Escherichia coli DNA Helicase I Catalyzes a Sequence-specific Cleavage/Ligation Reaction at the F Plasmid Origin of Transfer*

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Recent studies have shown that the Escherichia coli F plasmid-encoded tral gene product (TraIp), also known as DNA helicase I, catalyzes the formation of the site- and strand-specific nick that initiates F plasmid DNA transfer. Scission of the phosphodiester bond at the nic site within the origin of transfer (oriT) is accompanied by the covalent attachment of TraIp to the 5’-phosphate of the nicked DNA strand. This mechanism suggests that TraIp may also be capable of catalyzing a DNA ligation reaction using the energy stored in the protein-DNA intermediate. To test this possibility, an in vitro assay was designed that utilized short single-stranded DNA oligonucleotides of different lengths derived from the region within oriT that spanned the nic site. Purified TraIp was capable of efficiently cleaving single-stranded DNA that contained a nic site, and upon cleavage, the protein became covalently linked to the 5’-end of the nic site. When TraIp was incubated with two oligonucleotides of different length that contained the nic site, there was formation of novel recombinant products resulting from a TraIp-catalyzed cleavage/ligation reaction. Furthermore, the cleavage and ligation reactions were both sequence-specific. These data suggest that TraIp plays an important role in the initiation and termination of conjugal DNA transfer.

The transfer of DNA from one bacterial cell to another, mediated by transmissible plasmids, is an important mechanism for genetic exchange within a bacterial population. Classically, our understanding of the process of bacterial conjugation derives from genetic studies of transmissible plasmids such as F, R100, RP4, and others (for reviews see Refs. 1–3). In each case, a donor and a recipient cell establish cell-cell contact, and a single strand of DNA is transferred from the donor to the recipient cell with an overall 5’ to 3’ polarity. The transferred strand of DNA either circularizes to form an episome or is integrated, via recombination, into the chromosome of the recipient cell. Although well understood from a genetic perspective, the biochemical mechanism of this process remains poorly defined.

Molecular genetic studies of the Escherichia coli F plasmid have described 24 gene products of the tra family that are involved in conjugal DNA transfer from a donor F’ cell to a recipient F− cell (1, 4). Twenty of these genes are, to some extent, coordinately regulated in the large 35-kilobase pair tra operon (1, 4), and their functions can be divided into three categories: (i) proteins that form the pilus, (ii) proteins that recognize other F+ cells and discourage mating, and (iii) proteins that function in the DNA metabolic events associated with strand transfer. Only four of the tra gene products appear to interact directly with the F plasmid DNA during strand transfer, specifically the gene products of traY, traI, traM, and traD (3).

Biochemical studies have established that F plasmid DNA strand transfer is initiated by the formation of a site- and strand-specific nick at the origin of transfer (oriT) (2). The nicked strand is then unwound, presumably by a helicase, and transferred to the recipient cell. The products of the traD and traM genes have both been shown to be necessary for DNA transfer during conjugation (5, 6). The traD gene product has been shown to bind sequences near the nic site within oriT, but is not required for nicking to occur (7, 8). No well defined biochemical role has been assigned to the traD gene product, although it has been identified as an inner membrane protein (9). The traI gene product (TraIp) has been overexpressed, purified, and shown to bind a region of oriT that is near, but not coincident with, the site that is nicked to initiate strand transfer (10). This finding, in conjunction with genetic evidence indicating that TraIp is necessary for nicking the transferred strand (8), suggests that TraIp may play a role in regulating the site- and strand-specific nicking event. While there is speculation regarding the specific roles of the traM gene product, the traD gene product, and the traI gene product, the specific biochemical functions of these proteins are still poorly understood.

