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Cloning and Characterization of Genes Involved in Production of Mannose-Resistant, Neuraminidase-Susceptible (X) Fimbriae from a Uropathogenic O6:K15:H31 *Escherichia coli* Strain

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The uropathogenic *Escherichia coli* strain 536 (O6:K15:H31) exhibits a mannose-resistant hemagglutination phenotype (Mrh) with bovine erythrocytes and delayed Mrh with human and guinea pig erythrocytes. Neuraminidase treatment of the erythrocytes abolishes mannose resistant hemagglutination, which is typical for X fimbriae. *E. coli* strain 536 synthesizes two different fimbriae (Fim phenotype) protein subunits, 16.5 and 22 kilodaltons in size. In addition the strain shows mannose-sensitive hemagglutination and common type I (F1) fimbriae. The cosmid clone *E. coli* K-12(pANN801) and another nine independently isolated Mrh$^+$ cosmid clones derived from a cosmid gene bank of strain 536 express the 16.5-kilodalton protein band, but not the 22-kilodalton protein, indicating an association of the Mrh$^+$ property with the "16.5-kilodalton fimbriae." All cosmid clones were fimbriated, and they reacted with antiserum produced against Mrh$^+$ fimbriae of the *E. coli* strain HB101(pANN801) and lacked mannose-sensitive hemagglutination (F1) fimbriae. From the *Mrh fim* cosmid DNA pANN801, several subclones coding for hemagglutination and X fimbriae were constructed. Subclones that express both hemagglutination and fimbriae and subclones that only code for the hemagglutination antigen were isolated; subclones that only produce fimbriae were not detected. By transposon Tn*5* mutagenesis we demonstrated that about 6.5 kilobases of DNA is required for the Mrh$^+$ Fim$^+$ phenotype, and the 1.5- to 2-kilobase DNA region coding for the structural protein of the fimbriae has been mapped adjacent to the region responsible for the Mrh$^+$ phenotype. Two different regions can thus be distinguished in the adhesion determinant, one coding for hemagglutination and the other coding for fimbria formation. Transformation of plasmid DNA from these subclones into a Mrh$^-$ Fim$^-$ mutant of *E. coli* 536 and into a *galE* (rough) strain of *Salmonella typhimurium* yielded transformants that expressed both hemagglutination and fimbria production.

Escherichia coli strains cause about 80% of all urinary tract infections (UTI) and, as with other extraintestinal infections in general, several surface antigens (O and K) and extracellular proteins (e.g., hemolysin [Hly phenotype]) have been implicated in the determination of their virulence (12, 16, 28, 30, 31). In addition, the ability of strains to mediate agglutination of erythrocytes from different vertebrates in the presence of mannose contributes to virulence of these urinary pathogens (10, 14, 15; Marre et al., submitted for publication). In most cases the mannose-resistant hemagglutination phenotype (Mrh) is associated with the formation of specific protein frimbriae (pili). Both fimbriae and hemagglutination antigens enable bacterial attachment (adhesion) to the urinary tract epithelial cells (34). Whereas mannose-sensitive hemagglutination, which is connected with so called "common type I" (F1) fimbriae is found on most *E. coli* strains and other enterobacteria (23, 30), the presence of the Mrh fimbriae seems to be specific for enteric and urinary pathogens of *E. coli* (8, 18). In fact more than 90% of UTI *E. coli* strains agglutinate human or bovine erythrocytes (or both) in a mannose-resistant manner compared with only 9% of isolates from the normal fecal flora (10; Hacker et al., manuscript in preparation).

Two different types of chromosomal-encoded Mrh fimbriae from uropathogenic *E. coli* strains can be distinguished. The P fimbriae use a common receptor, the Gal-Gal globoside, which is identical to the human blood group P antigen and is normally present on urinary tract epithelial cells (20, 24, 36). Genetic determinants that code for different P fimbriae have been cloned from UTI *E. coli* strains (3, 5, 17, 33, 37); and one of them, the *pap* determinant, has been described in detail (2, 29). Mrh fimbriae from UTI *E. coli* strain which recognize receptors other than the P antigen have been termed X fimbriae. One of these X-specific fimbriae can interact with glycophorin A and shows blood group M specificity (35). Other X fimbriae recognize a neuraminic acid-containing receptor, which in the case of several strains investigated is a neuraminyl-α-(2-3) galactoside (32). This galactoside is present on human and bovine erythrocytes. After treatment of erythrocytes with neuraminidase, *E. coli* strains that produce these X fimbriae are not able to evoke hemagglutination.

