Tra Proteins Characteristic of F-Like Type IV Secretion Systems Constitute an Interaction Group by Yeast Two-Hybrid Analysis

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Using yeast two-hybrid screens, we have defined an interaction group of six Tra proteins encoded by the F plasmid and required by F+ cells to elaborate F pili. The six proteins are TraH, TraF, TraW, TraU, TrbI, and TrbB. Except for TrbI, these proteins were all identified as hallmarks of F-like type IV secretion systems (TFSSs), with no homologues among TFSS genes of P-type or I-type systems (T. Lawley, W. Klimke, M. Gubbins, and L. Frost, FEMS Microbiol. Lett. 224:1-15, 2003). Also with the exception of TrbI, which is an inner membrane protein, the remaining proteins are or are predicted to be periplasmic. TrbI consists of one membrane-spanning segment near its N terminus and an 88-residue, hydrophilic domain that extends into the periplasm. Hence, the proteins of this group probably form a periplasmic cluster in Escherichia coli. The interaction network identifies TraH as the most highly connected node, with two-hybrid links to TrbI, TraU, and TraF. As measured by transcriptional activation of lacZ, the TrbI-TraH interaction in Saccharomyces cerevisiae requires the TraH amino acid segment from residues 193 to 225. The TraU and TraF interactions are localized to C-terminal segments of TraH (amino acids 315 to 458 for TraF and amino acids 341 to 458 for TraU). The TrbI-TraH interaction with full-length (less the signal peptide) TraH is weak but increases 40-fold with N-terminal TraH deletions; the first 50 amino acids appear to be critical for inhibiting TrbI binding in yeast. Previous studies by others have shown that, with the exception of trbB mutations, which do not affect the elaboration or function of F pili under laboratory conditions, a mutation in any of the other genes in this interaction group alters the number or length distribution of F pili. We propose a model whereby one function of the TraH interaction group is to control F-pilus extension and retraction.

Type IV secretion systems (TFSSs) comprise a broadly distributed group of molecular machines that function to secrete macromolecules from gram-negative bacteria into other bacterial or eukaryotic cells (3, 4). These systems are responsible for the pathogenic effects of some bacterial species (4) and contribute to the dissemination of antibiotic resistance genes to pathogens of humans and domestic animals (37). Several classes of TFSSs are distinguishable by sequence comparisons (3, 6, 7, 26), and these systems may have arisen more than once during bacterial evolution.

TFSSs typically mediate DNA transfer (conjugation) (5, 14, 21). This activity has been broadly divided into two experimentally separable stages. The first is the establishment of secure cell-cell contacts, and the second is DNA transfer per se. These two stages are linked by coupling proteins, which are membrane or membrane-associated complexes responsible for substrate recognition and presentation to a channel or pore complex that spans the cell envelope (4, 27, 35). Individual functions associated with the first stage are designated Mpf (mating pair formation) functions and the corresponding proteins are Mpf proteins; proteins and functions associated with the second stage are designated Dtr (donor transfer replication). Most TFSS genes fall into the Mpf class (14, 17). However, these functional assignments are often based on mutant phenotypes. For proteins that function at more than one stage, null mutations would identify only the earliest stage. For this reason, the Mpf designation does not preclude a function at later stages of conjugal DNA transfer.

One feature common to all conjugal DNA transfer systems of gram-negative bacteria is the presence of conjugal pili (21, 32). These surface filaments function in the earliest stages of conjugation, when donor and recipient cells make initial contacts that eventually lead to DNA transfer. Insofar as they have been examined, conjugal pili are repeats of one quantitatively predominant subunit (12, 32). These subunits and the corresponding filaments are designated according to the conjugal DNA transfer system of which they are a part, e.g., F pilus(n), RP4 pilus(n), T pilus(n), etc. Notwithstanding their apparent structural simplicity, the formation of conjugal pili requires numerous Mpf proteins. For the 25-gene tra system borne by the F plasmid, a mutation in any of 16 genes abolishes the formation of extended F pilus or alters F-pilus length or number distribution (14).

After initial contacts, F pilus retract (8, 29), such that DNA transfer occurs primarily, if not exclusively, between cells that are firmly joined at their surfaces (11, 25, 33). Retraction also occurs when filamentous DNA bacteriophages bind to the F-pilus tip (22). It is unclear how widely distributed retraction is among TFSSs other than the F-like group.

