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MINIREVIEW

In the Driver's Seat: The *Bacteroides* Conjugative Transposons and the Elements They Mobilize

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THE DRIVERS: *Bacteroides* CONJUGATIVE TRANSPOSONS

Conjugative transposons are self-transmissible elements that are normally integrated into a chromosome or plasmid but can excise themselves and transfer by conjugation to a recipient (2, 3, 12, 18). These interesting elements, which have now been found in a variety of gram-positive and gram-negative bacteria, combine features of transposons, plasmids, and bacteriophages. They resemble transposons and temperate bacteriophages in that they integrate into DNA. In fact, the integrases of gram-positive conjugative transposons share some amino acid sequence similarity to bacteriophage integrases, and these same conjugative transposons encode a small protein that appears to be functionally equivalent to bacteriophage excisionases (1, 3, 12). Conjugative transposons resemble plasmids in that their transfer intermediate is a covalently closed circle, and this circular form contains a plasmid-like *oriT* sequence that allows it to be transferred by conjugation (3, 9). Conjugative transposons, like self-transmissible plasmids, can have very broad host ranges and clearly are contributing to the spread of antibiotic resistance genes among gram-positive bacteria and gram-negative anaerobes (1, 3, 4, 11, 18).

Conjugative transposons are capable not only of transferring themselves but also of driving the transmission of other elements. This is best established in the case of the *Bacteroides* conjugative transposons (Fig. 1). *Bacteroides* conjugative transposons can mobilize coresident plasmids either in *cis* or in *trans* (2, 14, 15, 21). In addition, they mediate the excision, circularization, and mobilization of unlinked integrated elements called NBUs (nonreplicating *Bacteroides* units) (16, 19). Two *Bacteroides* transposons, Tn*4399* (10) and Tn*4555* (17), also appear to be mobilized by conjugative transposons. Judging from what is currently known, the gram-positive conjugative transposons seem to be less versatile mobilizers of other elements than are the *Bacteroides* conjugative transposons. Tn*916*, for example, can mobilize plasmids in *trans* but appears to be unable to mobilize plasmids in *cis* (3, 12), and no activity analogous to excision and mobilization of NBUs has yet been reported for Tn*916*. Recently, however, Bannam et al. (1) have found what could be an NBU-type element in *Clostridium perfringens*, Tn*4551*, although it is not yet clear whether this element is capable of conjugal transfer and, if so, what other element is driving its transfer. Further investigations could reveal a greater repertoire of transfer activities for the gram-positive conjugative transposons than is currently envisioned.

The ability of *Bacteroides* conjugative transposons to mobilize elements other than themselves has important clinical implications. *Bacteroides* spp. are opportunistic human pathogens, and the incidence of antibiotic resistance among *Bacteroides* clinical isolates is increasing. The *Bacteroides* conjugative transposons appear to be the primary driving force responsible for the spread of resistance genes within the *Bacteroides* group. *tetQ*, a gene encoding a ribosome protection type of tetracycline resistance (Tc^r), is found widely on the *Bacteroides* conjugative transposons, and many also carry *ermF*, a gene encoding resistance to erythromycin (Em^r) and clindamycin (Cc^r; 13, 18). The extent to which conjugative transposons have spread within the *Bacteroides* group can be gauged from the observation that within the past three decades *Bacteroides* clinical isolates have gone from being uniformly susceptible to tetracycline to being uniformly resistant to it. The *ermF* gene has been found on plasmids as well as conjugative transposons, but at least one of these plasmids, pBFTM10, is transmissible only if it is mobilized by a conjugative transposon (14). Recently, a transmissible *Bacteroides* cefoxitin resistance gene, *cfxA*, has been found on an NBU-like element (Tn*4555*) (17), which is probably being excised and mobilized by the conjugative transposons.