In a previous paper we described the isolation of two different recombinant cosmids derived from the *E. coli* strains 536 (O6:K15:H31) and 764 (O18:K5:H$^-$) which exhibited mannose-resistant hemagglutination (3). Here we present evidence that one of these recombinant cosmids shows a neuraminidase-sensitive hemagglutination. This hemagglutination property is connected with X-specific fimbriae that consist of 16.5-kilodalton (kd) protein subunits. The genetic determinant for both fimbriae and hemagglutination is a region of about 6.5 kilobases (kb). Subcloning and transposon mutagenesis of this region indicate that the genes

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TABLE 1. Agglutination of *E. coli* strains 536 and HB101 carrying various recombinant DNAs that code for the *mrh fim* determinant or part of it

E. coli strain	Recombinant plasmid	Plasmid marker	Hemagglutination[a]					Receptor-specific agglutination[b]				Agglutination with:	
			Hu	Bv	Ch	Mk	Gp	p̄	NN	Neuraminidase		*S. cerevisiae* cells[c]	Serum against *E. coli* pANN801[d]
										Human	Bovine		
536			(R)	R	S	S	(R)	(R)	(R)	−	−	S	+++
HB101										−	−	−	−
HB101	pANN801	Ap^r *mrh fim*	R	*R*			R	R	R	−	−	−	++++
HB101	pANN802	Ap^r *mrh fim*	R	*R*			R	R	R	−	−	−	+++
HB101	pANN807	Ap^r *mrh fim*	R	*R*			R	R	R	−	−	−	+++
HB101	pANN801-13	Ap^r *mrh fim*	R	*R*			R	R	R	−	−	−	++++
HB101	pANN801-1	Tc^r *mrh*	R	*R*			R	R	R	−	−	−	++++

[a] Hemagglutination was done with the following erythrocytes: Hu, human; Bv, bovine; Ch, chicken; Mk, monkey; Gp, guinea pig. R, mannose-resistant hemagglutination; S, mannose-sensitive hemagglutination. [(R) is weak, R is normal, *R* is very strong].

[b] Human blood of the groups p̄ and NN was used to elucidate the P and M receptor. Human and bovine blood was treated with neuraminidase to find out neuraminic acid specifity. Abbreviation as in footnote *a*.

[c] Agglutination with *S. cerevisiae* cells indicates type I (Msh) fimbriae.

[d] The *E. coli* specific antibodies were removed from the serum by adsorption.

for fimbria formation and hemagglutinatinin are distinguishable, but closely linked, and are located on the chromosome.

MATERIALS AND METHODS

Bacteria. *E. coli* strain 536 (O6:K15:H31), isolated from a patient suffering from a UTI, was obtained from the Institut für Hygiene and Mikrobiologie, Würzburg (3). Strain 536 exhibits mannose-resistant hemagglutination with bovine erythrocytes which can be increased on ice. Additionally, delayed hemagglutination with human and guinea pig erythrocytes can be observed (Table 1). The hemagglutination, however, was abolished after the treatment of erythroctes with neuraminidase. Further, human erythrocytes missing the P blood group factors—the receptor of P fimbriae—agglutinate when mixed with *E. coli* 536 cells. Thus the hemagglutination of *E. coli* 536 is termed X specific with affinity to a neuraminic acid-containing receptor (32). In addition, the strain shows a mannose-sensitive agglutination with *Saccharomyces cerevisiae* cells, indicating the presence of common type I fimbriae (21, 29). The Hly$^-$ Mrh$^-$ mutant 21 of strain 536 (13), *S. typhimurium* strain SF1572 (*galE*), and *E. coli* K-12 strain HB101 were used for transformation.

The recombinant plasmids used here are listed in Table 1 and Fig. 4. For Tn*5* mutagenesis we used a lambda Tn*5* phage, kindly provided by A. Pühler, which was propagated on *E. coli* K-12 strain C600.

Media, chemicals, and enzymes. Cultures were grown in enriched nutrient broth or in Luria broth. For mannose-resistant hemagglutination, strains were grown on CFA plates as described previously (7). Antibiotics used were a gift from Bayer, Leverkusen, Federal Republic of Germany. Restriction enzymes and T4 ligase were purchased from New England Biolabs, Beveton, Mass. All other chemicals were obtained from E. Merck AG, Darmstadt, Federal Republic of Germany.

Isolation of chromosomal and plasmid DNA. Chromosomal DNA was isolated as described previously (13). Plasmid DNA from clones carrying recombinant DNA was screened by the cleared lysate procedure (4), and preparative DNA isolation was achieved as described previously (11).

Cosmid cloning procedure. Recombinant DNA was packaged in vitro as described previously (3, 6).

Restriction and ligation. Cleavage of the DNA by restriction enzymes was performed at 37°C in suitable buffers. Gel electrophoresis was carried out in 1% agarose. Ligation was achieved after heat inactivation of the restriction endonucleases at 65°C for 6 min as described previously (3).

Transformation. *E. coli* K-12 strains were transformed by the $CaCl_2$ method. Wild-type strains were transformed by a modified $CaCl_2$ method (26).

Tn*5* mutagenesis. Tn*5* mutagenesis was achieved after absorption of a lambda Tn*5* phage (multiplicity of infection 0.1) to the plasmid-bearing HB101 strain (10^8 CFU) as described previously (1). Transposon mutants were checked for hemagglutination and the presence of fimbriae.

Agglutination. Hemagglutination types were determined with erythrocytes obtained either locally (human, bovine, and guinea pig) or from Flow Laboratories, Bonn, Federal Republic of Germany. Agglutination was assayed in phosphate-buffered saline with and without 1% mannose. For detection of the P receptor we used human p̄ blood kindly obtained from S. Normark (Umea, Sweden). M-specific receptor agglutination was carried out with human NN blood obtained locally. For screening of neuraminic acid receptor, neuraminidase-treated human and bovine erythrocytes were used (31). Msh (common type I) fimbriae were further determined by agglutination with *Saccharomyces cerevisiae* cells (30).

