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Reciprocal Exchange of Minor Components of Type 1 and F1C Fimbriae Results in Hybrid Organelles with Changed Receptor Specificities

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Type 1 and F1C fimbriae are surface organelles of *Escherichia coli* which mediate receptor-specific binding to different host surfaces. Such fimbriae are found on strains associated with urinary tract infections. The specific receptor binding of the fimbriae is due to the presence of receptor recognition proteins present in the organelles as minor structural elements. The organization of the *fim* and *foc* gene clusters encoding these fimbriae, as well as the structures of the organelles, are very similar, although the actual sequence homology of the structural elements is not remarkable; notably, the sequence identity between the minor components of the type 1 and F1C fimbriae is only 34 to 41%. Type 1 fimbriae mediate agglutination of guinea pig erythrocytes, whereas F1C fimbriae do not confer agglutination of any types of erythrocytes tested. However, F1C fimbriae mediate specific adhesion to epithelial cells in the collecting ducts of the human kidney as well as to cells of various cell lines. This report addresses the question of fimbrial promiscuity. Our data indicate that minor fimbrial structural elements can be exchanged between the two fimbrial systems, resulting in hybrid organelles with changed receptor specificity. This is the first study on reciprocal exchange of structural components from two different fimbrial systems.

Bacterial adherence is normally a prerequisite for successful colonization of a specific host tissue. The best-characterized group of bacterial adhesins is constituted by fimbriae (for reviews, see references 12 and 23). The fimbriae found on *Escherichia coli* strains associated with urinary tract infections can be differentiated into three categories on the basis of their hemagglutinating ability. The first category encompasses fimbriae mediating D-mannose-resistant agglutination of human erythrocytes, e.g., P and S fimbriae (10, 19, 20, 34). The second group consists of the type 1 fimbriae and mediates D-mannose-sensitive agglutination of various erythrocytes, e.g., guinea pig erythrocytes (6, 33). To the third class belong fimbriae which do not possess any known hemagglutinating characteristics, notably F1C fimbriae (18, 36, 37).

Type 1 fimbriae are found on the majority of *E. coli* strains. It has been proposed that the biological role of type 1 fimbriae is to provide bacterial adhesion to mucus in the large intestine, which is the natural habitat of *E. coli* (28). Furthermore, several lines of evidence have pointed to a role of type 1 fimbriae as possible virulence factors in uropathogenic *E. coli* strains (7). F1C fimbriae are nonhemagglutinating. However, they do contribute to the adhesive properties of certain *E. coli* strains, since they mediate specific adherence to the collecting ducts and the distal tubules of the human kidney (37) as well as to cultured renal tubulus cells and various cell line cells (21, 26).

A single type 1 or F1C fimbria is a thin, 7-nm-wide, approximately 1-μm-long surface polymer. It consists of about 1,000 subunits of a major building element, the FimA protein or the FocA protein. Additionally, a few percent minor components are also present as integral parts of the fimbriae. In the case of type 1 fimbriae, it has been shown that the minor components, FimF, FimG, and FimH, are involved in the binding of the fimbriae to D-mannose-containing structures and in fimbrial length regulation (14, 25, 32). The FimH protein has been shown to be the actual receptor-binding molecule (24). The FimF and FimG components are probably required for integration of the FimH adhesin into the fimbriae. The minor components per se are not necessary for synthesis of the fimbriae, since recombinant bacteria entirely missing the corresponding genes are able to express pure FimA fimbriae (14). However, such organelles are devoid of adherence activity. The structure of F1C fimbriae closely resembles that of type 1 fimbriae (31, 36). They also contain minor components, i.e., the FocF, FocG, and FocH proteins. By analogy to type 1 fimbriae and the closely related S fimbriae, the putative adhesin of the F1C fimbriae is probably constituted by one of the minor components (27, 31, 34).

The major structural proteins of type 1 and F1C fimbriae exhibit about 60% sequence homology; consequently, type 1 and F1C fimbriae show weak immunological cross-reactivity. However, it is noteworthy that the minor components show only between 34 and 41% homology (35). The two gene clusters encoding the type 1 and F1C fimbriae, the *fim* and *foc* genes, are almost identical in genetic organization (Fig. 1). The major difference is found in the sectors encoding the genes required for regulation of expression.

The structural components of type 1 and F1C fimbriae are produced as precursors having an N-terminal signal sequence. This element is subsequently removed during export across the

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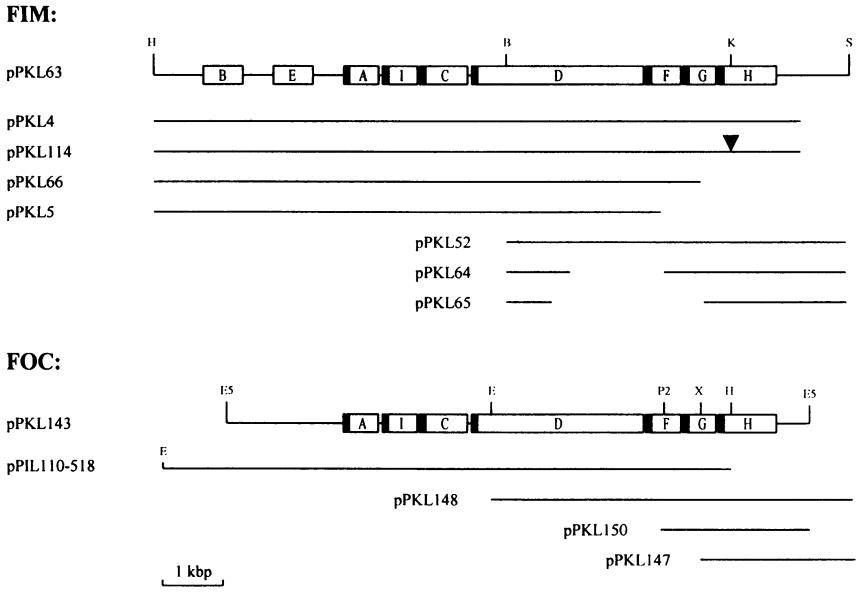