Type IV Mpf systems typically include core components that are recognizable by sequence similarities among classes (3, 26). Other components, however, appear to be class specific (26). The TFSSs encoded by F and the F-like R factors include several such components. Five of the 16 F-plasmid-encoded Tra proteins required for the formation of F pilus or for normal
TABLE 1. Primers used to amplify tra sequences of bait plasmids

<table>
<thead>
<tr>
<th>Bait construct</th>
<th>Primers&lt;sup&gt;a&lt;/sup&gt; (5′ primer, 3′ primer)</th>
<th>nt &lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAS1traH</td>
<td>GCAAGCGTCGcatGGATGTGACAGGCG, CGTCCAGGTCTCCATAAAGGCG</td>
<td>8090–8115, 19486–19466</td>
</tr>
<tr>
<td>pAS1traR</td>
<td>CCGCGCCcatgGGAAGATGACAGGCG, CATATAACAGATCCGGATTAAAAATTGG</td>
<td>15127–15150, 15843–15815</td>
</tr>
<tr>
<td>pAS1traW</td>
<td>GGAACCATGagATCGCCGGC, CACCCACAGGagTCGTCTCAT</td>
<td>10726–10742, 11381–11361</td>
</tr>
<tr>
<td>pAS1traU</td>
<td>CAGAGGAGGCCAgGAAATGAAGC, GCTTCATCATGagCCCTCAAGGAG</td>
<td>11345–11376, 12368–12346</td>
</tr>
<tr>
<td>pAS1trb</td>
<td>TACAGGAGGatGGCATGAGTGC, GCCCCGGATGTCATGTTCT</td>
<td>10335–10356, 10747–10727</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lowercase letters indicate mutations that were introduced to aid cloning.
<sup>b</sup> tra sequence coordinates were obtained from reference 15.

F-pilus number and length distributions appear not to have homologues in TFSs outside of the F and F-like families (26). These are TraF, TraH, TraW, TrbC, and TraU. There are no data regarding physical interactions among these proteins, but genetic data suggest that they have a common function(s). A mutation in tra<sub>F</sub>, tra<sub>H</sub>, tra<sub>W</sub>, or trb<sub>C</sub> abolished the ability of F<sup>C</sup> cells to form extended F pili that were visible by electron microscopy (14). However, TraF, TraH, and TraW mutants retained significant sensitivity to filamentous bacteriophages that bind to the F-pilus tip (1), suggesting that these Tra proteins are required for F-pilus extension. (The trb<sub>C</sub> mutant could not be tested.) Mutations in tra<sub>U</sub> also reduced the number of F pili per cell and the mean F-pilus length, but not as drastically as mutations in the other genes (31). (Such mutations reduced DNA donor activity more than expected from the reduction in F-pili, suggesting that TraU affects multiple stages of conjugation [31].)

Here we show by yeast two-hybrid analyses that TraH, TraF, TraW, TraU, and TrbB are components of the same Tra protein interaction group. An additional member of this group is TrbI. While trb<sub>B</sub> mutations had no effect on F-pilus functions in otherwise tra<sub>F</sub> cells (24), trb<sub>1</sub> mutants were reported to elaborate unusually long F pili (28). The properties of mutants in individual components of this interaction group suggest a role for the group in regulating F-pilus retraction and extension.

MATERIALS AND METHODS

Strains and plasmids. *Saccharomyces cerevisiae* strain Y190 (MATa gal4+ gal80Δ his3Δ trp1-901 ade2-101 ura3-52 leu2-1312 Cyh2+ Gal1- lacZ::URA3 Gal1::HIS3 + αLYS2) and plasmids pACTII and pAS1CYH2 were originally obtained from Steven Elledge, Baylor University College of Medicine (10). Bait and prey libraries derived from plasmid pTG801 (16) and constructed in plasmids pAS1CYH2 and pACTII, respectively, were described previously (18). Specific bait plasmids were constructed by PCR amplification of tra segments from JCFL0 (F<sup>C</sup> lac<sup>C</sup>- tra<sup>F</sup>) or pTG801 (tra<sub>U</sub>, tra<sub>W</sub>, and trb<sub>U</sub>), as described by Harris et al. (19). The primers used for each such construct are shown in Table 1. PCR products were purified from agarose gels by centrifugation of frozen (liquid N<sub>2</sub>) gel segments (10 min at 13,000 × g) through siliconized glass wool, digested with NcoI and BamHI, and cloned into pAS1CYH2 digested with the same enzymes. All constructs were tested alone for transcriptional activation; only the tra<sub>F</sub> construct gave low and variable levels of activation under some conditions (see Results).