Preparation of fimbriae. Fimbriae were isolated from bacteria grown on plates as described previously (19).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fimbria protein subunits. After disintegration of the fimbriae by boiling for 5 min in 10 mM Tris-hydrochloride (pH 7.8) containing (in 1 ml) 4% sodium dodecyl sulfate, 0.01 ml of mercaptoethanol, 0.2 ml of glycerol, and 0.002% bromophenol blue, the fimbria samples (15 μg protein) were run on slab gels as described previously (19, 25).

Preparation of antisera. Mrh fimbriae antisera were prepared from rabbits as described previously (19).

Electron microscopy. Cells were grown overnight and washed with water before application to Formvar-coated grids. After soaking in 1% phosphotungstic acid (pH 6.4) for 1 min, the grids were examined under a Zeiss-10A transmission electron microscope.

RESULTS

Analysis of recombinant cosmids determining mannose-resistant hemagglutination. In a previous paper (3) we reported the isolation of a recombinant Mrh$^+$ cosmid with an inserted segment of chromosomal DNA from strain 536 into the vector pJC74. This *E. coli* K-12 clone, carrying the recombinant cosmid pANN801, exhibited the same mannose-resistant hemagglutination pattern as the parent strain 536

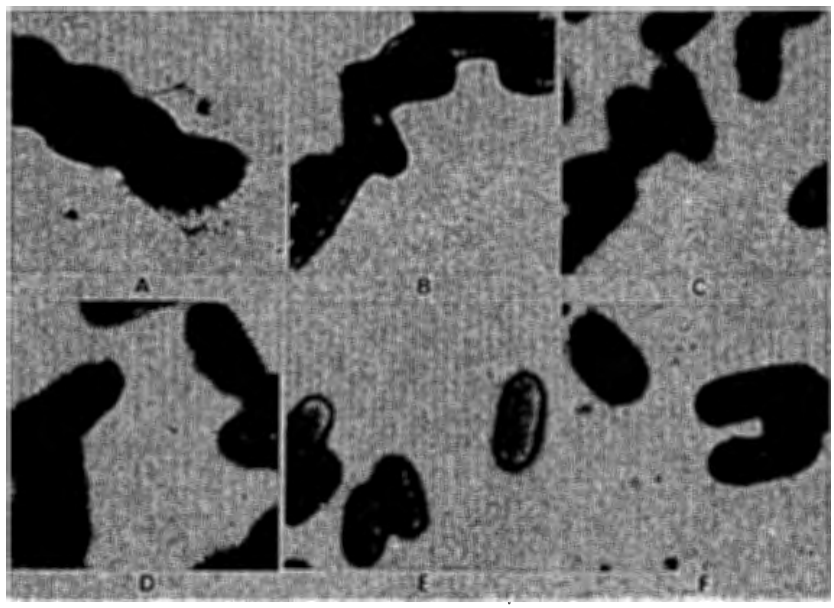

FIG. 1. Electron micrographs of the fimbriated and nonfimbriated *E. coli* strains. Panels: (A) *E. coli* 536, Mrh$^+$ Msh$^+$; (B) *E. coli* HB101; (C) *E. coli* HB101(pANN801-13), Mrh$^+$ Fim$^+$ (D) *E. coli* HB101(pANN801-13)::Tn*5*/O15, Mrh$^+$ Fim$^+$; (E) *E. coli* HB101(pANN801-1), Mrh$^+$ Fim$^-$; (F) *E. coli* HB101(pANN801-13::Tn*5*/742, reduced fimbria production, Mrh$^-$.

(Table 1). However, the intensity of hemagglutination of the *E. coli* K-12 clone was higher compared with that of the wild-type strain 536, suggesting an influence of the higher gene dose. More recently we constructed a new gene bank of strain 536 by cloning large partial *Sau*3A fragments (average size, 40 kb) into the vector pHC79. Among 800 colonies, we identified nine clones that were Mrh$^+$. All isolated clones exhibited the same hemagglutination pattern (e.g., pANN802 and pANN807 in Table 1). The presence of fimbriae could be demonstrated by electron microscopy in all 10 *E. coli* K-12 clones (3) (Fig. 1). When grown on agar, none of them had the ability to agglutinate *S. cerevisiae* cells, indicating the absence of common type I fimbriae. Further, the mannose-resistant hemagglutination of all 10 clones was sensitive to the treatment of erythrocytes with neuraminidase, suggesting the presence of neuraminic acid specific fimbriae on the respective bacteria (Table 1). Hemagglutination and fimbriae were optimally expressed after growing the strains at 37°C, but when cultivated at 18°C these properties were lost.

After purification of the fimbriae from the wild-type strain 536 and dissociation into protein subunits with sodium dodecyl sulfate, two different fimbria proteins were obtained on polyacrylamide gel electrophoresis, one 16.5 kd and the other 22 kd in size (Fig. 2, lane B), indicating that the strain 536 expresses two different fimbriae. All *E. coli* K-12 clones that code for mannose-resistant hemagglutination showed only the 16.5-kd protein band, but not the 22-kd protein band (see Fig. 2, lanes D and E, for pANN801 and pANN807 respectively). This indicates that the 16.5-kd pili are associated with the X-specific, mannose-resistant hemagglutination properties of the strain 536 and are produced independently of the 22-kd fimbriae and the mannose-sensitive hemagglutination (Msh) (F1) fimbriae.

