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Demonstration of regulatory cross-talk between P fimbriae and type 1 fimbriae in uropathogenic *Escherichia coli*

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The majority of *Escherichia coli* strains isolated from urinary tract infections have the potential to express multiple fimbriae. Two of the most common fimbrial adhesins are type 1 fimbriae and pyelonephritis-associated pili (Pap). Previous research has shown that induced, plasmid-based expression of a Pap regulator, *papB*, and its close homologues can prevent inversion of the *fim* switch controlling the expression of type 1 fimbriae. The aim of the present study was to determine if this cross-regulation occurs when PapB is expressed from its native promoter in the chromosome of *E. coli* K-12 and clinical isolates. The regulation was examined in three ways: (1) mutated alleles of the *pap* regulatory region, including *papB* and *papI*, that maintain the *pap* promoter in either the off or the on phase were exchanged into the chromosome of both *E. coli* K-12 and the clinical isolate *E. coli* CFT073, and the effect on type 1 fimbrial expression was measured; (2) type 1 fimbrial expression was determined using a novel *fimS*::*gfp*$^+$ reporter system in mutants of the clinical isolate *E. coli* 536 in which combinations of complete fimbrial clusters had been deleted; (3) type 1 fimbrial expression was determined in a range of clinical isolates and compared with both the number of P clusters and their expression. All three approaches demonstrated that P expression represses type 1 fimbrial expression. Using a number of novel genetic approaches, this work extends the initial finding that PapB inhibits FimB recombination to the impact of this regulation in clinical isolates.

INTRODUCTION

Urinary tract infections (UTIs) are common, affecting a large proportion of the population. It is estimated that 20 % of women develop a UTI in their lifetime, and antibiotic treatment results in approximately 110 000 prescriptions per million inhabitants per annum in Europe (Naber, 2000). *Escherichia coli* strains are the predominant cause of uncomplicated UTIs, responsible for between 60 and 80 % of the cases reported in the UK each year (Graham & Galloway, 2001). Many infections are asymptomatic, especially in the elderly (Nicolle, 2001), but others result in cystitis. If the infection ascends to the kidney, then pyelonephritis can occur. Such infections are a significant origin of Gram-negative sepsis.

Abbreviations: GFP, green fluorescent protein; HA, haemagglutination; Lrp, leucine-responsive regulatory protein; MRHA, mannose-resistant HA; MSHA, mannose-sensitive HA; Pap, pyelonephritis-associated pili; Prf, Pap-related fimbriae; RBC, red blood cell; Sfa, S-fimbrial adhesin; UPEC, uropathogenic *E. coli*; UTI, urinary tract infection.

Fimbrial adhesins are important virulence factors that allow binding of the bacteria to specific receptors on epithelial cells of the urinary tract. The two adhesins most commonly associated with UTI are type 1 fimbriae, and pyelonephritis-associated pili (Pap) and Pap-related fimbriae (Prf); the last two are collectively termed P fimbriae in this study. Type 1 fimbriae mediate binding to α-D-mannose-containing receptors and extracellular matrix components, whereas P fimbriae bind to glycoreceptors containing the αGal(1-4)βGal moiety (Lindberg *et al.*, 1984). Although type 1 fimbriae are common to the majority of *E. coli* isolates, the FimH adhesin has been shown to be important in a mouse model of UTI, and a degranulation response to the fimbriae is associated with renal scarring (Mizunoe *et al.*, 1997). There is good evidence from a number of epidemiological studies that P fimbriae are important in upper UTI (Johnson, 1991). While the mechanism is debatable (Hedlund *et al.*, 1999; Schilling *et al.*, 2003), P-fimbrial expression has been shown to induce inflammation in humans and in a mouse model. A current model provides evidence for P-fimbrial

adherence provoking inflammation in a cluster of differentiation number 14 (CD-14)-independent manner, probably by association with toll-like receptor number 4 (TLR-4) (Frendeus *et al.*, 2001).

To limit immune exposure and inflammation, the expression of type 1 and P fimbriae is phase variable: both are controlled by reversible genetic switches. The mechanisms that control the phase-variable expression are distinct, with type 1 fimbriae regulated by an invertible DNA element (*fim* switch), and P fimbriae by mutually exclusive protein complexes initiated by alternative DNA methylation patterns (Blomfield, 2001). Phase variation of type 1 fimbrial expression requires the activity of two recombinases; FimB promotes inversion in both directions, whereas FimE mediates predominantly on-to-off inversion (Gally *et al.*, 1996; Klemm, 1986). Previous work has demonstrated that a regulator from the P-fimbrial gene cluster, PapB, when expressed from a plasmid, is able to prevent inversion of the *fim* switch (Holden *et al.*, 2001; Xia *et al.*, 2000). Cross-talk by PapB has been shown to occur by inhibition of FimB-promoted recombination together with increasing *fimE* expression (Xia *et al.*, 2000). The implication of this finding is that PapB produced from an activated P-fimbrial gene cluster can act on the *fim* locus to prevent expression of type 1 fimbriae. The increase in FimE levels will turn the *fim* switch off and the inhibition of FimB-promoted recombination will maintain the *fim* switch in the off orientation. Subsequent work has shown that the closest homologues of PapB from other related fimbrial clusters have a similar activity (Holden *et al.*, 2001). As *E. coli* clinical isolates often carry multiple adhesin gene clusters, such co-ordinate control between clusters would prevent co-expression of adhesins at the single-cell level. This may be important to further limit immune exposure and also to prevent the physical interference of one adhesin with another. Moreover, such cross-talk may be important to define the progression of an infection (Gunther *et al.*, 2001; Holden & Gally, 2004).

The aim of this study was to determine whether cross-talk between P and type 1 fimbriae occurs in *E. coli* clinical isolates with the levels of PapB that are produced from its natural promoter on the chromosome. Fifty clinical *E. coli* isolates associated with pyelonephritis, cystitis or asymptomatic carriage were used for the study. A selective bias meant that all the isolates contained at least one P-gene cluster and so could potentially demonstrate cross-talk between P and type 1 fimbriae. Carriage of P-gene clusters and functional P-fimbrial expression was established for each group of isolates. Type 1 fimbrial expression was then examined at the genetic level through to the production of functional fimbriae, and cross-talk with P fimbriae was demonstrated.

METHODS

Strains and plasmids. The strains and plasmids used for this study are listed in Tables 1, 2 and 3.

Cloning and DNA manipulation. For genetic manipulation, *E. coli* strains were grown on LB medium supplemented with antibiotics as required. Single-copy fusions were as follows: *egfp* was amplified from peGFP using primers GFP 5′ and GFP 3′ (Roe *et al.*, 2003) and cloned into pAJR25, forming pAJR28, and into pKC8, forming pKC12. *pap* DNA from strains DL2121, DL 2496 (Nou *et al.*, 1995) and J96 (Hull *et al.*, 1981; Marklund *et al.*, 1992) which included *papI* ORF, *papB* ORF and the start codon of *papA* was amplified with the primers 177P and 178P (Table 4), cloned initially into pCR4TOPO-TA, then subcloned with *Bam*HI and *Kpn*I sites into either pAJR28 or pKC12, for exchange into *E. coli* K-12 or CFT073 backgrounds, respectively, as described previously (Blomfield *et al.*, 1991; Porter *et al.*, 2004). The plasmids constructed corresponded to the mutated *pap* regulatory regions as follows: pNJH83 (K-12) and pNJH86 (CFT073) contained P*papBA* ON from DL 2121, forming ZAP986 and ZAP989; pNJH84 (K-12) and pNJH87 (CFT073) contained P*papBA* OFF from DL 2496, forming ZAP987 and ZAP990; pNJH85 (K-12) and pNJH88 (CFT073) contained wild-type P*papBA* (J96), forming ZAP988 and ZAP991. All insertions were confirmed by specific PCR.

