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Nucleotide Sequences of the R1-19 Plasmid Transfer Genes *traM*, *finP*, *traJ*, and *traY* and the *traYZ* Promoter

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The complete nucleotide sequences of the R1 *drd-19* (R1-19) plasmid transfer genes *traM*, *finP*, *traJ*, and *traY* and the region encoding the *traYZ* promoter were determined. The *traM* protein from R1-19 was similar to the 127-amino-acid *traM* product from the conjugative plasmid F; only 28 residues were not identical. *finP*, a negative regulatory element of the *traJ* gene, contained a 12-base-pair inverted repeat identical to that found in the F plasmid, but differed in the 7 base pairs found between the repeats. The *traJ* gene and the *traYZ* promoter (the site of transcriptional stimulation by the *traJ* product) were completely different from the equivalent sequences in plasmid F. Galactokinase fusion studies of the *traYZ* promoter indicated that the R1-19 and F plasmids have analogous but not homologous *traYZ* promoter strengths and regulation. The *traY* protein from R1-19 was 44 residues shorter than the *traY* product from plasmid F, but there was some homology within the C-terminal halves of the *traY* gene products. The predicted translational start codon for the *traY* gene is GUG.

Resistance R factors are large, self-transmissible plasmids which encode antibiotic resistance genes. R1 is a member of the IncFII plasmid incompatibility group and contains a 35-kilobase (kb) transfer (*tra*) region which is highly homologous to that in plasmid F and other IncF plasmids (27). This *tra* region encodes the gene products necessary for pilus production, surface exclusion, and conjugal DNA metabolism and is controlled by several regulatory elements. Of the *tra* regions in the IncF plasmids, the F plasmid *tra* region has been studied most extensively (34; N. S. Willetts and J. Maule, Genet. Res., in press). R1 *drd-19* (R1-19) is a derepressed mutant of R1 (*finO*) and expresses its conjugation system constitutively (19).

Although the *tra* regions of the F and R1-19 plasmids are homologous, they are not identical. Sharp et al. (27) observed two small regions of nonhomology which mapped near the beginning of the *tra* regions of these two plasmids (A. J. Clark, *in Symposium on Origin and Evolution of Sex*, in press). Ostermann et al. (22) demonstrated that plasmids R1-19 and F have both homologous and nonhomologous sequences within their origin of transfer (*oriT*) regions. Frost et al. (9) found that, although the *traALE* regions of plasmids R1-19 and F were very similar, the DNA sequences diverged immediately upstream of *traA* (the pilin gene). The present DNA sequencing studies of the region between the *oriT* and *traA* genes from R1-19 confirmed that these regions are nonhomologous in the R1-19 and F plasmids.

This region contains several *tra* genes (*traM*, *finP*, *traJ*, and *traY*) and the *traYZ* promoter. The *traM* product is essential for DNA transfer and may be involved in the triggering of DNA transfer and conjugative DNA synthesis by binding to a plasmid-specific region of the *oriT* gene (33). Four alleles of the *traM* gene have been described for IncF plasmids (F-like, R1-like, R100-like, and ColB4-like). Similarily, these *traM* variants are found with plasmid-specific *oriT* sequences and four *traY* alleles (Willetts and Maule, in press). The *traY* product is thought to be a component of the *traYZ* endonuclease, which may be responsible for strand nicking at the gene *oriT* (33). It has been suggested that the *traY* product may be involved in plasmid-specific *oriT* recognition, whereas the *traZ* protein is probably responsible for nicking the DNA (Willetts and Maule, in press). The F plasmid *traJ* product is required for expression of the *traYZ* and *traM* operons of plasmid F (11, 20). It has been shown that the F plasmid *traJ* product increases transcriptional levels at the *traYZ* promoter (20), which is located immediately downstream of the *traJ* gene (8). The *traYZ* transcript proceeds from *traY* to *traZ* and is approximately 32 kb long. Four *traJ* alleles in the IncF plasmid group have been identified, three of which correspond to the four *traMY oriT* alleles. (Type II [ColB4-like] and type III [R1-like] *oriT* alleles both have a type III *traJ* allele, and R-386 has a unique *traJ* allele [Willetts and Maule, in press].) Expression of the *traJ* gene is repressed by the *finOP* (fertility inhibition) system. The *finO* gene is not plasmid specific, whereas there are at least six different *finP* types in the IncF group (Willetts and Maule, in press). The *finP* gene is located in the region between the *traM* and *traJ* genes, being transcribed in the opposite direction from that of *traJ* transcription, whereas the *finO* gene is not linked with the *tra* region. The lack of either the *finO* or *finP* gene relieves *traJ* repression and allows concomitant *traYZ* operon expression, producing a derepressed state.

