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Transcriptional Organization and Regulation of Expression of Region 1 of the *Escherichia coli* K5 Capsule Gene Cluster

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The transcriptional organization and regulation of region 1 expression of the *Escherichia coli* K5 capsule gene cluster were studied. Region 1 was transcribed as an 8.0-kb polycistronic mRNA which was processed to form a separate 1.3-kb transcript encoding the 3′-most gene *kpsS*. Transcription of region 1 of the *E. coli* K5 capsule gene cluster was directed from a single promoter 225 bp upstream of a previously unidentified gene, *kpsF*. The promoter had −35 and −10 consensus sequences typical of an *E. coli* σ 70 promoter, with no similarities to binding sites for other σ factors. Two integration host factor (IHF) binding site consensus sequences were identified 110 bp upstream and 130 bp downstream of the transcription start site. In addition, two AT-rich regions separated by 16 bp identified upstream of the region 1 promoter were conserved upstream of the region 3 promoter. The *kpsF* gene was 98.8% identical with the *kpsF* gene identified in the *E. coli* K1 antigen gene cluster and confirms that the *kpsF* gene is conserved among group II capsule gene clusters. An intragenic Rho-dependent transcriptional terminator was discovered within the *kpsF* gene. No essential role for KpsF in the expression of the K5 antigen could be established. The temperature regulation of region 1 expression was at the level of transcription, with no transcription detectable in cells grown at 18°C. Mutations in regulatory genes known to control temperature-dependent expression of a number of virulence genes had no effect on the temperature regulation of region 1 expression. Likewise, RfaH, which is known to regulate expression of *E. coli* group II capsules had no effect on the expression of region 1. Mutations in the *himA* and *himD* genes which encode the subunits of the IHF led to a fivefold reduction in the expression of KpsE at 37°C, confirming a regulatory role for IHF in the expression of region 1 genes.

In excess of 80 capsular polysaccharides or K antigens on the surface of *Escherichia coli* have been described (39). They have been divided into three groups, I, II, and III, on the basis of a number of biochemical and genetic criteria (25, 45). Group II K antigens differ from the other two groups in exhibiting temperature-dependent regulation, with capsule expression at 37°C but not at 18°C (24).

A number of group II capsule gene clusters have been cloned and subjected to a detailed molecular genetic analysis (53, 54, 60, 61, 66). These studies revealed that group II capsule gene clusters have a conserved genetic organization consisting of three functional regions (6, 54). A central serotype-specific region 2, which encodes enzymes for the synthesis and polymerization of the specific K antigen, is flanked by regions 1 and 3, which are conserved between different group II K antigen gene clusters (6, 46, 52, 54, 59).

The cloned K5 antigen gene cluster has been subjected to a detailed molecular genetic analysis to determine the roles played by the proteins encoded by regions 1 and 3. Mutations in region 3 result in cytoplasmic polysaccharide unlinked to phospholipid, which is associated with the inner face of the cytoplasmic membrane (6, 28). Determination of the nucleotide sequence of region 3 from both the K1 and K5 antigen gene clusters has identified two genes, *kpsM* and *kpsT*, which are organized in a single transcriptional unit (41, 62). Analysis of the predicted amino acid sequences of the KpsM and KpsT proteins suggested that they are members of the ABC transport superfamily (40, 41, 50, 62) and constitute a polysaccharide transport system for the export of polysaccharides across the cytoplasmic membrane. In this system, the KpsT protein acts as an ATPase to energize the transport process mediated by KpsM, an integral inner-membrane protein (40, 47). Proteins homologous to KpsM and KpsT have been identified in both *Haemophilus influenzae* and *Neisseria meningitidis* and are believed to play similar roles in the export of polysaccharides in these two bacteria (20, 27, 40).

Analysis of region 1 of the K5 antigen gene cluster identified five genes (*kpsEDUCS*) probably organized in a single transcriptional unit (43). In the K1 antigen gene cluster, an additional gene, *kpsF*, has been identified upstream of *kpsE*, although its function is unknown (11). Mutations in either the *kpsE* or *-D* gene result in periplasmic polysaccharide, suggesting a role for these two proteins in the export of polysaccharide across the periplasmic space and onto the cell surface (6, 7). The location of the KpsD protein within the periplasmic space (69) and the topology of the KpsE protein within the inner membrane with a large periplasmic domain (55) are in keeping with these roles. Mutations in either the *kpsC* or *-S* gene result in aggregates of polysaccharide within the cytoplasm and a reduction in membrane transferase activity (7, 8). It is possible that these proteins function to modify the polysaccharide prior to export, perhaps by the addition of phospholipid to the reducing end of the polysaccharide (52). The KpsU protein has been purified and shown to be a functional CMP–2-keto-3-deoxyoctulosonic acid (KDO) synthetase enzyme which catalyzes the formation of CMP-KDO (56). This explains why strains expressing group II capsules have elevated levels of CMP-KDO synthetase at capsule-permissive temperatures (18). The elevation of KpsU activity at 37°C compared with 18°C suggests that the expression of region 1 is regulated by temperature.

In this communication, we confirm the presence of a *kpsF*

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TABLE 1. Strains and plasmids used

Strain or plasmid	Relevant property(ies)	Source or reference
Strains		
LE392	F$^-$ *hsdR514 supE44 supF58 lacY1 galK2 galT2 metB1 trpR55*	36
L32706	*gutQ*::Tn*5*	M. H. Saier
MS101	K-12/K5$^+$	64
MS102	MS101 *hns*::Tn*10*	64
MS104	MS101 *himA*	64
MS105	MS101 *himD*	64
MS106	MS101 *rfaH*::Tn*5*	64
MS140	MS101 *hha*::Tn*5*	This work
MS150	MS101 Δ(*lac proAB*)	I. Roberts
MS151	MS101 *rimJ*	This work
MSFE100	MS101 Δ(*kpsE*)	I. Roberts
DS410	*minA minB ara xyl mtl azi thi*	16
W3110	F$^-$ IN(*rrnD-rrnE*) 1 λ$^-$	I. Roberts
2055	(W3110)*trpE9851 leu-277 rho-4*	35
Plasmids		
pCB192	*lacZ* promoter probe vector	58
pTZ18/19	Phagemid cloning-expression vector	31
pGEM5Z	Phagemid cloning-expression vector	Promega
pHV100	*luxAB* vector for the study of transcription termination	44
pKK223-3	Vector	10
pPC6	Cloned K5 capsule genes lacking *kpsF*	43
pGB110	K5$^+$ cosmid	53
pH18	*Hin*dIII fragment encoding region 1 except *kpsF* cloned in pUC18	43
pDSHB	1.4-kb *Hin*dIII-*Bam*HI fragment containing putative promoter 5′ to *kpsE* cloned in pCB192	This study
pDSHcH	1.2-kb *Hin*cII-*Hin*dIII fragment containing the region 1 promoter cloned in pCB192	This study
pDSHcS	1.5-kb *Hin*cII-*Sma*I fragment containing the region 1 promoter cloned in pCB192	This study
pDSB1	5.3-kb *Bam*HI fragment cloned in pCB192	This study
pDSB2	1.7-kb *Bam*HI fragment cloned in pCB192	This study
pDS110	2.2-kb *Eco*RI-*Sma*I fragment cloned in pTZ19R	This study
pDS111	2.5-kb *Eco*RV fragment cloned in pTZ19R containing the *kpsF* gene	This study
pDS130	0.35-kb *Vsp*I-*Sau*3A fragment spanning the region 1 promoter; cloned in pTZ19R	This study
pDS200	1.1-kb *Eco*RI fragment encompassing region 1; cloned in pKK223-3	This study
pDS200T	pDS200 with sequence upstream of the *Sph*I site in *kpsU* deleted	This study
pDS2001	pDS201 with transcriptional terminator inserted at the *Hpa*I site in *kpsC*	This study
pDS300	1.5-kb *Hin*dIII-*Bam*HI fragment spanning from *kpsF* to *kpsE*; cloned in pHV100	This study
pDS301	1.1-kb *Sma*I-*Bam*HI fragment spanning from *kpsF* to *kpsE*; cloned in pHV100	This study
pDS304	0.4-kb *Hin*dIII-*Dra*I fragment from within *kpsF*; cloned in pHV100	This study

