Structure and Function of the F Factor and Mechanism of Conjugation

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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 $5' \rightarrow 3'$ 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.

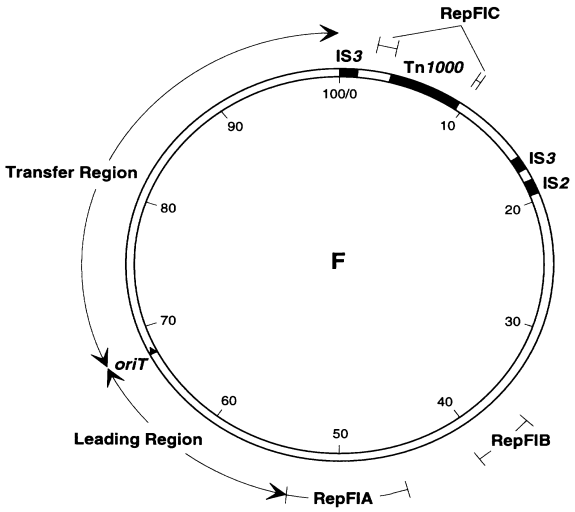

FIGURE 1 Physical and functional map of the F plasmid. Numbers within the map indicate kilobase coordinates based on the 100-kb F map. The transposable elements IS2, 1S3, and Tn1000 are represented by solid boxes. The extents of the replication (RepFIA, RepFIB, and RepFIC), transfer, and leading regions 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-*Eco*RI 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 Tn*1000* (also known as γδ) and IS*2* and two copies of IS*3* (Fig. 1) (129, 147–149) (see chapters 111 and 124 for descriptions of transposable elements). Tn*1000* appears to have interrupted RepFIC (300), whereas IS*3* 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 *Hin*dIII restriction cleavage site in IS*3* (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 *Bam*HI-*Hin*dIII 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 *Hin*dIII 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” *srnB* (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 $5' \rightarrow 3'$ 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).

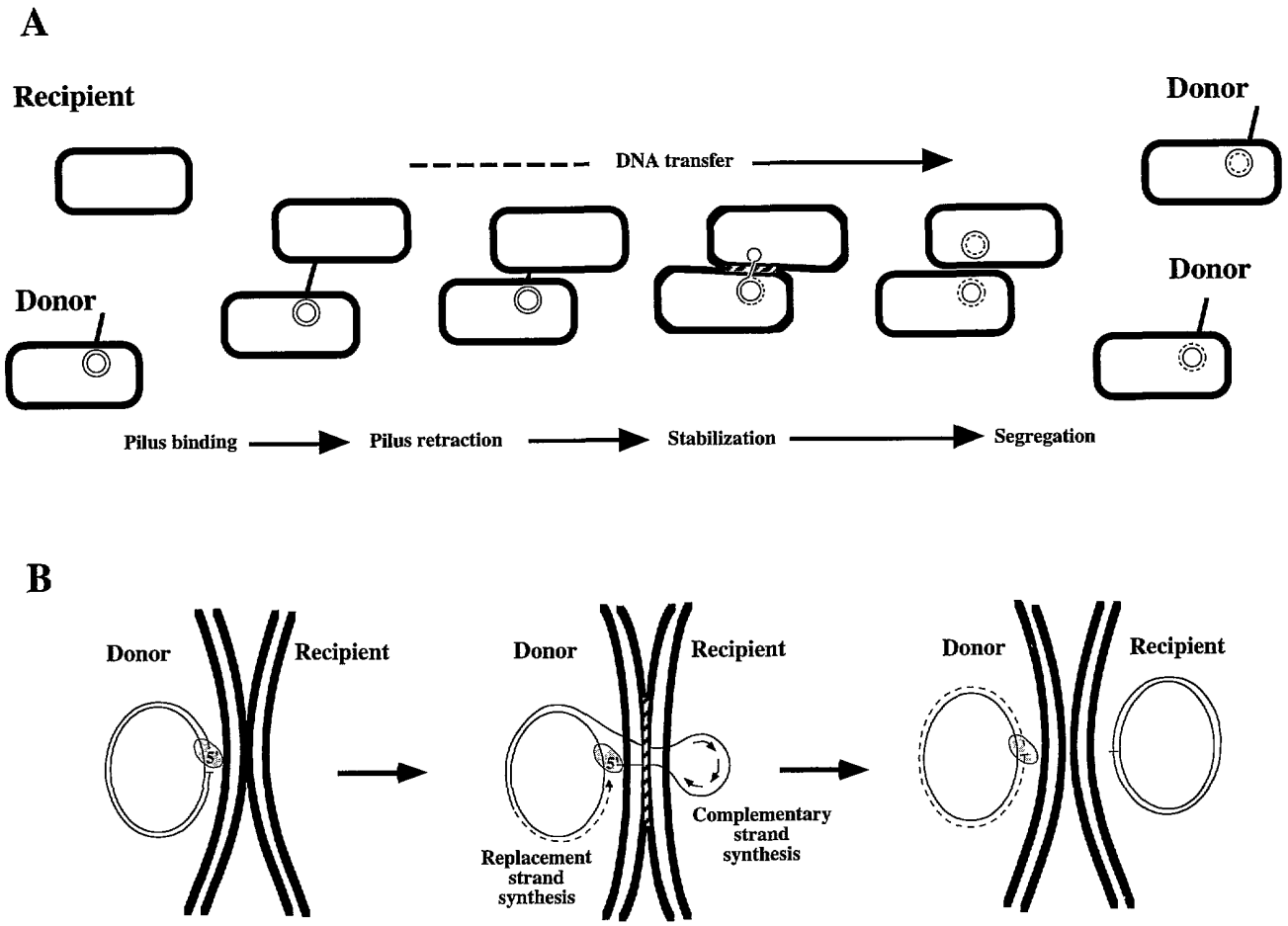

FIGURE 2 Stages of F-mediated conjugal-DNA transfer. (A) Intercellular contact begins when the tip of an F pilus extended from an F^+ donor interacts with the recipient cell. Depolymerization of filament subunits brings the cell surfaces together. Specific and progressive surface interactions then stabilize this association during DNA transfer. The electron-dense conjugative junction is represented by hatching. After F-DNA transfer is completed, an active process separates the cells, now both F^+. (B) DNA transfer events begin when contact(s) established or facilitated by the pilus transmits a "mating signal" to a protein complex, which includes the *traI* product, TraI (grey), associated with the F-*oriT* site. This stimulates the displacement of a single strand of F DNA, which is passed into the recipient cell. After nicking, the F-TraI relaxase/helicase remains attached to the 5′ *oriT* end and is imagined to locate at the site of intercellular connection. As the strand is unwound from *oriT* in the 5′ → 3′ direction and moved into the recipient cell, replacement strand synthesis in the donor and complementary strand synthesis in the recipient also usually occur. Transfer terminates when the transferred strand is again recircularized at *oriT.*

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 IS*3* (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 N^{α}-acetylation steps in propilin maturation depend on expression of *traQ* (163, 180, 256) and *traX* (252), respectively.

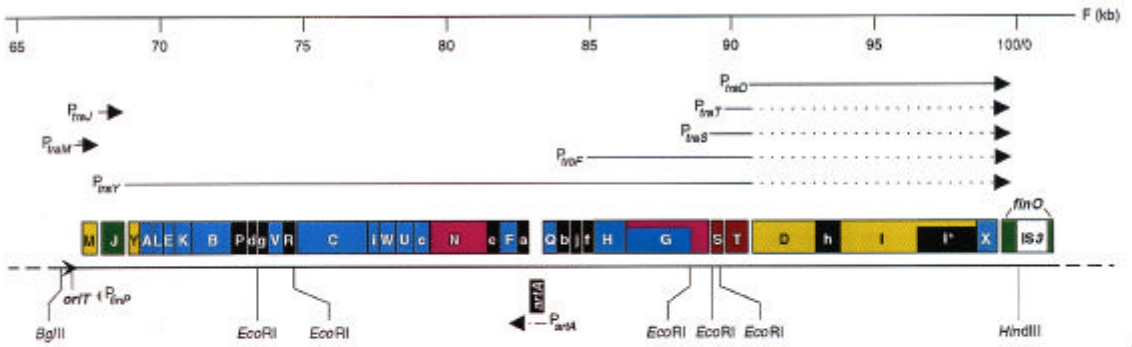

FIGURE 3 Physical and genetic map of the F-plasmid transfer region. F-plasmid map (Fig. 1) kilobase coordinates are shown at the top. Arrowed lines denote transcripts initiating at the indicated promoters; dotted segments signify uncertainty in the extremity of the transcript. Extents of the indicated genes are represented by boxes; the functional class associated with a particular gene is indicated by the color of its box and is discussed further in the text. Colors: blue, pilus biogenesis; red, surface exclusion; magenta, mating-aggregate stabilization; green, regulation; yellow, conjugal-DNA metabolism; black, unknown/nonessential. Capital and lowercase letters are used to label *tra* and *trb* genes, respectively. An arrowhead indicates the position of the origin of transfer (*oriT*) and the direction of conjugal DNA transfer. The copy of IS3 that interrupts the *finO* gene is also denoted. The positions of recognition sites for the restriction endonucleases *Eco*RI and *Hin*dIII are shown below the genetic map. Only the *Bgl*II site at 66.6F is shown for orientation purposes. The map is adapted from reference 164.

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 β-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 1 Transfer region genes involved in conjugation

Gene	Functional group[a]	Product length (aa)[b]	Product size (kDa)[b]	Product location[c]	Reference(s)
finO[d]	Regulation	186	21.0	*Cytoplasm*	59, 371
finP	Regulation	78 nt[e]		*Cytoplasm*	88, 339
traA	Pilus biogenesis	121 [70]	12.8 [7.2]	Inner membrane and extracellularly	106, 252, 255, 278
traB	Pilus biogenesis	475	50.5	*Inner membrane*	257; Frost, unpublished data cited in reference 103
traC	Pilus biogenesis	875	99.2	Cytoplasm/inner membrane[f]	306, 307
traD	DNA metabolism	717	81.7	Inner membrane	173, 280, 370
traE	Pilus biogenesis	188	21.1	Inner membrane	7, 106, 163, 200
traF	Pilus biogenesis	247 [228]	28.0 [*25.9*]	Periplasm	368
traG[g]	Pilus biogenesis and aggregate stabilization	938	102.4	Inner membrane	94, 230, 254, 356
traH	Pilus biogenesis	458 [*434*]	50.2 [*47.8*]	*Periplasm*	94, 132, 230
traI[g]	DNA metabolism	1,756	192.0	Cytoplasm	2, 40
traJ	Regulation	229	27.0	Cytoplasm	62, 328
traK	Pilus biogenesis	242 [*221*]	25.6 [*23.3*]	*Periplasm*	287
traL	Pilus biogenesis	91	10.4	*Inner membrane*	103, 106
traM	DNA metabolism	127	14.5	Cytoplasm	73, 328
traN	Aggregate stabilization	602 [*584*]	65.7 [*63.8*]	Outer membrane	224
traQ	Pilus biogenesis	94	10.9	Inner membrane	223, 367, 369
traS	Surface exclusion	149	16.9	Inner membrane	7, 172
traT	Surface exclusion	244 [*233*]	26.0 [23.8]	Outer membrane	6, 172, 245, 246, 248, 288
traU	Pilus biogenesis	330 [*308*]	36.8 [*34.2*]	Periplasm	253
traV	Pilus biogenesis	171 [*153*]	18.6 [*16.6*]	*Outer membrane*	76, 257
traW	Pilus biogenesis	210 [*193*]	23.6 [*21.7*]	Periplasm	225, 227
traX	Pilus biogenesis	248	27.5	Inner membrane	59, 222, 252
traY	DNA metabolism	131	15.2	Cytoplasm	95, 157, 199, 261
trbC	Pilus biogenesis	212 [*191*]	23.4 [*21.2*]	Periplasm	226
trbI	Pilus biogenesis	128	14.1	Inner membrane	227

[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 IS*3* 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 IS*3* 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).

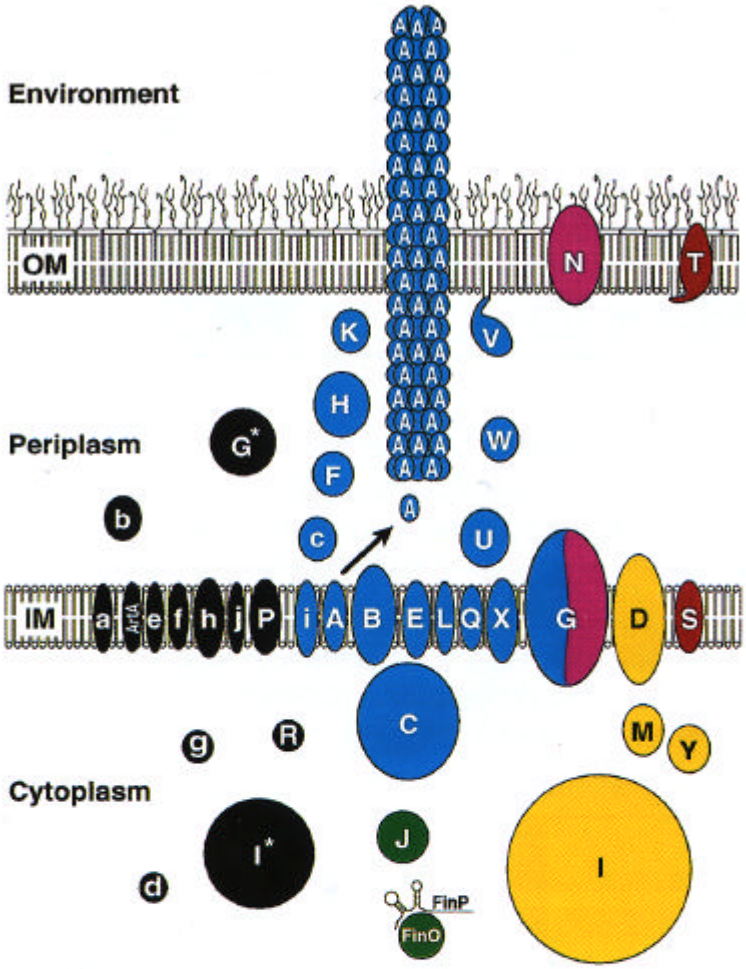

FIGURE 4 Cellular localization of F *tra* region products. A diagram of the *E. coli* inner (IM) and outer (OM) membranes is shown. Schematic representations of *tra* region products are superimposed on the diagram to indicate the probable cellular locations of the products; relative sizes are only approximated (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 *traX* gene is required for N^{α}-acetylation of the F-pilin polypeptide (252). The predicted product of the *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 *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 *traX* ORF, there may be more than one translation initiation site. *traX* codons 29 to 225 encode a region essential to pilin acetylation activity (Maneewannakul et al., unpublished). N^{α}-acetylation 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-*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 IncPα plasmid RP4 (282), the product of the *Dichelobacter nodosus* (formerly *Bacteroides nodosus*) *fimC* gene (145), and the deduced product of an ORF from the filamentous single-stranded DNA phage Cf1c of *Xanthomonas campestris* pv. citri (94a, 197). Paralleling what has been observed for *traX*, two polypeptides have been associated with expression of *fimC*, which is located immediately downstream of the major fimbrial subunit gene, *fimA*, in some *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^{α}-acetylation (223). Both forms of F pilin were found to exhibit considerable stability in vivo (223).