pAS1CYH2 tra<sub>H</sub> deletions were isolated from a pAS1CYH2 tra<sub>F</sub> fragment library (18) by colony lift hybridization. Radioactive tra<sub>H</sub> DNA was obtained by random primer labeling (13) with [α<sup>32</sup>P]dCTP. DNA was obtained by PCR amplification from JCFL0 with the forward primer 5′ GCAGAAATGATGC CACC (tra nucleotides [nt] 18024 to 18040) and the reverse primer 3′ TCATT CACCGGTGTGCT (nt 19410 to 19374). (Numbering of the tra<sub>H</sub> nucleotides was from reference 15.) The pAS1CYH2 tra<sub>F</sub> library was plated at 60,000 CFU/plate. Of 360,000 colonies screened, 85 potential positive colonies were isolated and sequenced. Several of these were selected based on the extent of the deletions and, where necessary, were frame shifted to correspond to the GAL4 sequence of pAS1CYH2 by a fill-in reaction catalyzed by the Klenow fragment of DNA polymerase I or by tung bean nuclelease. In all cases, a polypeptide of the appropriate size could be detected in yeast extracts analyzed by Western blotting with antibodies against the hemagglutinin (HA) epitope included in the GAL4 segment of pAS1CYH2 (18).

Media and growth conditions. Yeast extract-peptone-dextrose and synthetic complete dropout media were described previously (23). Yeast strains were routinely grown at 30°C with aeration, and growth was monitored by total cell counts in a hemocytometer or by measuring the optical density at 600 nm. For interaction screens, yeast colonies appearing within 5 days at 30°C on Leu<sup>+</sup> His<sup>+</sup> plates containing 40 mM 3-aminotriazole (added to reduce HIS3 [imidazole glycerol phosphate dehydratase] activity) were considered to be His<sup>+</sup> and those yielding a visible blue color upon colony lifting within 18 h at 41°C were considered to be LacZ<sup>+</sup>.

*Escherichia coli* was routinely cultured in Luria-Bertani medium at 37°C with aeration. Growth was monitored by measuring the optical density at 600 nm. Ampicillin was added, when necessary, at 100 μg/ml.

Methods. Yeast transformations were carried out by the lithium acetate-polyethylene glycol method, as described previously (23); transformation efficiencies with the pACTtra library were generally 10<sup>5</sup> to 10<sup>6</sup> Leu<sup>+</sup> Trp<sup>+</sup> colonies/μg of DNA. Plasmid DNAs were prepared from Zymolyase-treated yeast cells (23) and introduced into *E. coli* by electroporation. Otherwise, *E. coli* cells were transformed by the CaCl<sub>2</sub> method.

Beta-galactosidase activities in yeast were measured with chlorophenol-red-b-D-galactopranoside (CPRG; Boehringer-Mannheim) essentially as described previously (10). Cells in 5 ml of culture at an optical density at 600 nm of 0.6 to 0.8 were collected by sedimentation, suspended in 1 ml of H buffer (10), permeabilized with sodium dodecyl sulfate and CHCl<sub>3</sub>, and assayed. Chlorophenol-red released by hydrolysis was measured as the absorbance at 574 nm.

Western blot analyses of yeast extracts were performed as described previously (19).