On the other hand, the tral gene product (TraIp), also known as E. coli DNA helicase I, has been well studied biochemically. This protein was originally isolated as a DNA-dependent ATPase with helicase activity (11, 12) and has subsequently been shown to be encoded by the F plasmid tral gene (13). The purified protein catalyzes a processive 5’ to 3’ helicase reaction (12, 14), and it has been suggested that TraIp drives the transferred strand of DNA from the donor to the recipient cell during conjugation (3). More recently, TraIp has been shown to catalyze the site- and strand-specific nicking reaction at oriT that serves to initiate the transfer of F DNA (15, 16). It has also been shown that, subsequent to strand scission, TraIp remains covalently bound to the 5’-end of the nicked DNA strand (17).

In view of the covalent attachment of TraIp to the 5’-end of the nicked DNA strand, it seemed possible that TraIp might also mediate recircularization of the transferred strand via a ligation activity of the protein. The energy for this ligation event would come from the original phosphodiester bond energy preserved in the DNA-protein covalent intermediate. To test this idea, an intermolecular recombination assay was de-
Oligonucleotide sequences

The sequences for the oligonucleotides used in this study are shown. Each oligonucleotide was named based on its length in nucleotides. Only the 22-mer (32-mer) and the 30-mer (31-mer) contained the nic site. The sequences of the 3'-end labeled 22- and 31-mers, respectively, except a 3P-labeled ddATP was added on the 3'-end as described under "Experimental Procedures." The 1-nucleotide addition is represented by "(A)."

| Oligonucleotide sequences |
|---------------------------|
| 22-mer (32-mer)           |
| 30-mer (31-mer)           |
| 41-mer                    |
| 25-mer                    |
| 17-mer                    |
| 5'-TTGCGCTGGGGTGCTGCTAATT(C)-3' |
| 5'-CTGTCGGTCTGCGCGTATGACC(T)-3' |
| 5'-GTCGCGCTGGGGTGCTGCTAATT(C)-3' |
| 5'-TGTAATTCCTTCAAATAAAAATT-3' |
| 5'-GTAACACGAGCCGCA-T-3'    |


either 500 ng or 2 μg of TrafIp. Incubation was for 20 min at 37 °C, and the reactions were stopped by the addition of an equal volume of 2 × gel loading buffer (100 mM Tris-Cl (pH 6.8), 200 mM dithiothreitol, 4% SDS, 0.2% bromphenol blue, and 20% glycerol). The samples were desalted by boiling for 3 min prior to loading on a 9% SDS-polyacrylamide gel. Electrophoresis was at 100 V (constant voltage), until the bromphenol blue reached the bottom of the gel. The gel was fixed and stained with Comassie Blue. After destaining, the gel was dried and exposed to x-ray film.

Oligonucleotide Recombination Reactions—Reaction mixtures were the same as for the cleavage reaction, except that 1 pmol of a second unlabeled oligonucleotide was added. The specific second oligonucleotide used is noted in appropriate figure legends. When 5'-end labeled oligonucleotides were used, the reactions were terminated as described above for the cleavage reactions. When 3'-end labeled oligonucleotides were used, the reactions were stopped by the addition of SDS to 0.1% and 0.2 mg/ml proteinase K. Incubation was continued for 20 min at 37 °C. The products were resolved on a 16% polyacrylamide, 8 × urea denaturing gel. Electrophoresis conditions were as described above for the cleavage reactions.

To test the effect of protein denaturants on the cleavage/ligation reaction catalyzed by TrafIp, the combination assay reaction mixtures (using 5'-end labeled DNA) were increased to 51 μl. Upon completion of the 2-h incubation, each reaction was divided into three aliquots. One set of reactions was treated as described above for the cleavage reactions. One set of reactions was heated at 65 °C for 20 min prior to loading on the gel, and one set of reactions was directly loaded on the gel. All of the samples were resolved on a 16% polyacrylamide, 25% glycerol native gel. Electrophoresis was at 2.6 W for 8 h in 0.5 × TBE running buffer.