The minimal *fim*::*gfp*$^{+}$ construct (pNJH97) was cloned by amplifying *fim* DNA from pMM34 (Blomfield *et al.*, 1991) in the off orientation with primers 151F and 152F (Table 4), and cloned into pAJR145 using *Xba*I. The orientation was confirmed by restriction analysis. The minimal *fim* switch unit was cloned into pDG19, using primers 135F and 136F, generating pNJH79 for allelic exchange into strain BGEC144, generating strain ZAP973, as described in Gally *et al.* (1994). Insertion was confirmed by specific PCR. It should be noted that the plasmid was not successfully transformed into all of the clinical isolates, resulting in a reduced number of samples tested.

Fimbrial cluster deletions in *E. coli* strain 536 were carried out using the method described in Datsenko & Wanner (2000). The template plasmid pKD3 was used to insert the *cat* cassette in each operon individually using specific primers for *fim* (fim1 and fim2, Table 4), *prf* (prf1 and prf2, Table 4) and *sfa* (sfa1 and sfa2, Table 4). The *cat* cassette was excised using pCP20, as described in Datsenko & Wanner (2000). Deletion of the clusters was verified by Southern blotting and specific PCR.

Southern blotting. Southern hybridization was used to determine the number of P-fimbriae-related clusters carried by each *E. coli* isolate. CFT073, J96 and 536 reference strains, and pPap5 (Table 1), were used as controls for probe specificity. Genomic DNA from each isolate and plasmid DNA were digested overnight with *Hin*dIII or *Bgl*II restriction endonucleases, resolved on agarose gels and blotted onto nitrocellulose (Hybond-N^{+}; Amersham) using the method described in Sambrook *et al.* (1989). Two probes were used for hybridization to increase the probability of detecting all P-related clusters: the *papF* probe was amplified with primers 196P and 197P (Table 4), and the *papB* probe was amplified with papB1 and papB2, described in Holden *et al.* (2001); both were labelled with fluorescein-dUTP using the ECF Random Prime Labelling kit (Amersham Biosciences). Following hybridization, the membrane was washed with high-stringency buffers and the signal was amplified using an ECF Signal Amplification System (Amersham Biosciences). The signal was detected with a Fuji FLA-2000 scanner with a 580 nm filter.

Assay of *fim* switch recombination and orientation. FimB-promoted recombination was measured using a minimal MOPS X-Gal plating assay, essentially as described previously (Gally *et al.*, 1993; Holden *et al.*, 2001). In brief, for off-to-on recombination frequencies, a white colony was selected, diluted in PBS to a suitable cell number, and plated onto minimal MOPS X-Gal agar. The number of blue and white colonies was counted, and the frequency calculated as a function of the number of generations that the

Table 1. *E. coli* strains used in this study

Strain	Relevant genotype	Reference or source
MG1655	K-12 F^- λ^-	Guyer *et al.* (1981)
AAEC185A	F^- λ^- *supE44 hsdR17 mcrA endA1 thi-1* Δ(*fimBEACDFGH*) Δ*recA*	Blomfield *et al.* (1991)
AAEC370A	MG1655 Δ*lacZYA fimA*::*lacZYA fimE-am18*	Blomfield *et al.* (1993)
BGEC144	MG1655 Δ*lacZYA* Δ *fimE-fimA*Ω(*sacB*-Kan^r) *fimA-lacZYA fimE-am18*	Gally *et al.* (1994)
DL 2121	Lrp binding site 3 mutation in *pap*, results in a predominantly ON phenotype	Nou *et al.* (1995)
DL 2496	Lrp binding site 4 mutation in *pap*, results in a predominantly OFF phenotype	Nou *et al.* (1995)
J96	*pap*, *prf*	Hull *et al.* (1981); Marklund *et al.* (1992)
CFT073	*fim*, *pap1*, *pap2*, *sfr*	Mobley *et al.* (1990, 1993)
ZAP973	Allelic exchange of the minimal *fim* switch unit from pNJH79 exchanged into BGEC144	This study
ZAP986	Allelic exchange of *pap* regulatory DNA from DL2121 (phase on) into the *lac* locus of AAEC370A	This study
ZAP987	Allelic exchange of *pap* regulatory DNA from DL2496 (phase off) into the *lac* locus of AAEC370A	This study
ZAP988	Allelic exchange of *pap* regulatory DNA from J96 into the *lac* locus of AAEC370A	This study
ZAP989	Allelic exchange of *pap* regulatory DNA from DL2121 (phase on) into the *lac* locus of CFT073	This study
ZAP990	Allelic exchange of *pap* regulatory DNA from DL2496 (phase off) into the *lac* locus of CFT073	This study
ZAP991	Allelic exchange of *pap* regulatory DNA from J96 into the *lac* locus of CFT073	This study
536	*fim*, *prf*, *sfa*	Knapp *et al.* (1986)
536 *prf*	Strain 536 with *prf* deleted	This study
536 *sfa*	Strain 536 with *sfa* deleted	This study
536 *fim*	Strain 536 with *fim* deleted	This study
536 *prf sfa*	Strain 536 with *prf* and *sfa* deleted	This study
536 *prf fim*	Strain 536 with *prf* and *fim* deleted	This study
536 *prf sfa fim*	Strain 536 with *prf*, *sfa* and *fim* deleted	This study

original colony had been through, and was expressed as a value per cell per generation. Orientation of the *fim* switch was carried out essentially as described in Leathart & Gally (1998). *fim* DNA was amplified by PCR using primers 2535 and 3137, incorporating $7{\cdot}4 \times 10^5$ Bq [^{32}P]dATP for radiolabelling when necessary. The PCR-amplified products were digested asymmetrically with *Hinf*I, and the fragments were resolved on 4% acrylamide gels in sodium borate buffer (Brody & Kern, 2004). If radiolabelled, the gels were dried and exposed to X-ray film for at least 12 h. Exposure of more than 24 h was carried out to determine whether there was any *fim* DNA in the ON orientation for the lysate containing the PpapBA ON construct.

Haemagglutination (HA) and yeast-cell agglutination. Human red blood cells (RBCs) were isolated from 5 ml whole blood and suspended in 10 ml PBS. For HA assays, RBCs were used at 1×10^7 cells ml^{-1}. Bacteria were harvested from CFA plates [optimal

Table 2. *E. coli* clinical isolates used in this study

Associated symptoms	Isolate names	Reference
Asymptomatic	Hu 1690, Hu 1691, Hu 1740, Hu 1751, Hu 1756, Hu 1757, Hu 1758, Hu 1761	Hull *et al.* (1998)
	U43, U68, U105, U113, U126, U151	Graham *et al.* (2001)
	BR 2, BR 20	Keegan *et al.* (2003)
Cystitis	AUTI 4, AUTI 7, AUTI 12, AUTI 19, AUTI 31, AUTI 36, AUTI 43, AUTI 47, AUTI 48, AUTI 62, AUTI 64, AUTI 66, AUTI 72	Keegan *et al.* (2003)
Pyelonephritis	AUTI 8, AUTI 11, AUTI 20, AUTI 67, AUTI 68, AUTI 69, AUTI 70, AUTI 71	Keegan *et al.* (2003)
	AP4, AP7, AP18	Pere *et al.* (1987)
	IHE 1041, IHE 1049, IHE 1086, IHE 1106, IHE 1152, IHE 1190, IHE 1268, IHE 1402, IHE 1431	Pouttu *et al.* (2001)
	KS 71	Nowicki *et al.* (1984)