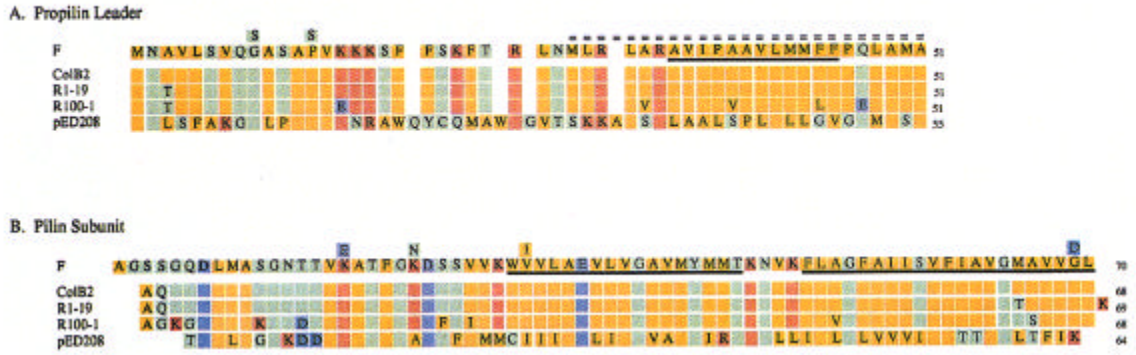

FIGURE 5 Amino acid sequences of the F-propilin leader peptide (A) and the F-pilin subunit (B) (106). A bar indicates the typical signal sequence within the leader peptide; hydrophobic residues proposed to form membrane-spanning regions of the signal and of membrane pilin subunits are underlined. The 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 *traA* missense mutations (105) are indicated above the F sequences; those affecting the propilin leader were detected in pilus-deficient F*lac* mutants, whereas those affecting subunit structure were detected in F*lac* 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 *traA* alleles of F-like plasmids (84, 102) are shown. The alignment is as presented by Frost et al. (103) 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 *traB*, *traC*, *traE*, *traG*, *traL*, and *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 *tra* region products. It is therefore presumed that TraC normally associates with one or more of the *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 *traC* mutations appear to abolish F-pilus outgrowth, the *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 f1 sensitivity is retained but F-pilus filaments are not detected (226). Donor cells unable to express *traU* were found to synthesize reduced numbers of otherwise apparently normal F pili (253). Mutation of *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 *trbI* mutants also synthesize unusually long pili (227).

The *traG* gene is unique in that mutations in this gene fall into either of two phenotypic classes. Whereas all *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 *traG* function essential to filament outgrowth can be expressed from a *Sma*I fragment carrying only *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 *traG* product is believed to reflect a defect in mating-aggregate stabilization (230; also see below). Present at 500 to 600 copies per F^+ 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 promoterdistal 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 conjugative 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 piliate *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).

COMPONENTS INVOLVED IN THE DNA TRANSFER STAGES OF CONJUGATION

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 *Bgl*II 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), RI (IncFII) (273), R100 (IncFII) (240), ColB4-K98 (IncFIII) (86), pSU316 (IncFIII) (218), pED208 (IncFV) (72), and pSU233 (IncFVII) (302). The DNA segment between the *Bgl*II 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

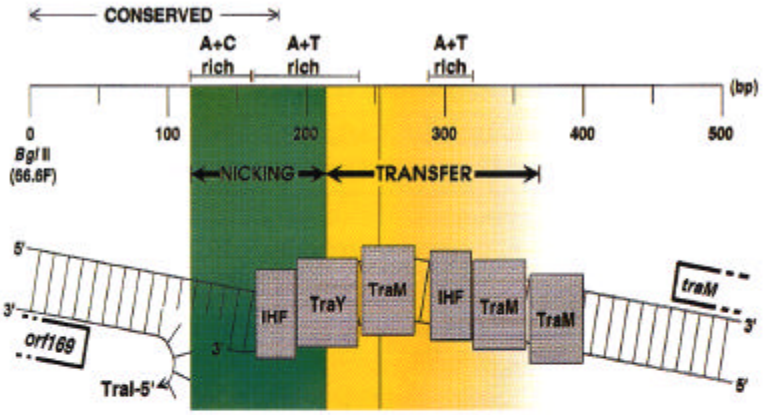

FIGURE 6 F-plasmid *oriT* region. Extents of regions of high sequence conservation among F-like plasmids and unusual base composition are shown above a scale that represents distance in base pairs from the *Bgl*II site at 66.6F. Kinks in the *oriT* DNA schematic representation denote intrinsic bends. Grey boxes superimposed on the diagram indicate the approximate positions of the sites bound by the named proteins. Covalent attachment of the 5′ terminus (arrowhead) of the nicked DNA strand to TraI is indicated, whereas the resulting 3′ terminus denotes the position of the nick. The minimal regions of *oriT* needed for nicking and transfer are shown in green and yellow, respectively. The yellow gradient signifies the progressive reduction in transfer efficiency resulting from deletion of those sequences from the right. The locations of the 5′ ends of the *orf169* and *traM* genes are indicated.

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^+ cell (186), the cytoplasmically located 192-kDa F protein, TraI, corresponds to the well-characterized enzyme *E. coli* DNA helicase I (2) and exhibits the 5′ → 3′ 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′ terminus of the nicked DNA strand (Fig. 2 and 6); no such linkage to the 3′ 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 *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 TraY protein may determine which TraM-binding sites can be occupied; expression of both TraY and IHF contributed to maximal *traM* product expression (Penfold and Frost, unpublished). The *traM* products of R1, R100, and pED208 also bind to multiple sites within their respective *oriT* regions (3, 72, 309), and autoregulation of the R1- and R100-*traM* alleles has been demonstrated (4, 310). TraM binding has also been suggested to regulate transcription of a promoter for gene *19* on plasmid R1 (192). As a mutant *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 *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 TraM may also be critical to DNA-binding activity (310).

Whereas the minimal *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 TraY-binding region, additional rightward sequences, including TraM-binding sites, are required for efficient transfer (Fig. 6) (107, 111). The segment between the AT-rich region and the *traM* gene shows considerable sequence divergence among the characterized F-like plasmids and, in addition to sites that bind 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 TraM-binding site closest to *oriT* completely abolish transfer (73, 107).

The characteristics of F 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 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). 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 TraI has led to the further suggestion that TraI might also energize the transmission of the DNA strand during conjugation (315, 360). Such models assume that TraI is in some way immobilized, perhaps in interaction with a *tra*-encoded or chromosomally encoded protein associated with the inner membrane (Fig. 2) (315); it has been suggested that TraD might provide such an association (64). In the absence of TraI-mediated unwinding activity but not nicking activity, some other helicase can apparently substitute, albeit at a lower transfer efficiency (335).

The F-*traD* gene encodes an 81.7-kDa inner membrane protein (280). The gene sequence indicates that TraD is 717 aa in length (40, 173). The deduced R100-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 TraD (370). Although *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 TraD is required only after mating-aggregate formation (279). These characteristics have led to the suggestion that TraD is involved in the transportation of single-stranded DNA across the cell envelope into the recipient (183). Several observations support the notion that TraD plays a role in transmembrane conveyance of nucleic acid. First, RNA phages, such as MS2, are able to adsorb to the pili of *traD* mutants but infection is aborted because of a defect in RNA penetration (284, 308). Second, *traD* is required for mobilization of the plasmid ColE1 and related plasmids, unlike the other DNA metabolism genes *traM*, *traI*, and probably *traY* (355). Finally, purified TraD has been found to bind DNA in a nonspecific manner (280).

Recently, amino acid sequence similarity has been detected between TraD and polypeptides encoded by transfer systems previously thought to be distinct from that of F, namely, TraG of RP4, TrwB of R388, and 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, P*finP* and P*artA*, 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 P_{traY} 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 P_{traY} 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 $P_{trbF,}$ P*traS*, P*traT*, and P*traD* 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 P_{traY} (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).

P_{traY} 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, P_{traY} (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 form a helix-turn-helix DNA-binding domain (74, 285). Zone sedimentation data indicate that TraJ may be dimeric (62).

P_{traY} has been shown to be utilized in vitro by the *E. coli* σ^{70} RNA polymerase (115). However, RNA polymerase was found to form a stable complex at P_{traY} 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 P_{traY} in the absence of TraJ (319), Gaudin and Silverman (115) have suggested that in the absence of a transcriptional activator, the sequences preceding P_{traY} contribute to local relaxation of the promoter region. In such a model, TraJ may invoke transcription from P_{traY} by facilitating the restoration of normal superhelicity (115). Assays employing *galK* transcriptional fusions have indicated that in the presence of TraJ, P_{traY} 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 P_{traY} 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_{traT}, and P_{traD} appear to be *traJ*-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_{traY} described above. Transfer of the F plasmid is derepressed as a result of insertional inactivation of the *finO* gene by the transposable element IS*3* (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_{finP}, 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 IS*3* 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 P_{traY} 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 *traD* 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 *tra* region transcription (113). Although the presence of JR66a was found to reduce transcription of distal 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 *tra* genes. However, the synthesis of all 11 *tra* products detected in the study was unaffected by FinU (109).

The FinV fertility inhibition system encoded by plasmid R485 inhibits F piliation and hence transfer but does not reduce surface exclusion, indicating that an effect on *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 *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 InfluencingF-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 F^+ cells, reinforcing the notion that conjugation is a cellular process. 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 *tra* products led Sowa et al. (323) to speculate that transcription of the *tra* region may be regulated by temperature.

The early finding that F^- phenocopies of donor cells (i.e., 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 F^- phenocopies also failed to elaborate normal numbers of F pili (42). It is therefore likely that growth into stationary phase has a general effect on *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 F-like plasmids, although the data available are very limited. Harwood and Meynell (136) found that addition of exogenous cAMP to *cya* mutants carrying various derepressed F-like plasmids reduced piliation, although this effect was not evident in cells harboring F. In contrast, the observed reduction in transfer-related properties expressed by *cya* and *crp* mutant hosts led Kumar and Srivastava (196) to suggest that F-*tra* expression is dependent on cAMP and its receptor protein. The identification of a sequence in the vicinity of P_{traJ} with similarity to the binding-site motif of the *crp* product, catabolite activator protein, lends credence to this proposal (285).

Several other chromosomal genes appear to play regulatory roles in the expression of F-transfer functions. The products of *cpxA* (mapping at 88.34 min on the *E. coli* chromosome; also called *ecfB, ssd,* and *eup*) (16, 17, 293) and *sfrA* (99.95 min; alternatively known as *arcA, fex, cpxC, msp,* and *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 *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 P_{traY} 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 *rfaH* 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 P_{traY} 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 P_{traJ} (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 P_{traY} 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 P_{traY} 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 $IncI_1$ plasmid, ColIb-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 facilites 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 vizualized 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

Gene	Product length (aa)[a]	Product size (kDa)[a]	Product location[b]	Comments[c] and reference(s)
artA	104	12.1	*Inner membrane*	Nonessential product yet to be identified (180); translation supported by *artA′-′lacZ* fusion (367).
traP	196	22.0	*Inner membrane*	Probable product identified but function unknown (257; Frost, unpublished data cited in reference 103)
traR	73	8.3	*Cytoplasm*	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)
trbA	115	12.9	Inner membrane	Identified product found to be nonessential (180, 367, 369)
trbB	179 [159]	19.5 [17.4]	Periplasm	Identified product found to be nonessential (180, 367, 369)
trbD	65	7.1	*Cytoplasm*	Product of unknown function yet to be identified (Frost, unpublished data cited in reference 103)
trbE	86	9.9	Inner membrane	Identified product found to be nonessential (224)
trbF	126	14.5	*Inner membrane*	Product of unknown function yet to be identified (132); translation supported by *trbF′-′phoA* fusions (Y. N. Lin, J. Tennent, N. Firth, and R. A. Skurray, unpublished data)
trbG	83	9.1	*Cytoplasm*	Product of unknown function yet to be identified (76)
trbH	239	26.3	*Inner membrane*	Nonessential product yet to be identified (40, 221)
trbJ	93	10.2	*Inner membrane*	Identified product found to be nonessential (221, 367)

[a]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.

