MICROBIOLOGICAL REVIEWS, Dec. 1995, p. 579–590 Vol. 59, No. 4
0146-0749/95/$04.00+0

Conjugative Transposons: an Unusual and Diverse Set of Integrated Gene Transfer Elements

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IT'S A PLASMID! IT'S A PHAGE! NO, IT'S A CONJUGATIVE TRANSPOSON!

Ancient mythologies are full of chimeric figures, such as sphinxes, centaurs, and griffons, that combine features of more than one animal. Conjugative transposons could be viewed as a molecular equivalent of these creatures, in the sense that conjugative transposons combine features of transposons, plasmids, and bacteriophages. Unlike the mythological chimeras, however, conjugative transposons are not figments of the human imagination. They are all too real gene transfer elements, which are contributing to the spread of antibiotic resistance genes in several clinically important groups of bacteria, including gram-positive cocci and *Bacteroides* spp. (11, 12, 58, 72). Conjugative transposons are discrete DNA elements that are normally integrated into a bacterial genome (3, 11, 12, 57, 58, 72). The steps thought to be involved in their transposition and transfer are illustrated in Fig. 1. The first step is excision and formation of a covalently closed circular intermediate, which can either integrate elsewhere in the same cell (intracellular transposition) or transfer itself by conjugation to a recipient, where it integrates into the recipient's genome (intercellular transposition).

Conjugative transposons are transposon-like in the sense that they excise from and integrate into DNA, but they appear to have a different method of excision and integration from that of well-studied transposons such as Tn*5* and Tn*10*, in their mechanism of excision and integration (3, 11, 52, 57). For example, conjugative transposons have a covalently closed circular transposition intermediate and do not duplicate the target site when they integrate into DNA. Conjugative transposons are plasmid-like in that they have a covalently closed circular transfer intermediate and are transferred by conjugation, but unlike plasmids, the circular intermediate of a conjugative transposon does not replicate, at least in hosts so far investigated (3, 36, 52, 60). This caveat is an important one, because there are plasmids in *Streptomyces* spp. that integrate into the chromosome in some hosts and replicate as plasmids in other hosts (30). At this point, the possibility that the circular forms of conjugative transposons are capable of replication in some hosts cannot be ruled out. In fact, conjugation itself could be viewed as a form of replication, because the single-stranded circle that remains behind in the donor and the single-stranded copy that enters the recipient must be made double stranded before integration occurs. Conjugative transposons are phage-like in that their excision and integration resembles excision and integration of temperate bacterio-

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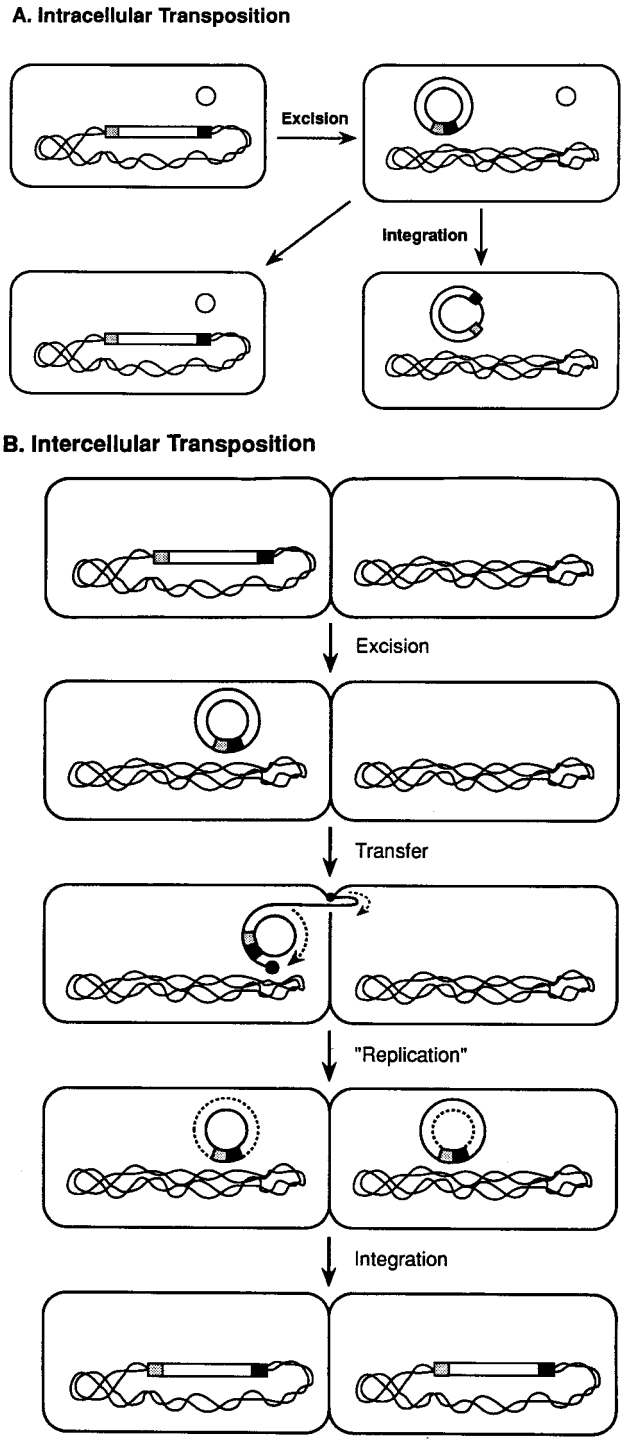

FIG. 1. (A) Steps thought to occur during intracellular transposition of a conjugative transposon. The integrated form of the conjugative transposon excises to form a covalently closed double-stranded DNA circle, which is the transposition intermediate. Filled boxes indicate the two ends of the integrated form of the conjugative transposon. The excised circular form does not replicate. It can integrate either back into the chromosome or into a plasmid. (B) Steps thought to occur during intercellular transposition of a conjugative transposon. The integrated form excises to form a covalently closed circle, which is the transfer intermediate. The circular form is nicked at an *oriT*, and a single strand is transferred by a process similar to plasmid transfer. The *oriT* is shown as internal in this figure. This is known to be the case for one type of *Bacteroides* conjugative transposon (36) and has been suggested (but not proven) to be the case for Tn*916* (59). In the recipient and in the donor, the double-stranded form of the circular intermediate is regenerated and the circular form integrates.

phages, which also have a circular intermediate. In fact, sequence analysis of integrases of some conjugative transposons suggests that they are members of the lambda integrase family (31, 48, 49, 51, 77). In contrast to the lambdoid phages, however, conjugative transposons do not form viral particles, and they are transferred by conjugation rather than by phage transduction. The purpose of this review is to survey recent work on the characteristics and activities of conjugative transposons.

WHERE ARE CONJUGATIVE TRANSPOSONS FOUND?

Gram-Positive Bacteria

Conjugative transposons were first discovered in the late 1970s by two different laboratories, both of which were working on gram-positive cocci (19, 20, 67). The conjugative transposon found in *Enterococcus faecalis* by Clewell's group was designated Tn*916* (19, 20). The conjugative transposon found in *Streptococcus pneumoniae* was originally called the Ω*cat*/*tet* element and is now called Tn*5253* (1, 67). Tn*916* and Tn*5253* differ considerably in size. Tn*916* is 18.5 kbp in size, whereas Tn*5253* is at least 60 kbp. Tn*5253* carries an inserted Tn*916*-like element, but the rest of the conjugative transposon appears to be unrelated to Tn*916*. Both Tn*916* and Tn*5253* carry the tetracycline resistance (Tc^r) gene *tetM*, which encodes a ribosome protection type of resistance protein (6). In the intervening years since Tn*916* and Tn*5253* were discovered, a number of other gram-positive conjugative transposons have been described (see, for example, references 16, 25, 26, 33, 50–52, and 80). Many of these have proved to be related to Tn*916*. Tn*916* and a closely related conjugative transposon, Tn*1545*, have been the most intensively studied of the gram-positive conjugative transposons. Tn*1545* is larger than Tn*916* (25 kbp) and carries kanamycin and erythromycin resistance genes in addition to *tetM*, but its ends and most of its interior are virtually identical to the corresponding regions of Tn*916* (7, 12).