RESULTS

TraH and TraF interact in yeast two-hybrid assay. TraH is one of several periplasmic Tra proteins that are required for the elaboration of F pili (14). We used tra<sub>H</sub> as bait in a two-hybrid screen of a library of tra fragments derived from plasmid pTG801 which includes all of the F plasmid tra genes required to elaborate functional F pili and only those genes, with a few exceptions (16). The tra<sub>H</sub> gene comprises 458 codons, with a predicted 24-amino-acid leader peptide (15). The bait plasmid included tra<sub>H</sub> codons 24 to 458. Of 250,000 Leu<sup>+</sup> Trp<sup>+</sup> transformants containing both the bait and prey plasmids, 45 were His<sup>+</sup> LacZ<sup>+</sup> as well, indicating transcriptional activation. Forty-four of these were sequenced, of which 40 contained tra<sub>F</sub> segments (Table 2). The remaining prey plasmids contained tra<sub>C</sub>, tra<sub>B</sub>, or tra<sub>L</sub>. The tra<sub>C</sub> and tra<sub>B</sub> inserts were in frame with respect to the upstream GAL4 sequence, whereas the tra<sub>L</sub> insert was not.

TraF is itself a periplasmic protein of 247 amino acids with a predicted 19-amino-acid leader peptide (15, 38). The 5′ termini of the tra<sub>F</sub> fragments contained in the 40 prey plasmids were all within the segment comprising tra<sub>F</sub> codons R64 to
The segment R64-W88 might border a region required for protein folding in yeast or might be amino acids that are directly required for the TraF-TraH interaction.

We next used a traF bait, comprising traF codons 19 to 247, to screen the tra fragment library. We performed two separate screens, which combined yielded His+ LacZ+ transformants at a frequency of $5.5 \times 10^{-3}$, or 30-fold higher than that in the traH screen (Table 2). Only two tra genes were identified in both screens, among 40 total sequences. Twelve contained traH segments, all in frame, and four contained traC sequences, with three in frame. Several other tra genes were identified in one screen or the other, but not both, along with reverse tra and unidentified, possibly vector, sequences (Table 2).

The high fraction of questionable positive results in the screen TraF is unusual in our experience. While we have no definitive explanation, we observed unusually variable levels of transcriptional activation in yeast cells containing both the traF bait and random prey plasmids. In our screens, we selected those with higher levels, estimated from the intensity of the blue color in colony lifts for $\beta$-galactosidase activity, but we could easily have chosen a significant number of false-positive transformants. For this reason, we are inclined for the time being to credit only the traH isolates, both because they still constituted nearly a third of the total sequences and because the traH screen identified exclusively traF preys. Also note that TraC is a cytoplasmic, peripheral membrane protein in tra+ cells (34), whereas TraF is periplasmic (38). These different cellular locations make it improbable, though certainly not impossible, that the TraC interaction is functionally significant.

In contrast to the results with traF preys isolated with the traH bait, the S' end points of the 12 traH preys were distributed throughout the central half of the coding region, from F135 to T326. These results suggest that a segment of TraH between T326 and the C terminus of the molecule, L458, is sufficient for TraF binding in yeast (Fig. 1). In a reciprocal experiment, we found that yeast cells with a bait plasmid containing trbI encoding amino acids 259 to 458 (traH259-458) and a prey plasmid containing traF75-247 were His+ and LacZ+ (data not shown), again suggesting that a C-terminal segment of TraH contains a TraF binding site.

**Interactions involving TrbI.** An additional interaction involving TraH was detected with bait plasmids lacking 5'-terminal traH codons beyond the 24 codons encoding the TraH signal peptide. We identified these deletions by colony hybridization with the bait plasmid library and by DNA sequence analysis, as described in Materials and Methods. Surprisingly, when these deletions were used in small-scale screens of the tra fragment prey library, the predominant prey in His+ LacZ+ yeast transformants was TrbI, a 128-amino-acid inner membrane protein in *E. coli* (28). A larger-scale screen with one of these deletions, traF199-458 (data not shown), yielded 16 trbI bait plasmids of 18 that were sequenced (Table 3). The overall frequency of positive transformants was fivefold higher in this screen than in the traH199-458 screen (Table 2), perhaps explaining why no traF preys were identified among the 18 that were sequenced from the traH199-458 screen. Most of the trbI segments identified in this screen contained the entire gene, which at 128 codons is smaller than the mean fragment length of 1 kb for the prey library (18). Four segments of the 16, however, began at codons N32, V35, I39, and R41. All of these are within or, in the case of R41, immediately adjacent to a putative membrane-spanning segment (codons W18 to V40 [15]). Hence, the interaction domain of TrbI for TraH is within the segment from R41 to the C-terminal P128. This region is relatively hydrophilic and extends into the periplasm (34).

