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Transcriptional analysis and regulation of the *sfa* determinant coding for S fimbriae of pathogenic *Escherichia coli* strains

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Abstract. The *sfa* determinant codes for S fimbrial adhesins which constitute adherence factors of pathogenic *Escherichia coli* strains. We have recently shown that the *sfa* determinant is transcribed from three promoters, pA, pB, and pC. In comparison with the promoters pB and pC, promoter pA, which is located in front of the structural gene *sfaA*, showed very weak activity. Here we have determined the exact positions of the mRNA start points by primer extension studies. We have also shown that mRNAs of 500, 700 and 1400 bases can be detected using oligonucleotide probes specific for the genes *sfaB*, *sfaC* and *sfaA*. SfaB and SfaC are positive regulators influencing fimbriation and the production of the S-specific adhesin which is encoded by the gene *sfaS* located in the distal half of the determinant. In addition, it is demonstrated that SfaB and SfaC interfere with the regulatory effect of the histone-like protein H-NS, encoded by a locus termed *drdX* or *osmZ*. In a *drdX*$^+$ strain the regulators are necessary for transcription of the *sfa* determinant. In contrast, *sfa* expression is activator-independent in a *drdX*$^-$ strain. In this latter genetic background, a substantial fraction of the *sfa* transcripts is initiated from promoter pA. On the basis of these data we discuss a model for the regulation of this adhesin-specific determinant.

Key words: Gene regulation – Fimbriae – Adhesion – Transcription – *trans*-activation

Introduction

Pathogenic *Escherichia coli* strains are able to attach to epithelial cells of the gut (intestinal *E. coli* isolates) and to uroepithelial cells of the urinary tract (extraintestinal *E. coli* strains; Orskov and Orskov 1985). *E. coli* isolates which produce the capsule antigen K1 may cause urinary tract infections (UTI) and newborn meningitis (NBM). Such isolates are able to bind to cells of the bladder and kidney and also to brain tissues (Korhonen et al. 1985; Ott et al. 1991). The majority of uropathogenic *E. coli* strains produce P fimbriae as the main attachment factor; the fimbriae bind to the α-Gal-1,4-β-Gal linkage in glycolipids (Jann and Hoschützky 1990). In contrast, NBM strains and UTI isolates of serotype 06 exhibit S fimbrial adhesins (Sfa) (Blum et al. 1991; Ott et al. 1991). S fimbriae recognize glycoproteins which terminate with α-sialyl-2,3-β-lactose-containing receptors (Parkkinen et al. 1986; Moch et al. 1987). Sfa plays a role in the pathogenesis of experimental UTI in rats and also in infections of the brain (Marre et al. 1986; Parkkinen et al. 1988).

We have cloned and analyzed the genetic determinants coding for S fimbriae of a UTI and of an NBM isolate (Hacker et al. 1985; Kestler et al., submitted). The *sfa* gene clusters consist of nine genes; four of these code for proteins that form the fimbrial adhesin complex (Schmoll et al. 1990b). While the protein SfaA (16 kDa) represents the major subunit protein, SfaS (14 kDa) is identical to the sialic acid-specific lectin (Moch et al. 1987; Schmoll et al. 1989; Morschhäuser et al. 1990).

S fimbriae as well as P fimbriae and other adherence factors are not expressed constitutively; they are strongly regulated by environmental factors such as temperature, osmolarity, growth rate, or sugars (Göransson et al. 1989; Schmoll et al. 1990a). Using *sfa-phoA* fusions it was demonstrated that at least three promoters, located upstream of the gene *sfaA*, direct transcription of the *sfa* determinant (Schmoll et al. 1990b). It was also shown that two proteins, SfaB and SfaC, which are encoded by genes located in the proximal (5′) half of the determinant, are necessary for *sfa* expression. These proteins exhibit strong homology to the regulatory proteins, PapB and PapI, which are involved in the regulation of the *pap* determinant that codes for the P fimbriae of serotype F13 (Baga et al. 1985; Göransson et al. 1988).

Here we present data on the physical mapping of the *sfa* promoter regions and on the mRNAs transcribed from the proximal part of the *sfa* gene cluster. In addition

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it is shown that the regulatory gene products, SfaB and SfaC, influence *sfa* transcription together with the histone-like protein H-NS (also known as H1) encoded by a locus termed *drdX* (Göransson et al. 1990) or *osmZ* (May et al. 1990).

Materials and methods

Media and reagents. Bacterial strains were grown on Luria Bertani (LB) agar plates or in liquid LB medium. For maintenance of plasmids 100 µg of ampicillin and/or 30 µg of chloramphenicol per ml were added. Antibiotics were obtained from Sigma (Deisenhofen) and Serva (Heidelberg). *Bss*HII was from New England Biolabs. Other restriction enzymes, Klenow enzyme, T4 polynucleotide kinase, AMV reverse transcriptase and RNase inhibitor were from Pharmacia, Freiburg, FRG. DNase I was from Boehringer, and T4 DNA ligase from Gibco. [^{32}P]ATP was purchased from NEN Research Products. [^{35}S]ATP was obtained from Amersham.

Recombinant DNA techniques. Plasmid DNA was isolated by the alkaline lysis method (Birnboim and Doly 1979). DNA fragments were purified by the freeze-squeeze method (Thuring et al. 1975) after digestion with appropriate restriction enzymes and separation in 0.8–1% agarose gels. Filling-in of 3′ recessed ends with Klenow enzyme and DNA ligation was performed according to standard protocols (Sambrook et al. 1989). *E. coli* strains were transformed by the $CaCl_2$ method (Lederberg and Cohen 1974).