gene as the first gene of region 1 of the K5 capsule gene cluster and locate the region 1 promoter 5′ to *kpsF*. We demonstrate that expression of region 1 is regulated in response to temperature at the level of transcription and that the transcript is processed to yield a smaller *kpsS*-specific transcript. A Rho-dependent transcriptional terminator was identified within the *kpsF* gene and may provide a mechanism for regulating transcription of region 1 in response to the physiological status of the cell.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were routinely grown in L broth supplemented with 100 μg of ampicillin or 25 μg of chloramphenicol per ml as required. The appropriate mutations in known regulatory genes were introduced into MS101 by P1 transduction (34).

DNA procedures. Recombinant DNA procedures were performed by standard methods (30). Restriction endonucleases and DNA-modifying enzymes were purchased from GIBCO BRL. Single-stranded M13 or denatured double-stranded DNA templates (71) were sequenced by the dideoxy chain-termination method (57) with [α-thio-^{35}S]dATP and modified T7 DNA polymerase, Sequenase version 2.0 (U.S. Biochemicals Corp.). The DNA fragments were analyzed on buffer gradient gels (3). The DNA sequence was analyzed with the programs of the Wisconsin Genetics Computer Group (15) at the SEQNET facility.

β-Galactosidase assays. The β-galactosidase activities of cell lysates were measured by cleavage of the chromogenic substrate *O*-nitrophenyl-β-D-galactoside (ONPG) as described by Miller (34).

Luciferase assays. Bacterial growth and expression of luciferase were measured as described by Peabody et al. (44). Cultures were grown overnight in L broth, and 0.1 ml was used to inoculate 15 ml of minimal media (M9) containing ampicillin (100 μg/ml) thiamine (5 μg/ml), 0.02% glucose, and appropriate amino acids (40 μg/ml). At an optical density at 600 nm (OD_{600}) of 0.1, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 100 mM. OD was measured at defined intervals thereafter, and culture samples were diluted to 0.1 OD_{600} with SSC (1× SSC is 0.15 M NaCl plus 0.15 M sodium citrate) buffer. The light emitted by 100 ml of the diluted sample upon the addition of 10 ml of the luciferase substrate *n*-decanal (Sigma) was measured in a Labsystems luminoskan RS.

RNA preparation. RNA was extracted by resuspending a mid-logarithmic-phase culture in lysis buffer (0.02 M sodium acetate [pH 5.2], 0.5% sodium dodecyl sulfate [SDS], 1 mM EDTA) and phenol equilibrated with 0.2 M sodium acetate (pH 5.2), both preheated to 60°C. Following further phenol and chloroform extractions, the RNA was precipitated and, when required, was treated with RNase-free DNase I (Pharmacia).

Northern (RNA) analysis. RNA was electrophoresed under denaturing conditions in a formaldehyde agarose gel and was transferred to a Hybond N filter (Amersham) according to the manufacturer's protocol. DNA probes were prepared from PCR products or restriction endonuclease fragments purified from agarose gels with a Sephaglass BandPrep kit (Pharmacia). Radiolabelled probes were generated by random hexanucleotide primer extension by using the Klenow fragment of DNA PolymeraseI (GIBCO BRL) in the presence of [^{32}P]dCTP. Filters were hybridized and washed at high stringency according to the Amersham protocol. Filters were stripped of hybridized probe by boiling in 0.1% SDS.

Primer extension. Oligonucleotide primers were 5′-end labelled with [γ-^{32}P]ATP by using T4 polynucleotide kinase (Promega). Each labelled oligonucleotide was hybridized with total cellular RNA extracts and used as a primer by avian myeloblastosis virus reverse transcriptase (Promega) as described by the

manufacturer. The resulting cDNAs were analysed on sequencing gels adjacent to a DNA sequencing ladder generated from the same oligonucleotide. The primers used were RT7 (5′-GCGAACAGAGGTAATTAGATATGG-3′) and RT9 (5′-GTCAGTCCATGCTTATATGCAGG-3′).

RNase protection. Radiolabelled RNA probes were synthesized by using the Promega Riboprobe system, a modification of the procedure described by Melton et al. (32). K5 DNA was cloned into vector pTZ18 or pTZ19 (31) downstream of the T7 promoter and was linearized with an appropriate restriction endonuclease. This DNA template was used to synthesize high specific activity RNA probes by incorporation of [α-^{32}P]UTP according to the Promega protocol. Full-length transcripts were purified by electrophoresis through and excision from polyacrylamide sequencing gels. The RNA was eluted in 2 to 3 volumes of Tris-EDTA–0.1% SDS–1 mM EDTA–proteinase K (10 mg/ml) at 37°C overnight and extracted with phenol and then chloroform before being precipitated with ethanol. The purified probe was hybridized with 50 to 100 μg of total RNA at 40°C for at least 6 h and digested with RNase One (Promega) at 25°C, and the undigested products were analyzed on a sequencing gel adjacent to a DNA sequencing ladder.

Protein analysis. Protein samples were prepared by boiling cultures in gel loading buffer prior to analysis by SDS-polyacrylamide gel electrophoresis (PAGE) (29). Western blotting (immunoblotting) was performed as previously described (65). Bound antibody was detected with the ECL Western blotting system (Amersham) according to the manufacturer's instructions. When appropriate, the signals were quantified with scanning densitometry by using a Bio-Rad GS-700 Imaging Densitometer with transparency frame 4200164. Minicells were prepared from strain DS410 as described previously (53), and the [^{35}S]methionine-radiolabelled proteins were visualized by autoradiography after SDS-PAGE.

Nucleotide sequence accession number. The nucleotide sequence of the entire ORF upstream of *kpsE* of the K5 antigen gene cluster has been deposited with EMBL under accession no. X95264.

RESULTS AND DISCUSSION

Identification of a *kpsF* gene upstream of region 1 of the K5 antigen gene cluster and location of the region 1 promoter. An open reading frame (ORF) terminating 71 bp upstream from the *kpsE* gene of region 1 of the K5 antigen gene cluster has been identified previously (42). Plasmid constructs carrying only the 3′ end of this ORF express a K5 capsule, suggesting that this ORF is not essential for K5 capsule production (42, 43). To locate the promoter upstream of *kpsE*, a *Hin*dIII-*Bam*HI fragment (Fig. 1) was cloned into the promoter probe vector pCB192 (58) to form plasmid pDSHB (Table 1; Fig. 1). No β-galactosidase activity could be detected in strains harboring pDSHB (data not shown), suggesting that the promoter for region 1 was not present on this fragment. To confirm this, an RNase protection assay was performed with a radiolabelled antisense RNA probe spanning this region (probe 1 in Fig. 1). The RNA probe was fully protected by transcripts present in RNA extracted from either LE392(pDS200), which contains sequences 5′ to the *Hin*dIII site (Fig. 1), or strain MS101 (data not shown). This confirmed that there are no promoters within this region.