RESULTS

Cleavage of ssDNA Oligonucleotides by TrafIp—Previous studies have shown that TrafIp catalyzes a site- and strand-specific nicking reaction in vitro at oriT (15, 16). This reaction depends on the presence of MgCl₂ and a supercoiled DNA substrate. To determine if TrafIp could cleave a ssDNA substrate in a manner similar to the site- and strand-specific nicking of supercoiled DNA, two ssDNA oligonucleotides, selected from a region within oriT that contained the nic site, were constructed (Table I, 30- and 22-mer). The 30-mer oligonucleotide contained the nic site after the 20th nucleotide, and the 22-mer oligonucleotide contained the nic site after the 14th nucleotide. The 30- and 22-mer were each radioactively labeled at their 5'-ends and incubated with an equimolar amount of purified TrafIp. The result of this experiment is shown in Fig. 1. After incubation with TrafIp, cleavage products were observed comigrating with the marker at 20 nucleotides for the 5'-end labeled 30-mer (Fig. 1A, lane 2) and at 14 nucleotides for the 5'-end labeled 22-mer (Fig. 1A, lane 5). In both cases the primary cleavage product observed indicated that cleavage occurred at the nic site. In addition, the reaction was dependent on the presence of MgCl₂ (Fig. 1A, lanes 3 and 9).

In addition to the expected cleavage products, other ssDNA cleavage products were also apparent. In the case of the 30-mer, there was cleavage between the 18th and 19th nucleotide to produce a 5'-end labeled product 18 nucleotides in length and cleavage between the 12th and 13th nucleotides to produce a 12-nucleotide product (Fig. 1A, lane 2). In the case of the 22-mer, there was cleavage between the 12th and 13th nucleotide
Cleavage/Ligation Reaction Catalyzed by Helicase I

A lane corresponding to phosphodiester bond scission at the 18th nucleotide and 18th nucleotide to produce a labeled product 17 nucleotides in length, and cleavage between the 18th and 19th nucleotide.

Site
tryptase

The labeled oligonucleotides were incubated separately with short single-stranded substrates. Experiments measuring reaction kinetics have shown that these sites are cleaved more frequently than cleavage at the 12- and 17-mers formed in the 22-mer cleavage reaction mixtures containing 5'-end labeled oligonucleotides by TraIp.

The polyacrylamide gel was subsequently exposed to x-ray film to visualize labeling of the protein by the covalently bound DNA (Fig. 2B). When TraIp was incubated with the 3'-end labeled 31-mer, transfer of the radioactive label to the protein was observed (Fig. 2B, lanes 3' and 4'). Incubation of TraIp with the 5'-end labeled DNA did not result in transfer of the radioactive label to the protein (Fig. 2B, lanes 5' and 6'). This confirms the covalent attachment of TraIp to the DNA upon cleavage of the phosphodiester bond, as these samples were boiled in SDS prior to being resolved on the polyacrylamide gel. Moreover, the covalent linkage must be to the 5'-end of the cleavage site since 3'-end labeled DNA is not. Thus, upon cleaving the 30-mer oligonucleotide, TraIp releases the 20-nucleotide fragment and remains covalently bound on the 5'-end of the 10-nucleotide fragment that lies 3' to the nic site.

TraIp Catalyzes a Cleavage | Ligation Reaction—To investigate the possibility of a cleavage/ligation activity intrinsic to TraIp, an intermolecular oligonucleotide recombination assay was devised (Fig. 3A). TraIp was incubated with both the 30-mer and the 22-mer; one of the oligonucleotides was 5'-end labeled. If TraIp catalyzes a cleavage/ligation reaction, then specific recombinant molecules of novel size should be formed from intermolecular rearrangements between the two oligonucleotides cleaved by TraIp. When the labeled oligonucleotide is the 30-mer, four ligation products can be envisioned. The 10-mer cleavage product, with TraIp covalently bound on the 5'-end, could be ligated to the 20-mer cleavage product to regenerate the starting substrate. Alternatively, it could be ligated to the unlabeled 14-mer produced by cleavage of the 22-mer. The latter event would not be observed since the 22-mer was not 5'-end labeled. Similarly, the 8-mer cleavage product, with TraIp covalently bound on the 5'-end, could be ligated to the 14-mer to regenerate the 22-mer. As before, this event would not be observed. However, the 8-mer could be ligated to the 5'-end labeled 20-mer that results from TraIp-catalyzed cleavage of the labeled 30-mer. This would generate a 5'-end labeled 28-mer resulting from an intermolecular recombination event promoted by TraIp. When the 22-mer is 5'-end labeled and the 30-mer is unlabeled, the predicted intermolecular recombinant molecule should be 24 nucleotides in length.