Table 3. Plasmids used in this study

Plasmid	Relevant genotype	Reference
pCR4 TOPO-TA	Commercial vector	Invitrogen
pACYC184	Commercial vector	NEB
pPap5	Complete *pap* gene cluster from J96	Lindberg *et al.* (1984)
pIB307	pMAK705-based vector for allelic exchange; temperature-sensitive replicon	Blomfield *et al.* (1991)
pDG19	pMAK705-based vector for allelic exchange into *fim*; temperature-sensitive replicon	Gally *et al.* (1994)
pMM36	pACYC with *fim* switch in the ON orientation	Mcclain *et al.* (1991)
pMM34	pACYC with *fim* switch in the OFF orientation	Mcclain *et al.* (1991)
peGFP	Commercial vector	Clontech
pKD3	Template plasmid for λ Red recombination	Datsenko & Wanner (2000)
pCP20	Helper plasmid for resistance gene excision	Datsenko & Wanner (2000)
pAJR25	MG1655 *lacI* and *lacA* regions in pIB307	Porter *et al.* (2004)
pAJR32	pAJR25 with *sacB-kan* cassette inserted between *lacIA*	Porter *et al.* (2004)
pAJR28	pAJR25 with promoterless *egfp* inserted between *lacIA*	This study
pAJR145	pACYC *rpsM*::*gfp*$^+$ transcriptional fusion	Roe *et al.* (2004)
pKC8	CFT073 *lacI* and *lacA* regions in pIB307	This study
pKC11	pCK8 with *sacB kan* cassette inserted between *lacIA*	This study
pKC12	pKC8 with promoterless *egfp* inserted between *lacIA*	This study
pNJH20	pBAD18 containing *papB* ORF from J96	Holden *et al.* (2001)
pNJH79	pDG19 containing the minimal *fim* switch in the OFF orientation	This study
pNJH83	pAJR28 with *pap* regulatory DNA from DL 2121	This study
pNJH86	pKC12 with *pap* regulatory DNA from DL 2121	This study
pNJH84	pAJR28 with *pap* regulatory DNA from DL 2496	This study
pNJH87	pKC12 with *pap* regulatory DNA from DL 2496	This study
pNJH85	pAJR28 with *pap* regulatory DNA from J96	This study
pNJH88	pKC12 with *pap* regulatory DNA from J96	This study
pNJH97	pAJR145, minimal *fim* switch fused to *gfp*$^+$, replacing *rpsM*	This study

Table 4. Primers used in the study

Primer	Sequence (5′–3′)
177P	CGC GGA TCC GCA TGC CCA CAG ATT GAG TTA
178P	CGG GTA CCC ATA AAT AAC AAC CTC TTT TTC ATT AC
196P	GTG CAG ATT AAC ATC AGG GG
197P	ATG CTC ATA CTG GCC GTG GT
135F	CCG GCG CAT GCT AAA TAC AAG ACA ATT GGG GCC AAA CTG TCC
136F	CCG GAT CCC CAA AAG ATG AAA CAT TTG GGG CC
151F	CTA GTC TAG ATG CAT GCT AAA TAC AAG ACA ATT GGG GCC AAA CTG TCC
152F	CTA GTC TAG ACC AAA AGA TGA AAC ATT TGG GGC C
2535	GCC GGA TTA TGG GAA AGA
3137	GCC GCT GTA GAA CTG AGG
prf1	GCA AGA ATC ATT ATT CTT TTG CCT GAA GCT ATC CGG CAT ACT CAG GCA TTT CAC GCT TTA GTG TAG GCT GGA GCT GCT T
prf2	CTG ATG TAA CTT TTA TCT GTT TCA GTG AAG CAT GTC CAC AGA TTG AGT TAT TAA CAT ATG AAT ATC CTC CTT AGT TCC TA
sfa1	AAT CTG CAC TCT GAT GTA ACT TTT ATC TGT TTC AGT GAA GTA TGC CCA CAG ATT GAG TTA GTG TAG GCT GGA GCT GCT T
sfa2	TCA CTA GGT CTT TCT GCA ACA CTA CTG CTT TCA ACA AGT CAG GCA TTT CAC ACT CAT ATG AAT ATC CTC CTT AGT TCC TA
fim1	TAT TGC TAA CCC AGC ACA GCT AGT GCG CGT CTG TAA TTA TAA GGG AAA AAC GAT GGT GTA GGC TGG AGC TGC TT
fim2	TTT AGC TTC AGG TAA TAT TGC GTA CCA GCA TTA GCA ATG TCC TGT GAT TTC TTT ACA TAT GAA TAT CCT CCT

for expression of colonization factor antigen (Evans *et al.*, 1977)], the OD_{600} adjusted to 1·0 (measured in a Cecil Aurius CE2021 spectrophotometer, path-length 10 mm), and diluted twofold in PBS. Equal volumes of RBCs were mixed with bacterial dilutions in a 96-well plate and allowed to agglutinate at 4 °C for at least 16 h. The agglutinating titre was determined as the lowest dilution that prevented formation of a defined pellet of RBC. Yeast-cell agglutination was carried out with baker's yeast (*Saccharomyces cerevisiae*). Clinical isolates were plated onto LB agar. Five single colonies of each strain were pooled, inoculated into 3 ml LB and incubated statically for 24 h. The cultures were reinoculated into a further 3 ml LB and incubated statically for another 24 h. Agglutination was carried out by mixing bacterial and yeast suspensions on glass slides and the degree of clumping was assessed. For all agglutinations, 1 % (w/v) mannose was added as required.

Flow cytometry. Bacteria transformed with pNJH97 were plated on CFA for two subsequent days, recovered as described for HA, and washed once in an equal volume of PBS, pre-warmed to 37 °C. The bacteria were then suspended in 1 ml PBS containing 0·1 % (v/v) formaldehyde. Fluorescence was measured at 488 nm using a BD FACS Caliber flow cytometer. At least 20 000 events were gated, and in each case the negative control was set to 0·5 %.

Statistical analysis. Statistical analysis of association of the number of P clusters with isolate class, correlation between number of P clusters and mannose-resistant HA (MRHA), and analysis of variance of fluorescence levels using pNJH97 between the isolate classes (Table 2) were carried out using the Minitab computer program. The threshold for statistical significance was $P<0·05$.

RESULTS

Single-copy papB represses recombination of the *fim* switch in *E. coli* K-12 and CFT073 backgrounds

To examine repression of *fim* recombination by *papB* from the Pap regulatory region on the chromosome, three variants of the sequenced urinary tract isolate *E. coli* CFT073 were constructed. Each of the constructs contained different *pap* regulatory alleles placed in single copy at the *lac* locus. The regulatory alleles included the ORFs of both regulators, *papI* and *papB*, with the intergenic region containing the divergent promoters for *papI* and *papB*. Alteration of two of the leucine-responsive regulatory protein (Lrp) binding sites within the intergenic region has previously been shown to result in apparent constitutive expression or repression of the *papBA* promoter (Nou *et al.*, 1995). We used these altered sequences to derive three variants of the regulatory alleles: P*papBA* ON, P*papBA* OFF and wild-type P*papBA* (phase variable). In each case, the regulatory alleles were fused to the enhanced green fluorescent protein (GFP) reporter (*egfp*) to verify expression status by fluorescence microscopy. In strains containing the locked ON allele, the majority of the bacterial population were found to be fluorescent, whereas the majority of bacteria were found to be non-fluorescent in strains containing the locked OFF allele (data not shown).

The status of type 1 fimbriae expression was then measured in the presence of these three different alleles using *fim* switch (*fimS*) orientational analysis and agglutination methods. PCR and restriction digestion analysis of the *fim* switch (Leathart & Gally, 1998) from the *E. coli* CFT073-derived strains was carried out with [^{32}P]dATP to increase the sensitivity with which the *fim* switch in the on orientation could be detected. The constructs were grown under conditions considered to optimize Pap expression (Evans *et al.*, 1980), which consistently resulted in low levels of type 1 fimbrial expression. When *papB* was locked in phase off in the majority of cells [ZAP990 (P*papAB* OFF, CFT073)], or was subject to wild-type phase-variable expression [ZAP991 (P*papAB* J96, CFT073)], *fimS* DNA in both on and off orientations was detected. However, when *papB* was expressed in the majority of cells [ZAP989 (P*papBA* ON, CFT073)], it was not possible to detect any *fimS* DNA in the on orientation (Fig. 1).

