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## AFA and F17 adhesins produced by pathogenic *Escherichia coli* strains in domestic animals

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**Abstract** – AFA and F17 are afimbrial and fimbrial adhesins, respectively, produced by pathogenic *Escherichia coli* strains in domestic animals. F17-related fimbriae are mainly detected on bovine and ovine *E. coli* associated with diarrhoea or septicaemia. The F17-G adhesin subunits recognize N-acetyl-D-glucosamine (GlcNAc) receptors present on bovine intestinal cells. Some F17 subtypes also bind to GlcNAc receptors present on human uroepithelial and intestinal Caco-2 cells or to the laminin contained in the basement of mammalian membranes. F17 is often associated with other virulence factors (aerobactin, serum resistance, CNF2 toxin, K99, CS31A or AFA adhesins) on pathogenic *E. coli*. A cluster of only four genes is required to synthesize functional F17-related fimbrial structures. The hypothesis of multifunctional F17 fimbrial subunits is supported by the fact that: i) the N-terminal part of the adhesin subunit participates in receptor recognition, whereas the C-terminal part is required for biogenesis of the fimbrial filament; and ii) the interaction between structural and adhesin subunits seems to be crucial for the initiation of monomer polymerization. Recently, determinants related to the *afa* gene clusters from human pathogenic *E. coli* associated with intestinal and extra-intestinal infections were identified in strains isolated from calves and piglets with diarrhoea and septicaemia. Two *afa*-related gene clusters, designated *afa-7* and *afa-8*, that encode afimbrial adhesins were cloned and characterized from bovine pathogenic *E. coli*. These animal *afa* gene clusters were plasmid and chromosome borne and were expressed by strains that produced other virulence factors such as CNF toxins, F17, PAP and CS31A adhesins. A high frequency of *afa-8* and a low prevalence of *afa-7* among bovine *E. coli* isolates were suggested by preliminary epidemiological studies. As with the human *afa* gene clusters, the animal ones encode an adhesive structure composed of two proteins: AfaE which mediates adhesion to epithelial cells and AfaD which is an invasin. © Inra/Elsevier, Paris.

**fimbrial adhesin / afimbrial adhesin / *Escherichia coli* / domestic animals / family of adhesins**

**Résumé** – Adhésines AFA et F17 produites par les souches d'*Escherichia coli* pathogènes chez les animaux. Les adhésines AFA et F17 de type respectivement afimbriales et fimbriales sont pro-

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duites à la surface de souches d'*Escherichia coli* pathogènes pour des animaux domestiques. Les fimbriae apparentés à la famille F17 sont le plus souvent produits par des souches isolées de diarrhée ou de septicémie chez les bovins ou les ovins. L'adhésine F17 reconnaît un récepteur de type N-acétyl-D-glucosamine (GlcNAc) présent sur les cellules intestinales bovines. Quelques variants F17 adhèrent également à des cellules épithéliales ou intestinales humaines et à la laminine des cellules de mammifères. Les fimbriae F17 sont souvent associés à d'autres facteurs de virulence (aérobactine, résistance au sérum, toxine CNF2 (*cytotoxic necrotizing factor 2*) ou adhésine K99, CS31A ou AFA). Seulement quatre gènes sont nécessaires à la synthèse des fimbriae. La nature multifonctionnelle des sous-unités fimbriales est suggérée par le fait que : 1) la partie N-terminale de l'adhésine est impliquée dans la reconnaissance du récepteur alors que la partie C-terminale participe à la biogenèse du fimbriae ; et 2) l'interaction entre la sous-unité majeure et l'adhésine est essentielle pour l'initiation de la polymérisation du fimbriae. Récemment des déterminants similaires aux opérons *afa* des souches associées chez l'homme à des infections intestinales et extraintestinales, ont été identifiés dans des souches pathogènes pour les veaux et les porcelets. Deux opérons, désignés *afa-7* et *afa-8*, codant pour des adhésines afimbriales ont été clonés à partir de souches isolées chez des veaux atteints de septicémie. Ces opérons sont portés par le chromosome ou par un grand plasmide dans des souches qui expriment d'autres facteurs de virulence telles les toxines CNF ou les adhésines F17, PAP ou CS31A. Des études épidémiologiques préliminaires ont suggéré une forte prévalence de *afa-8* parmi les souches isolées chez les veaux. Comme les opérons *afa* des souches humaines, les opérons *afa-7* et *afa-8* codent pour une structure adhésive composée de deux protéines : AfaE qui permet l'adhésion aux cellules épithéliales et AfaD qui est une invasive. © Inra/Elsevier, Paris.

#### adhésines fimbriales / adhésines afimbriales / *Escherichia coli* / animaux domestiques / familles d'adhésines

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## 1. INTRODUCTION

*Escherichia coli* is a normal inhabitant of the intestinal tract of humans and animals [59, 82]. However, pathogenic *E. coli* strains are also the causative agent of intestinal or extra-intestinal infections, such as urinary tract infection (UTI), meningitis or septicaemia. The mechanisms involved in the development of extra-intestinal infections include the ability to colonize mucosal surfaces, to traverse the epithelial cell layers, to resist the bactericidal effect of the complement, to escape phagocytosis and to survive and multiply in body fluids. Virulence factors, such as cytotoxins, adhesins, aerobactin, outer membrane proteins or lipopolysaccharides (LPS), are associated with the capacity of *E. coli* strains to cause extra-intestinal infections. A classification of diarrhoeagenic *E. coli* has been established, based on clinical aspects of the disease and on the identification of the virulence factors produced by the pathogenic strain [25, 59].

In any case, the capacity of the bacteria to adhere to host cells is a prerequisite step for the process of microbial colonization and invasion. The binding of the pathogenic bacteria to host receptors is usually mediated by fimbrial or afimbrial adhesins [25, 59, 82]. Fimbriae (or pili) are supramolecular structures produced at the bacterial cell surface. The bulk of the fimbriae is composed of the same repeated structural subunit and a specific adhesin subunit mediates the host receptor recognition. The adhesin subunit generally located at the top or within the fimbrial structure is presented away from the bacterial cell surface. The first *E. coli* adhesins studied were those associated with the presence of fimbriae which can be visualized by electron microscopy. Later on, other adhesins were reported not to be associated with fimbriae. These non-fimbrial (or afimbrial) adhesins were found in human and animal pathogenic isolates.

This article will specifically focus on the fimbrial and afimbrial adhesins, i.e. the F17

and AFA family of adhesins, which have been studied and associated with diarrhoea or septicaemia in animals over the last few years.

## 2. FIMBRIAL ADHESINS

Fimbriae could be classified by their receptor binding specificities. For example, the type I fimbriae mediating mannose-sensitive haemagglutination (MSHA) are found at the cell surface of both commensal and pathogenic strains. In contrast, most fimbriae of pathogenic *E. coli* cause mannose-resistant haemagglutination (MRHA) [25, 59].

Particular fimbriae are expressed by *E. coli* strains associated with human diarrhoea [25, 59]. Briefly, the colonization of the human intestinal epithelium is mediated by the colonization factor antigen (CFA) expressed by enterotoxigenic *E. coli* (ETEC). The fimbrial adhesins designated Bfp (bundle-forming pilus) are produced by enteropathogenic *E. coli* (EPEC) strains responsible for infantile gastroenteritis. Furthermore, AAF/I (aggregative adherence factor I) mediates the aggregative adherence phenotype associated with several enteroaggregative *E. coli* (EaggEC).

Fimbriae produced by *E. coli* strains associated with diarrhoea and/or septicaemia in domestic animals have also been intensively studied [59]. The K88 (F4) fimbriae expressed by ETEC strains bind to specific Gal $\beta$ 1-containing glycolipids present in the porcine small intestine mucus [23]. The release of heat-labile (LT) and/or heat-stable (ST) enterotoxins results in diarrhoea in neonatal and weaned piglets. The *E. coli* strains producing K99 (F5) adhesins bind to the small intestines of neonatal pigs, calves and lambs [18]. The K99 fimbrial adhesion factor recognizes a sialic acid residue of glycolipids in the intestinal mucus layer of neonatal animals [96]. In addition, the 987P (F6) fimbriae are expressed by porcine ETEC [44] and F41 fimbriae by

bovine and porcine ETEC strains [19]. Furthermore, the non-enterotoxigenic *E. coli* strains isolated from domestic animals have also been associated with diarrhoea and/or septicaemia or bacteraemia. For example, the F165 fimbrial complex has been isolated from piglets and calves with septicaemia or with various diseases for which diarrhoea is the most prominent clinical sign [24]. The F165<sub>1</sub> fimbriae included in the F165 complex mediate adhesion to the  $\alpha$ -D-Gal(1,4)- $\beta$ -Gal receptors present on sheep erythrocytes [42]. Throughout the last few years, the fimbriae included in the F17 family have been extensively detected among pathogenic *E. coli* strains isolated from domestic animals.