Specific rabbit antiserum was raised against the Mrh fimbriae by immunization with the Mrh$^+$ Fim$^+$ *E. coli* K-12 clone carrying the cosmid pANN801, followed by adsorption with the plasmid-free *E. coli* K-12 strain. This specific serum showed a strong reaction with the parental *E. coli* strain 536 and with all Mrh$^+$ Fim$^+$ *E. coli* K-12 clones, but not with the plasmid-free K-12 strain (Table 1) or with *E. coli* K-12 strains carrying cloned P-fimbria genes (data not shown).

Restriction map of the recombinant cosmids expressing the Mrh$^+$ property. To detect the existence of common DNA fragments in the Mrh$^+$ recombinant cosmids, we cleaved the

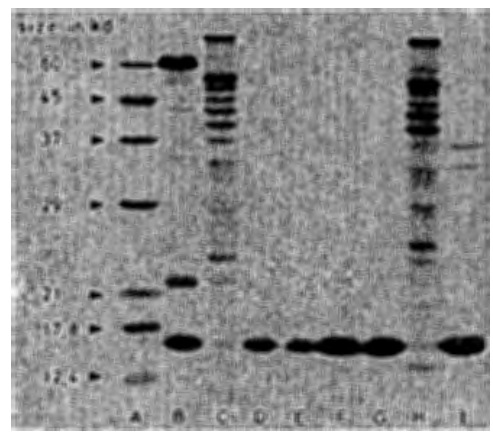

FIG. 2. Protein subunits of fimbriae from different *E. coli* strains on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. The fimbria proteins isolated from an equal number of cells were suspended in 200 μl of buffer. The volumes loaded on the gel are indicated. Lanes: (A) size marker proteins; (B) *E. coli* 536, Mrh$^+$ Fim$^+$ (20 μl; (C) *E. coli* HB101 (20 μl); (D), HB101(pANN801), Mrh$^+$ Fim$^+$ (1 μl); (E) HB101(pANN807), Mrh$^+$ Fim$^+$ (1 μl); (F) HB101(pANN801-13), Mrh$^+$ Fim$^+$ (1 μl); (G) HB101(pANN801-4) Mrh$^+$ Fim$^+$ (0.5 μl); HB101(pANN801-1), Mrh$^+$ Fim$^-$ (20 μl); (I) HB101(pANN801-13)::Tn*5*/742, with reduced fimbria production, Mrh$^-$ (7 μl).

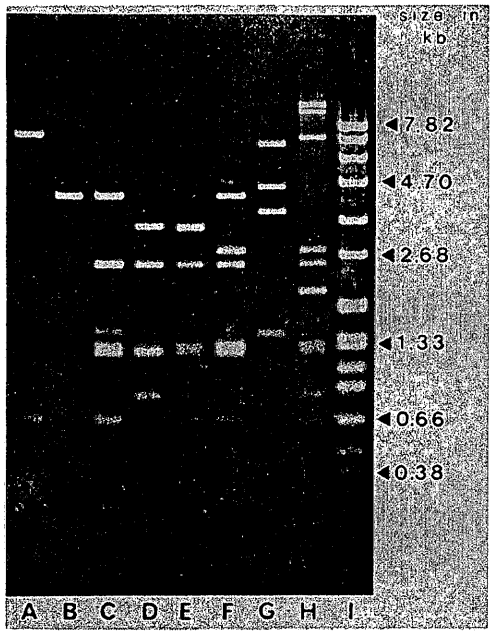

FIG. 3. Restriction patterns obtained after digestion of recombinant DNAs. Lanes: (A) pANN801-12 cleaved with *Pst*I, (B) pBR322 cleaved with *Pst*I, (C) pANN801-1 cleaved with *Pst*I, (D) pANN801-11 cleaved with *Pst*I, (E) pANN801-13 cleaved with *Pst*I, (F) pANN801-4 cleaved with *Pst*I, (G) pANN801-4 cleaved with *Eco*RI, (H) pANN801 cleaved with *Pst*I, (I) marker SPP1 phage DNA cleaved with *Eco*RI. The common *Pst*I fragments found in different DNAs tested are designated as P5, P8, P9, P10, P11, and P12 (for details, see the text).

DNA with the restriction enzymes *Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI, *Bg*lII, and *Sal*I. One *Eco*RI fragment of 4.7 kb and six *Pst*I fragments ranging in size from 0.5 to 2.9 kb were found to be present in all Mrh$^+$ recombinant cosmids tested (for pANN801, see Fig. 3). This led us to the assumption that these DNA fragments belong to the *mrh fim* determinant of the strain 536. To develop a suitable strategy for subcloning the *mrh fim* determinant, located on the 30-kb insert of the Mrh$^+$ Fim$^+$ cosmid pANN801, a physical map of this DNA was constructed. The six common *Pst*I fragments, designated P5 (2.6 kb), P9 (1.30 kb), P8 (1.35 kb), P11 (0.7 kb), P12 (0.5 kb), and P4 (2.9 kb), are linked together (Fig. 4). Two *Eco*RI sites flanking a 4.7-kb segment (see above; Fig. 4) were mapped in the region of the six *Pst*I fragments. The *Bam*HI site into which the chromosomal partial *Sau*3A fragment was ligated was still retained at one of the insert 0.2 kb from another *Pst*I fragment, p10.