Fig. 3B shows the results from such an experiment performed using a labeled 30-mer and an unlabeled 22-mer (lanes 1-4) or a labeled 22-mer and an unlabeled 30-mer (lanes 5-8). The labeled oligonucleotide was incubated with TraIp in the absence of the unlabeled oligonucleotide to demonstrate cleavage at the nic site. As expected, the 30-mer was cleaved to yield a 5'-end labeled 20-mer (lane 2), and the 22-mer was cleaved to produce a 5'-end labeled 14-mer (lane 6). When the second,
Fig. 3. Site-specific oligonucleotide recombination catalyzed by TraIp. A, schematic to illustrate the methodology employed to investigate the cleavage/ligation reaction catalyzed by TraIp. B, TraIp was incubated with 5'-end labeled oligonucleotides (1 pmol) (30-mer, lanes 1-4 and 22-mer, lanes 5-8) as described under “Experimental Procedures.” Lanes 1 and 5, no protein; lanes 2 and 6, 400 ng (2 pmol) of TraIp; lane 3, 400 ng (2 pmol) of TraIp and 1 pmol of unlabeled 22-mer; lane 4, 400 ng (2 pmol) of TraIp, no MgCl₂, 5 mM EDTA, and 1 pmol of unlabeled 22-mer; lane 7, 400 ng (2 pmol) of TraIp and 1 pmol of unlabeled 30-mer; lane 8, 400 ng (2 pmol) of TraIp, no MgCl₂, 5 mM EDTA, and 1 pmol of unlabeled 30-mer. Reactions were stopped with the addition of SDS to 0.1%, and incubation was continued at 37 °C for 10 min. The positions of the recombinant products are indicated at the right.

unlabeled oligonucleotide was added and incubated with TraIp, the predicted recombinant products were observed (28-mer, lane 3 or 24-mer, lane 7). The formation of recombinant products was dependent on the presence of MgCl₂ in the reaction (Fig. 3B, lanes 4 and 8). In each case, the intermolecular recombinant products were formed at a high frequency, indicating that TraIp does indeed catalyze an efficient cleavage/ligation reaction.

To ensure that the cleavage/ligation reaction observed was not a secondary effect due to the presence of denaturants, the following experiment was performed. Large scale oligonucleotide recombination reactions were incubated for 2 h at 37 °C using the 5'-end labeled 30-mer and the unlabeled 22-mer. Each reaction mixture was then divided into three aliquots. One set of reaction tubes was treated with 0.1% SDS as described previously, one set of reaction tubes received no further treatment, and one set of reaction tubes was incubated at 65 °C for 20 min. All of the samples were then resolved on a native polyacrylamide gel as described under “Experimental Procedures.” In each case, the cleavage/ligation reaction proceeded as before (data not shown). Thus, the cleavage/ligation reaction proceeds in the absence of any denaturing treatment, and therefore cannot be a secondary effect of protein denaturation.

