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Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation

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A chromosomal DNA fragment which mediates Pap (pili associated with pyelonephritis) pili formation, mannose-resistant hemagglutination (MRHA) and binding to uroepithelial cells has been isolated from the uropathogenic *Escherichia coli* clinical isolate J96, and genetically studied. Analysis of polypeptides expressed by the Pap DNA led to detection of a number of polypeptides ranging in mol. wt. from 13 000 to 81 000 daltons. The gene order and transcriptional orientation for four of the corresponding cistrons was: 13 000 *(papB)* 19 500 (*papA*, structural gene for the Pap pilus subunit), 81 000 *(papC)* and 28 500 *(papD)*. Analyses of a *lacZ-papA* gene fusion located a promoter upstream from *papA* within the cloned DNA. Transposon Tn*5* insertions in any of these four cistrons decreased or eliminated Pap pili formation. A number of transposon Tn*5* mutations were identified in a region distal to *papD* that expressed normal levels of the *papA* protein on the cell surface in the form of recognizable pili structures but did not agglutinate human erythrocytes or adhere to uroepithelial cells. This region expressed polypeptides of 15 000, 24 000, 26 000 and 35 000 daltons. This finding shows that Pap pili formation and binding properties can be genetically dissociated.
Key words: urinary tract infection/pili assembly/Tn*5* mutants/virulence factor

Introduction

Some pathogenic bacteria may localize to the site of infection by binding to receptors on mucosal membranes. In the unobstructed urinary tract for example, the severity of infection correlates with the adhesive capacity of the infecting *Escherichia coli* strain (Svanborg-Edén *et al.*, 1983b). The attachment of the majority of *E. coli* isolated from children with acute pyelonephritis to human urinary tract epithelial cells (Leffler and Svanborg-Edén, 1981; Källenius *et al.*, 1981; Väisänen *et al.*, 1981) may be explained by binding to globoseries glycolipid receptors with α-D-Gal*p*-(1-4)-β-D-Gal*p* in a terminal or internal position (Leffler and Svanborg-Edén, 1980). Bacterial binding to the same receptors in human erythrocytes results in hemagglutination (Källenius *et al.*, 1980). Morphological data which imply that pili function as adhesins on uropathogenic *E. coli* are based on the strong correlation between the presence or absence of pili seen by electron microscopy and the adhesive capacity of these bacteria (Duguid, 1968; Svanborg-Edén and Hansson, 1978). Biochemical data, using pili separated from the bacteria, have also suggested that pili possess the functional properties of adhesins in that such preparations retained hemagglutinating ability and binding specificity *in vitro* (Svanborg-Edén *et al.*, 1983a; O'Hanley *et al.*, 1983). To determine the actual role played by pili as a virulence factor, we have begun a molecular genetic analysis of uropathogenic *E. coli*. The aim is to define the essential genetic determinants, assembly pathway and regulation of pilus production.

A chromosomal DNA fragment which confers mannose-resistant hemagglutination (MRHA) and uroepithelial attachment to *E. coli* K12 has been isolated from the uropathogenic clinical *E. coli* strain J96 (Hull *et al.*, 1981). By subcloning into the plasmid vector pACYC184 (Chang and Cohen, 1978) a derivative, plasmid pRHU845, was constructed which carries 11.1 kb of the original chromosomal DNA. This hybrid plasmid, when harboured in *E. coli* K-12, mediates expression of Pap (pili associated with pyelonephritis) pili, MRHA, and binding to a digalactoside receptor analogue (Normark *et al.*, 1983). In minicells the chromosomal insert expresses at least 11 resolvable polypeptides. A 19 500 dalton polypeptide was identified as the Pap pilin subunit with anti-Pap antibodies, and its structural gene, *papA*, has been located on the physical map of pRHU845 (Normark *et al.*, 1983) and DNA sequenced (Båga *et al.*, 1984).

In this study we localize a number of *pap* cistrons and present a first characterization of their role in Pap pili formation. A number of transposon insertional mutants have been analyzed for expression of pili antigen, pili fiber formation and for adhesion to human erythrocytes and uroepithelial cells. Our results suggest that Pap pili formation alone may not be sufficient for expression of adhesion and that 'accessory' gene products which are non-essential for polymerization of pilin subunits into pili may be required for Pap pili to act as an adhesin.

Results

Identification of coding regions for proteins expressed in minicells by the Pap DNA of plasmid pRHU845

The hybrid plasmid pRHU845 carries an 11.1-kb chromosomal segment from the uropathogenic *E. coli* strain J96 and confers piliation and MRHA when harboured in *E. coli* HB101 (Normark *et al.*, 1983). The chromosomal DNA inserted in this plasmid-mediated expression of a large number of polypeptides most of which were shown to be produced in a precursor form (Normark *et al.*, 1983). To establish the organization of the different coding regions in the Pap DNA we performed SDS-polyacrylamide gel analyses of proteins produced by cut-back derivatives and subclones of pRHU845 (Figure 2), and by a series of Tn*5* insertional mutants (Figure 3). The positions for the Tn*5* insertional mutants and the gene localizations given in the text are map-