Large conjugative transposons other than Tn*5253* have also been found in gram-positive bacteria. Two examples are Tn*3701* (found in a *Streptococcus pyogenes* strain [33]) and Tn*5276* (found in a *Lactococcus lactis* strain [50, 51]). Tn*3701* and Tn*5276* are about the same size as Tn*5253*. Tn*3701*, like Tn*5253*, is a composite element that contains a Tn*916*-like element integrated into a larger element. The larger element has some homology with Tn*5253*. Tn*5276* appears to be unrelated to Tn*3701* and Tn*5253*. It carries a gene involved in sucrose metabolism and a gene encoding resistance to nisin, a bacteriocin used by the food industry to prevent growth of undesirable bacteria (26).

Gram-Negative Bacteria

Conjugative transposons are not confined to the gram-positive bacteria. Tn*916* and its relatives, or *tetM* (the marker carried on the Tn*916* type elements), have been found in gram-negative bacteria, such as *Neisseria gonorrhoeae* and *Kingella* spp. (32, 53, 79). Finding Tn*916* in both gram-positive and gram-negative bacteria is not surprising since Tn*916* can transfer between gram-positive and gram-negative bacteria (4, 12–14, 47). A distinctive group of conjugative transposons, which are completely unrelated to Tn*916*, have been found in the gram-negative anaerobes, especially *Bacteroides* spp. (22, 23, 40, 44, 62, 72). The *Bacteroides* conjugative transposons range in size from 65 to over 150 kbp (3, 44). Like Tn*916*, most of them carry a ribosome protection type of Tc^r gene, *tetQ*, which is distantly related to *tetM* (43), and they also appear to have a

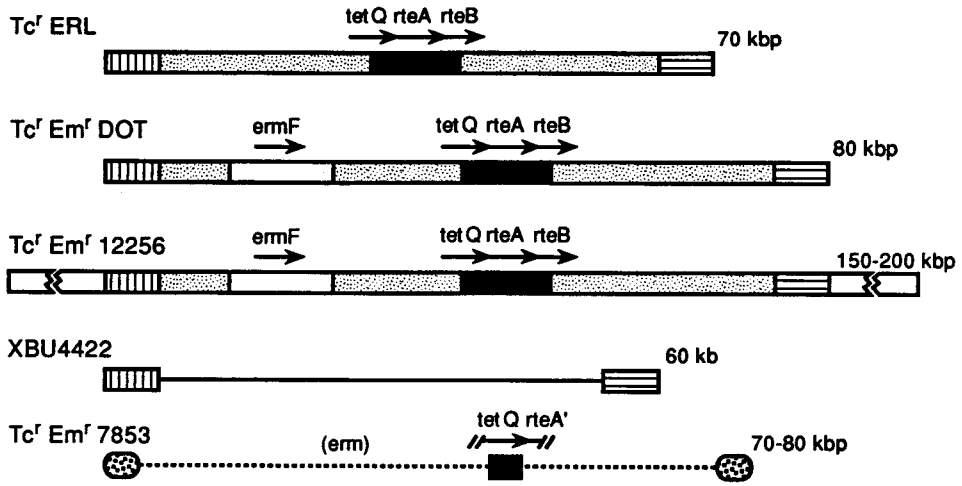

FIG. 2. Families of *Bacteroides* conjugative transposons. The Tc^r gene *tetQ* is located near the center of most of these element. In many of the elements, *tetQ* is linked to two genes, *rteA* and *rteB*, which are involved in regulation of transfer (73). In the case of Tc^rEm^r DOT, the transfer origin (*oriT*) is located about 1 kbp downstream of *rteB* (36). Shading indicates areas of the different conjugative transposons that are similar enough to cross-hybridize on Southern blots. The ends are shaded differently to indicate that they contain completely different DNA sequences. Some of the Tc^r ERL type elements have an additional segment (about 10 kbp) that contains and Em^r gene, *ermF* (23). Tc^rEm^r 12256 (also called Tn*5030* [23, 40]) is a hybrid element, which consists of a Tc^rEm^r DOT type element inserted into another conjugative transposon not related to the Tc^r ERL type conjugative transposons (3, 66). XBU4422 is a cryptic element, whose ends resemble those of Tc^r ERL but whose interior regions have only scattered regions of homology with Tc^r ERL (66). Tc^rEm^r 7853 appears to be completely unrelated to the Tc^r ERL type elements except for a region near its center that contains *tetQ* and a short segment of *rteA* (44). The *erm* gene on this element is not *ermF*.

circular transfer intermediate (3, 36, 62). The *Bacteroides* conjugative transposons, however, have features not found in any of the gram-positive conjugative transposons. These include the ability to insert into coresident plasmids and mobilize them in *cis*, the ability to excise and mobilize unlinked integrated elements, and an elaborate regulatory system that senses tetracycline and controls element transfer functions (see later sections). There are at least two distinct families of conjugative transposons in the *Bacteroides* species. One family is exemplified by a conjugative transposon called Tc^r ERL (Fig. 2) (62). This family has at least one cryptic member, XBU4422 (66). A second is exemplified by Tc^rEm^r 7853 (Fig. 2) (44), which appears to be completely unrelated to the Tc^r ERL family except in the region immediately around *tetQ*. A 150-kbp conjugative transposon, which we have called Tc^rEm^r 12256 (also called Tn*5030* [Fig. 2] [3, 40, 62]), is a compound element that consists of a Tc^r ERL type of element inserted into another element. This other element appears to be different from Tc^r ERL and Tc^rEm^r 7853 and could represent a third family of *Bacteroides* conjugative transposons.

The *Bacteroides* conjugative transposons provide a graphic example of how conjugative transposons can drive the spread of antibiotic resistance genes. *Bacteroides* spp. are opportunistic pathogens, which can cause life-threatening infections in people whose colons have been perforated or ruptured. At one time, *Bacteroides* infections could be treated with tetracycline. Today, virtually all *Bacteroides* clinical isolates are Tc^r, and all Tc^r isolates so far tested have proved to carry a conjugative transposon (44, 62). Resistance to other drugs of choice for treating *Bacteroides* infections, such as clindamycin and cefoxitin, is now being spread both by the conjugative transposons themselves and by the elements they mobilize.

One group of gram-negative bacteria, in which conjugative transposons have still not been reported, is the *Escherichia coli-Pseudomonas* group. Although it is conceivable that conjugative transposons do not occur in this phylogenetic group, it is more likely that the lack of reports of conjugative transposons simply reflects failure to look for them. Since Tn*916* and its relatives can transfer from gram-positive cocci to *E. coli* and *Pseudomonas* spp. under laboratory conditions (4, 14, 47), it would be surprising if Tn*916* were not found in natural isolates of the *E. coli-Pseudomonas* group of gram-negative bacteria. It is also possible, of course, that the *E. coli* group has its own unique group of conjugative transposons. Since conjugative transposons are integrated elements, except transiently during conjugal transfer, there is no systematic way to screen for them, analogous to plasmid preparation procedures. Accordingly, they are usually found by accident. Sometimes, a conjugative transposon announces its presence by integrating into a plasmid. In other cases, however, evidence suggesting the presence of a conjugative transposon is indirect. One indication that a conjugative transposon is present in a bacterial strain is conjugal transfer of chromosomal genes. Another is the apparent self-transfer of plasmids too small to encode the necessary transfer genes. This type of finding suggests the presence of a conjugative transposon, because many conjugative transposons can mobilize coresident plasmids in *trans.*

WHAT'S IN A NAME? THE NOMENCLATURAL DILEMMA

There is as yet no distinctive nomenclature for conjugative transposons. Groups working on the gram-positive conjugative transposons and some groups working on the *Bacteroides* conjugative transposons have given their elements transposon designations. This practice has the drawback that the designation does not indicate whether the element is a conjugative transposon or a nonconjugative transposon. As already indicated, conjugative transposons differ in a number of ways from most nonconjugative transposons, so the distinction is an important one. Further confusion arises because the Tn numbers for conjugative transposons and nonconjugative transposons can be quite similar. For example, Tn*1545* is a conjugative transposon and Tn*1546* is not. Our group has designated the *Bacteroides* conjugative transposons we study by the resistances they carry and the name of the strain in which they were first