## 2.1. F17-related fimbriae

### 2.1.1. F17-related fimbriae produced by *E. coli* strains

The family of the F17 fimbriae includes the F17a, F17b, F17c and F17d fimbriae expressed by pathogenic *E. coli* isolated from bovine with septicaemia and/or diarrhoea, and from bacteraemic lambs (*table 1*) [6, 7, 61, 84]. In addition, the G fimbriae also included in the F17 family are produced by *E. coli* strains associated with human urinary tract infection (UTI) (*table 1*) [90]. All the F17 subtype fimbriae mediate adhesion of the bacteria to a N-acetyl-D-glucosamine (Glc-NAc)-containing receptor present on host cells [6, 7, 34, 61, 84].

The fimbriae appear as fine and flexible protaneous filaments of 3–4 nm in diameter on the surface of bacterial cells cultured at 37 °C [7, 61]. The F17 fimbriae are heteropolymers composed of the F17-A and F17-G subunits with an apparent molecular mass of 20 and 36 kDa, respectively [6, 7, 22, 62, 63]. The bulk of the fimbriae are constituted of the same repeated F17-A (or GafA for G fimbriae) structural subunit. F17-G (or GafD) is the minor adhesin subunit conferring adhesive properties to the

fimbriae. The fimbriae included in the F17 family are serologic variants (or subtypes) based on differences in the amino acid composition of the F17-A or GafA structural subunits. Except for F17c-A and GafA subunits which are identical, the amino acid sequences of the different F17-A structural subunits show 72–85 % identity (*figure 1*) [22, 71].

The F17a fimbrial subtype (formerly called FY) was first identified on the 11a *E. coli* strain (O19:K32:H9) isolated from a diarrhoeic calf in France [13, 34]. These fimbriae were further identified as Att25 in Belgium [88] and characterized from the 25KH09 reference ETEC strain (O101:K+:H-) at the National Institute for Veterinary Research (Brussels) [61]. The F17a fimbrial subtype is chromosome-encoded, whereas the STa thermo-stable toxin also produced by the 25KH9 reference *E. coli* strain is plasmid-encoded [61]. The F17b fimbriae subtype, formerly called the Vir antigen, was first described on the S5 *E. coli* strain (O15:K+:H21) isolated from blood of a lamb with fatal bacteraemia in Great Britain [73]. The genes encoding the F17b fimbriae are located on the Vir transmissible plasmid [73] that also controls the synthesis of the cytotoxic necrotizing factor type 2 (CNF2) [22, 83, 84]. The F17c fimbriae subtype (originally named 20K fimbriae [7, 16]) was characterized from the *E. coli* strain 31A at the 'Institut national de recherche agronomique' of Clermont-Ferrand-Theix (France) [7]. The 31A reference strain (O153:K-:H-) isolated from the faeces of a calf with diarrhoea produces neither classical enterotoxins (STs or LTs) nor CNF toxins but causes experimental septicaemia in gnotobiotic calves [15]. The F17d serological variant (formerly named F111 fimbriae) was first identified in Belgium at the cell surface of the 111KH86 *E. coli* strain isolated from the intestinal content of a diarrhoeic calf [6]. In contrast to the other F17-related fimbriae, the apparent molecular weight of the F17d-A structural subunit is estimated to be 18 kDa by sulphate dode-

**Table I.** F17-related fimbriae produced by pathogenic *E. coli*, *P. mirabilis* or *H. influenzae* strains.

Fimbriae	Original appellation	Bacteria	Structural subunit	Adhesin subunit	Reference strain	Host	Pathogenicity	Adhesins or toxins associated on the reference strain
F17a	F(Y) and Att25	<i>E. coli</i>	F17a-A	F17a-G	11a 25KH9	bovine	diarrhoea	Sta enterotoxin; K99 adhesin Sta enterotoxin
F17b	Vir	<i>E. coli</i>	F17b-A	F17b-G	S5	ovine	bacteriemia	CNF2 toxin
F17c	20K	<i>E. coli</i>	F17c-A	F17c-G	31A	bovine	septicaemia	CS31A adhesin
F17d	Att111	<i>E. coli</i>	F17d-A	F17d-G	111KH86	bovine	diarrhoea	
G		<i>E. coli</i>	GafA	GafD	IH11165	human	pyelonephritis	M agglutinin
UCA		<i>P. mirabilis</i>	UcaA		HU1069	human	UTI	
UCA		<i>P. mirabilis</i>	UcaA		IVB247	canine	UTI	
Haf		<i>H. influenzae</i>	HafA	HafE	F3031	human	septicaemia	PMP fimbriae
Hif (Hib)	type b	biogroup <i>aegyptius</i> <i>H. influenzae</i>	HifA	HifE	770235f	human	meningitis	

```

                                +1
F17a-A      : *MQKIQFILGILAAAS*SSATLAY****DGKITFNGKVVQDTCVSTTE*SKNLTVKLPV 53
F17b-A      : *-R-----*-----*-----*-----N-T---A---A-G*-ES-S-V---
F17c-A/GafA : *-----*-----*-----*-----T-N-----AGTAP-A-T---
F17d-A      : *-R-----*-----*-----*-----T-----*-----T-----
UcaA        : MKR-VIALAT--S--F*AGSSM-----T---T---A---N-N*D---A-T---
HafA        : MEQFIMKKTLLGS-IL--F-GNVQ-ATTNAETS--V--F---EN--K-K-D*NQ-MS-V-ND-
HifA        : MKKTLGLS-IL--F-GNVQ-DINT**ETS--V--F---EN--K-K---H---S-V-ND-

F17a-A      : SANSLASSGKVVGLTPTFFILLEGCNTPAVT****GAQNVNAYFEPNANTDY*TTGNLTN 107
F17b-A      : -TTT-STDA-TA-----Q-T-----TVS-AS****VDK-----*-----
F17c-A/GafA : -TK--N-P---A-----S-S---A-A-*****S-S-K-----*-----
F17d-A      : -S---T-N-E-A-----S---TSP-SA*****E--K-----T-----*DSH--NI
UcaA        : -TTT-NENAATA-----H-T--AVG*MD*****S-KT---SSDI-V--H--K-
HafA        : GK--STK-NTAMP-----S-QD--NVTT**TANK*-SK-GV--YSWE-A-KDNDYT-K-
HifA        : GK--STKVNTAMP-----T-QN-DPTTANGTANK*-NK-GL--YSWK-V-KENNFT-K-

F17a-A      : *TASSG*ASNVIQQLLNADGVKAIKLG*****QAAAAQSVDTVAINDANVT***** 151
F17b-A      : *-----*-----*-----*-----TP-----*-----D-TG-N-TA-Q-DN-AMK*****
F17c-A/GafA : *-T-----*-----*-----*-----V-----*-----*-----*-----
F17d-A      : *ASG--N-T-----*-----*-----*-----TTP-L-----*-----TDS-G-A-TP---GT-----*
UcaA        : *-QTK*-D--V---S-AATT-Q-----*-----*-----TDS-T-D-HP-Q-DN---N*****
HafA        : *KM-NDF-TM-N--MES--TTP--VVGKTTEDFVHKG-NGSA-NST-VTSKHSIGNTAL
HifA        : EQTADY-T-N--MESN-T---SVVGKETEDFMHTNNGVALNQTHP-N-HISGSTQL

F17a-A      : *****LRYNAQYYATGVATAGDVTSTVNYIAYQ 180
F17b-A      : *****-----AP-----SA-----V--
F17c-A/GafA : *****-----
F17d-A      : *****-----R-----Q-----N-SA-----
UcaA        : *****-F-F-----QS-----KA--H-----E
HafA        : *NSNTSID-HFIS-----I---K-E-S--FQ---E
HifA        : TTGTNELP-HFI-----NK-----K-Q-S-DFQ---E

```

**Figure 1.** Comparison of the predicted amino acid sequences of the F17-related fimbrial structural subunits. The first amino acid residue of the mature protein is numbered +1. Gaps (\*) are introduced to obtain a maximal homology. Identical amino acid residues are identified by dashes. The sequences are compared using the Clustal W multiple alignment program. The accession numbers from NBRF or GenBank are A27625, I41206, L43373, L77091, U28420, U54780 and X16991, respectively.

cyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [6, 8]. The G fimbriae were purified and characterized from the *E. coli* strain IH11165 (O6 serogroup) present in the urine of a patient with acute pyelonephritis in Finland [90]. G fimbriae bind to erythrocytes treated with endo- $\beta$ -galactosidase, a treatment that exposes terminal Glc-NAc residues on the erythrocytes surface [90]. The G fimbriae on the reference strain are associated with an afimbrial haemagglutinin able to recognize the blood group M antigen [90].

