Molecular Characterization of the Type 3 (MR/K) Fimbriae of Klebsiella pneumoniae

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The expression of type 3 (MR/K) fimbriae by Klebsiella pneumoniae requires the production of at least four polypeptides with molecular masses of 20.5, 25, 34, and 78 kilodaltons. The genes encoding these polypeptides are located on a gene cluster 5,500 base pairs in length. The nucleotide sequence of the major fimbrial structural gene was determined, and its transcription was shown to initiate in vitro 157 base pairs upstream of the translational start site. The predicted amino acid sequence for the fimbrial subunit comprised 202 residues, including a signal peptide of 22 amino acids. Despite similarities in the organization of the gene cluster, the nucleotide and the amino acid sequence of the major fimbrial subunit revealed little agreement with other fimbrial subunit sequences.

Type 3 fimbriae were originally observed on strains of Klebsiella and were described as mediating a mannosereistant Klebsiella-like (MR/K) hemagglutination (7). Similar fimbrial appendages have since been found to be produced by many members of the Enterobacteriaceae (23, 24). These long, thin fimbriae (4 to 5 nm by 0.5 to 2 μm) are associated with hemagglutination of erythrocytes which have been treated with tannic acid, and hemagglutination occurs in the presence or absence of D-mannose. In addition, adherence mediated by type 3 fimbriae to plant roots, fungal hyphae, and glass surfaces has been documented (7, 13). More recently, Mobley and co-workers (21) have suggested that the MR/K hemagglutinin of Providencia stuartii facilitates adherence of urinary isolates to catheter surfaces. Serologic analyses indicate little antigenic cross-reactivity between type 3 and type 1 fimbriae of Klebsiella spp. (13, 24) or between the mannosereistant hemagglutinin of Proteus species (24). Purification of the type 3 fimbriae has allowed more detailed biochemical characterization of these appendages and determination of the major fimbrial subunit structure (4, 13). Purified type 3 fimbriae, isolated from different bacteria, possess molecular weights ranging from 18,500 to 21,500 (13, 25). In a detailed study of the type 3 fimbriae from different species of Klebsiella, Old and co-workers (25) demonstrated that the fimbria behaved like a serologically homogeneous group with minor variability in amino acid composition of the fimbrial subunit.

We recently described the molecular cloning of a gene cluster encoding phenotypic expression of K. pneumoniae type 3 fimbriae (11). We describe here the genetic organization of this gene cluster and the nucleotide sequence of the gene encoding the major fimbrial subunit.

MATERIALS AND METHODS

Bacterial strains and media. K. pneumoniae IA565 is a clinical isolate obtained from the clinical microbiology laboratory of the University of Iowa Hospitals and Clinics, Iowa City. Escherichia coli HB101 (16) and the nonfimbriated minicell-producing strain E. coli ORN103 (28) were used in all transformation experiments. All bacterial strains were grown in Luria broth or on Luria agar supplemented with the appropriate antibiotics (16).

Restriction endonuclease digestion of plasmid DNA, agarose gel electrophoresis, and construction of subclones. Restriction endonucleases were purchased from commercial sources and were used according to the manufacturers’ instructions. Restriction fragments were analyzed by agarose or acrylamide gel electrophoresis (16). Subcloning was performed by separation and subsequent ligation of the appropriate DNA fragments in low-gelling-temperature agarose (35). The construction of the recombinant plasmid pFK12, encoding phenotypic expression of type 3 fimbriae, has been described in detail elsewhere (11).

Purification of fimbriae and determination of the N-terminal amino acid sequence. Fimbriae were purified from K. pneumoniae IA565 as previously described (11). Subsequently, the first five N-terminal amino acids of the mature fimbrial subunit were determined by using a Beckman Applied Biosystems model 470A “Gas-Phase” sequencer.

Analysis of plasmid-encoded polypeptides. Minicells from E. coli ORN103 transformants were prepared as described elsewhere (22). Plasmid-encoded polypeptides were labeled with either [35S]methionine or a [14C]-labeled amino acid mixture. Minicells were lysed by boiling in a sodium dodecyl sulfate (SDS) electrophoresis buffer and analyzed by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE; 12% acrylamide, 0.3% bisacrylamide) by the method of Laemmli (14).