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

[c]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[a]

Conjugation							Mobilization			Protein secretion (Ptl)	Reference(s)
F (IncF1)	R64 (IncI1)	pMK101 (IncN)	RP4 (IncPα)	R388 (IncW)	Ti	pSK41	CloDF13	pTF-FC2	pS194		
Orf169 (p19)[b]		TraL	TrbN		VirB1						26a, 289
TraA		TraM	TrbC	TrwL[c]	VirB2					PtlA	58, 177, 210, 211, 289, 312
TraL		TraA	TrbD	TrwM[c]	VirB3					PtlB	58, 177, 210, 211, 289, 312, 347
TraC		TraB	TrbE	TrwK[c]	VirB4					PtlC	58, 178, 210, 211, 289, 313, 347
TraE		TraC	TrbF	TrwJ[c]	VirB5						178, 210, 211, 289
		TraD	TrbL	TrwI[c]	VirB6					PtlD	178, 211, 289, 347
		TraN		TrwH[c]	VirB7						178, 289
		TraE		TrwG[c]	VirB8					PtlE	178, 211, 289, 347
		TraO		TrwF[c]	VirB9					PtlF	178, 211, 289, 347
raB		TraF	TrbI	TrwE[c]	VirB10					PtlG	178, 210, 211, 289, 347
		TraG	TrbB	TrwD[c]	VirB11					PtlH	178, 210, 211, 259, 289, 347
			TraL		VirC1			MobD			188, 282, 299, 349
			TraM		VirC2			MobE			282, 299
	NikA		TraJ	TrwA	VirD1			MobB			108, 178, 213, 282, 299
TraI	NikB		TraI	TrwC	VirD2			MobA	R1x		26, 108, 153, 178, 189, 211, 213, 282, 283, 299
			TraH		VirD3						282
TraD			TraG	TrwB	VirD4		MobB				26, 46, 93, 178, 212, 213, 282, 373
						TraK					
			TraK					MobC			282, 299
TraX			TrbP								94a
		ArdB	KlcA								203
Ssb	Ssb		Ssb								93, 176
						TraM					

[a]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 Pt1 toxin secretion system of *Bordetella pertussis.* The table is adapted and extended from those of Kado (177, 178).

[b]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).

[c]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.

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LITERATURE CITED

1. **Abdel-Monem, M., H. Durwald, and H. Hoffmann-Berling.** 1976. Enzymic unwinding of DNA. II. Chain separation by an ATP-dependent DNA unwinding enzyme. *Eur. J. Biochem.* **65:**441–449.
2. **Abdel-Monem, M., G. Taucher-Scholz, and M. Q. Klinkert.** 1983. Identification of *Escherichia coli* DNA helicase I as the *traI* gene product of the F sex factor. *Proc. Natl. Acad. Sci. USA* **80:**4659–4663.
3. **Abo, T., S. Inamoto, and E. Ohtsubo.** 1991. Specific DNA binding of the TraM protein to the *oriT* region of plasmid R100. *J. Bacteriol.* **173:**6347–6354.
4. **Abo, T., and E. Ohtsubo.** 1993. Repression of the *traM* gene of plasmid R100 by its own product and integration host factor at one of the two promoters. *J. Bacteriol.* **175:**4466–4474.
5. **Achtman, M.** 1975. Mating aggregates in *Escherichia coli* conjugation. *J. Bacteriol.* **123:**505–515.
6. **Achtman, M., N. Kennedy, and R. Skurray.** 1977. Cell-cell interactions in conjugating *Escherichia coli:* role of *traT* protein in surface exclusion. *Proc. Natl. Acad. Sci. USA* **74:**5104–5108.
7. **Achtman, M., P. A. Manning, C. Edelbluth, and P. Herrlich.** 1979. Export without proteolytic processing of inner and outer membrane proteins encoded by F sex factor *tra* cistrons in *Escherichia coli* minicells. *Proc. Natl. Acad. Sci. USA* **76:**4837–4841.
8. **Achtman, M., P. A. Manning, B. Kusecek, S. Schwuchow, and N. Willetts.** 1980. A genetic analysis of F sex factor cistrons needed for surface exclusion in *Escherichia coli. J. Mol. Biol.* **138:**779–795.
9. **Achtman, M., G. Morelli, and S. Schwuchow.** 1978. Cell-cell interactions in conjugating *Escherichia coli:* role of F pili and fate of mating aggregates. *J. Bacteriol.* **135:**1053–1061.
10. **Achtman, M., and R. Skurray.** 1977. A redefinition of the mating phenomenon in bacteria, p. 234–279. *In* J. L. Reissig (ed.), *Microbial Interactions: Receptors and Recognition,* ser. B, vol. 3. Chapman & Hall, Ltd., London.
11. **Achtman, M., R. A. Skurray, R. Thompson, R. Helmuth, S. Hall, L. Beutin, and A. J. Clark.**

1978. Assignment of *tra* cistrons to *Eco*RI fragments of F sex factor DNA. *J. Bacteriol.* **133:**1383–1392.

12. **Achtman, M., N. Willetts, and A. J. Clark.** 1971. Beginning a genetic analysis of conjugational transfer determined by the F factor in *Escherichia coli* by isolation and characterization of transfer-deficient mutants. *J. Bacteriol.* **106:**529–538.
13. **Achtman, M., N. Willetts, and A. J. Clark.** 1972. Conjugational complementation analysis of transfer-deficient mutants of F*lac* in *Escherichia coli. J. Bacteriol.* **110:**831–842.
14. **Aguero, M. E., L. Aron, A. G. DeLuca, K. N. Timmis, and F. C. Cabello.** 1984. A plasmid-encoded outer membrane protein, TraT, enhances resistance of *Escherichia coli* to phagocytosis. *Infect. Immun.* **46:**740–746.
15. **Aksoy, S., C. L. Squires, and C. Squires.** 1984. Translational coupling of the *trpB* and *trpA* genes in the *Escherichia coli* tryptophan operon. *J. Bacteriol.* **157:**363–367.
16. **Albin, R., and P. M. Silverman.** 1984. Identification of the *Escherichia coli* K-12 *cpxA* locus as a single gene: construction and analysis of biologically-active *cpxA* gene fusions. *Mol. Gen. Genet.* **197:**272–279.
17. **Albin, R., and P. M. Silverman.** 1984. Physical and genetic structure of the *glpK-cpxA* interval of the *Escherichia coli* K-12 chromosome. *Mol. Gen. Genet.* **197:**261–271.
18. **Albin, R., R. Weber, and P. M. Silverman.** 1986. The Cpx proteins of *Escherichia coli* K12. Immunologic detection of the chromosomal *cpxA* gene product. *J. Biol. Chem.* **261:**4698–4705.
19. **Allaoui, A., R. Ménard, P. J. Sansonetti, and C. Parsot.** 1993. Characterization of the *Shigella flexneri ipgD* and *ipgF* genes, which are located in the proximal part of the *mxi* locus. *Infect. Immun.* **61:**1707–1714.
20. **Anthony, K. G., C. Sherburne, R. Sherburne, and L. S. Frost.** 1994. The role of the pilus in recipient cell recognition during bacterial conjugation mediated by F-like plasmids. *Mol. Microbiol.* **13:**939–953.
21. **Armstrong, G. D., L. S. Frost, P. A. Sastry, and W. Paranchych.** 1980. Comparative biochemical studies on F and EDP208 conjugative pili. *J. Bacteriol.* **141:**333–341.
22. **Armstrong, G. D., L. S. Frost, H. J. Vogel, and W. Paranchych.** 1981. Nature of the carbohydrate and phosphate associated with ColB2 and EDP208 pilin. *J. Bacteriol.* **145:**1167–1176.
23. **Bagdasarian, M., A. Bailone, J. F. Angulo, P. Scholz, M. Bagdasarian, and R. Devoret.** 1992. PsiB, an anti-SOS protein, is transiently expressed by the F sex factor during its transmission to an *Escherichia coli* K-12 recipient. *Mol. Microbiol.* **6:**885–893.
24. **Bailey, M. J., V. Koronakis, T. Schmoll, and C. Hughes.** 1992. *Escherichia coli* HlyT protein, a transcriptional activator of haemolysin synthesis and secretion, is encoded by the *rfaH* (*sfrB*) locus required for expression of sex factor and lipopolysaccharide genes. *Mol. Microbiol.* **6:**1003–1012.
25. **Bailone, A., A. Bäckman, S. Sommer, J. Célérier, M. M. Bagdasarian, M. Bagdasarian, and R. Devoret.** 1988. PsiB polypeptide prevents activation of RecA protein in *Escherichia coli. Mol. Gen. Genet.* **214:**389–395.
26. **Balzer, D., W. Pansegrau, and E. Lanka.** 1994. Essential motifs of relaxase (TraI) and TraG proteins involved in conjugative transfer of plasmid RP4. *J. Bacteriol.* **176:**4285–4295.

26a. **Bayer, M., R. Eferl, G. Zellnig, K. Teferle, A. Dijkstra, G. Koraimann, and G. Högenauer.** 1995. Gene *19* of plasmid R1 is required for both efficient conjugative DNA transfer and bacteriophage R17 infection. *J. Bacteriol.* **177:**4279–4288.

27. **Bayer, M. E.** 1976. Role of adhesion zones in bacterial cell-surface function and bio-genesis, p. 393–427. *In* A. Tzagoloff (ed.), *Membrane Biogenesis.* Plenum Publishing Corp., New York.
28. **Bayer, M. E.** 1991. Zones of membrane adhesion in the cryofixed envelope of *Escherichia coli. J. Struct. Biol.* **107:**268–280.
29. **Benz, I., and H. Müller.** 1990. *Escherichia coli* DNA helicase I. Characterization of the protein and of its DNA-binding properties. *Eur. J. Biochem.* **189:**267–276.

30. **Bergquist, P. L.** 1987. Incompatibility, p. 37–78. *In* K. G. Hardy (ed.), *Plasmids: a Practical Approach.* IRL Press, Oxford.
31. **Bergquist, P. L., S. Saadi, and W. K. Maas.** 1986. Distribution of basic replicons having homology with RepFIA, RepFIB, and RepFIC among IncF group plasmids. *Plasmid* **15:**19–34.
32. **Beutin, L., and M. Achtman.** 1979. Two *Escherichia coli* chromosomal cistrons, *sfrA* and *sfrB*, which are needed for expression of F factor *tra* functions. *J. Bacteriol.* **139:**730–737.
33. **Beutin, L., P. A. Manning, M. Achtman, and N. Willetts.** 1981. *sfrA* and *sfrB* products of *Escherichia coli* K-12 are transcriptional control factors. *J. Bacteriol.* **145:**840–844.
34. **Bitter-Suermann, D., H. Peters, M. Jurs, R. Nehrbass, M. Montenegro, and K. N. Timmis.** 1984. Monoclonal antibody detection of IncF group plasmid-encoded TraT protein in clinical isolates of *Escherichia coli*. *Infect. Immun.* **46:**308–313.
35. **Bowie, J. U., and R. T. Sauer.** 1990. TraY proteins of F and related episomes are members of the Arc and Mnt repressor family. *J. Mol. Biol.* **211:**5–6.
36. **Bradley, D. E.** 1980. Determination of pili by conjugative bacterial drug resistance plasmids of incompatibility groups B, C, H, J, K, M, V, and X. *J. Bacteriol.* **141:**828–837.
37. **Bradley, D. E.** 1980. Morphological and serological relationships of conjugative pili. *Plasmid* **4:**155–169.
38. **Bradley, D. E.** 1983. Specification of the conjugative pili and surface mating systems of Pseudomonas plasmids. *J. Gen. Microbiol.* **129:**2545–2556.
39. **Bradley, D. E., D. E. Taylor, and D. R. Cohen.** 1980. Specification of surface mating systems among conjugative drug resistance plasmids in *Escherichia coli* K-12. *J. Bacteriol.* **143:**1466–1470.
40. **Bradshaw, H. D., Jr., B. A. Traxler, E. G. Minkley, Jr., E. W. Nester, and M. P. Gordon.** 1990. Nucleotide sequence of the *tra*I (helicase I) gene from the sex factor F. *J. Bacteriol.* **172:**4127–4131.
41. **Breg, J. N., J. H. van Opheusden, M. J. Burgering, R. Boelens, and R. Kaptein.** 1990. Structure of Arc repressor in solution: evidence for a family of β-sheet DNA-binding proteins. *Nature* (London) **346:**586–589.
42. **Brinton, C. C., Jr.** 1971. The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. *Crit. Rev. Microbiol.* **1:**105–160.
43. **Broda, P.** 1975. Transience of the donor state in an *Escherichia coli* K-12 strain carrying a repressed R factor. *Mol. Gen. Genet.* **138:**65–69.
44. **Buxton, R. S., and L. S. Drury.** 1983. Cloning and insertional inactivation of the dye (*sfrA*) gene, mutation of which affects sex factor F expression and dye sensitivity of *Escherichia coli* K-12. *J. Bacteriol.* **154:**1309–1314.
45. **Buxton, R. S., and L. S. Drury.** 1984. Identification of the dye gene product, mutational loss of which alters envelope protein composition and also affects sex factor F expression in *Escherichia coli* K-12. *Mol. Gen. Genet.* **194:**241–247.
46. **Cabezón, E., E. Lanka, and F. de la Cruz.** 1994. Requirements for mobilization of plasmids RSF1010 and ColE1 by the IncW plasmid R388: *trwB* and RP4 *traG* are interchangeable. *J. Bacteriol.* **176:**4455–4458.
47. **Caro, L. G., and M. Schnös.** 1966. The attachment of the male-specific bacteriophage f1 to sensitive strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **56:**126–132.
48. **Carter, J. R., and R. D. Porter.** 1991. *traY* and *traI* are required for *oriT*-dependent enhanced recombination between *lac*-containing plasmids and λp*lac5*. *J. Bacteriol.* **173:**1027–1034.
49. **Cavalli, L. L., E. Lederberg, and J. M. Lederberg.** 1953. An effective factor controlling sex compatibility in *Bacterium coli*. *J. Gen. Microbiol.* **8:**89–103.
50. **Chase, J. W., B. M. Merrill, and K. R. Williams.** 1983. F sex factor encodes a single-stranded DNA binding protein (SSB) with extensive sequence homology to *Escherichia coli* SSB. *Proc. Natl.*