The Cleavage/Ligation Reaction Catalyzed by TraIp Is Sequence Specific—In an effort to begin to define the 3'-end sequence requirements for TraIp-mediated ligation, oligonucleotide recombination assays were performed using the 3'-end labeled 23-mer and unlabeled oligonucleotides that could not be cleaved by TraIp (see Table I). The 41-mer used in this experiment contains the sequence located immediately to the 5' side of the site nicked by TraIp at oriT. The 17-mer contains a GT dinucleotide at the 3'-end, as does the 41-mer, but is otherwise unrelated to the nic site at oriT. The 25-mer contains a TT dinucleotide at its 3'-end and is also otherwise unrelated to the nic site at oriT.

We reasoned that if TraIp recognized a specific 3'-end sequence prior to catalyzing the ligation of the two DNA strands then after cleavage of the 23-mer, the protein covalently bound on the 5'-end of the 9-mer should be able to ligate to the 41-mer to form a 50-mer. On the other hand, the protein should not be able to ligate to the 17-mer to form a 26-mer or to the 25-mer to form a 34-mer since the sequences on the 3'-end of these oligonucleotides were unrelated to oriT. This was in fact the case, as shown in Fig. 4. TraIp accurately cleaved the 3'-end labeled 23-mer to produce an unlabeled 14-mer, and a labeled 9-mer covalently bound by TraIp (Fig. 4, lane 2). When the unlabeled 30-mer, containing a complete nic site, was added to the reaction, there was efficient intermolecular recombination to yield a labeled 29-mer as expected (Fig. 4, lane 3). When the unlabeled 41-mer with a 3'-end identical with that of a cleaved nic site was added to the reaction we, observed formation of the predicted 50-mer recombinant molecule (Fig. 4, lane 4). There was no intermolecular recombination observed when either the 17- or 25-mer were added to the reaction (Fig. 4, lanes 5 and 6, respectively). However, in each case there was efficient cleavage of the 23-mer as evidenced by the ladder of DNA fragments.
Cleavage/Ligation Reaction Catalyzed by Helicase I

FIG. 4. The ligation reaction catalyzed by TraIp is sequence specific. TraIp was incubated with the 5'-end labeled 23-mer (1 pmol) as described under "Experimental Procedures." Lane 1, no protein; lane 2, 400 ng (2 pmol) of TraIp; lanes 3–6, 400 ng (2 pmol) of TraIp and 1 pmol of a second, unlabeled oligonucleotide (either the 30-mer (lane 3), the 41-mer (lane 4), the 25-mer (lane 5), or the 17-mer (lane 6). Reactions were stopped by the addition of SDS to 0.1% and 0.2 mg/ml proteinase K, and incubation was continued for 10 min at 37 °C. Markers (lanes M) were the 5'-end labeled 23-mer and 31-mer as well as the 5'-end labeled 41-mer and an 82-mer species formed from a ligation of two 5'-end labeled 41-mer oligonucleotides.

migrating at the position of a 16–17-mer corresponding to the 3'-end labeled 9-mer retarded in the gel by the covalent association of one or several amino acids resistant to the proteinase K digestion. We conclude that TraIp recognizes a specific sequence at the 3'-OH end prior to catalyzing a ligation reaction. Moreover, this recognition site must extend beyond the GT dinucleotide sequence present on two of the oligonucleotides used in this experiment.

DISCUSSION

Previous studies from this lab, and others, have demonstrated that the F plasmid-encoded TraIp, also known as DNA helicase I, catalyzes a site- and strand-specific nicking reaction at the nic site located within the F plasmid oriT (15, 16). Nicking requires a superhelical DNA substrate, and it has been suggested that the single-stranded character present in supercoiled DNA is important for recognition by TraIp (16). We have directly demonstrated that TraIp is able to cleavage of 5'-end labeled ssDNA oligonucleotides containing the nic site from oriT. Cleavage of the ssDNA oligonucleotides occurred rapidly (data not shown) and required the presence of MgCl₂ in the reaction mixture. This is consistent with previous results obtained using superhelical DNA substrates containing oriT (15, 16). Two ssDNA oligonucleotides of different length were used for these studies and, in each case, the phosphodiester bond scission occurred at the same site that is nicked in vivo to initiate conjugal DNA strand transfer (8).