*Trans*-complementation analysis shows that the genetic determinants of F17a, F17b and F17c fimbriae encode functionally interchangeable systems confirming the concept

of a family of fimbriae [22, 71]. Furthermore, a genotypic detection performed on a large collection of F17-producing *E. coli* strains demonstrates that the F17 family is very large and probably includes other closely related subtypes that have not yet been characterized [8].

### 2.1.2. F17-related fimbriae produced by *Proteus mirabilis* and *Haemophilus influenzae* strains

Fimbriae included in the F17 family are also related to the UCA fimbriae expressed by human or canine uropathogenic *Proteus mirabilis* strains (table I) [9, 17]. The predicted amino acid sequence of the UcaA

structural subunit shows a 55–58 % identity to those of the different F17-A subtypes [9, 17, 71]. Furthermore, the codon usage frequency of the gene encoding the F17a-A subunit is closely related to that of UCA and other *P. mirabilis* fimbriae, suggesting that UCA and F17 fimbrial genes may share a common ancestor [17]. A high sequence similarity (60 %) is also observed with the amino acid sequence of the Hif major fimbrial subunit (table I) [32, 98]. The Hif (or Hib) fimbriae are produced by a *Haemophilus influenzae* strain isolated from the throat of a child with meningitis and mediate adhesion of the bacteria to human buccal cells [32, 39]. Moreover, the F17-A subunits show an obvious homology with those of the Haf fimbriae produced by a *H. influenzae* biogroup *aegyptius* strain found to be the etiologic agent of Brazilian purpuric fever (BPF) and associated with pediatric purulent conjunctivitis and septicemia [89]. The identity between the structural subunit genes shows that UCA, Hif or Haf fimbriae are distant members of the F17 family. Phylogenetic analysis strongly suggests that the human *P. mirabilis* strain has acquired the genes necessary for the UCA fimbrial biosynthesis recently in evolution by a horizontal gene transfer, most probably from *E. coli* [9].

## 2.2. Prevalence of F17-related-producing *E. coli* strains

Epidemiologic studies have mainly been performed by immunological detection using antibodies directed against the F17a or F17c subtypes. Anti-F17c antibodies recognize common determinants of the F17a, F17b, F17c, F17d and G native fimbriae [7]. In contrast, the use of anti F17a antibodies results in a very weak reactivity with the F17b, F17c or G subtypes [7, 22]. Therefore, differences in the specificity of F17 detection can occur among the different studies depending on the anti-F17 antibodies used. Recently, a multiplex PCR method

was developed to detect the genes encoding the different F17 subtypes [8].

F17 are expressed by 15–46 % of bovine *E. coli* associated with diarrhoea or septicemia in France and Belgium [7, 87, 88]. In contrast, F17 fimbriae are detected among 3 % of *E. coli* isolated from healthy calves suggesting that F17 fimbriae are probably involved in the infectious process [87]. In addition, 11–23 % of F17-positive isolates are associated with diarrhoeic calves from Japan, Algeria and India [40, 72, 95]. Only 3.4 % of *E. coli* from diarrhoeic calves in Scotland and England are F17-positive [74]. However, 29 % of bacterial strains isolated in Scotland from the intestinal content of lambs with a severe tubular disease (nephropathy) [2] express F17 fimbriae [7]. A clinicopathological survey of lambs with nephropathy demonstrates that about half of the lambs investigated showed clinical evidence of diarrhoea [2]. In addition, F17 fimbriae are also produced by *E. coli* strains isolated from kids and lambs with diarrhoea in Spain (8.6 %) [11]. In humans, only 3 % of the *E. coli* strains isolated in France from diarrhoeic stools of hospitalized patients were F17-positive, suggesting that F17 fimbriae do not play an important role in infantile and adult diarrhoea [7].

In addition to bacterial strains associated with diarrhoea or septicemia, fimbriae included in the F17-family are also expressed by *E. coli* strains isolated from the milk of cows suffering from clinical mastitis in the Netherlands (55 %) [64]. The role of F17 fimbriae in the infectious process of *E. coli* associated with mastitis infection is unknown. However, in vitro adhesion experiments have demonstrated that the F17-producing strains adhere to cultured mammary epithelial cells [64].

## 2.3. Association of F17-related fimbriae with virulence factors

F17 fimbriae are often associated with virulence factors on pathogenic *E. coli*. A



recent report reveals that 45 % of the F17-positive *E. coli* strains isolated from bovine with diarrhoea are resistant to complement and produce aerobactin suggesting that the bacterial strains may cause septicaemia in calves [86]. Most of the bovine bacterial strains expressing the F17c subtype fimbriae also produce the afimbrial CS31A adhesin that mediates adhesion to a N-acetylneuraminic acid-containing receptor present in the human carcinoma cell line Caco-2 [7, 8, 21, 36]. In addition, F17-related fimbriae are detected on 9 % of the K99-positive bovine *E. coli* strains [14] and on 22 % of bovine *E. coli* strains associated with both the CNF2 toxin and AFA [56, 58, 68]. This suggests a strategy used by pathogenic *E. coli* strains to adhere to different host tissues and therefore increase their pathogenicity. The F17b subtype is prominent among bovine CNF2-producing strains with virulence properties associated with extra-intestinal infections (O78 serogroup, aerobactin and serum resistance) [8, 84]. Genes encoding the F17b fimbriae and CNF2 toxin are located on the Vir transmissible plasmid [73, 84].

Pathogenic *E. coli* producing F17c subtype fimbriae are prominent among bovine and ovine isolates (47 %) whereas F17a, F17b and F17d fimbriae subtypes are detected on 30, 11 and 11 % of the F17-positive bacterial strains, respectively [8]. The high occurrence of F17c subtype fimbriae in France and Belgium [7] can be explained by the use of antibiotics in animal care. Indeed, the bacterial strains producing the F17c subtype are strongly associated with a self-transmissible plasmid coding for virulence factors (CS31A adhesin and aerobactin production) and for antibiotic resistance [7, 20]. In addition, *E. coli* strains producing both CS31A and F17c fimbrial subtypes have a *m*-inositol-positive phenotype [7]. These strains may represent a new example of the association between bacterial clones and plasmid-encoded virulence factors [7].

The F17-producing *E. coli* strains isolated from bovine intestinal contents are sometimes referred to as ETEC strains but only when F41 and/or K99 fimbrial adhesins are also produced at the bacterial cell surface [86]. Although some bovine F17-positive strains producing STa and/or STb enterotoxins have been isolated in India [40], a recent study clearly demonstrated that K99/F41-negative isolates producing F17 fimbriae do not correspond to the definition of ETEC strains [86]. When associated with the K99 adhesin, F17a-positive isolates represent 10 % of bovine ETEC strains isolated in France and 30 % of those isolated in Belgium in surveys performed in the early 1980s [14, 88]. Experimental studies have revealed that colostral antibodies against both K99 and F41 adhesins or against F17 adhesins alone do not provide protection for calves inoculated with ETEC strains producing K99, F17 and F41 adhesins [12]. A protective effect is obtained only when antibodies directed against the three adhesins are associated in the colostrum of vaccinated cows [12]. The ETEC strains carrying both K99 and F17 fimbriae adhere more strongly to bovine enterocytes than to ETEC that only express K99 [35]. In contrast to ETEC that cause diarrhoea in lambs during the first days of life, non-ETEC strains expressing F17 fimbriae are isolated from calves between days 4 and 21 [14, 87, 102].

#### 2.4. Adhesive properties

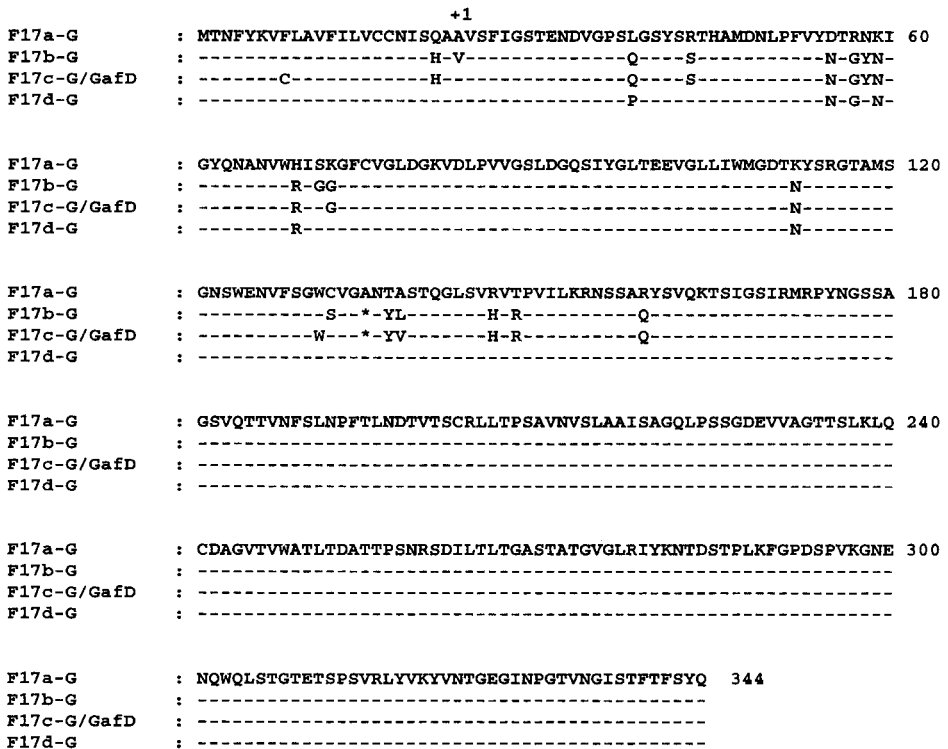
It is well documented that F17-related fimbriae mediate *in vitro* adhesion to N-acetyl-D-glucosamine (GlcNAc)-containing receptors present on bovine erythrocytes and intestinal cells [7, 34, 61]. The recognition of host receptors is mediated by the F17-G protein exported at the bacterial cell surface and located within the F17 fimbrial structures [22, 63, 71, 91]. Analysis of amino acid comparisons reveals that all the substitutions are located within the N-terminal part of the F17-G adhesin subunit. In