Hemagglutination test and competitive ELISA. A 25-μl portion of a 3% (vol/vol) suspension of tanned erythrocytes was mixed with an equal volume of bacterial suspension in phosphate-buffered saline (PBS) with or without 4% D-mannose. Erythrocytes (human, group A) were tanned by incubating equal volumes of 0.01% (wt/vol) tannic acid solution in saline and a 3% erythrocyte suspension in PBS. The erythrocytes were subsequently washed twice in PBS.

A competitive enzyme-linked immunosorbent assay (ELISA) (34) was used to quantitate the amount of type 3 fimbrial protein expressed by transformants. An appropriate dilution of a monoclonal antibody preparation raised against purified type 3 fimbriae (11) was incubated (37°C, 60 min) with equal volumes of serial twofold dilutions of bacterial suspension. Before dilution, all bacterial suspensions contained approximately 108 cells per ml. Following incubation of the bacterial-antibody suspension, 100 μl was added to plates holding purified type 3 fimbriae as the
solid-phase antigen. The reaction was developed by using a biotin-streptavidin-peroxidase system, and all tests were performed in duplicate.

**Nucleotide sequence analysis.** The method of Maxam and Gilbert (18) was used to determine the nucleotide sequence of the gene encoding the major fimbrial subunit as well as the immediate flanking sequences. Both strands of DNA were sequenced, and in addition, overlapping fragments were sequenced to avoid omission of short DNA sequences (see Fig. 3).

**Northern (RNA) blot analyses.** The phenol extraction method of Aiba et al. (3) was used to prepare RNA from *K. pneumoniae* IA565 and *E. coli* HB101. Cells were grown at 37°C without shaking for 3, 6, and 16 h. The incubation medium contained 50 mM Tris, 50 mM Na2HPO4, 10 mM K2HPO4, 20 mM NH4Cl, and 10 mM NaCl, pH 7.8, to which 1% glycerin and 0.3% Casamino Acids (Difco) were added. This medium facilitates the expression of type 3 fimbriae by *K. pneumoniae* IA565.

Northern blotting was carried out as described previously (16) with the use of formaldehyde-agarose gel electrophoresis and GeneScreen Plus membrane (New England Nuclear). An RNA molecular weight standard was used to determine the size of transcripts. The DNA probe was derived from a *TaqI* digest of the deletion derivative pFK40 and was composed almost entirely of nucleotides from within the type 3 fimbrial subunit gene (see Fig. 3).

**S1 mapping.** A DNA restriction fragment prepared from pFK40 was 5'-end labeled with polynucleotide kinase and strand-separated, and the complementary strand was used in hybridizations with RNA prepared from *K. pneumoniae* IA565. The location of the DNA strand is shown in Fig. 3. The double-stranded hybrids were treated with 300 U of S1 nuclease, and S1 mapping was performed as described previously (3).

**RESULTS**

Identification and localization of polypeptides encoded by the *K. pneumoniae* type 3 fimbrial gene cluster. Specific deletions of pFK12 were constructed (Fig. 1) and used to transform *E. coli* ORN103. Subsequent analysis of the plasmid-encoded polypeptides detected four gene products with molecular masses of 20.5, 25, 34, and 83 kilodaltons (kDa) that were implicated in phenotypic expression of type 3 fimbriae. After labeling with [35S]methionine, an 83-kDa polypeptide was expressed by minicells containing pFK14, pFK22, pFK25, and pFK28 (Fig. 2a). Minicells containing pFK21 expressed a possible truncated form of this polypeptide with a molecular mass of 30 kDa. The 34-kDa polypep-
tide (Fig. 2a), comigrating on SDS gels with a minor component of purified fimbriae (Fig. 2c), was expressed by minicells containing the deletion derivatives pFK22, pFK24, and pFK25 (Fig. 2a). Also, minicell lysates of pFK28 and pFK31 contained a 25-kDa polypeptide. This polypeptide was absent in lysates containing pFK30 and pFK34 and was not seen in other preparations due to the chloramphenicol acetyltransferase produced by the cloning vector. Minicells containing pFK12 and pFK40, after labeling with a 3H-acetate mixture, expressed a polypeptide with an electrophoretic mobility similar to that of the major type 3 fimbrial subunit. This protein was absent in transformants of pFK22 and pFK25 (Fig. 2b). When only 35S-labeled methionine was used, the major fimbrial subunit was not labeled, and this result is consistent with the absence of methionine residues, as predicted from the nucleotide sequence (see Fig. 4).