Bacterial strains and plasmids. The *sfa* determinant was originally cloned from *E. coli* strain 536, a urinary tract isolate (06:K15:H31), described elsewhere (Hacker et al. 1985). As hosts for plasmids the *E. coli* strains HB101 (F^-, *hsdS20* (r_B^-, m_B^-), *recA13, ara-14, proA2, lacY1, galK2, rpsL20* (Sm^r), *xyl-5, mtl1, supE44;* Boyer and Roulland-Dussoix 1969), MC1029 (*araD139, Δ(ara, leu)*7697, lacZΔM15, *galU, galK, strA, recA56;* Casadaban and Cohen 1980), and HMG5 (as MC1029, but *drdX;* Göransson et al. 1990) were used. Strains WK6 *Δ(lac-proAB) galE, strA,* F′, *lacI^{q}ZΔM15, proA$^+$B$^+$*) and BMH71-18 *mutS Δ(lac-proAB), thi, supE,* F′, *lacI^{q}ZΔM15, proA$^+$B$^+$, mutS215::*Tn*10*) were used for site-specific mutagenesis (Stanssens et al. 1989). Plasmid pANN801-13 carries the *sfa* determinant cloned in pBR322 (Hacker et al. 1985). Plasmid pANN801-15 contains the proximal *Eco*RV-*Cla*I fragment of the *sfa* gene cluster with the genes *sfaA, sfaB, sfaC* in vector pACYC184 (Chang and Cohen 1978).

Plasmid pANN81-1 *(sfaB$^-$, sfaC$^-$)* was obtained by linearizing pANN801-13 with *Bss*HII, partial cleavage with *Eco*RI, subsequent end-filling and religation. Plasmid pANN81-2 *(sfaB$^-$, sfaC$^-$)* is deleted for the *Eco*RV-*Sma*I fragment in the proximal part of the *sfa* determinant. Plasmid pANN81-3 has a frameshift mutation in *sfaB* that was obtained by cleavage of pANN801-13 with *Bss*HII, subsequent end-filling and religation. Plasmid pANN81-4 *(sfaC$^-$, sfaB$^+$)* is deleted for the *Eco*RV-*Nru*I fragment carrying *sfaC* and part of the intercistronic region between *sfaC* and *sfaB*. Plasmid pANN81-5 was constructed by deleting pANN81-3 for the *Eco*RV-*Nru*I fragment mentioned above, giving rise to a derivative that has a frameshift mutation in *sfaB* and a deletion of *sfaC* and part of the intercistronic regulatory region. Plasmid pANN81-6 carries a frameshift mutation in *sfaC* that was obtained by the insertion of an adenine between positions 191 and 192 of the published sequence (Schmoll et al. 1990b), resulting in a newly created *Pst*I site. Plasmid pANN81-7 is a derivative of pANN81-3 that is deleted for the *Eco*RV-*Bal*I fragment downstream of *sfaC*. Plasmid pANN801-153 was constructed by deleting the *Nru*I-*Cla*I fragment of pANN801-15, resulting in a plasmid that carries the *Eco*RV-*Nru*I fragment coding for *sfaC*. Plasmid pANN801-154 was obtained by deleting the *Nco*I-*Cla*I fragment of pANN801-15, giving rise to a construct that contains the *Eco*RV-*Nco*I fragment coding for *sfaC* and *sfaB*. Plasmid pANN801-155 is a derivative of pANN801-154 that is deleted for a fragment that extends from the *Nru*I site between *sfaC* and *sfaB* to an *Nru*I site downstream from *sfaC* in the vector pACYC184. pANN801-155 codes for *sfaB*.

Site-specific mutagenization. Construction of the frameshift mutation in *sfaC* was by the gapped-duplex DNA method of Stanssens et al. (1989). The 4.7 kb *Eco*RI fragment of plasmid pANN801-13 was cloned into the vectors pMa5-8 and pMc5-8. The pMa5-8 derivative was cleaved with *Eco*RV and *Nru*I, and after elution from an agarose gel, it was annealed to the single-stranded pMc5-8 derivative. For mutagenization, the oligonucleotide 5′-GGGAAAACCT*GCAGAAATTG-CGGAGG-3′ was used. The oligonucleotide is identical to the coding strand of *sfaC* with the exception of the additional thymidine (indicated by the asterisk). Plasmids were screened for the newly created *Pst*I site and the mutation was confirmed by sequencing. The mutagenized *Eco*RI fragment was used to replace the original fragment in pANN801-13 to create plasmid pANN81-6.

RNA isolation. Total RNAs were extracted from bacterial cells after growth on agar plates by the method of Brosius et al. (1982) or after growth in liquid medium to early logarithmic phase by the hot phenol method (see Baga et al. 1985).

Identification of mRNA start points by primer extension. 5′ labelling of oligonucleotide primers with γ-[^{32}P]ATP and primer extension analysis with AMV reverse transcriptase were performed according to standard protocols (Ausubel et al. 1987). The oligonucleotides 5′-CCCAAGGTCAGGGCTGAAAATACAG-CC-3′ (*sfaA* primer), 5′-CCGGGTAATAACTTCATG-CTGTGCC-3′ (*sfaB* primer) and 5′-CCGCCAGCGCC-TCCGCAATTTCTGCGG-3′ (*sfaC* primer), complementary to the 5′ regions of the coding strands of *sfaA, sfaB* and *sfaC,* respectively, were used. Each reaction contained 50 µg of RNA. Samples were loaded on a 6.7% polyacrylamide, 42% urea sequencing gel. For identifica-

tion of transcript start points, ^{32}P or ^{35}S sequencing reactions with double-stranded cloned DNA as template and the primers used for the extension reaction were run in parallel.

Northern hybridization. Total RNA was separated in 1.2% agarose / formaldehyde gels and blotted to nitrocellulose or Hybond N membranes according to standard protocols (Ausubel et al. 1987). Prehybridization and hybridization were performed in 6 × SSC, 5 × Denhardt's reagent, 0.1% SDS, 1 mM EDTA, 50 mM TRIS-HCl pH 7.5 and 100 µg/ml denatured salmon sperm DNA in 50% deionized formamide. As probes the ^{32}P-labelled oligonucleotides specific for *sfaA*, *sfaB* and *sfaC* were used. Filters were washed 3 × 5 min with 2 × SSC, 0.1% SDS under stringent conditions and exposed to X-ray film (Fuji RN-NIF) for 15 h to 5 days. As size markers 0.16-1.77 and 0.24-9.5 kb RNA ladders (Gibco) were run on the same gel and stained with ethidium bromide.