We report here the nucleotide sequences of the R1-19 *traM*, *finP*, *traJ*, and *traY* genes and the *traYZ* promoter, and we compare these sequences with the related but not identical genes from the F conjugative plasmid.

MATERIALS AND METHODS

Bacterial strains and plasmids. The R1-19 plasmid was carried in the host strain *Escherichia coli* JC6256 (F^- *lac trp*). pUC8 recombinants were transformed into *E. coli* JM83 (32), and galactokinase (*galK*) chimeras containing the promoter assessment vector pKO4 were transformed into *E. coli*

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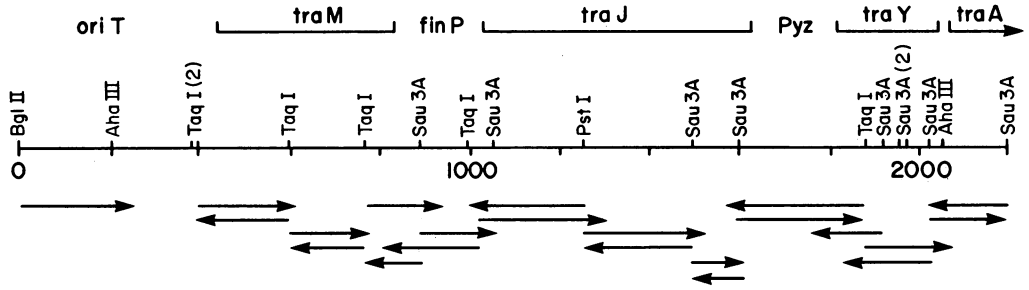

FIG. 1. Strategy for DNA sequencing of *traM*, *finP*, *traJ*, and *traY* genes and the *traYZ* promoter (Pyz) from plasmid R1-19. Restriction sites for *Aha*III, *Bgl*II, *Pst*I, *Sau*3A, and *Taq*I are marked, and the direction and length of sequenced DNA fragments are shown by arrows beneath the restriction map. The relative locations of the genes sequenced are also shown. (Sequence information for residues 25 to 474 was obtained from Ostermann et al. [22].)

ED5363 (*gal*) (18). pBF402 is an 8-kb *Eco*RI fragment from R1-19 ligated into pUC8 and is equivalent to the *Eco*RI f6 fragment from the F plasmid. pRS27 is the *Eco*RI f6 fragment from plasmid F ligated into pSC101 (29). pBF403 is a 320-base-pair R1-19 *Sau*3A fragment (see Fig. 2, nucleotides 1602 to 1922) ligated into pKO4-*Bam*HI (18), and pBF302 consists of the 682-base-pair *Sau*3A fragment from plasmid F (containing the *traYZ* promoter) (8) and the vector pKO4. pBF405 is a recombinant containing the 1,847-base-pair R1-19 *Aha*III fragment (see Fig. 2, nucleotides 200 to 2047) and pACYC184 (4).

Plasmid purification. R1-19 and the chimeras were isolated and purified by the method of Humphreys et al. (14), and small amounts of chimeric plasmids were obtained by the method of Birnboim and Doly (3).

Recombinant DNA techniques and DNA sequencing. Recombinant DNA techniques and DNA sequencing were performed as described previously (7, 9), except that M13mp18 and M13mp19 were used as the M13 vectors (35).

***galK* assay.** The *galK* assay was done exactly as described by McKenney et al. (18), except that the specific activity of the undiluted [^{14}C]galactose was 59.6 mCi/mmol. Units were calculated as described previously (18) and are expressed as nanomoles of galactose phosphorylated per minute per unit of optical density at 650 nm.