contrast, an absolute conservation is observed on the C-terminal part of the protein (figure 2) [22, 71]. Lintermans et al. speculate that F17a fimbriae may initiate the binding of bacteria to calf intestinal villi by hydrophobic interactions between the intestinal mucus and the F17a-A structural subunit that is characterized by a high hydrophobicity [63]. This interaction is then followed by a specific Glc-Nac-dependent adhesion to the epithelial carbohydrate receptors mediated by the F17a-G subunit [63].

The F17-fimbrial adhesin subtypes can be subdivided on the basis of their receptor specificities. Different studies demonstrate

that: i) F17a-producing *E. coli* strains bind to a specific receptor of calf brush border enterocytes but not to human receptors on human Caco-2 cells; ii) G fimbriae bind to human Caco-2 cells in culture but recognition of bovine enterocyte receptors appears to be influenced by the cell surface hydrophobicity; iii) F17c adhesin recognizes receptors on both the Caco-2 cells and bovine enterocyte brush borders; and (iv) only the F17a subtype fimbriae adhere to human uroepithelial cells [7, 63, 71, 84].

Mannose-resistant haemagglutination (MRHA) inhibition experiments show that GlcNAc binds to F17 agglutinins with different affinities in the preferential order:



**Figure 2.** Comparison of the predicted amino acid sequences of the F17-related adhesin subunits. The first residue of the mature protein is numbered +1. Gaps (\*) are introduced to obtain a maximal homology. Identical amino acid residues are identified by dashes. The sequences are compared using the Clustal W multiple alignment program. Accession numbers from GenBank are: M36649, L14319, L43374 and L77091, respectively.

F17c > G > F17b > F17a > F17d (table II) [7]. The high inhibitory potency of bovine submaxillary gland mucin (BSM) (containing 25 % sialic acid) and the influence of free N-acetylneuraminic acid, cerebrosides and glycophorin A on the binding of F17b and G fimbrial lectins to receptors present on bovine erythrocytes suggests that sialic acid may be involved in the binding of both lectins (table II) [7]. Porcine intestinal mucin (PGM) (containing 0.9 % of sialic acid) also induces a strong MRHA inhibition with the F17c, G and F17d subtypes (table II) [7]. The differences in the inhibitory potencies of BSM and PGM probably reflect various receptor specificities of the GlcNAc-binding fimbrial lectins.

Mucin is a high molecular weight glycoprotein constituting the mucus layer which covers the surface of the gastrointestinal tract [77]. The mucus layer may protect the host cells against the infectious process by preventing bacterial attachment. However, the host receptors recognized by the F17 adhesins are the O-glycosidically linked oligosaccharides of bovine intestinal mucin where the sequence GlcNAc $\beta$ 1-3Gal $\beta$ 1 is internally located [75]. In fact, the minimal GlcNAc $\beta$ 1-3Gal $\beta$ 1 sequence of the GlcNAc-containing receptors strongly binds to the F17a lectin when located in a terminal or internal position in carbohydrate moieties

[75]. The receptors recognized by the F17 lectins are present in both the mucus layer and the membrane of the epithelial brush-border. Several interactions established by F17 fimbriae are involved in the infection process: F17 adhesins first mediate the initial binding to the mucus layer and then facilitate the subsequent development of the second stage binding to the surface of intestinal villi [75, 93]. Furthermore, a decrease in adhesion of F17-producing strains is observed with older-calf ileal mucus suggesting that the density of receptors recognized by F17 adhesins are dependant on the age of the calf and the intestinal segment [75].

In addition, the G fimbriae mediate the adhesion of the bacterial strains to the laminin contained in the basement of mammalian membranes [92]. Laminin is a multi-domain glycoprotein produced by a variety of cell types, including epithelial and endothelial cells [70]. Terminal Glc-Nac residues in short side chains of the core structures of laminin are recognized by F17 adhesins [92]. It is well documented that laminin are recognized by other fimbrial adhesins: the mannose and sialyloligosaccharide chains of laminin are specific binding sites for *E. coli* strains producing type I and S fimbriae, respectively [52, 99]. In addition to the recognition of the GlcNAc

**Table II.** Comparative inhibitory activities of receptor analogs on MRHA of *E. coli* strains with N-acetyl-D-glucosamine binding fimbrial agglutinins.

Receptor analogs	Relative inhibitory potency <sup>a</sup>				
	F17a	F17b	F17c	F17d	G
N-acetyl-D-glucosamine (GlcNac)	0.13	0.5	1.0	0.03	0.34
Porcine intestinal mucin (PGM)	4.2	4.0	10	34	17
Bovine submaxillary gland mucin (BSM)	7.0	25	18	7	25
N-acetylneuraminic acid (pH 7.5)	— <sup>b</sup>	0.01	—	—	—
Glycophorin A from human blood	—	—	—	—	0.30

Values above 1.0 indicate a stronger affinity of the adhesin for the compound than free GlcNac [7].

<sup>a</sup> The relative inhibitory potency of free GlcNac on MRHA of the reference F17c-producing strain is assigned the value of 1.0 (0.19 mM is required for 50 % inhibition).

<sup>b</sup> No inhibition at the maximal concentration used (50 mM for glycolipids and glycoproteins).

receptors present in intestinal mucosal cells, the adhesion of F17 fimbriae to laminin may increase the bacterial colonization at damaged intestinal tissue sites and may, therefore, promote the translocation of the bacterial strains to extra-intestinal sites [92].

## 2.5. Genetic organization

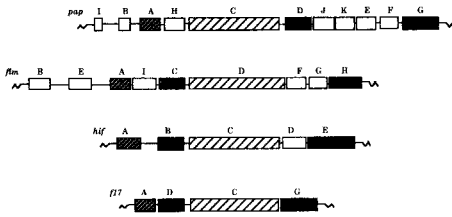
The organization of several fimbrial gene clusters has been intensively studied. For example, the biosynthesis of functional K99, type I or P fimbriae produced by *E. coli* strains requires the transcription of complex gene clusters of about 10 kb in size including eight to eleven genes each [25, 59]. On the basis of functional properties, the gene clusters can be divided into different groups of genes encoding: i) the proteins that regulate the expression of fimbrial genes clusters; ii) the structural, adhesin and minor subunits included in the heteropolymeric fimbrial structure exported at the cell surface; and iii) the periplasmic chaperon and the outer membrane 'usher'. The periplasmic chaperon binds to subunit proteins and prevents improper polymerization, aggregation and proteolytic activity in the periplasm [48]. The chaperon-subunit complex is then targeted to the 'usher' that acts as a molecular doorkeeper which receives the complexes and incorporates the subunits into the growing fimbriae [48].

### 2.5.1. Genetic organization of the *f17* gene clusters

A more simple genetic organization is observed for the different F17 fimbriae since the gene clusters required to synthesize functional fimbriae are 5 kb in size and include only four genes (*figure 3*) [8, 22, 62, 63, 71]. The analysis of the *f17a* gene cluster reveals the presence of *f17a-A*, *f17a-D*, *f17a-C* and *f17a-G* encoding a structural subunit, a periplasmic chaperon, an outer membrane usher and an adhesin subunit, respectively (*figure 3*) [63]. Few bovine *E. coli* strains

can express two distinct F17 structural subunits (F17c-A/F17d-A or F17b-A/F17d-A) encoded by two complete and separate gene clusters [8]. Moreover, in some cases, the two distinct structural subunits are produced at the bacterial cell surface [8]. It seems that the two F17-related gene clusters have evolved from each other in these bacterial strains by duplication [8]. The strains producing both F17c /F17d or F17b /F17d fimbriae subtypes possess two separate and complete gene clusters but the *f17d-A* structural subunit gene is associated with the *f17c-G* or *f17b-G* adhesin gene, respectively [8]. This finding suggests that the related gene clusters encoding the different F17 fimbriae subtypes are probably organized in mosaic operons constituted of F17-related structural and adhesin gene subunits [8].