While cleavage occurred primarily at the nic site on each ssDNA oligonucleotide, there was detectable cleavage at other sites. Kinetic studies showed that formation of products from these sites occurred at a lower rate than did formation of products from cleavage at the nic site (data not shown). We have also been able to alter cleavage specificity by changing the salt concentration in the reaction. Higher, more physiological salt concentrations (150–200 mM) favor cleavage at the nic site as do extremely low concentrations of salt (10 mM), like those used in our assays. At intermediate salt concentrations, the nic site and secondary sites are cleaved with equal efficiency (data not shown). In addition, we have not been able to detect any recombiant products resulting from TraIp cleavage at the secondary sites. So, while these products are formed in the cleavage reaction, we suggest that they are a consequence of the conditions of the experiment and may not have any biological relevance. Inspection of the DNA sequence surrounding several of these secondary cleavage sites revealed that they contained a 5'-GT-3' dinucleotide pair immediately 5' to the phosphodiester bond that was cleaved. Since the nic site also contains a 5'-GT-3' dinucleotide, it is likely that cleavage at the secondary sites is due to a relaxed interaction between TraIp and the ssDNA oligonucleotide under our in vitro conditions.

Cleavage reactions were also performed using ssDNA oligonucleotides labeled at the 3'-end (data not shown). In this case, the labeled cleavage product migrated as a series of bands with retarded mobility on a polyacrylamide gel (see Fig. 4, lanes 2–6). This result was expected since previous studies (17) have shown that TraIp covalently binds the 5'-end of the cleaved strand as it catalyzes phosphodiester bond scission. Thus the 3'-end labeled cleavage product, after proteinase K digestion, would be expected to have one or more amino acids covalently bound on the 5'-end of the DNA strand.

To directly demonstrate the covalent attachment of TraIp to the 5'-end of the cleavage site, a label transfer experiment was performed. The 32P label on the DNA was efficiently transferred to TraIp in a linkage that is apparently covalent since it is resistant to boiling in the presence of SDS. Moreover, transfer of 32P label from DNA to protein was observed only when a 3'-end labeled ssDNA oligonucleotide was used. Thus TraIp must covalently bind the 5'-side of the cleaved phosphodiester bond. This confirms previous results obtained using supercoiled plasmid DNA (17).

The data from these experiments also further our understanding of the DNA structure that TraIp prefers. It has been suggested that TraIp acts on DNA with single-stranded character that is produced by the supercoiling of the F plasmid. The data presented here show that TraIp can cleave ssDNA and that this reaction has biochemical properties identical with the reaction observed when supercoiled dsDNA is used as the substrate.

In addition to the ssDNA cleavage activity intrinsic to TraIp, we have directly demonstrated that the protein-DNA intermediate is able to mediate a ligation reaction when provided with an appropriate 3'-OH end (see Fig. 3A). Thus, TraIp catalyzes both a site- and strand-specific cleavage reaction and a site-specific ligation reaction. This reaction is analogous to the cleavage/ligation reaction catalyzed by the oX174 CisA protein (21), and a similar reaction has been demonstrated for the nicking proteins encoded by other transmissible plasmids (18, 19). This mechanism for endonucleolytic cleavage of the DNA followed by ligation of the transferred strand by TraIp-like proteins seems to be highly conserved among transmissible plasmids. Indeed, this conservation extends to the mechanism of transfer used by the Ti plasmid to transfer from Agrobacterium tumefaciens to plant cells (22). The significant difference in the F plasmid system is the existence of the cleavage/ligation activity on the same polypeptide as the helicase activity, which is presumably responsible for driving strand transfer. The other nicking enzymes that have been characterized do not
Cleavage/Ligation Reaction Catalyzed by Helicase I

contain a helicase activity. Moreover, the helicases involved in strand transfer in the RP4 system and the Ti plasmid system have yet to be identified.