Determination of fimbriation and hemagglutination. Determination of fimbriae production was done in an enzyme-linked immunosorbent assay with whole cells and the monoclonal antibody F1, which is directed against the major fimbrial subunit SfaA, as described previously (Schmoll et al. 1989). Hemagglutination was quantified by mixing serial dilutions of bacteria with bovine erythrocytes in microtiter plates as described (Jones and Rutter 1972).

Oligonucleotide synthesis. Oligonucleotides were synthesized with an Applied Biosystems 380A synthesizer by the phosphoramidite method of Beaucage and Caruthers (1981).

DNA sequencing. DNA sequencing was performed by the dideoxy chain-termination method (Sanger et al. 1977) with a T7 sequencing kit from Pharmacia as described by the manufacturer.

Results

Determination of mRNA start points of the sfa *determinant*

In a previous paper (Schmoll et al. 1990b) we demonstrated that the *sfa* determinant is transcribed from three promoters, pA, pB and pC, with are located upstream of the genes *sfaA*, *sfaB* and *sfaC*, respectively. The gene

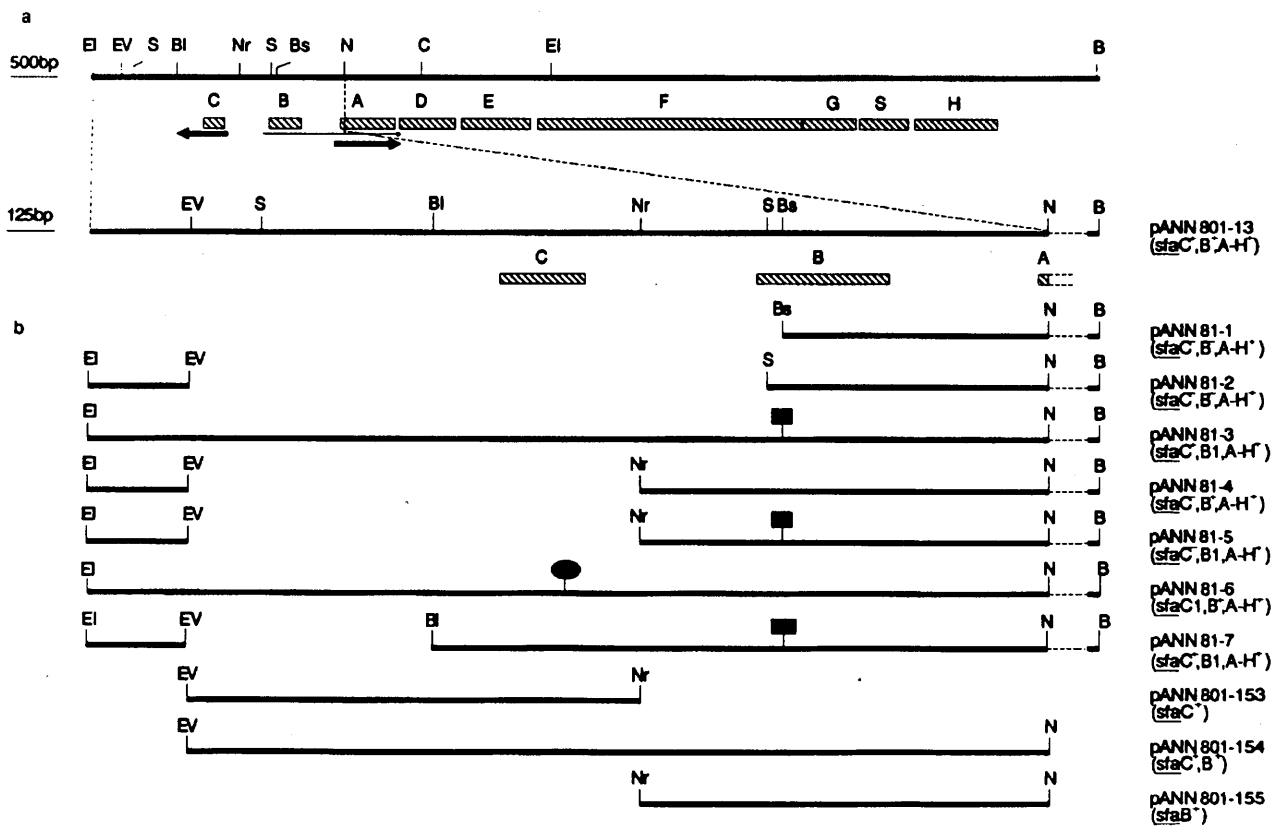

Fig. 1a–b. Genetic organization of the *sfa* determinant. *Hatched boxes* represent *sfa*-specific genes. Transcripts detected by Northern hybridization are indicated by *arrows*. **a** Plasmid pANN801-13 containing the *sfa* genes. **b** The regulatory region is shown enlarged. The genetic structure of mutant derivatives of pANN801-13 is given. Deletions are indicated by *gaps*, the frameshift mutation in *sfaB* is marked by a *square*, the frameshift mutation in *sfaC* by an *oval*. Plasmid designations and genotypes are shown on the right. Relevant restriction sites are indicated: B, *Bam*HI; Bl, *Bal*I; Bs, *Bss*HII; C, *Cla*I; EI, *Eco*RI; EV, *Eco*RV; N, *Nco*I; Nr, *Nru*I; S, *Sma*I