Acad. Sci. USA **80:**5480–5484.
51. **Cheah, K.-C., A. Ray, and R. Skurray.** 1986. Expression of F plasmid *traT:* independence of *traY→Z* promoter and *traJ* control. *Plasmid* **16:**101–107.
52. **Cheah, K.-C., and R. Skurray.** 1986. The F plasmid carries an IS*3* insertion within *finO. J. Gen. Microbiol.* **132:**3269–3275.
53. **China, B., T. Michiels, and G. R. Cornelis.** 1990. The *pYV* plasmid of Yersinia encodes a lipoprotein, YlpA, related to TraT. *Mol. Microbiol.* **4:**1585–1593.
54. **Clark, A. J.** 1985. Conjugation and its aftereffects in *E. coli,* p. 47–68. *In* H. O. Halvorson and A. Monroy (ed.), *The Origins and Evolutions of Sex.* Alan R. Liss, Inc., New York.
55. **Clewell, D. B. (ed.).** 1993. *Bacterial Conjugation.* Plenum Publishing Corp., New York.
56. **Cohen, A., W. D. Fisher, R. Curtiss III, and H. I. Adler.** 1968. DNA isolated from *Escherichia coli* minicells mated with F^+ cells. *Proc. Natl. Acad. Sci. USA* **61:**61–68.
57. **Collins, J., and R. H. Pritchard.** 1973. Relationship between chromosome replication and F′*lac* episome replication in *Escherichia coli. J. Mol. Biol.* **78:**143–155.
58. **Covacci, A., and R. Rappuoli.** 1993. Pertussis toxin export requires accessory genes located downstream from the pertussis toxin operon. *Mol. Microbiol.* **8:**429–434.
59. **Cram, D. S., S. M. Loh, K.-C. Cheah, and R. A. Skurray.** 1991. Sequence and conservation of genes at the distal end of the transfer region on plasmids F and R6–5. *Gene* **104:**85–90.
60. **Crawford, E. M., and R. F. Gesteland.** 1964. The adsorption of bacteriophage R-17. *Virology* **22:**165–167.
61. **Cullum, J., J. F. Collins, and P. Broda.** 1978. The spread of plasmids in model populations of *Escherichia coli* K12. *Plasmid* **1:**545–556.
62. **Cuozzo, M., and P. M. Silverman.** 1986. Characterization of the F plasmid TraJ protein synthesized in F′ and Hfr strains of *Escherichia coli* K-12. *J. Biol. Chem.* **261:**5175–5179.
63. **Curtiss, R., III.** 1969. Bacterial conjugation. *Annu. Rev. Microbiol.* **23:**69–136.
64. **Dash, P. K., B. A. Traxler, M. M. Panicker, D. D. Hackney, and E. G. Minkley, Jr.** 1992. Biochemical characterization of *Escherichia coli* DNA helicase I. *Mol. Microbiol.* **6:**1163–1172.
65. **Date, T., M. Inuzuka, and M. Tomoeda.** 1977. Purification and characterization of F pili from *Escherichia coli. Biochemistry* **16:**5579–5585.
66. **Datta, N.** 1975. Epidemiology and classification of plasmids, p. 9–15. *In* D. Schlessinger (ed.), *Microbiology—1974.* American Society for Microbiology, Washington, D.C.
67. **Dempsey, W. B.** 1987. Integration host factor and conjugative transfer of the antibiotic resistance plasmid R100. *J. Bacteriol.* **169:**4391–4392.
68. **Dempsey, W. B.** 1987. Transcript analysis of the plasmid R100 *traJ* and *finP* genes. *Mol. Gen. Genet.* **209:**533–544.
69. **Dempsey, W. B.** 1989. Sense and antisense transcripts of *traM,* a conjugal transfer gene of the antibiotic resistance plasmid R100. *Mol. Microbiol.* **3:**561–570.
70. **Dempsey, W. B.** 1993. Key regulatory aspects of transfer of F-related plasmids, p. 53–74. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
71. **Dempsey, W. B., and B. E. Fee.** 1990. Integration host factor affects expression of two genes at the conjugal transfer origin of plasmid R100. *Mol. Microbiol.* **4:**1019–1028.
72. **Di Laurenzio, L., L. S. Frost, B. B. Finlay, and W. Paranchych.** 1991. Characterization of the *oriT* region of the IncFV plasmid pED208. *Mol. Microbiol.* **5:**1779–1790.
73. **Di Laurenzio, L., L. S. Frost, and W. Paranchych.** 1992. The TraM protein of the conjugative plasmid F binds to the origin of transfer of the F and ColE1 plasmids. *Mol. Microbiol.* **6:**2951–2959.
74. **Dodd, I. B., and J. B. Egan.** 1990. Improved detection of helix-turn-helix DNA-binding motifs in protein sequences. *Nucleic Acids Res.* **18:**5019–5026.
75. **Dong, J., S. Iuchi, H. S. Kwan, Z. Lu, and E. C. C. Lin.** 1993. The deduced amino-acid sequence of the cloned *cpxR* gene suggests the protein is the cognate regulator for the membrane sensor, CpxA,

in a two-component signal transduction system of *Escherichia coli*. *Gene* **136:**227–230.
76. **Doran, T. J., S. M. Loh, N. Firth, and R. A. Skurray.** 1994. Molecular analysis of the F plasmid *traVR* region: *traV* encodes a lipoprotein. *J. Bacteriol.* **176:**4182–4186.
77. **Drury, L. S., and R. S. Buxton.** 1985. DNA sequence analysis of the *dye* gene of *Escherichia coli* reveals amino acid homology between the Dye and OmpR proteins. *J. Biol. Chem.* **260:**4236–4242.
78. **Dürrenberger, M. B., W. Villiger, and T. Bächi.** 1991. Conjugational junctions: morphology of specific contacts in conjugating *Escherichia coli* bacteria. *J. Struct. Biol.* **107:**146–156.
79. **Dutreix, M., A. Bäckman, J. Célérier, M. M. Bagdasarian, S. Sommer, A. Bailone, R. Devoret, and M. Bagdasarian.** 1988. Identification of *psiB* genes of plasmids F and R6–5. Molecular basis for *psiB* enhanced expression in plasmid R6–5. *Nucleic Acids Res.* **16:**10669–10679.
80. **Erickson, M. J., and R. J. Meyer.** 1993. The origin of greater-than-unit-length plasmids generated during bacterial conjugation. *Mol. Microbiol.* **7:**289–298.
81. **Everett, R., and N. Willetts.** 1980. Characterisation of an *in vivo* system for nicking at the origin of conjugal DNA transfer of the sex factor F. *J. Mol. Biol.* **136:**129–150.
82. **Farrand, S. K.** 1993. Conjugal transfer of Agrobacterium plasmids, p. 255–292. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
83. **Fee, B. E., and W. B. Dempsey.** 1986. Cloning, mapping, and sequencing of plasmid R100 *traM* and *finP* genes. *J. Bacteriol.* **167:**336–345.
84. **Finlay, B. B., L. S. Frost, and W. Paranchych.** 1986. Nucleotide sequence of the *traYALE* region from IncFV plasmid pED208. *J. Bacteriol.* **168:**990–998.
85. **Finlay, B. B., L. S. Frost, and W. Paranchych.** 1986. Nucleotide sequences of the R1–19 plasmid transfer genes *traM, finP, traJ,* and *traY* and the *traYZ* promoter. *J. Bacteriol.* **166:**368–374.
86. **Finlay, B. B., L. S. Frost, and W. Paranchych.** 1986. Origin of transfer of IncF plasmids and nucleotide sequences of the type II *oriT, traM,* and *traY* alleles from ColB4-K98 and the type IV *traY* allele from R100-1. *J. Bacteriol.* **168:**132–139.
87. **Finlay, B. B., L. S. Frost, W. Paranchych, J. M. R. Parker, and R. S. Hodges.** 1985. Major antigenic determinants of F and ColB2 pili. *J. Bacteriol.* **163:**331–335.
88. **Finlay, B. B., L. S. Frost, W. Paranchych, and N. S. Willetts.** 1986. Nucleotide sequences of five IncF plasmid *finP* alleles. *J. Bacteriol.* **167:**754–757.
89. **Finlay, B. B., and W. Paranchych.** 1986. Nucleotide sequence of the surface exclusion genes *traS* and *traT* from the $IncF_{0lac}$ plasmid pED208. *J. Bacteriol.* **166:**713–721.
90. **Finnegan, D., and N. Willetts.** 1972. The nature of the transfer inhibitor of several F-like plasmids. *Mol. Gen. Genet.* **119:**57–66.
91. **Finnegan, D., and N. Willetts.** 1973. The site of action of the F transfer inhibitor. *Mol. Gen. Genet.* **127:**307–316.
92. **Finnegan, D. J., and N. S. Willetts.** 1971. Two classes of F*lac* mutants insensitive to transfer inhibition by an F-like R factor. *Mol. Gen. Genet.* **111:**256–264.
93. **Firth, N., K. P. Ridgway, M. E. Byrne, P. D. Fink, L. Johnson, I. T. Paulsen, and R. A. Skurray.** 1993. Analysis of a transfer region from the staphylococcal conjugative plasmid pSK41. *Gene* **136:**13–25.
94. **Firth, N., and R. Skurray.** 1992. Characterization of the F plasmid bifunctional conjugation gene, *traG*. *Mol. Gen. Genet.* **232:**145–153.
94a. **Firth, N., and R. Skurray.** 1995. A protein family associated with filament biogenesis in bacteria. *Mol. Microbiol.* **17:**1218–1219.
95. **Fowler, T., L. Taylor, and R. Thompson.** 1983. The control region of the F plasmid transfer operon: DNA sequence of the *traJ* and *traY* genes and characterisation of the *traY-Z* promoter. *Gene* **26:**79–89.
96. **Fowler, T., and R. Thompson.** 1986. Shadow promoters in the F plasmid transfer operon. *Mol. Gen. Genet.* **202:**509–511.

97. **Frame, R., and J. O. Bishop.** 1971. The number of sex-factors per chromosome in *Escherichia coli*. *Biochem. J.* **121:**93–103.
98. **Freundlich, M., N. Ramani, E. Mathew, A. Sirko, and P. Tsui.** 1992. The role of integration host factor in gene expression in *Escherichia coli*. *Mol. Microbiol.* **6:**2557–2563.
99. **Frost, L., S. Lee, N. Yanchar, and W. Paranchych.** 1989. *finP* and *fisO* mutations in FinP antisense RNA suggest a model for FinOP action in the repression of bacterial conjugation by the F*lac* plasmid JCFL0. *Mol. Gen. Genet.* **218:**152–160.
100. **Frost, L. S.** 1993. Conjugative pili and pilus-specific phages, p. 189–223. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
101. **Frost, L. S., and D. P. Bazett-Jones.** 1991. Examination of the phosphate in conjugative F-like pili by use of electron spectroscopic imaging. *J. Bacteriol.* **173:**7728–7731.
102. **Frost, L. S., B. B. Finlay, A. Opgenorth, W. Paranchych, and J. S. Lee.** 1985. Characterization and sequence analysis of pilin from F-like plasmids. *J. Bacteriol.* **164:**1238–1247.
103. **Frost, L. S., K. Ippen-Ihler, and R. A. Skurray.** 1994. Analysis of the sequence and gene products of the transfer region of the F sex factor. *Microbiol. Rev.* **58:**162–210.
104. **Frost, L. S., J. S. Lee, D. G. Scraba, and W. Paranchych.** 1986. Two monoclonal antibodies specific for different epitopes within the amino-terminal region of F pilin. *J. Bacteriol.* **168:**192–198.
105. **Frost, L. S., and W. Paranchych.** 1988. DNA sequence analysis of point mutations in *traA*, the F pilin gene, reveals two domains involved in F-specific bacteriophage attachment. *Mol. Gen. Genet.* **213:**134–139.
106. **Frost, L. S., W. Paranchych, and N. S. Willetts.** 1984. DNA sequence of the F *traALE* region that includes the gene for F pilin. *J. Bacteriol.* **160:**395–401.
107. **Fu, Y.-H. F., M.-M. Tsai, Y. N. Luo, and R. C. Deonier.** 1991. Deletion analysis of the F plasmid *oriT* locus. *J. Bacteriol.* **173:**1012–1020.
108. **Furuya, N., T. Nisioka, and T. Komano.** 1991. Nucleotide sequence and functions of the *oriT* operon in IncI1 plasmid R64. *J. Bacteriol.* **173:**2231–2237.
109. **Gaffney, D., R. Skurray, and N. Willetts.** 1983. Regulation of the F conjugation genes studied by hybridization and *tra-lacZ* fusion. *J. Mol. Biol.* **168:**103–122.
110. **Gamas, P., L. Caro, D. Galas, and M. Chandler.** 1987. Expression of F transfer functions depends on the *Escherichia coli* integration host factor. *Mol. Gen. Genet.* **207:**302–305.
111. **Gao, Q., Y. Luo, and R. C. Deonier.** 1994. Initiation and termination of DNA transfer at F plasmid *oriT*. *Mol. Microbiol.* **11:**449–458.
112. **Gasson, M., and N. Willetts.** 1976. Transfer gene expression during fertility inhibition of the *Escherichia coli* K12 sex factor F by the I-like plasmid R62. *Mol. Gen. Genet.* **149:**329–333.
113. **Gasson, M. J., and N. S. Willetts.** 1975. Five control systems preventing transfer of *Escherichia coli* K-12 sex factor F. *J. Bacteriol.* **122:**518–525.
114. **Gasson, M. J., and N. S. Willetts.** 1977. Further characterization of the F fertility inhibition systems of "unusual" Fin^+ plasmids. *J. Bacteriol.* **131:**413–420.
115. **Gaudin, H. M., and P. M. Silverman.** 1993. Contributions of promoter context and structure to regulated expression of the F plasmid *traY* promoter in *Escherichia coli* K-12. *Mol. Microbiol.* **8:**335–342.
116. **Gerdes, K., A. Nielsen, P. Thorsted, and E. G. H. Wagner.** 1992. Mechanism of killer gene activation. Antisense RNA-dependent RNase III cleavage ensures rapid turn-over of the stable *hok*, *srnB* and *pndA* effector messenger RNAs. *J. Mol. Biol.* **226:**637–649.
117. **Göldner, A., H. Graus, and G. Högenauer.** 1987. The origin of transfer of P307. *Plasmid* **18:**76–83.
118. **Golub, E., A. Bailone, and R. Devoret.** 1988. A gene encoding an SOS inhibitor is present in different conjugative plasmids. *J. Bacteriol.* **170:**4392–4394.
119. **Golub, E. I., and K. B. Low.** 1985. Conjugative plasmids of enteric bacteria from many different

incompatibility groups have similar genes for single-stranded DNA-binding proteins. *J. Bacteriol.* **162:**235–241.

