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The role of *Bacteroides* conjugative transposons in the dissemination of antibiotic resistance genes

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Abstract. Investigations into the mechanisms of antibiotic resistance gene transfer utilized by *Bacteroides* species have led to a greater understanding of how bacteria transfer antibiotic resistance genes, and what environmental stimuli promote such horizontal transfer events. Although *Bacteroides* spp. harbor a variety of transmissible elements that are involved in the dissemination of antibiotic resistance genes, it is one particular class of elements, the conjugative transposons, that are responsible for most of the resistance gene transfer in *Bacteroides*. The potential for *Bacteroides* conjugative transposons to transfer antibiotic resistance genes extends beyond those genes carried by the conjugative transposon itself, because *Bacteroides* conjugative transposons are able to mobilize coresident plasmids in trans and in cis, and also stimulate the excision and transfer of unlinked integrated elements called mobilizable transposons. These characteristics of conjugative transposons alone have significant implications for the ecology and spread of antibiotic resistance genes, and in terms of biotechnology. A novel feature of the most widespread family of *Bacteroides* conjugative transposons, the CTnDOT/ERL family, is that their transfer is stimulated 100- to 1000-fold by low concentrations of tetracycline. This is significant because the use of antibiotics not only selects for resistant *Bacteroides* strains, but also stimulates their transfer. Other *Bacteroides* conjugative transposons do not require any induction to stimulate transfer, and hence appear to transfer constitutively. The constitutively transferring elements characterized so far appear to have a broader host range than the CTnDOT/ERL family of conjugative transposons, and the prevalence of these elements is on the increase. Since these constitutively transferring elements do not require induction by antibiotics to stimulate transfer, they have the potential to become as pervasive as the CTnDOT/ERL family of conjugative transposons.

Key words. *Bacteroides*; conjugative transposons; antibiotic resistance; CTnDOT; tetracycline.

Introduction

The human intestinal tract contains a resident microflora that comprises hundreds of different bacterial species. One of the most numerically predominant are the obligate anaerobes of the genus *Bacteroides*, which are estimated to account for 25–30% of the microflora in the human intestinal tract [1]. *Bacteroides* play a number of roles as part of the normal microflora, but some species of *Bacteroides*, including *B. fragilis* and *B. thetaiotaomicron*, are also opportunistic pathogens which can cause life-threatening infections if they escape the colon due to surgery or other trauma. In fact, *Bacteroides* spp. are the anaerobes most frequently isolated from human clinical specimens [2].

Bacteroides spp. are naturally resistant to aminoglycosides. Some also carry genes that confer resistance to penicillins and cephalosporins (β-lactams), 5-nitroimidazoles, tetracycline and the macrolide-lincosamide-streptogramin$_B$ (MLS) group of antibiotics, which includes erythromycin and clindamycin [3–9]. All of these resistance determinants have been found on transmissible genetic elements, and recent surveys show that *Bacteroides* spp. are becoming increasingly resistant to antibiotics, particularly the tetracyclines and the MLS group antibiotics [3, 10–12].

Like many other bacteria, *Bacteroides* harbor a variety of transmissible elements that are involved in the dissemination of antibiotic resistance determinants. These include

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mobilizable and conjugative plasmids, compound transposons, mobilizable transposons (MTns), and conjugative transposons (CTns) (table 1). Despite the variety of elements present in *Bacteroides* spp., it is the conjugative transposons that appear to be responsible for most of the antibiotic resistance transfer in the *Bacteroides* group. The most convincing evidence for this was provided in a recent survey of 289 *Bacteroides* strains in which the dramatic increase in tetracycline resistance (from 30 to 80%), and in MLS resistance (from < 2 to 23%) that has occurred during the last 30 years was found to be directly attributable to the spread of *Bacteroides* conjugative transposons [3].
The fact that these resistance determinants are prevalent and found on transmissible elements in *Bacteroides* spp., particularly elements as pervasive as conjugative transposons, is of particular concern. The concern is not only that opportunistic infections caused by *Bacteroides* spp. may become untreatable, but also that *Bacteroides* spp. may act as reservoirs of antibiotic resistance genes, and as such may be able to transfer them to pathogenic bacteria that are passing through the colon. Investigations into the origins of resistance genes found in *Bacteroides* spp., the presence of these resistance genes in different genera, and the ability of the *Bacteroides* elements carrying these resistance genes to transfer between different genera of animal and human origin, provides evidence that exchange of resistance genes can and does occur between *Bacteroides* spp. and other bacteria that may only transiently occupy the same sites [3, 13–15]. The problem with such transfer events is ultimately that certain bacterial infec-

Table 1. Transmissble elements of *Bacteroides* spp. carrying antibiotic resistance genes.

