INTRODUCTION

Conjugation, a process which promotes DNA transfer from a donor to a recipient cell mediated by physical contact (49, 139), occurs among both gram-negative and gram-positive bacteria and streptomycetes (for reviews of specific systems, see the book *Bacterial Conjugation* [55]). Donor ability is conferred by the presence of an infectious DNA element which disseminates to other cells. Commonly, genes encoding conjugative-transfer functions are associated with an extrachromosomal replicon, termed a self-transmissible or conjugative plasmid. In addition to self-transfer, the transfer systems of conjugative plasmids often facilitate the independent transfer of nonconjugative, mobilizable plasmids that are coresident in the donor cell. DNA sequences that become cointegrate with the conjugative plasmid can also be transferred; thus, integration and other recombinational rearrangements can result in transmission of sequences from the bacterial chromosome, of transposons, and of nonmobilizable plasmids. As a means of genetic exchange among individual cells and populations, both within and between bacterial species, conjugation is a phenomenon of fundamental evolutionary and ecological consequence (for a review, see reference 236). The significance of this process has been further highlighted by evidence that conjugation systems can also facilitate interkingdom transmission of genetic material. Ti plasmid-mediated T-DNA transfer from *Agrobacterium* species to plants appears to represent a novel form of bacterial conjugation (177, 211, 324, 364, 372). Transfer of both broad- and narrow-host-range plasmids (R751 and F, respectively) from *Escherichia coli* to *Saccharomyces cerevisiae* has also been demonstrated (142, 143).

Responsible for the earliest observation of genetic transfer (206), the F (fertility) factor of *E. coli* K-12 was the first plasmid to be described (49) and, as both subject and tool, has been studied ever since. The insight and ingenuity of early investigators allowed principal characteristics of F, F-mediated DNA transfer, and the circular configuration of plasmid and chromosomal DNAs to be determined solely through analysis of genetic crosses involving chromosomal markers (for an interesting account, see *The Genetics of Bacteria and their Viruses* [140]). Key deductions were (i) that F is a circular “episome” able either to replicate autonomously or to integrate in the bacterial chromosome and (ii) that the efficient transfer of chromosomal markers from Hfr (high-frequency recombinant) donor strains reflects a stable integration of F. The order and time of entry of markers transferred by Hfr donors could then be seen to reflect the position and orientation of a specific site, the F origin of transfer (*oriT*). It was perceived that transfer of DNA must always begin at this site and proceed unidirectionally around the circular genome. That a single strand of DNA (56, 124, 269) was transferred in the 5′ → 3′ direction (152, 269) was subsequently demonstrated. These appear to be basic precepts for conjugation, and *oriT* sites on many other subsequently studied conjugative elements have been found to function similarly in directing the transfer of contiguous DNA.

The observation of conjugation and the synchronous Hfr crosses necessary to time-of-entry experiments also depended on the efficient way in which F donors contact recipients. “Mating pairs” could form quickly in liquid suspensions, persist during gentle dilution, and be disrupted by severe
agitation. F pili, the filaments that extend from the donor cell surface to initiate these contacts, were discovered after bacteriophages that infected F\(^+\) but not F\(^-\) cells were isolated. The adsorption of RNA phages along the length of F pili distinguished them from other fimbrial appendages (60). Expression of a pilus filament has also proven to be essential for other enteric and pseudomonad plasmid conjugation systems (36, 38, 100, 161, 286), and all conjugative-plasmid transfer among these gram-negative organisms is thought to depend on contacts created by these structures.

**STRUCTURE OF THE F PLASMID**

The F plasmid is a circular DNA molecule, 100 kb in size (359). The various functional regions of F and the position of oriT (coordinate 66.7F) are indicated on the map shown in Fig. 1. These confer its basic properties, summarized briefly as follows.

![FIGURE 1](image-url)  
**FIGURE 1** Physical and functional map of the F plasmid. Numbers within the map indicate kilobase coordinates based on the 100-kb F map. The transposable elements IS2, IS3, and Tn1000 are represented by solid boxes. The extents of the replication (RepFIA, RepFIB, and RepFIC), transfer, and leading regions are indicated outside the map. The origin of conjugative transfer, and leading regions are indicated outside the map. The origin of conjugative transfer (oriT) is denoted by a triangle indicating the direction of single-stranded DNA transfer (leading region transferred first). The map is adapted from reference 359.

**Leading Region**

The F DNA sequences located between the origin of conjugal transfer, oriT, and RepFIA are presumed to be the first to enter the recipient cell during conjugation and have therefore been designated the leading region (296). Leading-region gene products are thought to assist in establishing F DNA in the recipient and are discussed in a subsequent section.
Autonomous Replication

The RepFIA region, believed to be primarily responsible for the typical replication properties of F, contains both unidirectional (oriS) and bidirectional (oriV) replication origins (201). The maintenance characteristics of mini-F plasmids that include only the F-EcoRI fragment f5 (44.6 to 53.7F) closely resemble those of F. Stringent regulation of RepFIA and associated maintenance and partitioning mechanisms act in concert to sustain the plasmid at one to two copies per cell (57, 97).

The secondary replication region, RepFIB, is independently functional and can sustain plasmid replication in the absence of RepFIA. The RepFIC region includes an incomplete remnant of a replication system that is used by some other related plasmids (31). The complexities of F replication have been reviewed elsewhere (184, 185, 359).

Transposable Elements

The F sequence includes a single copy of Tn1000 (also known as γδ) and IS2 and two copies of IS3 (Fig. 1) (129, 147–149) (see chapters 111 and 124 for descriptions of transposable elements). Tn1000 appears to have interrupted RepFIC (300), whereas IS3 inactivation of the transfer region regulatory gene, finO, is responsible for the constitutively high levels of conjugative transfer exhibited by F (52, 371). These elements also mediate the F-chromosomal integration events that form Hfr donors (see chapters 127 and 128) which, through imprecise excision, can subsequently generate F-prime (F′) plasmids (see chapter 129). The insertion of transposable elements into an F-plasmid progenitor was fortuitous for the efficient transfer of chromosomal markers crucial to the original detection of conjugation and to the subsequent utility of F (353; also see chapter 137).

Conjugative Transfer

Including the oriT site (map position 66.7F) and extending to the HindIII restriction cleavage site in IS3 (coordinate 100/0F), the transfer (tra) region encodes all of the F loci known to be required for efficient conjugative transfer; its 33.3-kb nucleotide sequence has recently been compiled (103). The mechanism of the transfer process and the characteristics and functions of tra products are detailed in subsequent sections.

Insertion of the 41-kb BamHI-HindIII F fragment (coordinates 59.3 to 100F) which includes the transfer region causes other replicons to become self-transmissible (174, 295). The circularized 55-kb HindIII fragment which includes RepFIA, as well as the tra and leading regions of F, forms a useful plasmid, pOX38 (130), that has F transfer and maintenance properties but lacks transposable elements, RepFIC and RepFIB sequences, and other loci between map positions 100/0F and 45F. The latter include the “killer gene” snrB (116) and pif loci responsible for inhibition of bacteriophage T7 development (249). The map positions of these and other named F loci have been tabulated previously (359).

F-LIKE PLASMIDS

The F-transfer system is the prototype for the various conjugation systems expressed by a large group of conjugative plasmids known collectively as F-like. The relatedness of these plasmids was initially indicated by the morphological and serological similarity of the pili they expressed and the bacteriophage sensitivities these conferred (66, 204). Relationships among F-like plasmids have been further subdivided on the basis of incompatibility (Inc), resulting in the seven Inc groups, IncFI through IncFVII. This subdivision is generally associated with a plasmid’s replicon(s), since plasmids are placed in the same Inc group if they cannot stably coexist in the same host cell (66). Incompatibility, which forms the basis of both F- and non-F-like plasmid classification, usually eventuates if plasmids share similar replication functions (30). The relatedness of F-like plasmids was further demonstrated by heteroduplex analysis (311) and, more recently, by DNA sequencing (for a review, see reference 103). In addition to clinically significant determinants, which include those for antibiotic resistances and production of hemolysins and toxins, F-like plasmids have been found to
encode a range of ecologically important factors, such as colicins and metabolic activities (see reference 164 for a tabulation). F-like plasmids are found throughout the family Enterobacteriaceae (171).

The classification of other conjugative plasmids from the family Enterobacteriaceae and the genus Pseudomonas, not known to have F-like conjugation systems, has been based primarily on incompatibility. However, as such transfer systems are examined, indications of broader similarities are emerging. The two structural types of conjugative pili detected have suggested that all conjugative plasmids in these Inc groups could belong to two evolutionary families. Whereas many, like F, express long, flexible pili, transfer efficiently in liquid cultures, and are frequently associated with phage f1 (M13, fd) and/or J sensitivity (e.g., IncF complex, IncD, IncC, and IncJ plasmids), a second group produces short, rigid pili, transfers more efficiently among cells on surfaces (unless assisted by other pili), and often confers sensitivity to phages PR4 and/or X (e.g., IncP, IncW, IncN, and IncI plasmids) (36–38; reviewed in references 100, 161, and 286). Analysis of transfer gene organization and DNA sequences has generally supported this two-family grouping (103, 128) and indicated that Agrobacterium Ti plasmid transfer systems also resemble those of the IncP, IncW, IncN, and IncI group of plasmids (210, 211, 213, 282, 344; S. Bolland, Ph.D. thesis, University of Cantabria, Spain, 1991). The characteristics of some plasmids blur even this distinction, however. As indicated in subsequent sections, evidence for relationships that span the two families has also begun to emerge (178, 212, 213, 283, 312, 313).

F-CONJUGATION PROCESS

The proficiency of the F-conjugative system in liquid matings has allowed the physiology of F-mediated cell contacts to be studied. Figure 2 depicts the stages of intercellular contact and DNA transfer thought to occur during F-mediated conjugation. These are as follows. Contact between F+ donor and F− recipient cells is believed to be instigated by an interaction between the tip of an F pilus and the recipient cell surface (144, 264, 274, 276). There is evidence for the occurrence of DNA transfer between cells that were not in surface contact (134, 276), but there is also both indirect (9, 279) and direct (78) evidence that conjugating cells are typically aggregated in close wall-wall association (5, 275). When DNA transfer is completed, mating cells actively disaggregate (9, 78) to yield two cells capable of donor activity.

The first surface association of donors and recipients in aggregates is thought to reflect pilus retraction, mediated by depolymerization of the pilus subunit, into the donor and/or recipient cell envelope(s) (63, 162, 265, 315). These donor and recipient contacts then become stabilized in a manner that renders the aggregate more resistant to shear forces (5, 9, 230). In thin sections, the cells exhibit relatively large, electron-dense regions of envelope association, termed conjugative junctions (78). The biochemical reactions involved and the pathway for DNA transport remain unclear. DNA entry into the recipient has been suggested to occur through a direct passage to the recipient cytoplasm (359) or via the recipient periplasm, where recipient transport components might facilitate DNA uptake (78).

A large number of the F products required for conjugative transfer are involved in F-pilus synthesis and aggregate stabilization. Recipient cells carrying mutations altering the structure of the lipopolysaccharide (20) or the outer membrane protein OmpA (232) also affect F-donor cell interactions, but it is unclear whether these cell surface components are involved in initial contacts with the pilus or at a subsequent stabilization stage (for reviews, see references 10, 161, and 358). The effects are plasmid specific, since ompA mutants which act as poor recipients for F+ donors mate efficiently with cells harboring R100-1 or R136 (231, 320) and since F, ColB2, and R100-1 transfers are affected differently by mutations in individual lipopolysaccharide core biosynthesis (rfa) genes (20). Furthermore, these specificities do not depend on the pilin subunit (20), and the transfer defects associated with conjugation-defective recipients can often be bypassed if matings are undertaken on a solid surface rather than in liquid media (137, 138).