RESULTS

Cloning and sequencing strategies. When R1-19 plasmid DNA-*Eco*RI was separated on an agarose gel, immobilized on nitrocellulose, and probed with a 1.4-kb *Pst*I fragment containing the *traALE* genes of the F plasmid transfer region (10), a single R1-19 *Eco*RI fragment (8 kb) showed homology (data not shown). This fragment corresponded to the 8-kb *Eco*RI f6 fragment from plasmid F which contains the F plasmid *traMJYALEKBP*, *finP*, and *oriT* genes (34). This *Eco*RI fragment from R1-19 was cloned into the vector pUC8 (32) and called pBF402. The 1.26-kb *Bgl*II-*Pst*I fragment and the adjacent 2.1-kb *Pst*I fragment were subcloned into pUC8 and sequenced by using the M13 dideoxy sequencing method (25). A restriction map, the sequencing strategy, and the putative gene products found in the *Bgl*II-*Pst*I fragment and in the first half of the 2.1-kb *Pst*I fragment are shown in Fig. 1. The remainder of the *Pst*I fragment contains the *traALE* genes, and the nucleotide sequence of the *traA* gene was reported previously (9). The R1-19 *traL* gene and most of the *traE* gene have been sequenced and are nearly identical to the corresponding sequence from the F plasmid (B. Finlay, unpublished results). The nucleotide sequences and the predicted products of the *traM*, *finP*, *traJ*, and *traY* genes are shown in Fig. 2. (Included in this figure is the 450-nucleotide sequence published previously which contains the R1 *oriT* region [22]. This sequence corresponds to nucleotides 25 to 474 in Fig. 2.) Recently Koronakis et al. (16) determined the nucleotide sequence of the *traM* gene from the R1 plasmid. This sequence is identical to the R1-19 *traM* sequence presented here except for the adenine at position 893 (Fig. 2), which is not present in the R1 sequence. This difference would not alter the predicted gene products.

By using heteroduplex mapping, Sharp et al. (27) found that the *tra* operons of the F and R1 plasmids are highly homologous, whereas the remainder of the plasmids show little similarity. Sequencing studies on the R1-19 pilin gene have demonstrated that, although the *traALE* sequences from the F and R1-19 plasmids are very similar, the sequences diverge immediately upstream of the *traA* gene (9). The heteroduplex mapping results of Sharp et al. reveal a 1.2-kb region of nonhomologous DNA upstream of the predicted location of the *traA* gene (64 kb [27]; 69 kb [34]). The nonhomologous region corresponds to map positions 60.0 to 61.2 in the R1 plasmid and 62.7 to 63.9 in the F plasmid (27). Upstream of this nonhomologous sequence is 0.4 kb of homologous DNA (map positions 60.0 to 59.6 in R1 and 62.7 to 62.3 in F, again using the map units of Sharp et al., preceded by 0.4 kb of nonhomologous DNA (map positions 59.6 to 59.2 in R1 and 62.3 to 61.9 in F). There are no other nonhomologous areas upstream until well beyond the *oriT* region, the beginning of the *tra* region.

Comparing the sequence from the R1-19 plasmid (Fig. 2) with the corresponding sequence from the transfer region of the F plasmid (8, 30, 31) revealed strong homology from nucleotides 1 to 173 in R1-19, which includes the three major nick sites for the *oriT* region (31) (located after positions 124, 127, and 130 in Fig. 2). From nucleotides 174 to 592 there was little similarity, but from 593 to 1053 there was again strong homology, except for the area between 854 and 936 where the homology was limited. From nucleotides 1054 to 2091 there was little homology, although the regions encoding the carboxyl termini of the *traY* gene products were similar. These results correlated well with the heteroduplex studies of Sharp et al. (27), accounting for the two nonhomologous regions seen by these investigators.

R1-19 *traM* gene. The R1-19 *traM* gene encodes a 127-amino-acid protein (14,448 daltons) which shows considerable homology with the *traM* gene product from plasmid F