120. **Golub, E. I., and K. B. Low.** 1986. Derepression of single-stranded DNA-binding protein genes on plasmids derepressed for conjugation, and complementation of an *E. coli ssb*$^-$ mutation by these genes. *Mol. Gen. Genet.* **204:**410–416.
121. **Golub, E. I., and K. B. Low.** 1986. Unrelated conjugative plasmids have sequences which are homologous to the leading region of the F factor. *J. Bacteriol.* **166:**670–672.
122. **Graus, H., A. Hödl, P. Wallner, and G. Högenauer.** 1990. The sequence of the leading region of the resistance plasmid R1. *Nucleic Acids Res.* **18:**1046.
123. **Graus-Göldner, A., H. Graus, T. Schlacher, and G. Högenauer.** 1990. The sequences of genes bordering *oriT* in the enterotoxin plasmid P307: comparison with the sequences of plasmids F and R1. *Plasmid* **24:**119–131.
124. **Gross, J. D., and L. G. Caro.** 1966. DNA transfer in bacterial conjugation. *J. Mol. Biol.* **16:**269–284.
125. **Gross, R., B. Arico, and R. Rappuoli.** 1989. Families of bacterial signal-transducing proteins. *Mol. Microbiol.* **3:**1661–1667.
126. **Grossman, T. H., L. S. Frost, and P. M. Silverman.** 1990. Structure and function of conjugative pili: monoclonal antibodies as probes for structural variants of F pili. *J. Bacteriol.* **172:**1174–1179.
127. **Grossman, T. H., and P. M. Silverman.** 1989. Structure and function of conjugative pili: inducible synthesis of functional F pili by *Escherichia coli* K-12 containing a *lac-tra* operon fusion. *J. Bacteriol.* **171:**650–656.
128. **Guiney, D. G.** 1993. Broad host range conjugative and mobilizable plasmids in Gram-negative bacteria, p. 75–104. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
129. **Guyer, M. S.** 1978. The γδ sequence of F is an insertion sequence. *J. Mol. Biol.* **126:**347–365.
130. **Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low.** 1980. Identification of a sex-factor affinity site in *E. coli* as γδ. *Cold Spring Harbor Symp. Quant. Biol.* **45:**135–140.
131. **Ham, L. M., D. Cram, and R. Skurray.** 1989. Transcriptional analysis of the F plasmid surface exclusion region: mapping of *traS, traT,* and *traD* transcripts. *Plasmid* **21:**1–8.
132. **Ham, L. M., N. Firth, and R. Skurray.** 1989. Nucleotide sequence of the F plasmid transfer gene, *traH:* identification of a new gene and a promoter within the transfer operon. *Gene* **75:**157–165.
133. **Ham, L. M., and R. Skurray.** 1989. Molecular analysis and nucleotide sequence of *finQ,* a transcriptional inhibitor of the F plasmid transfer genes. *Mol. Gen. Genet.* **216:**99–105.
134. **Harrington, L. C., and A. C. Rogerson.** 1990. The F pilus of *Escherichia coli* appears to support stable DNA transfer in the absence of wall-to-wall contact between cells. *J. Bacteriol.* **172:**7263–7264.
135. **Harrison, J. L., I. M. Taylor, K. Platt, and C. D. O'Connor.** 1992. Surface exclusion specificity of the TraT lipoprotein is determined by single alterations in a five-amino-acid region of the protein. *Mol. Microbiol.* **6:**2825–2832.
136. **Harwood, C. R., and E. Meynell.** 1975. Cyclic AMP and the production of sex pili by *E. coli* K-12 carrying derepressed sex factors. *Nature* (London) **254:**628–660.
137. **Havekes, L. M., and W. P. M. Hoekstra.** 1976. Characterization of an *Escherichia coli* K-12 F$^-$ Con$^-$ mutant. *J. Bacteriol.* **126:**593–600.
138. **Havekes, L. M., B. J. J. Lugtenberg, and W. P. M. Hoekstra.** 1976. Conjugation deficient *E. coli* K12 F$^-$ mutants with heptose-less lipopolysaccharide. *Mol. Gen. Genet.* **146:**43–50.
139. **Hayes, W.** 1953. Observations on a transmissible agent determining sexual differentiation in *Bacterium coli. J. Gen. Microbiol.* **8:**72–88.
140. **Hayes, W.** 1968. *The Genetics of Bacteria and Their Viruses.* Blackwell Scientific Publications, Oxford.

141. **Hedges, R. W., N. Datta, J. N. Coetzee, and S. Dennison.** 1973. R factors from *Proteus morganii. J. Gen. Microbiol.* **77:**249–259.
142. **Heinemann, J. A.** 1991. Genetics of gene transfer between species. *Trends Genet.* **7:**181–185.
143. **Heinemann, J. A., and G. F. Sprague, Jr.** 1989. Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature* (London) **340:**205–209.
144. **Helmuth, R., and M. Achtman.** 1978. Cell-cell interactions in conjugating *Escherichia coli:* purification of F pili with biological activity. *Proc. Natl. Acad. Sci. USA* **75:**1237–1241.
145. **Hobbs, M., B. P. Dalrymple, P. T. Cox, S. P. Livingstone, S. F. Delaney, and J. S. Mattick.** 1991. Organization of the fimbrial gene region of *Bacteroides nodosus:* class I and class II strains. *Mol. Microbiol.* **5:**543–560.
146. **Howland, C. J., C. E. D. Rees, P. T. Barth, and B. M. Wilkins.** 1989. The *ssb* gene of plasmid ColIb-P9. *J. Bacteriol.* **171:**2466–2473.
147. **Hu, S., E. Ohtsubo, and N. Davidson.** 1975. Electron microscopic heteroduplex studies of sequence relations among plasmids of *Escherichia coli:* structure of F13 and related F-primes. *J. Bacteriol.* **122:**749–763.
148. **Hu, S., E. Otsubo, N. Davidson, and H. Saedler.** 1975. Electron microscope heteroduplex studies of sequence relations among bacterial plasmids: identification and mapping of the insertion sequences IS*1* and IS*2* in F and R plasmids. *J. Bacteriol.* **122:**764–775.
149. **Hu, S., K. Ptashne, S. N. Cohen, and N. Davidson.** 1975. αβ sequence of F is IS*3*. *J. Bacteriol.* **123:**687–692.
150. **Hultgren, S. J., S. Normark, and S. N. Abraham.** 1991. Chaperone-assisted assembly and molecular architecture of adhesive pili. *Annu. Rev. Microbiol.* **45:**383–415.
151. **Igo, M. M., J. M. Slauch, and T. J. Silhavy.** 1990. Signal transduction in bacteria: kinases that control gene expression. *New Biol.* **2:**5–9.
152. **Ihler, G., and W. D. Rupp.** 1969. Strand-specific transfer of donor DNA during conjugation in *E. coli. Proc. Natl. Acad. Sci. USA* **63:**138–143.
153. **Ilyina, T. V., and E. V. Koonin.** 1992. Conserved sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaebacteria. *Nucleic Acids Res.* **20:**3279–3285.
154. **Inamoto, S., T. Abo, and E. Ohtsubo.** 1990. Binding sites of integration host factor in *oriT* of plasmid R100. *J. Gen. Appl. Microbiol.* **36:**287–293.
155. **Inamoto, S., H. Fukuda, T. Abo, and E. Ohtsubo.** 1994. Site- and strand-specific nicking at *oriT* of plasmid R100 in a purified system: enhancement of the nicking activity of TraI (helicase I) with TraY and IHF. *J. Biochem.* **116:**838–844.
156. **Inamoto, S., and E. Ohtsubo.** 1990. Specific binding of the TraY protein to *oriT* and the promoter region for the *traY* gene of plasmid R100. *J. Biol. Chem.* **265:**6461–6466.
157. **Inamoto, S., Y. Yoshioka, and E. Ohtsubo.** 1988. Identification and characterization of the products from the *traJ* and *traY* genes of plasmid R100. *J. Bacteriol.* **170:**2749–2757.
158. **Inamoto, S., Y. Yoshioka, and E. Ohtsubo.** 1991. Site- and strand-specific nicking *in vitro* at *oriT* by the *traY-traI* endonuclease of plasmid R100. *J. Biol. Chem.* **266:**10086–10092.
159. **Ippen, K. A., and R. C. Valentine.** 1967. The sex hair of *E. coli* as sensory fiber, conjugation tube, or mating arm? *Biochem. Biophys. Res. Commun.* **27:**674–680.
160. **Ippen-Ihler, K., M. Achtman, and N. Willetts.** 1972. Deletion map of the *Escherichia coli* K-12 sex factor F: the order of eleven transfer cistrons. *J. Bacteriol.* **110:**857–863.
161. **Ippen-Ihler, K., and S. Maneewannakul.** 1991. Conjugation among enteric bacteria: mating systems dependent on expression of pili, p. 35–69. *In* M. Dworkin (ed.), *Microbial Cell-Cell Interactions.* American Society for Microbiology, Washington, D.C.
162. **Ippen-Ihler, K., and E. G. Minkley, Jr.** 1986. The conjugation system of F, the fertility factor of *Escherichia coli. Annu. Rev. Genet.* **20:**593–624.

163. **Ippen-Ihler, K., D. Moore, S. Laine, D. A. Johnson, and N. S. Willetts.** 1984. Synthesis of F-pilin polypeptide in the absence of F *traJ* product. *Plasmid* **11:**116–129.
164. **Ippen-Ihler, K. A., and R. A. Skurray.** 1993. Genetic organisation of transfer-related determinants on the sex factor F and related plasmids, p. 23–52. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
165. **Iuchi, S., D. Furlong, and E. C. C. Lin.** 1989. Differentiation of *arcA, arcB,* and *cpxA* mutant phenotypes of *Escherichia coli* by sex pilus formation and enzyme regulation. *J. Bacteriol.* **171:**2889–2893.
166. **Iuchi, S., and E. C. C. Lin.** 1988. *arcA* (*dye*), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. *Proc. Natl. Acad. Sci. USA* **85:**1888–1892.
167. **Iuchi, S., and E. C. C. Lin.** 1991. Adaptation of *Escherichia coli* to respiratory conditions: regulation of gene expression. *Cell* **66:**5–7.
168. **Iuchi, S., and E. C. C. Lin.** 1992. Mutational analysis of signal transduction by ArcB, a membrane sensor protein responsible for anaerobic repression of operons involved in the central aerobic pathways in *Escherichia coli. J. Bacteriol.* **174:**3972–3980.
169. **Iuchi, S., and E. C. C. Lin.** 1992. Purification and phosphorylation of the Arc regulatory components of *Escherichia coli. J. Bacteriol.* **174:**5617–5623.
170. **Iuchi, S., Z. Matsuda, T. Fujiwara, and E. C. C. Lin.** 1990. The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulon. *Mol. Microbiol.* **4:**715–727.
171. **Jacob, A. E., J. A. Shapiro, L. Yamamoto, D. L. Smith, S. N. Cohen, and D. Berg.** 1977. Plasmids studied in *Escherichia coli* and other enteric bacteria, p. 607–638. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), *DNA Insertion Elements, Plasmids and Episomes.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
172. **Jalajakumari, M. B., A. Guidolin, H. J. Buhk, P. A. Manning, L. M. Ham, A. L. M. Hodgson, K.-C. Cheah, and R. A. Skurray.** 1987. Surface exclusion genes *traS* and *traT* of the F sex factor of *Escherichia coli* K-12. Determination of the nucleotide sequence and promoter and terminator activities. *J. Mol. Biol.* **198:**1–11.
173. **Jalajakumari, M. B., and P. A. Manning.** 1989. Nucleotide sequence of the *traD* region in the *Escherichia coli* F sex factor. *Gene* **81:**195–202.
174. **Johnson, D. A., and N. S. Willetts.** 1980. Construction and characterization of multicopy plasmids containing the entire F transfer region. *Plasmid* **4:**292–304.
175. **Jones, A. L., P. T. Barth, and B. M. Wilkins.** 1992. Zygotic induction of plasmid *ssb* and *psiB* genes following conjugative transfer of IncI1 plasmid ColIb-P9. *Mol. Microbiol.* **6:**605–613.
176. **Jovanovic, O. S., E. K. Ayres, and D. H. Figurski.** 1992. The replication initiator operon of promiscuous plasmid RK2 encodes a gene that complements an *Escherichia coli* mutant defective in single-stranded DNA-binding protein. *J. Bacteriol.* **174:**4842–4846.
177. **Kado, C. I.** 1993. *Agrobacterium*-mediated transfer and stable incorporation of foreign genes in plants, p. 243–254. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
178. **Kado, C. I.** 1994. Promiscuous DNA transfer system of *Agrobacterium tumefaciens:* role of the *virB* operon in sex pilus assembly and synthesis. *Mol. Microbiol.* **12:**17–22.
179. **Kanukollu, U., S. Bieler, S. Hull, and R. Hull.** 1985. Contribution of the *traT* gene to serum resistance among clinical isolates of enterobacteriaceae. *J. Med. Microbiol.* **19:**61–67.
180. **Kathir, P., and K. Ippen-Ihler.** 1991. Construction and characterization of derivatives carrying insertion mutations in F plasmid transfer region genes, *trbA, artA, traQ,* and *trbB. Plasmid* **26:**40–54.
181. **Kellenberger, E.** 1990. The 'Bayer bridges' confronted with results from improved electron microscopy methods. *Mol. Microbiol.* **4:**697–705.
182. **Kim, K., and R. J. Meyer.** 1989. Unidirectional transfer of broad host-range plasmid R1162