Bacteroides conjugative transposons range in size from 65 kbp to over 150 kbp (2, 13, 18). Although most of the *Bacteroides* conjugative transposons carry antibiotic resistance genes, one cryptic member of the family has been identified (XBU4422; 2, 15). The *Bacteroides* conjugative transposons studied to date have generally proved to be related enough to each other to cross-hybridize throughout their length on high-stringency Southern blots. This family of conjugative transposons is exemplified by Tc^r Em^r DOT, a 70-kbp conjugative transposon that carries both *tetQ* and *ermF* (Fig. 2). Recently, a conjugative transposon has been found that is not a member of the Tc^r Em^r DOT family (Tc^r Em^r 7853) (11). It carries *tetQ* but is otherwise unrelated to Tc^r Em^r DOT. Tc^r Em^r 7853 also differed from members of the Tc^r Em^r DOT family in that it mobilized coresident plasmids only at very low frequencies, it did not mediate NBU excision and transfer, and the *erm* gene it carries was not *ermF*. The existence of Tc^r Em^r 7853 suggests that there may be greater diversity among the *Bacteroides* conjugative transposons than was previously supposed.

Transfer of the *Bacteroides* conjugative transposons is thought to occur in three steps. The first step is precise or nearly precise excision from the chromosome to form a covalently closed circle. This circular intermediate usually contains a short, 4- to 5-bp segment from DNA adjacent to one end of the conjugative transposon, a feature reminiscent of Tn*916* excision and circularization (2, 3, 12). Evidence that the Tc^r Em^r DOT-type conjugative transposons have a circular intermediate comes from sequence analysis of excision and integration events and from the recent finding that the *oriT* of Tc^r Em^r DOT is near the middle of the element (Fig. 2) (9), but the excised circular forms have not yet been demonstrated

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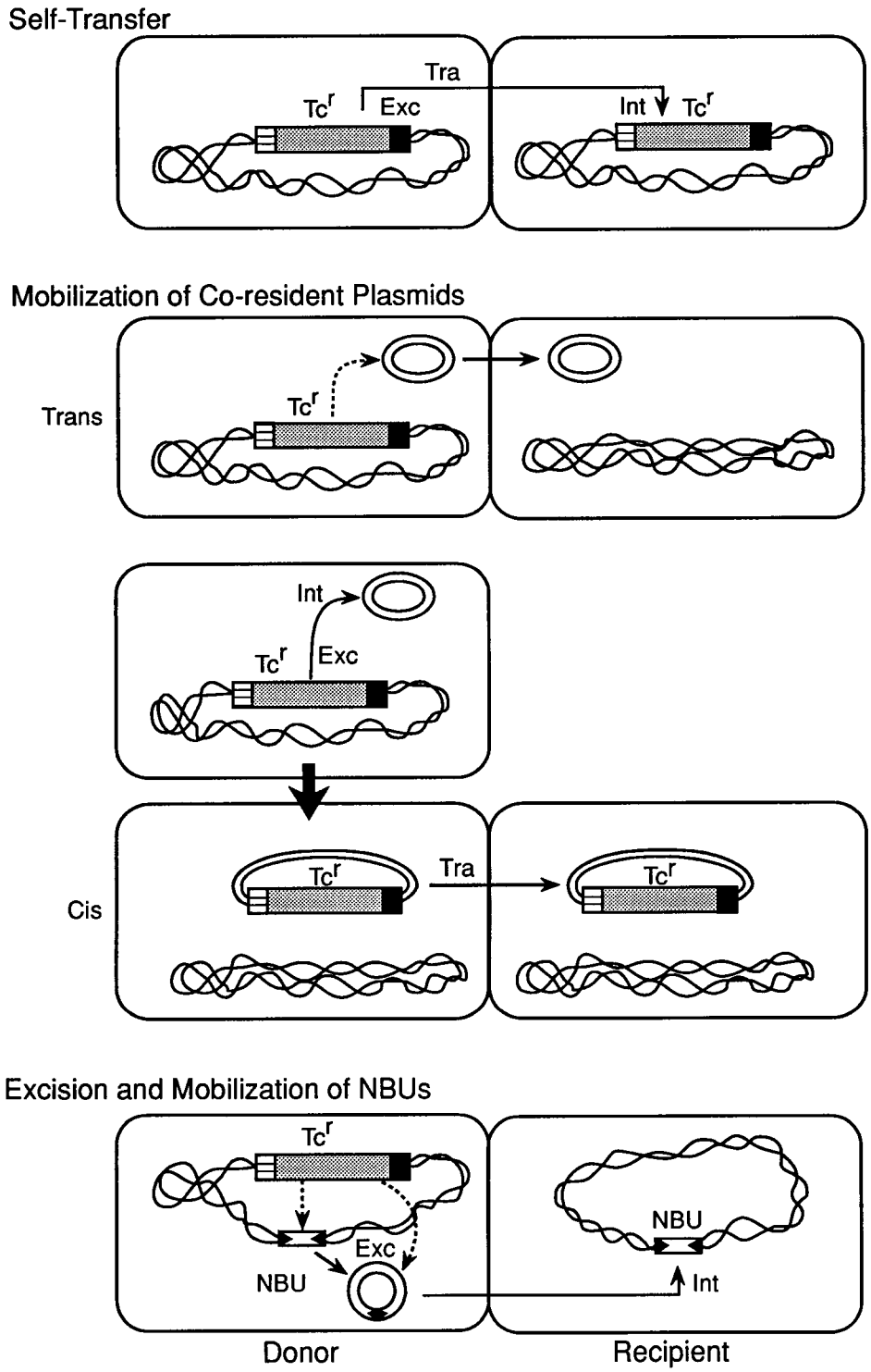

