INTRODUCTION

Conjugative pili are characteristic of type IV secretion systems that mediate horizontal gene transfer by Gram-negative bacteria (Valentine et al., 1969; Paranchych & Frost, 1988; Ippen-Ihler & Maneewannakul, 1991; Fullner et al., 1996; Lai & Kado, 1998). Insofar as they have been examined, these extracellular filaments are repeats of one quantitatively predominant subunit (Paranchych & Frost, 1988; Lai & Kado, 1998; Eisenbrandt et al., 1999). Conjugative pili and the corresponding pilin subunits are generally identified by the origin of the type IV secretion system of which they are components. The present report concerns F-pili, which are composed of F-pilin and elaborated by F\(^{+}\) strains of *Escherichia coli*.

F-pili are 8–9 nm in diameter and of indeterminate length. Structurally, they are hollow cylinders with a hydrophilic axial lumen that is accessible to the aqueous medium (Folkhard et al., 1979; Paranchych & Frost, 1988; Silverman, 1997). Functionally, F-pili make initial, SDS-sensitive contacts between donor and recipient cells (Achtman & Skurray, 1977; Manning & Achtman, 1979). Thereafter, F-pili retract so that donor and recipient cells are in direct surface-to-surface contact (Durrenberger et al., 1991). DNA transfer between closely apposed cells appears to be general (Samuels et al., 2000; Lawley et al., 2002), arguing against transfer through extended pili. In fact, once stable surface-to-surface contacts are formed, extended F-pili are no longer required for DNA transfer (Panicker & Minkley, 1985), although a role for very short filaments cannot be excluded. F-pili also serve as adsorption organelles for several classes of bacteriophage, notably the f2 and Q\(\beta\) classes of icosahedral RNA bacteriophage and the F1/M13 class of filamentous DNA, or Ff, bacteriophage. RNA bacteriophage bind to the sides of F-pili, whereas DNA bacteriophage bind to the tip (Valentine et al., 1969).

F-pilin (70 amino acids) first accumulates as an inner-membrane protein. It is derived from the *traA* gene product (121 amino acids), beginning with proteolytic cleavage between TraA amino acids A51 and A52 and catalysed by host leader peptidase B (Moore et al., 1981a; Frost et al., 1984; Majdalani & Ippen-Ihler, 1996; Majdalani et al., 1996). N\(^{\alpha}\)-Acetylation of A1 is required to yield ‘mature’ F-pilin,
although unacyetylated subunits still assemble and function (Grossman & Silverman, 1989; Grossman et al., 1990; Moore et al., 1993; Maneewannakul et al., 1995). Unlike T- and RP4-pilins (Eisenbrandt et al., 1999), F-pilin is not circular.

Ippe-Ilher and colleagues showed that formation of membrane F-pilin requires only the traA and traQ genes (Moore et al., 1981b; Maneewannakul et al., 1993) and provided evidence that membrane F-pilin is the precursor to filament F-pilin (Sowa et al., 1983). Our studies showed that TraQ interacts directly with the C-terminal domain IV of F-pilin (Paiva et al., 1992; Harris et al., 1999), and we proposed that TraQ acts catalytically to escort TraA into the inner membrane.

Once formed, membrane F-pilin is stable as such in cells unable to elaborate F-pili (Sowa et al., 1983). F-pilus assembly from membrane F-pilin substrate requires numerous additional Tra proteins that act at or in association with the cell envelope (Firth et al., 1996; Harris et al., 2001; Harris & Silverman, 2004). Altogether, about half the F DNA transfer (tra) genes essential for DNA transfer are required for F-pilus assembly and function (Grossman & Silverman, 1989; Firth et al., 1996). These additional proteins form an envelope-associated secretion machine (R. Harris and others, unpublished data), as is also true of other type IV systems (Thorstenson et al., 1993; Grahn et al., 2000; Gilmour et al., 2001; Kumar et al., 2000; Krall et al., 2002).