Therefore, the nucleotide sequence of the entire ORF upstream of *kpsE* and region 1 of the K5 antigen gene cluster was determined (Fig. 2). This revealed a gene 98.7% identical to the *kpsF* gene identified upstream of *kpsE* in the K1 antigen gene cluster (12, 13), confirming that this gene is conserved between different group II capsule gene clusters. The G+C ratio of this DNA was 49%, which is typical for that reported for *E. coli* (38) and similar to the rest of the K5 region 1 (43). The predicted amino acid sequence of the encoded protein was 95% identical to that of the KpsF protein identified in the K1 antigen gene cluster, suggesting that KpsF may play a conserved role in the expression of group II K antigens. Expression of the KpsF protein was confirmed by minicell analysis of plasmid pDS111, a subclone of the 2.5-kb *Eco*RV fragment containing the entire *kpsF* gene (Fig. 1; Table 1). This encoded a protein of the predicted molecular mass (35 kDa), whereas pDS110, which has the 3′ end of *kpsF* deleted (Fig. 1; Table 1), encoded a truncated protein of 22 kDa (Fig. 3).

To locate the site of transcription initiation upstream of

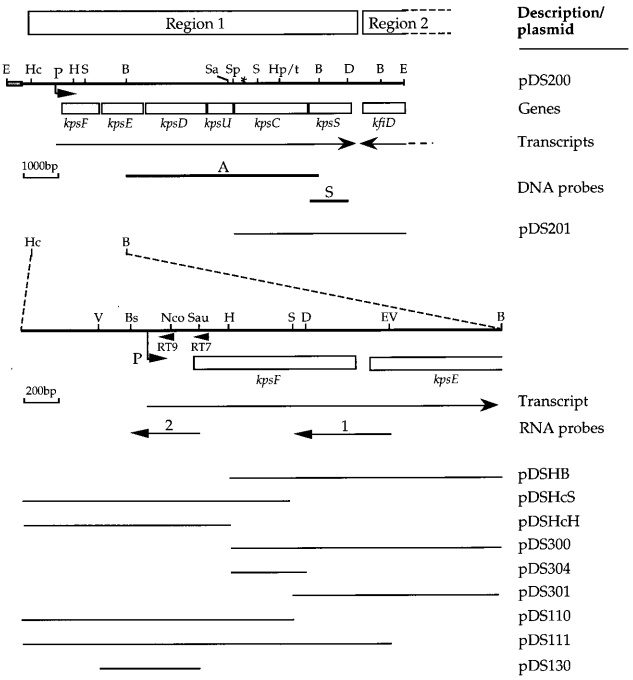

FIG. 1. Analysis of region 1. The top line is a restriction map of an 11-kb *Eco*RI fragment from pGB110 (the shaded box encodes cos4 sequences). This encodes region 1 (*kpsF*, *-E*, *-D*, *-U*, *-C*, and *-S*) and was subcloned to make pDS200. P, the proposed promoter; Hp/t, site of insertion of the *rrn* transcriptional terminator (9) in *kpsC*; open boxes, genes (with their designations and the proposed transcripts indicated below); bold lines, the restriction fragments and PCR products used as probes in Northern analysis; lines with arrowheads to the left, antisense RNA probes used in RNase protection assays. Sequences upstream of the *Sph*I site in *kpsU* were deleted from pDS200 to create pDS201. At the bottom, the promoter region is enlarged to show the fragments assayed for promoter activity (pDSHB, pDSHcS, and pDSHcH) or transcription termination (pDS300, -301, and -304). The DNA fragments cloned in pTZ19 to demonstrate expression of KpsF in minicells and generate antisense RNA probes are shown (pDS110, pDS111, and pDS130). The locations of primers RT7 and RT9 used to map the transcription start point are indicated by arrowheads. Abbreviations: B, *Bam*HI; Bs, *Bsa*BI; D, *Dra*I; E, *Eco*RI; EV, *Eco*RV; Hc, *Hin*cII; H, *Hin*dIII; Hp, *Hpa*I; Nco, *Nco*I; Sa, *Sal*I; Sau, *Sau*3A; S, *Sma*I; Sp, *Sph*I; V, *Vsp*I.

kpsF, primer extension was performed with oligonucleotide RT7 (Fig. 1 and 2), which is complementary to the 5′ end of the *kpsF* gene. This identified three potential transcriptional start points, approximately 40, 90, and 220 bp upstream of the gene (data not shown). To confirm that these represented three separate transcriptional initiation start sites and were not a result of stalling of the reverse transcriptase, an RNase protection experiment was performed by using radiolabelled antisense RNA probe 2 (Fig. 1). This was generated as a runoff transcript from plasmid pDS130, which contains a 0.35-kb *Vsp*I-*Sau*3A fragment spanning the region 1 promoter (Fig. 1) cleaved with *Bsa*BI. Only a single RNA fragment of 260 nucleotides was protected by RNA from MS101 (data not shown), the size of which was consistent with transcription initiating from the most 5′ of the three possible start sites.

The start site was mapped to 225 bp upstream of the ATG codon of *kpsF* by primer extension (Fig. 4) with primer RT9 (Fig. 1), which annealed 100 bp downstream of the estimated start site (Fig. 2). Upstream of the transcriptional initiation site are regions similar to the −35 and −10 consensus sequences for an *E. coli* σ^{70} promoter (Fig. 2). No similarities with binding sites for other σ factors were identified. Two integration host factor (IHF) binding site consensus sequences (19) are located 110 bp upstream and 130 bp downstream of the start

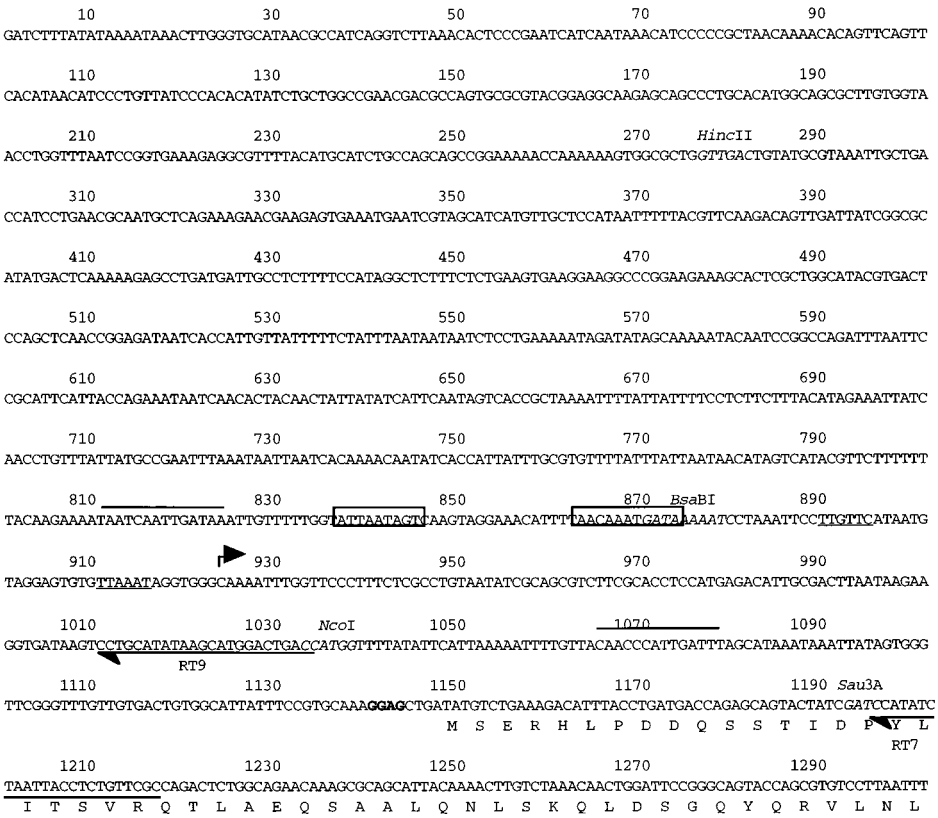

FIG. 2. Nucleotide sequence of the 5′ end of the *kpsF* gene and upstream regions. Italics, selected restriction enzyme sites; arrows, primers RT7 and RT9 used to determine the transcript start point; underlining, potential −35 and −10 regions; arrow, the first nucleotide transcribed; overlining, sequences matching the consensus IHF binding site; boxed areas, the conserved AT-rich regions that are also present in the region 3 promoter; boldface, Shine-Dalgarno sequence preceding *kpsF*; single-letter code, the predicted amino acid sequence of KpsF.