during conjugative mobilization. Evidence for genetically distinct events at *oriT*. *J. Mol. Biol.* **208:**501–505.
183. **Kingsman, A., and N. Willetts.** 1978. The requirements for conjugal DNA synthesis in the donor strain during F*lac* transfer. *J. Mol. Biol.* **122:**287–300.
184. **Kline, B. C.** 1985. A review of mini-F plasmid maintenance. *Plasmid* **14:**1–16.
185. **Kline, B. C.** 1988. Aspects of plasmid F maintenance in *Escherichia coli*. *Can. J. Microbiol.* **34:**526–535.
186. **Klinkert, M. Q., A. Klein, and M. Abdel-Monem.** 1980. Studies on the functions of DNA helicase I and DNA helicase II of *Escherichia coli*. *J. Biol. Chem.* **255:**9746–9752.
187. **Kolodkin, A. L., M. A. Capage, E. I. Golub, and K. B. Low.** 1983. F sex factor of *Escherichia coli* K-12 codes for a single-stranded DNA binding protein. *Proc. Natl. Acad. Sci. USA* **80:**4422–4426.
188. **Koonin, E. V.** 1993. A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. *J. Mol. Biol.* **229:**1165–1174.
189. **Koonin, E. V., and T. V. Ilyina.** 1993. Computer-assisted dissection of rolling circle DNA replication. *BioSystems* **30:**241–268.
190. **Koraimann, G., and G. Högenauer.** 1989. A stable core region of the *tra* operon mRNA of plasmid R1–19. *Nucleic Acids Res.* **17:**1283–1298.
191. **Koraimann, G., C. Koraimann, V. Koronakis, S. Schlager, and G. Högenauer.** 1991. Repression and derepression of conjugation of plasmid R1 by wild-type and mutated *finP* antisense RNA. *Mol. Microbiol.* **5:**77–87.
192. **Koraimann, G., C. Schroller, H. Graus, D. Angerer, K. Teferle, and G. Högenauer.** 1993. Expression of gene *19* of the conjugative plasmid R1 is controlled by RNase III. *Mol. Microbiol.* **9:**717–727.
193. **Koronakis, V., and G. Högenauer.** 1986. The sequences of the *traJ* gene and the 5′ end of the *traY* gene of the resistance plasmid R1. *Mol. Gen. Genet.* **203:**137–142.
194. **Koronakis, V. E., E. Bauer, and G. Högenauer.** 1985. The *traM* gene of the resistance plasmid R1: comparison with the corresponding sequence of the *Escherichia coli* F factor. *Gene* **36:**79–86.
195. **Kuhn, B., M. Abdel-Monem, H. Krell, and H. Hoffmann-Berling.** 1979. Evidence for two mechanisms for DNA unwinding catalyzed by DNA helicases. *J. Biol. Chem.* **254:**11343–11350.
196. **Kumar, S., and S. Srivastava.** 1983. Cyclic AMP and its receptor protein are required for expression of transfer genes of conjugative plasmid F in *Escherichia coli*. *Mol. Gen. Genet.* **190:**27–34.
197. **Kuo, T.-T., M.-S. Tan, M.-T. Su, and M.-K. Yang.** 1991. Complete nucleotide sequence of filamentous phage Cf1c from *Xanthomonas campestris* pv. *citri*. *Nucleic Acids Res.* **19:**2498.
198. **Lahue, E. E., and S. W. Matson.** 1988. *Escherichia coli* DNA helicase I catalyzes a unidirectional and highly processive unwinding reaction. *J. Biol. Chem.* **263:**3208–3215.
199. **Lahue, E. E., and S. W. Matson.** 1990. Purified *Escherichia coli* F-factor TraY protein binds *oriT*. *J. Bacteriol.* **172:**1385–1391.
200. **Laine, S., D. Moore, P. Kathir, and K. Ippen-Ihler.** 1985. Genes and gene products involved in the synthesis of F-pili, p. 535–553. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), *Plasmids in Bacteria.* Plenum Publishing Corp., New York.
201. **Lane, H. E. D.** 1981. Replication and incompatibility of F and plasmids in the IncFI group. *Plasmid* **5:**100–126.
202. **Lanka, E., and B. M. Wilkins.** 1995. DNA processing reactions in bacterial conjugation. *Annu. Rev. Biochem.* **64:**141–169.
203. **Larsen, M. H., and D. H. Figurski.** 1994. Structure, expression, and regulation of the *kilC* operon of promiscuous IncPα plasmids. *J. Bacteriol.* **176:**5022–5032.
204. **Lawn, A. M., G. G. Meynell, E. Meynell, and N. Datta.** 1967. Sex pili and the classification of

sex factors in the *Enterobacteriaceae. Nature* (London) **216:**343–346.
205. **Lederberg, J., L. L. Cavalli, and E. M. Lederberg.** 1952. Sex compatibility in *Escherichia coli. Genetics* **37:**720–730.
206. **Lederberg, J., and E. Tatum.** 1946. Gene recombination in *E. coli. Nature* (London) **158:**558.
207. **Lee, S. H., L. S. Frost, and W. Paranchych.** 1992. FinOP repression of the F plasmid involves extension of the half-life of FinP antisense RNA by FinO. *Mol. Gen. Genet.* **235:**131–139.
208. **Lerner, T. J., and N. D. Zinder.** 1979. Chromosomal regulation of sexual expression in *Escherichia coli. J. Bacteriol.* **137:**1063–1065.
209. **Lerner, T. J., and N. D. Zinder.** 1982. Another gene affecting sexual expression of *Escherichia coli. J. Bacteriol.* **150:**156–160.
210. **Lessl, M., D. Balzer, W. Pansegrau, and E. Lanka.** 1992. Sequence similarities between the RP4 Tra2 and the Ti VirB region strongly support the conjugation model for T-DNA transfer. *J. Biol. Chem.* **267:**20471–20480.
211. **Lessl, M., and E. Lanka.** 1994. Common mechanisms in bacterial conjugation and Ti-mediated T-DNA transfer to plant cells. *Cell* **77:**321–324.
212. **Lessl, M., W. Pansegrau, and E. Lanka.** 1992. Relationship of DNA-transfer-systems: essential transfer factors of plasmids RP4, Ti and F share common sequences. *Nucleic Acids Res.* **20:**6099–6100.
213. **Llosa, M., S. Bolland, and F. de la Cruz.** 1994. Genetic organization of the conjugal DNA processing region of the IncW plasmid R388. *J. Mol. Biol.* **235:**448–464.
214. **Loh, S., D. Cram, and R. Skurray.** 1989. Nucleotide sequence of the leading region adjacent to the origin of transfer on plasmid F and its conservation among conjugative plasmids. *Mol. Gen. Genet.* **219:**177–186.
215. **Loh, S., R. Skurray, J. Célérier, M. Bagdasarian, A. Bailone, and R. Devoret.** 1990. Nucleotide sequence of the *psiA* (plasmid SOS inhibition) gene located on the leading region of plasmids F and R6–5. *Nucleic Acids Res.* **18:**4597.
216. **Loh, S. M., D. S. Cram, and R. A. Skurray.** 1988. Nucleotide sequence and transcriptional analysis of a third function (Flm) involved in F-plasmid maintenance. *Gene* **66:**259–268.
217. **Loh, S. M., A. Ray, D. S. Cram, L. E. O'Gorman, and R. A. Skurray.** 1986. Location of a second partitioning region (ParL) on the F plasmid. *FEMS Microbiol. Lett.* **37:**179–182.
218. **López, J., L. Salazar, I. Andrés, J. M. Ortíz, and J. C. Rodríguez.** 1991. Nucleotide sequence of the *oriT-traM-finP* region of the haemolytic plasmid pSU316: comparison to F. *Nucleic Acids Res.* **19:**3451.
219. **Luo, Y., Q. Gao, and R. C. Deonier.** 1994. Mutational and physical analysis of F plasmid *traY* protein binding to *oriT*. *Mol. Microbiol.* **11:**459–469.
220. **Maher, D., R. Sherburne, and D. E. Taylor.** 1993. H-pilus assembly kinetics determined by electron microscopy. *J. Bacteriol.* **175:**2175–2183.
221. **Maneewannakul, K., and K. Ippen-Ihler.** 1993. Construction and analysis of F plasmid *traR*, *trbJ*, and *trbH* mutants. *J. Bacteriol.* **175:**1528–1531.
222. **Maneewannakul, K., S. Maneewannakul, and K. Ippen-Ihler.** 1992. Sequence alterations affecting F plasmid transfer gene expression: a conjugation system dependent on transcription by the RNA polymerase of phage T7. *Mol. Microbiol.* **6:**2961–2973.
223. **Maneewannakul, K., S. Maneewannakul, and K. Ippen-Ihler.** 1993. Synthesis of F pilin. *J. Bacteriol.* **175:**1384–1391.
223a. **Maneewannakul, K., S. Maneewannakul, and K. Ippen-Ihler.** 1995. Characterization of *traX*, the F plasmid locus required for acetylation of F-pilin subunits. *J. Bacteriol.* **177:**2957–2964.
224. **Maneewannakul, S., P. Kathir, and K. Ippen-Ihler.** 1992. Characterization of the F plasmid mating aggregation gene *traN* and of a new F transfer region locus *trbE*. *J. Mol. Biol.* **225:**299–311.
225. **Maneewannakul, S., P. Kathir, D. Moore, L.-A. Le, J. H. Wu, and K. Ippen-Ihler.** 1987.

Location of F plasmid transfer operon genes *traC* and *traW* and identification of the *traW* product. *J. Bacteriol.* **169:**5119–5124.
226. **Maneewannakul, S., K. Maneewannakul, and K. Ippen-Ihler.** 1991. Characterization of *trbC,* a new F plasmid *tra* operon gene that is essential to conjugative transfer. *J. Bacteriol.* **173:**3872–3878.
227. **Maneewannakul, S., K. Maneewannakul, and K. Ippen-Ihler.** 1992. Characterization, localization, and sequence of F transfer region products: the pilus assembly gene product TraW and a new product, TrbI. *J. Bacteriol.* **174:**5567–5574.
228. **Manning, P. A., L. Beutin, and M. Achtman.** 1980. Outer membrane of *Escherichia coli:* properties of the F sex factor *traT* protein which is involved in surface exclusion. *J. Bacteriol.* **142:**285–294.
229. **Manning, P. A., B. Kusecek, G. Morelli, C. Fisseau, and M. Achtman.** 1982. Analysis of the promoter-distal region of the *tra* operon of the F sex factor of *Escherichia coli* K-12 encoded by *Eco*RI restriction fragments f17, f19, and f2. *J. Bacteriol.* **150:**76–88.
230. **Manning, P. A., G. Morelli, and M. Achtman.** 1981. *traG* protein of the F sex factor of *Escherichia coli* K-12 and its role in conjugation. *Proc. Natl. Acad. Sci. USA* **78:**7487–7491.
231. **Manning, P. A., and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: differentiation of proteins 3A and 3B on acrylamide gels and further characterization of *con* (*tolG*) mutants. *J. Bacteriol.* **127:**1070–1079.
232. **Manoil, C., and J. P. Rosenbusch.** 1982. Conjugation-deficient mutants of *Escherichia coli* distinguish classes of functions of the outer membrane OmpA protein. *Mol. Gen. Genet.* **187:**148–156.
233. **Marvin, D. A., and W. Folkhard.** 1986. Structure of F-pili: reassessment of the symmetry. *J. Mol. Biol.* **191:**299–300.
234. **Matson, S. W., and B. S. Morton.** 1991. *Escherichia coli* DNA helicase I catalyzes a site- and strand-specific nicking reaction at the F plasmid *oriT*. *J. Biol. Chem.* **266:**16232–16237.
235. **Matson, S. W., W. C. Nelson, and B. S. Morton.** 1993. Characterization of the reaction product of the *oriT* nicking reaction catalyzed by *Escherichia coli* DNA helicase I. *J. Bacteriol.* **175:**2599–2606.
236. **Mazodier, P., and J. Davies.** 1991. Gene transfer between distantly related bacteria. *Annu. Rev. Genet.* **25:**147–171.
237. **McEwen, J., and P. Silverman.** 1980. Chromosomal mutations of *Escherichia coli* that alter expression of conjugative plasmid functions. *Proc. Natl. Acad. Sci. USA* **77:**513–517.
238. **McEwen, J., and P. Silverman.** 1980. Genetic analysis of *Escherichia coli* K-12 chromosomal mutants defective in expression of F-plasmid functions: identification of genes *cpxA* and *cpxB*. *J. Bacteriol.* **144:**60–67.
239. **McIntire, S. A., and W. B. Dempsey.** 1987. Fertility inhibition gene of plasmid R100. *Nucleic Acids Res.* **15:**2029–2042.
240. **McIntire, S. A., and W. B. Dempsey.** 1987. *oriT* sequence of the antibiotic resistance plasmid R100. *J. Bacteriol.* **169:**3829–3832.
241. **Meynell, E.** 1978. Experiments with sex pili: an investigation of the characters and function of F-like and I-like sex pili based on their reactions with antibody and phage, p. 207–233. *In* D. E. Bradley, E. Raizen, P. Fives-Taylor, and J. Ou (ed.), *Pili.* International Conferences on Pili, Washington, D.C.
242. **Meynell, E., G. G. Meynell, and N. Datta.** 1968. Phylogenetic relationships of drug-resistance factors and other transmissible bacterial plasmids. *Bacteriol. Rev.* **32:**55–83.
243. **Miki, T., T. Horiuchi, and N. S. Willetts.** 1978. Identification and characterization of four new *tra* cistrons on the *E. coli* K12 sex factor F. *Plasmid* **1:**316–323.
244. **Miller, J. F., E. Lanka, and M. H. Malamy.** 1985. F factor inhibition of conjugal transfer of broad-host-range plasmid RP4: requirement for the protein product of *pif* operon regulatory gene *pifC*. *J. Bacteriol.* **163:**1067–1073.