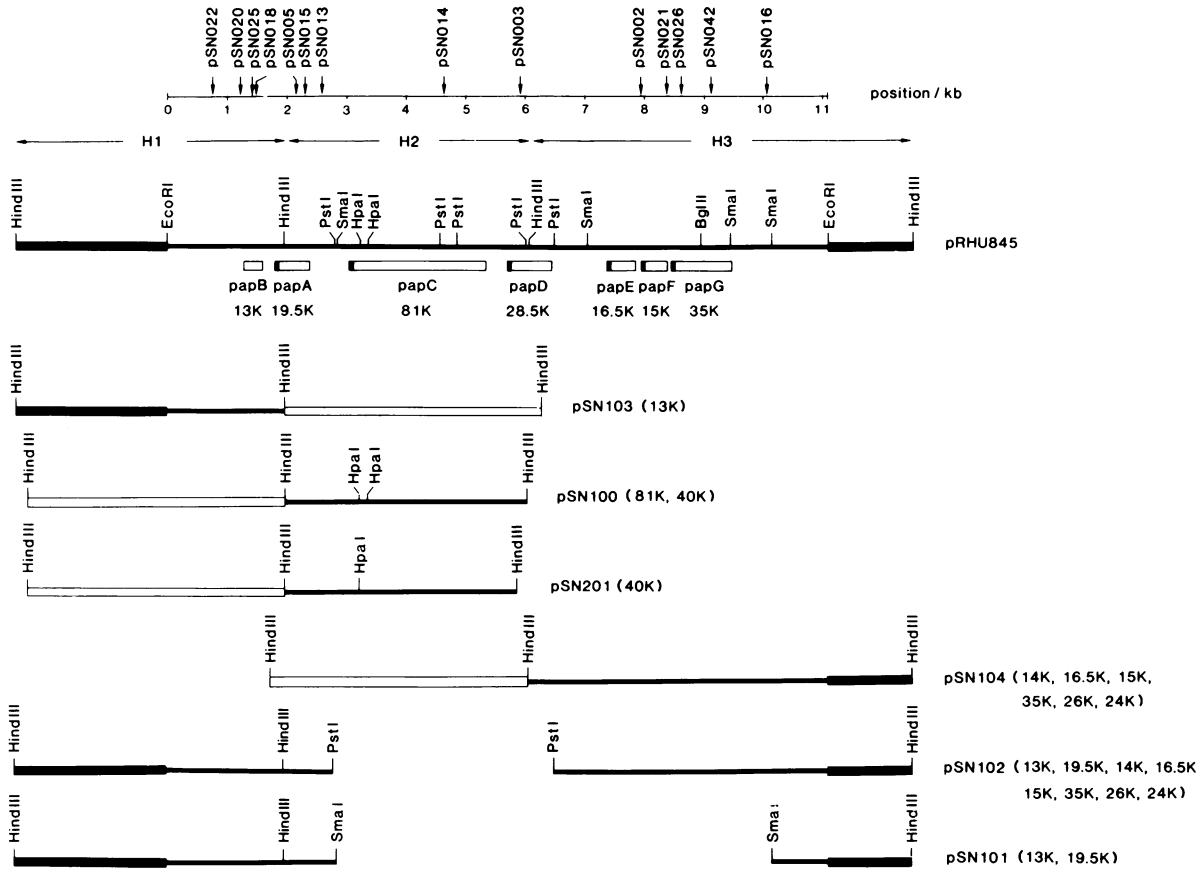

Fig. 1. Genetic organization of Pap DNA in pRHU845. The upper line shows the size (in kilobase pairs) of the *Eco*RI fragment inserted in plasmid pACYC184. The positions for various Tn*5* insertions are given above. The restriction map of pRHU845 and the positions for identified *pap* genes are given. The thick vertical bar represents the region coding for the signal peptide. The designations under the bars refer to the mol. wts. (x 10^3 dalton units) of the mature polypeptides. Subclone and cut-back derivatives of pRHU845 used in the identification of various *pap* genes are given below. The *pap* polypeptides expressed in the different constructs are given within brackets.

ped in relation to the left *Eco*RI site (Figure 1).

The structural gene *papA* coding for the 19 500-dalton Pap pilus subunit has recently been exactly localized by DNA sequencing (Båga *et al.*, 1984), and is positioned between 1.8 and 2.3 kb. Two Tn*5* insertions (pSN005 and pSN015) were mapped within *papA*. A 13 000-dalton polypeptide (P13) was located transcriptionally upstream from *papA*. This polypeptide was expressed from plasmids pSN101, pSN102 and pSN103 (Figure 2, lanes c, d and g), and its expression was inactivated by Tn*5* insertional mutants pSN020, pSN025 or pSN018 (Figure 3, lane o, and data not shown). The gene *papB* for P13 must therefore be positioned between 1.1 and 1.8 kb. This has recently been confirmed by DNA sequencing (Båga *et al.*, in preparation). pSN103 expressed no other polypeptides from pRHU845 than P13 (Figure 2, lane g). Moreover, Tn*5* insertions in pRHU845 <1 kb from the left *Eco*RI site did not affect the polypeptide pattern in minicells (data not shown).