F DNA transfer processes are believed to be precipitated by a “mating signal” generated by functional-pair formation (183, 277). Many aspects of the DNA-related events necessary for conjugative transfer have been characterized (for reviews, see references 202 and 349). Briefly, a protein complex (oriT complex) is associated with the origin of transfer of transmissible and mobilizable plasmids. One DNA strand in the oriT site is “nicked” by a relaxase that catalyzes the covalent attachment of the 5′ end of the DNA to the protein. The F-plasmid relaxase, TraI, is also a helicase and can unwind the nicked strand in the 5′ → 3′ direction.
During transfer, this protein is suggested to be associated with the site of intercellular connection through which this single strand of DNA is passed; because it is attached to the 5′ end of oriT, it may also catalyze recircularization of the transported strand to terminate transfer (202, 234, 235, 297, 349, 359, 360). Replacement strand synthesis in the donor and complementary-strand synthesis in the recipient depend on host enzymes (183, 350) and are not essential for DNA transfer per se (183, 304). Figure 2 shows synthesis in the donor by a rolling-circle mechanism (349); a variation in which both the 5′ and 3′ oriT ends remain associated with TraI has also been suggested (359, 360). The F-TraI protein forms a covalent linkage only with the 5′ oriT end; whether the 3′ end remains bound in some other persistent association is not yet clear (235).

Proteins encoded by the leading region may also contribute to establishing the plasmid DNA in the recipient, but F does not appear to encode or to transfer a primase such as that associated with IncI and IncP plasmid conjugation systems (202, 349).
ORGANIZATION OF THE TRANSFER REGION

The nucleotide sequence of the entire 33.3-kb F-tra region has revealed its genetic structure at maximal resolution (GenBank accession number U01159) (103). Figure 3 shows the organization and functional classification of the 36 open reading frames (ORFs) known or deemed likely to encode products. With one exception, artA, all translated genes are encoded on the same DNA strand. The finO sequence is interrupted by a copy of the transposable element IS3 (52, 371).

The functional classes depicted in Fig. 3 reflect the phenotype of the relevant mutation and determine the category in which each individual tra region gene product and activity is discussed in this chapter. Classical genetic techniques were initially used to define F-plasmid-encoded transfer functions (12, 13, 92, 160, 270, 271). Recombinant DNA techniques subsequently confirmed the autonomy of the region for conjugative DNA transfer (174, 214, 295) and facilitated the refinement of the genetic and physical map (11, 225, 229, 257, 321, 334). The involvement of loci that had not been represented in early mutant collections has, in most cases, been tested by insertion mutagenesis employing resistance gene cassettes prior to gene replacement via homologous recombination (180).

A variety of gene expression and protein analysis methods have now also been applied toward the detection of tra region gene products. In most cases, and particularly with genes known to be involved in conjugation, the products encoded have been identified and the subcellular location of many of these proteins has also been examined (Table 1). Figure 4 correlates the known or predicted location of tra region products with their size and function.

STRUCTURE AND BIOGENESIS OF F PILI

One to three F pili are typically visualized extending 1 to 2 mm from the surface of a donor cell (10, 162). Production of a thick, flexible filament similar to an F pilus in appearance has been associated with the capacity of F and F-like plasmid donors to conjugate efficiently in liquid (39, 100). Analysis of purified F pili has detected only a single type of protein subunit, F pilin (21, 42, 65, 144). Optical and X-ray diffraction studies indicate that these arrange helically to form a cylindrical pilus structure 8 nm in diameter, with a 2-nm axial hole (233). The basic helix contains 25 subunits in two turns and has a pitch of 16 nm. The unit rise is 1.28 nm, and the crystallographic repeat is 32 nm. Pilin subunits in F pili are related by fivefold symmetry around the pilus axis. Pili can therefore be envisioned as consisting of doughnut-like discs, composed of five segments that each correspond to a pilin subunit; the discs are stacked such that each is rotated 28.8° with respect to the disc below (100, 278). An alternative view is that five strands of polymerized pilin are wound together in a helical fashion. Treatments with Triton X-100 or sodium PPI have been used to visualize very fine fibers of pilin (42, 241).

In addition to their requirement for conjugal DNA transfer, F pili are utilized as receptors by male-specific bacteriophages; RNA phages, such as R17 (e.g., f2, MS2, and Qβ), attach to the sides of the pilus (60), whereas filamentous single-stranded DNA phages, like f1 (e.g., M13 and fd), adsorb to its tip (47). The inhibition of mating-pair formation by F-specific DNA phages provides evidence that the pilus tip is crucial for initial contacts between donor and recipient cells (159, 264), although nonspecific interactions involving the pilus side have also been suggested (274).

Synthesis of the F-Pilus Subunit

The products of three F genes, traA, traQ, and traX (Table 1), distinctly spaced within the tra region (Fig. 3), are involved in synthesis of F-pilin subunits. The traA gene encodes the 121-amino-acid (aa) precursor of the pilus subunit, propilin (TraA) (Fig. 5), a 12.8-kDa polypeptide that requires proteolytic processing to yield the 7.2-kDa pilin polypeptide (106, 163, 247). Mature F pilin has a sequence corresponding to that of the last 70 aa in TraA (Fig. 5) and possesses an acetylated N terminus, which forms its major antigenic determinant (87, 104). The processing and Nα-acetylation steps in propilin maturation depend on expression of traQ (163, 180, 256) and traX (252), respectively.
TraQ, a 94-aa inner membrane protein of 10.9 kDa (367, 369), appears to provide an efficient, secA-independent, and ethanol-resistant pathway for rapid insertion of propilin into the inner membrane (N. Majdalani and K. Ippen-Ihler, unpublished data; N. Majdalani, D. Moore, S. Maneewanakul, and K. Ippen-Ihler, unpublished data). The 51-aa propilin leader peptide is then removed in a single cleavage step which depends on signal peptidase I (Majdalani et al., unpublished). Although unusually long, the propilin leader does contain all of the features typical of signal sequences cleaved by this enzyme: positively charged residues are followed by a hydrophobic core region and a processing sequence, Ala$^{3}$-Met-Ala$^{51}$-Ala$^{+1}$, which conforms to the $-3,-1$ rule (Fig. 5) (106, 272, 342). In the absence of TraQ, propilin folding is thought to interfere with its membrane translocation; the traA product is rapidly degraded, and only a very small percentage becomes processed (223). The unusual N-terminal sequence and length of the propilin leader peptide are not, however, responsible for these effects. Recent results show that maturation of an altered traA product with a foreshortened leader sequence equivalent to propilin residues 28 to 51 is still TraQ dependent. Furthermore, when the 52-aa propilin leader sequence and processing site was joined to the mature portion of either $\beta$-lactamase or alkaline phosphatase, processing of the fusion protein and secretion of the enzyme into the periplasm occurred efficiently and entirely independently of TraQ (Majdalani et al., unpublished). Thus, sequences within the mature portion of propilin appear to dictate the TraQ dependence of pilin subunit maturation.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Functional group</th>
<th>Product length (aa)</th>
<th>Product size (kDa)</th>
<th>Product location</th>
<th>Reference(s)</th>
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<td>21.0</td>
<td>Cytoplasm</td>
<td>59, 371</td>
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<td>Regulation</td>
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<td></td>
<td>Cytoplasm</td>
<td>88, 339</td>
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<td>traA</td>
<td>Pilus biogenesis</td>
<td>121 [70]</td>
<td>12.8 [7.2]</td>
<td>Inner membrane and extracellularly</td>
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<td>475</td>
<td>50.5</td>
<td>Inner membrane</td>
<td>257; Frost, unpublished data cited in reference 103</td>
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<td>99.2</td>
<td>Cytoplasm/inner membrane</td>
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<td>102.4</td>
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<td>26.0 [23.8]</td>
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<td>Cytoplasm</td>
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<td>23.4 [21.2]</td>
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<td>226</td>
</tr>
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<td>14.1</td>
<td>Inner membrane</td>
<td>227</td>
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*a* Primary references for allocation of genes to functional groups are shown in the text.

*b* Sizes and lengths are calculated from the deduced amino acid sequence of each product. Sizes and lengths of processed products are shown in brackets. Values for processed products based on predicted cleavage sites (i.e., not N-terminal sequencing) are italicized.

*c* Product locations have been determined experimentally unless shown italicized, in which case the predicted location is listed.

*d* The *finO* gene of F is inactivated by an inserted IS3 element, and its complementation class is based on the phenotype of *finO* alleles from other F-like plasmids. The *finO* product characteristics are hypothetical values calculated after the IS3 sequence is removed (see text).

*e* Since *finP* encodes an antisense RNA molecule, its product length is in nucleotides.

*f* TraC is a cytoplasmic protein that fractionates with the inner membrane in the presence of other *tra* products (see the text).

*g* In addition to the products shown, *traG* and *traI* have been found to encode the smaller products TraG* and TraI*, respectively (see the text).
FIGURE 4  Cellular localization of F tra region products. A diagram of the E. coli inner (IM) and outer (OM) membranes is shown. Schematic representations of tra region products are superimposed on the diagram to indicate the probable cellular locations of the products; relative sizes are only approximated (see also Tables 1 and 2). Readers are referred to the text for a detailed commentary on the role and localization of most products. The lipoproteins, TraT and TraV, are shown with N-terminal tails linking them to lipid moieties in the outer membrane. The antisense RNA product of finP is also shown. The product of the finO gene is shown even though F does not express this product, since it can be provided in trans by other F-like plasmids. An arrow indicates the transition of F pilin from inner membrane protein to pilus constituent. The broad classes of function(s) associated with particular Tra (capital) and Trb (lowercase) products are indicated with color: blue, pilus biogenesis; red, surface exclusion; magenta, mating-aggregate stabilization; green, regulation; yellow, conjugal-DNA metabolism; black, unknown/nonessential. The figure is adapted from reference 103.
Expression of the \textit{traX} gene is required for N$\alpha$-acyetylation of the F-pilin polypeptide (252). The predicted product of the \textit{traX} ORF is 248 aa in length and appears to be a polytopic inner membrane protein (59). In vivo and in vitro analyses have detected two \textit{traX} products, TraX1 (24 kDa) and TraX2 (22 kDa), which associate with the inner membrane (222, 223a) but appear significantly smaller than the 27.5 kDa calculated from the sequence (59). Although both products seem to be translated from the \textit{traX} ORF, there may be more than one translation initiation site. \textit{traX} codons 29 to 225 encode a region essential to pilin acetylation activity (Maneewannakul et al., unpublished). N$\alpha$-acyetylation of pilin is a property common to all of the F-like systems characterized thus far (87, 104, 365, 366) but appears not to be essential for F-pilus biogenesis or function. Under typical laboratory conditions, an F-\textit{traX} mutant was found to be phenotypically normal for both conjugal DNA transfer and phage sensitivity (252). However, the antibody-binding characteristics of pili elaborated in the absence of TraX differ from those of wild-type pili (126, 127, 252).

Amino acid sequence similarity has been detected between TraX, TrbP of the IncP\(\alpha\) plasmid RP4 (282), the product of the \textit{Dichelobacter nodosus} (formerly \textit{Bacteroides nodosus}) \textit{fimC} gene (145), and the deduced product of an ORF from the filamentous single-stranded DNA phage Cf1c of \textit{Xanthomonas campestris} pv, citi (94a, 197). Paralleling what has been observed for \textit{traX}, two polypeptides have been associated with expression of \textit{fimC}, which is located immediately downstream of the major fimbrial subunit gene, \textit{fimA}, in some \textit{D. nodosus} serotypes (145).