FIG. 1. Overview of the *fim* and *foc* gene clusters as present in plasmids pPKL63 and pPKL4 and in plasmid pPKL143, respectively. Black boxes indicate signal peptide-encoding sectors. The extent of other recombinant plasmids is indicated by bars. Only DNA inserts are shown. Relevant restriction sites: B, *Bam*HI; E, *Eco*RI; E5, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; P2, *Pvu*II; S, *Sal*I; X, *Xho*I. The triangle indicates linker insertion in plasmid pPKL114.

inner membrane. All evidence indicates that this translocation is dependent on the normal *E. coli* export system (SecA, SecB, etc.) (5, 29, 30). However, further export to the cell exterior, i.e., from the periplasm and across the outer membrane, is dependent on specific export and assembly systems constituted by the FimC and FimD and the FocC and FocD proteins, respectively (13, 15, 27, 36).

In this report, we have addressed the question of reciprocal exchange of minor structural proteins, i.e., whether fimbrial building elements originating from one of these fimbria-encoding gene clusters can be accommodated by the other. Notably, we have investigated whether the exchange of minor constituents of the fimbriae results in organelles with changed receptor specificities.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* K-12 strains used in this study were HB101 (1, 2) and AAEC191A (λ^- F^- *recA* Δ*fimB-fimH*), a plasmid-cured version of AAEC191, generously provided by Ian Blomfield, Bowman Gray School of Medicine, Winston-Salem, N.C. Cells were grown on solid medium or in liquid broth supplemented with the appropriate antibiotics.

Plasmids. Plasmids pPKL4, pPKL5, pPKL66, pPKL52, pPKL63, pPKL64, and pPKL65 containing all or part of the *fim* gene cluster have been described previously (11, 14, 16, 17). Plasmid pPIL110-51 containing the total *foc* gene cluster and plasmid pPIL110-518 carrying a deletion in the *focH* gene have also been described previously (31, 36). Plasmid pPKL114 was made by insertion of an oligonucleotide containing translational stop codons in all three reading frames in the unique *Kpn*I site in the *fimH* gene of plasmid pPKL4. Plasmid pPKL143 was made by insertion of a 9-kbp *Eco*RV fragment from plasmid pPIL110-51 (36) into the *Eco*RV site of pBR322. Plasmids pPKL147 and pPKL148 were *Sal*I-*Xho*I and a partial *Eco*RI deletion of plasmid pPIL110-51, respectively. Plasmid pPKL150 was made by inserting a 2.3-kbp *Pvu*II-*Bam*HI fragment from pPKL143 into *Eco*RV- and *Bam*HI-restricted pACYC184 (3).

DNA techniques. Isolation of plasmid DNA was carried out by the mini-lysate method of Holmes and Quigley (8) or Del Sal et al. (4). Restriction endonucleases were used as specified by the manufacturer (New England Biolabs).

Hemagglutination. The capacity of bacteria to express a D-mannose binding phenotype was assayed by their ability to agglutinate guinea pig erythrocytes on glass slides. Aliquots of liquid bacterial cultures grown to an optical density of 5.0 and 5% erythrocytes were mixed, and the time until agglutination occurred was measured.

Cell adherence. As a model cell line for bacterial adherence to eucaryotic cells, the HEp-2 cell line (ATCC CCL-23) was used. This is a cell line of epithelial-like morphology and of human origin, easy to handle and well established in many laboratories. HEp-2 cells were maintained in Dulbecco's modified Eagle medium containing 10% fetal calf serum without antibiotics. Cells were harvested from confluent monolayers and cultured in 96-well flat-bottom microtiter plates in 100-μl aliquots for 24 h, after which the culture medium was removed and the cells were fixed by addition of 100 μl of glutaraldehyde (1.25% in phosphate-buffered saline [PBS]) at room temperature for 1 h.

Bacterial adhesion to epithelial cells was quantified by an enzyme-linked immunosorbent assay (ELISA) as previously described, using a rabbit anti-*E. coli* K-12 polyclonal antiserum and a second anti-rabbit antibody labelled with alkaline phosphatase (21, 22, 26). *p*-Nitrophenyl phosphate (Sigma) was used as a substrate. The color reaction was allowed to develop for 30 min and was read at 405 nm with a microplate reader (Behring EL 311). Each experiment consisted of at least 12 parallel measurements done in duplicate.

Antisera. Rabbit antisera were raised against purified type 1 and F1C fimbriae. The sera were adsorbed with HB101 cells expressing F1C fimbriae and type 1 fimbriae, respectively,

TABLE 1. Characteristics of plasmids used in this study when present in strain AAEC191A or HB101

Plasmid[a]	Relevant genotype	Hemagglutination[b]	Presence of fimbriae[c]
pPKL4 (B)	Total *fim* cluster	15	+
pPKL63 (A)	Total *fim* cluster	15	+
pPKL114 (B)	*fimH*	>600	+
pPKL66 (B)	*fimA*$^+$-*fimF*$^+$	>600	+
pPKL5 (B)	*fimA*$^+$-*fimF*$^+$	>600	+
pPKL52 (A)	*fimF*$^+$ *fimG*$^+$ *fimH*$^+$	>600	−
pPKL64 (A)	*fimG*$^+$ *fimH*$^+$	>600	−
pPKL65 (A)	*fimH*$^+$	>600	−
pPKL143 (B)	Total *foc* cluster	>600	+
pPIL110-518 (B)	*focH*	>600	+
pPKL148 (A)	*focF*$^+$ *focG*$^+$ *focH*$^+$	>600	−
pPKL150 (A)	*focG*$^+$ *focH*$^+$	>600	−
pPKL147 (A)	*focH*$^+$	>600	−

[a] A or B indicates pACYC184- or pBR322-based vector.
[b] Hemagglutination of guinea pig erythrocytes indicated in seconds before reaction occurred. Average values of four measurements are given.
[c] +, fimbriated phenotype; −, bald phenotype.

before use. A monoclonal antiserum specific for the FimH and FocH proteins was kindly provided by Maryvonne Dho-Moulin, Institut National de la Recherche Agronomique, Tours, France.