Element Type	Size (kb)	Identified in	Antibiotic resistance genes	Phenotype	Ref.
Plasmids					
pBFTM10 (or pCP1)	15.0	*B. fragilis*	*ermF*, *tetX** (Tn*4400*)	mob+, tra–	61, 71, 72
pBI143	2.8	*B. fragilis*	none	mob+, tra–	73
pB8 -51 (also pLV22a)	4.4	*B. eggerthii*	none	mob+, tra–	74
pBF4 (or pIP410)	41.0	*B. fragilis*	*ermF*, *tetX** (Tn*4351*)	mob+, tra+	59, 75, 76
pBI136	80.0	*B. ovatus*	*ermFS*, *aadS** (Tn*4551*)	mob+, tra+	77, 78
pRYC3373	39.5	*B. uniformis*	*catII*	mob+. tra+	79
pIP417	7.7	*B. vulgatus* BV-17	*nimA*	mob+, tra–	80, 81
pIP419	10.0	*B. thetaiotaomicron* BT13	*nimC*	mob+, tra–	67
pIP421	7.3	*B. fragilis* F239	*nimD*	mob+, tra–	82, 83
p5482A	45.0	*B. thetaiotaomicron*	none	unknown	37
non-MTns					
Tn*4351*	5.5	pBF4	*ermF*, *tetX**	n/a	84
Tn*4400*	5.7	pBFTM10	*ermF*, *tetX**	n/a	85
Tn*4551*	9.0	pBI136	*ermFS*, *aadS**	n/a	86
MTns					
NBU1	10.3	*B. uniformis*	none	mob+, tra–	28, 87–89
NBU2	11.1	*B. fragilis*	*mefE-linA*$_{N2}$*	mob+, tra–	90
NBU3	10.0	*B. fraglis*	none	mob+, tra–	22
Tn*4399*	9.6	*B. fragilis* TM4.2321	none	mob+, tra–	31
Tn*4555*	12.5	*B.vulgatus* CLA341	*cfxA*	mob+, tra–	29
Tn*5520*	4.7	*B. fragilis* LV23	none	mob+, tra–	32
CTns					
XBU4422	60	*B, uniformis* 1001	none	mob+, tra+	22
CTnERL	52	*B. fragilis* ERL	*tetQ*	mob+, tra+	91
CTnDOT	65	*B. thetaiotaomicron* DOT	*tetQ, ermF* *tetX**, *aadS**	mob+, tra+	91
CTn*12256* (also Tn*5030*)	150.0	*B. fragilis* 12256 (also *B. fragilis* V503)	*tetQ, ermF*, *tetX1**, *tetX2**, *aadS**	mob+, tra+	91, 92
CTnBst	100.0	*Bacteroides* strain WH207	*ermB*	mob+, tra+	[Gupta et al., unpublished]
CTnGERM1	85.0	*B. ovatus* DH3716	*ermG*	mob+, tra+	[Wang et al., unpublished]
CTn*7853*	70.0	*B. thetaiotaomicrion* 7853	*tetQ*, *ermG*	mob+, tra+	47

Resistance genes are specified where known and are as follows: cefoxitin (*cfxA*), chloramphenicol (*catII*), erythromycin and lincomycin (*mefE-linA*$_{N2}$), erythromycin and MLS$_B$-group antibiotics (*ermB*, *ermG*, *ermF, ermFS*), 5-nitroimidazole (*nimA*, *nimC*, *nimD*), streptomycin (*aadS*), tetracycline/minocycline (*tetM*, *tetQ*, *tetX*). An asterisk indicates antibiotic resistance genes that do not provide *Bacteroides* spp. antibiotic resistance at a level detectable in host cells. Other phenotypes associated with these elements are as follows: mob+, ability to be mobilized by conjugative transposons and plasmids from *E. coli* incompatibility group P; tra+, encode transfer functions necessary for self-transfer from a donor to a recipient via conjugation.

tions other than those caused by *Bacteroides* may become increasingly difficult to treat.