An 81 000-dalton protein (P81) was encoded by pRHU845 as well as by subclone pSN100 (Figure 2, lanes a, f, and i). A Tn*5* insertion at position 4.6 kb (pSN014) abolished expression of P81, whereas two other Tn*5* insertions within the *Hind*III fragment H2, pSN013 at 2.6 kb and pSN003 at 5.9 kb, did not. Expression of P81 was, however, much reduced in pSN013, suggesting that transcription of its corresponding gene, *papC*, must be the same as for *papA*, i.e., from left to right (Figure 3, lanes g, l, m and p). A deletion of a 130-bp *Hpa*I fragment at position 3.2 kb abolished expression of P81 (Figure 2, lane j), suggesting that the 5′ end of *papC* is located between this site and the Tn*5* insertion site in pSN013 at position 2.6 kb.

The coding size for an 81 000-dalton polypeptide is ~2.2 kb. The 3′ end of *papC* must, therefore, be located close to position 3.0 kb. A 40 000-dalton polypeptide P40 is also expressed from *Hind*III fragment H2. Since its expression in pSN100 is unaffected by the *Hpa*I cut-back deletion (Figure 2, lane j), we tentatively place the coding region for this polypeptide between the *Hpa*I sites at 3.2 kb and the *Hind*III site at 6.0 kb. At this stage we are not able to localize more precisely the coding region for P40. The Tn*5* insertion mutant pSN003 did not express a 28 500-dalton protein (P28.5) (Figure 3, lanes g and q). This insertion markedly decreased the abundance of other *pap* proteins such as the *papA* pilin. Neither the three *Hind*III subclones (pSN100, pSN103, pSN104) nor the *Pst*I cutback derivative (pSN102) expressed P28.5 (Figure 2) supporting our conclusion that the corresponding gene, *papD*, spans the *Hind*III site at 6.0 kb.

Comparing the expression in minicells from pSN101, pSN102 and pSN104 with that of pRHU845 (Figure 2) and the vector plasmids, pACYC184 and pBR322, reveals that polypeptides of 35 000 (P35), 26 000 (P26), 24 000 (P24), 16 500 (P16.5), 15 000 (P15) and 14 000 (P14) daltons in mol. wt. are specified by the right part of the chromosomal insert on pRHU845. P16.5 was expressed by pSN102 and pSN104

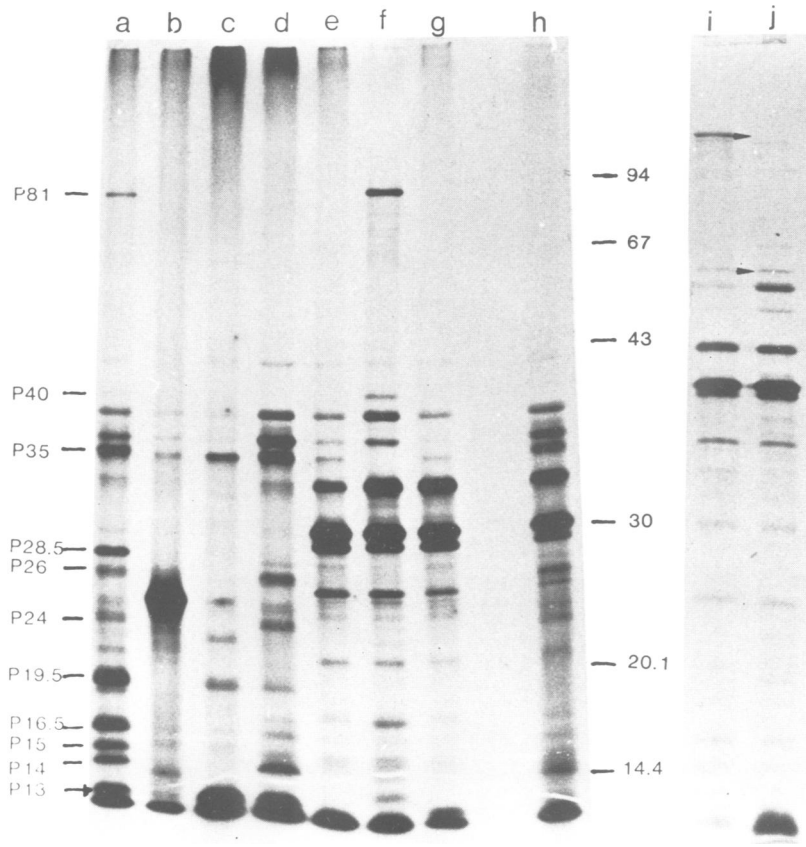

Fig. 2. Minicell analysis of cut-back and subclone derivatives of pRHU845 in strain P678-54. **(a)** pRHU845, **(b)** pACYC184, **(c)** pSN101, **(d)** pSN102, **(e)** pBR322, **(f)** pSN100, **(g)** pSN103, **(h)** pSN104, **(i)** pSN100, **(j)** pSN201. **Lanes a–h** show a fluorograph of a 12–17.5% polyacrylamide gradient gel whereas **lanes i** and **j** are from a 17.5% continuous gel. Positions of proteins discussed in the text are indicated on the left side. In **lanes i** and **j** relevant proteins are indicated by arrows. In the figure mol. wt. (x 10^3) dalton units of Pharmacia standard proteins are also indicated.