Subcloning of the *mrh fim* determinant from pANN801. The 4.7-kb *Eco*RI fragment and the six *Pst*I fragments, which were assumed to include the coding region of the *mrh fim* determinant, are located on a *Bam*HI fragment of pANN801 that spans from the vector *Bam*HI site to a *Bam*HI site 14.8 kb to the right (Fig. 4). For subcloning this fragment, pANN801 DNA was digested with *Bam*HI, and the fragments were ligated into the *Bam*HI site of pBR322. The resulting DNA was usd to transform the non-hemagglutinating *E. coli* K-12 strain HB101. Transformants were subsequently tested for Mrh$^+$ activity. One transformant which exhibited mannose-resistant hemagglutination and fimbria formation possessed a 19.1-kb recombinant plasmid (pANN 801-6) corresponding to the predicted size (14.8-kb *Bam*HI fragment inserted into the 4.3-kb vector pBR322; Fig. 4). Thus, it was demonstrated that the coding region for mannose-resistant hemagglutination and fimbria production is located on the 14.8-kb *Bam*HI fragment of pANN801.

We then subcloned the 5.3-kb *Bam*HI-*Eco*RI fragment, which spans the region 0.2 kb left of P10 to the middle of P8 (Fig. 4), into pBR322 (pANN801-11). The 4.7-kb *Eco*RI fragment to the right of the 5.3-kb *Bam*HI-*Eco*RI fragment was cloned into the single *Eco*RI site of pACYC184 (pANN801-12). Neither the 4.7-kb *Eco*RI fragment nor the 5.3-kb *Eco*RI-*Bam*HI fragment possessed the complete information for fimbria synthesis and hemagglutination. Ligation of the 4.7-kb *Eco*RI fragment with *Eco*RI-cleaved pANN801-11 yielded the recombinant plasmid pANN801-

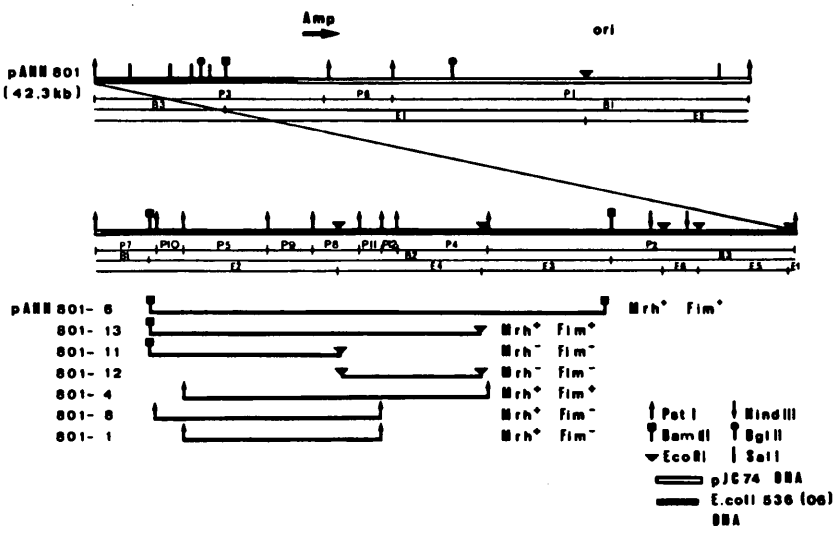

FIG. 4. Restriction map of the recombinant cosmid pANN801, which codes for the *mrh fim* determinant of strain 536 and of subcloned derivatives.

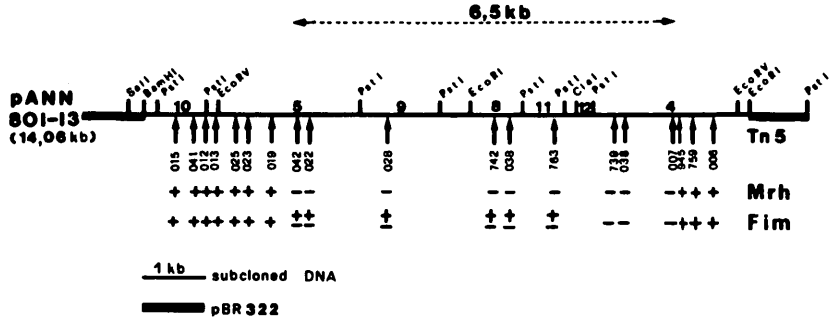

FIG. 5. Restriction map of the *mrh fim* subclone pANN801-13 and the location of Tn*5* insertions leading to the indicated mutant phenotypes (see the text for details).

13. Transformation of *E. coli* K-12 HB101 cells with this plasmid resulted in the production of fimbriae and mannose-resistant hemagglutination. This indicates that the 10.0-kb *Bam*HI-*Eco*RI fragment of pANN801 inserted into the vector plasmid pBR322 contains the genetic determinants encoding for hemagglutination and production of fimbriae.

