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Comparison of the Genetic Determinant Coding for the S-Fimbrial Adhesin (*sfa*) of *Escherichia coli* to Other Chromosomally Encoded Fimbrial Determinants

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DNA probes specific for different regions of the S-fimbrial adhesin (*sfa*) determinant were constructed and hybridized with DNA sequences coding for P (F8 and F13), mannose-sensitive hemagglutinating type 1 (F1A), and F1C fimbriae. While the *sfa* and F1C DNA determinants exhibited homology along their entire lengths, the P-fimbrial and type 1-fimbrial determinants exhibited homology to regions of the *sfa* cluster responsible for the control of transcription and, to a minor extent, to regions coding for proteins involved in biogenesis and/or adhesion of the fimbriae and for the N-terminal part of the fimbrillin subunit.

Fimbriae or pili are filamentous protein structures on the cell surfaces of different bacteria, including extraintestinal *Escherichia coli* strains which cause urinary tract infections, sepsis, or newborn meningitis (13, 20). Very often these fimbriae are associated with adhesins which enable the bacteria to attach to eucaryotic tissues or agglutinate erythrocytes (hemagglutination) (4). The *E. coli* fimbrial adhesins are characterized on the basis of their receptor specificity. P fimbriae, which are further subdivided into serologically distinct groups (F7 to F13), recognize the α-D-Gal-(1→4)-β-D-Gal region of globosides (11, 19). S-fimbrial adhesins interact with sialic acid-containing receptors (22). The binding of type 1-fimbrial adhesins can be blocked by adding 2% mannose to the medium (15, 19). Therefore, these fimbriae are termed mannose-sensitive hemagglutinating fimbriae. Another group, F1C fimbriae, is apparently devoid of receptor specificity (15).

In the last few years the genetic determinants coding for some of the above-mentioned fimbrial adhesins have been cloned and characterized on the molecular level (for reviews, see references 13 and 27). It has been shown that S-fimbrial adhesin (*sfa*) determinants produced by different *E. coli* strains represent a cluster of genes with a homogeneous genetic structure (21), and similar results have been found for type 1-fimbrial determinants (3). The different P-fimbrial determinants also show homology to each other but to a lesser extent than the gene clusters coding for S and mannose-sensitive hemagglutinating type-1 fimbriae (5, 17, 29). Here we present data on a comparison of the genetic determinants coding for S fimbriae with cloned determinants coding for P, type 1, and F1C fimbriae.

The plasmids used are shown in Table 1. pANN801-13 carries the *sfa* determinant of the O6 strain 536 (previously termed X [8]). All *sfa*-specific fragments (probes A to I, K, and L [Fig. 1]) were derived from pANN801-13. The P-specific plasmids pRHU845 and pANN921 were described previously (7, 18). pRHU845 was kindly supplied by S. Normark (University of Umea, Umea, Sweden). pMSH2980 carries the entire gene cluster for the mannose-sensitive hemagglutinating type 1 fimbriae of the O18 strain 2980 (F1A fimbriae) (G. Schmidt, J. Hacker and I. Orskov, unpublished results). The recombinant plasmid pPKL38, kindly supplied by P. Klemm (Technical University, Lynby, Denmark), carries nearly 4 kilobases of the 5′ end of the F1A determinant, which codes for the subunit protein and for proteins involved in biogenesis and the regulation of transcription (14). Cloning of DNA sequences coding for F1C fimbriae into pACYC184 resulted in the recombinant plasmid pPIL110-54 (28). The plasmids were transformed into *E. coli* K-12 strain HB101. The *E. coli* K-12 strains JM107 and JM103 were used as hosts for the M13 bacteriophage clones (10). The bacteria were cultivated in L broth or on L agar plates. Selective pressure was imposed by adding 50 μg of ampicillin, 20 μg of chloramphenicol, or 20 μg of tetracycline per ml (to avoid the loss of plasmids). The fimbriated *E. coli* K-12 clones were characterized by receptor-specific hemagglutination tests and agglutination with specific antisera as described previously (9, 16, 19).

Eleven different DNA fragments representing specific parts of the *sfa* determinant were cloned into M13 vectors. These well-characterized DNA sequences were used as probes (probes A to I, K, and L [Fig. 1]) to compare the *sfa* gene cluster with different fimbria-coding determinants in DNA-DNA dot blot experiments. In these experiments the recombinant M13 plasmids carrying *sfa*-specific DNAs were denatured by being boiled for 5 min and were then stored on ice. The samples were diluted (1 μg, 0.1 μg, or 0.01 μg of DNA per sample) with 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and dotted on nitrocellulose filters by using a Bio-dot apparatus. Washing and autoradiography were performed as described by Southern (26). Stringent conditions were used for the washing procedure (30 min at room temperature with 2× SSC–0.1% sodium dodecyl sulfate; three times for 45 min each time at 56°C with 0.1× SSC–0.1% sodium dodecyl sulfate). These conditions allowed a base-pair mismatch of about 30%. As radioactively labeled DNAs the recombinant plasmids pRHU845, pANN921, pMSH2980, pPKL38, and pPIL110-54 were used. They were labeled by nick translation with a mixture of all four α^{32}-P-labeled deoxynucleoside triphosphates and purified by ethanol precipitation (25). The fimbria-specific DNA fragments were cloned into the vector molecules pBR322, pACAC184, and pJC74. To detect vector-specific