245. **Minkley, E. G., Jr.** 1984. Purification and characterization of pro-TraTp, the signal sequence-containing precursor of a secreted protein encoded by the F sex factor. *J. Bacteriol.* **158:**464–473.
246. **Minkley, E. G., Jr., and K. Ippen-Ihler.** 1977. Identification of a membrane protein associated with expression of the surface exclusion region of the F transfer operon. *J. Bacteriol.* **129:**1613–1622.
247. **Minkley, E. G., Jr., S. Polen, C. C. Brinton, Jr., and K. Ippen-Ihler.** 1976. Identification of the structural gene for F-pilin. *J. Mol. Biol.* **108:**111–121.
248. **Minkley, E. G., Jr., and N. S. Willetts.** 1984. Overproduction, purification and characterization of the F *traT* protein. *Mol. Gen. Genet.* **196:**225–235.
249. **Molineux, I. J.** 1991. Host-parasite interactions: recent developments in the genetics of abortive phage infections. *New Biol.* **3:**230–236.
250. **Moll, A., P. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. *Infect. Immun.* **28:**359–367.
251. **Montenegro, M. A., S. D. Bitter, J. K. Timmis, M. E. Aguero, F. C. Cabello, S. C. Sanyal, and K. N. Timmis.** 1985. *traT* gene sequences, serum resistance and pathogenicity-related factors in clinical isolates of *Escherichia coli* and other gram-negative bacteria. *J. Gen. Microbiol.* **131:**1511–1521.
252. **Moore, D., C. M. Hamilton, K. Maneewannakul, Y. Mintz, L. S. Frost, and K. Ippen-Ihler.** 1993. The *Escherichia coli* K-12 F plasmid gene *traX* is required for acetylation of F pilin. *J. Bacteriol.* **175:**1375–1383.
253. **Moore, D., K. Maneewannakul, S. Maneewannakul, J. H. Wu, K. Ippen-Ihler, and D. E. Bradley.** 1990. Characterization of the F-plasmid conjugative transfer gene *traU*. *J. Bacteriol.* **172:**4263–4270.
254. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1981. The effect of *tra* mutations on the synthesis of the F-pilin membrane polypeptide. *Mol. Gen. Genet.* **184:**260–264.
255. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1981. Location of an F-pilin pool in the inner membrane. *J. Bacteriol.* **146:**251–259.
256. **Moore, D., B. A. Sowa, and K. Ippen-Ihler.** 1982. A new activity in the F *tra* operon which is required for F-pilin synthesis. *Mol. Gen. Genet.* **188:**459–464.
257. **Moore, D., J. H. Wu, P. Kathir, C. M. Hamilton, and K. Ippen-Ihler.** 1987. Analysis of transfer genes and gene products within the *traB-traC* region of the *Escherichia coli* fertility factor, F. *J. Bacteriol.* **169:**3994–4002.
258. **Morton, T. M., D. M. Eaton, J. L. Johnston, and G. L. Archer.** 1993. DNA sequence and units of transcription of the conjugative transfer gene complex (*trs*) of *Staphylococcus aureus* plasmid pGO1. *J. Bacteriol.* **175:**4436–4447.
259. **Motallebi-Veshareh, M., D. Balzer, E. Lanka, G. Jagur-Burdzy, and C. M. Thomas.** 1992. Conjugative transfer functions of broad-host-range plasmid RK2 are coregulated with vegetative replication. *Mol. Microbiol.* **6:**907–920.
260. **Mullineaux, P., and N. Willetts.** 1985. Promoters in the transfer region of plasmid F, p. 605–614. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), *Plasmids in Bacteria.* Plenum Publishing Corp., New York.
261. **Nelson, W. C., B. S. Morton, E. E. Lahue, and S. W. Matson.** 1993. Characterization of the *Escherichia coli* F factor *traY* gene product and its binding sites. *J. Bacteriol.* **175:**2221–2228.
262. **Nijkamp, H. J. J., R. de Lang, A. R. Stuitje, P. J. M. van den Elzen, E. Veltkamp, and A. J. van Putten.** 1986. The complete nucleotide sequence of the bacteriocinogenic plasmid CloDF13. *Plasmid* **16:**135–160.
263. **Nomura, N., H. Masai, M. Inuzuka, C. Miyazaki, E. Ohtsubo, T. Itoh, S. Sasamoto, M. Matsui, R. Ishizaki, and K.-I. Arai.** 1991. Identification of eleven single-strand initiation sequences (*ssi*) for priming of DNA replication in the F, R6K, R100 and ColE2 plasmids. Gene **108:**15–22.

264. **Novotny, C., W. S. Knight, and C. C. Brinton, Jr.** 1968. Inhibition of bacterial conjugation by ribonucleic acid and deoxyribonucleic acid male-specific bacteriophages. *J. Bacteriol.* **95:**314–326.
265. **Novotny, C. P., and P. Fives-Taylor.** 1974. Retraction of F pili. *J. Bacteriol.* **117:**1306–1311.
266. **O'Callaghan, R. J., L. Bundy, R. Bradley, and W. Paranchych.** 1973. Unusual arsenate poisoning of the F pili of *Escherichia coli*. *J. Bacteriol.* **115:**76–81.
267. **Ogata, R. T., and R. P. Levine.** 1980. Characterization of complement resistance in *Escherichia coli* conferred by the antibiotic resistance plasmid R100. *J. Immunol.* **125:**1494–1498.
268. **Ogata, R. T., C. Winters, and R. P. Levine.** 1982. Nucleotide sequence analysis of the complement resistance gene from plasmid R100. *J. Bacteriol.* **151:**819–827.
269. **Ohki, M., and J. Tomizawa.** 1968. Asymmetric transfer of DNA strands in bacterial conjugation. *Cold Spring Harbor Symp. Quant. Biol.* **33:**651–658.
270. **Ohtsubo, E.** 1970. Transfer-defective mutants of sex factors in *Escherichia coli*. II. Deletion mutants of an F-prime and deletion mapping of cistrons involved in genetic transfer. *Genetics* **64:**189–197.
271. **Ohtsubo, E., Y. Nishimura, and Y. Hirota.** 1970. Transfer-defective mutants of sex factors in *Escherichia coli*. I. Defective mutants and complementation analysis. *Genetics* **64:**173–188.
272. **Oliver, D.** 1985. Protein secretion in *Escherichia coli*. *Annu. Rev. Microbiol.* **39:**615–648.
273. **Ostermann, E., F. Kricek, and G. Högenauer.** 1984. Cloning the origin of transfer region of the resistance plasmid R1. *EMBO J.* **3:**1731–1735.
274. **Ou, J. T.** 1973. Inhibition of formation of *Escherichia coli* mating pairs by f1 and MS2 bacteriophages as determined with a Coulter counter. *J. Bacteriol.* **114:**1108–1115.
275. **Ou, J. T., and T. F. Anderson.** 1970. Role of pili in bacterial conjugation. *J. Bacteriol.* **102:**648–654.
276. **Ou, J. T., and T. F. Anderson.** 1972. Effect of Zn^{2+} on bacterial conjugation: inhibition of mating pair formation. *J. Bacteriol.* **111:**177–185.
277. **Ou, J. T., and R. L. Reim.** 1978. F^- mating materials able to generate a mating signal in mating with HfrH *dnaB*(Ts) cells. *J. Bacteriol.* **133:**442–445.
278. **Paiva, W. D., T. Grossman, and P. M. Silverman.** 1992. Characterization of F-pilin as an inner membrane component of *Escherichia coli* K12. *J. Biol. Chem.* **267:**26191–26197.
279. **Panicker, M. M., and E. G. Minkley, Jr.** 1985. DNA transfer occurs during a cell surface contact stage of F sex factor-mediated bacterial conjugation. *J. Bacteriol.* **162:**584–590.
280. **Panicker, M. M., and E. G. Minkley, Jr.** 1992. Purification and properties of the F sex factor TraD protein, an inner membrane conjugal transfer protein. *J. Biol. Chem.* **267:**12761–12766.
281. **Pansegrau, W., and E. Lanka.** 1991. Common sequence motifs in DNA relaxases and nick regions from a variety of DNA transfer systems. *Nucleic Acids Res.* **19:**3455.
282. **Pansegrau, W., E. Lanka, P. T. Barth, D. H. Figurski, D. G. Guiney, D. Haas, D. R. Helinski, H. Schwab, V. A. Stanisich, and C. M. Thomas.** 1994. Complete nucleotide sequence of Birmingham IncPα plasmids. Compilation and comparative analysis. *J. Mol. Biol.* **239:**623–663.
283. **Pansegrau, W., W. Schröder, and E. Lanka.** 1994. Concerted action of three distinct domains in the DNA cleaving-joining reaction catalyzed by relaxase (TraI) of conjugative plasmid RP4. *J. Biol. Chem.* **269:**2782–2789.
284. **Paranchych, W.** 1975. Attachment, ejection and penetration stages of the RNA phage infectious process, p. 85–111. *In* N. Zinder (ed.), *RNA Phages.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
285. **Paranchych, W., B. B. Finlay, and L. S. Frost.** 1986. Studies on the regulation of IncF plasmid transfer operon expression, p. 117–129. *In* S. B. Levy and R. P. Novick (ed.), *Antibiotic Resistance Genes: Ecology, Transfer, and Expression.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
286. **Paranchych, W., and L. S. Frost.** 1988. The physiology and biochemistry of pili. *Adv. Microb. Physiol.* **29:**53–114.

287. **Penfold, S. S., K. Usher, and L. S. Frost.** 1994. The nature of the *traK4* mutation in the F sex factor of *Escherichia coli. J. Bacteriol.* **176:**1924–1931.
288. **Perumal, N. B., and E. G. Minkley, Jr.** 1984. The product of the F sex factor *traT* surface exclusion gene is a lipoprotein. *J. Biol. Chem.* **259:**5357–5360.
289. **Pohlman, R. F., H. D. Genetti, and S. C. Winans.** 1994. Common ancestry between IncN conjugal transfer genes and macromolecular export systems of plant and animal pathogens. *Mol. Microbiol.* **14:**655–668.
290. **Pradel, E., and C. A. Schnaitman.** 1991. Effect of *rfaH* (*sfrB*) and temperature on expression of *rfa* genes of *Escherichia coli* K-12. *J. Bacteriol.* **173:**6428–6431.
291. **Pramoonjago, P., M. Kaneko, T. Kinoshita, E. Ohtsubo, J. Takeda, K. S. Hong, R. Inagi, and K. Inoue.** 1992. Role of TraT protein, an anticomplementary protein produced in *Escherichia coli* by R100 factor, in serum resistance. *J. Immunol.* **148:**827–836.
292. **Pugsley, A. P.** 1993. The complete general secretory pathway in gram-negative bacteria. *Microbiol. Rev.* **57:**50–108.
293. **Rainwater, S., and P. M. Silverman.** 1990. The Cpx proteins of *Escherichia coli* K-12: evidence that *cpxA, ecfB, ssd,* and *eup* mutations all identify the same gene. *J. Bacteriol.* **172:**2456–2461.
294. **Rashtchian, A., J. H. Crooks, and S. B. Levy.** 1983. *traJ* independence in expression of *traT* on F. *J. Bacteriol.* **154:**1009–1012.
295. **Ray, A., K.-C. Cheah, and R. Skurray.** 1986. An F-derived conjugative cosmid: analysis of *tra* polypeptides in cosmid-infected cells. *Plasmid* **16:**90–100.
296. **Ray, A., and R. Skurray.** 1983. Cloning and polypeptide analysis of the leading region in F plasmid DNA transfer. *Plasmid* **9:**262–272.
297. **Reygers, U., R. Wessel, H. Müller, and H. Hoffmann-Berling.** 1991. Endonuclease activity of *Escherichia coli* DNA helicase I directed against the transfer origin of the F factor. *EMBO J.* **10:**2689–2694.
298. **Riede, I., and M.-L. Eschbach.** 1986. Evidence that TraT interacts with OmpA of *Escherichia coli. FEBS Lett.* **205:**241–245.
299. **Rohrer, J., and D. E. Rawlings.** 1992. Sequence analysis and characterization of the mobilization region of a broad-host-range plasmid, pTF-FC2, isolated from *Thiobacillus ferrooxidans. J. Bacteriol.* **174:**6230–6237.
300. **Saadi, S., W. K. Maas, D. F. Hill, and P. L. Bergquist.** 1987. Nucleotide sequence analysis of RepFIC, a basic replicon present in IncFI plasmids P307 and F, and its relation to the RepA replicon of IncFII plasmids. *J. Bacteriol.* **169:**1836–1846.
301. **Sabelnikov, A. G.** 1994. Nucleic acid transfer through cell membranes—towards the underlying mechanisms. *Prog. Biophys. Mol. Biol.* **62:**119–152.
302. **Salazar, L., J. López, I. Andrés, J. M. Ortíz, and J. C. Rodríguez.** 1992. Characterization and nucleotide sequence of the *oriT-traM-finP* region of the IncFVII plasmid pSU233. *Mol. Gen. Genet.* **234:**442–448.
303. **Sambucetti, L., L. Eoyang, and P. M. Silverman.** 1982. Cellular control of conjugation in *Escherichia coli* K12. Effect of chromosomal *cpx* mutations on F-plasmid gene expression. *J. Mol. Biol.* **161:**13–31.
304. **Sarathy, P. V., and O. Siddiqi.** 1973. DNA synthesis during bacterial conjugation. II. Is DNA replication in the Hfr obligatory for chromosome transfer? *J. Mol. Biol.* **78:**443–451.
305. **Schandel, K. A., S. Maneewannakul, K. Ippen-Ihler, and R. E. Webster.** 1987. A *traC* mutant that retains sensitivity to f1 bacteriophage but lacks F pili. *J. Bacteriol.* **169:**3151–3159.
306. **Schandel, K. A., S. Maneewannakul, R. A. Vonder-Haar, K. Ippen-Ihler, and R. E. Webster.** 1990. Nucleotide sequence of the F plasmid gene, *traC,* and identification of its product. *Gene* **96:**137–140.
307. **Schandel, K. A., M. M. Muller, and R. E. Webster.** 1992. Localization of TraC, a protein