Attempts to confirm the suggestion that F pilin might be phosphorylated or glycosylated (42, 65, 144) have failed to identify any such covalent modification (22, 101). However, it has recently been shown that a minor proportion of pilin subunits, within both an inner membrane pool and assembled pili, do bear an uncharacterized modification which causes them to migrate on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slightly more slowly than the majority of subunits (223, 252). This modification, which is presumed to be performed by host-encoded products, appears to occur after signal peptide cleavage and does not interfere with, but is inhibited by, N$\alpha$-acyetylation (223). Both forms of F pilin were found to exhibit considerable stability in vivo (223).

![Figure 5](image_url) **Figure 5** Amino acid sequences of the F-propilin leader peptide (A) and the F-pilin subunit (B) (106). A bar indicates the typical signal sequence within the leader peptide; hydrophobic residues proposed to form membrane-spanning regions of the signal and of membrane pilin subunits are underlined. The nature of the amino acid side chain is indicated by color shading (red, basic; blue, acidic; green, polar, uncharged; yellow, hydrophobic). Amino acid substitutions resulting from \textit{traX} missense mutations (105) are indicated above the \(F\) sequences; those affecting the propilin leader were detected in pilus-deficient \textit{Flac} mutants, whereas those affecting subunit structure were detected in \textit{Flac} mutants exhibiting increased resistance to pilus-specific RNA phages. Below the \(F\) sequences, only the residues found to differ in the products encoded by the \textit{traA} alleles of F-like plasmids (84, 102) are shown. The alignment is as presented by Frost et al. (193) and includes small gaps, introduced to maximize similarity and accommodate differences in product size.
Topology of the F-Pilin Subunit

Under normal circumstances, the majority of pilin subunits in an F+ cell seem to be associated with the inner membrane rather than with assembled pili (127, 254, 255, 323). The topology of F pilin in the inner membrane has been analyzed by traA-′phoA gene fusions, which indicated that the protein contains two transmembrane segments and is oriented such that its N- and C-terminal residues are located in the periplasm (278). Immunogold labeling of spheroplasts has suggested that F-pilin subunits cluster at discrete locations in the inner membrane (278).

The sequences of F-traA mutations affecting phage sensitivity and of the traA genes carried by F-like conjugative plasmids (Fig. 5) have provided insights into the topology of assembled pilin and specificities associated with these filaments (100, 102, 103, 286). Both the long propilin signal sequence and the structure of pilin seem to be highly conserved among F-like plasmids (Fig. 5). Among F-like plasmids, differences in the N-terminal sequence of the mature pilin polypeptide alter the dominant epitope and confer plasmid specificities. However, this region of the protein, although exposed in membrane pilin and the basal knobs found on free pili, appears to be masked in the assembled filament except possibly at the tip (104, 278, 365). Residues at or near the pilin C terminus and in a region including mature pilin residues 12 to 22 (Fig. 5) appear to be important to RNA-phage binding and have been suggested to be available on the outside of the pilus (100, 105). Comparison of the rather different deduced pilin sequences of F and pED208 (IncFV), which both confer bacteriophage f1 sensitivity, has suggested that the binding site of these filamentous DNA phages could involve residues in the region of greatest identity (F-pilin residues 16 to 24 [Fig. 5]) (84). Another suggestion, however, is that residues closer to the F-pilin N terminus (near M9) are important to f1 binding at the pilus tip (100); antibody-binding studies indicate that both an F-pilin epitope including these residues and the N-terminal epitope of the pED208 pilin sequence are masked along the length of F pili but may be exposed at the end of the filament (104, 365). F-pilin residues K46 to K49 have been suggested to be located in the cytoplasm for membrane pilin and in the lumen of the assembled filament (278).

F-Pilus Assembly

Exhaustive genetic studies have demonstrated that the majority of genes in the F-tra region are involved in pilus assembly (Fig. 3 and 4). As discussed above, the propilin gene, traA, and the traQ and traX products involved in its maturation, accomplish the synthesis of membrane F pilin. However, mutations in traL, traE, traK, traB, traV, traC, traW, traU, traF, traH, traG, trbC, or trbI have each also been shown to have effects on piliation-associated phenotypes (13, 226, 227, 243, 252, 362). Since such mutations allow the accumulation of membrane F pilin (252, 255), the products of these genes are presumed to be involved in the assembly of subunits into the pilus filament. The known or predicted characteristics of these proteins are summarized in Table 1. Although little is known concerning their specific functions, each of these proteins appears to be associated with the cell envelope, lending credence to the notion that they constitute a pilus assembly complex (162, 200, 359). As Fig. 4 indicates, subcellular localization studies suggest that this complex could connect the inner and outer membranes of the cell.

The products of traW, traU, traF, and trbC possess typical N-terminal peptidase I signal sequences, and their cleavage and translocation to the periplasm have been experimentally demonstrated (Table 1) (226, 227, 253, 368). TraK and TraH are likewise thought to be periplasmically located, as the deduced amino acid sequence of each contains an identifiable N-terminal peptidase I signal peptide (132, 287). Globomycin inhibition of signal peptidase II processing has demonstrated that traV encodes a lipoprotein, as suggested by the characteristic signal peptide evident at the N terminus of the deduced TraV sequence (76). Lipid modification of TraV is presumed to account for the larger than predicted sizes of mature TraV and its precursor (16.6 and 18.6 kDa, respectively [Table 1]) calculated from their migration upon SDS-PAGE (20 and 21.5 kDa, respectively) (76, 257). As a lipoprotein, mature TraV is likely to be tethered to the outer membrane via covalent lipid modification and, as such, may represent the only tra product required for pilus biogenesis to be so located (Fig. 4) (76).
Cellular fractionation studies and/or hydropathy analyses of the additional six products encoded by \textit{traB}, \textit{traC}, \textit{traE}, \textit{traG}, \textit{traL}, and \textit{trbI} suggest that with the exception of TraC, these are integral inner membrane proteins (Fig. 4; Table 1) (103). Whereas TraC appeared to be localized in the cytoplasm when synthesized in isolation, Schandel et al. (306, 307) found that it fractionated with the inner membrane in the presence of other \textit{tra} region products. It is therefore presumed that TraC normally associates with one or more of the \textit{tra} inner membrane proteins (307), although its specific interactions have not been identified. Type A ATP/GTP-binding-site motifs which may be important to the energetics of pilus assembly have been identified in the deduced amino acid sequence of TraC and in that of one periplasmic assembly protein, TraH (103). Although the significance of these sequences is yet to be established experimentally, it should be noted that the agrobacterial T-DNA transfer protein, VirB4, which shares amino acid sequence similarity with TraC, has been shown to exhibit ATPase activity (313).

Whereas mutations in most of the genes of the pilus assembly class typically result in donor cells that lack pili and are completely resistant to pilus-specific phages and transfer deficient, some cause different phenotypes which may provide functional insights. Although all \textit{traC} mutations appear to abolish F-pilus outgrowth, the \textit{traC1044} missense mutation was found to cause only partial defects in mating-aggregate formation and filamentous single-stranded DNA phage infection, suggesting that at least the pilus tip is exposed in such a mutant (305, 306). Deletion/insertion mutations which eliminate expression of the periplasmic protein TrbC have been found to exhibit a similar phenotype in that significant \textit{f1} sensitivity is retained but F-pilus filaments are not detected (226). Donor cells unable to express \textit{traU} were found to synthesize reduced numbers of otherwise apparently normal F pili (253). Mutation of \textit{trbI} also allowed pilus production, and such mutations also had no discernible effect on DNA transfer efficiency but were found to alter male-specific phage sensitivities, indicative of altered pilus function; overproduction of TrbI was found to cause the same effect (227). Some \textit{trbI} mutants also synthesize unusually long pili (227).

The \textit{traG} gene is unique in that mutations in this gene fall into either of two phenotypic classes. Whereas all \textit{traG} mutations result in transfer deficiency, only the N-terminal region of the gene product is essential to pilus biogenesis. Mutation or deletion of the C-terminal region does not affect piliation (11–13, 160), and the \textit{traG} function essential to filament outgrowth can be expressed from a \textit{SmaI} fragment carrying only \textit{traG} codons 1 to 534 (94). The N-terminal region relevant to piliation is suggested to include a large periplasmic domain, anchored in the inner membrane by the surrounding membrane-spanning regions (94). Transfer deficiency resulting from mutation of the C-terminal portion of the \textit{traG} product is believed to reflect a defect in mating-aggregate stabilization (230; also see below). Present at 500 to 600 copies per F\textsuperscript{+} cell (230), TraG is therefore believed to be bifunctional (Fig. 3 and 4) (11, 13).

Electron microscopy of thin sections has indicated that F-pilus outgrowth occurs at regions of adhesion between inner and outer membranes (27). Although the existence and nature of such adhesion zones (also known as Bayer junctions) remain controversial (28, 181), the envelope locations associated with pilus assembly proteins (Fig. 4; Table 1) suggest that they could interact with each other and with pilin to create such connections, forming a site for filament extension and perhaps also a route for DNA.

Assembly of the filament is presumed to be energy dependent, since respiratory poisons, such as cyanide and arsenate, result in pilus retraction (265, 266). As suggested above, the ATP-binding sites on assembly proteins, TraC and TraH, could be important to this process. Although the possibility that F-pilin subunits add to the tip of the filament has been raised (315), there are data demonstrating that the thick, flexible conjugative pili of an IncH plasmid lengthen by subunit addition at the base (220), suggesting that F pilin may also be polymerized in this way. The analyses by Sowa et al. (323) suggested that F-pilin monomers in the membrane pool can be transiently and reversibly assembled into F pili. These authors proposed a model in which pilin is conserved in an inner membrane pool and recycled via pilus outgrowth and subsequent retraction (323). Filament assembly can be viewed as a transport process, engaged in the secretion and uptake of pilin, that is, as an energy-dependent “pump” which can move pilin from the inner membrane through the periplasm, excrete it as a polymer, and take it up again. Another view is to consider pilin assembly proteins to form a filament-organizing center...
and, like proteins that mediate polymerization of actin and tubulin, to organize, activate, and deactivate the polymerization of pilin. There is evidence suggesting that filaments may also be involved in other types of macromolecular transport in bacteria (292). The similarities detected among proteins involved in conjugation, as well as their possible implications, are discussed at the end of this chapter.

**MATING-AGGREGATE STABILIZATION**

Cells carrying F derivatives bearing mutations in the promoter-distal portion of traG or within traN elaborate apparently normal pili. However, they fail to transfer DNA (13, 230, 243), even though conjugal DNA metabolism can be initiated (183). This, together with the observation that such mutants form aggregates with recipient cells inefficiently, has led to the assignment of these genes to the mating-aggregate stabilization stage of the conjugal process (Fig. 3 and 4) (230). This stage, classically defined by the mating-aggregate phenotype of recipient ompA mutants (230), is believed to represent the conversion of initial unstable contacts between donor and recipient cells to a form which is resistant to disruption by shear forces (137, 138, 230). However, unlike transfer to recipient ompA mutants, transfer from traG donors shows no increase in efficiency when matings are conducted on a solid surface, and a traN donor defect is suppressed only to a very limited extent under such conditions (230). The inability of piliated traG donor cells to form stable mating aggregates was found not to be due to a defect in pilus retraction (265).