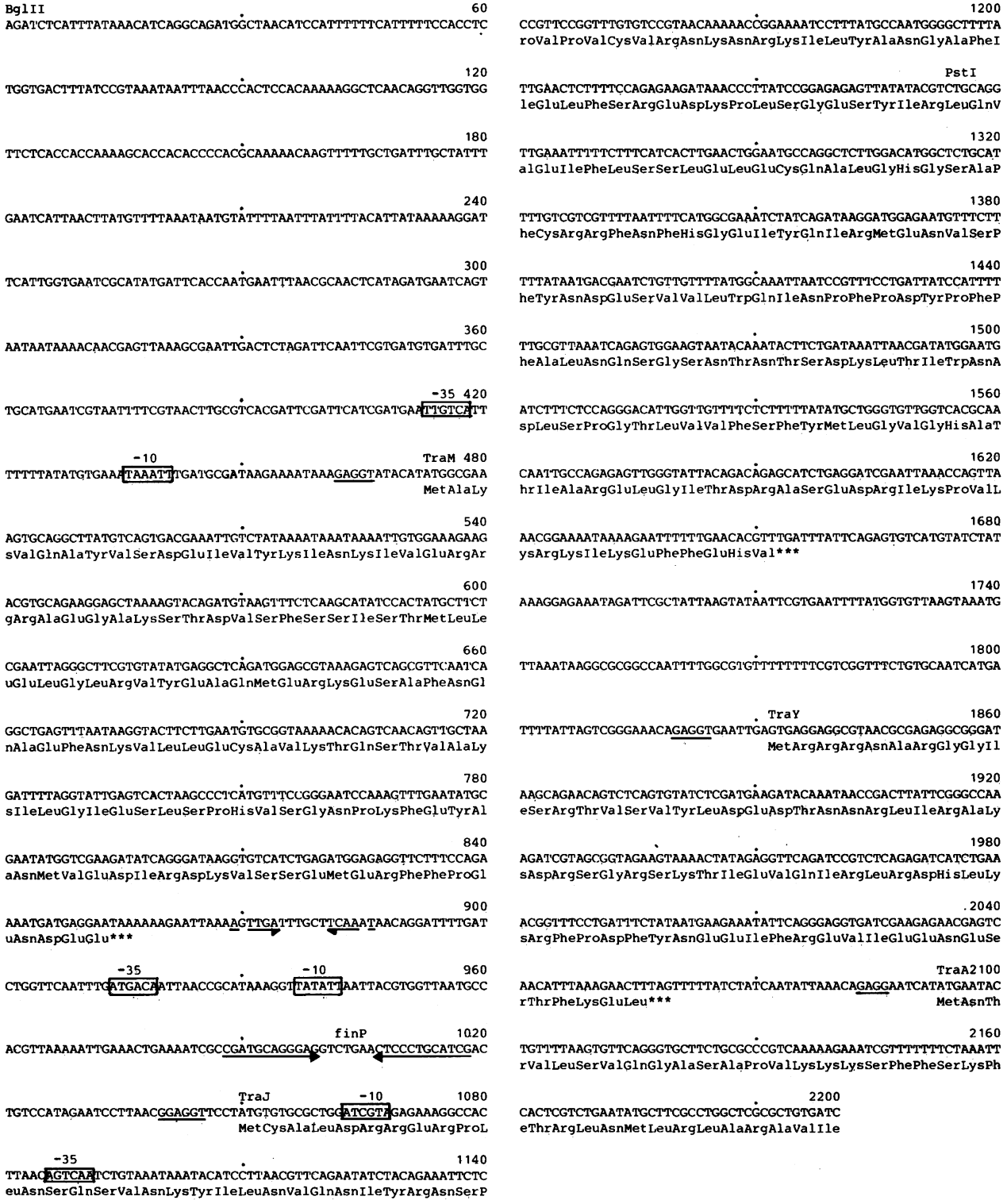

FIG. 2. Nucleotide sequences of *oriT*, *traM*, *finP*, *traJ*, and *traY* genes, and the *traYZ* promoter from plasmid R1-19. The sequence of nucleotides 25 to 474 (*oriT*) is from Ostermann et al. (22), and the entire *traA* sequence has been published previously (9). Potential −35 and −10 promoter regions for the *traM*, *traJ*, and *finP* genes are boxed, and inverted repeats are represented by arrows. Probable ribosome binding sites (28) are underlined. The *traYZ* promoter was localized between the *traJ* and *traY* genes, but its exact location was not determined.

