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F1C Fimbriae of a Uropathogenic *Escherichia coli* Strain: Genetic and Functional Organization of the *foc* Gene Cluster and Identification of Minor Subunits

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The genetic organization of the *foc* gene cluster has been studied; six genes involved in the biogenesis of F1C fimbriae were identified. *focA* encodes the major fimbrial subunit, *focC* encodes a product that is indispensable for fimbria formation, *focG* and *focH* encode minor fimbrial subunits, and *focI* encodes a protein which shows similarities to the subunit protein FocA. Apart from the FocA major subunits, purified F1C fimbriae contain at least two minor subunits, FocG and FocH. Minor proteins of similar size were observed in purified S fimbriae. Remarkably, some mutations in the *foc* gene cluster result in an altered fimbrial morphology, i.e., rigid stubs or long, curly fimbriae.

The first step in urinary tract infection is believed to be the adherence of bacteria, usually uropathogenic *Escherichia coli*, to the uroepithelium. In a number of cases, fimbriae have been shown to mediate this adherence (11, 33). Fimbriae of uropathogenic *E. coli* can be divided into three categories on the basis of their hemagglutinating activities. The first category consists of fimbriae involved in mannose-resistant hemagglutination of human erythrocytes, e.g., P, M, and S fimbriae (9, 15, 34, 35). The second category consists of type 1 fimbriae, which are responsible for mannose-sensitive hemagglutination of guinea pig erythrocytes (6, 14, 31). The third category consists of fimbriae that do not, as far as is known, mediate hemagglutination, e.g., F1C fimbriae (13). Although F1C fimbriae are not hemagglutinating, they do contribute to the adhesive properties of uropathogenic *E. coli* strains; Virkola et al. (42) stated that F1C fimbriae mediate specific adherence to the collecting ducts and the distal tubules of the human kidney.

The *foc* (fimbriae of serotype 1C) gene cluster involved in the synthesis of F1C fimbriae has been cloned, and its genetic organization has been partially elucidated (38). The gene cluster is highly homologous to the *sfa* gene cluster encoding S-fimbria adhesins (26, 27). The *sfr* gene cluster, which was recently described, is another member of this highly homologous family (29). The structure of the S fimbria, like those of P fimbriae and type 1 fimbriae, appears to be complex, as these fimbriae consist of major and minor subunits (2, 12, 19, 20, 24, 30). These observations suggest that F1C fimbriae might also carry more than one fimbrial subunit. In this paper, we present a detailed genetic study of the organization of the *foc* gene cluster and the composition of F1C fimbriae.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strain HB101 (5) and cultivation conditions for it (37) have been described previously. Antibiotics (ampicillin and chloramphenicol) were used at concentrations of 50 μg ml^{-1}.

Plasmid constructions. All plasmids carry mutant *foc* gene clusters derived from pPIL110-51 or pPIL110-543 (see Fig. 1A). pPIL110-543 was constructed by the introduction of the 10.5-kilobase (kb) *Eco*RI-*Sal*I fragment of pPIL110-54 in pBR322 (38). pPIL110-516 was constructed by the introduction of the 3.0-kb *Cla*I-*Eco*RI fragment of pPIL110-512 in pBR322 (4). pPIL110-518 was constructed from pPIL110-512 and pPIL110-516 by the introduction of the 6.2-kb *Cla*I-*Hin*dIII fragment of pPIL110-512 into pPIL110-516. Plasmids pPIL110-525 and pPIL-526 are both frameshift derivatives of pPIL110-518. The frameshifts were introduced by ligation of a 12-mer *Xho*I linker in the *Cla*I and *Kpn*I sites, respectively, after these sites were filled in with T4 DNA polymerase. pPIL110-527 was constructed by *Bal* 31 treatment of pPIL110-512 digested with *Kpn*I; a 12-mer *Xho*I linker was introduced. Exchange of the 0.4-kb *Xho*I-*Hin*dIII fragment of pPIL110-543 by the 14-base-pair *Sal*I-*Hin*dIII fragment of bacteriophage M13mp8 replicative-form DNA resulted in plasmid pPIL110-5424. pOU57-11 was constructed by exchange of the 1.0-kb *Bam*HI-*Eco*RI fragment of the runaway plasmid pOU57 (18) with the 10-base-pair *Bam*HI-*Eco*RI fragment of M13mp8 replicative-form DNA, followed by introduction of the 1.5-kb *Cla*I-*Eco*RI fragment of pPIL110-518.

Genetic techniques. Transformation was carried out as described by Kushner (17).

DNA techniques. Plasmid DNA was isolated essentially by the method of Holmes and Quigley (8). The conditions for restriction endonuclease reactions, the use of T4 DNA polymerase, and the ligation of plasmid DNA were as described by Maniatis et al. (23).