Electron microscopy. Bacteria from overnight cultures were resuspended in PBS. All bacterial clones were examined in parallel under exactly the same conditions. A 25-μl aliquot of bacterial suspension was placed on a Parlodion carbon-coated glow-discharged grid for 30 s. Grids were stained with 3 drops of 1% phosphotungstic acid (pH 7.5).

Immunoelectron microscopy. Twenty-five microliters of bacterial suspension in PBS was applied to glow-discharged carbon-coated 200-mesh copper grids for 1 min. The grids were washed with 3 drops of PBS, and excess binding capacity of the grids was blocked by soaking in 0.5% ovalbumin for 5 min. The grids were placed in the primary antibody solution (adsorbed anti-type 1, anti-F1C, or anti-FimH serum diluted 1:50 in 0.5% ovalbumin) for 45 min at 37°C and washed three times with PBS. The grids were then incubated for 45 min at 37°C with secondary antibodies (swine anti-rabbit immunoglobulins) conjugated with colloidal gold particles. After incubation, the grids were washed twice in 1% gelatin in PBS and twice in PBS. The grids were stained as described above. Electron microscopy was done on a JEOL 100B electron microscope.

RESULTS

In this study, we used two assays for observing the biological binding activity of the various clones, i.e., hemagglutination of guinea pig erythrocytes and binding to cells from the human epithelial cell line HEp-2, for monitoring of type 1 fimbria-mediated binding and F1C fimbrial-type binding activity, respectively. We used two *E. coli* K-12 *fim* mutants, AAEC191A and HB101, as hosts. The two strains have behaved similarly in our hands regarding the inability to express type 1 or F1C fimbriae or to exhibit binding activity before the relevant genes were introduced. When plasmids harboring either the *fim* or *foc* wild-type gene cluster, as contained in plasmids pPKL63 or pPKL4 and pPKL143 (Fig. 1 and Table 1), were introduced into AAEC191A or HB101 host cells, they became fimbriated (references 14 and 31 and this study). These hosts, containing either plasmid pPKL4 or pPKL63, also give good agglutination of guinea pig erythrocytes, indicating that the fimbriae are fully adhesive (Table 1). It was previously shown that purified fimbriae from such hosts, in addition to the major subunit protein, FimA, also contain the minor fimbrial components, FimF, FimG, and FimH (25). AAEC191A and HB101 hosts harboring plasmid pPKL143 (total *foc* gene cluster) are unable to agglutinate any known erythrocytes but adhere well to cells

TABLE 2. Complementation between the *fim* and *foc* gene clusters in *E. coli* K-12 hosts AAEC191A or HB101, with respect to the genes encoding the minor components originating from either system, including the adhesins[a]

Plasmid(s)	Hemagglutination[b]	Cell-binding activity (mean ± SE)[c]
pPIL110-518 (*focH*) + pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$)	37	
pPIL110-518 (*focH*) + pPKL64 (*fimG*$^+$ *fimH*$^+$)	29	
pPIL110-518 (*focH*) + *pPKL65* (*fimH*$^+$)	15	
pPKL143 (all *foc* genes) + pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$)	30	
pPKL143 (all *foc* genes) + pPKL64 (*fimG*$^+$ *fimH*$^+$)	180	
pPKL143 (all *foc* genes) + pPKL65 (*fimH*$^+$)	42	
pPKL143 (all *foc* genes)	>600	0.57 ± 0.06
pPKL114 (*fimH*)	>600	0.26 ± 0.02
None (*fim foc*)	>600	0.27 ± 0.01
pPKL114 (*fimH*) + pPKL148 (*focF*$^+$ *focG*$^+$ *focH*$^+$)	>600	0.49 ± 0.05
pPKL114 (*fimH*) + pPKL150 (*focG*$^+$ *focH*$^+$)	>600	0.33 ± 0.02
pPKL114 (*fimH*) + pPKL147 (*focH*$^+$)	>600	0.42 ± 0.02
pPKL66 (*fimA*$^+$-*fimF*$^+$)	>600	0.27 ± 0.03
pPKL66 (*fimA*$^+$-*fimF*$^+$) + pPKL148 (*focF*$^+$ *focG*$^+$ *focH*$^+$)	>600	0.50 ± 0.02
pPKL66 (*fimA*$^+$-*fimF*$^+$) + pPKL150 (*focG*$^+$ *focH*$^+$)	>600	0.35 ± 0.02
pPKL66 (*fimA*$^+$-*fimF*$^+$) + pPKL147 (*focH*$^+$)	>600	0.19 ± 0.01
pPKL5 (*fimA*$^+$-*fimD*$^+$)	>600	0.27 ± 0.02
pPKL5 (*fimA*$^+$-*fimD*$^+$) + pPKL148 (*focF*$^+$ *focG*$^+$ *focH*$^+$)	>600	0.52 ± 0.03
pPKL4 (all *fim* genes) + pPKL148 (*focF*$^+$ *focG*$^+$ *focH*$^+$)	15	
pPKL4 (all *fim* genes) + pPKL150 (*focG*$^+$ *focH*$^+$)	20	
pPKL4 (all fim genes) + pPKL147 (*focH*$^+$)	15	

[a] Adequate controls have been included for comparison. The resultant phenotypes were assayed by mannose-sensitive hemagglutination of guinea pig erythrocytes and binding to HEp-2 cells.
[b] Hemagglutination of guinea pig erythrocytes indicated in seconds before reaction occurred. Average values of four measurements are given.
[c] ELISA values (see Materials and Methods for assay conditions).

from the human cell line HEp-2 (Table 2). In this study, we used a number of plasmids which either do or do not contain the regions of the *fim* or *foc* gene clusters that encode the minor structural components (Fig. 1). A summary of the phenotypes conferred by these plasmids is given in Table 1.

