JOURNAL OF BACTERIOLOGY, Aug. 1991, p. 4603–4610
0021-9193/91/154603-08$02.00/0

Vol. 173, No. 15

Identification of Two Genes, *kpsM* and *kpsT*, in Region 3 of the Polysialic Acid Gene Cluster of *Escherichia coli* K1

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The polysialic acid capsule of *Escherichia coli* K1, a causative agent of neonatal septicemia and meningitis, is an essential virulence determinant. The 17-kb *kps* gene cluster, which is divided into three functionally distinct regions, encodes proteins necessary for polymer synthesis and expression at the cell surface. The central region, 2, encodes products required for synthesis, activation, and polymerization of sialic acid, while flanking regions, 1 and 3, are thought to be involved in polymer assembly and transport. In this study, we identified two genes in region 3, *kpsM* and *kpsT*, which encode proteins with predicted sizes of 29.6 and 24.9 kDa, respectively. The hydrophobicity profile of KpsM suggests that it is an integral membrane protein, while KpsT contains a consensus ATP-binding domain. KpsM and KpsT belong to a family of prokaryotic and eukaryotic proteins involved with a variety of biological processes, including membrane transport. A previously described *kpsT* chromosomal mutant that accumulates intracellular polysialic acid was characterized and could be complemented in *trans*. Results of site-directed mutagenesis of the putative ATP-binding domain of KpsT are consistent with the view that KpsT is a nucleotide-binding protein. KpsM and KpsT have significant similarity to BexB and BexA, two proteins that are essential for polysaccharide capsule expression in *Haemophilus influenzae* type b. We propose that KpsM and KpsT constitute a system for transport of polysialic acid across the cytoplasmic membrane.

Escherichia coli is the most common gram-negative organism causing sepsis and meningitis in neonates (41). Morbidity and mortality rates are high, and neurological sequelae are common (39). Most of the *E. coli* strains in these infections synthesize the K1 capsular polysaccharide as an essential virulence determinant (43, 54). The K1 polysaccharide is an α-2,8-linked linear homopolymer of sialic acid and is identical to the group B polysaccharide capsule of *Neisseria meningitidis* (30). Sialic acids are essential constituents of many mammalian glycoconjugates displaying a variety of biological functions (47, 49). While relatively rare among prokaryotes, sialic acids are frequent components of capsular polysaccharides associated with bacterial disease (44). Pathogenesis is correlated with the ability of terminal sialic acid residues to inhibit complement activation by the alternative pathway (14, 18, 27, 40). Purified K1 polysaccharide is also poorly immunogenic in humans (63), a consequence, perhaps, of similarities to structures found in host tissue (16, 57).

The 17-kb *kps* gene cluster of *E. coli* K1 encodes functions for the synthesis, activation, and polymerization of sialic acid, as well as translocation of the polymer to the bacterial cell surface (6, 7, 54). These genes are divided into three functional regions (8, 53). The 5.8-kb central region, 2, contains information for sialic acid synthesis, activation, and polymerization (7, 59). Regions 1 and 3 are thought to be involved in polymer assembly and transport and are conserved among *E. coli* strains that synthesize chemically distinct capsules (45, 46). We report that region 3 of the *kps* gene cluster contains two genes, *kpsM* and *kpsT*, that encode proteins predicted to be 29.6 and 24.9 kDa in size, respectively. KpsM and KpsT share sequence homology with BexB and BexA, proteins required for capsule expression in *Haemophilus influenzae* type b (32). Like BexA (31), KpsT contains a consensus ATP-binding site, and the results of site-directed mutagenesis are consistent with the view that nucleotide binding is important to KpsT function. *kpsM* appears to encode an integral membrane protein, and we propose that KpsM and KpsT constitute a system for transport of polysialic acid across the cytoplasmic membrane.

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophage, and media. The bacterial strains and plasmids used in this study are described in Table 1. Bacteriophage E is specific for *E. coli* strains that synthesize the K1 polysaccharide (20). Bacterial cultures were grown at 37°C in L broth or on L agar and were supplemented with appropriate antibiotics. Precipitin halo formation was assayed on antiserum agar plates as previously described (53).

DNA manipulations and sequencing. DNA manipulations were performed essentially as described by Maniatis et al. (38). Plasmid DNA was also prepared by the rapid alkaline extraction procedure of Ish-Horowicz and Burke (29), while genomic DNA was isolated via hexadecyltrimethylammonium bromide precipitation (3). Southern blotting was performed by capillary transfer, and hybridizations with the formamide method were done by using Zeta-Probe nylon membrane (Bio-Rad, Richmond, Calif.) as recommended by the manufacturer.