Interestingly, a cluster of five genes is required for the expression of the two most distantly related members of the F17-family: the Hif and Haf fimbriae produced by *H. influenzae* strains associated with meningitis and Brazilian purpuric fever (BPF), respectively [89, 98] (*figure 3*). In addition to the four proteins with functions similar to those required for F17 fimbrial biogenesis, the HifD minor subunit (predicted to be a lipoprotein) may take part in the formation of the adhesin-usher complex (HifE-HifC) implicated in the Hif fimbrial polymerization [98]. The lipoprotein properties of HifD probably serve to anchor the protein to the bacterial membrane [98]. HifD remains membrane associated during the fimbrial polymerization and the incorporation of HifD in the growing fibrillum would probably terminate the polymerization [98]. Moreover, *H. influenzae* strains associated with BPF carry two distinct fimbrial gene clusters (*haf1* and *haf2*) with more than 99 % identity, similarly to some F17-producing *E. coli* strains [89]. Read et al. [89] strongly suggest that: i) the duplication of the two gene clusters probably occurred by a recent intragenic recombination rather than by the acquisition of DNA from another strain through natural transformation; and



**Figure 3.** Maps of genes encoding the biogenesis of fimbriae. *pap*, *fim*, *hif* and *f17* cluster of genes code for the biogenesis of P, Type I, Hif (or Hib) and F17 fimbriae, respectively. Major structural subunit (▨); outer membrane usher (▧); periplasmic chaperon (■); adhesin subunit (■).

ii) these strains with two functional sets of pilus genes may produce significantly greater amounts of fimbriae on the cell surface.

### 2.5.2. Hypothesis of multifunctional F17 fimbrial proteins

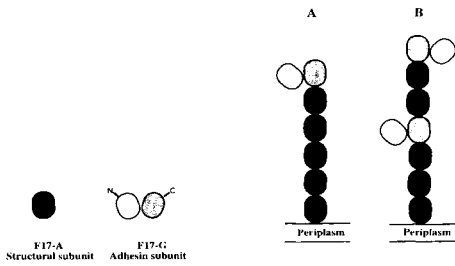
The comparison of amino acid sequences of the F17 adhesin subunits, reveals that the substitutions are located on the N-terminal halves of the respective proteins, whereas the C-terminal parts are identical (figure 2) [22, 71, 91]. Genetic analysis of G fimbriae biosynthesis is performed by means of deletion mutagenesis of the *gafD* adhesin subunit gene [91]. A deletion in the N-terminal half of GafD results in the biosynthesis of morphologically intact G fimbriae but the adhesion to the Glc-Nac-containing receptor is not retained [91]. In contrast, deletions in the C-terminal half of the protein abolish both Glc-Nac recognition and expression of G fimbriae [91]. The absolute conservation of the C-terminal part of the different F17-G proteins strongly suggests a strict structural or functional role of the F17 adhesin subunits. Saarela et al. [91] propose that the N-terminal part of GafD participates in receptor recognition, whereas the C-terminal part is required for the biogenesis of the fimbrial filament or for the integration of GafD in the fimbrial filament. This agrees

well with a secondary structure prediction analysis on the adhesin and structural subunits (including F17a-A and F17a-G proteins) showing a clear structural relatedness between the structural subunit and the C-terminal half of the adhesin [33]. The C-terminal-part of the adhesin, which folds as a structural subunit, can serve as an assembly module required for adhesin presentation at the fibrillum tip [33] (a model for the assembly of F17 fimbrial structures is proposed in figure 4). Moreover, the PapG adhesin is described as a two-domain protein in which the highly conserved C-terminal part is required for forming a complex with the PapD chaperon [43]. The interaction of PapD with PapG is required for the incorporation of the adhesin subunit into the fimbrial structure and for the enhancement of the cleavage of the PapG signal sequence [43].

In addition, the interactions between the GafA structural subunit and the GafD adhesin subunit seem to be crucial for the initiation of fimbrial monomer polymerization [91]. When compared to Hif fimbrial biogenesis, the additional HifD protein is implicated in the initiation of fimbrial polymerization by anchoring the adhesin-usher complex to the bacterial membrane [98]. Moreover, during P fimbrial biogenesis, the two distinct proteins PapK and PapF are required as polymerization initiators of P fimbrial monomers and for the correct presentation of the adhesin subunit within the fimbriae [46]. Genes encoding equivalent proteins are not present on the cluster of genes required to functionally express F17 fimbriae. A multifunctional nature of fimbrial proteins could explain why only four genes are necessary for the F17 fimbrial biogenesis [91].

## 3. AFIMBRIAL ADHESINS

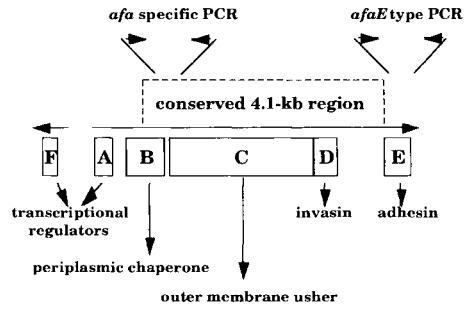
The first report of determinants encoding an adhesin that was not associated with the presence of visible fimbriae on the bacterial surface was that of an *afa* gene cluster



**Figure 4.** Model for the assembly of F17 fimbrial structures. The N-terminal half of F17-G adhesin subunit mediates eukaryotic receptor recognition. The C-terminal half can be involved in the assembly of the subunit within the fimbrial structure. The F17-A structural subunit shows a clear structural relatedness with the C-terminal part of F17-G [33]. The adhesin subunit is located within the fimbrial structure [63, 91] but its location at the tip of the fibre (A) has not been demonstrated. Interaction of F17-A with the C-terminal part of F17-G also permits the incorporation of one or several adhesin monomers within the fimbrial structure (B).

encoding an adhesive structure designated AFA, for afimbrial adhesin in 1984 [53]. The *afa* gene cluster was shown to be located on a 6.7-kb fragment cloned from the genomic DNA of a pyelonephritic *E. coli* strain (KS52). The cloned fragment expressed polypeptides with molecular masses of 13, 30, 100, 18.5 and 16 kDa and was encoded by five genes, *afaA* to *afaE*, respectively, organized in an operon (figure 5). Among the *afa* genes, only *afaB*, *afaC* and *afaE* were demonstrated to be required for MRHA expression and adherence to uroepithelial cells [53, 54]. The *afaE* gene has been identified as the structural adhesin gene which mediates specific binding to uroepithelial cells as well as to HEP-2 and HeLa cells with a so-called diffuse adherence pattern [100].

Hybridization experiments and western blot (immunoblot) analyses demonstrated the existence of gene clusters structurally related to the first *afa* operon to be described but encoding antigenically distinct adhesins. These experiments demonstrated that all the



**Figure 5.** Genetic and structural organization of the human *afa*-related gene clusters. Boxes represent genes and letters designate the different genes. The arrows refer to the direction of transcription of the *afa* genes. The conserved region of the *afa* operons defined by the presence of three *psfI* restriction fragments of 1.1, 0.4 and 2.6 kb is marked with dashed lines. The positions of the sets of primers used in the *afa* specific PCR and the *afaE* type PCR are indicated.

*afa* gene clusters harbour a highly conserved 4.1-kb DNA segment (carrying the *afaB*, *afaC* and *afaD* genes) and revealed heterogeneity for the *afaE* sequences (figure 5). Thus, it was proposed that at least four different *afa* operons exist, *afa-1*, *afa-2*, *afa-3* and *afa-4*, which encode variable adhesins first designated as AFA-I, AFA-II, AFA-III and AFA-IV, respectively [54]. Several years following these descriptions, the report that the afimbrial adhesive structure was composed of two proteins, AfaE and AfaD, allowed the renaming of the adhesins encoded by the various *afaE* genes as AfaE-I to AfaE-IV [28]. Recently, the AfaE-V type adhesin was described in human isolates associated with both intestinal or extra-intestinal disorders [27, 103]. Finally, the presence of *afa*-related sequences was detected among isolates from both diseased piglets and calves [41, 68] and two *afa*-related operons, designated *afa-7* and *afa-8*, encoding the AfaE-VII and AfaE-VIII type adhesins were characterized from bovine pathogenic *E. coli* [56] (table III).