Several indirect tests exist for the presence or absence of functional F-pili, including conjugal DNA donor activity and sensitivity to bacteriophage that use F-pili as adsorption sites. Direct assays for F-pili have included electron microscopy (Valentine et al., 1969; Curtiss et al., 1969), binding of RNA bacteriophage labelled with $^{32}$P (Valentine et al., 1969), and competitive ELISA (Frost et al., 1985). The last two assays are now rarely used, electron microscopy, though poorly suited to kinetic or other studies requiring high throughput, having by default become the assay of choice for F-pili. Here we describe alternative assays that employ fluorescent bacteriophage that bind specifically to F-pili. We show that fluorescence microscopy can be used to analyse F-pili number and length distributions within and between populations, whereas fluorescence measurements can be used for rapid, quantitative assays of cell cultures. We illustrate these advantages using a set of F-pilin missense mutants.

**METHODS**

**Strains, plasmids, bacteriophage, and culture conditions.** All strains were derivatives of *Escherichia coli* K-12. JC3272 and JC3272/F lac (JCFLO) have been described (Achtman et al., 1972). HfrH was originally obtained from the Coli Genetic Stock Center at Yale University. Strain AE2386 was derived from MC4100 (Casadaban, 1976). To abolish formation of type 1 pili, the fimD::lac gene of strain VL386 (Freitag & Eisenstein, 1983) was introduced into MC4100 by P1 transduction, selecting for a Lac+ phenotype. This strain switched to a Lac+ phenotype at high frequency and quickly became stably Lac+. A spontaneous NalR mutant was designated AE2386, F lac (JCFLO; Achtman et al., 1972) was introduced into AE2386 by conjugation with JC3272/F lac, selecting for NalR Lac+ transconjugants. XK1200/pOX38traA::cat (Anthony et al., 1994) was obtained from Dr Laura Frost, University of Alberta, Edmonton, Canada. TOP10 [relevant markers: araD139 (AaraA-leu)] was obtained from Invitrogen and used for tightly regulated traA expression (Guzman et al., 1995). pOX38traA::cat was introduced into TOP10 by conjugation with XK1200/pOX38traA::cat containing pWP901, a traA+ plasmid based on the pUC19 vector backbone (W. Paiva & P. Silverman, unpublished data). AE2248, used as a recipient in mating experiments, is an thi-34::Tn10 derivative of JC3272.

Bacteriophage R17 and the tet transducing bacteriophage F1Fus2 (Parmley & Smith, 1988) were from our laboratory stocks. R17 titres were measured by standard agar overlay with HfrH or JC3272/JCFLO as hosts. F1Fus2 titres were measured as transductant-forming units (t.f.u.) (tetracycline-resistance) with strain K91 as host (Parmley & Smith, 1988).

Where indicated, plasmid pMR119, encoding DsRed-Express (Otto et al., 2004), was introduced by transformation.

Bacteria were grown routinely in Luria–Bertani (LB) medium supplemented with antibiotics as necessary. For bacteriophage binding, the medium was also supplemented with 10 mM CaCl$_2$. Incubation was at 37°C with vigorous aeration. Growth was monitored by optical density at 600 nm. When used, nutrient broth contained, per litre: 10 g tryptone, 1 g yeast extract, 8 g NaCl and 0.2% (w/v) glucose.

**Preparation of fluorescent R17.** R17 was prepared from crude lysates by liquid polymer phase partition and isopycnic banding in step CaCl$_2$ gradients (Yamamoto et al., 1972). After dialysis against P buffer [50 mM TrisHCl (pH 7.6)/0.1 M NaCl/5 mM MgCl$_2$/0.1 mM EDTA], the suspension was made 50% (w/v) glycerol and stored at ~20°C. The final titre of the preparation used in these studies was 2.5 × 10$^{12}$ p.f.u. ml$^{-1}$ and has been stable for several years.