FIG. 1. Transfer activities of the *Bacteroides* conjugative transposons. The conjugative transposon excises itself from the donor genome (Exc) and transfers itself as a discrete unit (Tra) into a recipient where it integrates into the recipient's genome (Int). The conjugative transposon can mobilize plasmids in *trans*. The dashed arrow indicates proteins provided by the conjugative transposon which form the mating pore that is used by the plasmid to transfer to a recipient. Mobilization in *cis* occurs when a conjugative transposon excises from the chromosome and integrates into a plasmid, making the plasmid-conjugative transposon chimera self-transmissible. Tn*916* can mobilize plasmids in *trans* at low frequencies but does not mobilize plasmids in *cis* (3). Finally, the *Bacteroides* conjugative transposons excise (Exc) and mobilize in *trans* some unlinked integrated elements called NBUs. The dashed lines indicate proteins provided by the conjugative transposon. In the recipient, the NBU integrates into the recipient's genome (Int). NBUs can also integrate into plasmids (not shown) and provide an *oriT* and a mobilization protein that makes the NBU-plasmid chimera mobilizable by a conjugative transposon.

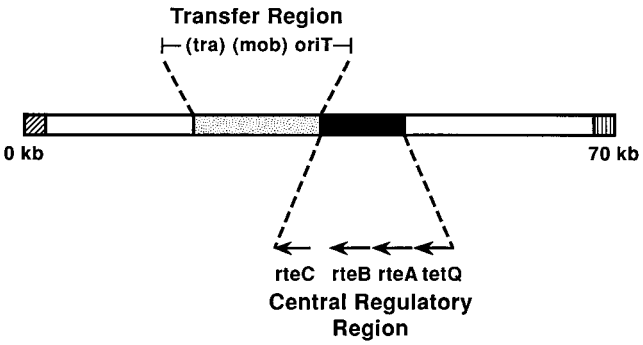

FIG. 2. Locations of known regulatory and transfer regions on a *Bacteroides* conjugative transposon. The central regulatory region contains *rteB*, which is essential for all conjugative transposon activities, and *rteC*, which is essential for self-transfer but not for plasmid mobilization or NBU excision and mobilization. The transfer region spans about 18 kbp and contains the element's *oriT*, genes required for initiation of transfer (*mob*), and genes that encode mating pore proteins (*tra*).