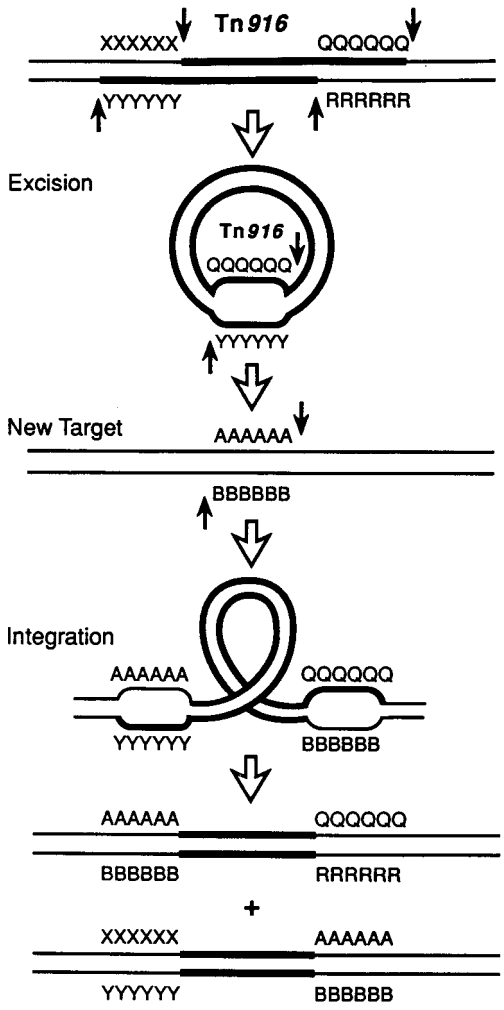

FIG. 3. Proposed mechanism for excision and integration of Tn*916* (9). Staggered cuts that occur 6 nucleotides from the ends of the element produce single-stranded overhangs (coupling sequences), which are joined covalently to produce the circular transposition intermediate. The bulged area indicates that the bases do not base pair in this region. Staggered cuts open up the circular form and its target site. Ligation produces two non-base-paired regions, which are resolved either by mismatch repair or by replication through the region.

found. For example, the conjugative transposon Tc^r ERL carries a Tc^r gene and was originally found in *B. fragilis* ERL. This nomenclature has the advantage that it distinguishes conjugative transposons from other transposons, but it is rather awkward. One solution, which would not require renaming all of the known conjugative transposons, would be to retain the transposon designations but use a prefix of "c," e.g., CTn*916*.

TRANSPOSITION MECHANISM

Tn*916* Transposition: the Phage Lambda Connection

Most of the work done to date on the mechanism of excision and integration of conjugative transposons has been done with the closely related gram-positive conjugative transposons Tn*916* and Tn*1545*. The model proposed for transposition of the Tn*916*-type elements is shown in Fig. 3 (57, 58). During excision, staggered cuts are made at each end of the element. These staggered cuts leave a single-stranded 6-nucleotide stretch at each end. These 6-nucleotide stretches are called coupling sequences. In early reports, the coupling sequences of Tn*916* were reported variously to be 5 to 7 nucleotides in length. Rudy and Scott (54) have now shown that the earlier suggestion by Poyart et al. (47, 48) that the coupling sequences of Tn*916* are 6 nucleotides in length was correct. This may not be true of all members of the Tn*916* family. Rice and Carrias (52) have presented evidence that a Tn*916*-like transposon, Tn*5381*, has coupling sequences of different lengths.

In the model depicted in Fig. 3, the single-stranded coupling sequences are joined covalently to produce a circular intermediate. The DNA molecule from which the conjugative transposon has excised is also resealed. The coupling sequences are not homologous and do not base pair with each other. During integration, the coupling sequences also form non-base-paired regions with the target site (Fig. 3). Subsequent DNA replication through the region either restores the coupling sequence of the original target site sequence or inserts a new 6-bp sequence. Alternatively, the mismatch repair system may resolve the mismatches. The same process occurs in the DNA segment from which the conjugative transposon excised, so that excision of a conjugative transposon can either lead to restoration of the exact sequence of the region prior to integration or can result in replacement of the original 6-bp sequence at the site of integration with a different 6-bp sequence. Integration of Tn*916* does not duplicate the target site.

Tn*916* integrates relatively randomly in most hosts, but it has a decided preference for long stretches of A's and T's (28, 59, 81). The site selectivity of Tn*916* has made it less useful as a tool for mutagenesis than it would have been if its integration were more random, but it has been used successfully for mutagenesis in some species (11, 21, 58). A convenient feature of Tn*916* is that it excises at high frequency in *E. coli*. Thus, if an interesting transposon insertion is generated, the region in which it occurred can be cloned and moved into *E. coli*, in which at least some of the excision events will restore the wild-type sequence (21).

The genes on Tn*916* and Tn*1545* that mediate integration (*int*) and excision (*xis*) have been identified and characterized (12, 18, 48, 49). The *int* and *xis* genes of Tn*1545* are very similar in sequence to the *int* and *xis* genes of Tn*916*. Both genes lie near one end of the element, downstream of *tetM*, and are transcribed in the same direction (Fig. 4) (18). The *int* gene product is essential for both excision and integration, whereas the *xis* gene stimulates excision but is not essential (48, 49, 58, 76). These roles are reminiscent of the roles of phage lambda Int and Xis proteins. In fact, the Tn*916* and Tn*1545* integrases have proved to be related to integrases of the phage lambda integrase family of site-specific recombinases at the amino acid sequence level (48, 49). They share the amino acid sequence signature near the active site that characterizes this family, but they have little sequence similarity to the lambda Int protein outside this region. The Xis of Tn*916*/*1545* has no sequence similarity to the Xis of phage lambda, but it resembles lambda Xis in that it is a small, basic protein. At first, it seemed surprising that an element whose mechanism of excision and integration appeared to be so different from that of phage lambda would have an integrase that is related to lambda integrase. In particular, the branch migration step that has always been a prominent feature in models of lambda integration would be impossible for Tn*916* because of lack of homology between the coupling sequences. Trieu-Cuot et al. (81) have pointed out that the lack of homology between the coupling sequences suggests that Tn*916* can transpose only when all four DNA strands are cleaved at the same time and that transposition does not occur by the sequential cleavage-branch migration mechanism ascribed to phage lambda.

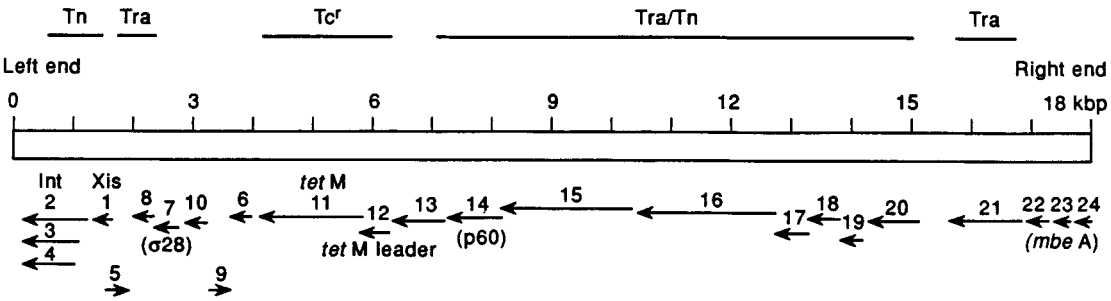

FIG. 4. Gene organization of Tn*916* (12, 18, 61, 77). Open reading frames revealed by sequence analysis are shown. Int and Xis are encoded by *orf2* and *orf1*, respectively. *orf3* and *orf4* are possible open reading frames that lie within *orf2*, whose significance is still unknown. *tetM* and the leader region that controls expression of *tetM* (74) are *orf11* and *orf12*, respectively. *orf7* has sequence similarly to the gene encoding sigma 28 of *Bacillus* spp. *orf14* has sequence similarity to the gene encoding a surface protein, p60, of *Listeria monocytogenes*. *orf23* has sequence similarity to the gene encoding a plasmid mobilization protein, MbeA, and might encode a mobilization protein involved in conjugal transfer of the circular form. The regions identified by transposon mutagenesis as being important for transfer (Tra), transposition (Tn), or both (Tra/Tn [56]) are indicated by horizontal lines at the top of the figure.