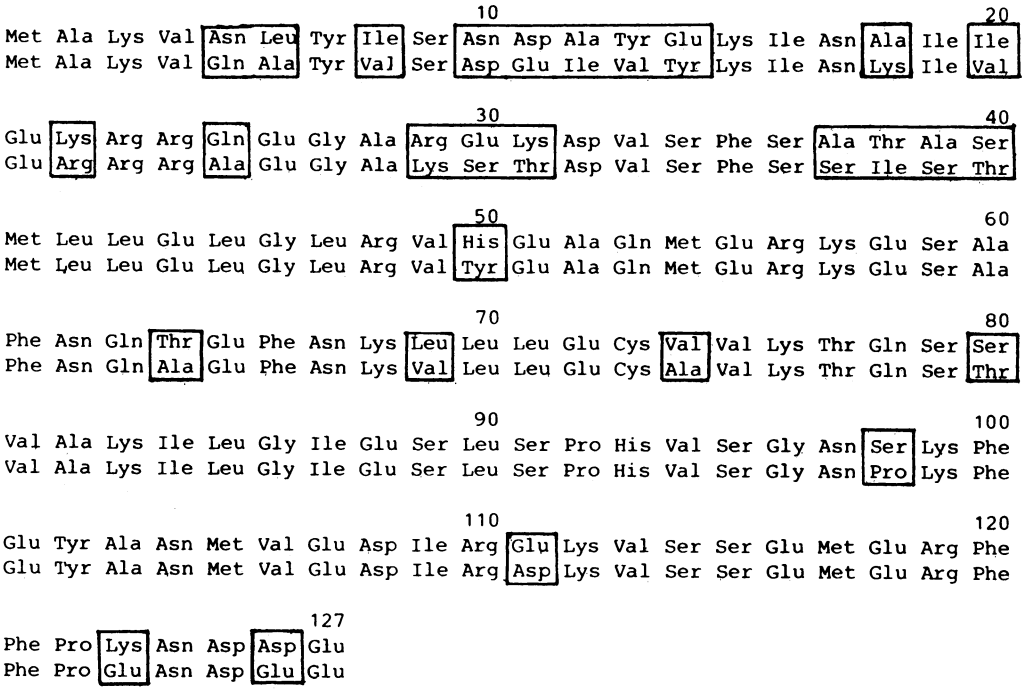

FIG. 3. Comparison of plasmid F (first line) and plasmid R1-19 (second line) *traM* open reading frames. Nonhomologous residues are boxed. The *traM* sequence for plasmid F is from Thompson and Taylor (30).

(31; Fig. 3). The DNA sequences encoding the amino-terminal regions of the *traM* gene products from the F and R1-19 plasmids differed quite significantly, and this difference was reflected in the protein sequences. Beyond residue 40 (nucleotide 592) there was a high level of protein homology, although there was a charge difference (Lys versus Glu) at residue 123.

The *traM* gene product from plasmid F is an inner membrane protein which does not undergo proteolytic processing (1). Although the amino-terminal protein sequences of the *traM* gene products from plasmid R1-19 and F are different, neither resembles the classical hydrophobic signal sequence (15), and it is not known how this protein enters the inner membrane.

Six nucleotides upstream of the *traM* start codon is a strong (five nucleotide) ribosome binding site (28). The RNA transcript and putative promoter for the F plasmid *traM* gene have been mapped (30), and there was no homologous sequence in the R1-19 plasmid. There was a possible promoter upstream of the *traM* gene in R1-19 (Fig. 2) which resembled the consensus promoter sequence (12, 24).

In plasmid F, immediately downstream of the *traM* stop codon, there is a region of dyad symmetry, followed by several thymine residues, which may be responsible for transcriptional termination of the *traM* transcript (30). The R1-19 plasmid had a small (5 base pair) region of dyad symmetry starting at position 869 (Fig. 2). It is possible that this was the R1-19 *traM* transcription terminator, but the resemblance to a rho-independent consensus terminator sequence (24) was weak.

R1-19 *finP* gene. The regions encoding the *finP* genes in the R1-19 and F plasmids were very similar. The inverted repeat sequences thought to be involved in *finOP* binding (*fisO*; [20]) were conserved, but the seven nucleotides between these areas of dyad symmetry (positions 1000 to 1006) were different. These changes would affect the proposed 24-amino-acid *finP* peptide (20) (starting at position 1027 and proceeding in the direction opposite to *traJ* transcription to position 955) by changing Thr-6 Ser-7 to Arg-6 Pro-7. If the *finP* product is an RNA molecule, these changes would affect the loop in a stem-and-loop structure, possibly providing the required *finP* plasmid specificity (see Discussion).

The proposed *finP* promoter in plasmid F is located within the sequence encoding the amino-terminal area of the *traJ* protein, with transcription proceeding in the opposite direction to that of *traJ* transcription (20). Because DNA homology between the F and R1-19 plasmids disappeared at position 1054, this should have affected the predicted *finP* promoter. However, the putative Pribnow box (TACGAT [Fig. 2]) was identical to that of plasmid F (30), and the −35 region (TTGACT) differed by only one nucleotide (T_{1086} in R1-19 is a G in plasmid F). The sequence between these areas showed no homology, nor did the surrounding regions. The opposite strand of the small inverted repeat previously mentioned as a possible termination signal for *traM* transcription (positions 886 to 869) may also serve as a *finP* terminator. When transcribed in the *finP* direction, this area of dyad symmetry is followed by several thymine residues, which are usually present in *rho*-independent terminators (24).