For DNA sequence determination, one strand of the 1.5-kbp fragment containing *kpsM* and *kpsT* was sequenced by using a nested set of deletions generated from pSR203 by the Erase-a-Base kit (Promega, Madison, Wis.). To sequence the complementary strand, oligonucleotides were synthesized by using an Applied Biosystems 380B DNA synthesizer and used as primers. Helper phage R408 was

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TABLE 1. Bacterial strains and plasmids

E. coli strain or plasmid	Relevant genotype or properties	Source or reference
K-12 strains		
HB101	F⁻ *hsds20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44 leu*	Laboratory collection
HB101F′	Same as above but F⁺ (Tn*5*)	Laboratory collection
XL-1 Blue	*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F⁺ *proAB lacZΔM15* Tn*10*)	Laboratory collection
JM107	Δ(*lac proAB*) *thi gyrA96 endA1 hsdR17 relA1 supE44 mcrA* (F⁺ *traD36 proAB lacI*^{q} *lacZ*ΔM15)	Laboratory collection
DH5α	F⁻ *endA1 hsdR17 supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*)U169 ϕ80d*lacZ*ΔM15	Laboratory collection
MG1656	F⁺ Tn*1000*; derivative of W1485	21
K1-K-12 strains		
EV1	PA360 (*kps*⁺ *serA*⁺ *malA*⁺)	60
EV13	EV1 *kps-24*	60
EV24	EV1 *neuB25*	60
EV95	EV36 *kpsT32*::Tn*10*	59
Plasmids		
Bluescript KS+	2.95 kb; Ap^r	Stratagene
pACYC184	4.0 kb; Tc^r Cm^r	10
pSR23	34-kb *E. coli* K1 *Bam*HI fragment containing the *kps* gene cluster cloned in pHC79	52
pSR46	pSR23::Tn*5* *kps*⁺	53
pSR64	11.5-kb *Eco*RI fragment from pSR46 cloned into pACYC184; includes Tn*5*	This study
pSR95	3.9-kb *Hin*dIII fragment from pSR46 cloned into pACYC184; includes 2.7 kb from *kps* locus and 1.2 kb of Tn*5*	51
pSR199	pSR95::Tn*1000*-12	This study
pSR203	2.4-kb *Bam*HI-*Bgl*II fragment containing *kpsM* and *kpsT* from pSR199 and cloned into *Bam*HI site of Bluescript KS+; includes 400 bp and *Bam*HI site from Tn*1000*	This study
pSR204	Same as pSR203 except in opposite orientation in Bluescript KS+ vector; *kpsM* and *kpsT* located downstream of *lac* promoter	This study
pSR210	Spontaneous deletion derivative of pSR64; contains 3.7 kb of the *kps* gene cluster (Fig. 1) and approximately 2.0 kb of Tn*5* and 3.5 kb of pACYC184; Tc^r	This study
pSR278	1.25-kb *Cla*I fragment from pSR210 cloned into Bluescript KS+	This study
pSR340	700-bp *Eco*RI-*Bam*HI PCR fragment containing wild-type *kpsT* cloned into Bluescript KS+	This study
pSR346	700-bp *Eco*RI-*Bam*HI PCR fragment containing *kpsT* with the KE44 mutation cloned into Bluescript KS+	This study

used to extract single-stranded DNA from cells harboring the Bluescript clones (Stratagene, San Diego, Calif.). DNA sequencing was done by the dideoxy-chain termination method (48) and the Sequenase sequencing kit (U.S. Biochemical Corp., Cleveland, Ohio). The University of Wisconsin Genetics Computer Group software package was used for sequence analysis (11).

In vitro transcription-translation. Purified plasmid pSR204 DNA (1 to 2 μg) was used as the template in an in vitro prokaryotic DNA-dependent transcription-translation kit (Amersham Corp., Arlington Heights, Ill.). The reactions were carried out by the method of the manufacturer.

Transposon mutagenesis. Insertions of Tn*1000* into pSR210 were isolated essentially as previously described (21). EV13 and EV24 are streptomycin resistant and were used as recipients in these experiments.

PCR amplification and cloning of wild-type *kpsT*. Amplification was accomplished by using a 1.25-kb *Cla*I fragment from pSR278 containing the entire wild-type *kpsT* gene. Primers (see Fig. 2) A (5′-ATCGGCCTGAATTCTACCGA ACGCG-3′) and B (5′-TATTGGAATGGATCCACTATAG GTC-3′) were used for amplification and introduction of unique *Eco*RI and *Bam*HI restriction endonuclease sites, respectively. The GeneAmp DNA kit (U.S. Biochemical Corp., Cleveland, Ohio) was used as suggested by the manufacturer, using approximately 100 ng of template DNA, 100 pmol of each primer, and 5 U of AmpliTaq DNA polymerase per reaction. The reactions were run on a Coy temperature cycler (Coy Laboratory Products Inc., Ann Arbor, Mich.) with an initial melt of 94°C for 2 min, followed by a 30-cycle sequence of 94°C for 20 s, reannealing at 55°C for 20 s, and polymerization at 72°C for 30 s. The polymerase chain reaction (PCR) products were visualized under long-wave UV light on a 0.7% agarose gel, and a 722-bp fragment was recovered via phenol freeze-squeeze (4). The fragment was checked by restriction endonuclease digestion and cloned into Bluescript KS+ by using the new restriction endonuclease sites.