What is a conjugative transposon?

Conjugative transposons are widely distributed in the microbial world, being found in three distantly related phylogenetic groups of bacteria including the Gram-positive bacteria, Gram-negative proteobacteria and in the Cytophage-Flexibacter-*Bacteroides* (CFB) group of Gram-negative bacteria [16–22].

Conjugative transposons are best defined in the most general terms, as discrete DNA segments that are normally integrated into the bacterial chromosome and transfer by conjugation from a donor to a recipient bacterium. Since these elements are integrated in the host cell chromosome except during transfer, there is no method for identifying conjugative transposons analogous to a plasmid DNA preparation. Consequently most have been identified because of their association with phenotypic traits such as antibiotic resistance, the ability to synthesize or catabolize various metabolites, or even roles in virulence [8, 16, 17, 23].

Mechanisms by which *Bacteroides* conjugative transposons transfer antibiotic resistance genes

Conjugative transposons begin conjugal transfer by excising from the chromosome to form a circular transfer intermediate. The next step in transfer appears to be similar to that of conjugative plasmid transfer, in that the circular intermediate is nicked at the origin of transfer (*oriT*), and a single-stranded copy of the conjugative transposon is then passed from a donor bacterium into a recipient bacterium. The single-stranded copy of the conjugative transposon present in both the donor and recipient is then made double stranded, and subsequently integrates into the donor and recipient chromosomes (fig. 1). In this way a conjugative transposon is able to transfer an antibiotic resistance determinant it encodes. The exact mechanism by which conjugative transposons excise from the donor chromosome and integrate into the recipient chromosome is the subject of another review herein, and consequently will not be discussed in this review. However, it should be noted that although the mechanism by which *Bacteroides* elements integrate appears to be similar to that of conjugative transposons from Gram-positive organisms, the mechanism for excision appears to be tightly regulated and much more complex than that of any other known integrated transmissible element [24].

The potential for *Bacteroides* conjugative transposons to transfer resistance genes extends beyond the conjugative transposon itself. In addition to transferring themselves, *Bacteroides* conjugative transposons also mobilize coresident plasmids in trans, or in cis by first integrating into a plasmid molecule and then transferring as a cointegrate (fig. 2). Although other conjugative transposons such as Tn*916* are also able to transfer plasmids in trans, they have not been observed to transfer plasmids in cis [17]. This is not surprising in the case of Tn*916*, because excision and circularization of Tn*916* are required in order for the transfer genes to be activated [25]. This is not true of *Bacteroides* conjugative transposons.