To explain why no trbI preys were identified among the 44 that were sequenced from the traF199-458 screen (Table 2), we considered the possibility that an N-terminal segment of TraH inhibits the TrbI-TraH interaction in yeast. To test this, we measured $\beta$-galactosidase activity in yeast strains containing different traH bait plasmids and the same trbIP-128 prey. This experiment confirmed that $\beta$-galactosidase activity increased about 40-fold when an N-terminal segment of TraH, between A21 and L137, was deleted from TraH (Fig. 2). A further deletion of TraH to G193 had no effect on the activity, which remained at a relatively high level, but deletion to A225 or further essentially abolished activity. These results indicate that the TraH segment from G193 to A225 is required for the binding of TrbI in the yeast two-hybrid assay and that this binding is inhibited by an N-terminal segment of TraH. Whether or not this also occurs in *E. coli* remains to be determined.

The putative TrbI binding segment of TraH is predicted to be very hydrophilic, with 12 charged and 8 polar amino acids (Fig. 2). It is therefore, in all likelihood, solvent exposed and hence available for protein-protein interactions.

We also performed a two-hybrid screen with a trbIP-128 bait plasmid. We sequenced each of the 31 His+ LacZ+ transformants. The fraction of questionable positive results in this screen was about 5-fold higher than that in the traH screen, despite the high levels of expression in yeast of the prey plasmids.
inactive fusion proteins (TraH225-458 and TraH15-330) \( (\text{Fig. 2}) \) cannot be attributed to different GAL4-TraH fusion protein fi
sulfpeptide, the data suggest that TraH amino acids 25 to 75 are
codons. Given that codons 1 to 24 encode the TraH signal
tent with previous results. First, all
codons 74 to 230. Two features of these segments are consis-
tment G193-A225. The
the N-terminal segment D24-L137 and the requirement for the seg-
be explained by effects of the 26-amino-acid difference be-
tween the two fusion proteins on folding, nuclear transport, or
other factors that are not directly related to transcriptional
activation.

**Interactions involving TraU and TraW.** We performed additional
two-hybrid screens to define new Tra protein interaction
groups. Unexpectedly, two of these screens expanded the
TraH/TraF/TrbI group. The first utilized \( \text{traU}^{58-330} \) as bait \((15, 31)\).
Positive transformants were obtained at a frequency of \( 5.6 \times 10^{-4} \)
(Table 4). Of those that were sequenced, 67% carried in-frame
\( \text{traH} \) segments; no other \( \text{tra} \) gene was represented more than
once. The 5’ termini of the \( \text{traH} \) segments were all between
codons T265 and V343 (of 458 codons), indicating that TraU
interacts with a C-terminal segment of \( \text{TraH} \), as does TraF
(\( \text{Fig. 1} \)).

We also performed a screen with a bait plasmid containing
\( \text{traH}^{48-210} \) \((15, 28)\). This screen yielded positive transformants at
a frequency of \( 5.1 \times 10^{-4} \) (Table 4). Of those prey plasmids
that were sequenced, 86% carried either of two \( \text{tra} \) genes, \( \text{trbB} \)
and \( \text{traU} \), at similar frequencies (Table 4). While
\( \text{trbB} \) insertion alleles had no effect on F-pilus-related functions under laboratory
conditions \((24)\), the \( \text{trbB} \) gene products encoded by the
F and R100 plasmids are 90% identical \((1)\), suggesting that there is some evolutionary pressure for conservation. The data
showing that the F \( \text{trbB} \) gene product is part of a Tra protein
interaction group also suggest a function for TrbB, albeit one
either not required in routine assays for conjugal DNA transfer
or donor-specific bacteriophage sensitivity or for which an-
other Tra protein is redundant \((24, 26)\).