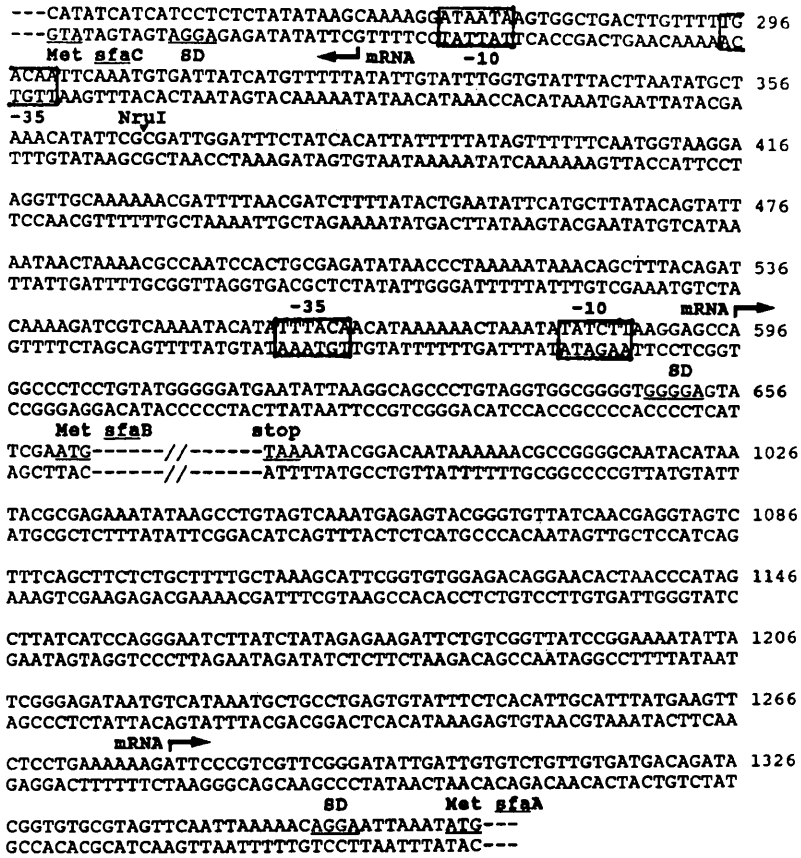

Fig. 2. DNA sequence of the intercistronic region between *sfaC* and *sfaB* and *sfaB* and *sfaA*, respectively. Start and stop codons and ribosome binding sites (SD) are *underlined*. The mRNA start sites are marked by *arrows*, the −10 and −35 regions are *boxed*. The *Nru*I site is indicated

sfaC is transcribed in the opposite direction to *sfaB* and *sfaA* (see also Fig. 1a). To determine the precise transcriptional start points, we performed primer extension experiments with total RNA extracted from strain HB101 (pANN801-13) carrying the entire *sfa* gene cluster (Fig. 1a) after growth overnight on agar plates. RNA from HB101 (pBR322) was used in a control reaction. Three main mRNA start points could be identified (data not shown). The predicted −10 and −35 regions of *E. coli* promoter consensus sequences found in front of *sfaC* and *sfaB* correspond well to the main transcriptional start sites (Fig. 2; McClure 1985). In the case of the mRNA start point in front of *sfaA*, no promoter consensus sequences could be identified.

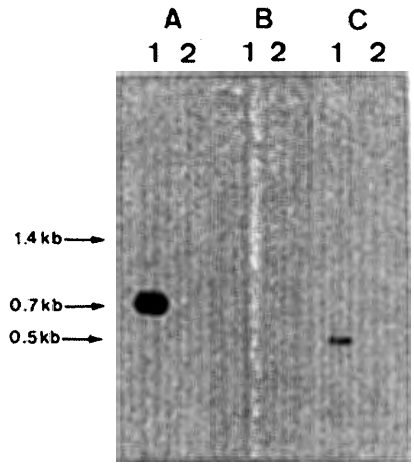

Fig. 3A–C. Detection of *sfa* mRNAs by Northern hybridization. RNA was isolated from strain HB101 containing plasmid pANN801-13 (lanes 1) and the vector pBR322 (lanes 2) and 10 µg of RNA was loaded in triplicate on a 1.2% agarose/formaldehyde gel and blotted to a nitrocellulose filter. The filter was cut in three equal parts and hybridized against ^{32}P-labelled oligonucleotides specific for *sfaA* (**A**), *sfaB* (**B**) and *sfaC* (**C**), respectively. Transcript sizes are indicated. The autoradiograph in **B** was exposed five times longer than in **A** and **C**

Identification of sfa *transcripts*

To identify mRNAs transcribed from the proximal part of the *sfa* gene cluster we performed Northern hybridization experiments with total RNA of clone HB101 (pANN801-13) after growth overnight on agar plates. Figure 3 shows the results of three Northern hybridizations with *sfaA*-, *sfaB*- and *sfaC*-specific probes. One transcript of about 500 nucleotides was identified, which initiates at pC and codes for SfaC (Fig. 3C). A second major transcript of about 700 nucleotides starting in front of and spanning the *sfaA* coding region was detected with the *sfaA* probe (Fig. 3A). After longer exposure

of the autoradigraph, a band at 1.4 kb could be visualized both with the *sfaB* (Fig. 3B) and the *sfaA* probes (Fig. 3A). This band corresponds to a transcript starting at pB and spanning both *sfaB* and *sfaA* (see Fig. 1a). The remaining signals obtained with the *sfaB* probe appear to represent degradation products.

Influence of sfaB *and* sfaC *on fimbriae formation and hemagglutination*

To determine the influence of mutations in *sfaB* and *sfaC* on Sfa production, fimbriation and hemagglutination of various clones (see Fig. 1b) were tested. As shown in Table 1, strain HB101 (pANN801-13), carrying the whole *sfa* determinant, exhibited S-specific fimbriation and hemagglutination. The plasmids pANN81-1 and pANN81-2, which were deleted for *sfaC, sfaB* and the intercistronic region, were unable to direct fimbriae formation and adhesin production. This ability was not restored by providing SfaC and SfaB *in trans*. The clone HB101 (pANN81-3) with the frameshift mutation in *sfaB* was also negative for fimbriation and hemagglutination, but these properties could be fully complemented by the plasmid pANN801-155, encoding SfaB. The *sfaC* frameshift mutation in pANN81-6, too, resulted in almost complete abolition of *sfa* expression, but this could also be complemented by providing SfaC *in trans*. Deletion of *sfaC* in pANN81-4 reduced both fimbriae production and hemagglutination, but to a lesser extent than in the *sfaC* frameshift mutant. This effect, too, could be reversed by providing SfaC *in trans*.