PCR mutagenesis of *kpsT*. Site-directed mutagenesis by overlap extension (25) was done by using the template described above. The first reactions, to make overlapping partial products, were done by using either amplification primer A with mutagenesis primer D (5′-CCGAAGTAAA GTTGACTCACCGGCTCC-3′) or amplification primer B with mutagenesis primer C (5′-GGAGCCGGTGAGTCAAC TTTACTTCGG-3′) (see Fig. 2). The PCR reactions and run parameters were the same as for the wild-type amplification described above. The resulting fragments were gel purified, recovered via phenol freeze-squeeze, and subjected to another PCR reaction as described above, by using approximately 2.5 pmol of each fragment with 100 pmol of amplification primers A and B. The run parameters this time

consisted of 1 initial melt cycle of 94°C for 5 min, followed by a 10-cycle extension-amplification sequence of 94°C for 1 min and 65°C for 1 min. Following this was a 25-cycle sequence of 94°C for 30 s and 65°C for 30 s. A 722-bp fragment was isolated, as described above, and checked by restriction endonuclease digestion. The desired mutation (lysine to glutamic acid at position 44) results in a unique *Hph*I site not found in the wild-type *kpsT* sequence.

SDS-PAGE. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was done by the method of Laemmli (37). Radiolabeled proteins were separated on a 15% gel for 3.0 h at 25 mA. The gel was fixed (40% methanol, 10% acetic acid, 3% glycerol) for 1.5 h, soaked in Amplify (Amersham Corp.) for 30 min, and vacuum dried at 60°C for 3.0 h. Autoradiography was performed with Kodak X-Omat film (Eastman Kodak, Rochester, N.Y.) at −70°C. Proteins in cell extracts were separated for 3 h at 25 mA on a 10% gel, which was then fixed (50% methanol, 10% acetic acid, 40% water) for 2 h, stained (fixative with 0.05% Coomassie brilliant blue) overnight, and destained (5% methanol, 7% acetic acid, 88% water) for 6 h. The gel was air dried between cellophane sheets at room temperature overnight.

Nucleotide sequence accession numbers. The nucleotide sequences for *kpsM* and *kpsT* are in the GenBank, EMBL, and DDBJ data bases under accession numbers M57382 and M57381, respectively.

RESULTS

Localization of region 3 coding sequences on pSR210. The junction between regions 3 and 2 of the *kps* gene cluster was more precisely defined by complementation analysis. For these experiments, two chromosomal mutations in the *kps* cluster of EV1, an *E. coli* K-12–K1 hybrid, were used (60). The mutation *kps-24* in EV13 was previously mapped by transductional analysis to the leftmost portion of region 3 (59). EV13 has a pleiotropic phenotype that results in reduced levels of CMP-NeuNAc synthetase and sialyltransferase activity (59, 60). The nature of the mutation in EV13 is not known but appears to be a defect in transcriptional regulation (64). A second mutant, EV24, harbors a region 2 mutation (59). EV24 has a defect in sialic acid synthesis and synthesizes a polymer only when grown in the presence of exogenously added sialic acid (59).

Plasmid pSR210, which includes most of region 3 and the proximal portion of region 2 (Fig. 1), complemented both the *kps-24* mutation of EV13 and the *neuB-25* mutation in EV24. Complementation was detected by precipitin formation on antiserum agar plates and sensitivity to K1-specific bacteriophage E. Tn*1000* insertions in pSR210 that blocked complementation of EV13 were isolated and mapped. These insertions clustered in a segment of pSR210 of approximately 2 kb (Fig. 1B, closed circles). Tn*1000* insertions in pSR210 which no longer complemented EV24 were also isolated and were distinct from those that blocked complementation of EV13. These insertions clustered to the right half of pSR210 (Fig. 1B, open circles). We conclude from these experiments that the Tn*1000* insertions in pSR210 define the junction between regions 3 and 2 of the *kps* gene cluster.