The presence of functional type 1 fimbrial expression was assessed in the *E. coli* CFT073-derived constructs by agglutination with yeast cells and with human RBCs. Expression of *papB* in ZAP989 (P*papBA* ON, CFT073) resulted in an absence of type 1-dependent yeast agglutination and a fourfold reduction in both MRHA and mannose-sensitive HA (MSHA) titres, relative to the wild-type. In contrast, constructs that contained the P*papAB* OFF, variable (ZAP990 and ZAP991) or wild-type CFT073 were all capable of rapid and complete yeast cell agglutination and a high level of MSHA and MRHA [titre of 4 ($\log_2$) or greater]. These results demonstrate the absence of type 1 fimbrial expression in the presence of a phase on regulatory allele expressing PapB.

The same mutated Pap regulatory alleles were exchanged into *E. coli* K-12 (AAEC370A, *fimE fimA*::*lacZYA*, Table 1), enabling measurement of FimB recombination frequencies using this established *lacZ* reporter system [described in Methods and in Gally *et al.* (1993)]. A 20-fold reduction in the frequency of FimB recombination in the off-to-on direction was observed in strain ZAP986 (Table 1) containing the phase on allele in which PapB is expressed in the majority of bacteria. A frequency of $3·31\times10^{-3}\pm1·05\times10^{-3}$ was measured in the wild-type strain AAEC370A compared to $0·18\times10^{-3}\pm0·4\times10^{-3}$ in ZAP986 (P*papBA* ON, K-12). In contrast, FimB switching was not affected in the strains containing either the phase OFF allele or the wild-type *pap* regulatory regions (ZAP987 and ZAP988, respectively, Table 1). The recombination frequency was shown to be $2·28\times10^{-3}\pm0·11\times10^{-3}$ for ZAP987 (P*papBA* OFF, K-12) and $3·74\times10^{-3}\pm0·56\times10^{-3}$ in ZAP988 (P*papAB* J96, K-12). Taken together, the results in the two different backgrounds prove that expression of *papB* from its natural promoter in the chromosome is able to inhibit FimB recombination and therefore prevent type 1 fimbrial expression.

Deletion of P-related fimbrial clusters in the clinical isolate *E. coli* 536 increases *fim* expression

A second approach to examine the impact of Pap [and the related S-fimbrial adhesin (Sfa)] clusters on *fim* was taken using a novel transcriptional GFP reporter system (GFP$^+$)

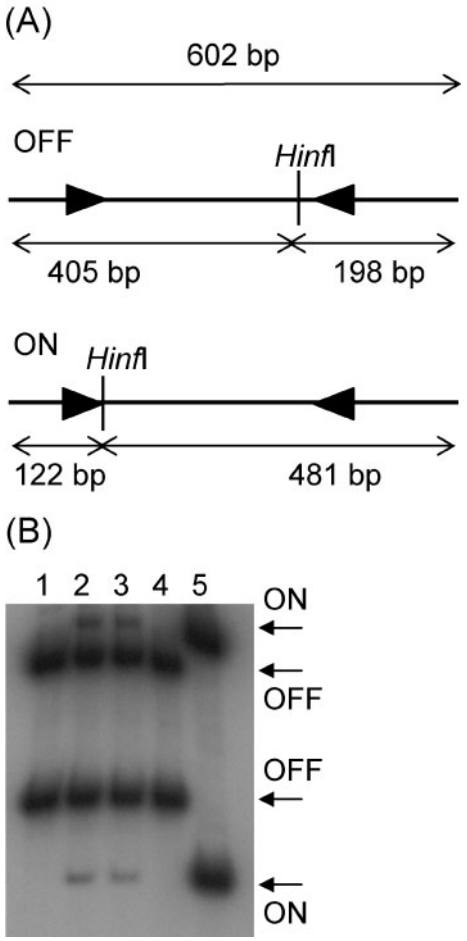

Fig. 1. Orientational analysis of the *fim* switch. (A) Diagrammatic representation of the orientational analysis (not to scale). The *fim* switch is amplified by PCR to give a 602 bp product. Digestion of the PCR amplicons with *HinfI* results in asymmetric products; in the off orientation, the resulting products are 198 and 405 bp in length, in the on orientation, the resulting products are 122 and 481 bp in length. (B) Autoradiograph showing *HinfI* restriction profiles of amplified *fim* switch regions from *E. coli* CFT073 constructs containing *papIB* regulatory regions. The bands corresponding to the *fim* switch in the OFF or ON orientation are shown with arrows. The templates for *fim* PCR were as follows: lane 1, ZAP989; lane 2, ZAP990; lane 3, ZAP991; lane 4, OFF; lane 5, ON.

fused to the *fim* switch (pNJH97, Table 3). The fusion consists of the *fim* switch region flanked by both outside binding sites for the *fim* recombinases, inverted repeat left (IRL) OUT and inverted repeat right (IRR) OUT, resulting in a sequence of 346 bp (Gally *et al.*, 1996). The responsiveness of this minimal *fim* switch unit to K-12 *fim* recombinases was confirmed in two ways. Firstly, chromosomal integration of the minimal *fim* switch unit into *E. coli* K-12 (strain ZAP973, Table 1), in which it is fused to *lacZ*, resulted in a FimB-promoted off-to-on recombination frequency of $2{\cdot}54 \times 10^{-3} \pm 0{\cdot}55 \times 10^{-3}$, similar to that measured from the full-length *fim* switch (strain AAEC370A, frequency of $3{\cdot}31 \times 10^{-3} \pm 1{\cdot}05 \times 10^{-3}$). Furthermore, the presence of *papB* induced from plasmid pNJH20 (Table 3) resulted in the complete absence of FimB-promoted recombination in strain ZAP973 containing the minimal *fim* switch construct. This demonstrates that the inhibitory function of PapB only requires the minimal *fim* switch unit. Secondly, the minimal switch readout plasmid (pNJH97) was transformed into two *E. coli* K-12 strains that contained mutations in either *fimB* or *fimE*. In the $fimB^+$, $fimE^-$ background, 90 % of the bacterial population was fluorescent, whereas in a $fimB^-$, $fimE^+$ background, fluorescence expression was negligible, indicating appropriate regulation by the two different recombinases. These data demonstrate that pNJH97 can provide a simple readout of *fim* recombinase activity which is subject to inhibition by PapB. The plasmid was then used to investigate cross-talk between Pap and type 1 clusters in different clinical isolate backgrounds.

The pyelonephritis isolate *E. coli* 536 contains a type 1 fimbrial cluster, a Pap-related fimbrial cluster, termed Prf (PapG class III) and an Sfa fimbrial cluster (Berger *et al.*, 1982). There is a high degree of homology between the Prf and Sfa clusters, in particular in the PapB- and PapI-like regulators (Dobrindt *et al.*, 2001; Holden *et al.*, 2001), and SfaB is also capable of repressing FimB-promoted recombination of the *fim* switch (Holden *et al.*, 2001). All three clusters were deleted to give combinations of single, double and triple knock-outs, using the λ-Red allelic exchange system (Table 1). Transformation of the *fim* readout plasmid (pNJH97, Table 3) into wild-type *E. coli* 536 resulted in a mean of 2·5 % of the bacterial population that expressed the $fim::gfp^+$ fusion. Deletion of the *fim* cluster (including the recombinase genes) led to only background levels of expression of the fusion, as anticipated (Fig. 2A). In contrast, deletion of the *prf* cluster resulted in a 1·8-fold increase in the percentage of fluorescent bacteria compared to the wild-type. Deletion of *sfa* resulted in a similar increase in the level of *fim* expression, although there appeared to be a larger degree of variation between replicates of the *sfa* strain. The combined *prf* and *sfa* deletions resulted in a 2·3-fold increase in the percentage of bacteria that expressed the $fim::gfp^+$ fusion (Fig. 2A, B). The level of *fim* expression in the double mutant was consistently higher than that in either of the single-mutant *prf* or *sfa* strains. Thus, repression of *fim* expression, even when present at multicopy levels on a plasmid, is detectable from chromosomal copies of Pap-related fimbrial clusters in a clinical isolate.