Most surprisingly, the plasmid pANN81-5, which in addition to the frameshift mutation in *sfaB*, is deleted for *sfaC* and part of the intercistronic region, retained some ability to produce fimbriae and adhesins. The presence of both SfaC and SfaB (pANN801-154) or SfaC alone (pANN801-153), but not SfaB alone (pANN801-155), could restore the full wild-type phenotype to clones carrying pANN81-5. It was concluded that the corresponding region has a negative *cis* effect on *sfa* expression. In contrast, the deletion of sequences located downstream of *sfaC* in pANN81-7 had no further effect on the phenotype of the *sfaB* frameshift mutant. Like the clone harboring pANN81-3, the phenotype of this clone could be complemented by SfaB *in trans*.

Influence of sfaB *and* sfaC *on transcription of* sfaA

To test the effect of the mutations in *sfaB* and *sfaC* on *sfaA* transcription, we determined the abundance of the transcript encoding the major fimbrial subunit. As shown in Fig. 4, the level of the *sfaA* transcript correlated well with the expression of S-specific fimbriae and hemagglutination (Table 1). The Sfa$^-$ clone carrying plasmid pANN81-2 did not produce detectable levels of the *sfaA* transcript, even when it was complemented with a plasmid carrying *sfaB*$^+$- and *sfaC*$^+$-specific sequences (Fig. 4a, lanes 2 and). In cells harboring plasmid pANN81-3 *(sfaB1)*, only a very small amount of the *sfaA* transcript

Table 1. S-specific fimbriation and hemagglutination of *sfa* clones

Resident plasmid	Coresident plasmid	Fimbriation	Hemagglutination
pANN801-13 (*sfaC*$^+$, *B*$^+$, *A*$^+$–*H*$^+$)	pACYC184 (Vector control)	+++[a]	+++[a]
pANN81-1 (*sfaC*$^-$, *B*$^-$, *A*$^+$–*H*$^+$)	pACYC184 (Vector control)	–	–
pANN81-1 (*sfaC*$^-$, *B*$^-$, *A*$^+$–*H*$^+$)	pANN801-154 (*sfaC*$^+$, *B*$^+$)	–	–
pANN81-2 (*sfaC*$^-$, *B*$^-$, *A*$^+$–*H*$^+$)	pACYC184	–	–
pANN81-2 (*sfaC*$^-$, *B*$^-$, *A*$^+$–*H*$^+$)	pANN801-154 (*sfa*C$^+$, B$^+$)	–	–
pANN81-3 (*sfaC*$^+$, *B1*, *A*$^+$–*H*$^+$)	pACYC184	–	–
pANN81-3 (*sfaC*$^+$, *B1*, *A*$^+$–*H*$^+$)	pANN801-155 (*sfa*B$^+$)	+++	+++
pANN 81-4 (*sfaC*$^-$, *B*$^+$, *A*$^+$–*H*$^+$)	pACYC184	++	++
pANN81-4 (*sfaC*$^-$, *B*$^+$, *A*$^+$–*H*$^+$)	pANN801-153 (*sfa*C$^+$)	+++	+++
pANN 81-5 (*sfaC*$^-$, *B1*, *A*$^+$–*H*$^+$)	pACYC184	+	+
pANN81-5 (*sfaC*$^-$, *B1*, *A*$^+$–*H*$^+$)	pANN801-153 (*sfa*C$^+$)	+++	+++
pANN81-5 (*sfaC*$^-$, *B1*, *A*$^+$–*H*$^+$)	pANN801-154 (*sfa*C$^+$, B$^+$)	+++	+++
pANN81-5 (*sfaC*$^-$, *B1*, *A*$^+$–*H*$^+$)	pANN801-155 (*sfa*B$^+$)	+	+
pANN81-6 (*sfaC1*, *B*$^+$, *A*$^+$–*H*$^+$)	pACYC184	(+)	(+)
pANN81-6 (*sfaC1*, *B*$^+$, *A*$^+$–*H*$^+$)	pANN801-153 (*sfa*C$^+$)	+++	+++
pANN81-7 (*sfaC*$^+$, *B1*, *A*$^+$–*H*$^+$)	pACYC184	–	–
pANN81-7 (*sfaC*$^+$, *B1*, *A*$^+$–*H*$^+$)	(pANN801-155 (*sfa*B$^+$)	+++	+++
pBR 322	pACYC184	–	–

[a] +++, strong reaction; ++, medium; +, weak; [(+) barely reaction detectable]; –, no reaction with anti-fimbrial antibody, no hemagglutination