Nucleotide sequence of region 3 of the *kps* gene cluster. We determined the nucleotide sequence of the segment of region 3 defined by the Tn*1000* insertions in pSR210 (Fig. 1). Two tandem open reading frames, designated *kpsM* and *kpsT*, were detected (Fig. 2). The *kpsM* termination codon overlaps the *kpsT* initiation codon by two nucleotides. Recent

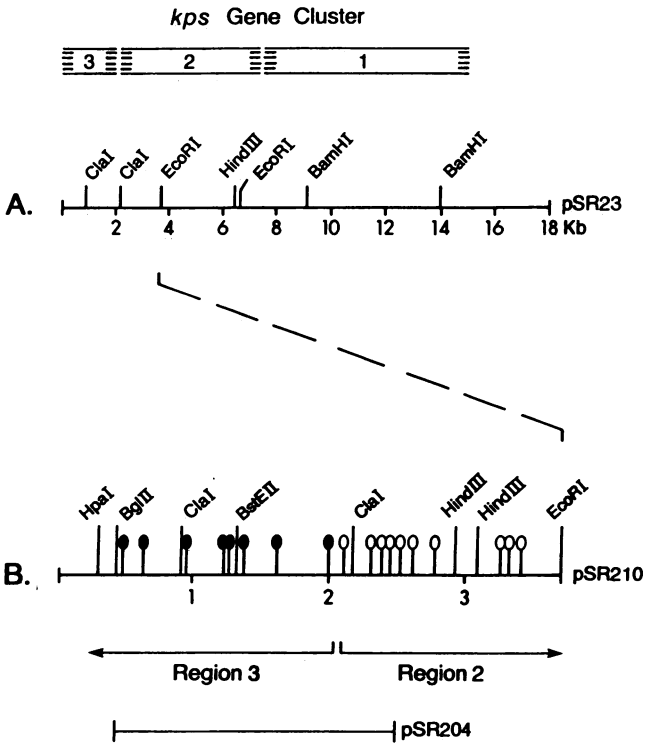

FIG. 1. (A) Restriction endonuclease map of pSR23 and the regional organization of the *kps* gene cluster. (B) Restriction endonuclease map of plasmid pSR210 and the Tn*1000* insertions that blocked complementation of EV13 (closed circles) or EV24 (open circles). Below is shown the boundary between regions 3 and 2, along with the position of plasmid pSR204, which was used in the in vitro transcription-translation system.

studies in this laboratory suggest that the two proteins are translationally coupled (62). *kpsM* and *kpsT* are predicted to encode proteins of 258 and 219 amino acids, with M_rs of 29,557 and 24,939, respectively. They constitute an operon that is transcribed from a promoter located 743 bp upstream of the putative initiation codon of *kpsM* (64).

To identify the *kpsM*- and *kpsT*-encoded gene products, we examined plasmid pSR204 (Fig. 1) in an in vitro transcription-translation system. In addition to the vector-encoded β-lactamase, two proteins with apparent molecular masses of 25.8 and 24.2 kDa were synthesized (Fig. 3). Both sizes are consistent with the predicted molecular mass of KpsT determined by translation of the DNA sequence. However, KpsM is predicted to have a molecular mass of 29,557 Da, and a protein of this size was not observed, suggesting that KpsM has a faster mobility in SDS-PAGE gels than expected from the calculated molecular mass. Alternatively, translation of KpsM may initiate at methionine codon 2 at position 160 in the nucleotide sequence (Fig. 2). Initiation at this codon would result in a protein with a predicted size of 23,482 Da, which is consistent with the smaller product observed in Fig. 3.

KpsT is a relatively hydrophilic molecule, while the KpsM protein has extensive hydrophobic stretches that could potentially form membrane-spanning helices (Fig. 4). By using the hydrophobic-transfer free-energy values of Engelman et al. (15), we determined that six of the predicted hydrophobic domains of KpsM gave values representing considerably more hydrophobicity than the −20-kcal/mol (1 cal = 4.184 J)