Analysis of protein synthesis in minicells. Analysis of plasmid-encoded proteins in minicells was performed as described previously (39).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assays, with intact cells as antigens, were

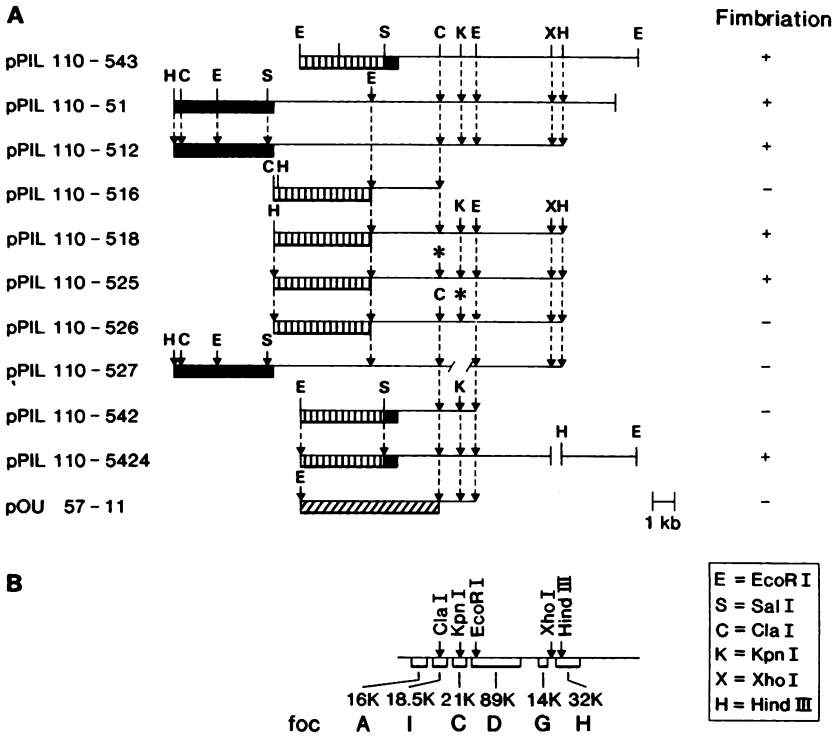

FIG. 1. Physical maps of the plasmids used in this study and their abilities to express F1C fimbriae in HB101. (A) Physical maps of pPIL110-543 and pPIL110-51, which harbor the complete *foc* gene cluster, and of mutant derivatives of these plasmids. Asterisks represent frameshift mutations. Symbols: ■, pACYC184; ▥, pBR322; ▨, pOU57. Fimbriation of HB101 carrying these plasmids was studied by electron microscopic examination of negatively stained whole cells. (B) Genetic map of the *foc* gene cluster. Open boxes show the locations of genes encoding F1C fimbriae. K, Kilodaltons (for the encoded proteins).

performed as described previously (40). The monoclonal antibody M9-6, a FocA-specific antibody, was used as an F1C-specific antibody (26).

Fimbria isolation. Fimbriae were isolated essentially as described by Moch et al. (24). Agar-grown bacteria were suspended in phosphate-buffered saline (pH 7.5) containing 100 mM NaCl and 30 mM glycine. The bacterial suspension (optical density at 660 nm, about 100) was agitated three times for 5 min each with an Omnimixer (setting 4) while cooling in an ice bath. After removal of the defimbriated bacteria and debris by centrifugation (27,000 × *g*, 30 min), fimbriae were precipitated from the supernatants with ammonium sulfate (10% saturation; pH 8) by incubation overnight at 4°C.

After centrifugation (27,000 × *g*, 30 min), the precipitate was dissolved and dialyzed against 25 mM Tris–30 mM glycine (pH 8.5). Insoluble material was removed by centrifugation (27,000 × *g*, 30 min). Fimbriae were spun down (250,000 × *g*, 3 h), dissolved in a small amount of water, and stored at 4°C.

Isolation of denatured FocC protein. A 200-ml liquid culture of HB101(pOU57-11) was grown at 30°C to an optical density at 660 nm of 0.2. The runaway replication of pOU57-11 was induced by a temperature shift to 37°C for 4 h (18). The periplasmic fraction of these cells was isolated by the method of Witholt et al. (43) and separated on a 5-mm-thick 11% (wt/vol) sodium dodecyl sulfate-polyacrylamide gel. The protein band representing the 21-kilodalton (kDa) FocC protein (according to the R_f value) was cut out of the gel. Finely cut gel slices were suspended in 50 ml of water and disrupted with a Potter tube. The gel fraction was isolated by centrifugation; the gel-free supernatant was freeze-dried and dissolved in 1.5 ml of water. This suspension contained 2.5 mg of denatured FocC protein (>99% pure). Antiserum against FocC protein was raised in rabbits as described by Overbeeke et al. (28).

Cryosectioning and immunocytochemical labeling. Cryosectioning and immunocytochemical labeling with protein A-gold complexes on ultrathin cryosections were carried out essentially as described by Van Bergen en Henegouwen and Leunissen (36). The efficiency of the labeling necessary to detect FocA in cryosections improved when cells were embedded in nonfixated 5% (wt/vol) gelatin.

Before use, rabbit antisera were preadsorbed with cell lysates of *E. coli* K-12 strain HB101.

Electron microscopy. Electron microscopy was performed with a Philips 201 microscope with nickel grids coated with Parlodion films. Preparations were negatively stained with 2% uranyl acetate for 2 min.

RESULTS

Construction and functional analysis of mutant *foc* gene clusters. Deletions and frameshift mutations were introduced at different sites in the *foc* gene cluster, and the effects of these mutations on the formation of fimbriae were studied.

The plasmids used in this study are shown in Fig. 1A. Some of the plasmids have been described before (38). The construction of the remaining plasmids is described in Materials and Methods.