The traN product was found to be a protein which is expressed as a precursor, undergoes signal sequence processing, and fractionates with the outer membrane in its mature form (Table 1). This gene encodes a 65.7-kDa, 602-residue polypeptide, with signal peptidase I cleavage predicted to remove 18 aa and to yield a 63.8-kDa product (224). Protease susceptibility experiments have also demonstrated that a portion of TraN is exposed extracellularly, raising the possibility that this protein interacts directly with a surface component of the recipient cell envelope (224). A large segment of the polypeptide is, however, resistant to external proteolytic digestion and may include a periplasmic domain(s). The deduced TraN amino acid sequence contains a type A ATP/GTP-binding site motif, although the significance of this site is unknown (103, 224). Thus far, TraN function has been defined by phenotypic characterization of only one traN mutant plasmid, in which an amber mutation truncates the product at residue 130 (224).

As indicated above, traG appears to be bifunctional, with sequences at the 5′ end of the gene being essential to piliation and those at the 3′ end of the gene being dispensable to the pilus assembly function but essential for its role in aggregate stabilization (Fig. 3 and 4; Table 1) (11, 13, 230). On the basis of protease susceptibility experiments and DNA sequence-based structural predictions, Firth and Skurray (94) have suggested a topology in which TraG is divided into two large periplasmic domains involved in performance of its dual roles: that nearest the N terminus is sufficient for the TraG pilus assembly function, and the second, composed of approximately half of the polypeptide length and including the C terminus, is additionally or separately required for stabilization. The detection of TraG* (Fig. 4), a 50-kDa periplasmic protein reactive with antibody raised against TraG C-terminal region sequences and suggested to be released from TraG by proteolytic cleavage, has raised the possibility that this portion of TraG functions in stabilization as an independent protein (94). Whether the N-terminal domain of TraG is also necessary to aggregate stabilization and whether TraG* release is influenced by early contact stage events and/or is necessary for aggregate stabilization is not yet clear. However, the phenotypes of traN and traG mutants, together with the envelope positions of TraN and TraG/TraG*, suggest that an interaction between periplasmic domains of these proteins might be needed to form a stable and functional connection between conjugating cells. Such an interaction may be associated with the formation of electron-dense conjugation junctions observed in thin sections (Fig. 2) (78).
Once mating contacts are made, the F-plasmid DNA undergoes processing and replicative events (Fig. 2) that result in the establishment of complete plasmid copies in both donor and recipient cells. Nicking of one DNA strand occurs as a prelude to these events; that strand is displaced and enters the recipient cell in a 5' → 3' direction (152). The dual nicking and DNA-unwinding activities demonstrated for F TraI indicate that its role is central to these events. However, there is evidence that the products of the F-traY, -traM, and -traD genes are also involved in the oriT nicking, strand displacement, and DNA transfer events that occur during conjugation (Table 1) (81, 183). As indicated above, synthesis of a replacement for the transferred strand (donor conjugal DNA synthesis) and generation of the complementary strand in the recipient (recipient conjugal DNA synthesis) are thought to occur concomitantly with conjugative DNA transfer (183). Since these processes appear to be undertaken by host-encoded activities and the transmission of DNA can apparently occur even if such replication is inhibited (183, 304, 350), they will not be discussed in detail here. Wilkins and Lanka (349) have recently reviewed the subject in depth.

Originally identified as a cis-acting site required for DNA transfer (361), the oriT region contains the site at which the single strand of DNA to be transferred is nicked and from which this strand is unwound and transported into the recipient. Sequences in the oriT region are also important to the recircularization of the transferred strand and termination of transfer of F (111) and other conjugative plasmids (80, 182, 202). For convenience, the oriT region has traditionally been defined as the segment between the BglII site at 66.6F and the traM gene (Fig. 3 and 6) (329), although deletion mapping has demonstrated that the minimal region required for oriT activity in cis is somewhat smaller than this (107). The nucleotide sequences of the oriT regions of eight F-like plasmids have been determined, namely, F (IncFI) (329), P307 (IncFI) (117, 123), R1 (IncFII) (273), R100 (IncFII) (240), ColB4-K98 (IncFIII) (86), pSU316 (IncFIII) (218), pED208 (IncFV) (72), and pSU233 (IncFVII) (302). The DNA segment between the BglII site at 66.6F and the AC-rich region that bounds the leftward limit of the minimal oriT region contains the promoters and 5' end of a leading region cistron known variously as orf169 or gene X (Fig. 6) (214, 329) and gene 19 on R1 (122).

Considerable functional specificity is associated with the conjugal DNA metabolism systems of F-like plasmids (357). The observed plasmid specificity of TraI, TraM, and TraY is presumed to result from DNA sequence differences within the various oriT regions at which these proteins act and corresponding variation in the sequences of the proteins. A component of such plasmid specificity may also reflect the interaction of the proteins themselves (357). Plasmid oriT regions typically contain a number of inverted and direct repeats; while some of these are recognized by proteins such as TraM (73), the functional significance of others is yet to be determined. The presence of intrinsic bends and binding sites for DNA-bending proteins indicates that the F-oriT region has a characteristic shape (219, 336). This notion is supported by correlations between the spacing and phase of the various oriT features (107). Notwithstanding the nucleotide sequence differences evident among the F-like oriT regions sequenced, a common structural and functional organization is apparent (Fig. 6).

**Nicking at oriT**

The site of nicking, which has been determined for plasmids F and R100 (158, 234, 330), falls within a region exhibiting a high degree of sequence conservation that extends at least 40 nucleotides to the left of the nick site and approximately 100 nucleotides to the right. The nick site is centered in and on the complementary strand to an AC-rich segment of approximately 40 nucleotides. The leftward limit of the F-oriT region is defined by the extent of this AC-rich region, as deletions into it from that side abolish nicking (107). The remainder of the conserved segment to the right of the nick site has a high A+T content and contains an intrinsic bend, a sequence bound by integration host factor (IHF), and a site (sbyA) to which the traY product binds (199, 219, 336); IHF- and TraY-binding sites in the R100-oriT region have
also been identified (154, 156). Removal of F sequences from the right has shown that only the left half of the sbyA TraY-binding sequence is essential for oriT nicking (107, 199). It was recently shown that bound TraY induces bending and that this protein can bind to additional sequences to the left of sbyA; the site, sbyC, overlaps the IHF-binding sequence (219).

The traI genes of plasmids F and R100 both encode a 1,756-aa polypeptide (40, 370); type A ATP/GTP-binding motifs occur in both the N-terminal and C-terminal regions of the deduced protein sequences (40). Present at about 600 copies per F\textsuperscript{+} cell (186), the cytoplasmically located 192-kDa F protein, TraI, corresponds to the well-characterized enzyme \textit{E. coli} DNA helicase I (2) and exhibits the 5\textsuperscript{′} → 3\textsuperscript{′} DNA unwinding activity appropriate for displacement of the transferred strand (1, 199). Assays with purified components have now demonstrated that the TraI protein of both F and R100 is responsible for introducing the single-strand break at oriT (158, 234, 297). A phosphodiester transferase, TraI becomes covalently linked to the 5\textsuperscript{′} terminus of the nicked DNA strand (Fig. 2 and 6); no such linkage to the 3\textsuperscript{′} end of the nick has been detected (155, 235, 297). F-oriT-nicking function has been shown to depend on the N-terminal sequences of TraI, some of which are dispensable to the unwinding function of the protein (335). Amino acid sequence similarity has been detected between this region of TraI and the TrwC, TraI, and VirD2 proteins, which are DNA-nicking proteins encoded by R388, RP4, and agrobacterial Ti plasmids, respectively (213, 282, 283; also see below); these proteins all belong to a superfamily of DNA-nicking enzymes (153, 189, 213). TraI*, an 88-kDa protein, originally known as 2b and then known as TraZ, was previously thought to derive from a genetically undefined cistron assigned the name traZ, and once suggested to function in oriT nicking (7, 81). However, this product appears to derive from a second translational start site within the traI reading frame and to lack the N-terminal TraI domains essential to nicking (334, 335). Thus, the significance of TraI* is unknown.

No F- or R100-encoded proteins other than TraI are essential to the oriT-nicking reaction in vitro (155, 234, 297). Although no F-TraY protein effect on the kinetics of the F-TraI-mediated in vitro reaction was detected (261), recent studies with purified R100 components have found the R100-nicking reaction to be stimulated by R100-TraY protein and IHF (155, 158). Other assays indicate that there is also an accessory requirement for F TraY in the in vivo reaction of TraI with oriT.
Expression of both *traI* and *traY* was necessary for the original observation of nicking in vivo (81) and for detection of recombination events stimulated by F *oriT* (48). In addition, the purified F- and R100-*traY* products have both been shown to bind to *oriT* region sites near the site of nicking (Fig. 6) (156, 199, 219, 261), and the major sequence determinants defined for TraY binding within the F site, *sbyA*, have been shown to be required for efficient nicking in vivo (111, 219). That bound TraY has been demonstrated to induce DNA bending at this sequence (219) indicates that TraY can affect *oriT* complex conformation.

The F- and R100-TraY polypeptides are cytoplasmic proteins of 15.2 and 8.5 kDa, respectively. Comparison of the deduced amino acid sequence of F TraY (95, 157) with itself and with alleles encoded by other IncFI (P307) (123), IncFII (R1 and R100) (85, 157, 193), IncFIII (ColB4-K98) (86), and IncFV (pED208) (84) plasmids has revealed that F *traY* has resulted from a gene duplication event (157); F TraY is 131 aa in length, whereas the products of other alleles range in size from 71 to 77 aa for the P307 and pED208 genes, respectively. Suggestive of a degree of regulation at the translational level, all of the *traY* genes sequenced thus far have been found to initiate translation at a GUG or UUG triplet rather than at the more frequently utilized AUG start codon (157).

Profile analysis has revealed that TraY is a member of a class of DNA-binding proteins that includes the Mnt and Arc repressors of phage P22 and the *E. coli* methionine repressor, MetJ (35, 41). Structural analyses of Arc and MetJ have indicated that two dimers of these proteins bind at their cognate operator sequences by using an N-terminal anti-parallel β-sheet structure (41, 322). The duplicate nature of F TraY led to the suggestion that dimerization might not be required for binding (261). Consistent with this notion, F TraY was found to exist as a monomer in solution (261), and binding data indicate that two F-TraY monomers bind separately to *sbyA* (219). The structural relationship to transcription repressors and the demonstration of TraY binding in the vicinity of the *P* *traY* promoter on both F (Fig. 3) and R100, albeit at lower affinity than at the *oriT* region site, *sby*, have raised the possibility that this protein negatively regulated its own transcription and hence that of most tra region genes (156, 261).

Nicking at *oriT* represents the initial step in the processing of plasmid DNA during conjugation, but there is no evidence to suggest that this is the decisive event precipitated by recognition of a mating contact. By definition, the *oriT* nicking demonstrated in vitro occurred in the absence of any signal generated by mating-pair formation. Likewise, a proportion of nicked molecules have been isolated from donors in the absence of recipients and from cells carrying mutations in tra genes required for the formation of mating aggregates (81). Conceivably, conditions used to detect nicked DNA could sidestep a signal requirement. However, the data imply an equilibrium of open-circular and covalently closed circular plasmid DNA in donor cell populations; thus, it was suggested that the signal may precipitate subsequent steps in conjugative DNA metabolism by triggering the initiation of DNA unwinding (81).