Why, then, is the Tn*916*/*1545* integrase a member of the lambda integrase family? A possible answer to this question has come, surprisingly enough, from recent work on phage lambda integration. Results of a recent study of the lambda integration mechanism have raised questions about the assumption that branch migration is a necessary step in lambda integration. Nunes-Duby et al. (46) have proposed a revised model for lambda integration that does not include branch migration and could accommodate the type of integration mechanisms proposed for Tn*916*/*1545*.

Progress toward an In Vitro System for Tn*916* Transposition

To prove the model illustrated in Fig. 3 conclusively, an in vitro system for transposition of Tn*916* is needed. The first step in this direction has been made by Lu and Churchward (38, 39), who have purified a maltose-binding protein fusion form of the Tn*916* Int and have shown that it footprints both ends of Tn*916*. The region protected by the bound maltose-binding protein integrase extended about 17 bp into the adjacent chromosomal DNA. This result may explain a puzzling observation of Jaworski and Clewell (29), who found that copies of Tn*916*, which had integrated in exactly the same site but differed in their coupling sequences, transposed at rates that varied by as much as 10^4. Since the Int-binding site includes the coupling sequence, some coupling sequences may bind the integrase better than others and thus promote excision from that site, and this could explain why some coupling sequences promote transposition better than others. In fact, Lu and Churchward (38) have now shown that the strength of Tn*916* Int binding to the junction regions of an integrant correlates well with the excision frequency of the integrant. Tn*916* Int must be recognizing something other than the primary sequence of the junction regions, because there is no clear sequence signature associated with the high-frequency coupling sequences (29, 38).

The DNA-footprinting experiments of Lu and Churchward (39) suggest that the Tn*916* Int has two DNA binding domains, one in its amino terminus and the other in its carboxy terminus. They propose that copies of Int bind initially to the ends of Tn*916* and then make contact with the other end via the second DNA binding domain when the two ends are brought together, much as lambda Int binds the phage arm-type and core sites during excision and integration. Their findings provide further support for the contention that integration of Tn*916*/*1545* occurs by a mechanism that is a variant of lambda integration and not a completely novel mechanism. The more things change, the more they remain the same. If Tn*916* integration and excision resembles that of phage lambda, it would seem likely that host factors, such as integration host factor and Fis, would be involved in excision and integration, but Poyart et al. (47) have shown that integration of Tn*1545* and a Tn*916* derivative in *E. coli* does not require integration host factor.

The integration and excision genes of some of the large gram-positive transposons have been cloned and sequenced. Like Tn*916* and Tn*1545*, these larger elements have *int* and *xis* genes, both of which are located near one end of the element (31, 33, 51, 83). Moreover, the Int proteins of the large elements also appear to be members of the lambda integrase family (31, 51). Integration of the larger conjugative transposons appears to be more site specific than integration of Tn*916* and Tn*1545*, and at least two of the elements appear to have a primary target site that is identical to a region at one end of the element.

Evidence that the Transposition Intermediate Is a Nonreplicating Circle

In early papers on Tn*916*, Clewell and Gawron-Burke (13) suggested that the transposition intermediate was a covalently closed circle. The circular form of Tn*916* was subsequently isolated by Scott et al. and shown to be capable of integration when introduced by transformation into *Bacillus subtilis* protoplasts (57, 60). This result suggests that the form of Tn*916* that integrates is a double-stranded circle, not the single-stranded form that is presumably transferred by conjugation in intercellular transposition. Sequence analysis of excision and integration events involving the large gram-positive conjugative transposons suggests that these elements also have a circular transposition intermediate, but the circular forms have not yet been isolated (50, 51, 83).

Excision and Integration of the *Bacteroides* Conjugative Transposons: Tn*916* Redux or a Novel Mechanism?

Analysis of excision and integration events involving the *Bacteroides* conjugative transposons has been done with a cryptic member of the Tc^r ERL family, XBU4422 (3). XBU4422, which has ends that are very similar to those of Tc^r ERL, was used because it is the only *Bacteroides* conjugative transposon that has been trapped on a plasmid. In *Bacteroides thetaiotaomicron*, the Tc^r ERL type of conjugative transposons integrated relatively site specifically, with three to seven sites per chromosome, depending on the element tested (3). The site

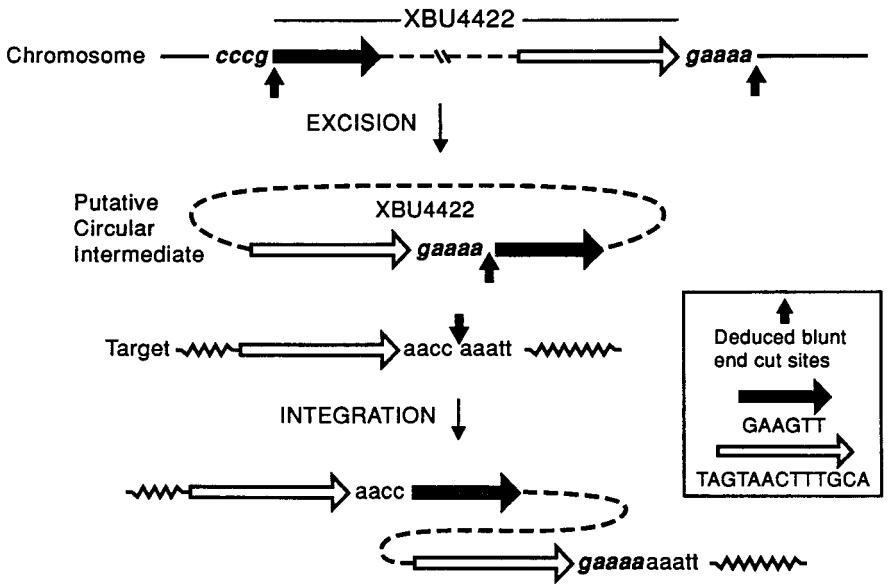

FIG. 5. Model proposed for excision and integration of the *Bacteroides* conjugative transposon, XBU4422 (3). The putative ends of XBU4422 are indicated by a solid horizontal arrow on the left and an open horizontal arrow on the right. The short stretches of DNA sequence these represent are provided in the boxed insert. Vertical arrows at the top of the figure indicate the sites of cleavage reactions that occur in over 90% of excision events. In one case, the cleavages occurred adjacent to CCCG and at the right end of XBU4422. The circular form of XBU4422 and the target site are cleaved at the positions indicated by the vertical arrowheads, and XBU4422 integrates to give the form shown at the bottom of the figure. An open horizontal arrow in the target indicates a 13-bp region that has high sequence identity (but not complete identity) with a 13-bp region adjacent to the right end of XBU4422. These similar regions may allow the circular form to align with its target, explaining the sequence selectivity and orientation-specific integration of XBU4422. The model shown here posits blunt-ended double-stranded cleavage events, but the possibility that excision and integration occur by a Tn*916*-like model, with CCCG and GAAAA as the coupling sequences, cannot be ruled out at this time (see text).

selectivity of integration presumably explains why attempts to trap *Bacteroides* conjugative transposons on a plasmid were not successful in most cases. Results of the sequence analysis of XBU4422 integration and excision events led us to suggest the model for integration and excision shown in Fig. 5. There was only one integration site on the plasmid used. Integration of XBU4422 into this site was orientation specific as well as site specific. The features of integration and excision events are best explained by assuming that there is a circular intermediate, as depicted in Fig. 5, but it is important to keep in mind that this putative circular intermediate has not yet been demonstrated directly by isolation of the circular form. There is a region of near identity between one end of the *Bacteroides* conjugative transposon and its target site (Fig. 5). This region may be responsible for the site selectivity of integration. Integration does not occur within this region, however, but at a point 4 bp from one end of it.