The expression of fimbriae encoded by the mutant *foc* gene clusters was studied by electron microscopic examination of negatively stained whole cells harboring these plasmids. HB101, an *E. coli* K-12 strain that does not form

TABLE 1. Plasmid-encoded proteins of mutant F1C fimbrial gene clusters

Plasmid	Expression of plasmid-encoded protein with molecular mass (kDa) of[a]:					Fimbriation
	16	18.5	21	32	89	
pPIL110-51[b]	+	+	+	+	+	+
pPIL110-512[b]	+	+	+	−	+	+
pPIL110-518	+	+	+	−	+	+
pPIL110-525	+	−	+	−	+	+
pPIL110-526	+	+	−[c]	−	+	−
pPIL110-527	+	+	−	−	−	−
pPIL110-516	+	−	−	−	−	−
pOU57-11[d]	−	−	+	−	−	−

[a] +, Protein or fimbriae expressed; −, protein or fimbriae not expressed.
[b] Published previously (38).
[c] Present in a truncated form with a mass of about 19 kDa.
[d] After induction of the runaway replicon by incubation for 1 h at 37°C.

fimbriae, was used as the host strain in all experiments. The results (summarized in Fig. 1A) show that pPIL110-518 is the smallest plasmid that still induces formation of fimbriae. A frameshift mutation in the *Kpn*I site of pPIL110-518 causes the formation of fimbriae to stop, whereas a mutation in the *Cla*I site does not affect the production of fimbriae.

Analysis of plasmid-encoded proteins in minicells. The synthesis of proteins encoded by mutant fimbrial gene clusters in minicells was analyzed. The results of these analyses are shown in Table 1. A representative gel, visualized as an autoradiogram, is shown in Fig. 2A. Minicells harboring pPIL110-526 do not express the 21-kDa protein, while minicells harboring pOU57-11 express only this protein; pPIL110-527 expresses neither the 21- nor the 89-kDa protein. The frameshift mutation present in the *Cla*I restriction site of pPIL110-525 abolishes the expression of the 18.5-kDa protein. The faint 16.5-kDa protein band in Fig. 2A represents a 16-kDa precursor protein, as has been shown before (38).

We have described elsewhere the arrangement of the genes encoding the 16-, 18.5-, 21-, and 89-kDa proteins (38), but the precise order of the genes encoding the 18.5- and 21-kDa proteins remained obscure. From the results presented here and the results described earlier (38), the locations and order of the four genes can be deduced (Fig. 1B).

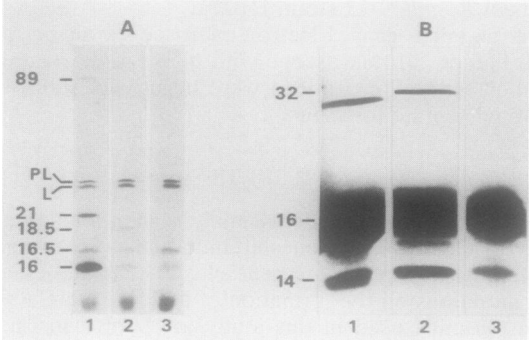

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of ^{14}C-labeled proteins and of fimbriae. (A) ^{14}C-labeled proteins, visualized on an autoradiogram, from minicells containing plasmid pPIL110-525 (lane 1), pPIL110-527 (lane 2), or pPIL110-516 (lane 3). L, β-Lactamase; PL, precursor of β-lactamase. (B) Fimbriae isolated from HB101 harboring pANN801-13 (lane 1), pPIL110-51 (lane 2), or pPIL110-518 (lane 3).

TABLE 2. Proteins present in fimbriae isolated from strain HB101 cells harboring mutant fimbrial gene clusters

Plasmid	Expression of fimbrial plasmid-encoded protein with molecular mass (kDa) of[a]:			
	14	16	30	32
pPIL110-51	+	+	−	+
pPIL110-543	+	+	−	+
pPIL110-512	+	+	−	−
pPIL110-518	+	+	−	−
pPIL110-525	+	+	−	−
pPIL110-5424	+	+	−	−
pANN801-13	+	+	+	−
pANN801-E4	+	+	−	+
pANN801-C25	+	+	+	−

[a] +, Protein expressed; −, protein not expressed.

Analysis of the protein composition of F1C and S fimbriae. The protein composition of F1C and S fimbriae was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified fimbriae. Gels were silver stained to allow detection of minor fimbrial components. The results (Fig. 2B and Table 2) show that both wild-type F1C and S fimbriae, encoded by cells harboring pPIL110-51 and pANN801-13 (7), respectively, contain at least two minor fimbrial components. F1C fimbriae are composed of a 16-kDa major subunit and 14- and 32-kDa minor subunits. S fimbriae contain a 16-kDa major subunit and a 14-kDa minor subunit (24 [referred to as a 12-kDa protein in that paper]) as well as a 30-kDa minor subunit. In agreement with those results, the *foc-sfa* hybrid gene clusters studied (24) (Fig. 3B) show the proper fimbrial composition: pANN801-E4 contained the 30-kDa Sfa minor subunit and pANN801-C25 contained the 32-kDa Foc minor subunit, next to the 14- and 16-kDa proteins, respectively. Recently, a 17-kDa protein was observed as a minor component in S fimbriae (T. Schmoll, H. Hoschützky, Y. Morschhäuser, F. Lottsplich, K. Jann, and J. Hacker, Mol. Microbiol., in press).

F1C fimbriae encoded by pPIL110-518 and pPIL110-5424 do not contain the 32-kDa minor protein, indicating that part of the gene coding for the 32-kDa protein is located between the *Hin*dIII and *Xho*I restriction sites in the gene cluster, distal to the gene encoding the 89-kDa protein. Recently, this distal DNA region has been sequenced completely. The data show the presence of three open reading frames coding for proteins with molecular masses of 17, 14, and 32 kDa, respectively. The DNA region shows a high homology (60 to 98%) to the corresponding *sfa* DNA region (I. van Die et al., manuscript in preparation). Despite the presence of an open reading frame which could encode a 17-kDa protein, we were unable to detect such a protein in minicell studies or in purified fimbriae.