**DNA Strand Displacement and Transfer**

The existence of a signal was deduced from the finding that replacement strand synthesis occurred only in donors which could elaborate F pili and which had been mixed with recipient cells (183). Such synthesis, which should reflect the progression of unwinding, was found also to depend on *traM* and to a lesser extent on *traD*, as well as on *traI* (183). The *traM* gene is dispensable to pilus assembly and aggregate stabilization, as well as to *oriT* nicking per se. As TraM was necessary for DNA transfer and accompanying donor strand replacement synthesis, it was suggested that TraM might transduce the signal that commits the *oriT* nicking machinery to unwind the DNA (81, 360). Alternatively, TraM may be required to anchor the *oriT* region to a transfer apparatus in the inner membrane (3) or to alter conformational properties of the DNA (103). These possibilities, which are not mutually exclusive, are consistent with the finding that a proportion of the otherwise cytoplasmically located products of the F-, R100- and pED208-*traM* genes is detected in inner membrane preparations; such a fractionation pattern is suggestive of an interaction with an intrinsic inner membrane protein (3, 72, 73).

Although the deduced 127-aa product of F-*traM* has a predicted size of 14.5 kDa (328), TraM migrates as a 10-kDa protein upon SDS-PAGE (73). In the form of a tetramer, TraM has been found to bind to three sites within the *oriT* region (Fig. 6) (73). The two binding sites closest to the F-*traM* gene
overlap the two \textit{traM} promoter sequences identified by transcript analysis, and expression of this F gene has been shown to be subject to negative autoregulation (S. S. Penfold and L. S. Frost, unpublished data). It appears that the presence of \textit{TraY} protein may determine which \textit{TraM}-binding sites can be occupied; expression of both \textit{TraY} and IHF contributed to maximal \textit{traM} product expression (Penfold and Frost, unpublished). The \textit{traM} products of R1, R100, and pED208 also bind to multiple sites within their respective \textit{oriT} regions (3, 72, 309), and autoregulation of the R1- and R100-\textit{traM} alleles has been demonstrated (4, 310). \textit{TraM} binding has also been suggested to regulate transcription of a promoter for gene 19 on plasmid R1 (192). As a mutant \textit{traM} product that lacks the eight C-terminal amino acid residues of the wild-type protein has been shown to form tetramers but to be unable to repress \textit{traM} or to bind to DNA, a role for the C-terminal region of the protein in DNA binding has been suggested (Penfold and Frost, unpublished). Other studies suggest that amino acid residues in the N-terminal region of \textit{TraM} may also be critical to DNA-binding activity (310).

Whereas the minimal \textit{oriT} region required for nicking is the approximately 100-nucleotide segment delimited by the leftward boundary of the AC-rich region and the first half of the \textit{TraY}-binding region, additional rightward sequences, including \textit{TraM}-binding sites, are required for efficient transfer (Fig. 6) (107, 111). The segment between the AT-rich region and the \textit{traM} gene shows considerable sequence divergence among the characterized F-like plasmids and, in addition to sites that bind \textit{TraM}, includes another intrinsic bend and a second IHF-binding site (73, 154, 156, 336). DNA sequence deletions extending from the right into this region result in decreasing transfer efficiencies as the length of the deletion increases; those affecting the left half of the \textit{TraM}-binding site closest to \textit{oriT} completely abolish transfer (73, 107).

The characteristics of F \textit{TraI} that contribute to strand displacement became apparent with the discovery that this protein corresponded to DNA helicase I (2), which exhibits ATP-dependent 5$'$ → 3$'$ DNA-unwinding activity (1, 64). An amino acid sequence motif characteristic of ATP-dependent helicases is present in the C-terminal region of the deduced protein sequence (370). Reports relating to the number of \textit{TraI} molecules required for maximal strand-unwinding activity have been conflicting; estimates have ranged from 5 to 90 per aggregate, and even a DNA/protein ratio of 1:1 has been found to result in significant unwinding (29, 64, 195, 198, 348). \textit{TraI} has been estimated to unwind DNA at a rate of approximately 1,200 bp/s (195). The ATP-dependent 5$'$ → 3$'$ helicase activity of \textit{TraI} has led to the further suggestion that \textit{TraI} might also energize the transmission of the DNA strand during conjugation (315, 360). Such models assume that \textit{TraI} is in some way immobilized, perhaps in interaction with a \textit{tra}-encoded or chromosomally encoded protein associated with the inner membrane (Fig. 2) (315); it has been suggested that \textit{TraD} might provide such an association (64). In the absence of \textit{TraI}-mediated unwinding activity but not nicking activity, some other helicase can apparently substitute, albeit at a lower transfer efficiency (335).

The \textit{F-\textit{traD}} gene encodes an 81.7-kDa inner membrane protein (280). The gene sequence indicates that \textit{TraD} is 717 aa in length (40, 173). The deduced R100-\textit{TraD} amino acid sequence is homologous to that of F but contains in its C terminus the three-residue sequence Gln-Gln-Pro reiterated 10 times instead of the single occurrence in F \textit{TraD} (370). Although \textit{traD} mutants elaborate apparently normal pili, form mating aggregates with recipients, and trigger conjugal DNA metabolism, they fail to transfer DNA (183, 279, 354). Furthermore, it appears that functional \textit{TraD} is required only after mating-aggregate formation (279). These characteristics have led to the suggestion that \textit{TraD} is involved in the transportation of single-stranded DNA across the cell envelope into the recipient (183). Several observations support the notion that \textit{TraD} plays a role in transmembrane conveyance of nucleic acid. First, RNA phages, such as MS2, are able to adsorb to the pili of \textit{traD} mutants but infection is aborted because of a defect in RNA penetration (284, 308). Second, \textit{traD} is required for mobilization of the plasmid \textit{ColE1} and related plasmids, unlike the other DNA metabolism genes \textit{traM}, \textit{traI}, and probably \textit{traY} (355). Finally, purified \textit{TraD} has been found to bind DNA in a nonspecific manner (280).

Recently, amino acid sequence similarity has been detected between \textit{TraD} and polypeptides encoded by transfer systems previously thought to be distinct from that of F, namely, \textit{TraG} of RP4, \textit{TrwB} of R388, and \textit{VirD4} of agrobacterial Ti plasmids (212, 213; also see below). Furthermore, it is likely that similar proteins are also involved in gram-positive DNA transfer mechanisms, because a member of this
protein family is encoded by the transfer region of conjugative staphylococcal plasmids (93, 258). Purified TraD has been shown to possess DNA-dependent ATPase activity (280). Consistent with this observation, the deduced F- and R100-TraD amino acid sequences contain both type A (370) and type B (212) nucleoside triphosphate-binding-site motifs. The type B motif is particularly well conserved in other members of the protein family to which TraD appears to belong (93, 212). It has been proposed that proteins of this family may link the conjugal DNA metabolism machinery to the DNA transport apparatus (202, 345).

Termination of F Transfer

The properties of TraI have suggested that this protein is also the mediator of transfer termination, and plasmids carrying two directly repeated oriT sites have been employed to test the DNA sequence requirements associated with this process (111, 202). Completion of transfer has been proposed to depend on recognition of the oriT site reconstituted by replacement strand synthesis, cleavage, and ligation of the newly generated 3′ end to the 5′ end of the transferred strand (Fig. 2) (202, 349). In support of this model, Gao et al. (111) have obtained data suggesting that termination can occur at F-oriT sequences which do not contain a preexisting nick. The sequence required for termination extends no more than 36 bases to the right of the nick site and includes phased poly(A) tracts which specify a sequence-determined bend (111). Since point mutations affecting the fourth and ninth base pairs to the right of the nick site affect both nicking and termination, a step involving TraI is suggested for both processes (111). Sequences preceding orf169 (Fig. 6) may also be involved in the termination step of conjugation, since sequence deletions in this region resulted in the transfer of plasmids of greater than unit length (107).

SURFACE EXCLUSION

The F-plasmid transfer region encodes two genes responsible for surface exclusion (Sfx). This property limits the host cell capacity to act as a recipient for the same or a closely related plasmid. The traS and traT genes (Fig. 3; Table 1) appear to be responsible for independent aspects of the phenomenon, acting in concert to reduce transfer efficiencies by several orders of magnitude (6, 8, 10). The expression of surface exclusion is probably a fundamental requisite of donor ability, since in its absence, donor cell populations would bear the metabolic cost of continuous and futile recipient-donor activity (54).

Five surface exclusion specificity classes, SfxI to SfxV, represented by F, ColB2-K98, R1, R100, and pED208, respectively, have been identified (89, 357). The two traS genes so far sequenced, those of F (172) and pED208 (89), encode products which differ markedly in primary sequence (103). This contrasts with the available data for the F (172), ColB2-K98 (325), R100 (268), and pED208 (89) traT alleles, in which only one or two amino acid differences appear to define the observed phenotypic specificities (135).

The traS gene encodes a 16.9-kDa inner membrane protein (Fig. 4), which, when present in the recipient, appears to prevent triggering of donor conjugal DNA metabolism (6, 172, 248). It has therefore been suggested that TraS blocks the transmission of a mating signal (230). Present at an estimated 20,000 to 30,000 copies per cell (6, 246), the 26-kDa lipoprotein product of the traT gene (172, 288) constitutes a major outer membrane component of F-containing cells (Fig. 4). Exposed on the cell surface (228), TraT is thought to span the outer membrane (325) in a multimeric form (135, 228, 245, 248). TraT appears to function by inhibiting the formation of mating aggregates (6). This, together with the observation that purified TraT could reduce transfer efficiency, led Minkley and Willetts (248) to propose that TraT may interact with the tip of the sex pilus, perhaps competing with the normal cellular receptor. However, the specificity between traT alleles and the corresponding transfer system that it inhibits has recently been found not to be associated with the cognate pilin sequence (20). It has also been suggested that TraT may exert its effect by masking a region of OmpA (298).

There is circumstantial evidence that the traT gene product may play a role in bacterial pathogenesis in addition to its surface exclusion function (for a review, see reference 325). Several studies have
demonstrated that at least in some hosts, TraT may contribute to bacterial serum complement resistance (250, 267, 268). It is suspected that TraT inhibits the functioning of complement membrane attack complex (291, 333). Reduced susceptibility to phagocytosis has also been attributed to the presence of TraT (14). Although surveys of clinical isolates have failed to establish an unambiguous link between traT and pathogenesis (34, 179, 251), the identification of traT homologs on nonconjugative virulence-associated Salmonella and Yersinia plasmids has lent credence to the proposition that the product of this gene may act as a virulence factor (53, 325).

TRANSFER GENE EXPRESSION

Transcription of the tra Region and Its Regulation

Transcriptional promoters have been identified preceding the F-plasmid traM, traJ, traY, trbF, traS, traT, and traD genes (Fig. 3) (96, 132, 328). In addition, two promoters, PfinP and PartA, have been found to initiate transcription in the opposite direction to that of the majority of the tra region genes (Fig. 3) (260, 367). The picture of tra region transcription that is emerging, while not complete, is consistent with a model in which PtraY is responsible for the initiation of a polycistronic mRNA that may encode 34 genes, from traY through finO, inclusively. This would represent an operon of approximately 32 kb. Although the 3'-extremity of the transcript originating from PtraY has not been precisely determined, recent analyses which have indicated that this promoter is required for normal levels of expression of the distal genes, traD and traI, support this notion (222). The promoters PtraB, PtraS, PtraT, and PtraD may, in combination with a proposed transcription terminator identified after the traT gene (131), serve to modulate and/or differentially regulate expression of distal tra operon genes. The expression of traT, in particular, appears to be somewhat independent of regulatory factors affecting transcription from PtraY (51, 294). From the accumulated data, the likely transcriptional units evident in the tra region are summarized in Fig. 3. Analysis of transcripts encoding traK has suggested that the unusual polarity of the amber mutation traK4 is due to the presence of a rho-dependent transcription termination element (287). Computer analysis suggests that such rho-dependent termination sequences are positioned at a number of sites within the tra region (287); transcription is therefore likely to be influenced by translational efficiency.