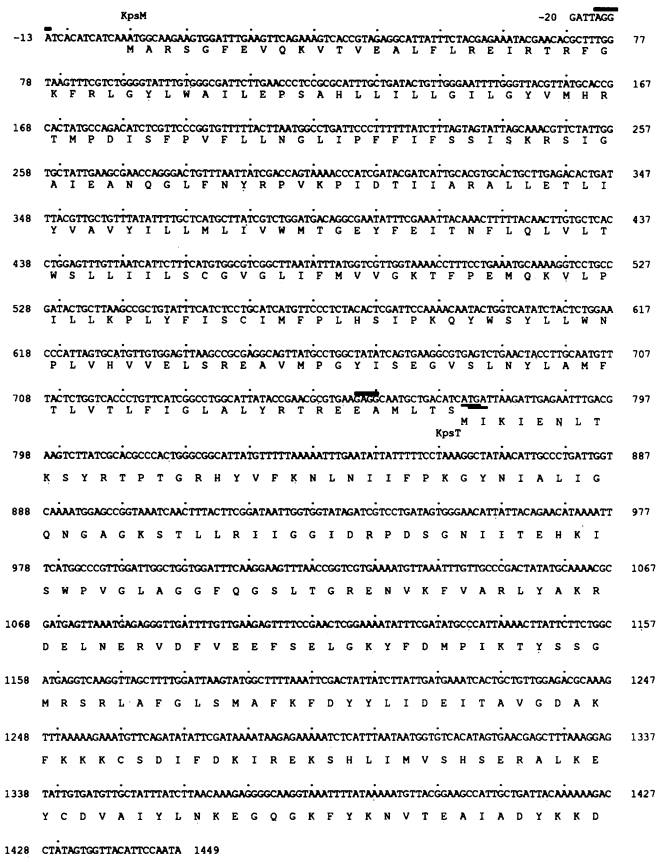

FIG. 2. Nucleotide sequences of *kpsM* and *kpsT*, with the deduced amino acid sequences. Each gene is indicated, as well as the putative Shine-Dalgarno (50) ribosome-binding sites. The overlap between the two reading frames is underlined. Primer A, used for PCR amplification, spans nucleotides 727 through 752, while primer B is complementary to the sequence from nucleotides 1425 through 1449. Mutagenesis primers C and D span nucleotides 894 through 920.

minimum value recommended for assigning potential transmembrane helices (indicated in Fig. 4). Results of Tn*phoA* insertion analysis are consistent with exposure of at least two regions of KpsM to the periplasm (data not shown).

Homology with *H. influenzae* type b capsule genes. Studies have revealed a common organization among *E. coli* K antigen biosynthetic genes (44, 45). Similar genetic organizations have also been observed for the polysialic acid gene cluster of *N. meningitidis* group B (17) and the capsular genes of *H. influenzae* type b (33), suggesting a common strategy for capsular polysaccharide genes among gram-negative bacteria. BexA is a 24.7-kDa protein essential for export of capsular polysaccharide in *H. influenzae* type b (31). KpsT shows 46% identity with the amino acid sequence of BexA (Fig. 5). Moreover, when identical and conserved amino acids are considered, the proteins are 70% similar. *kpsT* and *bexA* show 58.4% sequence similarity at the nucleotide level (data not shown). In addition, KpsM has 54.9% similarity and 26% identity at the amino acid level with BexB (32), another protein important for capsule expression in *H. influenzae* type b (data not shown).

Site-directed mutagenesis of the ATP-binding domain of KpsT. Kroll et al. (31) suggested that BexA belongs to a

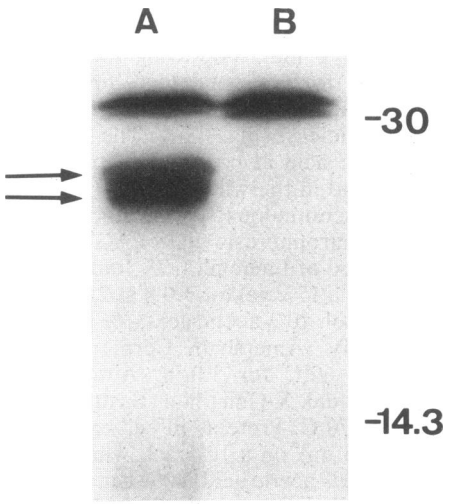

FIG. 3. Autoradiogram of [^{35}S]methionine-labeled proteins from the in vitro transcription-translation assay. The arrows indicate assay products unique to plasmid pSR204. These two products have apparent sizes of 25.8 and 24.2 kDa. The gel was exposed to film for 1.5 h. Lanes: A, pSR204; B, Bluescript KS+ vector. The following ^{14}C-labeled methylated proteins (CFA.626; Amersham) were used as standards: lysozyme, 14.3 kDa; carbonic anhydrase, 30 kDa; ovalbumin, 46 kDa; bovine serum albumin, 69 kDa; phosphorylase *b*, 92.5 kDa; myosin (heavy chain), 200 kDa.

family of ATP-binding proteins. These proteins share considerable sequence similarity and are involved with diverse biological processes, such as membrane transport, cell division, DNA repair, and glucan synthesis (5, 23, 24). Alignment of KpsT and various prokaryotic and eukaryotic sequences illustrating the conservation of amino acids in the ATP-binding sites of these proteins is shown in Fig. 6.

To test the hypothesis that ATP binding is important to KpsT function, a mutation in the ATP-binding domain of the protein was generated. For these experiments, a previously described chromosomal mutation in region 3 was further characterized. The mutant strain, EV95, is a Tn*10*-derived acapsular mutant of an *E. coli* K-12–K1 hybrid (59). EV95 is known to accumulate polysialic acid intracellularly (59). Complementation data (not shown) suggested that the transposon was inserted into the *kpsT* gene. To determine the location of the Tn*10* insertion in EV95 more precisely, Southern blot analysis was done. The results (data not shown) are consistent with a Tn*10* insertion into *kpsT* approximately 100 bp from the 3′ end of the gene.

The PCR was used to construct a DNA fragment containing *kpsT* that would be useful for overexpression of *kpsT* and site-directed mutagenesis. The primers used for amplification of the *kpsT* gene (Fig. 2) also generated an *Eco*RI site at the 5′ end and a *Bam*HI site at the 3′ end of the gene. The amplified product was cloned into Bluescript KS+ to utilize the *lac* promoter (Fig. 7). EV95 harboring this construct, designated pSR340, overexpressed the *kpsT* gene product. A Coomassie blue-stained SDS-PAGE gel of total cellular protein is shown in Fig. 7. Moreover, pSR340 complemented the *kpsT32*::Tn*10* mutation in EV95. Complementation was indicated by both precipitin halo formation on antiserum agar plates and sensitivity to K1-specific bacteriophage E.