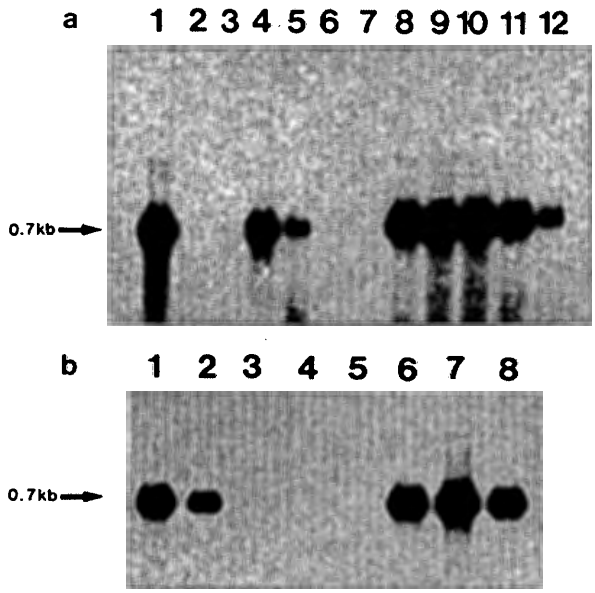

Fig. 4a–b. Detection of the *sfaA*-specific transcripts from various mutant clones by Northern hybridization against the *sfaA*-specific oligonucleotide. RNA was extracted from strain HB101 carrying the following plasmids: pANN801-13/pACYC-184 (**a, b** lane 1); pANN81-2/pACYC184 (**a** lane 2); pANN81-3/pACYC184 (**a** lane 3); pANN81-4/pACYC184 (**a** lane 4 and **b** lane 2); pANN81-5/pACYC184 (**a** lane 5); pBR322/pACYC184 (**a** lane 6 and **b** lane 5); pANN81-2/pANN801-154 (**a** lane 7); pANN81-3/pANN801-155 (**a** lane 8); pANN81-4/pANN801-153 (**a** lane 9 and **b** lane 6); pANN81-5/pANN801-153 (**a** lane 10); pANN81-5/pANN801-154 (**a** lane 11); pANN81-5/pANN801-155 (**a** lane 12); pANN81-6/pACYC184 (**b** lane 3); pANN81-7/pACYC184 (**b** lane 4); pANN81-6/pANN801-153 (**a** lane 7); pANN81-7/pANN801-155 (**a** lane 8). Samples of 20 µg of RNA were loaded in each lane

could be detected (Fig. 4a, lane 3). After providing SfaB *in trans*, the *sfaA* transcript was produced at about wild-type levels (Fig. 4a, lane 8). The same effect could be observed in cells carrying plasmid pANN81-7 with the additional deletion downstream of *sfaC* when they were complemented with SfaB *in trans* (Fig. 4b, lanes 4 and 8). A deletion of *sfaC* in the *sfaB1* frameshift mutant resulted in low-level production of *sfaA* mRNA (compare lanes 5 and 3 in Fig. 4a). Complementation with SfaB alone had only a small effect (lane 12), whereas addition of SfaC and SfaB (lane 11) or only SfaC (lane 10) led to a clear increase in the abundance of the *sfaA* transcript. The *sfaC* frameshift mutation resulted in production of a small amount of *sfaA* mRNA (observed after longer exposure of the autoradigraph in Fig. 4b) that could be elevated again by providing SfaC *in trans* (compare lanes 3 and 7 in Fig. 4b).

Detection of pA activity in sfa *gene clusters deleted for pB*

To detect *sfaA* transcription from pA alone, we performed primer extension experiments with RNA extracted from the clones carrying pANN81-1 or pANN81-2, which are deleted for *sfaB* and *sfaC* and the corresponding promoters pB and pC. It was evident from this analysis that in these clones the *sfaA*-specific transcript started at the same position as in clones carrying the wild-type gene cluster, although in amounts detectable only after long exposure of the autoradigoraph (compare lanes 3 and 4 with lane 1 in Fig. 5). In cells carrying the *sfaB1* frameshift mutation, the *sfaA* transcript was also produced at very low levels.

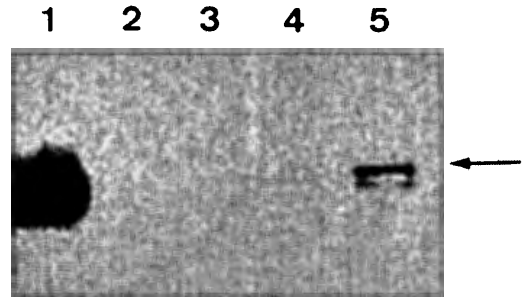

Fig. 5. Analysis of *sfaA* transcripts by primer extension. RNA was isolated from strain HB101 carrying the following plasmids: pANN801-13 (lane 1); pBR322 (lane 2); pANN81-1 (lane 3); pANN81-2 (lane 4); pANN81-3 (lane 5). The mRNA start point is marked by an *arrow*

Influence of a drd*X mutation on* sfa *expression*

The histone-like protein H-NS (also termed H1) encoded by the *drdX* locus influences expression of P fimbriae, colonization factor antigen I (CFAI) and other virulence determinants (Göransson et al. 1990; Dorman et al. 1990; Spears et al. 1986; Jordi et al. 1992; unpublished data). In order to decide whether H-NS plays a role in *sfa* expression, we introduced plasmid pANN801-13 and derivatives with mutagenized *sfaB* and/or *sfaC* loci into the $drdX^-$ strain HMG5 and its $drdX^+$ counterpart MC1029. As demonstrated in Table 2 the expression of S fimbrial adhesins from $sfaB^-$ and $sfaC^-$ mutants. Was restored in $drdX^-$ clones in comparison with $drdX^+$ cells.

Table 2. Influence of the *drd*X mutation on S-specific fimbriation and hemagglutination of *sfa* clones

Plasmid	Sfa production in	
	HMG5 (*drd*X$^-$)	MC1029 (*drd*X$^+$)
pANN801-13 ($sfaC^+$, B^+, A^+–H^+)	++++[a]	+++
pANN81-2 ($sfaC^-$, B^-, A^+–H^+)	+++	–
pANN81-3 ($sfaC^+$, *B1*, A^+–H^+)	+++	–
pANN81-4 ($sfaC^-$, B^+, A^+–H^+)	++++	++
pANN81-5 ($sfaC^-$, *B1*, A^+–H^+)	++++	+
pBR322 (Control)	–	–

[a] ++++, spontaneous and strong haemagglutination. Other evaluations are as in Table 1