Two promoters located between the oriT nick site and the traM gene, which apparently direct the transcription of gene 19, have recently been identified on R1 (192). These promoters are within the conserved segment of the oriT region (Fig. 6) and are in addition to tandem promoters located immediately upstream of orf169 and gene 19 of F and R1, respectively (192, 214).

PtraY appears to be regulated, either directly or indirectly, by several plasmid-encoded and chromosomally encoded proteins. The traJ gene (Fig. 3; Table 1) encodes a 27-kDa cytoplasmic product (62) that positively regulates transcription originating at the tra operon promoter, PtraY (109, 260, 318, 319, 351). A 229-aa protein (95), TraJ is estimated to be present at approximately 2,000 copies per F" cell (62). The F-, P307-, R1-, R100-, and pED208-encoded traJ products are quite distinct, the greatest degree of similarity being at their N termini (72, 85, 95, 123, 157), a region that has been predicted to contain a helix-turn-helix DNA-binding domain (74, 285). Zone sedimentation data indicate that TraJ may be dimeric (62).

PtraY has been shown to be utilized in vitro by the E. coli σ70 RNA polymerase (115). However, RNA polymerase was found to form a stable complex at PtraY only when the promoter sequence was in a supercoiled conformation (115). From this finding and the observed involvement of upstream sequences in the down-regulation of PtraY in the absence of TraJ (319), Gaudin and Silverman (115) have suggested that in the absence of a transcriptional activator, the sequences preceding PtraY contribute to local relaxation of the promoter region. In such a model, TraJ may invoke transcription from PtraY by facilitating the restoration of normal superhelicity (115). Assays employing galK transcriptional fusions have indicated that in the presence of TraJ, PtraY is a powerful promoter (260).

Purified TraY derived from both F and R100 has recently been found to bind to sequences overlapping the transcription initiation sites of their cognate PtraY promoters (156, 261). It is therefore likely that transcription of traY is autoregulated (156). If this is so, transcription of most tra genes is
subject to both positive and negative regulation mediated by *tra* region-encoded products, namely, TraJ and TraY, respectively.

TraJ has also been implicated in enhanced transcription from $P_{trbF}$, whereas $P_{traS}$, $P_{traF}$, and $P_{traD}$ appear to be *tra*-independent promoters (131, 172). Findings concerning the effect of TraJ on transcription of $traM$ have been conflicting. Analyses employing lacZ transcriptional fusions indicated that $P_{traM}$ was stimulated by TraJ (109), yet analogous experiments utilizing galK as a reporter gene revealed no such effect (260). Two initiation sites responsible for transcription of F *traM* (Penfold and Frost, unpublished) and for the *traM* alleles of plasmids R1 and R100 (4, 194) have been identified. Whereas binding of TraM affects both F-*traM* transcripts (Penfold and Frost, unpublished), only the more upstream of the R100-*traM* promoters is repressed by TraM binding; the second, weaker promoter appears to function constitutively (4). The R1-*traM* gene is also autoregulated (310).

**FinOP Fertility Inhibition**

In donor cells harboring most F-like plasmids, *tra* gene expression, and hence conjugative transfer itself, is repressed by a phenomenon known as fertility inhibition (*fin*) (90, 242). In combination, a small antisense RNA molecule, FinP, and a polypeptide encoded by the most distal *tra* gene, *finO*, inhibit the expression of the regulatory gene, *traJ* (Fig. 3) (99, 207, 351). The absence of TraJ, in turn, precludes transcription from the major transfer region promoter $P_{trbF}$ described above. Transfer of the F plasmid is derepressed as a result of insertional inactivation of the *finO* gene by the transposable element IS3 (Fig. 3) (52, 371). However, expression of a *finO* gene from a compatible coresident plasmid can repress F transfer in *trans* (92).

Approximately 78 nucleotides in length (339), the FinP RNA molecule is transcribed constitutively from a promoter, $P_{trbF}$, located within and in opposite orientation to the nontranslated leader of the *traJ* transcript (Fig. 3) (88, 260). Complementary base pairing between FinP and the *traJ* mRNA is thought to preclude translation of that mRNA into TraJ, because the duplex formed overlaps the translation initiation signals of *traJ* (83, 88). However, recent evidence indicates that RNase III-mediated cleavage of the duplex might be responsible for inactivation of *traJ* mRNA (339). FinP and the complementary region of the *traJ* transcript each fold into two stem-loop structures (339). Sequences within the secondary structure loops of the *traJ*/FinP RNA molecules appear to be critical to interactions between the RNA species (191) and appear to constitute the basis of the observed plasmid specificities (357).

The *finO* genes of F, ColB2, R6-5, and R100 have been sequenced and, after removal of intervening IS3 sequences and the target duplication from the F allele, found to code for highly homologous 186-aa products (59, 239, 337, 370, 371). The 21-kDa *finO* product is hydrophilic and therefore is presumed to reside in the cytoplasm, a location consistent with its regulatory role (Table 1) (332, 337, 371). FinO seems to exert its coregulatory effect on *traJ* expression by stabilizing the FinP antisense RNA (207). The FinO-mediated extension of FinP half-life, from $=2$ min to $>40$ min, occurs even in the absence of the complementary *traJ* messenger (207). This and the inability of FinO to protect FinP transcripts bearing a *fisO* mutation (91) have suggested that the FinO protein and FinP RNA might interact directly (99, 207). Recently, it was demonstrated that FinO is, in fact, an RNA-binding protein that interacts with one of the two stem-loops in FinP (Fig. 4) and with the complementary structure in the *traJ* mRNA (338). Allelic specificities attributed to the *finO* gene (357) have been found to result from differential expression by F-like plasmids (337).

The high-level fertility inhibition exhibited by plasmids such as R6-5 and R100 correlates with the presence on these plasmids of a gene known as *orfC* or *orf286*, located between *traI* and *finO* (59, 370). F-like plasmids such as ColB2 (and presumably the F progenitor) lack the sequences that encode *orf286* and express lower levels of fertility inhibition (59, 337, 357, 370). Cotranscription of *orf286* and *finO* leads to the synthesis of FinO at levels far in excess of that observed in the absence of *orf286* sequences and is believed to result from an increase in *finO* mRNA half-life (337). This enhancement of *finO* expression occurs only in cis, indicating that it is not mediated by the *orf286* translational product (337). Indeed, this stabilizing effect, resulting from cotranscription with the upstream gene, appears to be independent of *orf286* translation (337). Secondary structure in the mRNA as a result of base pairing
between sequences within orf286 and finO is thought to increase the resistance of the transcript to ribonucleolytic degradation (337).

A slightly different mechanism has been proposed for the regulation of the tra region of R100. Termed latch relay, this model is based on competition between transcripts initiating upstream of and within traM, and the untranslated traJ leader, for the antisense FinP molecules (69). Under such a scheme, the normal steady-state condition is “off”; any event that can affect this state may result in switching on of tra region transcription and hence transient derepression (70). Dempsey (68) has also described a second, upstream finP promoter on R100 and has identified a third potential stem-loop structure unique to the R100-FinP RNA molecule. A similar mechanism may operate on R1, since traM transcripts are also believed to regulate FinP activity expressed by this plasmid (194).

FinOP-mediated transfer repression is thought to reflect an evolutionary penalty associated with constitutive expression of conjugative functions. In addition to enduring the metabolic overhead associated with constitutive expression, host cells harboring derepressed F-like plasmids are vulnerable to infection by pilus-specific phages (141). Repressed plasmids are thought to escape such costs while maintaining transfer potential, because transient derepression in an individual donor may lead to “infectious spread” through a recipient population (43, 358). Such spread results from the high-frequency transfer exhibited by cells which have recently received the plasmid, because of the lag time required for synthesis of FinO and FinP to inhibitory levels and the ensuing dilution of TraJ and other tra products (337, 363). It has also been suggested that conjugative transfer may be required only to introduce a plasmid into a population, because any selected advantage conferred by that plasmid would facilitate its subsequent establishment via preferential host cell survival and growth (61).

Other Fertility Inhibition Systems

In addition to the normally endogenously encoded FinOP system, five other fertility inhibition systems have been identified on plasmids which, when coresident with F, reduce the efficiency of F transfer. These fertility inhibition systems also affect, to various degrees, the mating efficiency of other F-like plasmids (114). As well as the benefits associated with repression listed above, it has been suggested that plasmids encoding one of these trans-acting mechanisms may be able to compete more successfully for new hosts (109, 113). However, it is also possible that the repression caused by these systems is coincidental (162). The inhibition of the IncP plasmid RP4 transfer by F appears to represent an example of such an inadvertent interaction (326); the product of the autoregulated repC gene of F (also known as pifC), which regulates replication from oriV (184, 327), is thought to be responsible for this phenomenon (244).

The FinQ and FinW fertility inhibition systems are believed to act independently of traJ at the level of transcription (109, 112). FinQ is encoded by IncI1 (formerly IncIα) plasmids such as R62, R820a, TP102, and TP108 (109, 114). The finQ gene from R820a encodes a 40-kDa product, which is proposed to cause rho-independent termination of the transcript initiating at PtraY at several sites between traC and traD (109, 114, 133; L. M. Ham and R. A. Skurray, unpublished data). Encoded by the IncFI plasmid R455, FinW appears to act by reducing the transcription of traM (109, 113).

The FinC, FinU, and FinV fertility inhibition systems are thought to act posttranscriptionally. FinC is expressed by copy number mutants of the mobilizable bacteriocinogenic plasmid CloDF13, such as JN62 and JN77 (352). In addition to reducing the level of F transfer, FinC inhibition renders F-containing cells resistant to infection by RNA phage f2 but not to the filamentous DNA phage f1, a phenotype characteristic of F-traD mutants (352). FinC fertility inhibition results from overexpression of the CloDF13 mobA or rpi genes (262, 340). The product of mobA is a 58-kDa protein, MobB, involved in mobilization, whereas the only phenotype so far attributed to the 16-kDa rpi product is the FinC-mediated resistance to F-specific RNA phage infection described above (262). As transcription of traD was found to be unaffected by FinC, Willetts (352) suggested that this mechanism inhibits TraD. This contention has been strengthened by the recognition
of the CloDF13 MobB protein as a TraD homolog (26, 46; also see below). The presence of a gene encoding a TraD-like protein may also explain why \textit{tra}D is not required for mobilization of CloDF13 but is essential for transfer of the related plasmid ColE1, which encodes a different protein from an analogously located position in its genome (341, 352).

The basis of the FinU and FinV transfer inhibition systems, encoded by the plasmids JR66a (IncI1) and R485 (IncX), respectively, is unknown (113). FinU inhibits both pilus assembly and surface exclusion and was therefore suspected to affect \textit{tra} region transcription (113). Although the presence of JR66a was found to reduce transcription of distal \textit{F-tra} cistrons, the extent of the reduction was disproportional to the 7,000-fold transfer inhibition specified by the FinU system (109). On the basis of these findings, Gaffney et al. (109) suggested that although the effect on transcription may be responsible for the observed reduction in surface exclusion, the primary target of FinU inhibition was more likely to be the translation and/or function of one or more \textit{tra} genes. However, the synthesis of all 11 \textit{tra} products detected in the study was unaffected by FinU (109).