Interaction of F1C fimbriae missing the FocH protein with type 1 fimbrial minor components. Plasmid pPIL110-518 harbors the complete *foc* gene cluster except for the *focH* gene (Fig. 1). AAEC191A or HB101 cells containing this plasmid are fimbriated (Fig. 2A) and do not confer agglutination of erythrocytes (Table 1). Meanwhile, when plasmid pPKL65, encoding the FimH adhesin of type 1 fimbriae, was introduced into such hosts, a D-mannose binding phenotype resulted (Table 2). The ability to agglutinate guinea pig erythrocytes was comparable to that of hosts expressing wild-type type 1 fimbriae, indicating acceptance of the FimH adhesin by the F1C system. Electron microscopy showed the bacteria to be profusely fimbriated (Fig. 2B). Immunoelectron microscopy, performed with FimH-specific antiserum and colloid gold labelling, indicated the presence of the FimH protein as an integral part of the organelles (Fig. 3A).

To investigate the influence of the two other minor structural components, FimF and FimG, in addition to the FimH adhesin, two plasmids, pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$) and pPKL64 (*fimG*$^+$ *fimH*$^+$), were transformed into hosts already containing plasmid pPIL110-518 (*focH*). The presence of either pPKL64 or pPKL52, in addition to pPIL110-518, resulted in a hemagglutinating phenotype very reminiscent of that expressed by the combination of pPKL65 plus pPIL110-518 (Table 2). In both cases, i.e., pPKL64 or pPKL52 plus pPIL110-518, an even more lavish fimbriation was seen than when plasmid pPKL65 was complementing pPIL110-518 (Fig. 2C and D). This observation may indicate a more frequent initiation of fimbrial biosynthesis. Thus, as in the case of type 1 fimbriae, the minor components originating from the *fim* system may also play a role in initiation of synthesis of the hybrid organelles (see Discussion).

Interaction of an intact F1C fimbrial system with type 1 fimbrial minor components. We then investigated whether the presence of an intact *foc* gene cluster would interfere with the accumulation of minor components originating from the *fim* gene cluster, i.e., FimF, FimG, and FimH. When cells already containing plasmid pPKL143 were transformed with either plasmid pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$), pPKL64 (*fimG*$^+$ *fimH*$^+$), or pPKL65 (*fimH*$^+$), the resultant clones all exhibited a D-mannose binding phenotype, as assayed by mannose-sensitive hemagglutination of guinea pig erythrocytes (Table 2). However, the responses were generally considerably weaker than the titers obtained when the same plasmids were interacting with a *foc* gene cluster missing the *focH* gene, (pPIL110-518). This difference could indicate a preference of the F1C system for its own structural components in favor of minor components originating from type 1 fimbriae. Furthermore, the hemagglutination titer of the combination pPKL143 plus pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$) was higher than titers of the combinations pPKL143 plus pPKL64 (*fimG*$^+$ *fimH*$^+$) and pPKL143 plus pPKL65 (*fimH*$^+$), perhaps indicative of a more favorable cell surface exposure of the FimH adhesin, when the parental type 1 minor fimbrial components, FimF and FimG, also were present.

Electron microscopy showed that the presence of plasmid pPKL52, pPKL64, or pPKL65 in addition to plasmid pPKL143 in all cases conferred reduced fimbriation in comparison with the *foc* gene cluster alone (see, for example, Fig. 2F). Apparently, the presence of type 1 fimbrial minor components interfered with organelle biosynthesis when a full complement of F1C minor components also was present. Immunoelectron microscopy using colloidal gold labelling and anti-FimH serum indicated that the type 1 fimbrial adhesin was integrated at least to some extent in the fimbriae (Fig. 3B).

Interaction of type 1 fimbrial systems with F1C minor components. *E. coli* K-12 hosts AAEC191A and HB101, harboring plasmids pPKL114, pPKL66, and pPKL5, produce fimbriae that are missing one or more minor Fim components and are unable to agglutinate guinea pig erythrocytes. Furthermore, they do not adhere to HEp-2 cells any better than nonfimbriate controls do (Table 2). However, when such hosts in addition to the plasmids mentioned above also contained plasmids encoding one or more F1C minor components, the picture changed strikingly. In several cases, adhesion to HEp-2 cells comparable to that of bacteria expressing wild-type F1C fimbriae (pPKL143) was observed. Notably, introduction of plasmid pPKL148, encoding all Foc minor components, resulted in a very adhesive phenotype (Table 2). However, in some cases, for example, the combination of pPKL66 plus pPKL147, no rise in adhesive potential was observed. This might be an indication of competition between minor components originating from the two different fimbrial systems. A similar insignificant rise in adhesive potential was seen when hosts containing plasmid pPKL4 (total *fim* gene cluster) were compared with hosts which also contained plasmids encoding F1C minor components (data not shown). Again, this finding may indicate competition between the type 1 and F1C minor components.

Electron microscopy showed that the appearance of host cells harboring type 1 fimbria-encoding plasmids that were deficient in one or more of the minor components, i.e., pPKL5, pPKL66, and pPKL114, generally shifted toward a phenotype with more and shorter fimbriae, compared with the parental phenotype, when plasmid pPKL148, encoding the F1C minor components, FocF, FocG, and FocH, was also present (Fig. 4A and B), perhaps because of more frequent initiation of organelle synthesis caused by the F1C minor components. Immunoelectron microscopy of such clones with colloid gold-labelled F1C-specific antiserum indicated the presence of F1C minor components as integral parts of the organelles; however, the amount of detectable minor components seemed to be reduced compared with the wild-type phenotypes.