For conjugation with fluorescent dyes, a portion of the R17 suspension (12–15 ml) was dialysed at 4°C for 48 h against 2 litres of a solution containing 0.1 M NaHCO$_3$ (pH 8.5)/1 mM MgCl$_2$. The dialysis solution was changed once after 24 h. Alexa 488 carboxylic acid (succinimidyl ester; Molecular Probes) (1 mg) was dissolved in 0.1 ml anhydrous DMSO and added to 10 ml aliquots to the R17 suspension with gentle stirring and at ambient temperature over a 20–30 min period. After an additional 60 min with gentle stirring, the R17 suspension was loaded onto two linear CaCl$_2$ gradients (30 ml; $p = 1 – 663 – 1226$ g ml$^{-1}$) in P buffer. Centrifugation in the Beckman SW28 rotor was at 25 000 r.p.m. for 18 h at 5°C. The fluorescent band visible under UV illumination ($p = 1 – 45$ g ml$^{-1}$) was collected, dialysed against P buffer, diluted with an equal volume of 80% (w/v) glycerol, and stored at ~20°C. Titres were generally about 5 × 10$^{11}$ p.f.u. ml$^{-1}$ and Alexa 488-labeling corresponded to ~10$^6$ fluorescence intensity units (FIU) ml$^{-1}$. In earlier experiments, glycerol was removed by dialysis before the preparation was used, but this proved to be unnecessary.

**R17 binding and fluorescence measurements.** Cells (0–5–1 ml) and R17 (15–40 µl) were mixed at 4°C and incubated at that temperature for 10 min. Formaldehyde (50 µl of a 16% solution) was added and the samples incubated for 10 min at ambient temperature. Cells and bound bacteriophage were harvested by sedimentation for 4 min in a microcentrifuge at 10 000 g. Supernatant fractions were carefully removed by aspiration and discarded. Cell pellets were suspended in 1 ml 0.1% (w/v) SDS. Cells were sedimented, suspended in 1 ml 0.1% SDS, and sedimanted a last time. Supernatant fractions were combined.
Fluorescence was measured with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. A blank value consisting of the fluorescence from 0.1% SDS (40 FIU) or, depending on the experiment, from F− cells in 0.1% SDS (50–70 FIU) was subtracted from all readings.

Fluorescence microscopy. Cells were grown to an OD<sub>600</sub> of 0.3–0.4 in Medium E salts (Vogel & Bonner, 1956) containing 1% Casamino acids/1% tryptophan/1% glucose. Cultures were diluted to an OD<sub>600</sub> of 0.1 with PBS and 50 μl applied to a microscope dish (35 mm × 12 mm × 0.17 mm; World Precision Instruments). Before use, dishes were incubated for 10 min in normal goat serum diluted 100-fold in PBS and then rinsed three times with PBS. After 10 min incubation to allow cells to adhere, excess liquid was carefully removed and replaced with 50 μl R17 diluted fivefold in PBS. After 10 min, liquid was removed and an agar disc (2% in PBS, 0.16–0.19 mm thick) immediately placed over the cells. Fixation was with 2% formaldehyde in PBS (10 μl added to the surface of the agarose disc). After 2 min, excess liquid was removed and the cells were examined with a Zeiss LSM 510META laser scanning confocal microscope.

DNA donor activity and F1fus2 sensitivity. Cultures of TOP10/pOX38traA::cat containing traA genes cloned into a pBAD vector (Guzman et al., 1995) (see below) were grown to an OD<sub>600</sub> of 0.4. Where appropriate, traA expression was induced by addition of L-arabinose (final concentration 0.2%, w/v) 2.5–3 h before the cells were used. For donor activity, 0.5 ml of each culture was used to centrifugation. Supernatant fractions were aspirated and the cells suspended in 0.5 ml of a recipient cell culture (AE2248 grown to OD<sub>600</sub> 0.23). After incubation for 60 min at 37°C, the cell samples were diluted and 10 μl aliquots plated on media selective for transconjugants (chloramphenicol + tetracycline) and for donor cells (ampicillin + chloramphenicol).

For F1fus2 infectivity, we measured transduction of the bacteriophage <i>tet</i> gene. A portion (90 μl) of the cultures used to measure donor activity was mixed with 10 μl of an F1fus2 preparation containing 10<sup>9</sup> t.f.u. Samples were incubated at 4°C for 15 min, 37°C for 15 min, and subjected to centrifugation at 10 000 g for 2 min. Cell pellets were suspended in nutrient broth and incubated for 45 min at 37°C. Portions (10 μl) of serial dilutions were then spotted on LB/tetracycline plates.