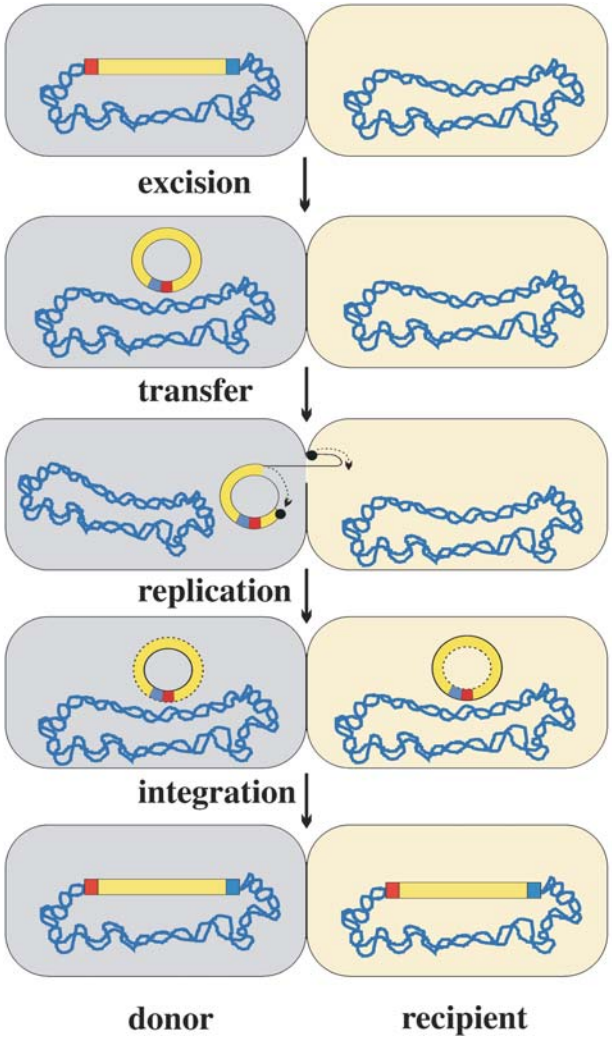

Figure 1. Steps involved in the conjugal transfer of a conjugative transposon. The integrated conjugative transposon (rectangle) excises from the chromosome of the donor to form a covalently closed circular transfer intermediate in which the left and right ends of the conjugative transposon are joined. A single-stranded nick is subsequently made at the origin of transfer (*oriT*, black circle) in the circular intermediate, and the nicked strand is presumed to be transferred from donor to recipient by a process similar to conjugal transfer of plasmid DNA. In the donor and recipient the single-stranded copy of the conjugative transposon is replicated, yielding a double-stranded form of the conjugative transposon which then integrates in the donor and recipient chromosomes, respectively.

