The F Pilus of Escherichia coli Appears To Support Stable DNA Transfer in the Absence of Wall-to-Wall Contact between Cells

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Separation of HfrC-F" mating pairs of Escherichia coli by a filter 6 μm thick with straight-through pores 0.01 to 0.1 μm in diameter did not prevent DNA transfer. We conclude that the F pilus alone is capable of acting as a stable conduit for cell-to-cell DNA transfer.

The most visible feature of the F-plasmid-mediated conjugation system of Escherichia coli is the F pilus, a long (up to 20-μm) (5), thin (about 8 nm in diameter) (3) tubular structure which extends from the bacterial cell surface. Conjugation seems to be initiated by contact of the distal end of the pilus with the intended recipient (reviewed in references 3, 5, and 7). The donor cells and the recipient cells are drawn into contact by contraction (probably depolymerization) of the pilus. The pilus, but not cells already drawn together by the pilus, can be disrupted by low concentrations of the detergent sodium dodecyl sulfate (6). Thus, it is not clear whether the hollow pilus (presumably protected from the detergent by the juxtaposed cell walls) is the conduit for DNA or whether cell-to-cell contact is a prerequisite for stable DNA passage via another path.

The availability of thin (6-μm) polycarbonate filters with pores of a uniform diameter in the submicrometer range going straight through the membrane (Nuclepore Corporation, Pleasanton, Calif.) suggested that it might be possible to limit conjugating cell-to-cell contact to the long, thin pilus. We utilized parabolic chambers (Bellco Glass, Vineland, N.J.), U-tubes in which the filter is clamped between ground-glass surfaces, to conduct the experiments. The halves of the chamber and the filter were sterilized separately; the ground-glass surfaces were then greased with sterilized vacuum grease, and the apparatus was assembled aseptically in a laminar-flow hood.

The recipient stain (F" ) was AB1157 (14), a multiple auxotroph (Table 1). Because it is λ-, a λ donor (KL226, HfrC, prototroph) (14) was used to prevent induction of λ during conjugation.

If conjugation were to occur, it would be limited to a surface of approximately 2.5 cm² (about 0.75 × 10⁹ to 1.5 × 10⁹ pores.) To fully cover the surface of the filter in the U-tube, about 5 ml of culture was introduced on either side. Because recombinants would initially be found on or near the filter surface, samples were taken with a loop. Because of this, and because of uncertain surface-to-volume relationships (to say nothing of uncertainties in the time necessary to initiate presumed pilial membrane penetration), primarily qualitative data are presented.

Strains were grown to a density of about 5 × 10⁸ cells per ml in rich broth (1% tryptone, 0.5% yeast extract, 1% NaCl, 0.004% thymine, 0.2% glucose) with very gentle shaking, and then 5 ml of the broth was aseptically introduced into the assembled chamber. Controls without bacteria were volumes of uninculturated rich broth. The chamber was agitated very gently (about 20 rpm), and the cells were allowed to remain in contact for 4 to 10 h (Table 2). The chambers were then shaken, and a loopful of broth from the recipient side (AB1157) was spotted on Davis-Mingioi minimal medium (2) modified by the omission of citrate and supplemented with 1.5% agar, 25 μg of L-proline, L-leucine, and L-arginine per ml, 2 μg of thiamine per ml, and 0.2% glucose. This modified medium selects for recipients recombinant for threonine and histidine.

In each attempted conjugation between the donor strain and the recipient strain, when they were separated by a Nuclepore filter with pores 0.015, 0.03, or 0.10 μm in diameter, recombination was observed (Table 2). Controls included the substitution of a filter of high tortuosity (Supor, 0.45-μm-diameter pore; Gelman Instrument Co., Ann Arbor, Mich.) and the omission of the donor or of the recipient. No growth was observed on the selective plate for any of the controls, indicating a lack of relevant spontaneous reversion, passage of a soluble factor, or leakage through the Nuclepore membrane (early trials without grease did leak.) In several cases, controls were incubated for several days without any increase in turbidity on the uninculturated side of the filter (data not shown).

Passage of the his locus was unexpected (we tested this locus after observing the transfer of the threonine locus) because of the length of time the cells must remain in contact. However, the environment of a cell pressed against a filter must be quite different from that of a cell in free solution (or even on agar), and the hydrodynamic stress could be much lower, thus accounting for the unusual stability of the conjugating pairs. Once transfer was de-

TABLE 1. Nutritional markers of AB1157 in relation to the origin of transfer of KL226

<table>
<thead>
<tr>
<th>Marker</th>
<th>Nutritional identity</th>
<th>Map position</th>
<th>Position relative to oriT</th>
<th>Presence in recipient plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>oriT</td>
<td>—a</td>
<td>13</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Δgpt-proA62</td>
<td>Proline</td>
<td>6</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>leuB6</td>
<td>Leucine</td>
<td>2</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>thr-1</td>
<td>Threonine</td>
<td>0</td>
<td>13</td>
<td>−</td>
</tr>
<tr>
<td>thi-1</td>
<td>Thiamine</td>
<td>90</td>
<td>23</td>
<td>+</td>
</tr>
<tr>
<td>argE3</td>
<td>Arginine</td>
<td>89</td>
<td>24</td>
<td>+</td>
</tr>
<tr>
<td>histG4</td>
<td>Histidine</td>
<td>44</td>
<td>79</td>
<td>−</td>
</tr>
</tbody>
</table>

Table: Data not applicable.

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tected, we used transfer of the thr and his loci as our standard.

The very small size of the pores (about 0.1 to 0.01 times the diameter of an E. coli cell) and the thickness of the Nuclepore membranes (about two to three times the length of an E. coli cell) virtually rule out the possibility of direct cell-to-cell contact in the experiment.

It is difficult to assess the number of successful mating pairs without knowing more about the kinetics. There are about $10^9$ pores, but about $1.2 \times 10^6$ E. coli cells with dimensions of about 1 by 2 µm would pack the filter 1 cell deep (a section of the filter about 18 mm in diameter was exposed). Assuming that 10 recombinants occur in each 50-µl sample plated, if the samples were uniformly mixed, if no growth occurred, and if all bacteria were rinsed from the membrane (conditions which were probably not met), then there were about $10^4$ matings that successfully transferred the his locus. It is obvious that the kinetics and geometry of the cell-to-cell transmembrane contact need further attention.

We conclude that stable DNA transfer can occur through the F pilus. We suspect that this is the sole route for DNA transfer, as it seems unlikely that a second route would be opened following cell-to-cell contact.

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**LITERATURE CITED**


