. Silver, manuscript in preparation). In these studies, a transcription antitermination mechanism has been implicated.

Neonatal meningitis caused by K1-producing *E. coli* remains a major health problem. The morbidity and mortality rates are high, and there are few normal survivors. Although there is much evidence that the K1 polysaccharide is an essential virulence determinant, purified K1 polysaccharide is a poor immunogen in humans and animals (47). This poor

immunogenicity has been explained by similarities between K1 and structures found in host tissue. For example, the K1 polysaccharide is identical to the polysialic acid found on the embryonic form of the neural cell adhesion molecule N-CAM (13, 44). As a consequence, an alternative to anticapsular immunity may be needed to render these organisms vulnerable to host defenses. We believe that an understanding of the products required for the export of polysaccharide may provide an alternative approach for prevention not only of K1 disease but also of infections caused by other encapsulated *E. coli*.

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LITERATURE CITED

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