Construction of F-pilin cysteine mutants by site-directed mutagenesis. With the exception of G64C, which was obtained by mutagenic PCR, cysteine mutations were introduced into traA of plasmid pWP901 using the USE Mutagenesis kit (Amersham Pharmacia Biotech). The primers used for the different mutations are listed in Table 1. We also found it necessary to introduce a third, wild-type primer for all mutations except A33C (Table 1). The reaction product was used to transform XK1200/pOX38traA::cat, selecting for Cam<sup>R</sup> Amp<sup>R</sup> transformants. Plasmid DNA was isolated and traA inserts sequenced in both directions. Following Manchak <i>et al.</i> (2002), we transferred the mutant and wild-type traA genes into the vector pBAD/Myc-His A (Invitrogen). These plasmids were introduced by transformation into TOP10/pOX38traA::cat.

**RESULTS**

Binding of fluorescent R17 bacteriophage to F-pili

Bacteriophage R17 was conjugated with the fluorescent dye Alexa 488 and purified as described in Methods. By denaturing gel electrophoresis, the R17 coat protein (~14 kDa; Weber & Konigsberg, 1975) contained essentially all of the fluorescence (Fig. 1). In addition to the coat protein, the preparation contained three other proteins visualized after Coomassie blue staining. These corresponded to ~28 kDa, ~37 kDa and ~48 kDa (Fig. 1). Only the 37 kDa protein, which corresponds in size to that of the bacteriophage A or maturation protein (Weber & Konigsberg, 1975), contained traces of fluorescence.

Biebricher & Duker (1984) first showed that fluorescent RNA bacteriophage bound to F-pili could be visualized by fluorescence microscopy. The result of a similar experiment but carried out with current imaging and image processing technology is shown in Fig. 2. The cells themselves, labelled internally by expression of the fluorescent protein DsRed-Express, are clearly visible. F-pili decorated with R17 showed up as fluorescent filaments in cultures of F<sup>+</sup> E' and Hfr strains but not of F<sup>−</sup> strains (not shown) or strains containing a <i>tra</i> mutation known to abolish F-pilus formation (Fig. 2).

**Table 1. Primers used to generate F-pilin cysteine mutants**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence*</th>
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<tbody>
<tr>
<td>S4C</td>
<td>CATCAGGTCTGACCAGAGGTGGGCGG</td>
</tr>
<tr>
<td>G5C</td>
<td>CCATCAAGTCCTGACAACTGTCGGC</td>
</tr>
<tr>
<td>S11C</td>
<td>CCGTGGTTGTACCAGCTGGCATCGG</td>
</tr>
<tr>
<td>S25C</td>
<td>CCATTTAACAACAGAGGTGCTCCTAC</td>
</tr>
<tr>
<td>A33C</td>
<td>CGACCAGAACTGACAGAAACACC</td>
</tr>
<tr>
<td>T45C</td>
<td>GACGTTCCTGCAATCATGTAC</td>
</tr>
<tr>
<td>G53C</td>
<td>GATGATGGCAAACAGGCGAGAATG</td>
</tr>
<tr>
<td>G69C</td>
<td>CCTGTCGACAGCAAGCAAGG</td>
</tr>
<tr>
<td>Common</td>
<td>CGACAGAACACTCAAGGAGAACAAC</td>
</tr>
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* Cysteine codons (reverse complement) are underlined.
† Wild-type sequence corresponding to the A33C mutagenic primer.

**Fig. 1.** Gel electrophoresis of proteins from fluorescent R17. An aliquot (15 μl) of R17 conjugated with Alexa 488 as described in Methods was denatured and the proteins separated by SDS gel electrophoresis. Lane 1, Coomassie blue stain; lane 2, illuminated with UV light; lane M, size markers.
Quantification of R17 binding to F-pili

Fluorescence microscopy can quickly provide information about F-pilus number per cell and length distributions, but for some applications a quantitative, sensitive and rapid assay for total cell-attached F-pili would also be useful. We describe such an assay here.