The FinV fertility inhibition system encoded by plasmid R485 inhibits \textit{F} piliation and hence transfer but does not reduce surface exclusion, indicating that an effect on \textit{tra} region transcription is unlikely (113). Subsequent transcriptional studies supported this contention (109). FinV is therefore thought to prevent the proper translation and/or function of one or more of the \textit{tra} products required for pilus biogenesis; Gaffney et al. (109) did in fact note a reduction in the amount of a 30-kDa protein synthesized in the presence of R485, although the origin of this polypeptide was unclear.

Environmental and Host Cell Factors Influencing F-Plasmid Transfer

In addition to host-encoded activities required for the DNA synthesis that accompanies transfer, a number of environmental and host-specified factors have been found to influence the donor ability of \textit{F}+ cells, reinforcing the notion that conjugation is a cellular process. \textit{F} pili are believed to retract when cultures are cooled below 25°C (265). Furthermore, the synthesis of the pilin subunit itself was found to diminish as the incubation temperature was lowered (265, 323). Parallel reductions in the synthesis of several other \textit{tra} products led Sowa et al. (323) to speculate that transcription of the \textit{tra} region may be regulated by temperature.

The early finding that \textit{F}− phenocopies of donor cells (i.e., \textit{F}+ or Hfr cell cultures with recipient ability) are produced by growth into late-stationary phase (205) suggests that expression of surface exclusion is affected by starvation or growth phase (6). Cultures of \textit{F}− phenocopies also failed to elaborate normal numbers of \textit{F} pili (42). It is therefore likely that growth into stationary phase has a general effect on \textit{tra} operon expression and may be associated with host-encoded cellular regulators, such as those discussed below (see also other chapters in this volume on growth and regulation of gene expression, e.g., chapter 93).

Intracellular levels of cyclic AMP (cAMP) have also been reported to influence the expression of transfer-related activities from \textit{F}-like plasmids, although the data available are very limited. Harwood and Meynell (136) found that addition of exogenous cAMP to \textit{cyt} mutants carrying various derepressed \textit{F}-like plasmids reduced piliation, although this effect was not evident in cells harboring \textit{F}. In contrast, the observed reduction in transfer-related properties expressed by \textit{cyt} and \textit{crp} mutant hosts led Kumar and Srivastava (196) to suggest that \textit{F-tra} expression is dependent on cAMP and its receptor protein. The identification of a sequence in the vicinity of \textit{P}_{\text{traJ}} with similarity to the binding-site motif of the \textit{crp} product, catabolite activator protein, lends credence to this proposal (285).

Several other chromosomal genes appear to play regulatory roles in the expression of \textit{F}-transfer functions. The products of \textit{cpxA} (mapping at 88.34 min on the \textit{E. coli} chromosome; also called \textit{ecfB}, \textit{ssd}, and \textit{eup}) (16, 17, 293) and \textit{sfrA} (99.95 min; alternatively known as \textit{arcA}, \textit{fex}, \textit{cpxC}, \textit{msp}, and \textit{dye}) (44, 165, 166, 208, 209, 314) represent transducer and effector homologs, respectively, of the two-component regulator protein families (18, 77, 346; for reviews, see references 125, 151, and 343). The SfrA+ phenotype is in fact an activity of the 29-kDa product (45, 77) encoded by the \textit{arcA} gene, which is
a transcriptional regulator of genes involved in aerobic metabolism (45, 77, 166, 167). The activity of ArcA is modulated, via histidine phosphorylation, by the signal transducer encoded by arcB (72.11 min), which senses the redox state of the cell (165, 168–170).

It has been shown that the Arc+ and Sfr+ functions of ArcA are genetically and physiologically separable (316). While it is possible that SfrA (ArcA) is acted on by CpxA in addition to ArcB, this relationship is yet to be clearly established (165). Evidence indicates that the 52-kDa cpxA product is required primarily for efficient traJ expression (18, 317, 346), whereas SfrA appears to be an activator of the PtraY promoter (109, 318). It is therefore possible that sfrA and cpxA act independently of each other. Several key questions relating to SfrA and CpxA remain to be answered, namely, the identity of the effector protein acted upon by CpxA, the possibility of a second transducer affecting SfrA, and the nature of the stimuli to which these systems respond. Encoded by a gene upstream of cpxA and exhibiting amino acid sequence similarity to regulatory proteins of other two component systems, the product of the recently identified cpxR is a candidate for the CpxA effector (75), as is the yet to be identified product of another chromosomal gene required for efficient tra expression, cpxB (41.01 min) (237, 238, 303, 317).

The sfrB gene (86.64 min; also known as tfaH and hlyT) encodes an 18-kDa product, which suppresses premature rho-dependent termination of tra region transcription (24, 33, 109). Two sites of SfrB-associated transcription termination have been localized within the tra region, between traC and traG and between traG and traS (33, 109). Mutations in sfrB are pleiotropic, also affecting flagellum formation, bacteriophage and antibiotic sensitivity, and lipopolysaccharide and hemolysin synthesis (24, 32, 290).

The sequence-specific, histone-like DNA-binding protein IHF is involved in a number of DNA-associated processes in E. coli, including replication, transposition, site-specific recombination, and gene expression (for reviews, see reference 98 and chapter 125). IHF has been shown to be necessary for efficient transcription of tra region genes from both F and R100 (67, 110; Penfold and Frost, unpublished); the amount and length of gene X, traM, and traJ transcripts synthesized by R100 were found to be affected in IHF− hosts (71). Transcription initiation from PtraY of F also appears to be reduced in such mutants (318), although this may represent an indirect effect due to reduced traJ expression.

IHF-binding sites have been localized in the oriT region, between the nick region and traM, from F and R100 (Fig. 6) (71, 156). The presence of these binding sites within the oriT region suggests that IHF also plays a role in the processes that lead to nicking and ultimately DNA transfer, perhaps via an interaction with TraM (4, 336). The IHF protein contributes to the nicking of R100 oriT in vitro (155) and can partially repress the most upstream R100-traM promoter (4). IHF binding has also been detected in the vicinity of PtraJ (71).

Posttranscriptional Modulation of tra Gene Expression

In addition to the regulatory controls governing translation of the traJ and finO mRNAs, posttranscriptional mechanisms involving mRNA processing and stability are believed to be involved in the regulation of expression of the long tra operon transcript. It has been proposed that 5′ endoribonucleolytic cleavage and protection from 3′ exoribonucleases leads to the accumulation of small, stable R1–19 mRNA species which derive from the polycistronic mRNA initiating at PtraY but encode only the traA gene product (propilin) (190). The inverted repeats presumed to form the RNA stem-loop secondary structures responsible for exoribonuclease resistance of the R1–19-traA transcripts are conserved on F (103). Like traA, the F-traT gene is also expressed in abundance and has similarly been found to be located on stable mRNAs (131). The expression of R1 gene 19 has been shown to be controlled by the endoribonuclease RNase III. A transcript initiating upstream of and in the opposite orientation to traM seems to be similarly processed (192).

The translation initiation codons of the majority of the genes that are thought to be coded for by the major tra transcript initiating at PtraY overlap with or are in close proximity to the stop codon of the previous cistron (40, 76, 94, 103, 131, 173, 224, 225, 227, 253, 306, 367, 368, 370). Such an
arrangement is suggestive of translational coupling (15). Although active coupling within the *tra* region has not been detected, juxtapositioning of translation control signals probably serves to maximize expression via translation reinitiation.

**OTHER PLASMID-ENCODED GENES POTENTIALLY INVOLVED IN CONJUGATION**

Nucleotide sequencing within the *tra* region revealed a number of ORFs presumed to correspond to previously undetected genes (40, 76, 103, 132, 224, 227, 367). The involvement in the conjugative process of many of these cistrons and several other *tra* genes identified only on the basis of product detection has been investigated by using resistance-gene cassette mutagenesis followed by homologous recombination into F-plasmid derivatives (180, 221, 222, 224, 226, 227, 253). Subsequent analyses have indicated that several of these mutants exhibit phenotypes indiscernible from that of wild-type F, as judged by standard laboratory assays. Characteristics of the *tra* region genes of unknown function are shown in Table 2. It has been suggested that such apparently dispensable genes may contribute to conjugative transfer from different hosts or under alternative conditions (164). It is also possible that some of these genes encode products that perform functions unrelated to DNA transfer; the presence of an additional gene, *orfE*, between *traT* and *traD* and the absence of *trbH* in the R100-*tra* region would seem to support this view (40, 370).

Although the autonomy of the F-*tra* region has been demonstrated by the transfer proficiency of a recombinant plasmid containing the segment 66.6 to 100F (214), there is accumulating evidence that genes in the 13-kb leading region (Fig. 1) may also normally play a role in conjugation. In addition to being conserved on F-like plasmids (121, 214, 311), leading-region sequences have been found to hybridize to non-F-like plasmids (118, 119, 146, 214, 311). A number of ORFs have been identified in the leading region, but only three functions have so far been associated with this DNA segment (for reviews, see references 164 and 349), namely, a plasmid maintenance system designated *flm* (F leading region maintenance; formerly called *parL* and also known as *stm*, and homologous to the *parB* hok/sok system of R1) (216, 217), a single-stranded DNA-binding protein encoded by the *ssb* gene (also known as *ssf*) (50, 187), and an inhibitor of the RecA-mediated SOS response, expressed by the *psiB* gene (25, 79, 215). Roles have been envisaged for all of these functions in the recipient subsequent to conjugative transfer (164).

Supporting the notion that the leading region may play a role in conjugative transfer, expression of the *ssb* and *psiB* genes of the conjugative IncI1 plasmid, Collb-P9, has been shown to be induced upon transfer into a recipient cell (175). The *psiB* gene of F also appears to be zygotically induced (23). Furthermore, the *ssb* gene of R1 is believed to be coordinately regulated with the *tra* genes of that plasmid (120). Genes encoding single-stranded DNA-binding proteins have been identified on a number of conjugative plasmids (see below and reference 176).

Although the leading-region gene *orf169* (Fig. 6) does not appear to be essential for F conjugation (214), experiments with the F-like R1-plasmid transfer system suggest that the corresponding gene *19* product is required for efficient transfer of that plasmid (26a). The gene *19* product is synthesized as a precursor which is subsequently processed, presumably by signal peptidase I, to a 17-kDa mature form (192, 214). Amino acid sequence similarity has been detected between the deduced product of *orf169/gene 19* and IpgF encoded by the *mxi* locus of the *Shigella flexneri* virulence plasmid, pWR100 (19) and, more recently, with R64 PilT, pMK101 TraL, RP4 TrbN, Ti VirB1 (Table 3), and Slt70, the soluble lytic transglycosylase of *E. coli* (26a; G. Koraimann, personal communication). In addition to other ORFs of unknown function in the leading region (122, 214), two single-stranded initiation sequences, designated *ssiD* and *ssiE*, have been identified in the F-leading region; these sites are likely to play a role in recipient conjugal DNA synthesis (263).
BACTERIAL CONJUGATION: A MACROMOLECULAR TRANSPORT MECHANISM

Similarities have recently been noted between proteins encoded by genes from a number of bacterial DNA transfer systems (Table 3). Most striking is the homology evident between the T-DNA transfer system encoded by the *vir* genes of agrobacterial Ti and Ri plasmids and the conjugation systems of the IncN, IncP, and IncW plasmids pMK101, RP4, and R388, respectively. The likeness of these systems, which extends to the levels of protein function and genetic organization, has given strong support to the hypothesis that the transfer of T-DNA to plants represents a modification of bacterial conjugation (324). Furthermore, amino acid sequence similarities have also been detected between products from these systems and those from IncF and IncI plasmids and even with transfer-associated proteins encoded by plasmids from gram-positive organisms (illustrated by pSK41 and pS194 in Table 3). The products of plasmids such as pED208 (IncFV) (103) and R751 (IncPβ) (128), which would appear to have diverged relatively recently from the lineages represented by F and RP4, respectively, are omitted from Table 3 because of constraints of space, but they serve to remind of the extent of variation that exists. In some cases, the homology evident between DNA metabolism proteins from different systems is paralleled by recognizable similarity in the nucleotide sequences of their respective nick sites; the *oriT* regions of R64, RP4, pTF-FC2, and pS194 and Ti-plasmid border sequences are examples of this duality (202, 281, 282, 299). The similarities described above have led to the realization that DNA transfer mechanisms from seemingly diverse host origins share genes of common evolutionary ancestry.