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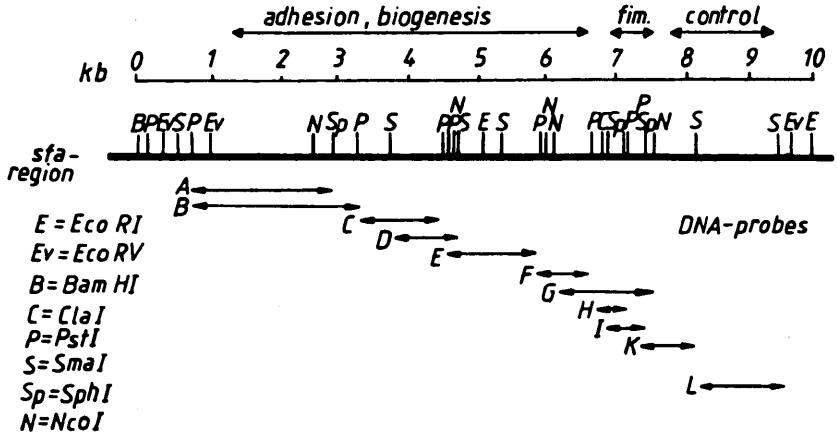

FIG. 1. Physical map of the *sfa* determinant. The regions involved in transcriptional control, production of fimbrillin (fim.), adhesion, and biogenesis are indicated. The arrows in the lower part of the figure represent the *sfa*-specific hybridization probes A to I, K, and L (see text). kb, Kilobases.

hybridization, we used these DNAs as controls. None of the three vector DNAs reacted with the *sfa*-specific probes.

It was demonstrated very recently (6, 25a; T. Schmoll et al., manuscript in preparation) that the *sfa* determinant consists of different regions involved in the production of fimbriae and adhesin, the biogenesis of fimbriae, and the control of transcription. Of the 11 *sfa*-specific probes, 7 mark the adhesion and/or biogenesis region (probes A to G). Probes H and I are specific for the fimbrillin protein. Probe K consists of DNA which codes for the N-terminal part of the fimbrillin protein and for protein SfaB, which is involved in the control of transcriptional activity. Probe L represents parts of the control region and *sfa*-flanking sequences, including the *sfaC* gene (Schmoll et al., in preparation). These *sfa*-specific probes were dotted on nitrocellulose filters and hybridized with radioactively labeled DNAs representing P-, F1A (mannose-sensitive hemagglutinating)-, and F1C-specific sequences.

In Fig. 2 dot blot experiments showing the hybridization of pRHU845 and pMSH2980 DNAs with five different *sfa*-specific probes is shown, and the results of all experiments are summarized in Table 1. The experiments with pPIL110-54 (F1C) confirmed our previous observations (21) that the F1C and *sfa* determinants show homology over their complete coding regions, as indicated by a positive reaction with all 11 probes. In contrast, the two P-fimbrial determinants (pRHU845 and pANN921) and the determinants coding for type 1 fimbriae (pMSH2980 and pPKL38) showed homology only to particular regions of the *sfa* determinant. Both *sfa*-specific probes representing the control region (probes K and L) gave positive signals after hybridization with the two P-specific DNAs (Table 1). The type 1-specific DNA strongly reacted only with probe K. In addition, minor homologies (i.e., homologies over a small region) were found between pRHU845, pMSH2980, and pPKL38 DNAs and *sfa* probes D and E, which mark the region involved in biogenesis and/or adhesion of the fimbriae. Furthermore, pANN921 and pMSH2980 reacted with probe G, representing parts of the biogenesis and/or adhesion region and the fimbrial structural gene.

The dot blot procedure used here is a very sensitive method for detecting homologies on the DNA level (5). Cloned adhesin determinants were used in this study instead of DNA isolated from wild-type strains, certainly advantageous since most strains carry more than one fimbrial determinant on their chromosomes (8, 24; G. Boulnois et al.,

TABLE 1. Homology of different determinants to *sfa*-specific probes in DNA-DNA dot blot experiments