**TABLE 4. TraU and TraW interactions by yeast two-hybrid screen**

<table>
<thead>
<tr>
<th>( \text{tra} ) bait ( \text{gene} )</th>
<th>No. of codons</th>
<th>No. of Leu(^+) Trp(^+) transformants</th>
<th>No. of His(^+) LacZ(^+) transformants</th>
<th>( \text{tra} ) gene (no. of sequences/total sequences)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{traW}^{4-210} )</td>
<td>210</td>
<td>236,000</td>
<td>121</td>
<td>( \text{traH} ) (39/58) ( \text{traU} ) (16/44) ( \text{trbB} ) (22/44)</td>
</tr>
<tr>
<td>( \text{traW}^{58-330} )</td>
<td>330</td>
<td>169,000</td>
<td>95</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The data presented here establish a new interaction group among F-plasmid Tra proteins required for the elaboration of F pili. This group consists of six proteins: they are TraH, TraF, TrbI, TraU, TraW, and TrbB. Except for TrbI, the other proteins of this group are or are predicted to be periplasmic (14). TrbI is an inner membrane protein but has a large periplasmic domain (34). Hence, interactions among the six proteins are consistent with their cellular localization.

The two-hybrid data identify TraH as the most highly connected member of the group, with two-hybrid links to TrbI, TraF, and TraU (Fig. 4). At 434 amino acids, periplasmic TraH is also the largest protein in this interaction group. These data suggest a central role for TraH in the group’s function(s).

Of the six Tra proteins in the TraH interaction group, five (TraH, TraF, TraW, TraU, and TrbB) were reported by Lawley et al. (26) to be characteristic of F-like TFSSs, insofar as sequence database searches returned no homologues among P-type or I-type TFSSs (26). Conversely, using virB query sequences, Cao and Saier found no homologues among F-plasmid tra genes for virB6-virB9 or virB11 (3). They did find tra homologues of virB2-5 and virB10, but none were among the genes found by Lawley et al. to be characteristic of F-like tra systems (3, 26). Cao and Saier attributed this to incomplete sequencing of the F tra region (3), but perhaps a more plausible hypothesis is that gram-negative TFSSs, at least in part, arose more than once during bacterial evolution.

A mutation in traH, traF, traW, traU, or trbI altered the F-pilus length or number distribution, suggesting that there are functional relationships among the corresponding Tra proteins. (The numbers of F pili per cell and F-pilus length distribution could amount to the same thing, since factors leading to shorter F pili might favor filamentation that are too short to be visible by electron microscopy. This would be scored as a decrease in the number of F pili per cell [2, 9].) The effects of mutations in traH, traF, and traW were similar (reference 14 and references therein). Each abolished the formation of F pili that were visible by electron microscopy of negatively stained cells. None affected the amount of membrane F pilin (30). All reduced DNA donor activity by several orders of magnitude (1 to 100%) of sensitivity to a fi bacteriophage at their tips.

FIG. 4. Protein interactions of the TraV/TraK/TraB and TraH/TraF/TraW/TraU/TnbI/TtrbB interaction groups. Arrows connect proteins that were shown to interact in yeast two-hybrid assays; arrowheads point to the prey. The interaction map was overlaid on the intracellular location of each protein in E. coli, as described previously (14). The TraV/TraK/TraB data are from reference 19; other data are from the present communication.

A mutation in traH, traF, or traU altered the F-pilus length or number distribution, suggesting that there are functional relationships among the corresponding Tra proteins. (The numbers of F pili per cell and F-pilus length distribution could amount to the same thing, since factors leading to shorter F pili might favor filamentation that are too short to be visible by electron microscopy. This would be scored as a reduction in the number of F pili per cell [2, 9].) The effects of mutations in traH, traF, and traW were similar (reference 14 and references therein). Each abolished the formation of F pili that were visible by electron microscopy of negatively stained cells. None affected the amount of membrane F pilin (30). All reduced DNA donor activity by several orders of magnitude (1 to 100%) of sensitivity to a fi bacteriophage at their tips. (TraW, TraF, and TraU have overlapping functions [26], which might explain why trbB mutations had no effect in a traF+ background [24].) TrbI would have to be tested in E. coli, in which the complete F-pilus assembly system can be analyzed (16).

A summary of this and our previous work on Tra protein interactions related to F-pilus formation is shown in Fig. 4. The TraB/TraK/TraV interaction group (19) consists of proteins with homologues, or at least obvious functional equivalents, among other TFSSs (20, 26). The TraH interaction group described here consists of Tra proteins that are characteristic of F and F-like systems (1, 26). How these interaction groups function in F-pilus formation and perhaps other stages of conjugal DNA transfer and especially whether the TraH and...
TraV/TraK/TraB groups interact with each other are topics of immediate interest.

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