To determine whether the 34-kDa polypeptide was required for fimbrial expression, double transformants from compatible cloning vectors were constructed and tested for MR/K hemagglutination and their ability to react with the monoclonal antibody raised against type 3 fimbriae. Transformants containing both pFK28 and pFK20 exhibited a phenotype indistinguishable from that of pFK12, Fim+ MR/K+. Transformants containing pFK28 and pFK26, however, which did not contain the entire gene encoding the 34-kDa polypeptide, did not hemagglutinate despite an ability to react with the monoclonal antibody (Table 1). Transformants containing pFK28 were also MR/K- and did not react with the monoclonal antibody. Interestingly, it was demonstrated that pFK14 transformants of E. coli HB101 appear to express type 3 fimbriae in lower amounts than transformants containing pFK12 (Table 1). The recombinant molecule pFK22, containing a deletion in the fimbrial structural gene, produced transformants which were Fim- MR/K-. Complementation of this transformant with pFK40 did not fully restore the Fim+ MR/K+ phenotype (Table 1).

Nucleotide and amino acid sequences. The DNA-sequencing strategy for the fimbrial gene is outlined in Fig. 3, and the results of the nucleotide sequence analysis are shown in Fig. 4. The coding sequences of the fimbrial gene consisted of 606 base pairs (bp). The predicted amino acid sequence consisted of a signal peptide of 22 amino acids, and the mature subunit protein, of 180 amino acids, had a calculated molecular weight of 18,477. The predicted cleavage point between two alanine residues was verified by amino acid sequence analysis of the first five N-terminal residues of fimbriae purified from K. pneumoniae IA565. The nucleotide sequence upstream of the fimbrial structural gene contains an open reading frame of 687 nucleotides, with a possible initiation codon at position 30 and a termination codon at position 714. Two inverted repeat sequences of 10 and 9 bp were identified; one of each of the repeat units

TABLE 1. Hemagglutination and competitive ELISA showing the phenotypic expression of the MR/K hemagglutinin and the type 3 fimbrial filament in E. coli HB101 transformed with various deletion derivatives of pFK14

<table>
<thead>
<tr>
<th>Recombinant plasmid in E. coli HB101</th>
<th>MR/K hemagglutinationa</th>
<th>ELISA inhibitionb (10⁸ bacteria/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFK14</td>
<td>++</td>
<td>12</td>
</tr>
<tr>
<td>pFK12</td>
<td>+++</td>
<td>3</td>
</tr>
<tr>
<td>pFK22</td>
<td>−</td>
<td>&gt;100</td>
</tr>
<tr>
<td>pFK28</td>
<td>−</td>
<td>&gt;100</td>
</tr>
<tr>
<td>pFK22 + pFK40</td>
<td>+</td>
<td>50</td>
</tr>
<tr>
<td>pFK28 + pFK20</td>
<td>++</td>
<td>3</td>
</tr>
<tr>
<td>pFK29 + pFK26</td>
<td>−</td>
<td>37</td>
</tr>
</tbody>
</table>

a−, No hemagglutination after shaking for 20 min; +, hemagglutination after shaking for 20 min; ++++, hemagglutination after shaking for 5 min; ++++, immediate hemagglutination.

b Inhibition is stated as the bacterial concentration (10⁸ bacteria per ml) required to decrease the reaction of the monoclonal antibody with solid-phase antigen by 50%. 
was located upstream of the fimbrial transcription initiation site (Fig. 4).

Transcription of the fimbrial gene. Semiquantitative determination of the levels of fimbrial gene transcripts by Northern blot analysis indicated that transcription levels increased through the late logarithmic phase of cell growth (Fig. 5). The DNA fragment protected from S1 nuclease digestion indicated that the transcription start site was at position 573 (Fig. 6a) which is 156 bp upstream from the initiation codon. By using RNA derived from cells transformed with pFK12, however, transcription initiation was placed at position 502 (Fig. 6b). The length of the transcript in K. pneumoniae was approximately 750 bases. Two sites with dyad symmetry and the potential to form a stem-loop structure were located downstream of the fimbrial structural gene at positions 1336 and 1489 (Fig. 4).

DISCUSSION

The type 3 fimbrial gene cluster of K. pneumoniae has recently been cloned, and these fimbriae were expressed in an Escherichia coli host (11). In the present study we analyzed the organization of the gene cluster and the sequence of the major type 3 fimbrial subunit.