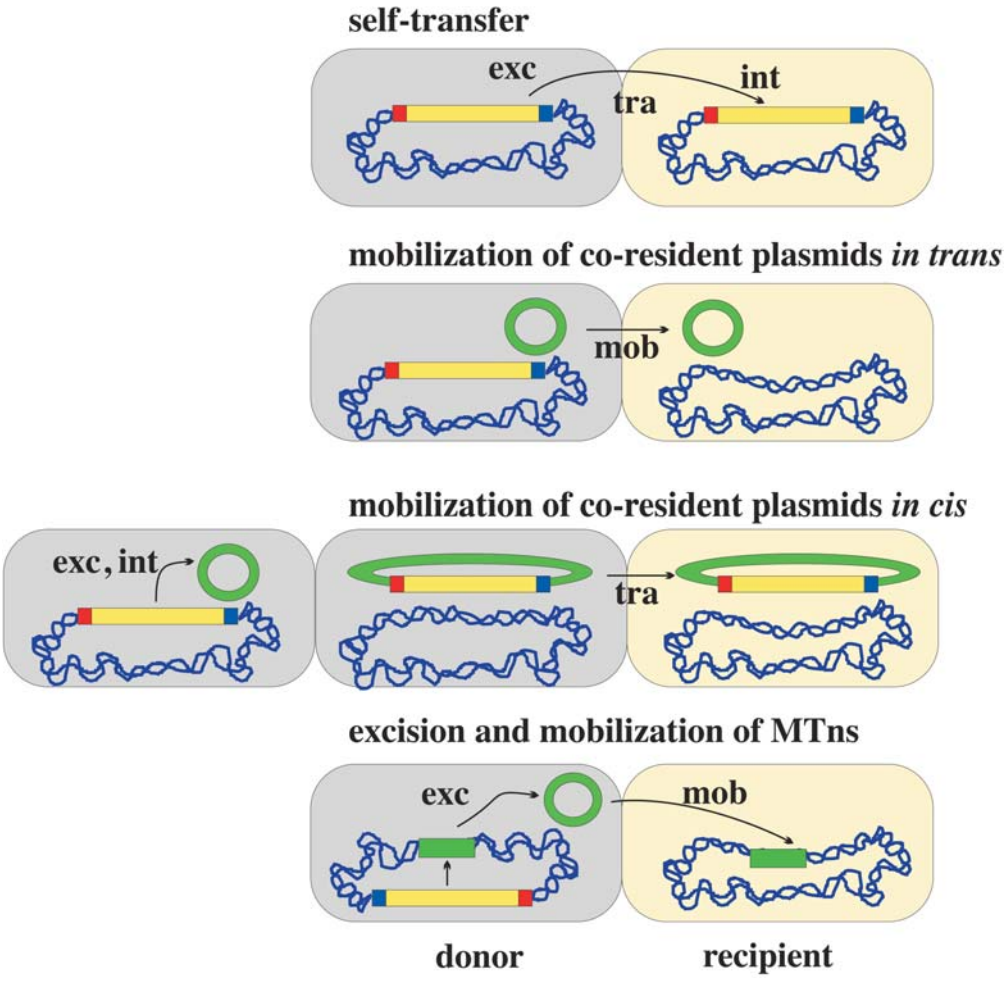

Figure 2. Mechanisms by which *Bacteroides* conjugative transposons can transfer antibiotic resistance genes from a donor to a recipient. *Bacteroides* conjugative transposons can excise (exc) from the donor chromosome and self-transfer to a recipient and subsequently integrate (int) into the recipient chromosome. Alternatively, *Bacteroides* conjugative transposons can mobilize (mob) plasmids carrying antibiotic resistance genes in trans or in cis. In the latter case, the conjugative transposon first excises from the donor chromosome and integrates into the coresident plasmid forming a chimeric plasmid, which can then be transferred to a recipient cell. Finally, *Bacteroides* conjugative transposons can stimulate the excision and mobilization of mobilizable transposons from a donor to a recipient. All of these transmissible elements are known to carry various antibiotic resistance genes in *Bacteroides* spp. Conjugative transposons are represented by a yellow box in which the left (red) and right (blue) ends are distinguished. Green shading indicates mobilizable plasmids and transposons.

Bacteroides conjugative transposons are unique in that they are able to stimulate the excision and transfer of unlinked integrated DNA elements called mobilizable transposons (MTns). These elements are much smaller than conjugative transposons and contain genes required for excision, mobilization and integration. Nonetheless, they rely on transfer proteins supplied by coresident conjugative elements in order to transfer intercellularly, and on regulatory proteins that somehow stimulate excision (fig. 2). The exact mechanism by which the conjugative transposons stimulate the excision and transfer of mobilizable transposons is unknown; however it is known that RteA and RteB encoded by genes located within the central regulatory region of the CTnDOT/ERL family of conjugative transposons are essential for the excision and mobilization of NBUs (fig. 3) [26]. The mechanism is currently under study. NBUs (non-replicating *Bacteroides* units) are the best characterized of the *Bacteroides* MTns, but there are others, including Tn*4555*, Tn*4399* and Tn*5520* [26–32]. Almost all *Bacteroides* plasmids and mobilizable transposons characterized so far have been shown to be mobilizable by *Bacteroides* conjugative transposons and also by the broad-host-range plasmids from the *Escherichia coli* IncP group [33, 34]. In addition, one of the mobilizable transposons, NBU1, has also been shown to integrate nonspecifically into the *E. coli* chromosome [28]. Some *Bacteroides* plasmids and MTns harbor antibiotic resistance genes (table 1), and so their mobilization by *Bacteroides* conjugative transposons adds to the antibiotic resistance problem. The broad host range IncPβ plasmid R751 can mobilize itself from *E. coli* into *Bacteroides*. Although R751 cannot be maintained in *Bacteroides,* if it