directly. The second step is transfer of the circular form by conjugation to the recipient by a process similar to plasmid transfer. That is, the circular form is nicked at an internal transfer origin (*oriT*) and a single-stranded copy of the conjugative transposon is transferred to the recipient through a mating pore (5, 6, 9). Recent unpublished work suggests that the Tn*916 oriT* is also internal (3). The third step in the transfer process occurs in the recipient, where the circular intermediate integrates into the genome of the recipient cell. In theory, the copy of the excised circular form that remains in the donor cell could reintegrate into the donor's genome, but this type of intracellular transposition has not been well documented.

Integration of *Bacteroides* conjugative transposons is orientation specific and relatively site specific. A comparison of the sequences of three integration sites with sequences near the ends of the conjugative transposon identified a 14-bp segment located 4 bp from one end of the conjugative transposon that had sequence similarity (but not complete identity) to a 14-bp segment adjacent to the insertion site (2). Alignment between the corresponding regions on the circular form of the conjugative transposon and the insertion site could be responsible for the site and orientation specificity of the integration event. Conjugative transposons do not exclude each other, so a single strain can acquire more than one conjugative transposon. The presence of another conjugative transposon in a donor does not lower the transfer frequency of a conjugative transposon and may even enhance it (2, 3, 12a).

STEPPING ON THE GAS: REGULATION OF TRANSFER BY TETRACYCLINE

An unusual feature of the *Bacteroides* conjugative transposons is that self-transfer and other activities are stimulated 100- to 1000-fold by low levels of tetracycline (ca. 1 μg/ml; 19, 20). The regulatory locus responsible for this stimulation is located near the middle of the element and contains a three-gene operon, *tetQ/rteA/rteB* (Fig. 3). *tetQ* encodes a ribosome protection type of tetracycline resistance (18). The deduced amino acid sequences of *rteA* and *rteB* suggest that *rteA* encodes the sensor of a two-component regulatory system and that *rteB* encodes the putative transcriptional activator component (20). At first, this observation suggested the hypothesis that RteA was the tetracycline sensor, but subsequent work suggests that this is not the case. Instead, tetracycline acts by increasing transcription of the entire *tetQ/rteA/rteB* operon by an unknown mechanism that does not require the participation of either RteA or RteB (unpublished data). RteB activates expression of another regulatory gene, *rteC*, which is essential for element self-transfer (19). RteC contains none of the known DNA-binding motifs and probably does not function as a transcriptional activator, but it may act as an antirepressor that counters the action of a repressor, which normally prevents expression of Tc^r Em^r DOT transfer genes (9). RteB also appears to act as an antirepressor (9). The putative repressor protein, with which RteB and RteC presumably interact, has not yet been identified.