(Figure 2), and the expression was not affected by insertional mutants pSN021, pSN002, pSN042 or pSN016. P15 was not expressed in mutant pSN021 and probably not in pSN002. This tentatively maps the genes for these two *papE, papF* in the region 6.5–8.3 kb on pRHU45 (Figure 1). In the insertional mutant pSN042, proteins P35, P26 and P24 were not expressed. Instead, new polypeptides with mol. wts. of 25 500, 24 500 and 14 500 daltons were detected (Figure 3, lane c). The fact that a Tn*5* insertion (pSN016) only 1 kb distal to that in pSN042 expressed P35, P26, as well as P24 (data not shown) strongly argues that the region specifying all these polypeptides is located proximal to the Tn*5* insertion in pSN016. Mutant pSN026 carries, a Tn*5* insertion 0.5 kb upstream from that in pSN042. The former clearly expressed P26, and P24 but not P35 (Figure 3, lane d). Consequently, a number of polypeptides on pRHU845 must be specified from a region not larger than 1.7 kb, which is the distance between the Tn*5* insertions in pSN021 and pSN016. The gene *papG* coding for P35 is tentatively positioned in Figure 1. The positions for the three genes *papE, papF* and *papG* are more exactly localized in the accompanying paper (Lindberg *et al.*).

To determine if the Pap DNA insert in pRHU845, contains initiation signals for transcription and translation we utilized the *lacZ* gene fusion methodology (Casadaban *et al.*, 1983). Since the nucleotide sequence of *papA* has been determined (Båga *et al.*, 1984), and the exact location and orientation of the gene therefore is known, we constructed a *papA-lacZ* hybrid gene by inserting the 2-kb *Eco*RI-*Hind*III fragment of pRHU845 (Figure 1) into the *lacZ* fusion vector pSKS107. The Lac^+ phenotype of the construction (plasmid pBEU80) demonstrated that the 2-kb *Eco*RI-*Hind*III fragment indeed promotes transcription and translation. Expression is therefore not dependent on some vector promoter in pRHU845. Recent experiments monitoring β-galactosidase expression with plasmid pBEU80 under different growth conditions, have demonstrated that the 2-kb *Eco*RI-*Hind*III fragment contains Pap regulatory functions determining temperature-dependent expression of the pili (Göransson *et al.*, in preparation),

Expression of Pap antigen, surface pili, and MRHA by Tn5 mutant derivatives of pRHU845

Expression of Pap antigen, surface-bound pili and MHRA were monitored after introduction of different Tn*5* insertion derivatives of pRHU845 into the *E. coli* K-12 strain HB101. As shown in Figure 4A, Tn*5* insertions between 0 and 0.5 kb did not affect the amount of Pap antigen expressed on intact cells suggesting that the promoter for *papA* is located downstream from this region. The fact that insertions in *papB* (pSN020, pSN025, pSN018) markedly decreased the amount of Pap antigen expressed argues that these insertions either cause polarity effects on *papA* and/or that *papB* itself is involved in Pap regulation. As expected, Tn*5* insertions in *papA* abolished expression of Pap antigen as well as formation of morphologically detectable pili. Insertional inactivation of either *papC* or *papD* resulted in no detectable surface expressed Pap antigen. No pili could be seen on cells carrying these mutants. However, low, but significant, amounts of