Site-directed mutagenesis by overlap extension with the PCR was used to change the lysine residue at position 44 of the KpsT protein. Conserved lysine residues in ATP-binding

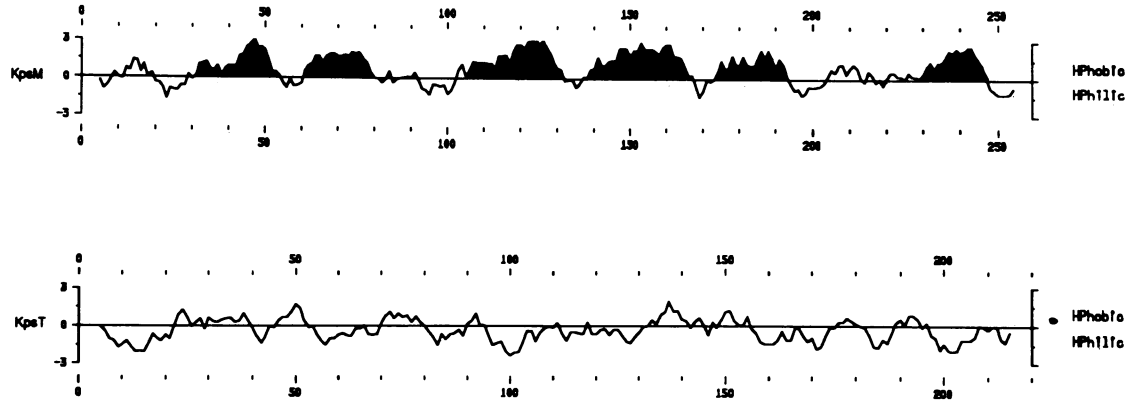

FIG. 4. Hydropathy profiles of KpsM (top) and KpsT (bottom) determined by the method of Kyte and Doolittle (36). The shaded areas indicate the six putative membrane-spanning regions.

domains have been shown to be important for interaction of ATP with protein-tyrosine kinases and rho protein (12, 13, 26, 28). The mutation not only changed the lysine to a glutamic acid residue but also introduced a new restriction endonuclease site, *Hph*I. The resultant mutant gene, KE44, was cloned into Bluescript KS+ with the unique *Eco*RI and *Bam*HI restriction endonuclease sites as described above. Restriction endonuclease digestion of this clone revealed the presence of the unique *Hph*I site, while DNA sequence analysis determined that no additional mutations were introduced during the PCR. Cells harboring the resultant plasmid, pSR346, overexpressed the mutant protein (Fig. 7). In contrast to pSR340, however, as assayed above, pSR346 did not complement the *kpsT36*::Tn*10* mutation in EV95.

DISCUSSION

Production of capsular polysaccharides in gram-negative bacteria is a complex process involving synthesis, activation, and polymerization of subunits into a large polymer which must be transported from a cell and anchored to its outer surface. The molecular, genetic, and biochemical events involved in these processes have not been fully elucidated. However, considerable progress has been made in our understanding of capsule synthesis and genetic organization from studies with the K1 and K5 polysaccharide capsules of *E. coli* (7, 54, 59).

The *kps* gene cluster of *E. coli* strains that synthesize antigenically diverse capsular antigens have a common genetic organization which defines three functional regions (45, 46, 59). The central region, 2, contains information for synthesis, activation, and polymerization of the specific sugars that determine the primary structure of the polymer and is unique for a given polysaccharide antigen (7, 59). In contrast, regions 1 and 3 function in more general aspects of capsule synthesis. These have been postulated to include postpolymerization functions, such as assembly of the polymer into a functional capsule and transport to the cell surface (6, 7, 59). In addition, regions 1 and 3 from chemically distinct capsular polysaccharides, such as K1 and K5, are functionally equivalent and highly conserved (45, 46).