Figure 3A shows the genetic localization (deduced from these data) of the genes coding for the minor subunits in the *foc* gene cluster and a comparison of the *foc*, *pap*, *sfa*, and *fim* gene clusters. We propose a lettering for the individual genes of the *foc* gene cluster in accordance with the lettering of the *fim* gene cluster.

Role of Foc proteins in biogenesis of F1C fimbriae. The formation of fimbrial filaments depends on the presence of at least three gene products (Fig. 1), i.e., FocA, FocC, and FocD. The lack of FocH and FocI does not abolish fimbria formation.

Minicells harboring plasmid pPIL110-526 or pPIL110-527, each of which lacks *focC*, show a decreased expression of FocA (Fig. 2A). This suggests that the 21-kDa FocC protein

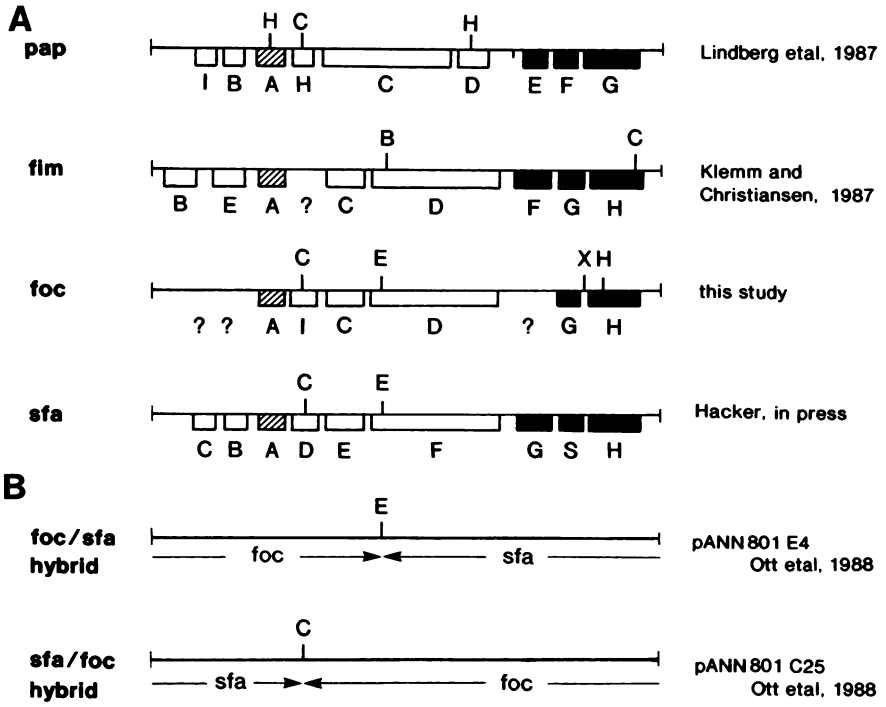

FIG. 3. Physical and genetical maps of gene clusters. C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; X, *Xho*I. (A) *pap*, *fim*, *foc*, and *sfa* gene clusters. Symbols: ■, genes coding for minor proteins; ▨, genes coding for major fimbrial subunits. (B) Hybrid *foc-sfa* gene clusters pANN801-E4 and pANN801-C25. The DNA fragments derived from the *sfa* and *foc* gene clusters are indicated.

is needed to stabilize FocA protein, as has been described for the 25-kDa FsoD and PapD proteins in the P-fimbria system (19, 30). Minicells harboring pPIL110-516, which codes only for the 16-kDa major subunit, do, however, produce detectable amounts of stable FocA, which is in contrast to the P-fimbria system. Immunocytochemical labeling of ultrathin cryosections of HB101 cells harboring pPIL110-516 or pPIL110-526 with polyclonal antiserum recognizing FocA shows that label accumulates in the periplasm of these cells (Fig. 4A). These observations also indicate that in the absence of FocC protein, a certain amount of FocA protein is stably present in the periplasm.

In cryosections of HB101 cells that overproduce the FocC protein from pOU57-11, a clear labeling of the periplasmic space is observed when the cryosections are labeled with polyclonal antiserum raised against denatured FocC protein (Fig. 4B). However, the FocC antiserum does not label cryosections of HB101 cells harboring the complete *foc* gene cluster, nor does it label whole cells. Apparently, the amount of FocC protein in HB101 cells harboring the wild-type *foc* gene cluster is too low to be detected by this technique. pOU57-11, however, contains only the *focC* gene; therefore, the periplasmic localization of FocC expressed from this plasmid does not necessarily reflect the localization of FocC in cells carrying the complete gene cluster. For this reason, the cellular localization of FocC was determined by Western blotting (immunoblotting) of fractionated HB101 cells harboring pPIL110-51 or pPIL110-518 with the polyclonal antiserum raised against FocC. The results of this analysis (Fig. 5) suggest a periplasmic localization of the FocC protein. We suppose that the FocC protein in the periplasm might be involved in stabilization or in the transport of the subunits from the inner membrane to the outer membrane.

The absence of the FocI protein, as in plasmid pPIL110-525, does not abolish the formation of fimbriae. Immunocytochemical labeling of cryosections of HB101 cells harboring pPIL110-525 with FocA-specific polyclonal antiserum reveals accumulation of label in the periplasm, which is not observed in cryosections of HB101 cells harboring pPIL110-518 (results not shown). Apparently, the absence of FocI protein results in a less efficient assembly of FocA subunits. This suggests a role for FocI in the initiation or assembly process.