Electron microscopy of host cells which harbored both plasmid pPKL4, encoding the entire *fim* gene cluster, and plasmid pPKL148, encoding a full ensemble of F1C minor components, showed that these cells tended to have shorter and more fimbriae than the parental host (Fig. 4C and D), whereas hosts containing the combination pPKL4 plus pPKL147 seemed to have fewer fimbriae (Fig. 4E). One can perhaps envision that the presence of all F1C minor components, in addition to the type 1 minor components, results in a more frequent initiation of organelle synthesis, whereas the presence of just FocH but not FocF and FocG perhaps induces production of defective initiation complexes by competition between FimH and FocH.

DISCUSSION

In this work, we have investigated the interaction between the structural components of the type 1 and F1C fimbriae. We examined whether minor structural components originating from one fimbrial system could be accommodated by the other system when the incoming foreign minor components interacted with a system deficient in one or more of the minor components and also in the case of interaction of heterologous components with intact parental organelle systems, having

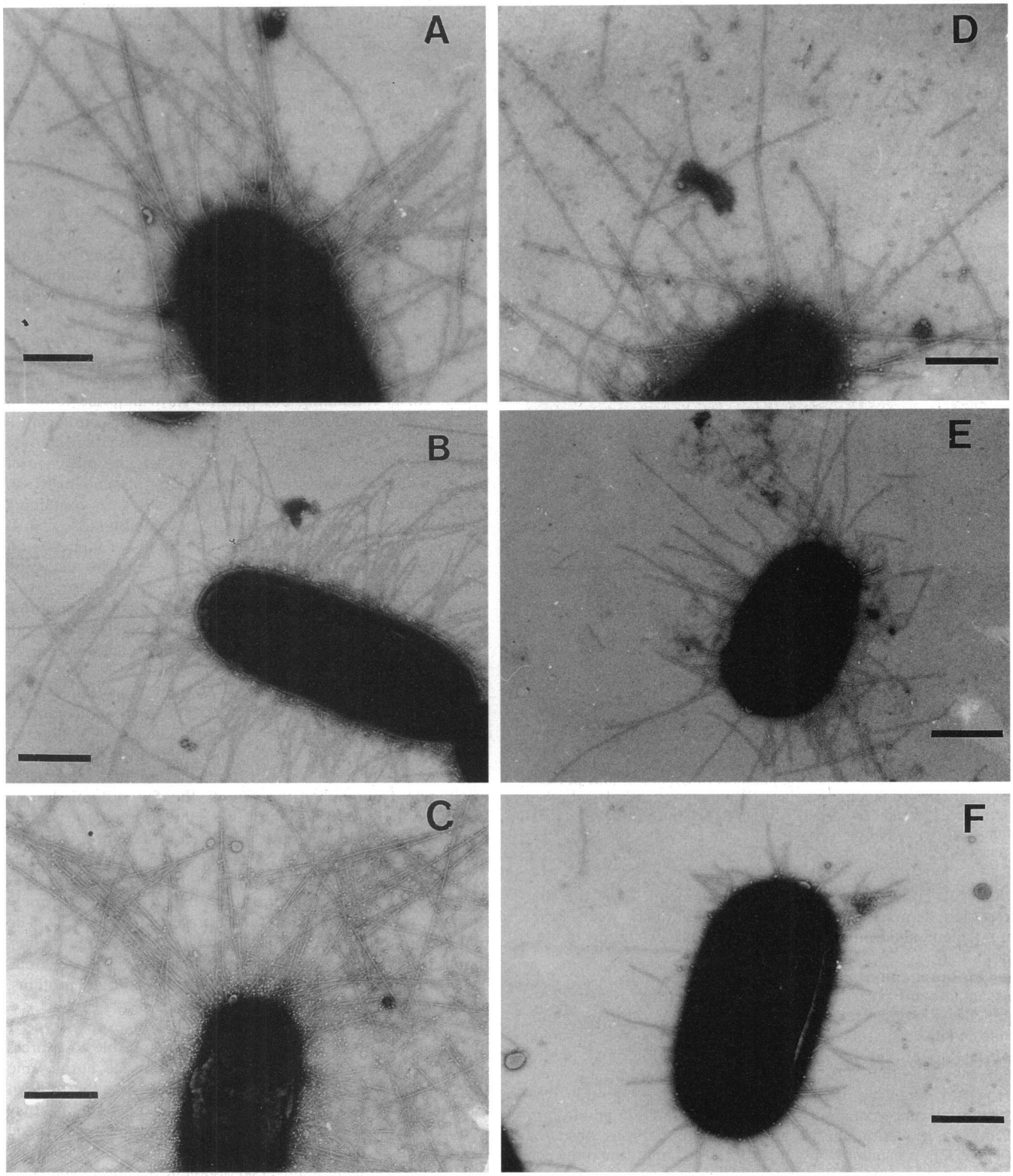

FIG. 2. Electron micrographs showing the effects of interaction of F1C fimbriae with incoming type 1 minor fimbrial components. *E. coli* K-12 *fim* hosts contained plasmids pPIL110-518 (*focH*) (A), pPIL110-518 plus pPKL65 (*fimH*$^+$) (B), pPIL110-518 plus pPKL64 (*fimG*$^+$ *fimH*$^+$) (C), pPIL110-518 plus pPKL52 (*fimF*$^+$ *fimG*$^+$ *fimH*$^+$) (D), pPKL143 (total *foc* gene cluster) (E), and pPKL143 plus pPKL65 (*fimH*$^+$) (F). Bars indicate 0.5 μm.