The detection of four polypeptides that are necessary for the expression of hemagglutinating type 3 fimbriae was done by minicell analysis (Fig. 2). A polypeptide comigrating with the major fimbrial subunit on SDS-PAGE was expressed by minicell lysates containing pFK40 and not by those containing pFK22. Therefore, the major fimbrial subunit gene is located on the 1.1-kb insert of pFK40 (Fig. 1). A 25-kDa polypeptide was present in cells transformed with pFK28 and pFK31 but absent in lysates of pFK30 and pFK34 transformants. In transformants containing pACYC184 as the cloning vehicle (Fig. 2a), this polypeptide was masked by the gene product of the chloramphenicol resistance determinant. The gene encoding the 25-kDa protein was located to the left of the SphI site on pFK14 (Fig. 1). An 83-kDa protein was visible in minicell lysates containing pFK22, pFK25, and pFK28 but not in the remaining preparations. Therefore, this determinant is predicted to be located to the right of the SmaI site of pFK14. A polypeptide with an electrophoretic mobility identical to that of a minor component present in hemagglutinating type 3 fimbriae was present in transformants of pFK24 but not in those of pFK21 and pFK28. This indicates that the gene encoding this polypeptide is located to the right of the PstI site of pFK14 and may contain the EcoRI site (Fig. 1).

An additional minor component of hemagglutinating type 3 fimbriae with a molecular weight of 40,000 (Fig. 2c) was not detected in the minicell preparations. This protein could be removed from fimbrial preparations by column chromatography with a Sepharose 4B CL column without loss of adhesive function (data not shown). This suggest that this protein is not a necessary structural or functional component of type 3 fimbriae.

The requirement for the 20.5- and 34-kDa gene products described above for the phenotypic expression of hemagglutinating type 3 fimbriae is indicated by the results shown in Table 1. pFK22 transformants possessing the entire gene cluster except an intact fimbrial structural gene did not hemagglutinate or react with antifimbrial antibodies. Complementation with pFK40 to restore the Fim” MR/K” phenotype was possible, but fimbrial expression was significantly lower than that by the parental strain. This may suggest a possible cis-acting function of the fimbrial gene. Although a mutant which only lacked the ability to encode the 25-kDa polypeptide was not constructed, this gene product is most likely necessary for fimbrial expression because of the position of its determinant in the gene cluster. This determinant is flanked by two genes which can be shown to affect fimbrial expression.