site (Fig. 2). IHF is a histone-like DNA-bending protein (19), and binding of IHF at these sites would change the DNA conformation and thereby alter the efficiency of transcription and/or mediate the action of other regulatory factors. To determine if IHF played any role in the expression of region 1, Western blot analysis with antisera to the KpsE was performed on lysates of strains MS104 and MS105. These strains have mutations in the *himA* and *himD* genes, respectively, which encode the two subunits of IHF (Table 1). Strain MSFE100, which has the *kpsE* gene deleted, was included as a negative control (Table 1). The intensity of the signal obtained was quantified by scanning densitometry. Equal loading of protein was confirmed by scanning densitometry of the same gel stained with Coomassie blue (data not shown). Expression of KpsE was reduced fivefold in MS104 and sixfold in MS105 strains and was absent in MSFE100 (Fig. 5), suggesting that although IHF is not essential for expression of region 1, it does play a regulatory role. These results are in accordance with previous results which showed that a functional IHF is not essential for group II capsule expression (64). Alignment of the nucleotide sequence upstream of *kpsF* with that upstream of

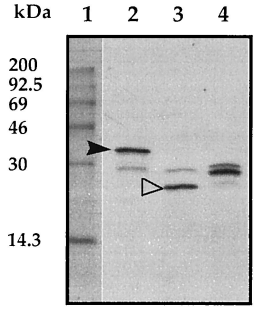

FIG. 3. Expression of KpsF in minicells. The proteins expressed by minicells harboring the following plasmids were analyzed by SDS-PAGE: pDS111, encoding KpsF (solid arrow) with a predicted molecular mass of 35.6 kDa (lane 2); pDS110, encoding a truncated KpsF (open arrow) with a predicted molecular mass of 22.1 kDa (lane 3); and pTZ19 (lane 4). The molecular masses of markers (lane 1) are indicated in kilodaltons.

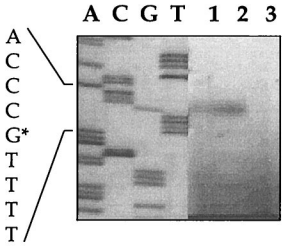

FIG. 4. Primer extension reactions were performed with oligonucleotide RT9 (Fig. 1 and 3) annealed with RNA extracted from MS101 (lane 1), LE392 (pDS200) (lane 2), and LE392(pKK223-3) (lane 3), all grown at 37°C. The products were analyzed by gel electrophoresis adjacent to a DNA sequencing ladder generated with the same primer. The bands observed with LE392 (pDS200) and MS101 show that transcription starts at cytosine residue 924 (marked in Fig. 3).

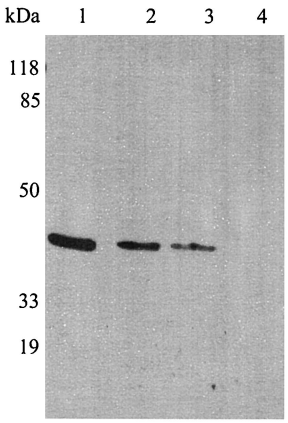

FIG. 5. Western blot analysis of KpsE expression. In each case, 50 μg of total cell protein was loaded and separated by SDS-PAGE prior to transfer and Western blotting with KpsE antisera. Lanes: 1, MS101; 2, MS104; 3, MS105; and 4, MSFE100.

region 3 revealed two conserved AT-rich sequences (Fig. 2) located at the same distance upstream of the respective transcription start sites. However, the JUMPstart sequence located 5′ to the *kpsM* gene in region 3 and postulated to play a role in regulation of group II capsule expression by RfaH (64) was not present upstream of region 1, suggesting that expression of this region is not regulated by RfaH. This was confirmed by Western blotting, which showed no detectable difference in the levels of KpsE expression in strains MS101 and MS106 (data not shown).

Transcriptional organization of region 1 of the K5 antigen gene cluster. In Northern blots of RNA extracted from LE392(pGB110) grown at 37°C, the radiolabelled region 1-specific DNA probe A (Fig. 1) hybridized to a large transcript of approximately 8.0 kb and to two bands corresponding to the positions of the two rRNA species (Fig. 6A, lanes 1 and 2). Such bands are commonly observed in Northern blots in which the probe binds to a larger transcript and in which large amounts of prokaryotic RNA are loaded onto the gel (37). A radiolabelled *kpsS*-specific DNA probe (Fig. 1) hybridized strongly to a small transcript of approximately 1.3 kb and weakly to the larger 8.0 kb transcript (Fig. 6B, lane 1) among RNA from LE392(pDS200) grown at 37°C. The detection of a 1.3-kb RNA transcript suggests either that there may be two transcriptional units within region 1 or that transcription originates 3′ to the *kpsS* gene on the opposite DNA strand, thereby generating an antisense RNA molecule. The presence of two transcriptional units within region 1 would require a large transcript originating from the promoter upstream of *kpsF* and terminating 3′ to *kpsC* and a second *kpsS*-specific transcript originating from a promoter located either within the intergenic gap between the *kpsC* and -*S* genes or in the 3′ end of the *kpsC* gene (Fig. 1). Indeed, previous studies of region 1 of the K1 antigen gene cluster suggest that the *kpsS* gene may be a transcriptional unit separate from the remainder of region 1 (13, 63). To attempt to identify a promoter in this region, a 5.3-kb *Bam*HI fragment corresponding to DNA probe A (Fig. 1) was cloned into plasmid pCB192 upstream of the *lacZ* gene, creating pDSB1 (Fig. 1). No detectable β-galactosidase activity was expressed by strains carrying this plasmid (data not shown), indicating that a promoter is not located within this region. To assay for transcription in the opposite direction, the adjacent 1.7-kb *Bam*HI fragment which spans into *kfiD*, the last gene in region 2 of the K5 capsule gene cluster (45) (Fig. 1), was cloned into pCB192, creating pDSB2 (Table 1). No detectable β-galactosidase activity was expressed by strains carrying this plasmid (data not shown).

To confirm that expression of *kpsS* is dependent on transcription of the upstream genes, two additional plasmids were constructed. The transcriptional terminator *rrnT1T2* (9) was inserted into the *kpsC* gene (Fig. 1) to generate plasmid pDS200T (Fig. 1), and sequences upstream of *kpsC* were deleted to make plasmid pDS201 (Fig. 1). Northern blot analysis with a radiolabelled *kpsS*-specific DNA probe with RNA from cells harboring pDS201 and pDS200T demonstrated that expression of the small 1.3-kb *kpsS*-specific transcript was abolished (Fig. 6B, lanes 2 and 3). To demonstrate that the intergenic space between *kpsC* and -*S* is transcribed, reverse-transcriptase PCR was performed on RNA extracted from LE392 (pDS200). A 2.0-kb fragment was amplified (data not shown), confirming that the intergenic space is transcribed.