R1-19 *traJ* gene. The F plasmid *traJ* gene product is required for transcription of the *traYZ* operon (Willetts and Maule, in press). The region encoding the *traJ* gene in F plasmid and the equivalent area in R1-19 showed no homology, which was in accordance with the findings of Alfaro and Willetts (2), who demonstrated that R1-19 was unable to complement an F plasmid *traJ* mutant (F *lac traJ90*). R1-19 encodes a 201-amino-acid (23,241 dalton) polypeptide (positions 1050 to 1652) in this nonhomologous area.

In plasmid F, the gene product of *traJ* is required for

traYZ operon transcription (34). Fowler et al. (8) demonstrated that a recombinant containing the *traJ* gene could stimulate the *traYZ* promoter approximately 2.1-fold above uninduced levels. To test whether the R1-19 plasmid has analogous *traJ*-like activity, a 1,847-base-pair R1-19 *Aha*III fragment (pBF405; positions 200 to 2047) which contained the *traM* and *finP* genes, the *traYZ* promoter, most of the *traY* gene, and the putative *traJ* polypeptide was cloned into pACYC184. This chimera was able to increase transcription of the R1-19 *traYZ* promoter (*traYZp*) 3.7-fold (Table 1). Based on this increased transcription and the fact that functions have been assigned to the remaining genes on this fragment, we suggest that this chimeric protein is the *traJ* equivalent in the R1-19 plasmid. It was similar in size to the F plasmid *traJ* protein (27,031 daltons [8]). This R1-19 *traJ* gene had ribosome-binding and translational start sites which were homologous to those of the F plasmid *traJ* gene (GGAGGTTCCTATG). Except for the *finP* promoter, there was no homology at the DNA or protein level beyond position 1054 (Met-1). F and R1-19 *traJ* proteins showed no similarity in the secondary structures predicted by the algorithms of Chou and Fasman (5) or Hopp and Woods (13) (data not shown).

The F plasmid *traJ* protein is believed to be incorporated into the outer membrane of bacteria without proteolytic processing (1). Examination of the F plasmid protein sequence revealed neither a signal at the amino terminus nor any apparent hydrophobic regions that would anchor the protein in the outer membrane (8). The R1-19 *traJ* sequence also showed no apparent signal sequence or hydrophobic domain. Fowler et al. (8) suggested that the tertiary structure of the F plasmid *traJ* protein could bring together small hydrophobic regions which could then form a hydrophobic membrane-anchoring domain. The role of the *traJ* protein in the outer membrane is currently unknown, and the paradox of how an outer membrane protein can regulate a promoter remains unresolved.

R1-19 *traYZp*. In plasmid F, *traYZp* is located between the *traJ* and *traY* genes (8, 20). Although there was no DNA homology between the R1-19 and F plasmids in this area, the gene products downstream of the *traY* gene were functionally interchangeable, and an analogous control system for R1-19 was expected. To test for *traYZp*-like activity, a 320-base-pair *Sau*3A fragment (nucleotides 1602 to 1922), which extends from the end of the *traJ* gene to the beginning of the *traY* gene, was cloned into the *galK* expression vector pKO4-*Bam*HI (18) and assayed for promoter strength. This region had promoter activity by itself (Table 1). However, when the R1-19 plasmid was present (i.e., a functional *traJ* gene was supplied), this promoter was stimulated threefold. When a chimera containing the *traJ* gene (pBF405) was present, transcription from this promoter was increased 3.7-fold over uninduced levels. These results are very similar to the results obtained with the F plasmid *traYZp* (8), which showed a 2.1-fold increase when the F plasmid *traJ* gene was present. (In our assay, a 1.9-fold increase was seen.) This suggests that the DNA between the *traJ* and *traY* genes contains the R1-19 *traYZp*. The F plasmid *traJ* gene was unable to stimulate this promoter, and the R1-19 *traJ* gene was unable to affect the F plasmid *traYZp* activity (Table 1), confirming that plasmids R1-19 and F have different *traJ traYZp* alleles (Willetts and Maule, in press).

The F plasmid *traYZp* has been sequenced (8) and encompasses a *Bst*EII site between the *traJ* and *traY* genes (20). The proposed −35 and −10 regions (CTGCAA and GAAGAT) do not resemble the procaryotic promoter consensus sequences (TTGACA and TATAAT) (12). This is not surprising because *traYZp* must contain a site for *traJ* product interaction and positive control promoters usually differ significantly from the procaryotic promoter consensus sequence (23). When the R1-19 sequence between the *traJ* and *traY* genes was compared with the consensus promoter sequence, no homologous regions were found. There was also no DNA homology between the R1-19 and F plasmids in the *traYZp* region. Therefore, the R1-19 *traYZp* sequence remains undefined but could be localized between the *traJ* and *traY* genes on a 320-base-pair *Sau*3A fragment.