The existence of non-fimbrial adhesins encoded by different gene clusters was then described at the cell surface of *E. coli* strains belonging to various pathotypes (table III). CS6 is a colonization factor (CF) of human enterotoxigenic *E. coli* (ETEC) strains that was characterized in 1985. It is one of the seven most commonly found CFs on human ETEC strains. The structure of CS6 remains undefined. It is composed of the C<sub>ss</sub>A and C<sub>ss</sub>B subunits which may constitute either a single fine fibrillar structure, or individual fine fibrillae, or an afimbrial adhesive sheath [101]. The M-specific agglutinin was identified in 1986 from a strain isolated from the urine of a patient with acute pyelonephritis [47, 90, 97]. This adhesin specifically recognizes the blood group M substance glycoprotein AM. Epidemiological studies showed that the production of the M-agglutinin is very infrequent in the strains isolated in pyelonephritis [3]. The NFA adhesins (NFA-I to NFA-VI) were characterized from human uropathogenic strains [1, 51]. The AIDA-I adhesin was identified in a diarrhoea-associated *E. coli* strain. This 100-kDa afimbrial adhesin is plasmid encoded and mediates, like the AfaE

adhesins, a diffuse adherence phenotype of the bacteria on HeLa and HEp-2 cells [5].

The CS31A adhesin has been identified among bovine and human enterotoxigenic or septicaemic *E. coli* [36]. The CS31A antigen that has been described as a capsule-like structure around the bacteria, is composed of the ClpG protein. The CS31A adhesin mediates adhesion to a N-acetylneuraminic acid-containing receptor present in the human carcinoma cell line Caco-2 [36]. It is mainly produced by bovine pathogenic strains also producing the F17c subtype (see above).

Among the afimbrial adhesins, those encoded by the *afa* gene clusters have been more-extensively studied.

### 3.1. The *afa* family of gene clusters

The *afa* family includes closely related gene clusters that are expressed by uropathogenic and diarrhoea-associated *E. coli* (table IV). The *dra* determinants encoding the Dr adhesins produced by uropathogenic strains [78, 85] as well as the *daa* determinants encoding the fimbrial F1845 adhesin produced by a strain isolated

**Table III.** Afimbrial adhesins produced by pathogenic *E. coli* strains.

<i>E. coli</i> pathotype	Adhesive structure	Adhesin-encoding gene	Cellular receptor	Genetic location	References
Human UPEC, human DAEC, and bovine pathogenic <i>E. coli</i>	AFA	<i>afaE</i>	DAF*	pl/ch	[56, 58]
Human DEAC	AIDA-I	nd	nd	pl	[5]
Human ETEC	CS6	<i>cssA</i> and <i>cssB</i>	nd	pl	[101]
Human UPEC	M	<i>bmaE</i>	blood group M antigen	ch	[90]
Human UPEC	NFA	<i>nfaA</i>	N-acetyl-D-glucosamine	ch	[1, 51]
Bovine and human pathogenic <i>E. coli</i>	CS31A	<i>clpG</i>	N-acetylneuraminic acid containing receptor	pl	[36]

Abbreviations: ETEC, enterotoxigenic *E. coli*; UPEC: uropathogenic *E. coli*; DAEC: diffuse adherent *E. coli* associated with diarrhoea in human beings; pl: plasmid; ch: chromosome; nd: not determined.

\* The decay-accelerating factor (DAF) is the receptor for AfaE adhesins produced by human pathogenic *E. coli* strains.

Table IV. Adhesins encoded by *afa*-related gene clusters.

Adhesin	Original appellation	Operon	Genetic location	Reference strain	Host	Pathogenicity	AfaE-I	AfaE-II	AfaE-III	% identity (AA level) with		M- agglutinin
										AfaE-V	F1845	NFA-I
AfaE-I	AFA-I	<i>afa-1</i>	ch	KS52	human	UTI, D	100	29	38	32	44	- <sup>4</sup>
AfaE-II	AFA-II	<i>afa-2</i> 1, 2	ch	A11	human	UTI, D		100	31	28	30	- <sup>4</sup>
AfaE-III	AFA-III	<i>afa-3</i>	pl/ch	A30	human	UTI, D			100	51	57	- <sup>4</sup>
AfaE-V		<i>afa-5</i>	pl/ch	AL851	human	UTI, D				100	48	- <sup>4</sup>
Dr	O75X	<i>dra</i>	ch	IH11128	human	UTI <sup>3</sup>	38	31	98	51	57	- <sup>4</sup>
Dr-II		<i>dra2</i>	ch	7732	human	UTI <sup>3</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	24	99
Drb121		NI			human	UTI <sup>3</sup>	43	25	46	39	46	- <sup>4</sup>
Drb122		NI			human	UTI <sup>3</sup>	32	31	47	77	43	- <sup>4</sup>
F1845		<i>dau</i>	pl/ch	C1845	human	UTI/D					100	25
NFA-I		<i>nfaI</i>			human	UTI <sup>3</sup>						100
NFA-111		NI			human	UTI <sup>3</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	63
NFA-116		NI			human	UTI <sup>3</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	99
AfaE-VII		<i>afa-7</i>	ch	262KH89	bovine	D <sup>3</sup>	- <sup>4,5</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>
AfaE-VIII		<i>afa-8</i>	pl/ch	239KH89	bovine, human	D,S, UTI	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>	- <sup>4</sup>

Abbreviations: pl: plasmid; ch: chromosome; NI: not identified; UTI: urinary tract infection; D: diarrhoea; S: septicæmia.

The sequence alignments were performed with the GAP program by using the Genetics Computer Group Sequence Analysis Software Package.

<sup>1</sup> Strains carrying the *afa-2* gene cluster were initially described as encoding the AFA-IV adhesins [54].

<sup>2</sup> Strains carrying both the *afa-2* and *afa-3* gene clusters were initially identified as encoding the AFA-II adhesins [54].

<sup>3</sup> The presence of AfaE-VII among human pathogenic *E. coli* was not investigated.

<sup>4</sup> Too different to obtain an identity comparison.

<sup>5</sup> 26 % identity with the AAF/I adhesin that is a fimbrial adhesin produced by enteroaggregative *E. coli*.



from stools of a child with persistent diarrhoea [10] have been demonstrated to belong to the *afa* family [26, 28]. All these operations shared a very similar genetic organization and were closely related at the DNA level [26, 58]. Consequently, probes and PCR assays used to detect the presence of *afa* gene clusters in a strain also detect the presence of *dra* and *daa* operons (figure 5). The *afaldaa*-specific DNA probes that consist of sequences internal to the highly conserved region (*afaB*, *afaC* and *afaD* genes) were first defined [3, 10]. Then, a specific PCR assay using primers chosen from the sequence of the DNA probes was developed [57] (figure 5).

High degrees of similarities were found between the nucleotide sequence of the *afa-3* gene cluster and the partial sequence of the non-fimbrial NFA-I-encoding operon [1, 26]. Whereas the structural gene encoding the NFA-I adhesin had no homology with any of the genes encoding the AfaE adhesins (nor was the NFA-I related to AfaE-III at the peptide level (table IV)), the AfaB chaperon exhibited 95.9 % identity with the homologous NfaE protein. Moreover, the non-coding region located upstream of the adhesin-encoding genes (*afaE* and *nfaA*) displayed strong similarities (79.5 % identity over 138 nucleotides) [26]. Such conservation at the DNA level suggests that both the *nfa* and *afa* determinants are closely related. Recently, the description of NFA-related adhesins, designated NFA111, NFA116 and Dr-II [85, 103] (table IV) encoded by strains that gave positive amplification using the *afa* specific PCR strongly enhanced this hypothesis.

The organization of the genes encoding the M-specific haemagglutinin blood group was studied with a cloned 6.5-kb DNA fragment from the *E. coli* strain IH11165 [90]. This DNA segment was shown to contain five genes (*bmaA* to *bmaE*) which code for polypeptides of 12.5, 30, 80, 18.5 and 21 kDa, respectively. Although the genetic organization of the M-agglutinin gene cluster resembles those of other afimbrial

adhesins, no relation could be deduced since only the *bmaE* gene which was demonstrated to encode the M-agglutinin subunit has been sequenced. Until the cloning and sequence analysis of the *afa-8* gene cluster was obtained, no significant sequence homology between the M-agglutinin subunit and other adhesin subunits was evidenced. The observation that the AfaE-VIII afimbrial adhesin encoded by the *afa-8* gene cluster exhibits strong homology with the M-agglutinin (97 % identity) [56] (table IV) suggests that the *bma* determinants belong to the same phylogenetic family of gene clusters as the *afa* and *nfa* operons.