The model for integration and excision of the *Bacteroides* conjugative transposons appears at first glance to be quite different from that proposed for Tn*916* (Fig. 3), but we cannot rule out the possibility that the *Bacteroides* elements actually use a Tn*916*-type excision-integration mechanism. The reason is that in one excision event, the CCCG from the left side of the element was taken along rather than the GAAAA shown in Fig. 5 (3). Thus, it is conceivable that CCCG and GAAAA play the role of coupling sequences. If so, the fact that the coupling sequences have different lengths might explain why GAAAA is taken along preferentially over CCCG, rather than GAAAA and CCCG appearing with approximately equal frequency, as is seen in the case of Tn*916*. A finding that supports, very indirectly, the possibility that the *Bacteroides* conjugative transposons are actually excising and integrating by a mechanism that resembles that of Tn*916*, rather than the one depicted in Fig. 5, is that a variant of the integration site shown in Fig. 5 has been found; this variant is identical to that shown in the figure except that the 4-bp segment adjacent to the integration site has been replaced by a different 4-bp segment. This is a type of excisant expected for an element that uses a Tn*916*-type excision/integration mechanism. It is not clear which element was responsible for this 4-bp change, and it may not have been a member of the XBU4422 family. All of the excision events involving XBU4422 resulted either in precise excision or excision that left behind one of the A residues of GAAAA, so the type of sequence replacement seen in some Tn*916* excision events was not detected during examination of XBU4422 excision from the target plasmid. Localization and sequencing of the *Bacteroides* conjugative transposon genes involved in excision and integration is under way, and information about these genes could help to resolve the question whether the *Bacteroides* elements integrate and excise similarly to Tn*916*.

Interactions between Conjugative Transposons: Cooperation rather than Antagonism

Conjugative transposons are a very sociable set of elements. The Tn*916*-type conjugative transposons do not exclude each other as do plasmids and some standard transposons (12, 45). This is also true for the *Bacteroides* conjugative transposons (62, 66). Thus, a strain can accumulate more than one conjugative transposon. The only limitation is that in the case of relatively site-specific elements, such as some of the *Bacteroides* conjugative transposons, occupation of a site by one copy of the conjugative transposon can limit the ability of incoming conjugative transposons to use the same site. Flannagan and Clewell (17) have observed that the presence of two copies of Tn*916* in the same strain results in a stimulation of transposition. This phenomenon has been called transactivation, and it might involve in *trans* action of Int and Xis pro-

duced by one element on the ends of the other, but the mechanism is still unknown. There are some indications that the *Bacteroides* conjugative transposons stimulate each other's transposition as well. Overexpression of a regulatory gene, *rteC*, which is involved in the control of transfer genes (see later sections) has been shown to stimulate transfer of a *Bacteroides* conjugative transposon when provided in *trans* in multiple copies (36). Thus, in the case of the *Bacteroides* conjugative transposons, interactions between different elements could be due to *trans* action of regulatory proteins rather than to *trans* action of proteins involved in excision.

Another example of how two conjugative transposons interact with each other is provided by Tn*5253*, the large composite conjugative transposon that has a Tn*916* type element integrated in it (1). The integrated Tn*916*-type element is very stable and does not excise from the larger element unless the region containing Tn*916* is cloned away from the rest of the element. This suggests that a gene on the larger element is repressing excision of Tn*916*, but the gene responsible for this effect has not yet been located and characterized.

TRANSFER OF THE CONJUGATIVE TRANSPOSONS

Transfer Intermediate and Transfer Origin

Little is known about the transfer process for any of the gram-positive conjugative transposons. Scott et al. (59) analyzed the sequences of Tn*916* integrants after conjugative transfer and integration in a new host. The results suggest that the circular transposition intermediate of Tn*916* is also the transfer intermediate and that a single-stranded copy of Tn*916* is transferred during conjugation. Although the paper reporting these results contains a figure that depicts a circular form of Tn*916* with an internal transfer origin (*oriT*), no evidence that Tn*916* has an internal *oriT* was presented. A feature of Tn*916* that would seem to argue against an internal *oriT* is that Tn*916* is unable to mobilize plasmids in *cis* (13, 58). That is, if Tn*916* is integrated into a plasmid in the donor, Tn*916* itself will transfer to a recipient but the plasmid markers are not cotransferred. If the circular intermediate of Tn*916* is, in fact, transferred like a plasmid from an internal *oriT*, Tn*916* ought to be able to mobilize plasmids in *cis*. One possible explanation for failure to mediate *cis* mobilization is that the *oriT* of Tn*916* is at or near an end of the element and that formation of the circular transposition intermediate is necessary to generate an active *oriT* region. Nonetheless, a recent review by Clewell et al. (12) mentions unpublished results that suggest that the Tn*916 oriT* is in fact internal and is located near an open reading frame near one end of Tn*916*. The deduced amino acid sequence of this open reading frame is similar to that of a ColE1 mobilization protein, MbeA (Fig. 4). Why, then, is Tn*916* unable to mobilize plasmids in *cis*? A possible explanation is that the process of conjugal transfer somehow enhances the rate of Tn*916* excision, so that transfer of the excised form of Tn*916* masks the transfer of Tn*916*::plasmid cointegrates. One way to resolve this issue would be to clone a large segment of Tn*916*, which lacks *int* and *xis*, into a nonmobilizable plasmid and to determine whether the clone is now self-transmissible. If there is an internal *oriT*, the resulting plasmid should transfer as an intact unit.

An interesting controversy has arisen as to whether Int is needed both in the donor and in the recipient or only in the donor. It is clear that Int is essential for both integration and excision. Storrs et al. (76) found that a mutant of Tn*1545* with an inactivated *int* gene would perform intercellular transposition only if a wild-type *int* gene was provided in *trans* in both the donor and the recipient (76). Subsequently, Bringel et al. (5) presented evidence that seemed to suggest that Int was required only in the donor. There are some problems with this more recent paper, however. Bringel et al. (5) found that when *int* was provided in *trans* on a plasmid and was provided only in the donor, intercellular transposition was detected but its frequency was 10^4-fold lower than the frequency seen when *int* was provided both in the donor and in the recipient. By contrast, if *int* was provided in *trans* from a wild-type integrated Tn*916* in the donor, with no *int* provided in the recipient, a somewhat higher frequency of intercellular transposition was seen (5). The problem with this set of experiments is that they appear not to have been done with recombination-deficient donor and recipient strains. Since a high degree of cotransfer of the mutant and wild-type forms of Tn*916* was observed, it is possible that cointegrates of wild-type and mutant Tn*916* formed prior to transfer via homologous recombination and were resolved in the recipient. Also, since wild-type Tn*916* was being cotransferred with the mutant element, Int could well have been provided in the recipient by the wild-type element, which was also being transferred.

The *Bacteroides* conjugative transposon XBU4422, unlike Tn*916*, is capable of mobilizing plasmids in *cis*, as expected if it has an internal *oriT* (3, 66). The *oriT* of XBU4422 has not been located, but the *oriT* region of a related conjugative transposon, Tc^rEm^r DOT, has been cloned and characterized (36). It is located near the middle of the conjugative transposon. This *oriT* region, cloned into a nonmobilizable plasmid, rendered the plasmid mobilizable by a chromosomal copy of Tc^rEm^r DOT. This result supports the hypothesis that the transfer intermediate of the *Bacteroides* conjugative transposons is a covalently closed circle, which is transferred similarly to a plasmid. That is, transfer starts with a single-stranded nick in the *oriT* region, and a single-stranded copy of the element is transferred through a mating pore to the recipient (34, 84, 85). The *oriT* region of Tc^rEm^r DOT has none of the consensus features found in *oriT* regions of conjugal *E. coli* group plasmids and at the ends of T-DNA (34). Although *oriT* regions are usually located near genes encoding the mobilization proteins that bind the *oriT* and form the relaxosome that nicks at the *oriT*, the genes encoding the mobilization proteins of Tc^rEm^r DOT are located at least 3 kbp from the *oriT* region. These genes have not yet been identified. The open reading frame located adjacent to the *oriT* of Tc^rEm^r DOT appears to encode a regulatory protein, RteC (see the next section), which could be controlling expression of the mobilization gene(s).