Variation in expression and morphology of F1C fimbriae. HB101 cells carrying pPIL110-51, pPIL110-512, pPIL110-518, or pPIL110-525 all produce fimbriae (Fig. 1A). Figure 4 shows electron micrographs of negatively stained HB101 cells harboring these plasmids. There is a striking difference in morphology among these fimbriae. HB101(pPIL110-51) produces stubs (length, <0.5 μm), many of which are found detached from the cell surface (Fig. 4C); HB101(pPIL110-512) produces rigid fimbriae with lengths of about 1.0 μm (Fig. 4D); HB101(pPIL110-518) produces long (>1.0 μm), curly fimbriae (Fig. 4E); and HB101(pPIL110-525) produces long but rigid fimbriae (Fig. 4F).

The difference in composition between the fimbriae encoded by pPIL110-51 and pPIL110-512 is the presence or absence of the FocH protein; the difference between pPIL110-518 and pPIL110-525 is the presence or absence of the FocI protein. Apparently, the presence or absence of these proteins influences fimbrial morphology. This variation in morphology is accompanied by a difference in the level of expression of fimbriae (Fig. 4). The expression of fimbriae from HB101(pPIL110-512) is four times higher than the expression from HB101(pPIL110-51) as revealed by enzyme-linked immunosorbent assays, while the expression from HB101(pPIL110-518) or HB101(pPIL110-525) is two to four times higher than the expression from HB101(pPIL110-512).

These results indicate that the DNA region located upstream of the *focA* gene, as well as the DNA region at the

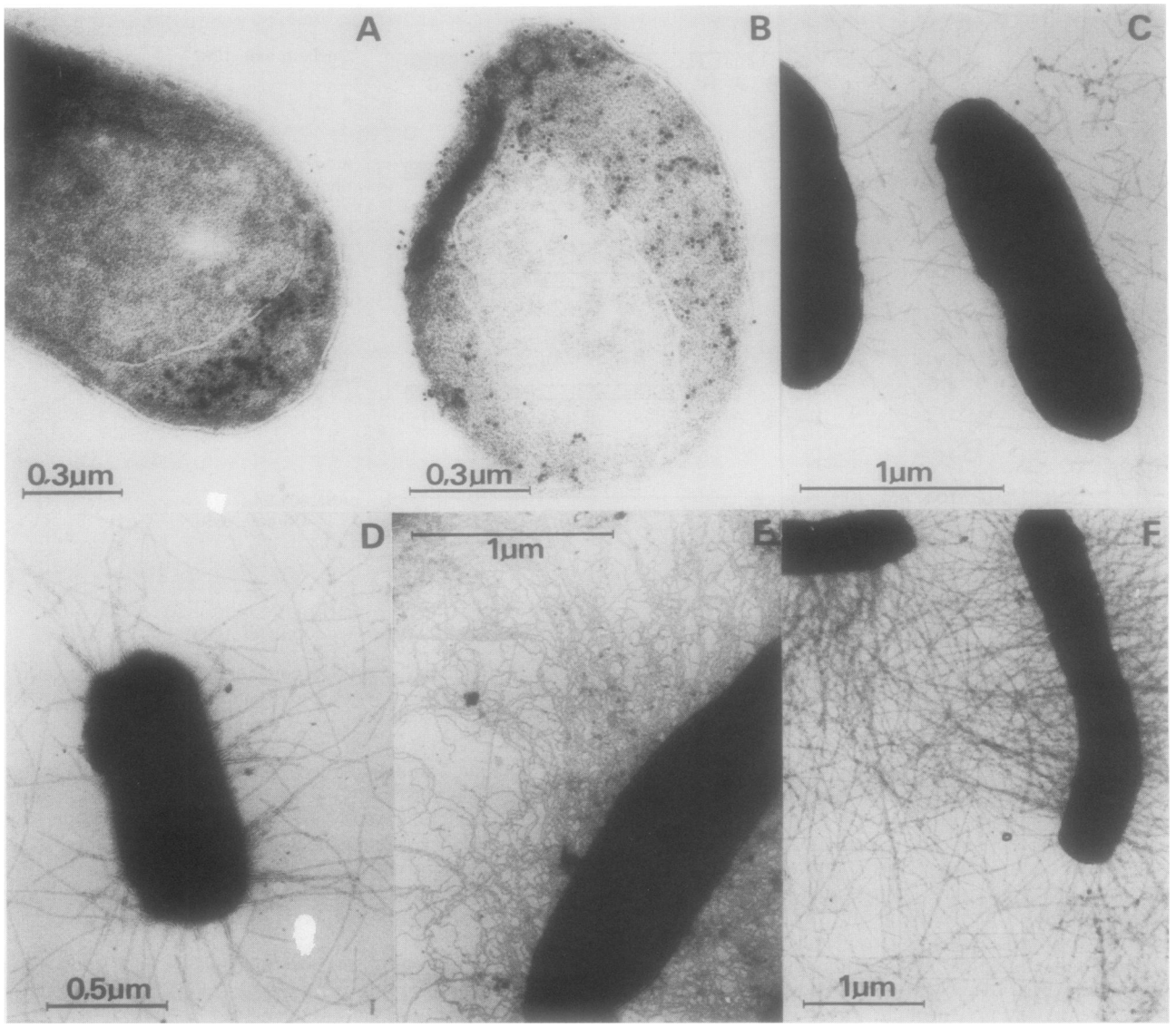

FIG. 4. Electron micrographs of HB101 harboring mutant F1C gene clusters. (A and B) Immunoelectron micrographs of ultrathin cryosections of HB101(pPIL110-516) labeled with polyclonal anti-F1C antiserum (A) and HB101(pOU57-11) (after a temperature shift from 30 to 37°C for 4 h) labeled with polyclonal anti-FocC antiserum (B). (C through F) Electron micrographs of negatively stained whole cells of HB101(pPIL110-51) (C), HB101(pPIL110-512) (D), HB101(pPIL110-518) (E), and HB101(pPIL110-525) (F).

distal part of the *foc* gene cluster, influences both the morphology and the level of expression of F1C fimbriae.