Probe	Coordinates[a] (kilobases)	*sfa* region	Homology[b] to:				
			pRHU845 (P and F13)	pANN921 (P and F8)	pPIL110-54 (F1C)	pMSH2980 and pPKL38[c] (F1A)	pANN801-13 (*sfa*)
A (*Pst*I-*Sph*I[d])	0.7–2.9	Flanking; A/B	−	−	+	−	+
B (*Pst*I-*Pst*I; P5)	0.7–3.2	Flanking; A/B	−	−	+	−	+
C (*Pst*I-*Pst*I; P9)	3.2–4.5	A/B	−	−	+	−	+
D (*Sma*I-*Sma*I; S6)	3.8–4.7	A/B	(+)	−	+	(+)	+
E (*Pst*I-*Pst*I; P8)	4.5–5.9	A/B	(+)	−	+	(+)	+
F (*Pst*I-*Pst*I; P11)	5.9–6.7	A/B	−	−	+	−	+
G (*Nco*I-*Nco*I)	6.1–7.6	A/B; fimbrillin	−	(+)	+	(+)	+
H (*Pst*I-*Pst*I; P12)	6.8–7.2	Fimbrillin	−	−	+	−	+
I (*Sph*I-*Sph*I)	6.9–7.4	Fimbrillin	−	−	+	−	+
K (*Pst*I-*Sma*I)	7.4–8.2	Fimbrillin control	+	+	+	+	+
L (*Sma*I-*Sma*I; S4)	8.2–9.3	Control flanking	+	+	+	−	+
M13 (vector control)			−	−	−	−	−

[a] See Fig. 1.
[b] +, Major homology; (+), minor homology (see text); −, no homology.
[c] pPKL38 was hybrized with probes F to I, K, and L.
[d] P5 to P12 represent the *Pst*I fragments of the *sfa* determinant, and S4 and S6 represent the *Sma*I fragments of the *sfa* determinant.

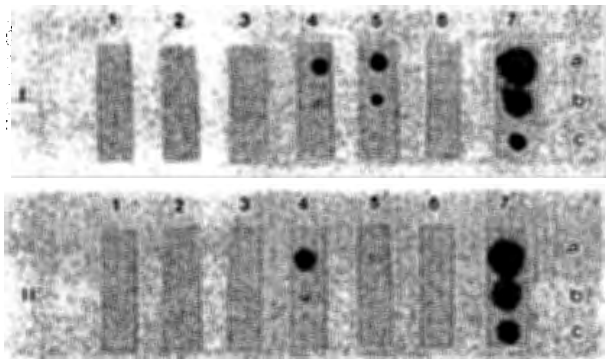

FIG. 2. DNA-DNA dot blot hybridization of plasmids pRHU845 (*pap*, F13, experiment I) and pMSH2980 (type 1, experiment II). Samples in rows a, b, and c contained 1, 0.1, and 0.01 μg of DNA, respectively. The following probes were used: M13 (control, lanes 1), probe F (lanes 2), probe H (lanes 3), probe K (lanes 4), probe L (lanes 5), probe I (lanes 6) and plasmids pRHU845 (experiment I, lane 7) and pMSH2980 (experiment II, lane 7) as controls.

manuscript in preparation). We conclude from the reported data that regions of P- and S-fimbrial determinants are very similar functionally but that the coding DNA sequences are mainly nonhomologous. On the other hand, the control regions of the two determinants show a high degree of similarity. The homology observed on the basis of the DNA-DNA dot blots was confirmed by a comparison of the DNA sequences of the *pap* (P and F13) determinant (1, 2) and the *sfa* genes (25a; Schmoll et al., in preparation), which showed that the *sfa*-specific DNAs coding for protein SfaB, which is involved in the transcriptional regulation of the *sfa* determinant, and for protein SfaC are almost identical to the P-specific regulatory genes *papB* and *papI*.

In addition, homologies over small DNA regions (minor homologies) were found among the F13, type 1, and *sfa* determinants in a region involved in biogenesis and/or adhesion (probes D and E). Minor homology between the gene clusters coding for F8 and type 1 fimbriae and *sfa* probe G (Table 1) may occur, at least between the sequences of the type 1 and *sfa* determinants coding for the N-terminal parts of the fimbriae. This is indicated by the recently published DNA compositions of both fimbrillin genes (12, 25a). The strong signal after hybridization between type 1-specific DNA and *sfa* probe K also seem to indicate this homology between the fimbrial structural genes and short fragments of similar DNA sequences in both control regions.

It should be pointed out that the homology detected by the dot blots does not necessarily indicate identity of the gene clusters under study; this is also true for the determinants coding for F1C and S fimbriae. Differences in the DNA sequences which code for the corresponding fimbrial subunits and a restriction site polymorphism in the 5′ region of both determinants have been observed recently (21, 25a, 28), and similar deviations have also been observed in the 3′ region of the two gene clusters (M. Ott, I. Van Die, and J. Hacker, unpublished results). Nevertheless, it is very interesting that two fimbrial determinants which are normally located on the chromosomes of strains which belong to different *E. coli* serotypes and are the causes of different diseases (S fimbriae are mostly associated with strains causing meningitis or cystitis, and F1C fimbriae are associated with pyelonephritogenic isolates [16, 23]) are highly related on the DNA level. The minimal differences in the DNA sequences of the corresponding determinants mentioned above must have, however, important functional consequences, since S fimbriae but not F1C fimbriae possess the capacity to adhere to sialic acid receptors. It will be important to determine the sequence differences between the *sfa* and F1C determinants, because they may answer some questions about the evolution of fimbrial determinants.

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