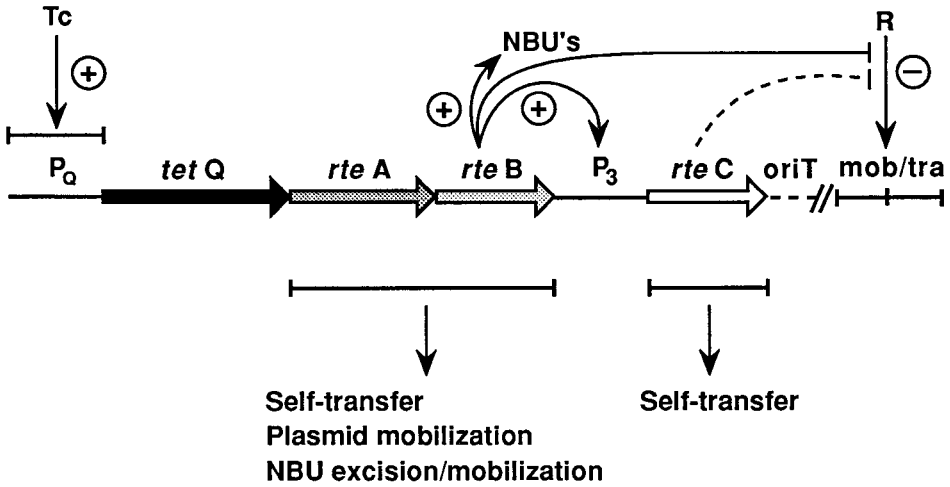

FIG. 3. Current model for tetracycline-dependent regulation of genes on the conjugative transposon (9). The promoter of the *tetQ-rteA-rteB* operon senses tetracycline by an unknown mechanism, and transcription from this promoter is enhanced at least 20-fold in the presence of tetracycline. Neither RteA nor RteB is required for this enhancement of transcription. Increased *rteB* message gives rise to increased levels of RteB protein. RteB activates transcription of *rteC* (19). RteB also triggers excision of the NBUs by an unknown mechanism. Although RteB appears to act as a transcriptional activator in the case of *rteC* expression, it also appears to act as an antirepressor to counteract the action of a repressor (R) that prevents transfer gene expression. RteC too appears to act as an antirepressor, but its effect is not as strong as that of RteB. This is indicated by the dashed line in Fig. 3. The repressor gene has not yet been located. Neither repressor function nor the interaction of RteB or RteC with the repressor appears to require binding of tetracycline to one or more of the regulatory proteins (9; unpublished results).

THE DRIVEN: CORESIDENT PLASMIDS, NBUs, AND MOBILIZABLE TRANSPOSON Tn*4399*

As indicated in Fig. 1, *Bacteroides* conjugative transposons can transfer plasmids either in *trans* or in *cis*. When a conjugative transposon mobilizes a plasmid in *trans*, the conjugative transposon provides the proteins needed to form the mating pore, and the plasmid provides the proteins that nick at the plasmid's *oriT* and initiate transfer of the plasmid through the mating pore. If a conjugative transposon integrates into a plasmid (*cis* mobilization), the conjugative transposon not only provides the mating pore proteins but may also provide the *oriT* and proteins that initiate the transfer of the plasmid chimera if the plasmid lacks an *oriT* and mobilization proteins.

NBUs are 10- to 12-kbp elements. The ends of NBUs are different from those of the Tc^r Em^r DOT-type elements, and the interior regions of the NBUs have little similarity to those of Tc^r Em^r DOT (2, 16; unpublished data). That is, the NBUs are not simply smaller transfer-defective versions of the Tc^r Em^r DOT-type elements. Nonetheless, NBUs require a coresident Tc^r Em^r DOT-type element to provide the *trans*-acting functions that allow them to excise and transfer. NBUs parasitize Tc^r Em^r DOT functions at two levels. RteB, a protein provided by Tc^r Em^r DOT, is required for NBU excision to occur, but the mechanism by which RteB triggers excision of the NBUs is still unknown. NBU excision and integration resemble excision and integration of phage lambda, i.e., the NBU excises from its chromosomal site to form a circular intermediate. The joined ends of the NBU circular form have a 14-bp sequence (*attN*) that is identical to their chromosomal integration site (*attB*), and insertion of the element occurs within this region of identity (16). Preliminary results suggest that the NBU integrase gene, like the *int* of Tn*916*, is a member of the lambda integrase family.