DISCUSSION

A number of fimbriae have been shown to contain minor components that specify their adhesive capacity. In this paper, we show that F1C fimbriae as well as S fimbriae carry 14- and 30- to 32-kDa minor proteins. We were not able to detect the 14-kDa protein in minicells; apparently, the expression of this protein was too low to be detected by the technique we used. Very recently, it was found that S fimbriae also contain a 17-kDa protein, which we did not observe (Schmoll et al., in press). On the basis of the overall homology of the *foc* and *sfa* gene clusters, it is likely that F1C fimbriae contain a similar protein. It is possible that we have not distinguished a 17-kDa protein in the F1C and S fimbriae because of comigration of the protein with the 16-kDa major subunit protein, which is present in very high amounts.

The genetic organization of the distal parts of the *pap*, *fim*, *sfa*, and probably *foc* gene clusters encoding the minor fimbrial components of P, type 1, S, and F1C fimbriae appears to be very much the same (Fig. 3A). The specific adhesion properties of S fimbriae have been shown to reside in the 14-kDa minor protein (24; Schmoll et al., in press). The adhesive properties of both type 1 and P fimbriae, however, appear to reside in a 35-kDa protein encoded by a gene located distally in the corresponding gene clusters (1, 2, 12, 19, 20, 30). Apparently, in different fimbrial families, different minor subunits have evolved as adhesins, and it will be interesting to see which of the different subunits of the *foc* gene cluster is responsible for the binding to kidney cells shown by Virkola et al. (42).

The genetic organization of the *foc* gene cluster is in good

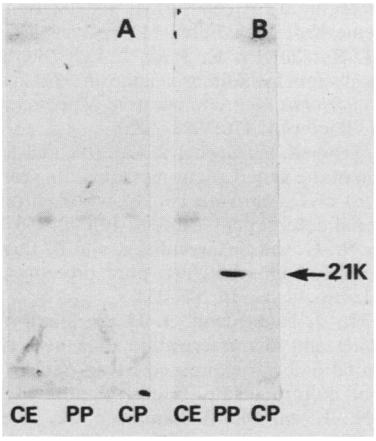

FIG. 5. Western blots of fractionated HB101 (A) and fractionated HB101 harboring pPIL110-518 (B). CE, Cell envelope fraction; PP, periplasmic fraction; CP, cytoplasmic fraction. All fractions were probed against anti-FocC polyclonal antiserum.

agreement with the genetic organization of the *sfa* and *fim* gene clusters (Fig. 3A) (12; J. Hacker, Curr. Top. Microbiol. Immunol., in press). The genetic organization of the *pap* gene cluster differs only in the order of the genes encoding the 21-kDa periplasmic protein and the 89-kDa protein (19). The *foc* and *sfa* gene clusters have been shown to be highly homologous at the DNA level, and genes can be exchanged between the clusters without loss of function (26, 27). The overall homology between the *foc* and *sfa* gene clusters on the one hand and the *pap* and *fim* gene clusters on the other hand appears to be low (27). The *focA*, *sfaA*, and *fimA* genes, however, share a sequence homology of about 60% (10, 32, 38). Preliminary data have shown that some genes can be exchanged between the *foc* and *fim* gene clusters without complete loss of function (P. Klemm et al., manuscript in preparation). These data indicate a functional relationship between the *foc* and *fim* gene clusters, which is, however, less close than the relationship between the *foc* and *sfa* gene clusters. Functional complementation between the *foc* or *sfa* gene cluster and the *pap* and *fso* gene clusters coding for P fimbriae could not be observed (26). On the other hand, the regulatory genes *sfaB* and *sfaC* have been shown to be highly homologous to the regulatory region of the P-fimbria gene cluster and are clearly different from the *fim* regulatory region (27).

The biogenesis of F1C fimbriae is critically dependent on the presence of the FocA and FocC gene products. Although it does not directly follow from our results, we suppose that the 89-kDa FocD protein is also a prerequisite for normal biogenesis, since a similar gene has been found in all gene clusters that have been studied in detail (11, 25, 41). FocC appears to be a periplasmic protein. Periplasmic proteins of similar molecular weight are encoded by the P-fimbria gene clusters (25). These proteins appear to function in the transport of the fimbrial subunits to the outside of the cell; in P-fimbria biogenesis, they have been implicated in the stabilization of the subunits in the periplasm. Although we observed a similar stabilizing activity of the FocC protein to some extent in minicell experiments, our experiments also show that the FocA major subunit can exist stably on its own in the periplasm.

Our results show that FocI and FocH are not necessary for the expression of fimbriae. FocH has been shown to be a minor fimbrial component. The localization and function of the FocI protein are not clear. The *focI* gene has been sequenced (I. van Die et al., manuscript in preparation). The deduced amino acid sequence of this sequence shows an open reading frame with typical fimbrial-subunit features, e.g., a hydrophobic N-terminal sequence and two cysteine residues at positions 42 and 85 from the first amino acid of the preprotein (in FocA, two cysteine residues are present, at positions 43 and 83). This suggests that the 18.5-kDa FocI protein is a minor subunit, although it could not be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified F1C fimbriae. The *focI* gene is located at a region of the gene cluster similar to that of *papH* in the *pap* cluster. The PapH protein is a subunitlike protein that has been implicated in the termination of fimbrial growth (3). Our data, however, do not indicate a role for FocI in growth termination. HB101 cells harboring pPIL110-525, which lacks *focI*, accumulate FocA, which suggests a role for FocI in the initiation or assembly of fimbriae.