To investigate whether the type 3 fimbrial structural gene constituted one determinant of an operon, with a second gene located immediately downstream, a Northern blot analysis was performed. However, the size of the mRNA (750 bases, Fig. 5) indicates that this is unlikely unless the mRNA of the second gene is less stable. In addition, two regions of dyad symmetry existed downstream from the predicted translation stop codon that may be involved in transcription termination of the fimbrial subunit gene. The first area of symmetry, located between positions 1336 and 1369, has the potential of forming a sizable hairpin structure which is not rich in GC content. This region is similar to those seen in some rho-dependent termination structures (2).
GAGTCGCTTGG TCATGAGCGGAG AAAAGATGAAAG TATATCTTGTGG AGGATGAGTTC TGCCUCACGAGG 72
AGCTCTCGGCG TTATACACGCCT ATAGCCGATAGG AGATCGTGCGCA GCTTCGACGAGG GCTTTGAGGTCG 144
TGAATTCTCC AGCACAAGAAG TGCGACACCTT TCCTGACTATCA ATATGCCGATTG CTCACACGCTTC 216
TGCTGCACAGA ACATCGACAGT TGCCATCAAAC CTTTATTACTCT TTTACCATCGGT GAAAGAACATG 288
CGGCTGACCTT GCATGCGGAGG CTTCTGACTACA CACGTAATCGCA GCAGTACTGCA ATATTTATATG 504
TGCTGCAAAGG TGACCCCGCCT GGGGCGACGCA AATAACCGCTTT TGGCATCGCGG TGAACATTATCTG 576
GCCGCCCAATG AAAGATGCGAT TCCTGCTATACC GCCGGCGCTATC TTCGATCAGCAG CCAGTATTCATG 648
GCATGCGAAATC TGCTGACTTT TGGCAGTATGTA CGTCTGCCCTT GCACGCGCAGT GCTACGAGTGA 720
GAAATGCGAAATG AAAAGGTTCTT CTCTCTCTGCA ATGCGCAGCGG TTTTGTGTGAT GCTTCGCGAAT 792
GCTGCGTACATG AACGTCGAGGG GCATGCGGATTA TCTTTCCCTGAAA GTTACCGAGTA TCTGATCTGTT 864
AAAT ATN VGG GGG GGV VFGF VGT VDT VST CV -1,+1 20 1 353
TCCGTAAGGGC AGGGGCAGGAT GGGCGCAGTTAT CTGTCCACAGG GCATTAACGAGG GTAAAGTCCGC 936
S V N G Q S D S A N VY L S P V T L T E V K A A C l a s 1 40 2
GCCGCGCATACC TATCTGAAACG AAATCTTCTC GACGATGTTTCT TCTGCTGCTCTGG TGTGAGCGGAG 1080
AAACGAGATAT GCACCCGCACT CTCTGTCGCGG GCCAAGCCGAAA 1080
CAGCAGGCCTAC CTCTGCTACACC GAGGCCCGCGCC GCCGAGAATAG CAGCTGTTTCTC TCCACCGATAC 1152
Q Q Q G Y L A N T E A A A A A G A Q N Q I L V L S T D N 100
GCCACCGGCTG ACCAAGAAATAC ATCCCGCCGGC AGCCACCCGCT GCCATGCTGCTC 1224
A T A L T N K I I P G D S T Q P K A G D A S A 160
GTCAAGGAGGCC GGGCGCTTAC TACTACGCGGC TATGAGGCCAC ACCGGCAGGCGG GTACCACCGGTT 1296
V Q D G A R F T Y Y V G Y A T S T P T T V T G T 160
TCTTAAACG GACGGCAGACT GAAAATATTTAT CAGAATAATGG CAGAAGATGAAA GCCGCTGCCGC 1368
V V N S Y A T T E I Y Q 180
CGGCCGCGTTCA TACCCGCTGAC TGGAAATCGCC AGGGCGCGCCG CTCTGCTCCGC CCACTCAGCTC 1440
TGGCCGCGTTT GCAGGCGGTCG AATTCGAGCT TAGACAAATTAC AAAGCCGGCTC GCCGCGAGTT 1512
TTTTTATTCTG AGCTGACAGTA AGCGGTTGGCGG CTTTTGACTACA TTTCGAATGGCAC AGGTCAGCATG 1584
CCAAATATATAT TTGTTAACG 1605
FIG. 4. Nucleotide sequence of the K. pneumoniae IA565 type 3 fimbrial structural gene and the predicted amino acid sequence of the encoded polypeptide. The nucleotides are numbered on the right, and the first amino acid of the mature fimbrial subunit is labeled +1. The site of cleavage of the processed fimbrial subunit is marked with an arrow, and the termination codon is indicated by the asterisk. The Shine-Dalgalno consensus sequence is labeled SD. The putative Klebsiella transcription initiation site, as determined in vivo, is marked with a triangle. The -10 and -35 regions are indicated. Transcription in the recombinant plasmid pFK12, which only contains the Klebsiella-derived nucleotide sequences to the right of the indicated Sau3A site, may initiate at a fusion promotor that is shown with open diamond (Fig. 6b). The two series of inverted repeat sequences are underlined and labeled 1 and 2. The regions of dyad symmetry positioned downstream from the termination codon are labeled a and b. The initiation and termination codons of the open reading frame upstream of the fimbrial structural gene are marked by arrowheads.

The second area of symmetry, stretching from position 1489 to 1503, is very similar in sequence and potential secondary structure to rho-independent termination sites (2, 29). This region possesses a small hairpin loop with a GC-rich stem and is flanked by a string of thymines on the 3' side and an adenine-rich area 5' to this region. The Northern blot analysis further revealed that relatively large proportions of mRNA specific for type 3 fimbriae were only present in the stationary phase of growth (Fig. 5), and therefore optimal expression of this gene occurs late in the growth phase.

E. coli transformants containing pFK28 are Fin- MR/K-, and complementation with pFK26 caused a low level of surface fimbrial expression (Table 1) but no hemagglutinating activity. Complementation of pFK28 with pFK20 fully restored the Fin+ MR/K+ phenotype. Such results do not exclude a possible cis-activity of the fimbrial structural gene, since pFK28 lacks only the transcription start site but contains the entire structural gene of the fimbrial subunit. Furthermore, these results suggest that fimbrial filaments can be expressed in the absence of the 34-kDa polypeptide.
The presence of this fimbrial component, however, appears to be necessary for the expression of hemagglutinating activity. A similar distinction between the fimbrial structural gene and that of the adhesive moiety has been reported for *E. coli* (20) and *K. pneumoniae* (5) type 1 fimbriae as well as the pap pili (15). Furthermore, Maurer and Orndorff (17) have reported that the gene product determining receptor specificity of the *E. coli* type 1 fimbriae is located at the opposite end of the gene cluster from the fimbrial structural gene. An analogous organization has been reported for the pap pili (15).