The NBUs encode a single mobilization gene (*mob*), which allows them to take advantage of the mating pore provided by the conjugative transposon (7, 8). This Mob protein is unusual in that it appears to have both *oriT*-binding and nicking activity (8). By contrast, conjugative plasmids generally have more than one mobilization protein, with the *oriT*-binding activity and nicking activity mediated by different proteins (5, 6, 22). The *oriT* of the NBUs is internal, as would be expected if the covalently closed circular form is the transfer intermediate, and NBU DNA integrated into a plasmid makes that plasmid mobilizable by the conjugative transposons (7, 8, 16). Although the *Bacteroides* conjugative transposons have so far not been reported to transfer themselves to *E. coli* and related bacteria, they can mobilize plasmids and NBUs from *Bacteroides* spp. to *E. coli* (14, 16), and the NBUs integrate in *E. coli*. NBU circle forms are mobilized not only by *Bacteroides* conjugative transposons but also by the IncP plasmids of the *E. coli-Pseudomonas* group. Apparently, the NBU Mob protein is able to interact with the mating pore formed by the IncP plasmids as well as with that formed by the *Bacteroides* conjugative transposons. Thus, the NBUs are at least as promiscuous as such notably promiscuous plasmids as IncQ and IncP plasmids.

Another unusual type of transmissible *Bacteroides* integrated element is the mobilizable transposon Tn*4399* (10). Tn*4399* differs from the Tc^r Em^r DOT-type conjugative transposons in that it has 13-bp inverted repeats at its ends and creates a small target site duplication when it integrates (2, 3, 12). Like the conjugative transposons, however, Tn*4399* brings along a 5-bp sequence from its previous site when it transposes (10) and could thus be using a similar integration mechanism. The interior of Tn*4399* contains two mobilization genes (*mocA* and *mocB*) and an *oriT* region, which allow it to be mobilized by conjugative transposons. Since the *oriT* of Tn*4399* is internal, the transfer intermediate is presumably a circle, although there is as yet no direct evidence for such a circular intermediate. Despite the fact that some features of Tn*4399* resemble those of the NBUs, Tn*4399* appears to be unrelated to the NBUs at the DNA sequence level, except in an area upstream of the mobilization genes, which is virtually identical to a DNA segment on the NBUs (8). This DNA segment has high sequence similarity with bacterial primase genes, but its function in transposition is unknown.

REACHING THE END OF THE LINE: CONCLUSIONS AND IMPLICATIONS

The activities of the *Bacteroides* conjugative transposons have broad implications for conjugal transfer of genes in the environment. First, plasmids are clearly not the only kind of element capable of conjugal transfer, nor are they necessarily the main driving force behind the spread of antibiotic resistance genes. The very broad host range of the conjugative transposons allows them to move between distantly related hosts, and since they survive by integrating rather than replicating, they can be maintained in strains that might not allow replication of a broad-host-range plasmid. Second, the presence of a conjugative transposon in a bacterial strain can increase considerably its gene transfer potential, because conjugative transposons not only transfer themselves but also mobilize coresident plasmids and mobilizable integrated elements. The fact that NBUs, Tn*4399*, and *Bacteroides* conjugative transposons can mobilize plasmids in *cis* means that there is no such thing as a safe plasmid. Even a plasmid that has had its *oriT* and *mob* genes removed can become transmissible if a conjugative transposon, an NBU, or a Tn*4399*-type element integrates into it, a fact that should be taken into account in the design of genetically engineered organisms that are to be released into the environment. Third, the fact that tetracycline acts as an inducer of transfer gene expression illustrates how use of an antibiotic could accelerate the spread of antibiotic resistance genes, not only by selecting for their acquisition but also by stimulating their transfer. Finally, since at least some conjugative transposons are cryptic and since the circular intermediates of the large conjugative transposons may not be visible in plasmid preparations, there is no way to be sure whether a bacterial strain harbors a conjugative transposon. The only indication that a conjugative transposon is present may be the unexplained transfer of a plasmid that seems too small to be able to mediate its own transfer or transfer of a chromosomally encoded gene. This raises the possibility that conjugative transposons are even more widely distributed among bacterial species than is suggested by the current literature. One could well be lurking in your favorite bacterial strain!

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