Transfer Genes

The region of Tn*916* that is required for transfer has been mapped and sequenced (12, 18) (Fig. 4). An interesting finding was that none of the open reading frames in the transfer region had significant sequence similarity to sex pilus genes of conjugal plasmids. Also, the number of possible genes in this region is relatively small compared with that in the transfer regions of conjugal plasmids. This raises the possibility that the Tn*916* conjugal transfer system is much simpler than that of conjugal plasmids such as F or RP4 and may lack a sex pilus. There is a precedent for this in the gram-positive cocci. A group of plasmids, whose transfer is triggered by peptide pheromones, employs a surface protein receptor on the donor, rather than a sex pilus, to initiate the close contact with a recipient that leads to formation of the mating pair (10). The region of the *Bacteroides* conjugative transposon Tc^rEm^r DOT, which is necessary and sufficient for conjugal transfer of the element, has been localized to an 18-kbp region of the element (36), and sequenc-

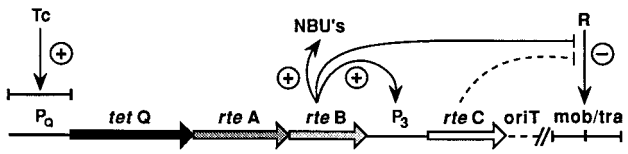

FIG. 6. Regulatory genes involved in control of transfer activities of the *Bacteroides* conjugative transposon Tc^{r}Em^r DOT (36, 56, 73–75). Tetracycline causes a 20-fold enhancement of transcription from the *tetQ* promoter (indicated by a circled + sign), thus causing enhanced production of RteA and RteB. RteB activates excision of unlinked elements called NBUs (see Fig. 7) and also causes a downstream gene on Tc^{r}Em^r DOT, *rteC*, to be expressed. RteB and RteC are essential for element self-transfer. The role of RteA is unknown. Neither RteA nor RteB is required for expression of the *tetQ/rteA/rteB* operon. Both RteB and RteC appear to act as antirepressors which bind to and inactivate a repressor (R) that normally represses transcription of some essential transfer genes (indicated by a circled − sign). The effect of RteB is stronger than that of RteC, as indicated by a dashed line from *rteC*. The *oriT* region of Tc^{r}Em^r DOT lies immediately downstream of *rteC*.

ing of this region is under way. So far, no homologs of Tn*916* transfer region *orf* genes have been found (unpublished results). It remains to be seen whether the Tc^{r}Em^r DOT type conjugal transfer system resembles that of Tn*916* or is more like that of conjugal plasmids.

REGULATION OF TRANSFER GENES: HOW AN ANTIBIOTIC CAN FUNCTION AS AN APHRODISIAC FOR BACTERIA

The transfer of many conjugative plasmids is regulated, but only in a few cases is the signal known (see, for example, references 10, 27, and 86). In the case of the *Bacteroides* conjugative transposons, the signal is tetracycline. A brief exposure of a *Bacteroides* donor carrying Tc^{r}Em^r DOT to low levels of tetracycline stimulates self-transfer of the conjugative transfer by 10,000-fold (73, 75, 82). That is, tetracycline stimulates mating and thus acts as the bacterial equivalent of an aphrodisiac. The stimulation of transfer is probably not due to stress caused by tetracycline inhibition of protein synthesis. For one thing, it occurs in cells carrying *tetQ*, which allows protein synthesis to proceed in the presence of tetracycline. For another, autoclaved chlorotetracycline, a nontoxic analog of tetracycline, stimulates transfer. Finally, only very short exposure to tetracycline (<30 min) and low levels of tetracycline (1 μg/ml) are needed for stimulation of transfer.

At least some of the genes responsible for tetracycline stimulation of transfer have been identified and characterized (Fig. 6) (36, 56, 74). Tetracycline stimulates transcription of an operon that contains *tetQ* and two regulatory genes, *rteA* and *rteB*, by about 20-fold. The mechanism by which transcription of the *tetQ-rteA-rteB* operon is stimulated is not known, but it is clear that RteA and RteB are not required for expression of the operon. Possibly, some host protein interacts with tetracycline to stimulate transcription, but there is as yet no evidence for or against this hypothesis. Increased transcription results in increased production of RteA and RteB. RteA has amino acid sequence similarity to sensor proteins of two-component regulatory systems (73), but recent work in our laboratory indicates that RteA is not sensing tetracycline and may not be required for the tetracycline stimulation of transfer (unpublished data). RteB has amino acid sequence similarity to activator proteins of two-component systems. RteB is essential for transfer of the conjugative transposon (36, 73). One activity of RteB is to activate expression of a downstream gene, *rteC*, although the mechanism by which RteB does this is unknown (Fig. 6) (74). RteB also appears to act as an antirepressor to counter the effect of a repressor that normally prevents expression of transfer genes (Fig. 6) (36). The repressor has not yet been cloned and characterized. Its existence was inferred from the fact that the transfer region of Tc^{r}Em^r DOT, cloned away from *rteA*, *rteB*, *rteC*, and the rest of the conjugative transposon, was now capable of constitutive self-transfer. The mode of action of RteC is also unclear. A disruption in *rteC* completely abolishes transfer of the conjugative transposon but has little effect on mobilization of coresident plasmids. This leads us to suspect that RteC controls expression of the relaxosome genes and/or genes essential for excision and circularization in the conjugative transposon. RteC, like RteB, appears to be acting as an antirepressor, but it is not clear whether RteC interacts with the same repressor as RteB or with a different one (36).

An interesting question that arises in connection with this complex regulatory system is: why is tetracycline the signal? Tetracycline has been in use as an antibiotic for only a relatively short time, too short for such a complex system to develop in response to clinical use of this antibiotic. The hypothesis that tetracycline produced in nature by the actinomycetes may have contributed to the evolution of this system seems unlikely, because actinomycetes are aerobic soil bacteria whereas *Bacteroides* spp. are obligate anaerobes that would be inactive in those regions of soil where the actinomycetes are producing antibiotics. Similarly, in the anaerobic regions of soil and in the human colon, where *Bacteroides* spp. are normally found, the actinomycetes would be expected to be metabolically inactive. One possibility is that the conjugative transposons now found in *Bacteroides* spp. actually evolved in soil bacteria, such as *Flavobacterium* spp., which are related to *Bacteroides* spp., and were transferred to *Bacteroides* spp. by these soil bacteria when they were passing through the intestine. Another, more intriguing possibility is that tetracycline is not the real inducer of this system but, rather, that the inducer is some plant phenolic compound that resembles part of the tetracycline molecule. *Bacteroides* conjugative transposons transfer only when the donor and recipient are mated on solid surfaces. They do not mate in liquid medium. In the colon, *Bacteroides* spp., which rely on plant polysaccharides for much of their carbon and energy, may have means of sensing the surfaces of plant particles that reach the colon. It is possible that plant phenolics are used by *Bacteroides* spp. carrying conjugative transposons, as a signal that a surface suitable for mating is present. The problem with this hypothesis is that we have so far failed to find a plant phenolic compound that stimulates transfer of the conjugative transposons (unpublished results).

There have been three reports that transfer of Tn*916*-related conjugative transposons is stimulated by tetracycline (15, 70, 80). In two of these studies, tetracycline increased the transfer frequency by about 10-fold (15, 80), whereas in the third, a stimulation of over 100-fold was observed (70). This level of stimulation could be an indirect result of antibiotic-induced stress responses. Another possible explanation is suggested by the fact that the *int* and *xis* genes of Tn*916* are located downstream of *tetM* (Fig. 6). Su et al. (78) have shown that tetracycline stimulates transcription of *tetM* by an attenuation-type mechanism. Since *tetM*, *int*, and *xis* are all transcribed in the same direction, it is possible that tetracycline stimulation of *tetM* transcription results in increased readthrough transcription of *int* and *xis* and thus leads to a small increase in transposition frequency. Clewell et al. (12) have identified a possible regulatory gene, which they designate *traA*, that might also be contributing to regulation of transposition. This gene is located immediately upstream of *xis* and *int* and downstream of *tetM*. The model for TraA control of Tn*916* transposition which has

been proposed by Clewell et al. (12) is still largely speculative, and the role of tetracycline is unknown. If this model proves to be correct, it is completely different from the regulatory system found on the *Bacteroides* conjugative transposons.