Taken together, these data suggest that a separate transcript for *kpsS* does not originate from a promoter 5′ to the gene. It would appear that the smaller *kpsS*-specific message is derived by processing of the larger RNA molecule which spans the entire region 1.

Temperature regulation of region 1 transcription. To identify whether temperature-dependent expression of region 1 proteins is regulated at the level of transcription, Northern blots were performed on RNA extracted from LE392(pGB110) grown at 37 and at 18°C. A large RNA transcript of 8.0 kb detectable at 37°C was absent at 18°C (Fig. 6A, lanes 1 and 2), indicating that transcription of region 1 is regulated by temperature. Two additional experiments confirmed this conclusion. First, in an RNase protection assay, antisense RNA probe 1 (Fig. 1) was fully protected by RNA extracted from LE392 (pDS200) or MS101 grown at 37°C but degraded completely when hybridized to RNA extracted from cultures grown at 18°C (data not shown). Second, a *Hin*cII-*Hin*dIII fragment predicted to contain the region 1 promoter was cloned into

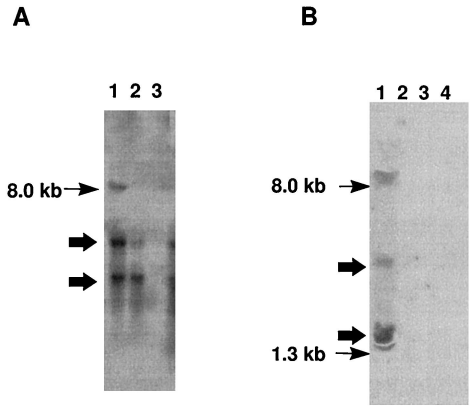

FIG. 6. (A) Northern blot analysis with probe A, which spans region 1 (Fig. 1). In all cases, 50 μg of total RNA was loaded into each track. An 8.0-kb region 1-specific transcript (thin arrow) among RNAs extracted from LE392(pGB110) grown at 37°C (lane 1) and at a much reduced level at 18°C (lane 2) was detected. Solid arrows, rRNA bands. No transcripts were detected from LE392 grown at 37°C (lane 3). (B) Northern blot with probe S, a PCR product encoding *kpsS* (Fig. 1). Among RNAs from LE392(pDS200) grown at 37°C (lane 1), the *kpsS* probe hybridized strongly to an approximately 1.3-kb transcript (open arrow), although larger transcripts were faintly detected. This *kpsS*-specific transcript was abolished by deletion of sequences upstream of *kpsC*, LE392(pDS201) (lane 2), or by insertion of a transcriptional terminator in *kpsC*, LE392(pDS200T) (lane 3). No transcripts were detected from LE392(pKK223-3) (lane 4). Solid arrows, the artifactual bands often observed at the position of the rRNA. The sizes of the hybridizing mRNA molecules are given in kilobases.

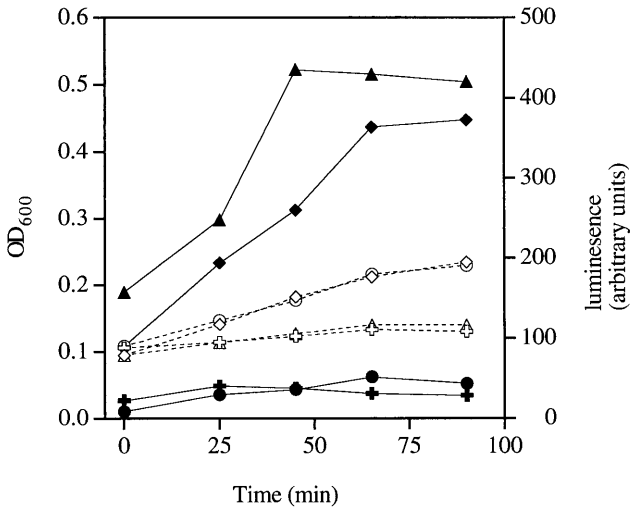

FIG. 7. Assays for transcriptional termination. Open symbols, OD_{600} values on the left-hand scale; closed symbols, the luminescence values on the right-hand scale. △, pHV100; ○, pDS300; ◇, pDS301; and +, pDS304. IPTG was added at time zero to induce the *lac* promoter.

pCB192 to generate plasmid pDSHcH (Fig. 1; Table 1). Plasmid pDSHcH was introduced into MS150, and β-galactosidase activity (Miller units) was assayed from cells grown at 37°C (996 ± 43) and 18°C (236 ± 5) (values are the means of four readings ± standard deviations). This confirmed that transcription from the region 1 promoter is regulated in response to temperature.

Mutations in regulatory genes known to be involved in the temperature regulation of virulence genes were introduced into MS101 (Table 1), and their effect on region 1 expression was monitored by Western blotting with antisera to the KpsE protein. Mutations in the *hns* gene which affects DNA topology (23) and is a pleiotropic regulator implicated in the temperature regulation of virulence gene expression in *Shigella flexneri* (48), in the *rimJ* gene, which is involved in thermoregulation of the *pap* operon (67), or in the *hha* gene, which influences DNA topology and regulates virulence genes in response to temperature (14, 21, 33) had no effect on expression of region 1 as measured by RNA dot blots and Western blotting (data not shown).

Identification of a *kpsF* intragenic transcriptional terminator. The observation that MS150 cells carrying pDSHcH expressed high levels of β-galactosidase activity compared with those for pDSHcS (Table 1; Fig. 1) at 37°C (996 ± 43 and 117 ± 4, respectively) was surprising, since pDSHcS contains only an additional 300 bp of the *kpsF* gene (Fig. 1). This suggests that significant transcriptional termination occurs in this region of the *kpsF* gene. To confirm this, restriction enzyme fragments were cloned into the transcriptional termination vector pHV100 (44). This vector allows transcriptional terminators to be identified by cloning fragments between the *lac* promoter and the *luxAB* genes of *Vibrio harveyi* (44). The presence of a transcriptional terminator reduces *luxAB* gene expression which can be assayed by a reduction in bioluminescence (22). Both the 1.5-kb *Hin*dIII-*Bam*HI fragment spanning from *kpsF* to *kpsE* (pDS300 [Fig. 1]) and the 415-bp *Hin*dIII-*Dra*I fragment from within the *kpsF* gene (pDS304 [Fig. 1]) demonstrated significant transcriptional termination activity when cloned into pHV100 (Fig. 7). However, when the 1,120-bp *Sma*I-*Bam*HI fragment (Fig. 1) was cloned in pHV100 to generate plasmid pDS301 (Fig. 1), it showed no significant transcriptional termination activity (Fig. 7). This suggests that the transcriptional terminator is located within the 330-bp *Hin*dIII-*Sma*I fragment (Fig. 1).

To investigate whether transcriptional termination was Rho dependent, the plasmids pDSHcH and pDSHcS were introduced into strain W3110 and the isogenic *rho* mutant strain 2055 (Table 1), and the ratio of β-galactosidase activity was measured. In W3110, the ratio of β-galactosidase activity from pDSHcH (650 ± 25) to that from pDSHcS (217 ± 7) was 3:1. In the *rho* strain, the ratio of β-galactosidase activity from pDSHcH (606 ± 16) to that from pDSHcS (580 ± 15) was 1:1. This demonstrates that the transcriptional termination occurring within the *kpsF* gene is Rho dependent. Analysis of the sequence in this region identified a cytosine-rich, guanosine-poor region characteristic of Rho-dependent terminators (2). Such intragenic terminators have been described previously and enable Rho to prevent continued elongation when the mRNA is not being actively cotranslated (1). This provides a mechanism to prevent the synthesis of untranslated transcripts during times of physiological stress (51). It is possible that the presence of this Rho-dependent terminator allows the transcription of region 1 to be modulated in response to the physiological state of the cell, such that under conditions of physiological stress expression of region 1 is reduced.