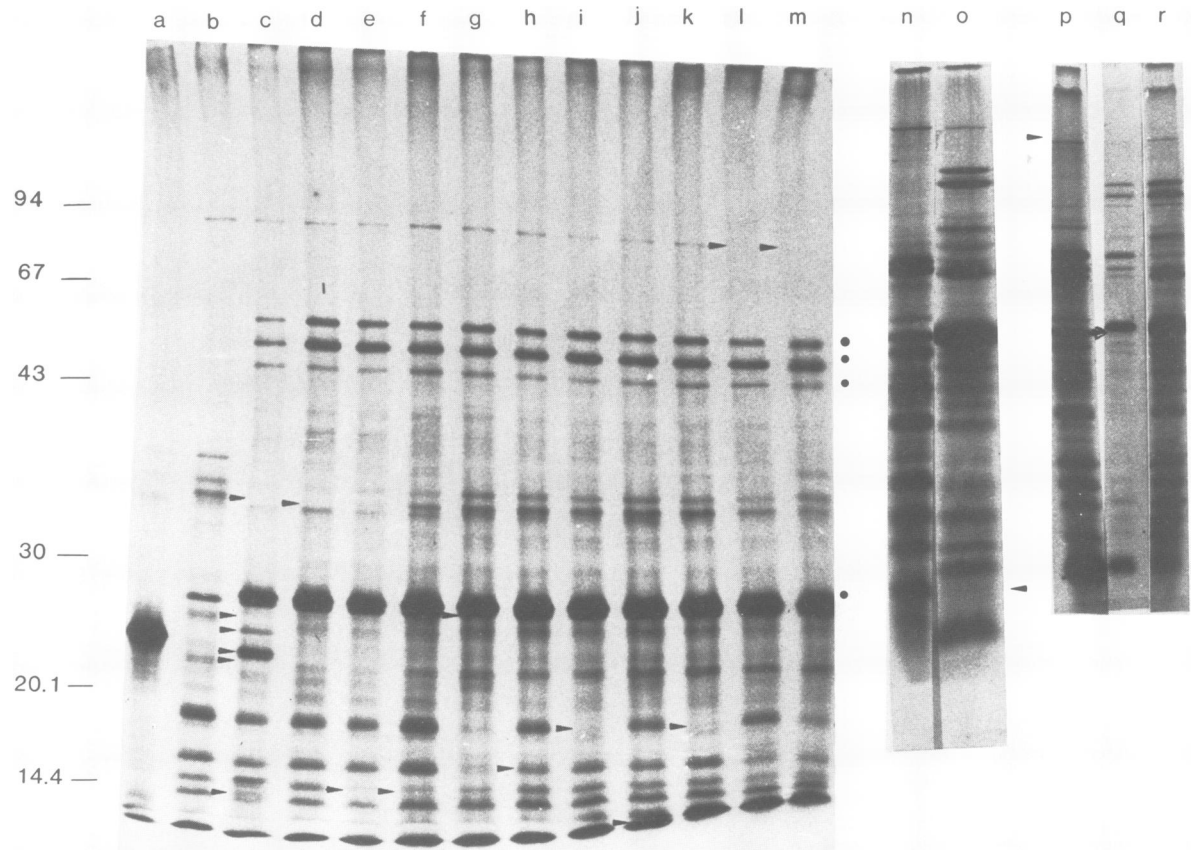

Fig. 3. Minicell analysis of Tn*5* insertional mutants of pRHU845 derivatives in strain P678-54. **(a)** pACYC184, **(b)** pRHU845, **(c)** pSN042, **(d)** pSN026, **(e)** pSN021, **(f)** pSN002, **(g)** pSN003, **(h)** pSN023, **(i)** pSN015, **(j)** pSN020, **(k)** pSN005, **(l)** pSN014, **(m)** pSN013, **(n)** pRHU845, **(o)** pSN020, **(p)** pSN013, **(q)** pSN003, **(r)** pRHU845. **Lanes a–l** show fluorographs of a 12–17.5% polyacrylamide gradient gel, **lanes n** and **o** are from a 15% continuous gel and lanes **p–r** are from a 10–17% polyacrylamide gradient gel. Positions of proteins discussed in the text are indicated by arrows and mol. wts. (x 10^3) dalton units of Pharmacia standard proteins are indicated on the left side. Proteins encoded by transposon Tn*5* are indicated by filled dots.

Pap antigen were detected in extracts of *E. coli* HB101 harboring such mutants (Figure 4A). These data suggested that *papC* and *papD* may be important for surface localization of the *papA* gene product.

All Tn*5* insertions within the region coding for *papB, papA, papC* and *papD* were negative for hemagglutination when harboured in *E. coli* HB101. Tn*5* insertions in the 7.8–9.5 kb region also completely abolished MHRA. However, the amount of Pap antigen was not affected by these insertions (Figure 4). Moreover, pili were readily visualized on HB101 cells harbouring representatives of this class of Tn*5* insertions (Figure 5). Pili purified from pSN021 showed the same subunit mol. wt. as pRHU845 (data not shown). Consequently, the distal *pap* region is not required for Pap pili formation but evidently encodes for gene products essential for mannose-resistant hemagglutination.

Attachment to human uroepithelial cells in vitro

The finding that there were MRHA-negative mutants of pRHU845 which still expressed morphologically recognizable pili, prompted us to test how such a phenotype might affect the adhesive properties of host bacterial cells for human uroepithelial cells. Human cells exfoliated into the urine were employed for this analysis (Svanborg-Edén *et al.*, 1977). Neither of the *papA* mutants tested, pSN005 and pSN015, nor the MRHA-negative mutants, pSN021 and pSN042, showed significant binding to the epithelial cells, as compared with controls (Table I). Similar results, i.e., no attachment, were obtained with the Tn*5* insertion mutants pSN003, pSN013, pSN014, pSN023, pSN025 and pSN026, whereas pSN016, mutated outside the Pap cistrons, showed the same binding as pRHU845. The results with pSN021 and pSN042 demonstrated that functional attachment to uroepithelial cells requires genetic information located outside the cistrons required for Pap pili formation.

Discussion

We have characterized a group of genes, which appear to act in concert to bring about the biosynthesis of a unique pilus, and the specific adherence of host bacterial cells to a receptor found on human erythrocytes and uroepithelial cells. The structural subunit of the pilus is encoded by the structural gene *papA* (Normark *et al.*, 1983). Pap pili isolated from *E. coli* K-12 harbouring pRHU845 retained the binding attributes of the clinical isolate from which the gene cluster was isolated. Thus, isolated pili preparations agglutinate human erythrocytes and digalactoside-coated latex beads (O'Hanley *et al.*, 1983). The isolated pili also bound to a bovine serum albumin-digalactoside conjugate in a solid phase binding assay (O'Hanley, personal communication). Not unexpectedly two Tn*5* insertions, within the *papA* structural gene pSN005 and pSN015, had profound effects on pili formation and antigenicity. Furthermore, pSN015, was completely negative for hemagglutination in all *E. coli* backgrounds tested. However, pSN005, carrying a Tn*5* insertion ~100 bp further 'upstream' in the *papA* gene, mediated a weak