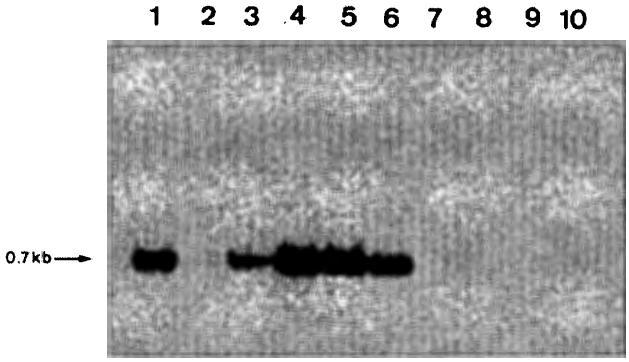

Fig. 6. Transcription of *sfaA* in strains HMG5 (*drdX*$^-$, lanes 1-5) and its isogenic wild-type strain MC1029 (*drdX*$^+$, lanes 6-10), containing the following plasmids: pANN801-13 (lanes 1, 6); pANN81-2 (lanes 2, 7); pANN81-3 (lanes 3, 8); pANN81-4 (lanes 4, 9); pANN81-5 (lanes 5, 10). Samples of 20 µg of RNA were separated in a 1.2% agarose/formaldehyde gel, blotted to Hybond N membrane and hybridized against the *sfaA*-specific oligonucleotide

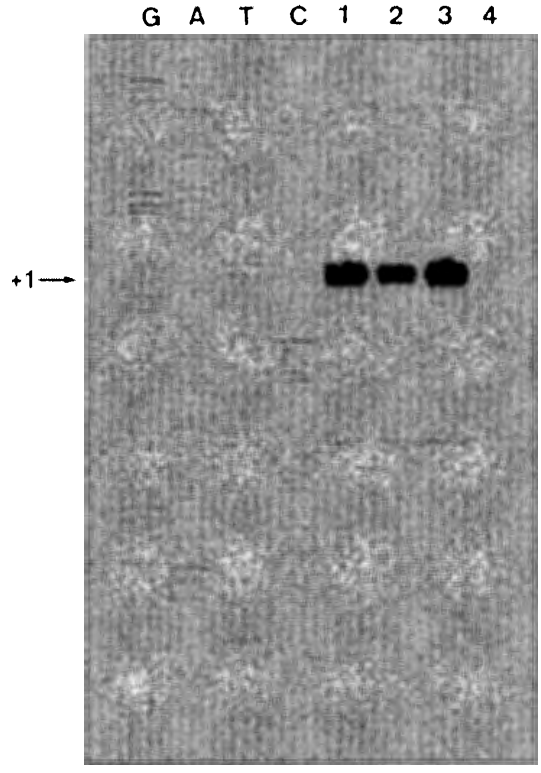

Fig. 7. Analysis of *sfaA* transcripts by primer extension in strains HMG5 (*drdX*$^-$, lanes 1, 2) and MC1029 (*drdX*$^+$, lanes 3, 4) carrying plasmids pANN801-13 (lanes 1, 3) or pANN81-2 (lanes 2, 4). A ^{35}S-labelled sequencing reaction with the primer used for the extension analysis was run in parallel

Transcription of *sfaA* in the different clones was monitored in a Northern hybridization experiment with RNA extracted from the cells after growth in liquid medium to a concentration of 50 Klett units at 37° C. The results of the experiment are given in Fig. 6. It is shown that the relative amount of the *sfaA* transcript produced in the *drdX*$^+$ strain MC1029 carrying the various *sfa*-coding plasmids was similar to that in strain HB101 (compare Fig. 6, lanes 6-10 with Fig. 4, lanes 1-5). In the *drdX*$^-$ strain HMG5, however, *sfaA* transcription from the *sfaB*$^-$ and *sfaC*$^-$ mutant plasmids was strongly increased relative to that in the *drdX*$^+$ strains (Fig. 6, lanes 1-5). This was true for the *sfaC*$^-$ clone as well as for the clone carrying the *sfaB1* frameshift mutation. Surprisingly, *sfaA* was transcribed at readily detectable levels even from the plasmid that was deleted for *sfaC*, *sfaB* and the intercistronic region (lanes 2 and 7 in Fig. 6; lane 2 in Fig. 4).

To confirm that the *sfaA* transcript produced from promoter pA in *drdX*$^-$ cells indeed starts at the previously characterized site we performed a primer extension analysis with RNA extracted from strains HMG5 (*drdX*$^-$) and MC1029 (*drdX*$^+$) carrying plasmids pANN801-13 and pANN81-2, (*sfaC*$^-$, *sfaB*$^-$) after growth to 50 Klett units in liquid medium at 37° C. As shown in Fig. 7, the *sfaA* mRNA produced in strain HMG5 (*drdX*$^-$) carrying the derivative pANN81-2 (Fig. 7, lane 2) started at the same site as the transcript produced from the wild-type *sfa* determinant (pANN801-13) in both the *drdX*$^+$ (lane 3) and *drdX*$^-$ (lane 1) backgrounds, indicating an activation of the promoter pA in *drdX*$^-$ cells.

Discussion

In this paper we describe the transcriptional regulation of the *sfa* determinant coding for the S fimbriae of the pathogenic *E. coli* strain 536. The *sfa* determinant is transcribed from three promoters pA, pB and pC, located in front of the genes *sfaA*, *sfaB*, and *sfaC*, respectively. One major transcript of 700 bases starts in front of *sfaA* and spans the coding region for the major fimbrial subunit, and two other mRNAs of 500 bases and 1400 bases, specific for *sfaC* and *sfaBA*, respectively, could also be detected by Northern hybridization. Two possible stem loop structures (see Schmoll et al. 1990b) beyond the *sfaA* coding region might act as barriers against degradation of the 700 and 1400 base transcripts by 3′–5′ exonucleases.