TABLE 1. Galactokinase assay of plasmids F and R1-19 *traYZ* promoter activity

Plasmid(s)	Promoter	*traJ* product	No. of galactokinase units[a]
pKO4	None		63
pBF403	R1-19 *traYZp*		175
pBF403, R1-19	R1-19 *traYZp*	R1-19	547
pBF403, pBF405	R1-19 *traYZp*	R1-19	658
pBF403, pRS27	R1-19 *traYZp*	F	246
pBF302	F *traYZp*		433
pBF302, pRS27	F *traYZp*	F	807
pBF302, pBF405	F *traYZp*	R1-19	373
pKL200	*lac*		646

[a] Nanomoles of galactose phosphorylated per minute per unit of optical density at 650 nm.

In plasmid F, the *traJ* product is not only required for *traYZ* operon transcription, but also possibility for *traM* transcription (11). In vitro studies on the *traM* promoter (*traMp*), which are contradictory to the above results, suggest that the *traM* promoter is constitutive (20). If this promoter was under *traJ* control, a homologous sequence between *traMp* and *traYZp* that the *traJ* product could recognize would be expected. In the R1-19 plasmid, there is an identical nine-nucleotide sequence 131 nucleotides upstream of the *traM* gene (nucleotides 342 to 350) and 122 nucleotides preceding the *traY* gene (nucleotides 1710 to 1718). These regions may be involved in *traJ* protein recognition and interaction with these two promoters.

R1-19 *traY* gene. The F plasmid *traY* gene product, believed to be a component of the *traY* endonuclease (33), is encoded by the region of DNA between *traYZp* and the *traA* gene (8). When the analogous area of the R1-19 plasmid was examined, a single open reading frame was found which spanned nucleotides 1832 to 2257 (75 residues). Although this region contained no AUGs, a GUG at nucleotide 1832 was preceded by a strong Shine-Dalgarno sequence (GAGGT [29]) 7 residues upstream. Although AUG is the usual start codon, there are several examples of GUG being used as an initiation codon (17). This *traY* polypeptide was 75 residues in length (9,037 daltons) and could be aligned with F plasmid *traY* gene product (Fig. 4), suggesting that it is the R1-19 *traY* gene product.

The product of the *traY* gene from the R1-19 plasmid was 44 amino acids shorter than that of the F plasmid, leading to a truncated amino-terminal region in the R1-19 protein. In addition, the amino termini of the two gene products were completely different. The protein homology between the *traY* products from plasmids F and R1-19, starting at residue 62 in the F *traY* protein, was reflected in the DNA sequence; there was extensive DNA homology in the latter half of the *traY* genes of plasmids F and R1-19 which continues into the *traALE* sequences (9).