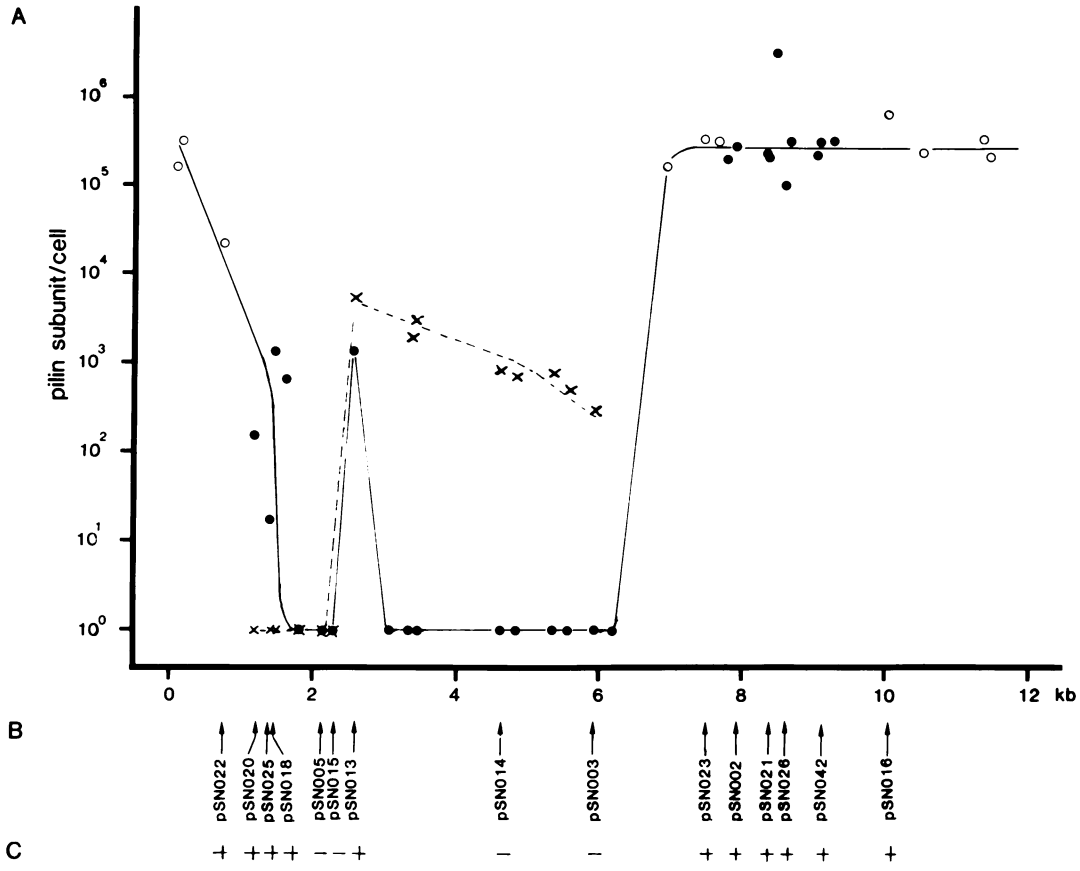

Fig. 4. **(A)** Expression of Pap antigen and MHRA by Tn*5* insertional mutants. The Y-axis indicates the number of pili subunits expressed on the cell surface or in cell extracts of various Tn*5* insertional mutants in HB101 background, as calculated from a competitive ELISA. The Tn*5* insertions further discussed in the text are given below. Purified Pap pili were used as a standard to convert adsorption to amount of pili antigen. The number of pilin subunits per cell was calculated from the known mol. wt. of the Pap pilin and Avogadros number. ● indicate MRHA-negative mutants; ○ = MRHA-positive mutants; x results from an ELISA assay with lysed cells. **(B)** Location of selected Tn*5* insertional mutants mentioned in the text. **(C)** Pili fiber phenotype as detected by electron microscopy.

MRHA$^+$ phenotype in one *E. coli* derivative (Normark *et al.*, 1983). Nevertheless, no binding to uroepithelial cells was found for this mutant.

In addition to *papA* we have defined three other cistrons *papB, papC* and *papD* encoding for 13 000-, 81 000- and 28 500-dalton polypeptides, respectively, that are essential in Pap pili biosynthesis (Figure 1). The cistron coding for P13 seems to be involved in the regulation of either pili or Pap antigen expression since insertional mutants within this cistron showed reduced levels of pilus-specific protein (Figure 4). Insertional mutations within the *papC* and *papD* genes abolished pili formation although pilin antigen could still be detected in cell extracts (Figure 4). We believe that the P81 and P28.5 products from these two genes mediate the assembly and/or anchorage of pilin subunits during the secretion and polymerization process. Repeatedly we have found that a Tn*5* inactivation of *papD* reduces the abundance of *papA* pilin in minicells. We do not know if this reflects a decreased expression from *papA* or if the stability of its gene product is reduced in a *papD* mutant. The MRHA$^-$ phenotype of *papC* and *papD* mutants suggests that expression of hemagglutination also requires these gene functions. It should be noted that proteins of similar size have been implicated in such roles for the biosynthesis of other adhesion pili, e.g., the K88 adhesin (Gaastra and de Graaf, 1982). Unlike the cloned K88 operon (Gaastra and de Graaf, 1982) we believe the *pap* DNA on pRHU845 is transcribed from its normal promoter. This will certainly facilitate regulatory studies.