MOBILIZATION OF CORESIDENT PLASMIDS

Both Tn*916* and the *Bacteroides* conjugative transposons are capable of mobilizing coresident plasmids in *trans* (11, 55, 64, 82). That is, the conjugative transposons provide the mating pore, which can be used by coresident plasmids that encode mobilization proteins to transfer to the recipient. In the case of the *Bacteroides* conjugative transposons, mobilization of coresident plasmids by these elements is clearly contributing to the spread of clindamycin resistance (due to *ermF*) among *Bacteroides* clinical isolates (64) and may be contributing to the spread of other resistance genes as well. Early in the study of *Bacteroides* plasmids, it was noted that many *Bacteroides* strains, representing a variety of distantly related species, carried identical small cryptic plasmids. The reason for this is now evident: the *Bacteroides* conjugative transposons have been shown to be capable of mobilizing all of the small cryptic plasmids so far tested (56, 64, 82). The widespread presence of the same cryptic plasmids in many different *Bacteroides* spp. provides clear evidence that the *Bacteroides* conjugative transposons have been an active force driving DNA exchange among *Bacteroides* strains in the colon. It is interesting that the same cryptic plasmids that are mobilized between *Bacteroides* spp. by the *Bacteroides* conjugative transposons are also mobilized between different strains of *E. coli* by the IncP plasmids R751 and RK2 (64). The ability of the *Bacteroides* conjugative transposons to mobilize coresident plasmids in *cis* has already been mentioned. This activity has important practical implications for the release of genetically engineered microorganisms into the environment. According to conventional wisdom, a plasmid used to introduce a gene into a genetically engineered microorganism can be rendered nontransmissible by eliminating any mobilization genes and *oriT* regions. Integration of a conjugative transposon, which is capable of *cis* mobilization, into such a plasmid would render it self-transmissible. The moral is clear: there is no such thing as a "safe" plasmid.

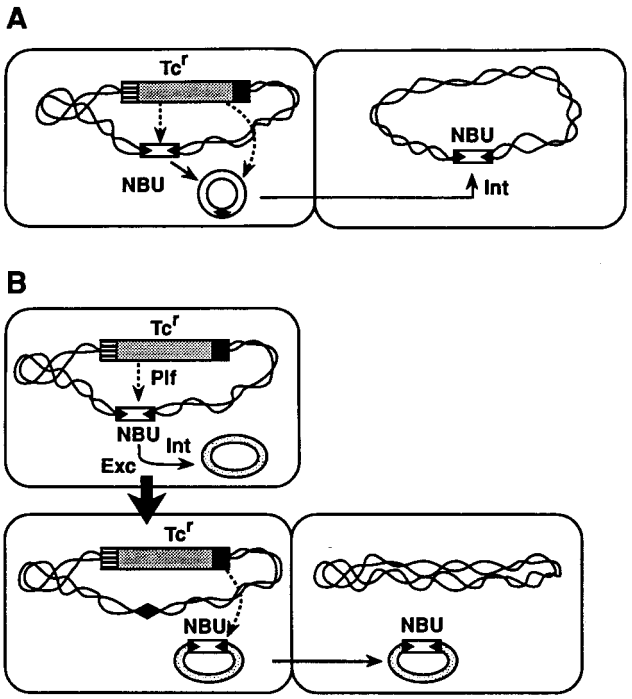

FIG. 7. (A) Excision and transfer of NBUs mediated by the *Bacteroides* conjugative transposons (69). The conjugative transposon is the large shaded element labeled with Tc^r. The NBU is the open element with triangular ends. The ends of the NBU and the conjugative transposon are shaded differently to indicate that they are completely different at the DNA sequence level (3, 69). RteB, provided by the conjugative transposon, acts in *trans* (dashed line) to activate the excision gene(s) on the NBU, thereby stimulating formation of the NBU circular form (73). Transfer genes provided by the conjugative transposon act in *trans* (dashed line) to allow transfer of the NBU circular form by providing the mating pore proteins. The NBU provides a protein, Mob (not shown), which initiates the transfer process (35, 37). In the recipient, the NBU circular form integrates into the genome. (B) The excised NBU (Exc) can also integrate (Int) into a coresident plasmid. The NBU-plasmid cointegrate becomes mobilizable in *trans* by the conjugative transposon because the integrated NBU provides an *oriT* and a mobilization protein that are capable of initiating transfer of the cointegrate (35, 37).

EXCISION AND TRANSFER OF UNLINKED INTEGRATED DNA SEGMENTS: AN UNUSUAL ACTIVITY OF THE *BACTEROIDES* CONJUGATIVE TRANSPOSONS

NBUs

An activity that has so far been reported only for the *Bacteroides* conjugative transposons is excision in *trans* of unlinked integrated elements and mobilization of these elements to recipients. One such integrated element has been designated NBU (nonreplicating *Bacteroides* unit) (65, 69). NBUs are a heterogeneous family of integrated elements that are 10 to 12 kbp in size and share highest homology in an internal region that contains a mobilization gene (*mob*) and the *oriT* region (35, 37, 65, 69). NBUs, like the conjugative transposons, excise to form a covalently closed circular intermediate (Fig. 7). NBUs are not simply small, transfer-defective forms of the conjugative transposons that excise and mobilize them but appear to be unrelated to the conjugative transposons (35, 69). NBU excision and circularization are triggered by RteB provided by the conjugative transposon (56, 75). How RteB does this is still not clear. The excised NBU circle form is then mobilized by the conjugative transposon. NBUs are unusual in that they encode only a single mobilization protein that appears to be capable of binding to the NBU *oriT*, nicking, and initiating the transfer of a single-stranded copy of the NBU through the mating pore provided by the conjugative transposon (35, 37). NBU circle forms are mobilized not only by *Bacteroides* conjugative transposons but also by the IncP plasmids R751 and RK2 (35, 37, 69). Moreover, NBUs can be transferred from *Bacteroides* to *E. coli* and integrate in *E. coli* (unpublished data). Thus, NBUs could in theory move from the *Bacteroides* group into the *E. coli* group and back again. Transfer to gram-positive bacteria has not been tested.

NBUs integrate by a mechanism that appears to be different from the integration mechanism of the *Bacteroides* conjugative transposons and resembles integration of the lambdoid phages (69). Evidence for this comes from the study of one NBU, NBU1. The joined ends of NBU1 form a 14-bp segment that is identical to a 14-bp target site in the chromosome of the recipient where NBU1 integrates. Integration occurs within this region of identity (69). The chromosomal target site in *Bacteroides* spp. lies in the 3′ end of a leucine-tRNA gene (unpublished data). Many lambdoid phages integrate near the ends of tRNA genes (8). The integrase gene of NBU1 has now been identified and sequenced and appears to be a member of the lambda integrase family (unpublished results). That is, it

has the conserved tyrosine and other amino acids normally found near the carboxy terminus of integrases of the lambda family, but its deduced amino acid sequence differs considerably from that of lambda integrase in other portions of the protein.

In *Bacteroides* spp., NBUs integrate relatively site specifically (3, 69), but in the absence of a primary site, they will integrate into other sites. For example, NBU1 integrates into several sites on *E. coli* plasmid R388, which shared only scattered identity with the primary site (unpublished data). Thus, NBUs can integrate into plasmids as well as into the chromosome. If an NBU integrates into a plasmid, it renders the plasmid mobilizable by a *Bacteroides*-type conjugative transposon or by IncP plasmids. This provides another illustration of the general statement that there is no such thing as a safe plasmid. The first NBUs to be identified were cryptic, but recently an integrated *Bacteroides* element (called Tn*4555*) has been reported, which is probably a member of the NBU family because it has considerable sequence similarity to NBU1 and NBU2 and which carries a cefoxitin resistance gene (71). Thus, NBUs appear to be contributing to the spread of antibiotic resistance genes in the *Bacteroides* group.