### 3.2. Genetic and structural organization of the *afa* gene clusters

#### 3.2.1. *afa* Gene clusters from human pathogenic *E. coli* strains

The information deduced from the sequence analysis of the *afa-3* gene cluster [26], encoding the AfaE-III adhesin, has shown that the *afa* gene clusters are composed of six genes (*afaA* to *afaF*) organized in two divergent transcriptional units corresponding to *afaA-afaE* and *afaF*, respectively (figure 5). Five of the six Afa products showed marked homologies with proteins encoded by previously described adhesion systems which allowed the attribution of a putative function in the biogenesis of the afimbrial adhesive sheath to each of them. The biogenesis of the AFA adhesive structure, like that of a fimbrial one [45], requires proteins with specialized functions such as a periplasmic chaperon (AfaB protein), an outer membrane anchor protein (AfaC), and transcriptional regulators (AfaA and AfaF) [26]. The *afaA* and *afaF* genes were found to encode products that belong to the family of the PapI-PapB regulators commonly involved in the regulation of *E. coli* fimbrial adhesin expression [26]. The *afaE* gene encoded the structural adhesin. No function

could be attributed to the AfaD product encoded by a gene known to be non-essential for the expression of MRHA and for the adherence to epithelial cells [55]. The role of AfaD in the biogenesis of the afimbrial structure was then assessed by immunogold and immunofluorescence experiments. Similar to AfaE, AfaD was found to be a surface-exposed protein as well as an adhesin; both AfaD and AfaE are concomitantly expressed by the bacterial cell [28]. The roles of the two components of the AFA afimbrial sheath during the association of *afa*-expressing strains with epithelial cells was investigated by ultrastructural and genetic analyses. These experiments revealed that bacteria producing both AfaD and AfaE could be internalized into HeLa cells. Then, the examination of mutants and polystyrene beads coated with either AfaD or AfaE demonstrated that AfaE and AfaD are involved in initial binding to HeLa cells and in the internalization process, respectively [49]. These data demonstrate that the *afa* gene cluster is unique among bacteria, as it alone encodes both the adhesion to and the invasion of epithelial cells.

The sequence of the *afaE* structural adhesin-encoding gene is highly heterogeneous among the *afa* gene clusters, leading to the production of antigenically distinct adhesins. The determinants encoding the adhesin subunit were cloned from various *afa*-expressing strains. The sequences of the genes encoding the AfaE-I, AfaE-II, AfaE-III and AfaE-V type adhesins as well as those encoding the Dr (Dr-I and Dr-II), F1845 and other adhesin variants called Drb121, Drb122, NFA111, NFA116 adhesins have been reported [10, 26, 27, 53, 54, 58, 78, 85, 103]. The homologies between the known AfaE subtypes are presented in *table IV*. A large number of AfaE adhesins still, however, remain to be identified. PCR assays have been developed for typing of the gene encoding the AfaE-I, AfaE-II, AfaE-III, AfaE-V and F1845 adhesin variants [27, 103] (*figure 5*). The AfaE-III adhesin encoded by the *afa-3* gene

cluster is closely related to the F1845 adhesin encoded by the *daa* operon (53.6 % identity); however, unlike the AfaE-III adhesin, F1845 is a fimbrial adhesin. Creation of recombinant chimeras between the *afa-3* and the *daa* gene clusters have revealed that the production of an afimbrial AfaE-III or a fimbrial F1845 adhesin is solely dependent on the nucleotide sequence of the adhesin-encoding gene [28]. These findings suggest that the respective architectural differences among the adhesive materials are determined by differences in the packing of AfaE-III or F1845 subunits into three-dimensional macromolecular structures. Epidemiological studies have revealed that the fimbrial state is exceptional among adhesive sheaths encoded by the *afa* family of gene clusters [103].

### 3.2.2. *afa* Gene clusters from animal pathogenic *E. coli* strains

Pathogenic *E. coli* isolates of porcine and bovine origin possess adhesin-encoding gene clusters (*pap*, *sfa*, *afa*) related to extra-intestinal infections in human beings. The presence of *afa*-related sequences was detected among isolates from both diseased piglets and calves [41, 68]. However, under high stringency hybridization conditions or in the *afa*-specific PCR derived from the sequence analysis of the conserved region of the human *afa* operons, the *afa* animal strains were negative. This suggests that the *afa* operons of animal pathogenic *E. coli* are structurally different from the operons of human *E. coli* isolates [68]. Additionally, hybridization experiments suggested that the *afa* animal operons are various [69]. Recently, two different *afa*-related gene clusters, designated *afa-7* and *afa-8* respectively, were cloned from *E. coli* strains isolated from calves with diarrhoea and septicaemia [56]. They encode afimbrial adhesins. The genetic organization of *afa-7* and *afa-8* was found to be similar to that of the *afa* gene clusters from human strains. Partial sequence determination of these gene

clusters indicated that the C-terminus of the AfaC proteins from *afa-7*, *afa-8* and *afa-3* are highly conserved (82 % identity over 79 amino acids between AfaC peptides from *afa-7* and *afa-3*; 67 % identity over 79 amino acids between AfaC from *afa-7* and *afa-8*). The AfaD peptides from the animal gene clusters display only 46 % identity with each other and with the AfaD invasin from *afa-3*. The AfaD products encoded by *afa-7* and *afa-8* complement the invasion of HeLa cell deficiency of an *afa-3*-expressing strain with a mutated *afaD* gene, suggesting that they are also invasins [56]. The AfaE-VII product displayed a weak similarity (26 % identity) with the fimbrial AAF/I adhesin encoded by enteroaggregative *E. coli* implicated as an agent of pediatric diarrhoea in the developing world [76]. AfaE-VII encodes a mature protein of 151 amino acids that includes two cysteine residues located 33 amino acids from each other as in the previously studied AfaE adhesins. In contrast, the *afaE* gene from *afa-8* encodes an adhesin that exhibits very strong homology with the M-agglutinin [90], an afimbrial adhesin described in uropathogenic human *E. coli* (97 % identity).

### 3.2.3. Dissemination of the *afa*-related gene clusters

The location of gene clusters of the *afa* family is dual. By subtyping, the *afa-3*, *afa-5*, *afa-8* and *daa* gene clusters were found both on large plasmids and on the chromosome [56, 58, 103]. Some *afa-8* determinants were located on the Vir plasmid which also carry sequences encoding CNF2 [56]. All the other identified subtypes were found only on the chromosome [10, 53, 54, 56, 103]. The plasmid-borne *afa-3* gene clusters were found to be flanked by two IS1 elements in direct orientation and two in opposite orientations [26]. The *afa-3* gene cluster, flanked by two directly oriented IS1 elements, was shown to translocate from a recombinant plasmid to the *E. coli* chromo-

some. This movement occurred via IS1-specific recombination mediated by a *recA*-independent mechanism [26]. Whereas such a situation has already been reported for drug resistance genes and for genes encoding toxins, this was the first evidence that a pathogenic determinant responsible for adhesion properties is capable of translocation from one replicon to another, allowing us to speculate on the possible dissemination of such determinants among pathogenic *E. coli*.

## 3.3. Prevalence of AFA-producing strains

### 3.3.1. Prevalence among human pathogenic *E. coli* strains

Several studies suggest that *afa*-positive strains play an important role in urinary tract infection pathogenesis. The Dr blood antigen component of the decay-accelerating factor that is the receptor of the AfaE adhesins from human operons [79, 80] is widely distributed along the urinary tract, underlying the importance of the AFA-producing strains in the ascending colonization of the urinary tract. The AFA strains occur frequently among *E. coli* isolates from pregnant women [81] and children [3, 4] with UTI and are also associated with recurring UTI [103]. Moreover, development of an animal model for experimental chronic pyelonephritis has provided evidence for the importance of expression of the *dra* gene cluster, an *afa*-related operon, in establishing persistent colonization of the urinary tract [38].

Human diarrhoeagenic *E. coli* comprises a heterogeneous group of pathogenic bacteria classified into six different pathotypes based on clinical aspects of the disease and identification of virulence factors produced by the isolates [76]. The frequency of each pathotype depends on the geographic area and varies among epidemiological studies. Diffusely adherent *E. coli* (DAEC) are one of these categories. The only known viru-

lence factor of DAEC strains is their diffuse pattern of adherence to cultured epithelial cells. Two families of gene clusters encoding diffuse adherence have been identified in DAEC strains: *afa/daa* [10, 58] and the AIDA (adhesin involved in diffuse adherence)-encoding genes [5]. Recent epidemiological studies have implicated DAEC strains carrying *afa* genes as a cause of acute and persistent diarrhoea in 2–6-year-old children in developing countries [30, 37, 60]. In New Caledonia, AFA strains were found to be the first bacterial pathogens associated with diarrhoea in children (corresponding to 22 % of the bacterial pathogens isolated from stools) (Y. Germani, pers. comm.). In contrast, several epidemiological studies have demonstrated that the AIDA-encoding genes are rare (0–1 %) in DAEC [30, 60].

Type-specific PCR were used to investigate the distribution of AfaE subtypes in uropathogenic and diarrhoeagenic *afa*-expressing *E. coli*. The data indicated that the adhesin subtype did not determine the pathotype (M.I. Garcia, pers. comm.). An *E. coli* isolate carrying an *afa* gene cluster encoding an AfaE-I adhesin has recently been reported to be the causative agent of both diarrhoea and cystitis in the same child [31]. *afa-1*, *afa-3* and *afa-5* gene clusters predominate in both uropathogenic and diarrhoeagenic strains (M.I. Garcia, pers. comm. and [103]).