Cells harboring mutations in region 2 do not synthesize a polymer, while intracellular polysaccharide accumulates in cells harboring mutations in either region 1 or 3 (7, 59). Immunoelectron microscopy and biochemical studies of region 1 mutants of the K5 *kps* gene cluster showed an intracellular polymer that was full length, carried a phosphatidic acid substitution, and was localized to the periplasmic space (34). These results are consistent with the concept that region 1 encodes genes involved in transport of the mature polysaccharide across the outer membrane (7, 51, 59). In contrast, with colloidal-gold-labeled K5 monoclonal antibodies, the K5 polysaccharide was localized to the cytoplasm of cells harboring mutations in region 3 (34). This material was

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KPST  MIKIENLTKSYRTPTGRHYVFKNLNIIFPKGYNIALIGQNGAGKSTLLRI 50
      ||:::|:.|.|:| .|:. |:|||:|: :.|| .|:::|.|||||||:|:
BEXA  MIRVNNVCKKYHTNSGWKTVLKNINFELQKGEKIGILGRNGAGKSTLIRL 50

      IGGIDRPDSGNIITEHKISWPVGLAGGFQGSLTGRENVKFVARLYAKRDE 100
      ::|:::.|.||.|  . .||||:::.|:|||||||.:|::|:.|||. .:
      MSGVEPPTSGTIERSMSISWPLAFSGAFQGSLTGMDNLRFICRLYDVDPD 100

      LNERVDFVEEFSELGKYFDMPIKTYSSGMRSRLAFGLSMAFKFDYYLIDE 150
      . .|  |..||||||.|:  |:|.|||||:.||||:||:...||:|||||
      YVTR..FTKEFSELGDYLYEPVKKYSSGMKARLAFALSLSVEFDCYLIDE 148

      ITAVGDAKFKKKCS.DIFDKIREKSHLIMVSHSERALKEYCDVAIYLNKE 199
      :.||||.:| .||. ::|:| :::| :|:|||||..|:|.||| |: |: :
      VIAVGDSRFAEKCKYELFEKRKDRS.IILVSHSPSAMKSYCDNAVVLE.N 196

      GQGKFYKNVTEAIADYKKD 218
      |  . :.::..|.. |...
      GIMHHFEDMDKAYQYYNET 215
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FIG. 5. Alignment of the amino acid sequences of KpsT and BexA of *H. influenzae* type b. Lines indicate identical amino acid residues, while double and single dots represent similar residues with comparison values of ≥0.50 and ≥0.10, respectively (56). The two proteins show 46.7% identity and 70% similarity at the amino acid level.

KpsT	(31-60)	GYNIALIGQNGAGKSTLLRIIGGIDRPDSG
BexA	(31-60)	GRKIGILGRNGAGKSTLIRLMSGVEPPTSG
MalK	(29-58)	GEFVVFVGPSGCGKSTLLRMIAGLETITSG
HlyB	(496-525)	GEVIGIVGRSGSGKSTLTKLIQRFYIPENG
ChvA	(361-390)	GETVAIGVPTGAGKTTLINLLQRVYDPDSG
Mdr	(1062-1091)	GQTLALVGSSGCGKSTVVQLLERFYDPMAG
Consensus sequence		GE-----G--G-GKST------G------G

FIG. 6. Amino acid homology between KpsT and representatives of a family of proteins with a consensus ATP-binding sequence. The consensus sequence shown is motif A of the adenine nucleotide-binding fold of Walker et al. (61). The position of each sequence within its respective protein is shown in parentheses. References: BexA, 31; MalK and HlyB, 24; ChvA, 9; and Mdr, 19. The lysine residue chosen for mutagenesis is underlined.

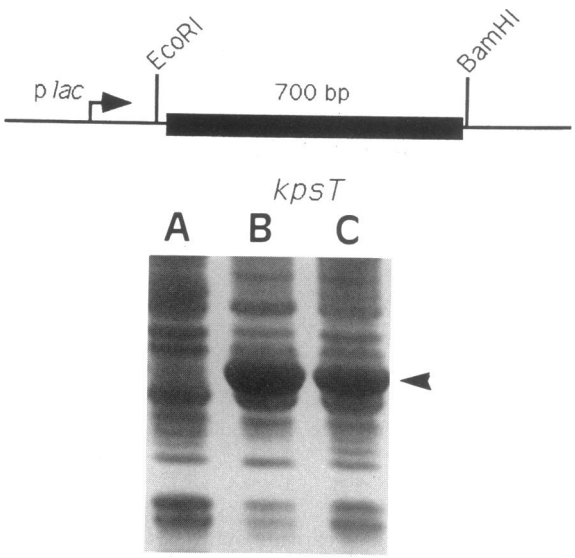

FIG. 7. Plasmid pSR340, with the orientation of the *lac* promoter with respect to *kpsT* in the cloned insert, is shown at the top. Below is an SDS-PAGE gel of total cellular proteins stained with Coomassie brilliant blue. A 1.5-ml volume of an overnight culture was spun down, suspended in 200 μl of cracking buffer (3), and boiled for 3 min. A 20-μl portion of each sample was loaded per well. Lanes: A, EV95/Bluescript KS+ vector; B, EV95/pSR340; C, EV95/pSR346 (KE44). KpsT is indicated by the arrowhead. The following prestained high-range protein standards (GIBCO-BRL, Gaithersburg, Md.) were used: lysozyme, 14.3 kDa; β-lactoglobulin, 18.4 kDa; carbonic anhydrase, 29 kDa; ovalbumin, 43 kDa; bovine serum albumin, 68 kDa; phosphorylase *b*, 97.4 kDa; and myosin (heavy chain), 200 kDa.