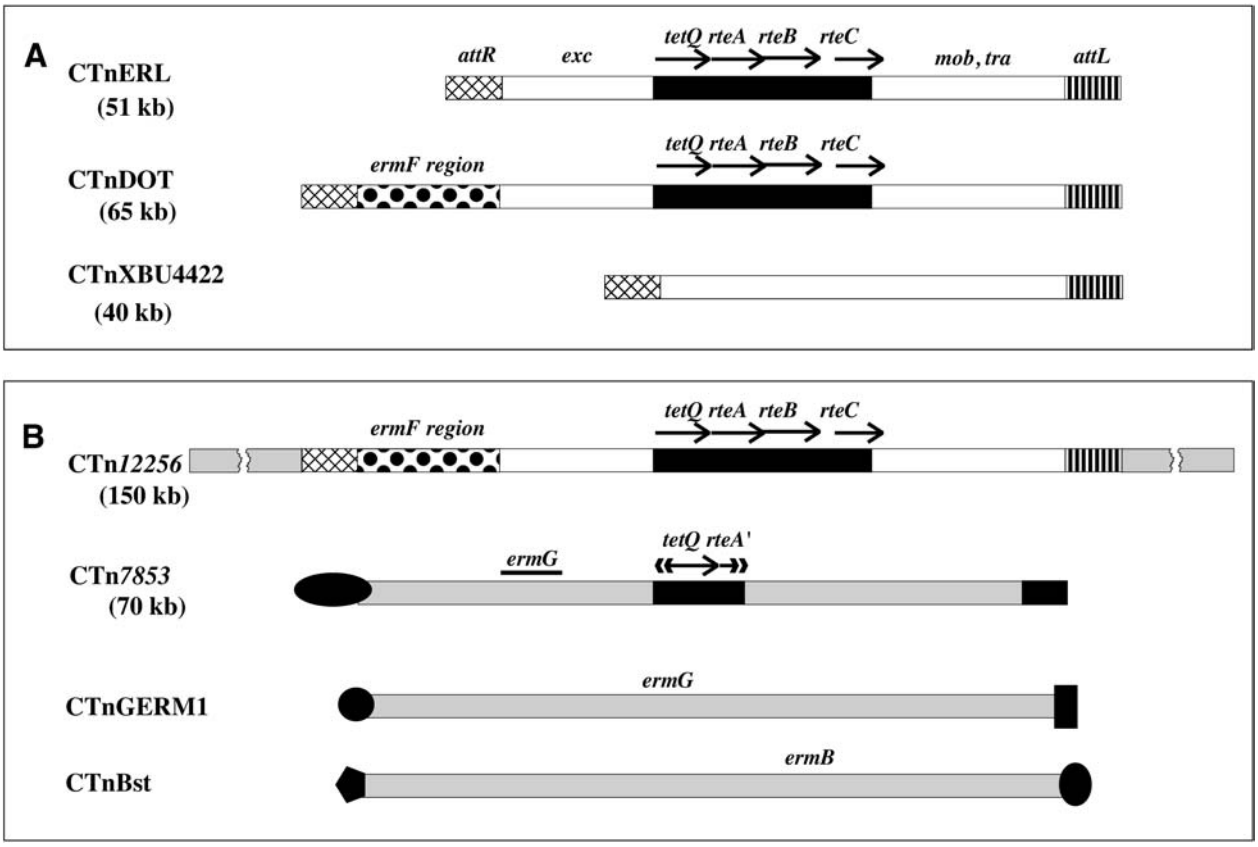

Figure 3. Schematic representation of five families of *Bacteroides* conjugative transposons. (*A*) The CTnDOT/ERL family of conjugative transposons so far includes three elements, CTnERL, CTnDOT and CTnXBU4422, and is characterized by having the same right (*attR*, hatched box) and left (*attL*, striped box) end sequences. The left-end sequence is different from the sequence found at the right end of the element. In addition, the CTnDOT/ERL family contains homologous excision (*exc*), mobilization (*mob*) and transfer (*tra*) modules, and in most but not all cases (CTnXBU4422) contains a central regulatory region (*tetQ*, *rteA*, *rteB*, *rteC*) that mediates the tetracycline induction of excision and transfer. CTnERL and CTnDOT are almost identical except that CTnDOT contains a 13-kb insertion (spotted box) comprising regions from other *Bacteroides* mobilizable and nonmobilizable transposons that encodes the macrolide resistance gene *ermF*. (*B*) Shows *Bacteroides* conjugative transposons that do not belong to the CTnDOT/ERL family (gray shading) or to each other, and includes CTn*12256*, CTn*7853*, CTnGERM1 and CTnBst. CTn*12256* is a hybrid element that comprises a CTnDOT element inserted into another conjugative transposon not related to the CTnDOT/ERL family of conjugative transposons. The lack of homology between the mother element and the fact that CTn*12256* does not require tetracycline induction for transfer has resulted in its exclusion from the CTnDOT/ERL family of conjugative transposons [14, 22]. CTn*7853* is completely unrelated to the CTnDOT/ERL elements except for a region near its center that contains *tetQ* and a truncated copy of *rteA* [47]. Similarly, the *ermG*- and *ermB*-containing resistance elements CTnGERM1 and CTnBst do not appear to be related to each other or any other of the *Bacteroides* conjugative transposons so far characterized based on hybridization studies [A. Gupta et al., unpublished; Y. Wang et al., unpublished].

carries the *Bacteroides* compound transposon Tn*4351*, it can be integrated into the *Bacteroides* chromosome, and in the presence of CTnERL can be mobilized from *Bacteroides*, back into in *E. coli* [35].