A model for the transcriptional regulation of the *sfa* gene cluster is given in Fig. 8. Analysis of clones with mutations in the regulatory region of the *sfa* determinant revealed the role of SfaB and SfaC as positive regulators of fimbriae expression, as suggested previously (Schmoll et al. 1990b). It was also shown that mutations in *sfaB* and *sfaC* not only affected the gene *sfaA*, coding for the major fimbrial subunit protein, but also the adhesin-specific locus *sfaS* located in the distal part of the determinant. Frameshift mutations in *sfaB* or *sfaC* resulted in very low levels of *sfa* transcription, as monitored by the quantitation of the *sfaA* mRNA. The level of transcription in the *sfaB1* mutant was insufficient to direct detectable fimbriae and adhesin production. Introduction of the *sfaB*$^+$ and *sfaC*$^+$ genes on a compatible plasmid *in trans*, however, restored transcription and fimbriae production to the level of the wild-type determinant. Interestingly, deletion of a DNA fragment comprising the *sfaC* coding

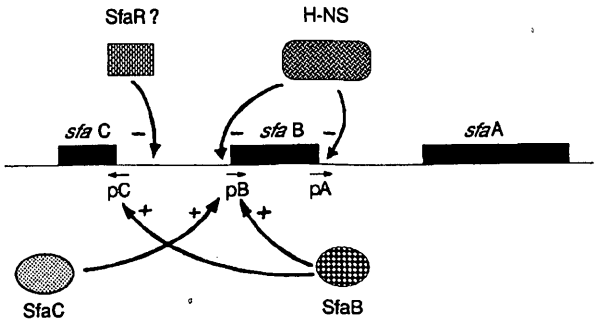

Fig. 8. Model of the transcriptional regulation of the *sfa* genes

region and part of the intercistronic region between *sfaC* and *sfaB* did not reduce *sfa* expression to the same extent as the *sfaC* frameshift mutation. The same deletion in addition to the *sfaB1* frameshift mutation even resulted in partial restoration of *sfa* transcription and synthesis of fimbriae and adhesins, indicating a negative effect of the *sfaC-sfaB* intercistronic region *in cis* on *sfa* expression. This result is in striking contrast to the situation in the *pap* gene cluster where the corresponding region in necessary for *pap* transcription. The binding of the PapB activator and the CRP-cAMP complex to this DNA region is responsible for this effect (Göransson et al. 1989). In the case of the *sfa* genes, an as yet unidentified repressor, indicated as SfaR in Fig. 8, may inhibit *sfa* expression by binding to this region in the absence of SfaB and SfaC.

While deletions of the region upstream of *sfaA*, including *sfaC* and *sfaB* and the promoters pC and pB, completely abolished fimbriae expression, transcription from pA could be detected at very low levels after long exposure of the autoradiograph from a primer extension experiment (see Fig. 6). Low but clearly detectable pA activity in *sfaB*$^-$ and *sfaC*$^-$ clones has already been demonstrated by using *sfaA-phoA* fusions (Schmoll et al. 1990b) or *sfaA-lacZ* fusions (Hacker 1990; J. Morschhäuser, unpublished results). SfaB and SfaC proteins, however, could not restore *sfa* expression *in trans* by directly acting on pA. This observation agrees with our previous results that SfaB acts on pB and pC, while SfaC activates pB but not pA (Schmoll et al. 1990b; see Fig. 8). The difference in production of the *sfaA* transcript between clones carrying the wild-type determinant and the derivatives deleted for *sfaB, sfaC* and the promoters pB and pC results from the fact that the *sfaA* transcript is produced from precursors that initiate at pB and pA by several processing steps (J. Morschhäuser et al., in preparation), as has already been shown for the *papA* transcript (Baga et al. 1988; Nilsson and Uhlin 1991). Most of *sfa* transcription normally initiates at pB, whereas pA is active only at very low levels.

In a search for a repressor that acts negatively on *sfa* expression by binding to the *sfaC-sfaB* intercistronic region, we investigated whether the *drdX* locus, which encodes the histone-like protein H-NS and has a role in thermoregulation of the *pap* operon (Göransson et al. 1990), also influences *sfa* transcription. In the *drdX*$^-$ strain HMG5, *sfa* transcription was partially independent of the activators SfaB and SfaC. Transcription in the *sfaB* frameshift mutant increased and deletion of *sfaC* alone or in addition to the *sfaB* mutation resulted in *sfa* transcription at wild-type levels. Even in the clone that was deleted for *sfaB, sfaC* and the intercistronic region, including pB and pC, the promoter pA was strongly activated. An activator-independent expression of fimbriae in a *drdX*$^-$ background has also been demonstrated for the *pap* and *cfaI* genetic determinants (Jordi et al. 1992; Forsman et al., submitted). As shown in Fig. 8 the *sfa* genes are another example of virulence genes that are under the negative control of the H-NS protein, which is counteracted by the fimbriae-specific activators SfaB and SfaC.

A tandem arrangement of genes coding for *trans*-regulatory proteins in the proximal region of adhesin determinants has also been described for adhesin gene clusters of intestinal (de Graaf 1990) and other extraintestinal *E. coli*, including the *fim* gene cluster coding for type I fimbriae (Klemm 1986) and the *pap* determinant (Baga et al. 1985; Forsman et al. 1989). Sequence comparisons between the regulatory genes of these determinants and the genes *sfaB* and *sfaC*, however, show a high degree of homology only between the loci *papI* and *papB* and the corresponding *sfa*-specific sequences (Schmoll et al. 1990b). This relatedness is further corroborated by the fact that the regulatory genes of *pap* and *sfa* genes clusters are able to *trans*-complement each other (Göransson et al. 1988; Morschhäuser et al., in preparation). In the case of the *sfa* determinant, an additional promoter in front of the major subunit-encoding gene *sfaA* was identified that is not found in front of *papA*. This promoter can be activated by a mutation in *drdX*, indicating that it might have a role in *sfa* expression under certain circumstances.

An effect of a *drdX* mutation on P fimbriae was recently described (Görannson et al. 1990). Our data support the recent finding that expression of the *sfa* determinant is influenced by the osmolarity of the environment (Schmoll et al. 1990a). The fact that the regulation of both adhesin determinants, *pap* and *sfa*, depends on the action of the osmoregulator H-NS confirms our view that adhesin gene clusters are part of global biological networks that are coordinately regulated by signals from the environment (see Miller et al. 1989).

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