To obtain more information on the organization of the *mrh fim* determinant we partially digested pANN801 DNA with *Pst*I and ligated the resulting fragments into the *Pst*I-cleaved pBR322. One of the recombinant plasmids, pANN801-4, which led to mannose-resistant agglutination and fimbria production, consisted of the *Pst*I fragments P5, P9, P8, P11, P12, and P4. As demonstrated by double digestions and by cleavage with the enzyme *Eco*RI (Fig. 3, lane G), the plasmid pANN801-4 carries a second copy of the P8 and P9 fragments between P5 and the vector *Pst*I site. In addition, subclones were obtained that carried only *Pst*I fragments P5, P9, P8, and P11. These coded only for hemagglutination, but not for the production of fimbriae (pANN801-1, pANN801-8). Thus, a region comprising P5, P8, P9, and P11 of pANN801 is necessary for the binding property to erythrocytes, but is not sufficient for the production of fimbriae.

Isolation of TN*5* mutants of pANN801-13. To define more precisely the region required for the Mrh$^+$ or Fim$^+$ phenotype (or both), a number of Tn*5* transposon insertion mutants of the Mrh$^+$ Fim$^+$ plasmid pANN801-13 were isolated and examined. After infection of strain HB101(pANN801-13) with a phage lambda Tn*5*, plasmid DNA was isolated and retransformed into HB101. Ap^r Km^r transformants were screened for hemagglutination and production of fimbriae. Mutants were obtained that were either negative in hemagglutination or in hemagglutination and fimbria production. From the location of the Tn*5* insertions in the mutants DNAs that are Mrh$^-$ or Fim$^-$ (or both), we conclude (Fig. 5) that the *mrh fim* determinant spans a region of about 6.5 kb. Tn*5* insertions located outside of this region did not alter the wild-type level of hemagglutination and fimbria production.

A region of almost 2 kb (proximal to the *Cla*I site in the P12 fragment) seems to be required for fimbria formation. Whole cells and cell extracts from Fim$^-$ mutants that contain Tn*5* insertions in this region were found to be devoid of the fimbria protein. Mutants with Tn*5* inserted in the *Pst*I fragments P9 and P8 and in part of P5 (nearly 4 kb in size) were phenotypically Mrh$^-$ Fim$^+$. The clones harboring plasmids with Tn*5* insertions in these *Pst*I fragments produce less fimbriae than does the original clone, but the subunit protein of these fimbriae had the same molecular size (16.5 kd) as the normal X fimbriae (Fig. 1F; Fig. 2, lane I). Surprisingly, Tn*5* insertions in the *Pst*I fragment P4 abolish mannose-resistant hemagglutination (in addition to fimbria production). On the other hand, subclone pANN801-1, which carries P5, P9, P8, and P11, but not P4 (Fig. 4), expresses this phenotype, indicating that P4 is actually not required for its determination. We therefore assume that P4 may carry a function(s) essential for the transcription of the genes involved in mannose-resistant hemagglutination that is likely to be located on P5, P9, P8, and P11. When these latter *Pst*I fragments are inserted into the *Pst*I site of the cloning vector pBR322, the promoter for the β-lactamase gene (*bla*) may enably the transcription of these genes.

Expression of hemagglutination and fimbria synthesis in strains possessing the subclones pANN801-1 and pANN801-13. The non-hemagglutinating strain *E. coli* K-12 was transformed with the recombinant DNAs pANN801-1 (Mrh$^+$ Fim$^-$) and pANN801-13 (Mrh$^+$ Fim$^+$). Both plasmids conducted the same hemagglutination pattern as the recombinant cosmid pANN801 from which they were derived (Table 1). The mannose-resistant hemagglutination was 5 to 10 times stronger than that of the wild-type strain 536. Hemagglutination was sensitive to treatment of erythrocytes with neuraminidase, and the strains reacted strongly with serum raised against pANN801-harboring cells. Mannose-sensitive agglutination of *S. cerevisiae* cells was not detectable. Strains carrying pANN801-13 produced fimbriae visible by electron microscopy, whereas a strain with pANN801-1 expressed only hemagglutination, but not visible fimbriae (Fig. 1C, E). In *E. coli* pANN801-13, both hemagglutination and fimbria production were fully expressed at 37°C, but a Mrh$^-$ Fim$^-$ phenotype was observed after incubation of the strain at 18°C. In contrast to the recombinant cosmid pANN801 and other Mrh$^+$ Fim$^+$ cosmids tested, the subclones pANN801-13 and pANN801-1 were stable in all *recA* transformants.

The two recombinant plasmids, pANN801-13 and pANN 801-1, were transformed into the Mrh$^-$ Fim$^-$ Hly$^-$ *E. coli* O6 mutant strain 536/21 (13) and into the *Salmonella typhimurium galE* (rough) strain SF1572. In both hosts the two plasmids exhibited the same phenotype as in the *E. coli* K-12 strains, e.g., mannose-resistant hemagglutination and fimbria production (pANN801-13) or mannose-resistant hemagglutination (pANN801-1) as determined again by agglutination of erythrocytes in the presence of mannose, electron microscopy, and precipitation of fimbria protein from extracts of these strains by specific antiserum.

DISCUSSION

Extensive work has been done in characterizing the cloned genetic determinants for the plasmid-encoded K88 and K99

adhesion factors isolated from *E. coli* strains that cause intestinal infections in animals (8, 9, 21, 22). More recently the chromosomally encoded genetic determinants of at least two different P fimbriae, often found in uropathogenic *E. coli* isolates were studied in detail (5, 29). Here we present a first genetic analysis of another chromosomally encoded adhesion factor from *E. coli* UTI strains, the so called X fimbriae.