Both FocI and FocH seem to influence fimbrial morphology. The stubs that we observed could be generated by breakage of fragile fimbriae due to the presence of minor subunits in the fimbriae. A similar fragility in type 1 fimbriae was suggested as being due to the presence of minor subunits interspersed at intervals along the fimbriae (12, 16). An alternative or additional explanation could be that stubs are formed as consequences of an increased number of initiation and termination events in fimbrial growth. In fact, it has been shown that minor subunits of P fimbriae play an important role in the initiation and termination of fimbrial growth (19, 30).

The curly shape of the fimbriae expressed by pPIL110-518 is difficult to explain. The only difference between pPIL110-518 and pPIL110-512, which yields a normal fimbrial shape, is the presence in pPIL110-512 of a DNA region from the 5′ distal part upstream of the regulatory region of the gene cluster. No structural function has been found for this region, but the presence of this region influences both the degree of expression and the shape of the fimbriae. When the *focI* gene in pPIL110-518 is inactivated, as in pPIL110-525, fimbrial shape is normal again. We suppose that the curly shape is generated by an imbalance between major and minor subunits (e.g., FocA and FocI) expressed in the *foc* gene cluster.

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LITERATURE CITED

1. **Abraham, S. N., J. D. Goguen, and E. H. Beachey.** 1988. Hyperadhesive mutant of type 1 fimbriated *Escherichia coli* associated with formation of FimH organelles (fimbriosomes). Infect. Immun. **56**:1023–1029.
2. **Abraham, S. N., J. D. Goguen, D. Sun, P. Klemm, and E. H. Beachey.** 1987. Identification of two ancillary subunits of *Escherichia coli* type 1 fimbriae by using antibodies against synthetic oligopeptides of *fim* gene products. J. Bacteriol. **169**:5530–5536.
3. **Båga, M., N. Norgren, and S. Normark.** 1987. Biogenesis of *Escherichia coli* Pap pili: PapH, a minor pilin subunit involved in cell anchoring and length modulation. Cell **49**:241–251.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L.**

Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.