Fluorescent R17 (2000–3000 FIU) was incubated with F\(^9\)lac or isogenic F\(^2\) cells (0.4–0.7 OD\(_{600}\) units) to allow bacteriophage adsorption. Cells and bound R17 were collected by sedimentation. The pellets were suspended in 0.1% SDS, which dissolves F-pili but leaves cells intact. Cells were removed by sedimentation and washed once with SDS. Fluorescence of the combined supernatant fractions was then measured. In preliminary assays, fluorescence in supernatant fractions from F\(^9\) or Hfr cells corresponded to 500–800 FIU per OD\(_{600}\) unit, depending on the strain. Fluorescence in equivalent fractions from F\(^-\) cells was 50–70 FIU per OD\(_{600}\) unit. Fluorescence increased linearly with increasing amounts of F\(^+\) cells except at very low levels (< 0.1 OD\(_{600}\) unit) (Fig. 3) and it was proportional to culture density during exponential growth (Fig. 4). As the culture left exponential growth (OD\(_{600}\) > 1.5), the ratio FIU/OD\(_{600}\) unit began to diminish (Fig. 4); a stationary-phase culture of the F\(^-\) strain used in this experiment (OD\(_{600}\) 3.2) measured 450 FIU per OD\(_{600}\) unit. This reduced level of F-piliation undoubtedly reflects the well-established F\(^-\) phenocopy effect.

Induction of traA assayed by R17 binding

Rapidity and sensitivity, the two main advantages of the fluorescence assay described above, are especially useful for kinetic experiments. An example is the formation of F-pili after traA induction. We first constructed a plasmid in which the traA gene was expressed from the araBAD promoter (see Methods). This construct was introduced into TOP10/pOX38traA::cat cells. A culture was then induced by addition of arabinose (0.2%), and R17 binding was followed as functions of time and culture density. An uninduced culture served as a control. R17 binding could be
observed in the induced culture within 60 min of arabinose addition and reached a maximum specific activity (FIU per OD₆₀₀ unit) after 3–4 h (Fig. 5a). In contrast, no binding above background was observed with uninduced cells (Fig. 5a). Note also that traA induction in these conditions drastically curtailed growth (Fig. 5b), an effect probably related to F-pilin overexpression.

Cysteine-containing F-pilin mutants

Similar to studies by Manchak et al. (2002), we have been constructing and characterizing F-pilin missense mutants, in conjunction with structural studies of F-pili necessary to interpret their effects. Here, we use a subset of such mutants to illustrate the utility and one limitation of the methods described above.

F-pilin, which is only 70 amino acids in length, normally lacks cysteines (Frost et al., 1984). We constructed several mutant traA genes, each with a cysteine codon at a different site (Table 2). Four of the mutations were in domain I, one in domain II, one in domain III and three in domain IV (Table 2) (Paiva et al., 1992). (Note that the traA525C gene also contained a second mutation, A55V.) These mutant traA genes were cloned into a pBAD vector as described in Methods.

All of the mutants accumulated high levels of membrane F-pilin (Fig. 6). Accumulation was dependent on arabinose induction (Fig. 6, lanes 1 and 2). The S4C and G5C mutants appear to accumulate less than the others (Fig. 6, lanes 3 and 4), but we attribute this to the fact that these mutations are within the wild-type sequence used to raise the anti-peptide antibody used in these experiments (Paiva et al., 1992) and hence have altered epitopes.

Even though these mutant F-pilins accumulated to normal or nearly normal levels, not all formed F-pili. Of the nine mutants tested, only three were positive by R17 binding and only one mutant, containing the G64C mutation, supported as much R17 binding as the wild-type control (Table 2). Tests based on conjugal DNA donor activity and filamentous bacteriophage F1 fus2 sensitivity largely confirmed these results (Table 2). Two mutations, A33C and T45C, led to significantly reduced R17 binding; fluorescence microscopy showed that both these mutations, but especially T45C, led to shorter-than-normal filaments (data not shown). Short filaments would not be expected to affect functions, such as Ff phage binding, that require only an F-pilus ‘tip’, and both the A33C and T45C mutants remained sensitive to F1 fus2 and active as DNA donors (Table 2). One mutation, G69C, abolished R17 binding without affecting DNA donor activity or F1 fus2 sensitivity (Table 2). This mutation appears to have specifically altered R17 adsorption sites along the F-pilus side.