Expression of type 1 fimbriae is inversely related to P-related fimbrial expression in uropathogenic *E. coli* (UPEC) isolates

We expanded the study to examine whether cross-talk between Pap and Fim occurs in a collection of UPEC isolates, all containing at least one P-related adhesin gene cluster. The UPEC isolates were grouped into three classes determined by their associated clinical UTI symptoms: pyelonephritis, cystitis or asymptomatic. Firstly, the UPEC

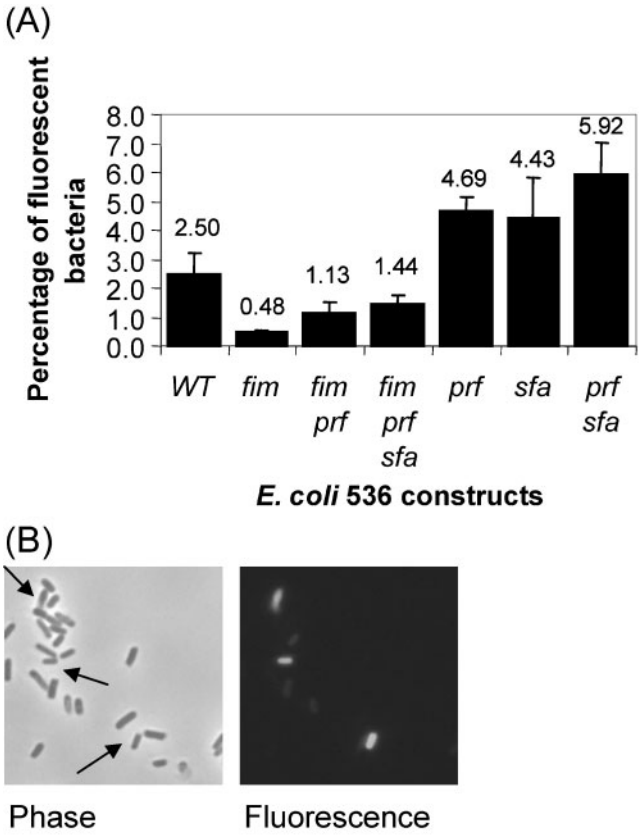

Fig. 2. *fim*::*gfp*$^+$ expression in *E. coli* 536 derivatives. (A) Percentage of fluorescent bacteria (expressing *fim*::*gfp*$^+$) in *E. coli* 536 and adhesin gene cluster mutants (mean of four replicates; error bars show standard deviation). (B) Micrographs (phase-contrast and fluorescence) of *E. coli* 536 *prf sfa* (pNJH97). Arrows highlight those bacteria that contain transcribed *fim*::*gfp*$^+$.

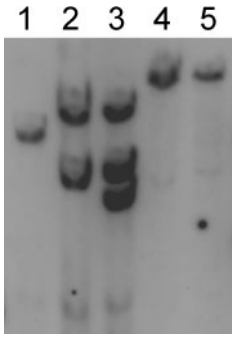

Fig. 3. *prf* copy number determined by Southern blotting. Genomic DNA digests from a representative selection of clinical isolates (Table 2) that show the presence of 1 (lanes 1, 4 and 5), 2 (lane 2) and 3 (lane 3) *prf* sequences (probe for *papB*). Detection of multiple *prf* clusters was verified with reference strains CFT073, J96 and 536 (data not shown). Fainter bands probably represent fimbrial clusters with high *papB* sequence homology, such as S fimbriae (*sfaB*) and F1C fimbriae (*focB*).

isolates were characterized for Pap-related adhesin gene clusters to determine both genetic carriage and fimbrial expression. The number of Pap-related clusters in each of the clinical isolates was determined by Southern blotting with probes against *papB* and *papF* (Fig. 3, Table 5). Despite selective bias of the presence of at least one P-related cluster (normally asymptomatic isolates tend not to carry P-related clusters), analysis of association using the chi square test revealed that the pyelonephritis isolates were more likely to possess multiple P fimbrial clusters than the asymptomatic isolates ($P=0{\cdot}009$). The cystitis isolates also differed from the pyelonephritis isolates, since they were shown to be more likely to carry only one P fimbrial cluster ($P=0{\cdot}056$).

Functional expression of the Pap-related adhesin gene clusters was assessed by MRHA of human RBCs, a measure of P-fimbrial binding capacity (Norgren *et al.*, 1984). Analysis of variance showed that the mean level of MRHA was significantly higher in the cystitis group compared to the asymptomatic group ($P=0{\cdot}037$). In addition, the median level of MRHA was shown to be higher for both the pyelonephritis and cystitis groups compared to the asymptomatic group (Fig. 4). The asymptomatic group contained the highest number of isolates that did not demonstrate MRHA, 31·25 %, while only 7·70 % of the cystitis and 14·29 % of the pyelonephritis isolates did not show MRHA. There was significant positive correlation between the level of MRHA and the number of P clusters for the isolates, Pearson correlation (r^2) of 0·321, $P=0{\cdot}023$, showing that those isolates with more P clusters tended to show an increased level of MRHA, as expected from other studies (Blanco *et al.*, 1997; Hull *et al.*, 1994). It should be noted that although the use of human RBCs alone limits recognition to functional expression of PapG class II, since PapG class III binds preferentially to the Forssman antigen present in other species (Marklund *et al.*, 1992), the majority of isolates were positive for agglutination, so MRHA with human RBCs was judged to be sufficient for the purposes of this study. The fact that the pyelonephritis and cystitis groups were more likely to carry a greater number of P clusters than asymptomatic groups and to express P fimbriae increased the probability of *papB* expression in each cell.

Table 5. Number of Prf clusters determined by Southern blot

The number of isolates carrying one, two or three Prf adhesin gene clusters is given, together with the value as a percentage in parentheses.

Isolate group	1 Cluster	2 Clusters	3 Clusters
Asymptomatic	11 (69)	2 (12)	3 (19)
Cystitis	8 (62)	4 (30)	1 (8)
Pyelonephritis	11 (52)	9 (43)	1 (5)

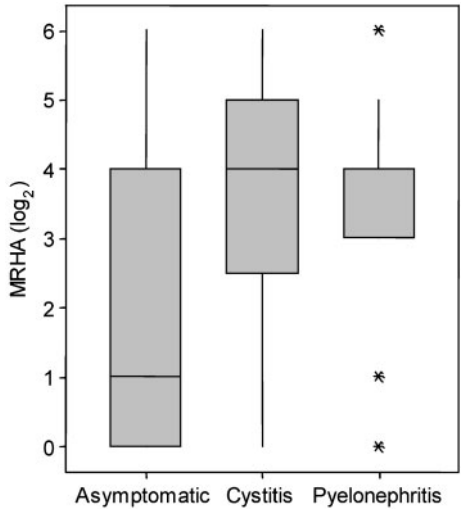

Fig. 4. MRHA levels for the clinical isolate groups. Boxplot showing levels of MRHA for the three clinical isolate groups, expressed as log_2 of the maximum agglutination titre. In each case, the top of the box shows the third quartile and the bottom shows the first quartile. The lines extend to the upper and lower limits, outliers are shown by an asterisk, and the middle line represents the median value (the median MRHA for the pyelonephritis group is the same as the first quartile).