Functions of KpsF in expression of group II capsules in *E. coli*. The identification of *kpsF* as the first gene in both the K1 and the K5 capsule gene clusters suggests a conserved role in the expression of group II capsules. Three homologs to the KpsF protein were identified by database searches. The GutQ protein, as previously reported for the K1 KpsF protein (11), was 41% identical over 292 amino acids. Two homologs, ORF 328 from *E. coli* (EMBL accession no. U18997), which was 41% identical over 307 amino acids, and a hypothetical KpsF protein from *H. influenzae* (EMBL accession no. U32841), which was 50% identical over 308 amino acids, were also detected.

A hydropathy plot of the predicted amino acid sequence of the *E. coli* K5 KpsF suggests that it is a cytoplasmic protein with no domains of significant hydropathy (data not shown). KpsF and all three homologs share the features of a nucleotide binding domain, namely, a glycine-rich loop preceded by a hydrophobic beta-strand. This region is similar to the Walker A ATP-binding site; however, the spacing of the hydrophobic patch and conserved glycines is more in keeping with dinucleotide (NAD/FAD) binding motifs (67). The significance of this possible dinucleotide binding fold for the function of the KpsF protein is unclear.

The predicted amino acid sequence of KpsF was 70% homologous over a stretch of 69 amino acids to a number of glutamine-dependent amidotransferases (data not shown). These enzymes catalyze the transfer of an amino group from glutamine to fructose-6-P to form glucosamine-6-P, which is the first step in the hexosamine biosynthetic pathway required for the synthesis of extracytoplasmic polysaccharides (17). Glutamine-dependent amidotransferases can be divided into two functional domains. The N-terminal domain of 200 amino acids is postulated to be involved in amide transfer, whereas the remaining 400 amino acids are involved in sugar phosphate binding (17). The short stretch of homology between KpsF and this family of enzymes suggests that KpsF is unlikely to act as a functional glutamine-dependent amidotransferase enzyme. However, the homology between KpsF and these enzymes is located in the C-terminal domain and may reflect a sugar binding site.

It has been reported that insertion of a kanamycin resistance gene cassette into the *kpsF* gene of the K1 capsule gene cluster

resulted in small K1 bacteriophage plaques and, therefore, that the KpsF protein is involved in the transport of K1 polysaccharide (11). However, previous observations that bacteria with a cloned copy of the K5 capsule gene cluster lacking a functional *kpsF* gene still produced a capsule comparable to that of wild-type K5 strains appear to conflict with this role for the KpsF protein (7, 8, 43). The mapping of the region 1 promoter upstream of the *kpsF* gene for the K5 capsule gene cluster, together with the recent identification of the region 1 promoter from the K1 capsule gene cluster at the same location (13), suggests that the observed phenotype seen with the insertion mutant in the *kpsF* gene of the K1 antigen gene cluster is as a result of polarity on transcription of downstream region 1 genes.

To preclude the possibility that the lack of a detectable phenotype in *E. coli* strains harboring the K5 capsule gene cluster lacking a functional *kpsF* gene was due to complementation by the *gutQ* gene on the chromosome, plasmid pPC6, which contains the K5 capsule gene cluster minus just *kpsF* (7), was introduced into strain L32706 (Table 1), which has a mutation in the *gutQ* gene. The introduction of pPC6 resulted in a capsule indistinguishable from that of MS101 when analyzed by immunoelectron microscopy (data not shown), suggesting that GutQ was not acting in *trans* to complement the *kpsF* deletion. This does not preclude the possibility that ORF 328 complements the *kpsF* deletion.

The GutQ protein has been postulated to act as a regulator of expression of the glucitol operon (70). To identify whether the KpsF protein had a similar regulatory role on region 1 expression, plasmid pDS111, carrying a functional copy of the *kpsF* gene, was introduced into strain MS150 harboring plasmid pDSHcH, and β-galactosidase activity was measured. The presence of the *kpsF* gene in multicopy had no effect on transcription from the region 1 promoter at either 37 or 18°C (data not shown), indicating that KpsF itself does not regulate transcription of region 1. The lack of an obvious function for KpsF is in agreement with the recent findings of Cieslewicz and Vimr (13), who likewise could not assign a ready function to the KpsF protein of the K1 capsule gene cluster.

Conclusions. The *kpsF* gene has been identified as the first gene of region 1 of the K5 capsule gene cluster. It was found to be homologous to the *kpsF* gene reported in region 1 of the K1 antigen gene cluster (11), suggesting that this gene is maintained between different group II capsule gene clusters and that the KpsF protein plays a conserved role in the expression of group II polysaccharides. No obvious function could be assigned to the KpsF protein, and deletion of the *kpsF* gene had no detectable effect on K5 antigen expression. Region 1 was transcribed as a single transcriptional unit from a promoter 225 bp 5′ to the *kpsF* gene. The large transcript was processed to yield a smaller transcript of 1.3 kb specific for the *kpsS* gene which is at the 3′ end of the transcript. The processing of mRNA has been implicated in the differential expression of a number of bacterial genes (4, 5, 26). The major F1845 fimbrial subunit gene of *E. coli* is located downstream of the other assembly genes but is expressed at high levels from a stable 1.3-kb processed transcript (4). This processing is independent of the two major endoribonucleases RNaseIII and RNaseE (4). The lack of either an RNaseIII or RNaseE consensus sequence (5) 5′ to *kpsS* suggests that the processing of the *kpsS*-specific transcript is also independent of these two endoribonucleases. The generation of a separate *kpsS* transcript may enable differential expression of KpsS from the other region 1 gene products. Control of the level of the KpsS protein might enable regulation of capsular polysaccharide expression via a role for KpsS in the modification of the polysaccharide prior to export (52).

An intragenic Rho-dependent transcriptional terminator was identified within the *kpsF* gene. This terminator may permit transcriptional regulation of region 1 in response to physiological stress in a manner analogous to that suggested for other intragenic terminators (51). In this system, under conditions of physiological stress in which the RNA message is not translated efficiently, transcription of region 1 would cease at the terminator within the *kpsF* gene and reduce the level of expression of region 1. The observation that mutations in region 1 lead to reductions in membrane transferase activity (7, 8) means that the overall effect could be to reduce capsule expression under physiologically stressful conditions.

The transcription of region 1 was regulated by temperature, with no transcription at 18°C being detectable in Northern blots. This is in contrast to the promoter probe data, which showed that significant promoter activity was still taking place at 18°C. A likely explanation for this discrepancy is the copy number of the plasmid and the presence of multiple copies of the region 1 promoter. The regulation of region 1 transcription in response to temperature was not mediated by either the *hns*, *rimJ*, or *hha* gene products, all of which have been implicated in temperature-dependent regulation of gene expression in other systems. Likewise, expression of region 1 was not mediated by RfaH, which is required for expression of region 3 at 37°C (64) and the expression of *rfa* genes (49).

Two AT-rich sequences located at the same relative position upstream of the region 1 and region 3 transcription initiation sites were conserved. These conserved sites could potentially mediate the coordinated regulation of regions 1 and 3 and may define operator sites at which a regulatory protein binds. The observation that two IHF binding site consensus sequences were located upstream from the transcription start point and that mutations in the *himA* and *himD* genes reduced the level of expression of region 1 indicates a role for IHF in mediating expression of region 1. Although the binding of IHF is not essential for region 1 expression, it may facilitate the action of other regulatory proteins. Experiments are currently under way to identify the regulator(s) of region 1 transcription.