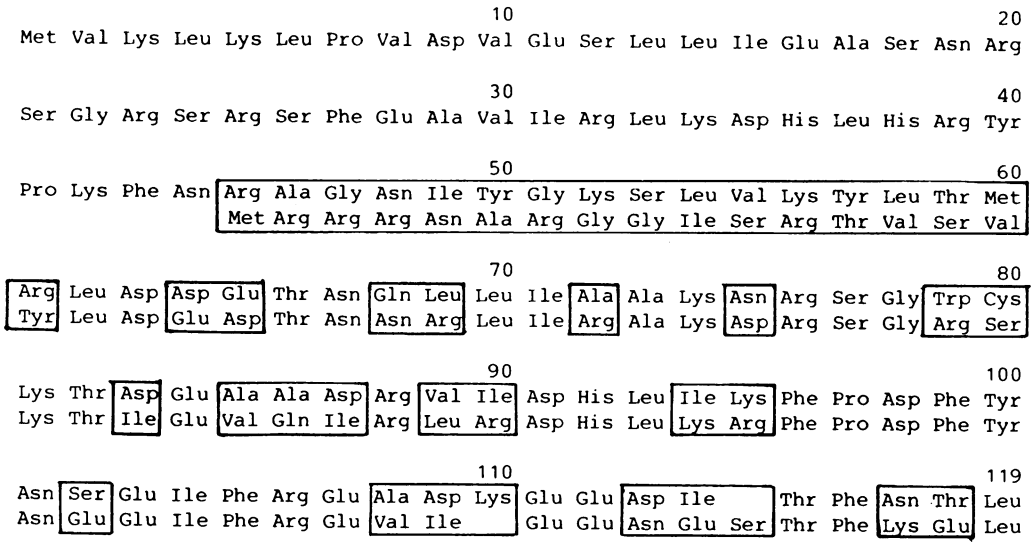

FIG. 4. Comparison of plasmid F (first line) and plasmid R1-19 (second line) *traY* open reading frames. the *traY* product from plasmid F is 44 amino acids larger than the equivalent product from plasmid R1-19. Nonhomologous residues are boxed, and spaces were inserted to allow maximum alignment. The *traY* sequence for plasmid F is from Fowler et al. (8).

DISCUSSION

In the IncF plasmid group, there are four *oriT* types (F-like, R1-like, ColB4-like, and R100-like [Willetts and Maule, in press]). The sequences of the F and R1-19 *oriT* genes have been published (22, 31). There is a highly homologous region that contains the three proposed nick sites (31), corresponding to nucleotides 1 to 173 in Fig. 2, followed by a sequence between nucleotide 174 and the *traM* gene that is nonhomologous and may be involved in binding of the plasmid-specific gene products of *traY* and *traM* as part of the process of DNA metabolism during DNA transfer (33). The *traY* gene products of the F, R1-19, and R100 plasmids are not interchangable (Willetts and Maule, in press), and because the *traZ* products of the R1-19 and F plasmids can complement each other (6), Willetts and Maule have suggested that the conserved *traZ* protein is the component of the *traYZ* endonuclease that actually nicks at the homologous *oriT* sequences, whereas the plasmid-specific *traY* product binds to the DNA at the nonhomologous sequence within the *oriT* region.

The *traM* gene product is also *oriT* specific and not interchangeable among F-like plasmids (Willetts and Maule, in press). It is believed to be involved in triggering conjugal DNA replication, possibly by binding at or near the *oriT* region (33). Since the *traM* gene product is plasmid specific, it may also recognize this area of nonhomology. Comparisons of the protein sequences of the *traM* proteins from the F and R1-19 plasmids revealed several nonhomologous areas (Fig. 3) which may be responsible for determining the plasmid specificity, although the majority of the differences are located within the first 40 residues.

Mullineaux and Willetts (20) have suggested that the product of the *finP* gene is a short RNA molecule that is capable of preventing transcription or translation or both of the *traJ* gene by interacting with its complementary RNA or DNA molecule, similar to the control of plasmid replication (26). The presence of a nonhomologous 7-nucleotide loop flanked by homologous inverted repeats suggests that this loop could provide the *finP* plasmid specificity, possibly by allowing complementary base pairing at the loop when the *finP* gene is present, much like the RNA I interactions with the RNA primer in ColE1 plasmid replication (26).

The site of *finOP* action (*fisO*) has been reported to include both inverted repeats and the sequence immediately downstream (20). Because this area (except the loop) was conserved between the F and R1-19 plasmids, the *finO* product, which is relatively nonspecific (Willetts and Maule, in press), probably recognizes this homologous area.

Although the −10 (nucleotide 1062) and −35 (nucleotide 1086) promoter regions of the R1-19 *finP* gene were highly homologous to those of the F plasmid (11 to 12 nucleotides), the intervening and surrounding sequences (in the amino-terminal area of the *traJ* gene) were completely different. This is also true for the R100-1 plasmid (unpublished results). The significance of this promoter conservation is unclear at present.

The plasmid specificity of the *traJ traYZp* allele is closely linked to that of the *oriT traY*, *traM*, and *finP* alleles (Willetts and Maule, in press). When *traJ traYZp* sequences from the F (type I) and R1-19 (type III) plasmids were compared, no homology was found, although they are analogous systems. Both *traJ* products, when supplied in *trans*, increased transcription from their respective *traYZp* by similar levels, yielding similar amounts of highly homologous *traYZ* transcript. Because the F and R1-19 *traJ* products interacted with their *traYZp* DNA, we examined both proteins for a possible DNA binding site by using the consensus sequence compiled by Ohlendorf et al. (21). However, we were unable to identify any predicted DNA binding domains in either protein sequence.

We are currently sequencing the remaining *finP*, *traJ traYZp*, and *oriT traM traY* alleles identified for IncF plasmids (Willetts and Maule, in press), and this should provide information regarding the structure and function of these homologous or analogous genes and their products.

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