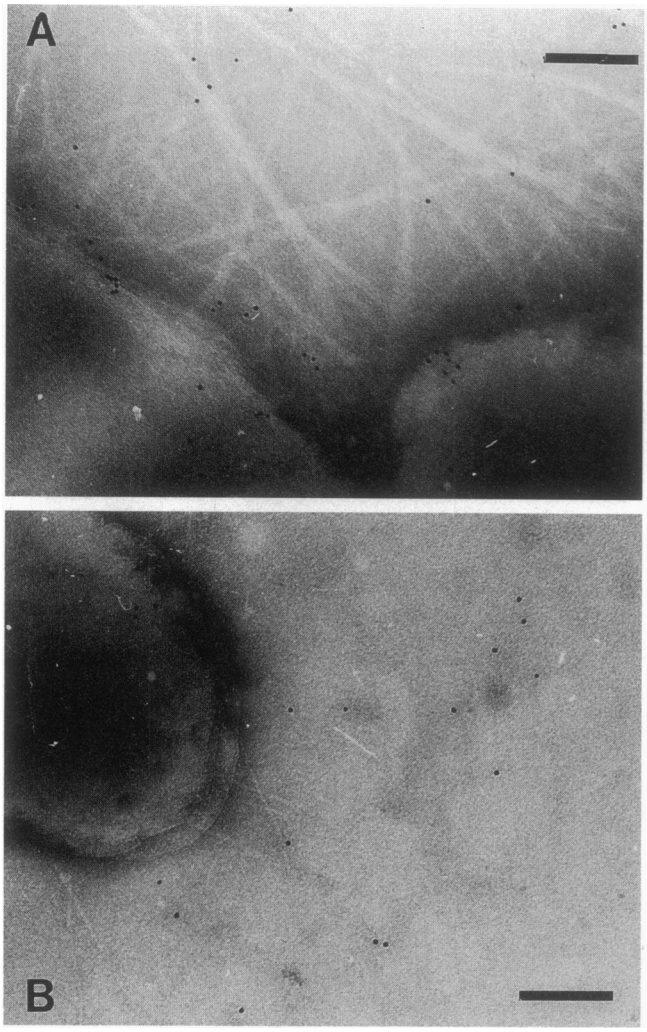

FIG. 3. Immunoelectron microscopy with colloid gold labelling of *E. coli* K-12 *fim* cells containing plasmids pPIL110-518 plus pPKL65 (A) and pPKL143 plus pPKL65 (B), using extensively adsorbed anti-type 1 serum (adsorbed with bacterial hosts expressing F1C fimbriae). Bars indicate 0.25 μm.

their full complement of structural elements. It is the first reported demonstration of complementation between structural elements from two fimbrial systems exhibiting limited homology.

Our observations indicate that FimH apparently is accepted as an integral part of F1C fimbriae, resulting in hybrid organelles with new receptor specificity, namely, for D-mannosides. This was seen in the combination of the plasmids pPIL110-518 and pPKL65 (Table 2 and Fig. 3A), which behaved essentially like the wild-type type 1 fimbrial system. However, when the incoming plasmid encoded the other type 1 fimbrial minor components, FimF and FimG, in addition to FimH, a reduction in the adhesive potential was observed (Table 2), presumably as a result of competition between minor components originating from different parental systems. A significant reduction of fimbriation was observed when combinations of type 1 fimbrial minor components interacted with an intact *foc* gene cluster, as shown in the combination of plasmid pPKL143 plus either plasmid pPKL52, pPKL64, or pPKL65 (compare Fig. 2E and F). This effect could probably also be ascribed to competition between the minor components from the two systems. Apparently, the *fim* components interfered with the *foc* system when the latter provided all of the components itself (plasmid pPKL143 encodes the wild-type *foc* gene cluster), resulting in a severe reduction of the amount of fimbriae on the cell surface. One could imagine that the presence of both FocH and FimH at the same time in the context of an intact *foc* system might result in formation of faulty initiation complexes, resulting in reduced fimbriation.

Similar results were observed going in the opposite direction, i.e., with F1C minor components in a type 1 background. The heterologous components seemed in general to be reasonably well accepted by type 1 systems deficient in one or more of the parental minor components, resulting in hybrid organelles with F1C receptor specificity and able to adhere to HEp-2 cells. However, accommodation of F1C minor components by intact type 1 organelle systems was less evident.

Minor component complexes have been shown to be involved in the initiation of fimbrial biosynthesis (14, 31, 32). Plasmid pPIL110-518 does not encode the minor component FocH. Hosts containing this plasmid form fimbriae fewer in number and longer than those formed by cells harboring a complete *foc* gene cluster, presumably because they initiate fimbrial biogenesis less efficiently. However, the presence of the minor component FimH, encoded by plasmid pPKL65, evidently partly alleviates this handicap, since hosts containing this plasmid in addition to pPIL110-518 exhibit a more fimbriated phenotype (Fig. 2C). Similar indications of influence on organelle number and length were also seen with incoming F1C minor components in a type 1 background.

There seems to exist a preference of F1C and type 1 fimbriae for integration of parental minor components. However, both systems are promiscuous; specifically, when parental components are not available, each system will readily accept available nonparental but compatible partners. This observation could perhaps also answer the more fundamental question of how wild-type strains, capable of expressing several fimbrial species, are able to avoid mixing these up during biosynthesis. However, there is no doubt that the minor components originating from either the *fim* or the *foc* system, despite showing only 34 to 41% identity, were integrated in the context of a foreign major structural protein. Furthermore, it is also noteworthy that heterologous minor components apparently were accepted by the nonparental biosynthesis machinery of F1C or type 1 fimbriae constituted by the FocC and FocD proteins, i.e., the FimC and FimD proteins, respectively. The molecular domains in the Fim and Foc minor components, required for recognition and interaction with the FocC/FimC chaperones and the FocD/FimD outer membrane proteins, must therefore be very similar.