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REFERENCES

1. **Adhya, S., and M. Gottesman.** 1978. Control of transcription termination. Annu. Rev. Biochem. **47:**967–996.
2. **Alifano, P., F. Rivellini, D. Limauro, C. B. Bruni, and M. S. Carlomagno.** 1991. A consensus motif common to all rho-dependent prokaryotic transcription terminators. Cell **64:**553–563.
3. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:**3963–3965.
4. **Bilge, S. S., J. M. Apostol, M. A. Aldape, and S. L. Moseley.** 1993. mRNA processing independent of Rnase III and Rnase E in the expression of the F1845 fimbrial adhesin of *E. coli*. Proc. Natl. Acad. Sci. USA **90:**1455–1459.
5. **Bilge, S. S., J. M. Apostol, K. J. Fullner, and S. L. Moseley.** 1993. Transcriptional organization of the F1845 fimbrial adhesin determinant of *Escherichia coli*. Mol. Microbiol. **7:**993–1006.
6. **Boulnois, G. J., I. S. Roberts, R. Hodge, K. R. Hardy, K. B. Jann, and K. N. Timmis.** 1987. Analysis of the K1 capsule biosynthesis genes of *Escherichia coli*: definition of three functional regions for capsule production. Mol. Gen. Genet. **208:**242–246.
7. **Bronner, D., V. Sieberth, C. Pazzani, I. S. Roberts, G. J. Boulnois, B. Jann, and K. Jann.** 1993. Expression of the capsular K5 polysaccharide of *Escherichia coli*: Biochemical and electron microscopic analyses of mutants with

defects in region 1 of the K5 gene cluster. J. Bacteriol. **175:**5984–5992.
8. **Bronner, D., V. Sieberth, C. Pazzani, A. Smith, G. Boulnois, I. Roberts, B. Jann, and K. Jann.** 1993. Synthesis of the K5 (groupII) capsular polysaccharide in transport-deficient recombinant *Escherichia coli*. FEMS Microbiol. Lett. **113:**279–284.
9. **Brosius, J., T. J. Dull, D. D. Sleeter, and H. F. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. **148:**107–127.
10. **Brosius, J., and A. Holy.** 1984. Regulation of ribosomal RNA promoters with a synthetic *lac* operator. Proc. Natl. Acad. Sci. USA **81:**6929–6933.
11. **Cieslewicz, M. J., S. M. Steenbergen, and E. R. Vimr.** 1993. Cloning, sequencing, expression, and complementation analysis of the *Escherichia coli* K1 *kps* region 1 gene, *kpsE*, and identification of an upstream open reading frame encoding a protein with homology to GutQ. J. Bacteriol. **175:**8018–8023.
12. **Cieslewicz, M. J., and E. R. Vimr.** 1994. Identification of *kpsF* as the first gene of region 1 in the *kps* cluster for polysialic acid capsule synthesis in *Escherichia coli* K1, abstr. D-232, p. 137. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology. 1994 American Society for Microbiology, Washington, D.C.
13. **Cieslewicz, M. J., and E. R. Vimr.** 1996. Thermoregulation of *kpsF*, the first region 1 gene in the *kps* locus for polysialic acid biosynthesis in *Escherichia coli* K1. J. Bacteriol. **178:**3212–3220.
14. **De la Cruz, F., M. Carmona, and A. Juárez.** 1992. The Hha protein from *Escherichia coli* is highly homologous to the YmoA protein from *Yersinia enterocolitica*. Mol. Microbiol. **6:**3451–3452.
15. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programmes for the VAX. Nucleic Acids Res. **12:**387–395.
16. **Dougan, G., and D. J. Sherratt.** 1977. The use of transposon I as a probe of the structure and function of plasmid ColE1. Mol. Gen. Genet. **151:**151–160.
17. **Fernandez-Herrero, L. A., M.-A. Badet-Denisot, and J. Berenguer.** 1995. *glmS* of *Thermus thermophilus* HB8: an essential gene for cell-wall synthesis identified immediately upstream of the S-layer gene. Mol. Microbiol. **17:**1–12.
18. **Finke, A., B. Jann, and K. Jann.** 1990. CMP-KDO-synthetase activity in *Escherichia coli* expressing the capsular polysaccharides. FEMS Microbiol. Lett. **69:**129–134.
19. **Freundlich, M., N. Ramani, E. Mathew, A. Sirko, and P. Tsui.** 1992. The role of integration host factor in gene expression in *Escherichia coli*. Mol. Microbiol. **6:**2557–2563.
20. **Frosch, M., U. Edwards, K. Bousset, B. Kraube, and C. Weisgerber.** 1991. Evidence for a common molecular origin of the capsule gene loci in gram-negative bacteria expressing group II capsular polysaccharides. Mol. Microbiol. **5:**1251–1263.
21. **Godessart, N., F. J. Muñoa, M. Regue, and A. Juárez.** 1988. Chromosomal mutations that increase the production of a plasmid-encoded haemolysin in *Escherichia coli*. J. Gen. Microbiol. **134:**2779–2787.
22. **Hill, P. J., C. E. D. Rees, M. K. Winson, and G. S. A. B. Stewart.** 1993. The application of *lux* genes. Biotechnol. Appl. Biochem. **17:**3–14.
23. **Hulton, C. S. J., A. Seirafi, J. C. D. Hinton, J. M. Sidebotham, L. Waddell, G. D. Pavitt, T. Owen-Hughes, A. Spassky, H. Buc, and C. F. Higgins.** 1990. Histone-like protein H1 (H-NS), DNA supercoiling, and gene expression in bacteria. Cell **63:**631–642.
24. **Jann, B., and K. Jann.** 1990. Structure and biosynthesis of the capsular antigens of *Escherichia coli*. Curr. Top. Microbiol. Immun. **150:**19–42.
25. **Jann, K., and B. Jann.** 1992. Capsules of *Escherichia coli*, expression and biological significance. Can. J. Microbiol. **38:**705–710.
26. **Klug, G.** 1993. The role of mRNA degradation in the regulated expression of bacterial photosynthesis genes. Mol. Microbiol. **9:**1–7.
27. **Kroll, J. S., B. Loynds, L. N. Brophy, and E. R. Moxon.** 1990. The *bex* locus of encapsulated *Haemophilus influenzae*: a chromosomal region involved in capsule export. Mol. Microbiol. **4:**1853–1862.
28. **Kröncke, K., G. J. Boulnois, I. S. Roberts, D. Bitter-Suermann, J. R. Golecki, B. Jann, and K. Jann.** 1990. Expression of the *Escherichia coli* K5 capsular antigen: immunoelectron microscopic and biochemical studies with recombinant *E. coli*. J. Bacteriol. **172:**1085–1091.
29. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Mead, D. A., E. Szczesna-Skorupa, and B. Kemper.** 1986. Single-stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Prot. Eng. **1:**67–74.
32. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridisation probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
33. **Mikulskis, A. V., and G. R. Cornelis.** 1994. A new class of proteins regulating gene expression in enterobacteria. Mol. Microbiol. **11:**77–86.
34. **Miller, J. H.** 1972. (ed.), Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Morse, D. E., and M. Guertin.** 1972. Amber *suA* mutations which relieve polarity. J. Mol. Biol. **63:**605–608.
36. **Murray, N. E., W. J. Brammer, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of *in vitro* recombinants. Mol. Gen. Genet. **150:**53.
37. **Newbury, S. F., N. H. Smith, E. C. Robinson, I. D. Hiles, and C. F. Higgins.** 1987. Stabilization of translationally active mRNA by prokaryotic REP sequences. Cell **48:**297–310.
38. **Ørskov, F.** 1984. The genus *Escherichia*, p. 420–423. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore.
39. **Ørskov, F., and I. Ørskov.** 1992. *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. **38:**699–704.
40. **Pavelka, M. S., Jr., S. F. Hayes, and R. P. Silver.** 1994. Characterization of KpsT, the ATP-binding component of the ABC-transporter involved with the export of capsular polysialic acid in *Escherichia coli* K1. J. Biol. Chem. **269:**20149–20158.
41. **Pavelka, M. S., Jr., L. F. Wright, and R. P. Silver.** 1991. Identification of two genes, *kpsM* and *kpsT*, in region 3 of the polysialic acid gene cluster of *Escherichia coli* K1. J. Bacteriol. **173:**4603–4610.
42. **Pazzani, C.** 1992. Molecular and genetic analysis of the *Escherichia coli* K5 capsule gene cluster. PhD thesis. University of Leicester, Leicester, United Kingdom.
43. **Pazzani, C., C. Rosenow, G. J. Boulnois, D. Bronner, K. Jann, and I. S. Roberts.** 1993. Molecular analysis of region 1 of the *Escherichia coli* K5 antigen gene cluster: a region encoding proteins involved in cell surface expression of capsular polysaccharide. J. Bacteriol. **175:**5978–5983.
44. **Peabody, D. S., C. L. Andrews, K. W. Escudero, J. H. Devine, T. O. Baldwin, and D. G. Bear.** 1989. A plasmid vector and quantitative techniques for the study of transcription termination in *Escherichia coli*. Gene **75:**289–296.
45. **Pearce, R., and I. S. Roberts.** 1995. Cloning and analysis of gene clusters for production of the *Escherichia coli* K10 and K54 antigens: identification of a new group of *serA*-linked capsule gene clusters. J. Bacteriol. **177:**3992–3997.
46. **Petit, C., G. P. Rigg, C. Pazzani, A. Smith, V. Sieberth, M. Stevens, G. Boulnois, K. Jann, and I. S. Roberts.** 1995. Region 2 of the *Escherichia coli* K5 capsule gene cluster encoding proteins for the biosynthesis of the K5 polysaccharide. Mol. Microbiol. **17:**611–620.
47. **Pigeon, R. P., and R. P. Silver.** 1994. Topological and mutational analysis of KpsM, the hydrophobic component of the ABC-transporter involved in the export of polysialic acid in *Escherichia coli* K1. Mol. Microbiol. **14:**871–881.
48. **Porter, M. E., and C. J. Dorman.** 1994. A role for H-NS in thermo-osmotic regulation of virulence gene expression in *Shigella flexneri*. J. Bacteriol. **176:**4187–4191.
49. **Pradel, E., and C. A. Schnaitmann.** 1991. Effect of *rfaH* (*sfrB*) and temperature on expression of *rfa* genes of *Escherichia coli* K-12. J. Bacteriol. **173:**6428–6431.
50. **Reizer, J., A. Reizer, and M. H. Saier, Jr.** 1992. A new subfamily of bacterial ABC-type transport systems catalyzing export of drugs and carbohydrates. Prot. Sci. **1:**1326–1332.
51. **Richardson, J. P.** 1991. Preventing the synthesis of unused transcripts by Rho factor. Cell **64:**1047–1049.
52. **Roberts, I. S.** 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. **50:**285–315.
53. **Roberts, I. S., R. Mountford, N. High, D. Bitter-Suermann, K. Jann, K. Timmis, and G. J. Boulnois.** 1986. Molecular cloning and analysis of genes for production of K5, K7, K12, and K92 capsular polysaccharides in *Escherichia coli*. J. Bacteriol. **168:**1228–1233.
54. **Roberts, I. S., R. Mountford, R. Hodge, K. Jann, and G. J. Boulnois.** 1988. Common organization for gene clusters for production of different capsular polysaccharides (K antigens) in *Escherichia coli*. J. Bacteriol. **170:**1305–1310.
55. **Rosenow, C., F. Esumeh, I. S. Roberts, and K. Jann.** 1995. Characterization and localisation of the KpsE protein of *Escherichia coli* K5, which is involved in polysaccharide export. J. Bacteriol. **177:**1137–1143.
56. **Rosenow, C., I. S. Roberts, and K. Jann.** 1995. Isolation from recombinant *Escherichia coli* and characterisation of CMP-Kdo synthetase, involved in the expression of the capsular K5 polysaccharide (K-CKS). FEMS Microbiol. Lett. **125:**159–164.
57. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
58. **Schneider, K., and C. F. Beck.** 1986. Promoter-probe vectors for the analysis of divergently arranged promoters. Gene **42:**37–48.
59. **Silver, R. P.** 1994. Genetic analysis of the *E. coli* K1 capsule gene cluster, p. 313–327. *In* V. L. Miller, J. B. Kaper, D. A. Portnoy, and R. R. Isberg, (ed.), Molecular genetics of bacterial pathogenesis. American Society for Microbiology, Washington, D.C.
60. **Silver, R. P., C. W. Finn, W. F. Vann, W. Aaronson, R. Schneerson, P. J. Kretschmer, and C. F. Garon.** 1981. Molecular cloning of the K1 capsular polysaccharide genes of *E. coli*. Nature (London) **289:**696–698.
61. **Silver, R. P., W. F. Vann, and W. Aaronson.** 1984. Genetic and molecular analyses of *Escherichia coli* K1 antigen genes. J. Bacteriol. **157:**568–575.
62. **Smith, A. N., G. J. Boulnois, and I. S. Roberts.** 1990. Molecular analysis of the *Escherichia coli* K5 *kps* locus: identification and characterization of an