To assess the impact of P-related clusters on *fim* in the clinical isolates, the *fim*::*gfp*$^+$ plasmid (pNJH97) was transformed into each of the clinical UPEC isolates and expression of the fusions assessed by flow cytometry. The values for each isolate were grouped and analysed for variance between the median values for each isolate class by the Kruskal–Wallis test for nonparametric data (Table 6). Expression of *fim*::*gfp*$^+$ at the population level was clearly lower in the pyelonephritis group, determined from the median values. In contrast, the highest overall level of *fim*::*gfp*$^+$ expression was seen in the asymptomatic group. The rank values for both the asymptomatic and cystitis isolate classes were closest to the overall rank, whereas the corresponding value for the pyelonephritis class was 15·5, indicating that there was a difference from the other two classes, although the *P* value for the difference is not significant (0·145).

Cross-talk was analysed further in these isolates by HA and orientation analysis of the *fim* switch (Graham *et al.*, 2001; Lim *et al.*, 1998). Assessment of the potential of each isolate to express *fim* when grown in static broth, conditions considered to be optimal for type 1 fimbrial expression, resulted in positive mannose-sensitive yeast agglutination (MSYA) for 13 of the 18 pyelonephritis isolates, seven of the 11 cystitis isolates and nine of the 13 asymptomatic isolates (Fig. 5B). Under different culture conditions known to induce P expression (Evans *et al.*, 1980), the HA titres showing the level of type 1 fimbrial expression were reduced (Fig. 5A). Even so, under these conditions, expression of type 1 fimbriae was significantly lower in both the cystitis and pyelonephritis groups compared to the asymptomatic group ($P=0·002$). This difference was confirmed by PCR and restriction digestion of the *fim* switch (data not shown). In summary, when P-fimbrial expression is promoted this correlates with a reduction in type 1 fimbrial expression.

Table 6. Kruskal–Wallis test for *fim*::*gfp*$^+$ expression

Isolate group (*n*)	Median	Mean rank
Asymptomatic (12)	13·87	22·4
Cystitis (9)	8·92	23·1
Pyelonephritis (17)	6·91	15·5
Overall (38)		19·5

DISCUSSION

At the single-cell level, expression of adhesins, flagella, capsules and other surface components is likely to be co-ordinated by bacteria to prevent co-expression of competing factors or occlusion of one factor by another. In turn, such co-ordination should limit immune stimulation. Our previous work has demonstrated the potential for such co-ordination between the P/S and type 1 fimbrial clusters. The work showed that PapB is a repressor of *fimA* expression, acting at the level of FimB recombinase activity and *fimE* transcription. The majority of the published work has been carried out in *E. coli* K-12, and uses multicopy levels of *papB* from an inducible promoter (Holden *et al.*, 2001; Xia *et al.*, 2000). The aim of the present study was to test whether the regulation is apparent when *papB* is expressed from its native promoter on the chromosome and whether this regulatory cross-talk can be detected in clinical isolates. The research used three different approaches to investigate cross-talk in UPEC. Two required modification of well-characterized clinical isolates, *E. coli* CFT073 and *E. coli* 536, while the third looked at fimbrial expression in a collection of isolates associated with different classes of UTI. Data generated by all three methods support the conclusion that PapB expressed at wild-type levels does inhibit type 1 fimbrial expression in clinical isolates.

Cross-talk was proven by the use of Lrp-binding-site mutations within the *papI–papB* intergenic region, originally constructed by David Low's group (Nou *et al.*, 1995). These mutations limit transition between the phase on and phase off states of P expression, resulting in a bacterial population the majority of which is in either the off or the on expression state. For this study, these mutated regions covering *papI–papB* were cloned in front of eGFP and then inserted into both *E. coli* K-12 and CFT073 backgrounds. In the on state, the majority of the bacteria expressed GFP, as determined by fluorescence microscopy, and will contain PapB; in the off state, the converse is true. A twentyfold reduction in FimB inversion of the *fim* switch from the off to the on orientation was measured in the presence of the 'on' P

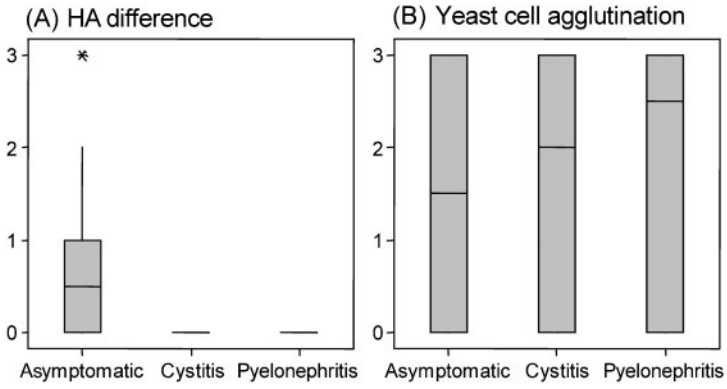

Fig. 5. HA levels for the clinical isolate groups. (A) Boxplot showing the level of human RBC HA attributable to type 1 fimbrial expression for the three clinical isolate groups. The difference was calculated by subtracting the level of MRHA (log_2) from the level of MSHA (log_2). The isolates were grown on CFA media, described in Methods. (B) Boxplot showing the level of mannose-sensitive yeast-cell agglutination for the three clinical isolate groups. The level of agglutination is expressed as strong (value of 3), medium (value of 2) or weak (value of 1). Isolates were grown statically in LB broth, to promote type 1 fimbrial expression. The boxplots are as described in Fig. 4.

promoter region, while no difference was measured in the presence of the 'off' P region. As described, these Lrp-binding mutations do not completely lock P phase variation, and so a proportion of the bacterial population does still undergo phase transition. A completely locked on P regulatory region is likely to demonstrate an increased repression of FimB switching, perhaps achieving the complete repression observed for induced levels of the regulator cloned on a plasmid (Holden *et al.*, 2001).

A further demonstration of the repression of type 1 fimbriae by P/S clusters was provided by using the *fim*::*gfp*$^+$ fusion in different *E. coli* 536 mutants that had had *fim*, *prf*, *sfa* clusters deleted in various combinations. This work showed that the proportion of bacteria expressing type 1 fimbriae increased by 2·3-fold in a strain that had had the Prf and S clusters deleted, and by 1·8-fold in a strain deleted for Prf. While these differences are subtle at the population level, they were reproducible and support the additive repressive effect of PapB homologues on type 1 fimbrial expression. This regulation was measurable despite the fact that the PapB/SfaB regulators from the chromosome were acting on a plasmid-based *fim*::*gfp*$^+$ construct.

To examine cross-talk in other *E. coli* isolates, a set of 50 human clinical isolates that contained P and type 1 fimbrial gene clusters were selected and grouped according to their associated UTI symptoms into the categories asymptomatic, cystitis and pyelonephritis. The isolates chosen for the study all contained at least one P cluster, so that an assessment of cross-talk with type 1 fimbriae could be carried out. Southern blotting revealed that pyelonephritis isolates were more likely to possess two or more P-related clusters when compared to the asymptomatic isolates. The number of P clusters present in the cystitis isolates was also higher than in the asymptomatic group. Functional expression of the P gene clusters was assessed using MRHA, and this correlated significantly with the number of P clusters present in each isolate and therefore with the disease associated with the isolate. While all bacteria assayed contained at least one P cluster, the asymptomatic group had the highest proportion of bacteria that had no clear MRHA, implying either that in these strains the expression of P fimbriae is repressed or that mutations have occurred in the operons that prevent expression. This is consistent with asymptomatic strains not expressing factors that provoke an inflammatory response (Graham *et al.*, 2001; Hull *et al.*, 1999; Lim *et al.*, 1998).

Expression of type 1 fimbriae in the different isolate groups was examined at the level of the DNA (*fim* switch orientation), promoter activity and functional fimbrial binding. These methods were selected to demonstrate fimbrial expression in the absence of a suitable generic antibody that would recognize fimbriae consistently for a large range of clinical isolates. In each case, the overall trend was the same: type 1 fimbrial expression was repressed in the pyelonephritis group compared to the asymptomatic isolates. This was confirmed using a novel plasmid-based GFP reporter fusion for measurement of *fimA* expression, supporting the concept that the higher the level of P/S expression, the lower the level of type 1 fimbrial expression.