Tn*4399*: a Mobilizable Transposon

NBUs are not the only integrated elements that are mobilized by the *Bacteroides* conjugative transposons. A mobilizable transposon, Tn*4399*, has been found in *Bacteroides fragilis* (24, 41). Tn*4399* has some features commonly found in nonconjugative transposons. For example, it creates a short target site duplication when it integrates (24). Tn*4399* resembles the conjugative transposons, however, in that it brings along a 5-bp segment from its former integration site when it transposes. Tn*4399* encodes two mobilization genes and an *oriT* (41). The fact that the *oriT* is internal raises the possibility that Tn*4399*, like the conjugative transposons and NBUs, also has a circular transfer intermediate. Transfer of Tn*4399* from a donor to a recipient is enhanced at least 1,000-fold by the presence of a *Bacteroides* conjugative transposon in the same strain, but it is not clear whether this enhancement is due to facilitation of the transfer process or whether the conjugative transposon plays some role in the excision of Tn*4399* as well. Tetracycline induction is also essential for this stimulation of transfer, suggesting a possible role for RteB and/or RteC. Originally, Tn*4399* appeared to be completely unrelated to the NBUs, but recently we have found a region of shared DNA sequence between Tn*4399* and the NBUs. It is nearly 1 kbp in length and lies upstream of the *oriT* regions of Tn*4399* and the NBUs (37). This shared region has high sequence similarity to bacterial primases and could play some role in regenerating the double-stranded form of the element after transfer of the single-stranded intermediate.

At the beginning of this section, the statement was made that transfer of integrated elements was so far unique to the *Bacteroides* conjugative transposons. An indication that such events might also be mediated by gram-positive conjugative elements is provided by the recent paper of Bannam et al. (2), which describes a *Clostridium perfringens* transposon, Tn*4551*, that has a circular intermediate. The transposon also contained open reading frames whose DNA sequences were homologous to plasmid mobilization genes, raising the possibility that Tn*4551* is mobilizable. Mobilization of the circular form was not demonstrated, nor was the identity of the element responsible for mobilization identified. Tn*4551* could be the first example of an NBU-like or Tn*4399*-like element in gram-positive bacteria. There was no sequence similarity between Tn*4551* and Tn*4399* or NBUs, however, and the primase homolog that ties Tn*4399* and the NBUs together was not evident on Tn*4551.*

The IncP Plasmid R751

Another case in which the *Bacteroides* conjugative transposons have been shown to mobilize integrated elements involves the IncP plasmid R751. R751 transfers itself from *E. coli* to *Bacteroides* spp., but it cannot replicate in *Bacteroides* spp. (63). Accordingly, we have used R751 as a delivery vehicle for the *Bacteroides* transposon Tn*4351*, which has been used widely for mutagenizing *Bacteroides* and related genera. Tn*4351* can either transpose on its own or cointegrate R751 into the *Bacteroides* chromosome (63). R751, integrated via Tn*4351* into the *Bacteroides* chromosome, was not capable of excising and transferring itself out of *Bacteroides* spp., probably because essential transfer genes are not expressed in *Bacteroides* spp. The *Bacteroides* conjugative transposon, Tc^r ERL, however, was able to transfer integrated R751 out of a *Bacteroides* donor into *E. coli* (63). Also, the *oriT* mobilization region of R751, cloned into a plasmid, was mobilized by Tc^r ERL.

This example calls into question a widely accepted assumption, i.e., that if a plasmid cannot replicate in a recipient, it will not be maintained in that host. Clearly, R751 can pass through a host in which it cannot replicate and return in fully replication-proficient form to a host in which its replication genes function. Another feature of this example is also instructive. Occasionally, the form of R751 that came out of *Bacteroides* spp. had picked up either one or two NBUs. A rolling plasmid does gather moss, or at least new DNA.

THE CALL OF THE WILD: NATURAL TRANSFER EVENTS INVOLVING CONJUGATIVE TRANSPOSONS

Under laboratory conditions, conjugative transposons transfer at relatively high frequencies (10^{-4} to 10^{-5} per recipient) and can move readily across genus lines, but does this type of transfer actually occur in nature? Tn*916*-type elements have been shown to transfer between different genera of gram-positive bacteria in the intestinal tracts of germ-free mice (15). Tn*916* also transfers between gram-positive and gram-negative bacteria, although the frequencies of such transfers are generally much lower than those of transfers between gram-positive bacteria (4, 14, 47). Evidence that such transfers can occur in nature comes from the finding that *tetM*, the resistance gene carried on Tn*916*, is widely disseminated in natural isolates, including many genera of gram-positive bacteria and such gram-negative species as *Neisseria gonorrhoeae* and *Kingella* spp. (14, 79). It is interesting that when Tn*916* is introduced into *N. gonorrhoeae*, it suffers deletions at its ends that render it nontransmissible (79).

The *Bacteroides* conjugative transposons have a similarly broad host range and can transfer plasmids from *Bacteroides* to *E. coli*, which are as distant from each other phylogenetically as *E. coli* is from the gram-positive bacteria. At least two of the *Bacteroides* conjugative transposons, Tc^rEm^r 12256 and Tc^rEm^r 7853, can transfer between *Bacteroides* spp. and *Prevotella* spp. under laboratory conditions (44, 68). From studies of natural isolates, it is appears that the *Bacteroides* conjugative transposons have spread widely in nature. Today, virtually all clinical isolates are resistant to tetracycline. All of the tetracycline-resistant *Bacteroides* clinical isolates we have tested to date proved to carry a conjugative transposon, most commonly one of the Tc^r ERL type (44, 55). Finding essentially the same conjugative transposon in a number of different *Bacteroides*

spp. demonstrates that transfer of these elements must occur quite frequently in the colon. Conjugative transposons that carry *tetQ* and may be members of the Tc^r ERL family have also been found in clinical isolates of the oral anaerobes *Prevotella* (formerly *Bacteroides*) *intermedia* and *Prevotella* (formerly *Bacteroides*) *denticola* (22). More recently, we showed that an allele of *tetQ* found in the oral anaerobe *Prevotella intermedia* had a sequence that was identical to that of a *tetQ* allele found in a *Bacteroides* clinical isolate (42). These findings suggest that transfer between oral and colonic bacteria is occurring.

Perhaps more surprising was our finding that alleles of *tetQ*, which were at least 94% identical to *tetQ* of *Bacteroides* clinical isolates, could be demonstrated in natural isolates of *Prevotella ruminicola*, a species found primarily in the rumen and intestine of livestock animals (42). This suggests that DNA transfer between the resident microfloras of humans and animals occurs in nature. The *tetQ* alleles in *P. ruminicola* strains were not located on a Tc^r ERL type element (42). It is still uncertain whether any of these *tetQ* alleles are located on a Tc^{r}Em^r 7853 type element, but if so, the sequence in the immediate vicinity of the *tetQ* gene is completely different from that in the *tetQ* region of Tc^{r}Em^r 7853 (42). The *tetQ* alleles found in the chromosome of *P. ruminicola* strains did not transfer under the conditions tested in this study. This could indicate that they are not on a transmissible element, but it should be kept in mind that most of the Tc^r ERL type elements would have been classified as nontransmissible if tetracycline had not been included in the medium. Perhaps the right inducing conditions have not been found. Thus, the possibility that the chromosomal alleles of *tetQ* in *P. ruminicola* strains are associated with conjugative transposons cannot be ruled out at this point. One plasmid-encoded *tetQ* allele was found and proved to be transmissible (42).

In the story of Oedipus Rex, the sphinx posed Oedipus a riddle that Oedipus had to answer or be slain by the sphinx. The riddle was: what animal goes on four legs at dawn, on two legs at noon, and on three legs in the evening? The answer was, of course, humans, who crawl as babies, walk upright as adults, and hobble along with a cane in old age. The conjugative transposons, as modern sphinxes, are challenging us with a riddle we may have to answer in order to survive: what can be done to slow or stop the transfer of antibiotic resistance genes by conjugative transposons and the elements they mobilize? Features of the conjugative transposons that are now being elucidated may help us to understand what practices stimulate transmission of these elements and what steps might be effective in limiting their spread.

ACKNOWLEDGMENTS

This work was supported by grant AI22383 from the U.S. National Institutes of Health. A.M.S. was supported by NIH traineeships T32GM07283 and T32AI07343.

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