involved in assembly of the F conjugative pilus. *J. Bacteriol.* **174:**3800–3806.
308. **Schoulaker-Schwarz, R., and H. Engelberg-Kulka.** 1983. Effect of an *Escherichia coli traD* (ts) mutation on MS2 RNA replication. *J. Gen. Virol.* **64:**207–210.
309. **Schwab, M., H. Gruber, and G. Högenauer.** 1991. The TraM protein of plasmid R1 is a DNA-binding protein. *Mol. Microbiol.* **5:**439–446.
310. **Schwab, M., H. Reisenzein, and G. Högenauer.** 1993. TraM of plasmid R1 regulates its own expression. *Mol. Microbiol.* **7:**795–803.
311. **Sharp, P. A., S. N. Cohen, and N. Davidson.** 1973. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli.* II. Structure of drug resistance (R) factors and F factors. *J. Mol. Biol.* **75:**235–255.
312. **Shirasu, K., and C. I. Kado.** 1993. Membrane location of the Ti plasmid VirB proteins involved in the biosynthesis of a pilin-like conjugative structure on *Agrobacterium tumefaciens. FEMS Microbiol. Lett.* **111:**287–294.
313. **Shirasu, K., Z. Koukolikova-Nicola, B. Hohn, and C. I. Kado.** 1994. An inner-membrane-associated virulence protein essential for T-DNA transfer from *Agrobacterium tumefaciens* to plants exhibits ATPase activity and similarities to conjugative transfer genes. *Mol. Microbiol.* **11:**581–588.
314. **Silverman, P., K. Nat, J. McEwen, and R. Birchman.** 1980. Selection of *Escherichia coli* K-12 chromosomal mutants that prevent expression of F-plasmid functions. *J. Bacteriol.* **143:**1519–1523.
315. **Silverman, P. M.** 1987. The structural basis of prokaryotic DNA transfer, p. 277–310. *In* M. Inouye (ed.), *Bacterial Outer Membranes as Model Systems.* John Wiley & Sons, Inc., New York.
316. **Silverman, P. M., S. Rother, and H. Gaudin.** 1991. Arc and Sfr functions of the *Escherichia coli* K-12 *arcA* gene product are genetically and physiologically separable. *J. Bacteriol.* **173:**5648–5652.
317. **Silverman, P. M., L. Tran, R. Harris, and H. M. Gaudin.** 1993. Accumulation of the F plasmid TraJ protein in *cpx* mutants of *Escherichia coli. J. Bacteriol.* **175:**921–925.
318. **Silverman, P. M., E. Wickersham, and R. Harris.** 1991. Regulation of the F plasmid *traY* promoter in *Escherichia coli* by host and plasmid factors. *J. Mol. Biol.* **218:**119–128.
319. **Silverman, P. M., E. Wickersham, S. Rainwater, and R. Harris.** 1991. Regulation of the F plasmid *traY* promoter in *Escherichia coli* K12 as a function of sequence context. *J. Mol. Biol.* **220:**271–279.
320. **Skurray, R. A., R. E. Hancock, and P. Reeves.** 1974. Con mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of a bacteriophage. *J. Bacteriol.* **119:**726–735.
321. **Skurray, R. A., H. Nagaishi, and A. J. Clark.** 1976. Molecular cloning of DNA from F sex factor of *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. USA* **73:**64–68.
322. **Somers, W. S., and S. E. V. Phillips.** 1992. Crystal structure of the *met* repressor-operator complex at 2.8 Å resolution reveals DNA recognition by β-strands. *Nature* (London) **359:**387–393.
323. **Sowa, B. A., D. Moore, and K. Ippen-Ihler.** 1983. Physiology of F-pilin synthesis and utilization. *J. Bacteriol.* **153:**962–968.
324. **Stachel, S. E., and P. C. Zambryski.** 1986. *Agrobacterium tumefaciens* and the susceptible plant cell: a novel adaptation of extracellular recognition and DNA conjugation. *Cell* **47:**155–157.
325. **Sukupolvi, S., and C. D. O'Connor.** 1990. TraT lipoprotein, a plasmid-specified mediator of interactions between gram-negative bacteria and their environment. *Microbiol. Rev.* **54:**331–341.
326. **Tanimoto, K., and T. Iino.** 1983. Transfer inhibition of RP4 by F factor. *Mol. Gen. Genet.* **192:**104–109.
327. **Tanimoto, K., and T. Iino.** 1984. An essential gene for replication of the mini-F plasmid from origin I. *Mol. Gen. Genet.* **196:**59–63.
328. **Thompson, R., and L. Taylor.** 1982. Promoter mapping and DNA sequencing of the F plasmid transfer genes *traM* and *traJ. Mol. Gen. Genet.* **188:**513–518.
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.

330. **Thompson, T. L., M. B. Centola, and R. C. Deonier.** 1989. Location of the nick at *oriT* of the F plasmid. *J. Mol. Biol.* **207:**505–512.
331. **Thorstenson, Y. R., G. A. Kuldau, and P. C. Zambryski.** 1993. Subcellular localization of seven VirB proteins of *Agrobacterium tumefaciens:* implications for the formation of a T-DNA transport structure. *J. Bacteriol.* **175:**5233–5241.
332. **Timmis, K. N., I. Andrés, and M. Achtman.** 1978. Fertility repression of F-like conjugative plasmids: physical mapping of the R6–5 *finO* and *finP* cistrons and identification of the *finO* protein. *Proc. Natl. Acad. Sci. USA* **75:**5836–5840.
333. **Timmis, K. N., G. J. Boulnois, S. D. Bitter, and F. C. Cabello.** 1985. Surface components of *Escherichia coli* that mediate resistance to the bactericidal activities of serum and phagocytes. *Curr. Top. Microbiol. Immunol.* **118:**197–218.
334. **Traxler, B. A., and E. G. Minkley, Jr.** 1987. Revised genetic map of the distal end of the F transfer operon: implications for DNA helicase I, nicking at *oriT*, and conjugal DNA transport. *J. Bacteriol.* **169:**3251–3259.
335. **Traxler, B. A., and E. G. Minkley, Jr.** 1988. Evidence that DNA helicase I and *oriT* site-specific nicking are both functions of the F TraI protein. *J. Mol. Biol.* **204:**205–209.
336. **Tsai, M.-M., Y.-H. F. Fu, and R. C. Deonier.** 1990. Intrinsic bends and integration host factor binding at F plasmid *oriT*. *J. Bacteriol.* **172:**4603–4609.
337. **van Biesen, T., and L. S. Frost.** 1992. Differential levels of fertility inhibition among F-like plasmids are related to the cellular concentration of *finO* mRNA. *Mol. Microbiol.* **6:**771–780.
338. **van Biesen, T., and L. S. Frost.** 1994. The FinO protein of IncF plasmids binds FinP antisense RNA and its target, *traJ* mRNA, and promotes duplex formation. *Mol. Microbiol.* **14:**427–436.
339. **van Biesen, T., F. Soderbom, E. G. H. Wagner, and L. S. Frost.** 1993. Structural and functional analysis of the FinP antisense RNA regulatory system of the F conjugative plasmid. *Mol. Microbiol.* **6:**771–780.
340. **van de Pol, H., E. Veltkamp, and H. J. J. Nijkamp.** 1979. CloDF13 plasmid genes affecting F*lac* transfer and propagation of male specific RNA phages. *Mol. Gen. Genet.* **168:**309–317.
341. **van Putten, A. J., G. J. Jochems, R. de Lang, and H. J. J. Nijkamp.** 1987. Structure and nucleotide sequence of the region encoding the mobilization proteins of plasmid CloDF13. *Gene* **51:**171–178.
342. **von Heijne, G.** 1984. How signal sequences maintain cleavage specificity. *J. Mol. Biol.* **173:**243–251.
343. **Wanner, B. L.** 1992. Is cross regulation by phosphorylation of two-component response regulator proteins important in bacteria? *J. Bacteriol.* **174:**2053–2058.
344. **Waters, V. L., K. H. Hirata, W. Pansegrau, E. Lanka, and D. G. Guiney.** 1991. Sequence identity in the nick regions of IncP plasmid transfer origins and T-DNA borders of *Agrobacterium* Ti plasmids. *Proc. Natl. Acad. Sci. USA* **88:**1456–1460.
345. **Waters, V. L., B. Strack, W. Pansegrau, E. Lanka, and D. G. Guiney.** 1992. Mutational analysis of essential IncPα plasmid transfer genes *traF* and *traG* and involvement of *traF* in phage sensitivity. *J. Bacteriol.* **174:**6666–6673.
346. **Weber, R. F., and P. M. Silverman.** 1988. The Cpx proteins of *Escherichia coli* K12. Structure of the *cpxA* polypeptide as an inner membrane component. *J. Mol. Biol.* **203:**467–478.
347. **Weiss, A. A., F. D. Johnson, and D. L. Burns.** 1993. Molecular characterization of an operon required for pertussis toxin secretion. *Proc. Natl. Acad. Sci. USA* **90:**2970–2974.
348. **Wessel, R., H. Müller, and H. Hoffmann-Berling.** 1990. Electron microscopic analysis of DNA forks generated by *Escherichia coli* DNA helicase II. *Eur. J. Biochem.* **192:**695–701.
349. **Wilkins, B., and E. Lanka.** 1993. DNA processing and replication during plasmid transfer

between Gram-negative bacteria, p. 105–136. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.

350. **Wilkins, B. M., and S. E. Hollom.** 1974. Conjugational synthesis of F*lac*$^+$ and Col I DNA in the presence of rifampicin and in *Escherichia coli* K12 mutants defective in DNA synthesis. *Mol. Gen. Genet.* **134:**143–156.
351. **Willetts, N.** 1977. The transcriptional control of fertility in F-like plasmids. *J. Mol. Biol.* **112:**141–148.
352. **Willetts, N.** 1980. Interactions between the F conjugal transfer system and CloDF13::Tn*A* plasmids. *Mol. Gen. Genet.* **180:**213–217.
353. **Willetts, N.** 1993. Bacterial conjugation: a historical perspective, p. 1–22. *In* D. B. Clewell (ed.), *Bacterial Conjugation.* Plenum Publishing Corp., New York.
354. **Willetts, N., and M. Achtman.** 1972. Genetic analysis of transfer by the *Escherichia coli* sex factor F, using P1 transductional complementation. *J. Bacteriol.* **110:**843–851.
355. **Willetts, N., and J. Maule.** 1979. Investigations of the F conjugation gene *traI: traI* mutants and λ*traI* transducing phages. *Mol. Gen. Genet.* **169:**325–336.
356. **Willetts, N., and J. Maule.** 1980. Characterisation of a λ transducing phage carrying the F conjugation gene *traG. Mol. Gen. Genet.* **178:**675–680.
357. **Willetts, N., and J. Maule.** 1985. Specificities of IncF plasmid conjugation genes. Genet. Res. **47:**1–11.
358. **Willetts, N., and R. Skurray.** 1980. The conjugation system of F-like plasmids. *Annu. Rev. Genet.* **14:**41–76.
359. **Willetts, N., and R. Skurray.** 1987. Structure and function of the F factor and mechanism of conjugation, p. 1110–1133. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* American Society for Microbiology, Washington, D.C.
360. **Willetts, N., and B. Wilkins.** 1984. Processing of plasmid DNA during bacterial conjugation. *Microbiol. Rev.* **48:**24–41.
361. **Willetts, N. S.** 1972. Location of the origin of transfer of the sex factor F. *J. Bacteriol.* **112:**773–778.
362. **Willetts, N. S.** 1973. Characterization of the F transfer cistron, *traL. Genet. Res.* **21:**205–213.
363. **Willetts, N. S.** 1974. The kinetics of inhibition of F*lac* transfer by R100 in *E. coli. Mol. Gen. Genet.* **129:**123–130.
364. **Winans, S. C.** 1992. Two-way chemical signaling in *Agrobacterium*-plant interactions. *Microbiol. Rev.* **56:**12–31.
365. **Worobec, E. A., L. S. Frost, P. Pieroni, G. D. Armstrong, R. S. Hodges, J. M. R. Parker, B. B. Finlay, and W. Paranchych.** 1986. Location of the antigenic determinants of conjugative F-like pili. *J. Bacteriol.* **167:**660–665.
366. **Worobec, E. A., W. Paranchych, J. M. R. Parker, A. K. Taneja, and R. S. Hodges.** 1985. Antigen-antibody interaction. The immunodominant region of EDP208 pili. *J. Biol. Chem.* **260:**938–943.
367. **Wu, J. H., and K. Ippen-Ihler.** 1989. Nucleotide sequence of *traQ* and adjacent loci in the *Escherichia coli* K-12 F-plasmid transfer operon. *J. Bacteriol.* **171:**213–221.
368. **Wu, J. H., P. Kathir, and K. Ippen-Ihler.** 1988. The product of the F plasmid transfer operon gene, *traF,* is a periplasmic protein. *J. Bacteriol.* **170:**3633–3639.
369. **Wu, J. H., D. Moore, T. Lee, and K. Ippen-Ihler.** 1987. Analysis of *Escherichia coli* K-12 F factor transfer genes: *traQ, trbA,* and *trbB. Plasmid* **18:**54–69.
370. **Yoshioka, Y., Y. Fujita, and E. Ohtsubo.** 1990. Nucleotide sequence of the promoter-distal region of the *tra* operon of plasmid R100, including *traI* (DNA helicase I) and *traD* genes. *J. Mol. Biol.* **214:**39–53.
371. **Yoshioka, Y., H. Ohtsubo, and E. Ohtsubo.** 1987. Repressor gene *finO* in plasmids R100 and F:

constitutive transfer of plasmid F is caused by insertion of IS*3* into F *finO*. *J. Bacteriol.* **169:**619–623.

372. **Zambryski, P.** 1988. Basic processes underlying *Agrobacterium*-mediated DNA transfer to plant cells. *Annu. Rev. Genet.* **22:**1–30.

373. **Ziegelin, G., W. Pansegrau, B. Strack, D. Balzer, M. Kroger, V. Kruft, and E. Lanka.** 1991. Nucleotide sequence and organization of genes flanking the transfer origin of promiscuous plasmid RP4. *DNA Seq.* **1:**303–327.