About 80% of *E. coli* strains are able to express type 1 fimbriae. Additionally, many such strains are able to express other fimbrial species, e.g., F1C, S, and P fimbriae, at the same time (9, 28). Since our study indicates that the adhesin from one fimbrial species can piggyback on another system, one may wonder why *E. coli* uses a strategy employing complete gene clusters for each system instead of just having one flexible system functioning as a universal organelle acceptor capable of exposing various different adhesins. One possible explanation for this seemingly paradoxical behavior of a microbe known to be very resource minded could be that in vivo, the major fimbrial subunit protein is submitted to intense immunological pressure. This could explain why a more resource-demanding

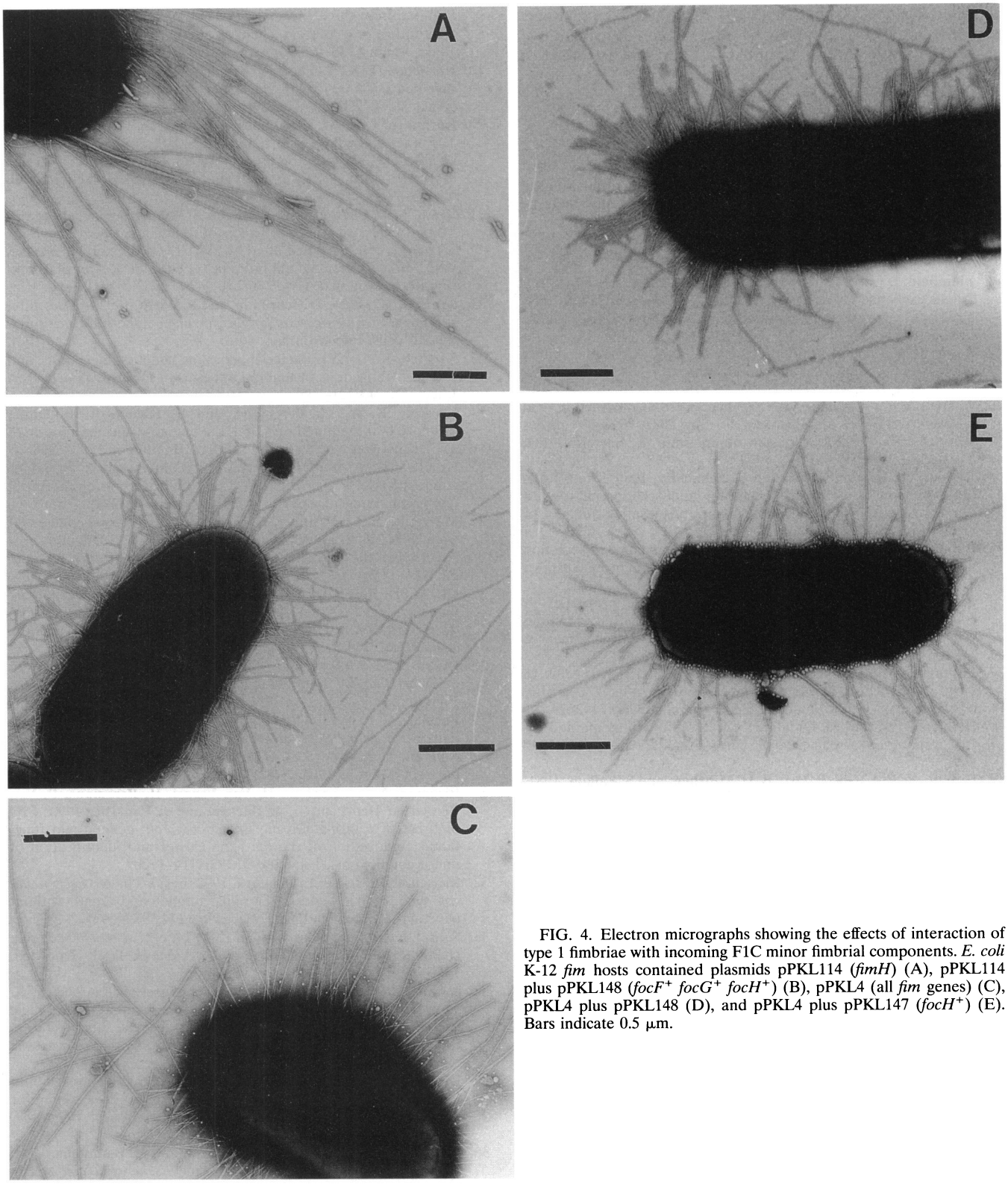

FIG. 4. Electron micrographs showing the effects of interaction of type 1 fimbriae with incoming F1C minor fimbrial components. *E. coli* K-12 *fim* hosts contained plasmids pPKL114 (*fimH*) (A), pPKL114 plus pPKL148 (*focF*$^+$ *focG*$^+$ *focH*$^+$) (B), pPKL4 (all *fim* genes) (C), pPKL4 plus pPKL148 (D), and pPKL4 plus pPKL147 (*focH*$^+$) (E). Bars indicate 0.5 μm.

approach, using several complete fimbria-encoding systems, might be worthwhile.

This study is an example of the ability of incomplete gene clusters to be activated by a different but homologous system, leading to expression of foreign adhesins by such systems. We think that this promiscuous behavior is highly significant in a biological context, since it demonstrates that a virulence factor, even when it is encoded by an incomplete gene cluster, can be

activated in a new host background. It is perhaps also an aspect that one should bear in mind in connection with deactivation of virulence factors in, for example, bacteria to be used for vaccines.