A major observation stemming from our current study is that the primary structure of the pilin subunit (that is, the product of the *papA* gene) is not in itself sufficient to confer adhesive properties upon bacterial host cells. Rather an additional region encompassing cistrons encoding for the biosynthesis of polypeptides of mol. wt. 15 000, 24 000, 26 000 and 35 000 are required for the adhesion. Cells carrying insertions within this region, represented by pSN021 and pSN042, synthesized pili as determined by electron microscopy (Figure 4C and Figure 5) as well as antigenically normal pilin subunits (Figure 4A). Yet such strains failed to show the MRHA$^+$ phenotype or to adhere to uroepithelial cells (Table I). Moreover, recent experiments (unpublished observations) have shown that purified pili preparations from *E. coli* harbouring pSN042 did not hemagglutinate human erythrocytes although the pilin subunits had the same mol. wt. and antigenicity as pili purified from *E. coli* K-12 HB101 carrying pRHU845.

The present data show that expression of binding activity requires the presence of accessory gene products which are non-essential for polymerization of pilin subunits into pili. They are further characterized in the accompanying paper (Lindberg *et al.*). We hope that our continuing examination of the genetic control, synthesis, processing, export and binding of pili will allow a better understanding of the mechanism by which pili contribute to bacterial pathogenicity.

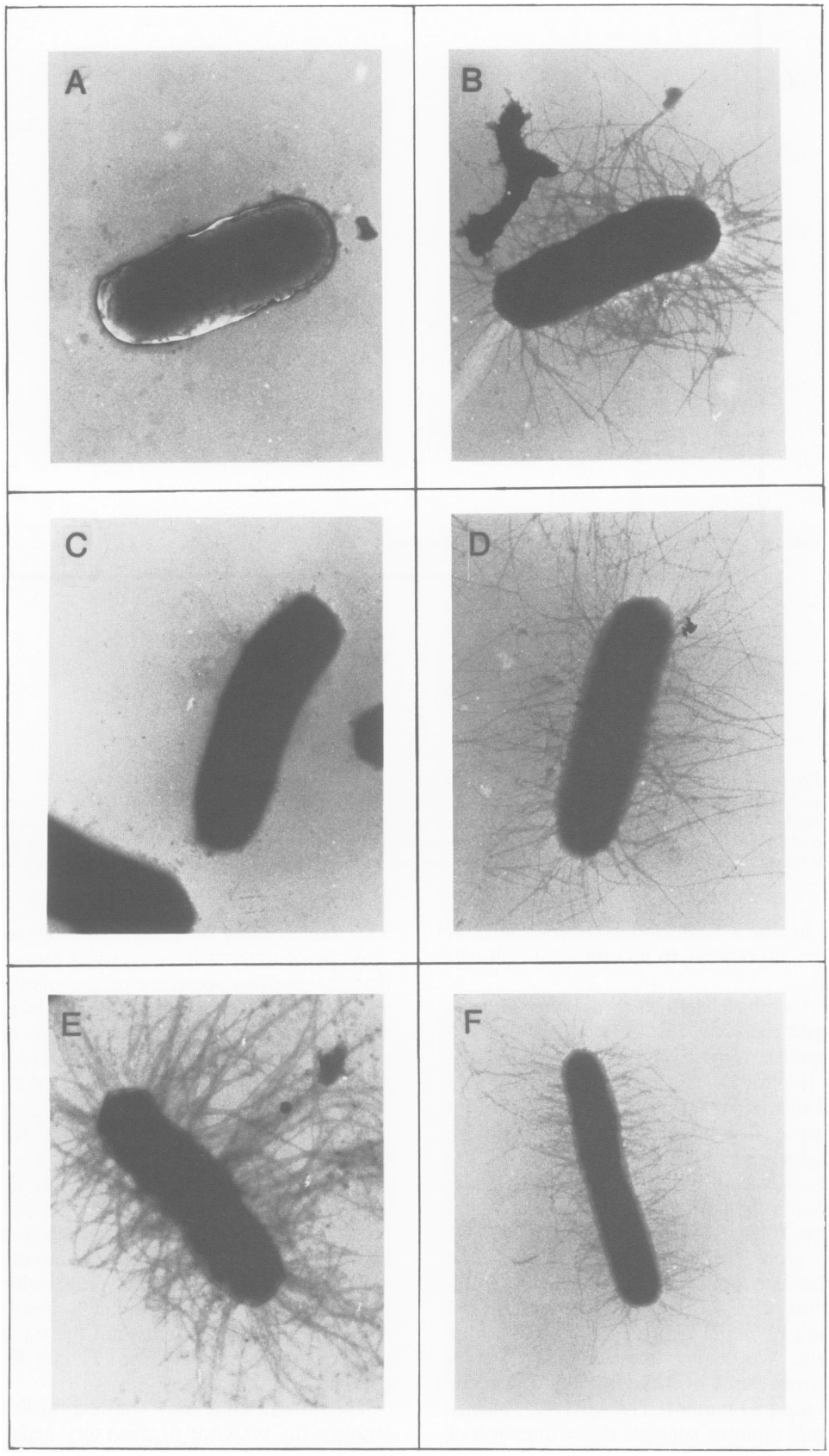

Fig. 5. Electron micrographs (24 000-fold magnification) of HB101 derivatives. **A**, plasmid-free HB101; **B**, HB101/pRHU845; **C**, HB101/pSN005, **D**, HB101/pSN021; **E**, HB101/pSN042; **F**, HB101/pSN016.