The DNA sequence analysis of the fimbrial structural gene revealed an open reading frame of 606 bp (Fig. 4). The initiation codon was preceded by the Shine-Dalgarno consensus sequence AGGA at position –8. Transcription in vivo was found to initiate 157 bp upstream of the translational start site. The putative –10 region had only limited sequence agreement with the consensus sequences in *E. coli* promoters (31). The –35 region had a sequence agreement of 75% with the equivalent *lac* promoter sequence (6). Furthermore, an AT-rich sequence in the –50 region, frequently found in *E. coli* promoters (31), was present. Poor recognition of the *Klebsiella* fimbrial gene promoter by *E. coli* is supported by the result that transcription of the fimbrial structural gene in pFK12 may be due to a hybrid promoter containing, in part, *Klebsiella*-derived sequences and, in addition, vector-derived sequences of the tetracycline resistance determinant of pACYC184 located upstream of the *BamHI* site (Fig. 6B). This construct is most likely the reason for the stronger phenotypic expression of type 3 fimbriae in pFK12 transformants than in those containing pFK14. In addition, the region immediately upstream of the fimbrial structural gene may have regulatory activity, as has been described for comparative locations in other fimbrial gene clusters (15, 26, 31).

The transcription of the *E. coli* *fimA* gene is regulated by an invertible region which contains the promoter sequence and is flanked by inverted repeats (1, 9, 12). Identical inverted repeats are present upstream of the *K. pneumoniae* type 1 fimbrial structural gene (30). The promoter for the type 3 fimbrial structural gene is likewise located on a DNA fragment flanked by two series of inverted repeats 10 and 9 bp in length. However, the nucleotide sequences of these fragments differ from those in *E. coli*. The predicted length of the amino acid sequence of the fimbrial subunit is 202 residues. The signal peptide had a length of 22 amino acids, compared with 23 amino acids for the *E. coli* and the *Klebsiella* type 1 fimbriae (27, 30), 20 amino acids for the *E. coli* K88 protein subunit (10), and 22 residues for the K99 fimbriae (32). Although this region fulfilled all the parameters predicted for signal peptides (19), it had very limited similarity with other fimbrial signal peptides. The calculated molecular mass of the mature fimbrial polypeptide was 18,477 Da, and this is less than that predicted from its electrophoretic mobility. A possible reason for this divergence is the presence of stable secondary structures in the fimbrial subunit. Furthermore, glycosylation, which is frequently found in exported polypeptides, could occur and thus cause a change in the electrophoretic mobility (8).
The predicted amino acid composition is in good agreement with the data of Old and co-workers (25) and Aleksic and Aleksic (4) for the type 3 fimbriae of \textit{K. oxytoca}, \textit{Salmonella enteritidis}, and \textit{Yersinia enterocolitica}. The presence of two cysteine residues, which could form a disulfide bond, is common in all other fimbrial structural polypeptides known among the \textit{Enterobacteriaceae} except in the K88 fimbrial protein. The absence of methionine in the mature fimbrial polypeptide explains its apparent absence in \textsuperscript{35}S-labeled minicell lysates.

The amino acid sequence of the type 3 fimbrial gene shows no significant sequence agreement with the morphologically similar K88 and K99 fimbriae, with pap pili, or with the \textit{K. pneumoniae} type 1 fimbriae of the same parent strain. Thus, despite a clear similarity between the type 3 and other fimbrial gene clusters in respect to the general organization, the type 3 fimbrial structural gene appears to be distinct in its nucleotide and amino acid composition. The precise function and regulation of \textit{K. pneumoniae} type 3 fimbriae, as well as the distribution of similar organelles among the \textit{Enterobacteriaceae}, remain to be investigated.

ACKNOWLEDGMENTS

G.-F.G. is a fellow of the postdoctoral program of the Federal Republic of Germany. B.L.A. is supported, in part, by Medical Scientist Training Program grant GM07337 from the National Institutes of Health.

LITERATURE CITED