### 3.3.2. Prevalence among bovine and porcine pathogenic *E. coli* strains

A preliminary study of the frequency of *afa-7* and *afa-8* gene clusters among the bovine and porcine *E. coli* isolates previously reported to carry *afa*-related sequences, suggests a high incidence of the *afa-8* gene cluster and a low prevalence of *afa-7* among these strains [56]. *afa-8* has been identified in bovine necrotogenic strains (producing a cytotoxic necrotizing factor (CNF1 or CNF2)) associated with intestinal and extra-intestinal infections in

farm animals. Most of these strains also hybridized with the F17 and/or PAP and CS31A probes [56]. The sequences of the *afaD* and *afaE* genes are highly conserved among these *afa-8*-positive strains [56]. The *afa-8* gene cluster has also been detected in CNF-negative bovine isolates and in F165-positive bovine and porcine *E. coli* isolates associated with intestinal infections (J. Fairbrother, pers. comm.).

## 3.4. Adhesion properties

### 3.4.1. AfaE adhesins produced by human pathogenic *E. coli* strains

The AfaE adhesins mediate a mannose-resistant agglutination of human erythrocytes (MRHA) expressing the Dr blood group antigen. The AfaE receptor has been more precisely identified as the short consensus repeat-3 (SCR-3) domain of the decay-accelerating factor (DAF or CD55) [80], one of the cell membrane proteins that regulate the complement cascade and protect eucaryotic cells from being lysed by an autologous complement. DAF is a 70-kDa glycoprotein widely distributed on haematopoietic cells, intestinal and urinary epithelia and endothelial cells [65–67]. The density of DAF molecules, however, is dependent on the cellular type and DAF molecules show tissue and host tropisms. AfaE adhesins from human pathogenic strains mediate a specific attachment to uroepithelial cells and to HEP-2, HeLa, Caco-2 and T84 epithelial cells with a so-called diffuse adherence pattern [49, 50].

### 3.4.2. AfaE adhesins produced by animal pathogenic *E. coli* strains

The AfaE adhesin produced by the *afa-7* operon mediates a mannose-resistant agglutination of human, bovine and porcine erythrocytes [56]. Preliminary data suggest that AfaE-VII also recognizes DAF molecules as a receptor (L. Lalioui, pers. comm.).

AfaE-VII mediates a specific attachment to human and bovine uroepithelial cells (Urotsa, and Mabin-Darby bovine kidney cell lines, respectively), and HeLa, Caco-2 cell lines [56].

The *afa-8* gene clusters mediate a man-nose-resistant agglutination of human erythrocytes [56]. AfaE-VIII adhesins are incapable of haemagglutinating bovine, porcine, sheep, cat, dog and horse erythrocytes (J. Fairbrother, pers. comm.). Since the AfaE-VIII adhesins displayed a high degree of homology (97 % identity) with the M-agglutinin, the blood group M substance glycophorin AM which is the receptor of the M-agglutinin [47] was supposed to also be the receptor of AfaE-VIII. The AfaE-VIII adhesins mediate attachment to human and canine uroepithelial cells (Urotsa and Mabin-Darby canine kidney cell lines, respectively) [56]. The receptor of AfaE-VIII on epithelial cells remains to be identified.

### 3.5. Interaction of *afa*-expressing *E. coli* with epithelial cells

The *afa* gene clusters have the unique feature of encoding both an adhesin and an invasin that seem to be involved in a two-stage process. AfaE is an adhesin that is absolutely required for adhesion to eukaryotic cells whereas AfaD is an invasin promoting internalization of adherent bacteria into these cells [28, 49] (*figure 6*).

During bacterium–epithelial cell interactions, it has been observed that adhesion of bacteria to cells is accompanied by a massive recruitment of DAF molecules at the adhesion site, with DAF molecules being particularly concentrated in the membrane extensions of the epithelial cells that tend to enwrap the adherent bacteria (*figure 6*) [50]. The DAF molecule is a glycosyl phosphatidylinositol (GPI)-anchored protein that has been reported to function in signal transduction in leucocytes [94]. Identification of the putative signal transduced to epithelial cells following AfaE-DAF interactions

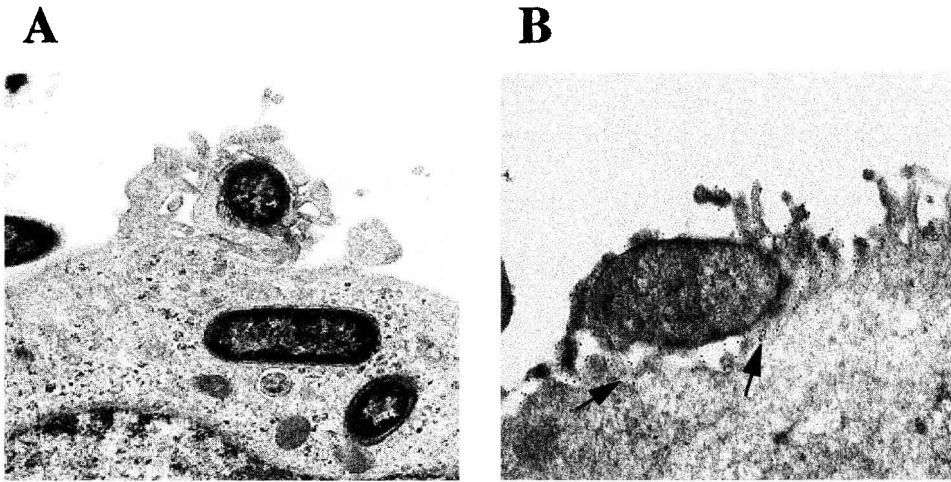
should elucidate the pathological process involved in diseases caused by the *afa*-expressing *E. coli* strains.

The *afaD* genes are structurally and functionally conserved among human *afa*-expressing *E. coli* isolates [29]. The AfaD variants produced by the *afa-7* and *afa-8* gene clusters and the AfaD homologue (AggB) produced by enteroaggregative *E. coli* (which are associated with persistent diarrhoea in humans) were also found to be invasins [29, 56]. Strains carrying an *afa* gene cluster are associated with acute and persistent forms of diarrhoea and urinary tract infections in human beings and the human AFA strain internalization occurs in both intestinal and urothelial cell lines [50]. The role, if any, of the invasion of epithelial cells in the pathogenesis of these diseases remains to be established. Epithelial cell penetration possibly provides a protected niche for the organism so that it can survive and persist within the host.

## 4. CONCLUSION

In conclusion, the F17-related fimbrial adhesins and AFA-related afimbrial adhesins produced by pathogenic *E. coli* strains have been extensively detected among domestic animals with diarrhoea and/or septicaemia. In addition, afimbrial adhesins are produced by pathogenic *E. coli* associated with various pathologies in humans. High percentages of identity are observed between F17 fimbrial proteins not only produced by different bacterial species (*E. coli*, *P. mirabilis*, *H. influenzae*), but also by bacteria from different infectious sites (intestinal tract, blood, urinary tract or throat) and different hosts (human, bovine, ovine or canine). The F17-family seems to be very large and probably includes other uncharacterized subtypes.

The study of the *afa*-related family of gene clusters demonstrated that most of the afimbrial adhesin-encoding operons belong to the same phylogenetic family of deter-



**Figure 6.** Interaction of a recombinant HB101 strain expressing the *afa-3* gene cluster with HeLa cells. A, Transmission electron micrograph of HeLa cells incubated for 3 h. Bacteria were embedded in microvillar extensions of the HeLa cells. Intracellular bacteria were observed in membrane-bound vacuoles. B, Electron micrograph of immunogold staining of HeLa cells infected for 3 h. DAF molecules (arrows) were detected with anti-DAF antibodies followed by anti-mouse IgG antibodies conjugated with 10-nm gold particles. A recruitment of DAF molecules was evidenced at the bacterial-HeLa cell interaction site. Magnifications, A:  $\times 15\,000$ , B:  $\times 17\,000$ . Photographs were from the 'Station centrale de microscopie électronique', Pasteur Institute, Paris.

minants. The *afa* gene cluster is unusual in that a single operon encodes both adhesion of the bacteria to the epithelial cells and the invasion of these cells. Consequently, the *afa* gene clusters are good models for studying bacterium-cell interaction mechanisms. The AFA family seems to be large and probably includes other uncharacterized variants. In human pathogenic *E. coli* associated with intestinal and extra-intestinal infections, at least three subtypes of *afa* gene clusters (*afa-1*, *afa-3*, and *afa-5*) are predominant. In contrast, only one *afa* gene cluster, the *afa-8* operon, seems to be predominant in bovine pathogenic *E. coli*.

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