Table I. Attachment *in vitro* of bacteria to human uroepithelial cells

Strain	Experiment number[a]	MRHA	Epithelial cell attachment (bacteria/cell)[b]	
			Sq	Tr
HB101	1–4	Neg	0	0
HB101/pRHU845	1	Pos	17	23
	2	Pos	75	0
	3	Pos	33	0
	4	Pos	55	30
HB101/pSN005	1–4	Weakly pos	0	0
HB101/pSN015	1	Neg	5	0
	2–4	Neg	0	0
HB101/pSN021	1–4	Neg	0	0
HB101/pSN042	1	Neg	3	6
	2–4	Neg	0	0

[a]The strains were tested on four different occasions to account for variability in cell preparations.
[b]Shows the mean number of bacteria attached to 20 squamous (Sq) and 20 transitional cells (Tr).

Materials and methods

Bacterial strains and plasmids, media and chemicals

The following *E. coli* K-12 strains were used: P678-54 (Adler *et al.*, 1967), HB101 (Boyer and Roulland-Dussaix, 1969), and MC1061 (Casadaban and Cohen, 1980). Construction of the Pap plasmid pRHU845 and its Tn*5* mutant derivatives has recently been described (Normark *et al.*, 1983). Plasmids pSN101 and pSN102 are *Sma*I and *Pst*I cut-back derivatives, respectively, of pRHU845 as described (Normark *et al.*, 1983) Plasmid pSN201 is a *Hpa*I cutback deletion derivative of pSN100. Plasmids pSN100, pSN103 and pSN104 are pBR322 derivatives carrying *Hind*III fragment H2, H1 and H3 respectively of pRHU845 (Normark *et al.*, 1983; Figure 1). The plasmid pSKS107 is a derivative of pBR322 and carries the *lacZ, lacY* and *lacA* genes without the promoter and is therefore Lac$^-$ (Shapira *et al.*, 1983). The *papA-lacZ* gene fusion was constructed by inserting the 2-kb *Eco*RI-*Hind*III fragment of pRHU845 into pSKS107. The resulting plasmid was denoted pBEU80 (see Results). The β-galactosidase activity expressed by MC1061/pBEU80 (232 units/A_{420} = 1) was about half the level of a *lac* promoter carrying derivative MC1061/pSKS106 (566 units/A_{420} = 1) whereas MC1061/pSKS107 expresses at least 1000-fold less of the enzyme (Shapira *et al.*, 1983). The media used was LA, Luria agar, and tryptic soy agar. Antibiotics were used in plates at the following concentrations: ampicillin 50 μg/ml, kanamycin 50 μg/ml and tetracycline 15 μg/ml. [^{35}S]Methionine was purchased from Amersham, UK. Alkaline phosphate-conjugated goat anti-rabbit antisera were obtained from Miles Yeda Ltd., Israel.

Protein analysis

Minicells were prepared essentially according to Thompson and Achtman (1978) and labelled with [^{35}S]methionine. The polypeptides were separated on 17.5% SDS-polyacrylamide gels, 10–17.5% or on 12–17.5% gradient gels essentially as described by Grundström *et al.* (1980). After fixation, staining and destaining (to visualize mol. wt. markers) the gel was examined by fluorography (Grundström *et al.*, 1980).

Quantitation of pilus antigen

The amount of pilus protein expressed was measured by a competitive enzyme-linked immunosorbent assay (ELISA) as previously described (Normark *et al.*, 1983). Surface antigen was quantitated using whole cells, whereas total expression of pilus antigen (surface exposed as well as non-exposed) was determined in cell extracts after lysis with 1% Nonidet P-40-1% sodium deoxycholate-0.1% SDS-0.15 M NaCl-0.01 M Tris-hydrochloride (pH 7.2) containing 1 mg/ml lysozyme.

Electron microscopy

Electron microscopy was performed using a JEOL 100B microscope with 400 mesh copper grids coated with thin films of 2% formvar. Bacteria were resuspended in P-buffer (Gibbs *et al.*, 1973) placed on the grid and then immediately removed by aid of a filter paper. Grids were then washed with P-buffer followed by negative staining with 3.55% ammonium molybdate for 5 s, and washed with re-distilled H_2O.

Bacterial binding assays

The binding properties of the bacterial strains grown on tryptic soy agar were tested by agglutination of human erythrocytes (Normark *et al.*, 1983) and by attachment to human uroepithelial cells (Svanborg-Edén *et al.*, 1977). For MRHA, human erythrocytes were washed in 50 mM phosphate-buffered saline (PBS), pH 7.4 and resuspended in PBS containing 3% (w/v) α-methyl-D-mannoside to a final erythrocyte concentration of 3%. The assay was performed on glass slides with one drop of erythrocyte suspension to which ~10 bacterial cells were added. Agglutination was read by the naked eye within 1 min.

For attachment assays epithelial cells of the squamous (vaginal epithelium) and transitional (bladder and ureter) types from the sediment of the urine of two female donors were resuspended in PBS. To 10^5 epithelial cells were added 10^8 bacteria and PBS (with α-methyl-D-mannoside) to a final volume of 1 ml. After gentle rotation for 60 min at 37°C, unattached bacteria were eliminated by repeated washing. The number of bacteria attached to the epithelial cells was counted by interference contrast microscopy with a Bürker chamber. Adhesion is given as the number of bacteria attached to 20 squamous and 20 transitional cells.

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