The hemagglutination ability of this adhesin is not inhibited by the addition of mannose and cannot be observed after treatment of erythrocytes with neuraminidase, indicating that the Mrh fimbriae belong to the class of X fimbriae that recognize the neuraminic acid receptor molecule and are often found on O2, O6, and O18:K1 strains (32; Hacker et al., in preparation). The expression of the 16.5-kd protein subunit of the X fimbriae seems to require a nearly 2-kb region of the 6.5-kb *mrh fim* determinant, whereas the region coding for the hemagglutination is located on the 4-kb segment adjacent to the fimbria-encoding sequence. Two discrete regions for hemagglutination and fimbria production, respectively, can thus be distinguished in the X adhesion determinant.

These data suggest that the general organization of the X-fimbria determinant is similar to that of the *pap* determinant (P fimbriae) analyzed by Normark and co-workers (2, 29). They have also identified separate, but closely linked, loci for P-specific hemagglutination and fimbria synthesis (these fimbriae consists of 19.5-kd protein subunits) and regions involved in the regulation of the two functions. Although the basic organization of the adhesion determinants for P and X fimbriae seems to be similar, no similarity in the restriction patterns was found. Furthermore, Southern hybridization showed no homology between these two determinants (S. Knapp and B. Lund, unpublished results). The difference in the DNA sequence of *pap* and X-fimbria determinants is further reflected by differences in the size of the fimbria protein subunit and the different receptor specificities of these adhesins.

The X-fimbria determinant described here was isolated from the uropathogenic *E. coli* strain 536 belonging to the serotype O6:K15:K31. In addition to this X fimbria, the strain 536 exhibits two other fimbriae a presumably non-hemagglutinating fimbria, which consists of a 22-kd protein subunit, and the common type I (F1) adhesin, indicated by a strong mannose-sensitive agglutination of *S. cerevisiae* cells. All cosmid clones screened for their ability to agglutinate bovine and human erythrocytes in a mannose-resistant manner expressed fimbriae consisting of the 16.5-kd protein subunit, but not the 22-kd protein subunit. None of the 10 independently isolated Mrh^+ clones showed mannose-sensitive hemagglutination, as evidenced by their failure to agglutinate *S. cerevisiae* cells. Thus, strain 536 expresses at least three different adhesins with separate coding regions on the chromosome. The expression of two or three different types of fimbrial subunits was also described recently for P-fimbriated UTI *E. coli* strains (33, 37). For a O18:K5:H^-:F1:F8 strain, two cloned fimbria determinants have been mapped on different regions on the chromosome of that strain (38; G. Schmidt et al., manuscript in preparation). Nothing is known up to now on the regulation of the expression of these different fimbriae. The occurence of spontaneous mutants of strain 536 which have lost, together with the hemolysin determinant(s), the mannose-resistant (and even mannose-sensitive) hemagglutination has been recently described (13). Their analysis may help to understand the organization and regulation of these virulence determinants, which may often be linked in a block on the chromosome (27).

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LITERATURE CITED

1. **Ausubel, F. M., and G. B. Ruvleun.** 1981. A general method for site-directed mutagenesis in gram-negative procaryotes: isolation of nitrogen fixation mutants in *Rhizobium meliloti*, p. 124–127. *In* D. Schlessinger (ed.), Microbiology—1981. American Society for Microbiology, Washington, D.C.
2. **Baga, M., S. Normark, J. Hardy, P. O'Hanley, D. Lark, O. Olssson, G. Schoolnik, and S. Falkow.** 1984. Nucleotide sequence of the *pap*A gene encoding the Pap pilus subunit of human uropathogenic *Escherichia coli* J. Bacteriol. **157**:330–333.
3. **Berger, H., J. Hacker, A. Juarez, C. Hughes, and W. Goebel.** 1982. Cloning of the chromosomal determinants encoding hemolysin production and mannose-resistant hemagglutination in *Escherichia coli*. J. Bacteriol. **152**:1241–1247.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1522.
5. **Clegg, S., and J. K. Piece.** 1983. Organization of genes responsible for the production of mannose-resistant fimbriae of a uropathogenic *Escherichia coli* isolate. Infect. Immun. **42**:900–906.
6. **Collins, J., and B. Hohn.** 1978. Cosmids: a type of plasmid gene-cloning vector that is packageable in vitro in bacteriophage lambda heads. Natl. Acad. Sci. U.S.A. **75**:4242–4246.
7. **Evans, D. J., and D. G. Evans.** 1983. Classification of pathogenic *Escherichia coli* according to serotype and the production of virulence factors with special reference to colonization-factor antigens. Rev. Infect. Dis. **5**(Suppl. 4):S692–S701.
8. **Gaastra, W., and F. K. DeGraaf.** 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. **46**:129–161.
9. **Gaastra, W., F. R. Mooi, A. R. Stuitje, and F. K. DeGraaf.** 1981. The nucleotide sequence of the gene encoding the K88 ab protein subunit of porcine enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **12**:41–46.
10. **Green, C. P., and V. L. Thomas.** 1981. Hemagglutination of human type O erythrocytes, hemolysin production and serogrouping of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis, and asymptomatic bacteriuria. Infect. Immun. **31**:309–315.
11. **Grinstedt, J., P. M. Bennett, S. Higginson, and M. H. Richmond.** 1978. Regional preference of insertion of Tn501 and Tn802 into RP1 and its derivatives. Mol. Gen. Genet. **166**:313–320.
12. **Hacker, J., C. Hughes, H. Hof, and W. Goebel.** 1983. Cloned hemolysin genes from *Escherichia coli* that cause urinary tract infection determine different levels of toxicity in mice. Infect. Immun. **42**:57–63.
13. **Hacker, J., S. Knapp, and W. Goebel.** 1983. Spontaneous deletions and flanking regions of the chromosomally inherited hemolysin determinant of an *Escherichia coli* O6 strain. J. Bacteriol. **154**:1146–1152.
14. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg-Eden.** 1983. Contribution of adhesion to bacterial persistance in the mouse urinary tract. Infect. Immun. **40**:265–272.
15. **Hagberg, L., U. Jodal, R. K. Korhonen, G. Lidin-Janson, U. Lindberg, and C. Svanborg-Eden.** 1981. Adhesion, hemagglutination, and virulence of *Escherichia coli* causing urinary tract infections. Infect. Immun. **31**:564–570.
16. **Hughes, C., J. Hacker, A. Roberts, and W. Goebel.** 1983. Hemolysin production as avirulence marker in symptomatic and asymptomatic urinary tract infections caused by *Escherichia*