The site specificity of the ligation reaction was investigated using three different acceptor oligonucleotides in the recombination assay. One of these oligonucleotides corresponded precisely to the sequence found on the 3'-side of the nic site. Recombination between this oligonucleotide and a TraIp-bound cleavage product proceeded to essentially the same extent as did recombination between oligonucleotides containing the nic site. Recombination between the TraIp-bound cleavage product and oligonucleotides that contained sequences that diverge from those found at the 3' side of the nic site, was not detected. One of these potential acceptor molecules contained a GT dinucleotide on the 3'-end as does the 3'-side of the nic site. Since this oligonucleotide failed to recombine with the TraIp-bound cleavage fragment, we conclude that TraIp recognizes a specific 3' acceptor sequence and the recognition site extends beyond the terminal dinucleotide. These findings are further supported by Gao et al. (23) who found that termination of strand transfer requires sequence determinants extending at least 9 nucleotides 5' of the nic site. Experiments are in progress to further define the sequence requirements for TraIp catalyzed ligation. It will be interesting to determine if the site recognized by TraIp for cleavage is identical with the site recognized for ligation.

The studies described above have demonstrated yet another enzymatic activity associated with the F plasmid TraIp. It is now clear that TraIp is a multifunctional protein with multiple roles in the DNA metabolism associated with conjugative DNA strand transfer (Fig. 5). TraIp is a DNA-dependent NTPase and a helicase. Presumably these two coupled activities are involved in catalyzing the unwinding event required to generate the ssDNA that is transferred to the recipient cell (Fig. 5d). In addition, TraIp is an endonuclease/ligase capable of introducing a site- and strand-specific nick in the F plasmid oriT and then resealing that nick via a ligation reaction. We suggest that the ligation activity has two roles in the strand transfer process in vivo. Initially TraIp binds a specific site within oriT on a supercoiled plasmid in the presence of MgCl2 (Fig. 5a). The bound protein catalyzes a site- and strand-specific nicking reaction within oriT that results in covalent attachment of the protein to the 5'-end of the cleaved strand (17). We suggest that the nicked (although not relaxed) species exists in equilibrium with the covalently closed form of the plasmid as a result of the endonuclease/ligation activity of TraIp (Fig. 5b). This idea is consistent with the observation that upon isolation of the F plasmid from cells, and subsequent to treatment with agents that disrupt protein structure, both supercoiled and nicked DNA molecules were recovered (Ref. 24; for review, see Ref. 25). For clarity of presentation, the 3'-end of the cleaved strand has been depicted as not associated with the protein. In fact, the 3'-end of the cleaved strand is tightly held by TraIp such that the superhelical density of the nicked and covalently closed forms of the TraIp-bound plasmid are identical (17). Some as yet unidentified protein-protein interaction presumably drives this equilibrium in the direction of the nicked DNA with subsequent relaxation of the plasmid when an appropriate mating signal is received (Fig. 5c). Then, the molecule of TraIp covalently bound on the 5'-end of the cleaved strand, acting as a helicase, unwinds the double-stranded DNA to generate ssDNA for transfer (Fig. 5d). This reaction requires the concomitant hydrolysis of ATP and could, in fact, be catalyzed by other molecules of helicase I. This remains to be determined. The 3'-OH on the cleaved strand could be utilized in donor-conjugal DNA synthesis, but this is not a requirement since strand transfer is not obligatorily coupled with donor-conjugal DNA synthesis (6). To simplify this model, synthesis from the 3'-OH has not been shown. Finally, the molecule of TraIp covalently bound on the 5'-end of the cleaved strand is poised to ligate the

2 J. A. Sherman and S. W. Matson, unpublished observations.
two ends of the "transferred" strand as the unwinding reaction is completed (Fig. 5e). The result is two ssDNA circles that are complementary to one another (Fig. 5f). Thus, TraIp is involved in both the initiation and termination of strand transfer through the catalysis of the cleavage/ligation described here.

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