inner-membrane capsular polysaccharide transport system. Mol. Microbiol. **4:**1863–1869.
63. **Steenbergen, S. M., T. J. Wrona, and E. R. Vimr.** 1992. Functional analysis of the sialyltransferase complexes in *Escherichia coli* K1 and K92. J. Bacteriol. **174:**1099–1108.
64. **Stevens, M. P., P. Hanfling, B. Jann, K. Jann, and I. S. Roberts.** 1994. Regulation of *Escherichia coli* K5 capsular polysaccharide expression: evidence for involvement of RfaH in the expression of group II capsules. FEMS Microbiol. Lett. **124:**93–98.
65. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
66. **Vimr, E. R., W. Aaronson, and R. P. Silver.** 1989. Genetic analysis of chromosomal mutations in the polysialic acid gene cluster of *Escherichia coli* K1. J. Bacteriol. **171:**1106–1117.
67. **White-Ziegler, C. A., L. B. Blyn, B. A. Braaten, and D. A. Low.** 1990. Identification of an *Escherichia coli* locus involved in thermoregulation of the *pap* operon. J. Bacteriol. **172:**1775–1782.
68. **Wierenga, R. K., J. Drenth, and G. E. Schulz.** 1986. Comparison of the three-dimensional protein and nucleotide structure of the FAD-binding domain of *p*-hydroxy-benzoate hydroxylase with the FAD-as well as NADPH-binding domains of glutathione reductase. J. Mol. Biol. **167:**725–739.
69. **Wunder, D. E., W. Aaronson, S. F. Hayes, J. M. Bliss, and R. P. Silver.** 1994. Nucleotide sequence and mutational analysis of the gene encoding KpsD, a periplasmic protein involved in transport of polysialic acid in *Escherichia coli* K1. J. Bacteriol. **176:**4025–4033.
70. **Yamada, M., Y. Yamada, and M. H. Saier, Jr.** 1990. Nucleotide sequence and expression of the *gutQ* gene within the glucitol operon. J. DNA Seq. Map. **1:**141–145.
71. **Yannisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.