5. **Boyer, H. W., and D. Roullard-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
6. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of Enterobacteriaceae, p. 186–217. *In* E. H. Beachey (ed.), Receptors and recognition, series B, vol. 6. Bacterial adherence. Chapman & Hall, Ltd., London.
7. **Hacker, J., G. Schmidt, C. Hughes, S. Knapp, M. Marget, and W. Goebel.** 1985. Cloning and characterization of genes involved in production of mannose-resistant, neuraminidase-susceptible (X) fimbriae from a uropathogenic O6:K15:H31 *Escherichia coli* strain. Infect. Immun. **47**:434–440.
8. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**: 193–197.
9. **Källenius, G., R. Möllby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svensson, and B. Cedergren.** 1980. The pk antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
10. **Klemm, P.** 1984. The *fim*A gene encoding the type I fimbrial subunit of *Escherichia coli*. Eur. J. Biochem. **143**:395–399.
11. **Klemm, P.** 1985. Fimbrial adhesins of *Escherichia coli*. Rev. Infect. Dis. **7**:321–340.
12. **Klemm, P., and G. Christiansen.** 1987. Three *fim* genes required for the regulation of length and mediation of adhesion of *Escherichia coli* type 1 fimbriae. Mol. Gen. Genet. **208**:439–445.
13. **Klemm, P., I. Ørskov, and F. Ørskov.** 1982. F7 and type 1-like fimbriae from three *Escherichia coli* strains isolated from urinary tract infections: protein chemical and immunological aspects. Infect. Immun. **36**:462–468.
14. **Korhonen, T. K.** 1979. Yeast cell agglutination by purified enterobacterial pili. FEMS Microbiol. Lett. **6**:421–425.
15. **Korhonen, T. K., V. Väisänen-Rhen, M. Rhen, A. Pere, J. Parkkinen, and J. Finne.** 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. J. Bacteriol. **159**:762–766.
16. **Krogfelt, K., and P. Klemm.** 1988. Investigation of minor components of *Escherichia coli* type 1 fimbriae: protein chemical and immunological aspects. Microb. Pathog. **4**:231–238.
17. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with Col E1 derived plasmids, p. 17–23. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier Biomedical Press, Amsterdam.
18. **Larsen, J. E. L., K. Gerdes, J. Light, and S. Molin.** 1984. Low-copy-number plasmid-cloning vectors amplifiable by derepression of an inserted foreign promotor. Gene **28**:45–54.
19. **Lindberg, F., B. Lund, J. Johansson, and S. Normark.** 1987. Localization of the receptor-binding adhesin at the tip of the bacterial pilus. Nature (London) **328**:84–87.
20. **Lindberg, F. P., B. Lund, and S. Normark.** 1986. Gene products specifying adhesion of uropathogenic *Escherichia coli* are minor components of pili. Proc. Natl. Acad. Sci. USA **83**:851–860.
21. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands. FEBS Lett. **58**:254–258.
22. **Lund, B., F. Lindberg, and S. Normark.** 1988. Structure and antigenic properties of the tip-located P pilus proteins of uropathogenic *Escherichia coli*. J. Bacteriol. **170**:1887–1894.
23. **Maniatis, T., E. F. Fritsch, and J. C. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Moch, T., H. Hoschützky, J. Hacker, K.-O. Krönke, and K. Jann.** 1987. Isolation and characterization of the α-sialyl-β-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:3462–3466.
25. **Norgren, M., M. Båga, J. M. Tennent, and S. Normark.** 1987. Nucleotide sequence, regulation and functional analysis of the *papC* gene required for cell surface localization of Pap pili of uropathogenic *Escherichia coli*. Mol. Microbiol. **1**:169–178.
26. **Ott, M., H. Hoschützky, K. Jann, I. Van Die, and J. Hacker.** 1988. Gene clusters for S fimbrial adhesin (*sfa*) and F1C fimbriae (*foc*) of *Escherichia coli*: comparative aspects of structure and function. J. Bacteriol. **170**:3983–3990.
27. **Ott, M., T. Schmoll, W. Goebel, I. Van Die, and J. Hacker.** 1987. Comparison of the genetic determinant coding for the S-fimbrial adhesin (*sfa*) of *Escherichia coli* to other chromosomally encoded fimbrial determinants. Infect. Immun. **55**:1940–1943.
28. **Overbeeke, N., G. van Scharrenburg, and B. Lugtenberg.** 1980. Antigenic relationships between pore proteins of *Escherichia coli*. Eur. J. Biochem. **110**:247–254.
29. **Pawelzik, M., J. Heesemann, J. Hacker, and W. Opferkuch.** 1988. Cloning and characterization of a new type of fimbria (S/F1C-related fimbria) expressed by an *Escherichia coli* O75:K1:H7 blood culture isolate. Infect. Immun. **56**:2918–2924.
30. **Riegman, N., I. van Die, J. Leunissen, W. Hoekstra, and H. Bergmans.** 1988. Biogenesis of $F7_1$ and $F7_2$ fimbriae of uropathogenic *E. coli*: influence of the FsoF and FstFG proteins and localization of the Fso/FstE protein. Mol. Microbiol. **2**:73–80.
31. **Salit, I. E., and E. C. Gottschlich.** 1977. Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. **146**:1182–1194.
32. **Schmoll, T., J. Hacker, and W. Goebel.** 1987. Nucleotide sequence of the *sfa*A gene coding for the S-fimbrial protein subunit of *Escherichia coli*. FEMS Microbiol. Lett. **41**:229–235.
33. **Svanborg Edén, C., and H. A. Hansson.** 1978. *Escherichia coli* pili as possible mediators for attachment to human urinary tract epithelial cells. Infect. Immun. **21**:229–237.
34. **Väisänen, V., T. K. Korhonen, M. Jokinen, C. G. Gahmberg, and C. Ehnholm.** 1982. Blood group M specific haemagglutination in pyelonephritogenic *Escherichia coli*. Lancet **ii**:11.
35. **Väisänen-Rhen, V., J. Elo, E. Väisänen, A. Siitonen, I. Ørskov, F. Ørskov, S. B. Svenson, P. H. Mäkelä, and T. K. Korhonen.** 1984. P-fimbriated clones among uropathogenic *Escherichia coli* strains. Infect. Immun. **43**:149–155.
36. **Van Bergen en Henegouwen, P. M. P., and J. L. M. Leunissen.** 1986. Controlled growth of colloidal gold particles and implications for labeling efficiency. Histochemistry **85**:81–87.
37. **Van Die, I., C. van den Hondel, H.-J. Hamstra, W. Hoekstra, and H. Bergmans.** 1983. Studies on the fimbriae of an *Escherichia coli* O6:K2:H1:F7 strain: molecular cloning of a DNA fragment encoding a fimbrial antigen responsible for mannose-resistant haemagglutination of human erythrocytes. FEMS Microbiol. Lett. **19**:77–82.
38. **Van Die, I., B. van Geffen, W. Hoekstra, and H. Bergmans.** 1985. Type 1C fimbriae of an uropathogenic *Escherichia coli* strain: cloning and characterization of the genes involved in the expression of the 1C antigen and nucleotide sequence of the subunit gene. Gene **34**:187–196.
39. **Van Die, I., I. van Megen, W. Hoekstra, and H. Bergmans.** 1984. Molecular organization of the genes involved in the production of $F7_2$ fimbriae, causing mannose resistant haemagglutination, of a uropathogenic *Escherichia coli* O6:K2:H1:F7 strain. Mol. Gen. Genet. **194**:528–533.
40. **Van Die, I., M. Wauben, I. Van Megen, H. Bergmans, N. Riegman, W. Hoekstra, P. Pouwels, and B. Enger-Valk.** 1988. Genetic manipulation of major P-fimbrial subunits and consequences for formation of fimbriae. J. Bacteriol. **170**:5870–5876.
41. **Van Doorn, J., B. Oudega, F. R. Mooi, and F. K. de Graaf.** 1982. Subcellular localization of polypeptides involved in the biosynthesis of K88a,b fimbriae. FEMS Microbiol. Lett. **13**:99–104.
42. **Virkola, R., B. Westerlund, H. Holthöfer, J. Parkkinen, M. Kekomäki, and T. K. Korhonen.** 1988. Binding characteristics of *Escherichia coli* adhesins in human urinary bladder. Infect. Immun. **56**:2615–2622.
43. **Witholt, B., M. Boekhout, N. Brock, J. Kingma, H. van Heerikhuizen, and L. de Ley.** 1976. An efficient and reproducible procedure for the formation of spheroplast from variously grown *Escherichia coli*. Anal. Biochem. **74**:160–170.