Taken together with our previous work, the current research demonstrates that P/S expression in clinical isolates leads to type 1 fimbrial repression through the activity of PapB/SfaB. While recent work has uncovered environmental cues in the host that down-regulate type 1 fimbriae, possibly in response to inflammation (El-Labany *et al.*, 2003; Roesch *et al.*, 2003; Schwan *et al.*, 2002), very little is known about the signals that stimulate P expression (Blomfield, 2001). Recently, Snyder *et al.* (2005) have demonstrated that locking the *fim* switch in the off orientation in *E. coli* CFT073 leads to an increase in the expression of one of the P-fimbrial clusters. This provides evidence that cross-talk can work in both directions to prevent multiple fimbrial expression at the level of the single bacterial cell. Other fimbrial and non-fimbrial adhesins, along with flagella and capsule, are also likely to be regulated co-ordinately at the single-cell level to achieve systematic expression that prolongs the infection and maximizes the number of bacteria produced (Barnich *et al.*, 2003; Schwan *et al.*, 2005). Therefore, while phase

variation is considered a stochastic process, it is controlled by other regulatory networks that govern organelle expression and environmental responses. Understanding these regulatory networks will be important in the development of novel strategies to treat UTIs, such as deliberately providing signals that down-regulate or switch off adhesin expression.

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REFERENCES

Barnich, N., Boudeau, J., Claret, L. & Darfeuille-Michaud, A. (2003). Regulatory and functional co-operation of flagella and type 1 pili in adhesive and invasive abilities of AIEC strain LF82 isolated from a patient with Crohn's disease. *Mol Microbiol* **48**, 781–794.

Berger, H., Hacker, J., Juarez, A., Hughes, C. & Goebel, W. (1982). Cloning of the chromosomal determinants encoding haemolysin production and mannose-resistant haemagglutination in *Escherichia coli*. *J Bacteriol* **152**, 1241–1247.

Blanco, M., Blanco, J. E., Alonso, M. P., Mora, A., Balsalobre, C., Munoa, F., Juarez, A. & Blanco, J. (1997). Detection of *pap*, *sfa* and *afa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains: relationship with expression of adhesins and production of toxins. *Res Microbiol* **148**, 745–755.

Blomfield, I. C. (2001). The regulation of *pap* and type 1 fimbriation in *Escherichia coli*. *Adv Microbiol Physiol* **45**, 1–49.

Blomfield, I. C., Mcclain, M. S. & Eisenstein, B. I. (1991). Type 1 fimbriae mutants of *Escherichia coli* K12 – characterization of recognized afimbriate strains and construction of new *fim* deletion mutants. *Mol Microbiol* **5**, 1439–1445.

Blomfield, I. C., Vaughn, V., Rest, R. F. & Eisenstein, B. I. (1991). Allelic exchange in *Escherichia coli* using the *Bacillus subtilis sacB* gene and a temperature-sensitive pSC101 replicon. *Mol Microbiol* **5**, 1447–1457.

Blomfield, I. C., Calie, P. J., Eberhardt, K. J., Mcclain, M. S. & Eisenstein, B. I. (1993). Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K12. *J Bacteriol* **175**, 27–36.

Brody, J. R. & Kern, S. E. (2004). Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. *Biotechniques* **36**, 214–216.

Datsenko, K. A. & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**, 6640–6645.

Dobrindt, U., Blum-Oehler, G., Hartsch, T., Gottschalk, G., Ron, E. Z., Funfstuck, R. & Hacker, J. (2001). S-fimbria-encoding determinant *sfa*(I) is located on pathogenicity island III_{536} of uropathogenic *Escherichia coli* strain 536. *Infect Immun* **69**, 4248–4256.

El-Labany, S., Sohanpal, B. K., Lahooti, M., Akerman, R. & Blomfield, I. C. (2003). Distant *cis*-active sequences and sialic acid control the expression of *fimB* in *Escherichia coli* K-12. *Mol Microbiol* **49**, 1109–1118.

Evans, D. G., Evans, D. J., Jr & Tjoa, W. (1977). Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. *Infect Immun* **18**, 330–337.

Evans, D. J., Jr, Evans, D. G., Young, L. S. & Pitt, J. (1980). Hemagglutination typing of *Escherichia coli*: definition of seven hemagglutination types. *J Clin Microbiol* **12**, 235–242.

Frendeus, B., Wachtler, C., Hedlund, M., Fischer, H., Samuelsson, P., Svensson, M. & Svanborg, C. (2001). *Escherichia coli* P fimbriae utilize the Toll-like receptor 4 pathway for cell activation. *Mol Microbiol* **40**, 37–51.

Gally, D. L., Bogan, J. A., Eisenstein, B. I. & Blomfield, I. C. (1993). Environmental-regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12 – effects of temperature and media. *J Bacteriol* **175**, 6186–6193.

Gally, D. L., Rucker, T. J. & Blomfield, I. C. (1994). The leucine-responsive regulatory protein binds to the *fim* switch to control phase variation of type 1 fimbrial expression in *Escherichia coli* K12. *J Bacteriol* **176**, 5665–5672.

Gally, D. L., Leathart, J. & Blomfield, I. C. (1996). Interaction of FimB and FimE with the *fim* switch that controls the phase variation of type 1 fimbriae in *Escherichia coli* K-12. *Mol Microbiol* **21**, 725–738.

Graham, J. C. & Galloway, A. (2001). ACP Best Practice No 167: the laboratory diagnosis of urinary tract infection. *J Clin Pathol* **54**, 911–919.

Graham, J. C., Leathart, J. B. S., Keegan, S. J., Pearson, J., Bint, A. & Gally, D. L. (2001). Analysis of *Escherichia coli* strains causing bacteriuria during pregnancy: selection for strains that do not express type 1 fimbriae. *Infect Immun* **69**, 794–799.

Gunther, N. W., Lockatell, V., Johnson, D. E. & Mobley, H. L. T. (2001). *In vivo* dynamics of type 1 fimbria regulation in uropathogenic *Escherichia coli* during experimental urinary tract infection. *Infect Immun* **69**, 2838–2846.

Guyer, M. S., Reed, R. R., Steitz, J. A. & Low, K. B. (1981). Identification of a sex-factor-affinity site in *Escherichia coli* as a gamma delta. In *Cold Spring Harbor Symposium on Quantitiative Biology*, vol. 45, pp. 135–140. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Hedlund, M., Wachtler, C., Johansson, E., Hang, L., Somerville, J. E., Darveau, R. P. & Svanborg, C. (1999). P fimbriae-dependent, lipopolysaccharide-independent activation of epithelial cytokine responses. *Mol Microbiol* **33**, 693–703.

Holden, N. J. & Gally, D. L. (2004). Switches, cross-talk and memory in *Escherichia coli* adherence. *J Med Microbiol* **53**, 585–593.

Holden, N. J., Uhlin, B. E. & Gally, D. L. (2001). PapB paralogues and their effect on the phase variation of type 1 fimbriae in *Escherichia coli*. *Mol Microbiol* **42**, 319–330.

Hull, R. A., Gill, R. E., Hsu, P., Minshew, B. H. & Falkow, S. (1981). Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. *Infect Immun* **33**, 933–938.

Hull, R. A., Nowicki, B., Kaul, A., Runyan, R., Svanborg, C. & Hull, S. I. (1994). Effect of Pap copy number and receptor specificity on virulence of fimbriated *Escherichia coli* in a murine urinary-tract colonization model. *Microb Pathog* **17**, 79–86.

Hull, R. A., Rudy, D. C., Wieser, I. E. & Donovan, W. H. (1998). Virulence factors of *Escherichia coli* isolates from patients with symptomatic and asymptomatic bacteriuria and neuropathic bladders due to spinal cord and brain injuries. *J Clin Microbiol* **36**, 115–117.