DISCUSSION

Notwithstanding the essential role of conjugative pili in horizontal DNA transfer mediated by type IV secretion systems, their structure, function and assembly are not well understood. The work we describe here was undertaken as part of a broader effort to determine how F-pili are formed and how they function. We were interested in developing simple assays that would yield a statistical profile of F-pili in bacterial populations (F-pilus number per cell and length distributions) and allow for comparative studies of total

Fig. 4. F-piliation as a function of culture density. AE2386/F lac was grown in LB medium at 37 °C with aeration. Portions of the culture at the indicated times were assayed for R17 binding (■) and OD₆₀₀ (▲).

Fig. 5. F-pili as a function of time after traA induction. Strain TOP10 containing pOX38 traA::cam and pBADtraA+ was incubated with aeration from a starting OD₆₀₀ of 0.02 for 45 min. To one culture, arabinose was added to 0.1%; a second culture served as uninduced control. At the indicated times, samples were removed for measurement of F-pili by R17 binding (a) and culture density (b). ●, No arabinose added; ○, arabinose added.
Table 2. Structure and functions of F-pilin cysteine mutants

Functional measurements were as described in Methods.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Sequence*</th>
<th>R17 binding (FIU per OD&lt;sub&gt;600&lt;/sub&gt; unit)</th>
<th>DNA donor activity, F1fus2 sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>600</td>
<td>+ , +</td>
</tr>
<tr>
<td>S4C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>- , -</td>
</tr>
<tr>
<td>G5C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>- , -</td>
</tr>
<tr>
<td>S11C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>- , -</td>
</tr>
<tr>
<td>S25C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>- , -</td>
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<tr>
<td>A33C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>81</td>
<td>+ , +</td>
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<tr>
<td>T45C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>39</td>
<td>+ , +</td>
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<tr>
<td>G53C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>- , -</td>
</tr>
<tr>
<td>G64C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>400</td>
<td>+ , +</td>
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<tr>
<td>G69C</td>
<td>AGSSQDLMASGNTTVKATFGKDSVVKVLDAEVLVGAVYMTTYMKNVKFLAGFAIISVFIAVGMVGL</td>
<td>&lt;10</td>
<td>+ , +</td>
</tr>
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</table>

*Domain designations I–IV were as described by Paiva et al. (1992).

F-pilus levels in different cell populations or in the same population at different times or after different physiological perturbations. The main advantage of the fluorescence assays we describe here over previous assays (Valentine et al., 1969; Curtiss et al., 1969; Frost et al., 1985) is that all the information can be obtained with only a single reagent. Fluorescent R17 is straightforward and inexpensive to prepare and stable for years, unlike bacteriophage containing [32P]RNA used in filter binding assays. Sample preparation for fluorescence microscopy is certainly no more time consuming or complicated than that for negative staining and electron microscopy, and a single field can yield data on scores of cells.

As with other assays for F-pili, the ones we describe here rely on the lateral binding of RNA bacteriophage to F-pili. This can be misleading. As we have shown here for the G69C mutant, and as Grossman & Silverman (1989) and Manchak et al. (2002) have also shown, altered F-pilins can be incorporated into filaments that fail to bind RNA bacteriophage. Such ambiguities might be minimized by using cysteine-reactive fluorescent dyes, rather than fluorescent bacteriophage, in conjunction with cysteine-containing F-pili.

Manchak et al. (2002), examining the effects of single missense mutations of F-pilin, found that in general DNA donor activity and sensitivity to filamentous DNA bacteriophage tracked together. In contrast, several mutations abolished RNA bacteriophage sensitivity with less of an effect on the other two functions. The G69C mutation we describe here evidently belongs in this class. The effects of the G69C mutation can not be attributed to the presence of cysteine at this locus since the G69D mutation had much the same effects (Frost & Paranchych, 1988). Interestingly, neither we nor Manchak et al. (2002), nor the more limited study by Frost & Paranchych (1988), identified F-pilin missense mutants that significantly reduced Ff bacteriophage sensitivity and DNA donor activity without also reducing or abolishing RNA bacteriophage sensitivity. One explanation for these data is that donor activity and DNA bacteriophage sensitivity are relatively robust functions with respect to modest alterations to F-pilin structure, whereas RNA bacteriophage infection is more sensitive.

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