coli. Infect. Immun. **39**:546–551.
17. **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. **33**:933–938.
18. **Hull, R. A., S. I. Hull, and S. Falkow.** 1984. Frequency of gene sequence necessary for pyelonephritis-associated pili expression among isolates of enterobacteriaceae from human extraintestinal infections. Infect. Immun. **43**:1064–1067.
19. **Jann, K., B. Jann, and G. Schmidt.** 1981. SDS polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* from different pathogenic origin. FEMS Microbiol. Lett. **11**:21–25.
20. **Källenius, G., R. Möllby, S. B. Svenson, J. Winberg, A. Lundblad, and S. Svensson.** 1980. The p^k antigen as receptor of pyelonephritic E. coli. FEMS Microbiol. Lett. **7**:297–300.
21. **Kehoe, M., M. Winther, and G. Dougan.** 1983. Expression of a cloned K88ac adhesin antigen determinant: identification of a new adhesin cistron and role of a vector-encoded promoter. J. Bacteriol. **155**:1071–1077.
22. **Kehoe, M., M. Winther, G. Dowd, P. Morrissey, and G. Dougan.** 1982. Nucleotide sequence homology between the K88 and K99 adhesion system of enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **14**:129–132.
23. **Korhonen, T. K., K. Lounatmaa, H. Ranta, and N. Kuusi.** 1980. Characterization of type 1 pili of *Salmonella typhimurium* LT2. J. Bacteriol. **144**:800–805.
24. **Korhonen, T. K., V. Väisänen, H. Saxen, H. Hultberg, S. B. Svensson.** 1982. P-antigen-recognizing fimbriae from human uropathogenic *Escherichia coli* strains. Infect. Immun. **37**:286–291.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
26. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072–1074.
27. **Low, D., V. David, D. Lark, G. Schoolnik, and S. Falkow.** 1984. Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotype O4 and O6 isolates from urinary tract infections. Infect. Immun. **43**:353–358.
28. **Minshew, B. H., J. Jorgensen, G. W. Counts, and S. Falkow.** 1978. Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos at extraintestinal *Escherichia coli* isolates. Infect. Immun. **20**:50–54.
29. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Baga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside-binding adhesin from a uropathogenic *Escherichia coli* strain. Infect. Immun. **41**:942–949.
30. **Ørskov, I., and F. Ørskov.** 1983. Serology of *Escherichia coli* fimbriae. Prog. Allergy *33*:80–105.
31. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41**:667–710.
32. **Parkkinen, J., J. Finne, M. Achtman, V. Väisänen, and T. K. Korhonen.** 1983. *Escherichia coli* strains binding neuraminyl alpha 2-3 galactosides. Biochem. Biophys. Res. Commun. **111**:456–461.
33. **Rhen, M., J. Knowles, M. E. Penttilä, M. Sarvas, and T. K. Korhonen.** 1983. P. fimbriae of *Escherichia coli*: molecular cloning of DNA fragments containing the structural genes. FEMS Microbiol. Lett. **19**:119–123.
34. **Svanborg-Edén, C., L. Hagberg, L. Å. Hanson, S. Hall, R. Hull, U. Jodal, H. Leffler, H. Lomberg, and E. Straube.** 1983. Bacterial adherence–a pathogenetic mechanism in urinary tract infections caused by *Escherichia coli*. Prog. Allergy **33**:175–188.
35. **Väisänen, V., T. K. Korhonen, M. Jokinen, C. G. Gahmberg, and C. Ehnholm.** 1982. Blood group M specific haemagglutination in pyelonephritogenic *Escherichia coli*. Lancett **ii**:1192.
36. **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.
37. **Van Die, J., C. Vanden Hondel, H. J. Hamstra, W. Hoekstra, and H. Bergmanns.** 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 hemagglutination of human erythrocytes. FEMS Microbiol. Lett. **19**:77–82.
38. **Wevers, P., R. Picken, G. Schmidt, B. Jann, K. Jann, J. R. Golecki, and M. Kist.** 1980. Characterization of pili associated with *Escherichia coli* O18ac. Infect. Immun. **29**:685–691.