Hull, R. A., Rudy, D. C., Donovan, W. H., Wieser, I. E., Stewart, C. & Darouiche, R. O. (1999). Virulence properties of *Escherichia coli* 83972, a prototype strain associated with asymptomatic bacteriuria. *Infect Immun* **67**, 429–432.

Johnson, J. R. (1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* **4**, 80–128.

Keegan, S. J., Graham, C., Neal, D. E., Blum-Oehler, G., N'Dow, J., Pearson, J. P. & Gally, D. L. (2003). Characterization of *Escherichia coli* strains causing urinary tract infections in patients with transposed intestinal segments. *J Urol* **169**, 2382–2387.

Klemm, P. (1986). 2 regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. *EMBO J* **5**, 1389–1393.

Knapp, S., Hacker, J., Jarchau, T. & Goebel, W. (1986). Large, unstable inserts in the chromosome affect virulence properties of uropathogenic *Escherichia coli* O6 strain 536. *J Bacteriol* **168**, 22–30.

Leathart, J. B. S. & Gally, D. L. (1998). Regulation of type 1 fimbrial expression in uropathogenic *Escherichia coli*: heterogeneity of expression through sequence changes in the *fim* switch region. *Mol Microbiol* **28**, 371–381.

Lim, J. K., Gunther, N. W., Zhao, H., Johnson, D. E., Keay, S. K. & Mobley, H. L. T. (1998). *In vivo* phase variation of *Escherichia coli* type 1 fimbrial genes in women with urinary tract infection. *Infect Immun* **66**, 3303–3310.

Lindberg, F. P., Lund, B. & Normark, S. (1984). Genes of pyelonephritogenic *Escherichia coli* required for digalactoside-specific agglutination of human cells. *EMBO J* **3**, 1167–1173.

Marklund, B. I., Tennent, J. M., Garcia, E., Hamers, A., Baga, M., Lindberg, F., Gaastra, W. & Normark, S. (1992). Horizontal gene transfer of the *Escherichia coli* Pap and Prs pili operons as a mechanism for the development of tissue-specific adhesive properties. *Mol Microbiol* **6**, 2225–2242.

Mcclain, M. S., Blomfield, I. C. & Eisenstein, B. I. (1991). Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. *J Bacteriol* **173**, 5308–5314.

Mizunoe, Y., Matsumoto, T., Sakumoto, M., Kubo, S., Mochida, O., Sakamoto, Y. & Kumazawa, J. (1997). Renal scarring by mannose-sensitive adhesin of *Escherichia coli* type 1 pili. *Nephron* **77**, 412–416.

Mobley, H. L., Green, D. M., Trifillis, A. L., Johnson, D. E., Chippendale, G. R., Lockatell, C. V., Jones, B. D. & Warren, J. W. (1990). Pyelonephritogenic *Escherichia coli* and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. *Infect Immun* **58**, 1281–1289.

Mobley, H., Jarvis, K. G., Elwood, J. P., Whittle, D. I., Lockatell, C. V., Russell, R. G., Johnson, D. E., Donnenberg, M. S. & Warren, J. W. (1993). Isogenic P-fimbrial deletion mutants of pyelonephritogenic *Escherichia coli:* the role of alpha-gal(1-4)beta-gal binding in virulence of a wild-type strain. *Mol Microbiol* **10**, 143–155.

Naber, K. G. (2000). Survey on antibiotic usage in the treatment of urinary tract infections. *J Antimicrob Chemother* **46** (Supplement 1), 49–52.

Nicolle, L. E. (2001). Epidemiology of urinary tract infection. *Infect Med* **18**, 153–162.

Norgren, M., Normark, S., Lark, D., O'Hanley, P., Schoolnik, G., Falkow, S., Svanborg-Eden, C., Baga, M. & Uhlin, B. E. (1984). Mutations in *Eschericha coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. *EMBO J* **3**, 1159–1165.

Nou, X. W., Braaten, B., Kaltenbach, L. & Low, D. A. (1995). Differential binding of Lrp to 2 sets of *pap* DNA-binding sites mediated by *papI* regulates *pap* phase variation in *Escherichia coli*. *EMBO J* **14**, 5785–5797.

Nowicki, B., Rhen, M., Vaisanenrhen, V., Pere, A. & Korhonen, T. K. (1984). Immunofluorescence study of fimbrial phase variation in *Escherichia coli* KS71. *J Bacteriol* **160**, 691–695.

Pere, A., Nowicki, B., Saxen, H., Siitonen, A. & Korhonen, T. K. (1987). *Escherichia coli* in the urine of patients with acute urinary tract infection. *J Infect Dis* **156**, 567–574.

Porter, M. E., Mitchell, P., Roe, A. J., Free, A., Smith, D. G. & Gally, D. L. (2004). Direct and indirect transcriptional activation of virulence genes by an AraC-like protein, PerA from enteropathogenic *Escherichia coli*. *Mol Microbiol* **54**, 1117–1133.

Pouttu, R., Westerlund-Wikstrom, B., Lang, H., Alsti, K., Virkola, R., Saarela, U., Siitonen, A., Kalkkinen, N. & Korhonen, T. K. (2001). *matB*, a common fimbrillin gene of *Escherichia coli*, expressed in a genetically conserved, virulent clonal group. *J Bacteriol* **183**, 4727–4736.

Roe, A. J., Yull, H., Naylor, S. W., Woodward, M. J., Smith, D. G. E. & Gally, D. L. (2003). Heterogeneous surface expression of EspA translocon filaments by *Escherichia coli* O157 : H7 is controlled at the posttranscriptional level. *Infect Immun* **71**, 5900–5909.

Roe, A. J., Naylor, S. W., Spears, K. J. & 8 other authors (2004). Co-ordinate single-cell expression of *LEE4*- and *LEE5*-encoded proteins of *Escherichia coli* O157 : H7. *Mol Microbiol* **54**, 337–352.

Roesch, P. L., Redford, P., Batchelet, S., Moritz, R. L., Pellett, S., Haugen, B. J., Blattner, F. R. & Welch, R. A. (2003). Uropathogenic *Escherichia coli* use D-serine deaminase to modulate infection of the murine urinary tract. *Mol Microbiol* **49**, 55–67.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schilling, J. D., Martin, S. M., Hunstad, D. A., Patel, K. P., Mulvey, M. A., Justice, S. S., Lorenz, R. G. & Hultgren, S. J. (2003). CD14- and toll-like receptor-dependent activation of bladder epithelial cells by lipopolysaccharide and type 1 piliated *Escherichia coli*. *Infect Immun* **71**, 1470–1480.

Schwan, W. R., Lee, J. L., Lenard, F. A., Matthews, B. T. & Beck, M. T. (2002). Osmolarity and pH growth conditions regulate *fim* gene transcription and type 1 pilus expression in uropathogenic *Escherichia coli*. *Infect Immun* **70**, 1391–1402.

Schwan, W. R., Beck, M. T., Hultgren, S. J., Pinkner, J., Woolever, N. L. & Larson, T. (2005). Down-regulation of the *kps* region 1 capsular assembly operon following attachment of *Escherichia coli* Type 1 fimbriae to D-mannose receptors. *Infect Immun* **73**, 1226–1231.

Snyder, J. A., Haugen, B. J., Lockatell, C. V., Maroncle, N., Hagan, E. C., Johnson, D. E., Welch, R. A. & Mobley, H. L. (2005). Coordinate expression of fimbriae in uropathogenic *Escherichia coli*. *Infect Immun* **73**, 7588–7596.

Xia, Y., Gally, D., Forsman-Semb, K. & Uhlin, B. E. (2000). Regulatory cross-talk between adhesin operons in *Escherichia coli*: inhibition of type 1 fimbriae expression by the PapB protein. *EMBO J* **19**, 1450–1457.