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REFERENCES

1. **Blomfield, I. C., V. Vaughn, R. F. Rest, and B. I. Eisenstein.** 1991. Allelic exchange in *Escherichia coli* using the *Bacillus subtilis sacB* gene and a temperature-sensitive pSC101 replicon. Mol. Microbiol. **5**:1447–1457.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementary analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
3. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
4. **Del Sal, G., G. Manfioletti, and C. Schneider.** 1989. The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. BioTechniques **7**:514–519.
5. **Dodd, D. C., and B. I. Eisenstein.** 1984. Dependence of secretion and assembly of type 1 fimbrial subunits on normal protein export. J. Bacteriol. **159**:1077–1079.
6. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of *Enterobacteriaceae*, p. 186–217. E. Beachey (ed.), Receptors and recognition, series B, vol. 6. Bacterial adherence. Chapman & Hall, London.
7. **Fujita, K., T. Yamamoto, T. Yokoda, and R. Kitagava.** 1989. In vitro adherence of type 1 fimbriated uropathogenic *Escherichia coli* to human ureteral mucosa. Infect. Immun. **57**:2574–2579.
8. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
9. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **51**:693–695.
10. **Källenius, G., R. Möllby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svenson, and B. Cedergren.** 1980. The P^k antigen as receptor for the haemagglutinin of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
11. **Klemm, P.** 1984. The *fimA* gene encoding the type 1 fimbrial subunit of *Escherichia coli*, nucleotide sequence and primary structure of the protein. Eur. J. Biochem. **143**:395–399.
12. **Klemm, P.** 1985. Fimbrial adhesins of *Escherichia coli*. Rev. Infect. Dis. **7**:321–339.
13. **Klemm, P.** 1992. *FimC*, a chaperone-like periplasmic protein of Escherichia coli involved in biogenesis of type 1 fimbriae. Res. Microbiol. **143**:831–838.
14. **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.
15. **Klemm, P., and G. Christiansen.** 1990. The *fimD* gene required for cell surface localization of *Escherichia coli* type 1 fimbriae. Mol. Gen. Genet. **220**:334–338.
16. **Klemm, P., B. J. Jørgensen, I. van Die, H. de Ree, and H. Bergmans.** 1985. The *fim* genes responsible for synthesis of type 1 fimbriae in *Escherichia coli*. Mol. Gen. Genet. **199**:410–414.
17. **Klemm, P., K. A. Krogfelt, L. Hedegaard, and G. Christiansen.** 1990. The major subunit of *Escherichia coli* type 1 fimbriae is not required for D-mannose specific adhesion. Mol. Microbiol. **4**:553–559.
18. **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.
19. **Korhonen, T. K., V. Väisanen-Rhen, M. Rhen, A. Pere, J. Parkkinen, and J. Finne.** 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. J. Bacteriol. **159**:762–766.
20. **Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Vaisanen-Rhen, J. Finne, F. Ørskov, and I. Ørskov.** 1985. Serotype, hemolysin production and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. **48**:486–491.
21. **Kreft, B., O. Carstensen, E. Straube, S. Bohnet, J. Hacker, and R. Marre.** 1992. Adherence to and cytotoxicity of *Escherichia coli* for eucaryotic cell lines quantified by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Zentralbl. Bakteriol. Infektionskr. Parasitenkd. Hyg. **276**:231–242.
22. **Kreft, B., R. Marre, O. Schramm, and R. Wirth.** 1992. Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured tubular cells. Infect. Immun. **60**:25–30.
23. **Krogfelt, K. A.** 1991. Bacterial adhesion: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Escherichia coli*. Rev. Infect. Dis. **13**:721–735.
24. **Krogfelt, K. A., H. Bergmans, and P. Klemm.** 1990. Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. Infect. Immun. **58**:1995–1998.
25. **Krogfelt, K. A., and P. Klemm.** 1988. Investigation of minor components of *Escherichia coli* type 1 fimbriae: protein chemical and immunological aspects. Microb. Pathog. **4**:231–238.
26. **Marre, R., B. Kreft, and J. Hacker.** 1990. Genetically engineered S and F1C fimbriae differ in their contribution to adherence of *Escherichia coli* to cultured renal tubulus cells. Infect. Immun. **58**:3434–3437.
27. **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.
28. **Ørskov, I., F. Ørskov, and A. Birch-Andersen.** 1980. Comparison of *Escherichia coli* fimbrial antigen F7 with type 1 fimbriae. Infect. Immun. **27**:657–666.
29. **Pugsley, A. P.** 1993. The complete secretory pathway in Gram-negative bacteria. Microbiol. Rev. **57**:50–108.
30. **Randall, L. L., and S. J. S. Hardy.** 1984. Export of protein in bacteria. Microbiol. Rev. **48**:290–298.
31. **Riegman, N., R. Kusters, H. Van Veggel, H. Bergmans, P. Van Bergen en Henegouwen, J. Hacker, and I. Van Die.** 1990. F1C fimbriae of a uropathogenic *Escherichia coli*: genetic and functional organization of the *foc* gene cluster and identification of minor components. J. Bacteriol. **172**:1114–1120.
32. **Russell, P. V., and P. E. Orndorff.** 1992. Lesions in two *Escherichia coli* type 1 pilus genes alter pilus number and length without affecting receptor binding. J. Bacteriol. **174**:5923–5935.
33. **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.
34. **Schmoll, T., H. Hoschützky, J. Morschhäuser, F. Lottspeich, K. Jann, and J. Hacker.** 1989. Analysis of genes coding for the sialic acid-binding adhesin and two other minor fimbrial subunits of the S-fimbrial adhesin determinant of *Escherichia coli*. Mol. Microbiol. **3**:1735–1744.
35. **Van Die, I., C. Kramer, J. Hacker, H. Bergmans, W. Jongen, and W. Hoekstra.** 1991. Nucleotide sequence of the genes coding for the minor fimbrial subunits of the F1C fimbriae of *Escherichia coli*. Res. Microbiol. **142**:653–658.
36. **Van Die, I., B. Van Geffen, W. Hoekstra, and H. Bergmans.** 1984. 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.
37. **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.