shorter than the surface polysaccharide and lacked the phosphatidic acid substitution. Additional work (35) using the energy uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone showed immunogold-labeled polysaccharide in the cytoplasm, not on the surface or in the periplasm, of wild-type K5 organisms. On the basis of these studies (34, 35), Boulnois and Jann (6) proposed that polysaccharide synthesis occurs on the cytoplasmic face of the inner membrane and region 3 products function in transport of the polymer across the cytoplasmic membrane in an energy-dependent process. Furthermore, the observation that the functional domain of the sialyltransferase complex of *E. coli* K1 is located on the cytoplasmic surface of the inner membrane (58) is in agreement with that view, supporting the idea that the growing polymer must in some way traverse the cytoplasmic membrane before being transported to the outer surface of the bacterial cell.

In the present study, we identified two genes, *kpsM* and *kpsT*, in region 3 of the *kps* gene cluster of *E. coli* K1. We believe that KpsM and KpsT form a transport system used to move polysialic acid across the cytoplasmic membrane. They appear to belong to a large family of prokaryotic and eukaryotic membrane translocators that perform many different functions (2, 5, 23). These include the histidine (His), oligopeptide (Opp), and maltose (Mal) permeases of enteric bacteria (22, 24); the cystic fibrosis conductance regulator (42); P-glycoprotein (mammalian multiple drug resistance pump) (19); and ChvA, which is required for export of β-1,2-glucan in *Agrobacterium tumefaciens* (9). Since binding and hydrolysis of ATP are believed to energize the movement of substances across a membrane by this family of proteins, Ames proposed they be referred to as "traffic ATPases" (2). Results presented in this report indicate that nucleotide interaction is important to KpsT function.

The structures of KpsM and KpsT are consistent with the basic organizational model of these transport systems. They consist of a hydrophobic component situated in a membrane and a hydrophilic component containing the ATP-binding fold of Walker et al. (61) that is located on the cytoplasmic side of the membrane. In certain groups within this family, the dual organization manifests itself as two domains within one large protein, with the two domains repeated in some cases (5), while in other groups (the enterobacterial periplasmic permeases, in particular) the motif is represented by individual homo- or heterodimers of each component (22). Furthermore, KpsD, a 60-kDa periplasmic protein required for capsule expression in *E. coli* K1, may be analogous to the periplasmic binding component seen in some of the bacterial transport systems (51).

The various transport systems move substances both ways across membranes and exhibit various degrees of substrate specificity. For example, the maltose and histidine transporters in bacteria are very specific and move their substrates into the cell (1), while the mammalian multiple drug resistance export pump appears to have a wide range of substrates (19). Since region 3 DNA from one *E. coli* serotype can complement region 3 mutations in a different serotype, KpsM and KpsT are able to transport a range of acidic polysaccharides.

The *kpsM* and *kpsT* genes from *E. coli* capsular serotype K5 have recently been cloned and sequenced (55). The sequences (nucleotide and translated amino acid) reported here were compared to the K5 sequences by using the GAP program of the University of Wisconsin Genetics Computer Group software package (11). The *kpsM* and *kpsT* nucleotide sequences from K1 are 94.8 and 69.3% identical, respectively, to the genes from the K5 serotype. At the amino acid level, KpsM from the K1 and K5 serotypes share 97.6% identity and 99.2% similarity, while the *kpsT* gene products are 72% identical and 84% similar. Furthermore, the KpsT protein from *E. coli* K5 is 5 amino acids longer at the carboxy terminus than the K1 gene product. The observation that KpsM and KpsT share significant similarity between *E. coli* serotypes and with BexB and BexA from *H. influenzae* type b suggests a common origin for capsule genes not only among *E. coli* strains but among gram-negative bacteria.

Further support for the idea that KpsM and KpsT constitute a transport system for polysialic acid will require additional characterization of both components. More information about the membrane topology and subcellular location of KpsM and KpsT is essential for proper development of a transport model. In addition, our view of the involvement of KpsM and KpsT in polymer transport necessitates interaction between the two proteins, which can be assessed by both physical and genetic methods. It should also be possible to test various aspects of the model by studying the properties of the system in subcellular inverted vesicles or in reconstituted proteoliposomes with purified components. Investigations into the KpsM-KpsT transport system can add to our understanding of not only capsule expression in *E. coli* but cellular transport processes in a variety of organisms.

ACKNOWLEDGMENTS

We gratefully acknowledge Laura Hales, who isolated and characterized plasmid pSR210 as part of an undergraduate research

project. We also thank Simon Kroll for generously providing the sequence of *bexB* prior to its publication.

This work was supported by Public Health Service grants AI 26655, from the National Institute of Allergy and Infectious Diseases, and S07RR05403-29, from the National Institutes of Health.

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