The role of the F pilus in the conjugative process has been the subject of debate for several decades (10, 42, 63, 353). The hypothesis that the pilus acts as the conduit for the passage of DNA from donor to recipient has gradually given way to the prevalent notion that the pilus acts as a “grappling hook” that retracts to bring mating cells into close physical contact, thereby enabling the establishment of conjugation junctions (78), the sites at which DNA transfer is presumed to occur. However, there are several reasons to think that the latter suggestion may represent an oversimplification of pilus function and that that the pilus or a vestige thereof remaining after retraction may indeed represent a specialized pore which facilitates DNA transmission across the donor and possibly the recipient cell envelope(s). Such a structure might include at least some of the *tra* products currently defined as pilus assembly proteins (Fig. 4; Table 1) and may correspond to structures visualized recently by freeze-fracture and freeze-etch electron microscopy (301).

First, the observed mating, albeit at low efficiency, between physically separated cells indicates that the lumen of the pilus can support the transmission of single-stranded plasmid DNA (134, 275). Second, mutations in the *traD* gene, which is believed to be involved in the transmission of single-stranded plasmid DNA (see above), result in a slightly increased level of host cell piliation (21), suggesting a link between the conjugal DNA processing machinery and pilus function. Indeed, TraD homologs from other DNA transfer systems (Table 3) have been suggested to mediate an interaction between the conjugal DNA metabolism proteins and the DNA transfer pore (202, 345). Third, the apparent complexity of F-pilus biogenesis may reflect a multifunctional role for the pilus and associated proteins. No less than 16 gene products appear to be involved in the elaboration of the F pilus, and representatives of these proteins are believed to be associated with the cytoplasm, inner membrane, periplasm, and outer membrane, with the pilus itself extending into the external environment of the cell (Fig. 4). In contrast, only about half this number of proteins seem to be directly involved in the elaboration of nonconjugative *E. coli* P and type 1 pili, and a number of those represent subunits of the pilus itself (150).
### TABLE 2  Transfer region genes of unknown function

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product length (aa)</th>
<th>Product size (kDa)</th>
<th>Product location</th>
<th>Comments and reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>artA</td>
<td>104</td>
<td>12.1</td>
<td>Inner membrane</td>
<td>Nonessential product yet to be identified (180); translation supported by artA'-lacZ fusion (367).</td>
</tr>
<tr>
<td>traP</td>
<td>196</td>
<td>22.0</td>
<td>Inner membrane</td>
<td>Probable product identified but function unknown (257); Frost, unpublished data cited in reference 103)</td>
</tr>
<tr>
<td>traR</td>
<td>73</td>
<td>8.3</td>
<td>Cytoplasm</td>
<td>Identified product found to be nonessential (221, 257); deduced product shares amino acid sequence similarity with the E. coli dosage-dependent dnaK suppressor, DksA, and coliphage 186 and P2 proteins of unknown function (76)</td>
</tr>
<tr>
<td>trbA</td>
<td>115</td>
<td>12.9</td>
<td>Inner membrane</td>
<td>Identified product found to be nonessential (180, 367, 369)</td>
</tr>
<tr>
<td>trbB</td>
<td>179 [159]</td>
<td>19.5 [17.4]</td>
<td>Periplasm</td>
<td>Identified product found to be nonessential (180, 367, 369)</td>
</tr>
<tr>
<td>trbD</td>
<td>65</td>
<td>7.1</td>
<td>Cytoplasm</td>
<td>Product of unknown function yet to be identified (Frost, unpublished data cited in reference 103)</td>
</tr>
<tr>
<td>trbE</td>
<td>86</td>
<td>9.9</td>
<td>Inner membrane</td>
<td>Identified product found to be nonessential (224); translation supported by trbF'-'phoA fusions (Y. N. Lin, J. Tennent, N. Firth, and R. A. Skurray, unpublished data)</td>
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<tr>
<td>trbF</td>
<td>126</td>
<td>14.5</td>
<td>Inner membrane</td>
<td>Product of unknown function yet to be identified (40, 221)</td>
</tr>
<tr>
<td>trbG</td>
<td>83</td>
<td>9.1</td>
<td>Cytoplasm</td>
<td>Product of unknown function yet to be identified (76)</td>
</tr>
<tr>
<td>trbH</td>
<td>239</td>
<td>26.3</td>
<td>Inner membrane</td>
<td>Nonessential product yet to be identified (221, 367)</td>
</tr>
<tr>
<td>trbI</td>
<td>93</td>
<td>10.2</td>
<td>Inner membrane</td>
<td>Nonessential product yet to be identified (221, 367)</td>
</tr>
</tbody>
</table>

*Sizes and lengths are calculated from the deduced amino acid sequence of each product. Sizes and lengths of processed products are shown in brackets. Values for processed products are based on predicted cleavage sites.*

*Product locations have been determined experimentally unless shown italicized, in which case the predicted location is listed.*

*The term “nonessential” indicates that a mutation in this gene was found not to cause any identifiable effect on the transfer-related phenotypes examined.*

Compelling support for the above contention that pilus components form a mating channel has been provided by sequence similarities to the F-pilus assembly proteins TraB, TraC, TraE, and TraL and the F-pilus subunit precursor itself, TraA (Table 3). Kado and his colleagues (178, 313) not only identified amino acid sequence similarity between these proteins and VirB products believed to form an envelope-spanning mating structure that facilitates agrobacterial T-DNA transfer to plant cells (331) but also demonstrated that VirB2 is processed in an analogous fashion to F propilin (312). These evolutionary relationships have been extended to include products known or suspected to be involved in pilus biogenesis/mating-pair formation encoded by other conjugative plasmids, such as pMK101, RP4, and R388 (Table 3) (210, 289; Bolland, Ph.D. thesis). Since no traditional pilus-like structure has been attributed to agrobacterial Ti plasmids (82), the relationships between “pilus-associated” proteins and the T-DNA transfer system are particularly intriguing. Perhaps even more provocative, however, have been the similarities detected between such pilus-associated proteins and the Ptl proteins responsible for the export of Bordetella pertussis multiple-subunit toxin (Table 3) (58, 347). The detection of an evolutionarily and functionally related apparatus involved in the export of a molecule other than DNA (namely, B. pertussis toxin) has led to the realization that the DNA transfer mechanisms discussed here, including that encoded by F, represent members of a family of pilus-related macromolecular transport systems.
TABLE 3  Similarities between proteins of macromolecular transport systems

<table>
<thead>
<tr>
<th>Conjugation</th>
<th>Mobilization</th>
<th>Protein secretion (Ptl)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (IncF1)</td>
<td>R64 (IncI1)</td>
<td>pMK10 (IncP)</td>
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<td></td>
<td></td>
<td>RP4 (IncP)</td>
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<td></td>
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<td>R388 (IncW)</td>
<td>Ti</td>
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<td></td>
<td></td>
<td>pSK41</td>
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<td></td>
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<td>CloDF13</td>
<td>pTF-FC2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>pS194</td>
</tr>
<tr>
<td>Orf169(p19)</td>
<td>TrAL</td>
<td>TrbN</td>
<td>VirB1</td>
</tr>
<tr>
<td></td>
<td>TrbC</td>
<td>TrwL&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>TrbD</td>
<td>TrwM&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>TrbE</td>
<td>TrwK&lt;sup&gt;c&lt;/sup&gt;</td>
<td>VirB4</td>
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<td>TrbF</td>
<td>TrwJ&lt;sup&gt;c&lt;/sup&gt;</td>
<td>VirB5</td>
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<tr>
<td></td>
<td>TrbL</td>
<td>TrwF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>VirB6</td>
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<td>TrbD</td>
<td>TrwH&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Ssb</td>
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</tbody>
</table>

<sup>a</sup>The proteins shown are from the gram-negative conjugation systems of plasmids F, R64, pMK101, RP4, and R388; the T-DNA transfer system of *Agrobacterium tumefaciens* Ti plasmids; the gram-positive conjugation system of the *Staphylococcus aureus* plasmid pSK41; the mobilization systems of the gram-negative plasmid CloDF13, the *Thiobacillus ferrooxidans* plasmid pTF-FC2, and the gram-positive plasmid pS194; and the Ptl toxin secretion system of *Bordetella pertussis*. The table is adapted and extended from those of Kado (177, 178).

<sup>b</sup>Similarities were noted to the deduced gene 19 product (p19) encoded by the IncFII plasmid, R1 (26a); gene 19 is homologous to F orf169 (see the text).

<sup>c</sup>The data for the relationships between R388 Trw and Ti VirB proteins are from Bolland (Ph.D. thesis), kindly provided by F. de la Cruz.
In recent years, considerable advancements have been made in our understanding of the biochemical basis of conjugal DNA metabolism, as exemplified by F and related DNA transfer systems. However, insights into the mechanistic basis of cell-cell DNA transport have so far proven more elusive. It is hoped that the recently identified relationships between F-pilus-associated proteins and those of other macromolecular transport mechanisms, which highlight a role for the pilus in the transmission of DNA, will stimulate progress on this key biological process in ensuing years. Studies of the F factor will doubtless remain central to these efforts.

DEDICATION

Karin Ippen-Ihler died on 17 March 1995 at the age of 53, shortly after the completion of this chapter, following a courageous battle with cancer. Karin’s intellect, strength, and determination were a hallmark of her scientific endeavors and were no less evident in the preparation of this chapter. Her commitment was, and is, an inspiration to all her colleagues. Karin is remembered with the highest regard and deepest affection.

ACKNOWLEDGMENTS

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LITERATURE CITED


119. **Golub, E. I., and K. B. Low.** 1985. Conjugative plasmids of enteric bacteria from many different


182. **Kim, K., and R. J. Meyer.** 1989. Unidirectional transfer of broad host-range plasmid R1162


204. Lawn, A. M., G. G. Meynell, E. Meynell, and N. Datta. 1967. Sex pili and the classification of


Location of F plasmid transfer operon genes \textit{traC} and \textit{traW} and identification of the \textit{traW} product. \textit{J. Bacteriol.} \textbf{169}:5119–5124.


329. **Thompson, R., L. Taylor, K. Kelly, R. Everett, and N. Willetts.** 1984. The F plasmid origin of
transfer: DNA sequence of wild-type and mutant origins and location of origin-specific nicks. *EMBO J.* **3**:1175–1180.


349. **Wilkins, B., and E. Lanka.** 1993. DNA processing and replication during plasmid transfer


constitutive transfer of plasmid F is caused by insertion of IS\(\text{3}\) into F \(\text{finO}\). *J. Bacteriol.* **169**:619–623.