Bacteroides conjugative transposons can transfer themselves and mobilize elements into distantly related bacteria such as *E. coli*, which suggests that the host range of *Bacteroides* elements is only limited by maintenance of the elements and expression of the transfer proteins in the recipient cell, and not by conjugative transposon-encoded transfer functions from *Bacteroides* donors [8, 34].

The ability of *Bacteroides* conjugative transposons to mobilize plasmids and mobilizable transposons also has implications for biotechnology, because the insertion of a *Bacteroides* conjugative transposon or even a mobilizable transposon into a previously nontransmissible recombinant plasmid could convert such a plasmid into a transmissible element. Examples of both scenarios have been observed under laboratory conditions. A mobilizable transposon, NBU1, and a cryptic conjugative transposon, CTnXBU4422, were identified after they serendipitously inserted into a nonmobilizable plasmid to form a chimera that was subsequently transmissible [36, 37].

Unlike bacteriophage and plasmids, *Bacteroides* conjugative transposons do not exclude each other; many *Bacteroides* strains contain more than one conjugative transposon, even conjugative transposons of the same type [38]. This means that a single host cell is able to acquire more than one conjugative transposon, which may in-

crease the potential of the host bacterium to increase its level of resistance and to donate genes. Also, conjugative transposons appear to be stably maintained even in the absence of selection [8].

Types of *Bacteroides* conjugative transposons

Bacteroides conjugative transposons range in size from 45 to 150 kb and can be divided into several families based on homology (fig. 3). Unlike Tn*916*, which integrates almost randomly into AT rich regions in most hosts, *Bacteroides* conjugative transposons are more site selective, having five to eight preferred sites per chromosome [22, 39].

The two best characterized of the *Bacteroides* conjugative transposons, CTnERL and CTnDOT, are almost identical except that CTnDOT contains a 13-kb insertion that encodes an erythromycin resistance gene (*ermF*) which confers resistance to MLS group antibiotics (fig. 3) [24, 38, 40–44]. This 13-kb insertion is designated the *ermF* region and appears to be a composite of *Bacteroides* MTns and non-MTns [38].

Bacteroides conjugative transposons that belong to the CTnDOT/ERL family are characterized by having left and right ends that are conserved, and by having extensive sequence identity in transfer, mobilization and excision genes (fig. 3). An unusual feature of the CTnDOT/ERL family of conjugative transposons is that self-transfer, mobilization of coresident plasmids and MTns are all induced 100- to 1000-fold by pregrowth in a medium containing low concentrations (1 μg/ml) of tetracycline. In the absence of tetracycline, excision and transfer of these elements is not detectable at all [8]. A central regulatory region encoding four genes designated *tetQ*, *rteA*, *rteB* and *rteC* mediates this tetracycline-induction effect [45]. Thus, not only does the use of tetracycline select for resistant strains of bacteria, but it also induces transfer of conjugative transposons. Once integrated, conjugative transposons are stably maintained in the host chromosome even in the absence of selection. Consequently, once resistance is acquired, loss of the resistant phenotype is rare. This is perhaps why over 80% of *Bacteroides* strains are now tetracycline resistant, even in people with no recent history of antibiotic use. It will be interesting to learn if other antibiotics or environmental stimuli also enhance transfer of such elements.

A cryptic element that belongs to the CTnDOT/ERL family, called CTnXBU4422, has also been identified, and although its ends cross-hybridize with the CTnDOT/ERL ends, and although there is extensive homology between mobilization and transfer modules, the central regulatory region found on CTnDOT and CTnERL is not present in this element [37]. The contribution of such cryptic elements to the spread of antibiotic resistance is often ignored. Yet, such elements could have important clinical effects since they may still play a role in the mobilization of elements that do harbor resistance determinants. Also, they could acquire resistance genes.

So far there are four *Bacteroides* conjugative transposons that do not belong to the CTnDOT/ERL family based on hybridization studies, including CTn*12256*, CTn*7853*, CTnGERM1 and CTnBst (fig. 3). CTn*12256* is the largest of the *Bacteroides* conjugative transposons, being a 150-kb composite element that contains a copy of the CTnDOT element integrated into another conjugative transposon that does not cross-hybridize with sequences from the CTnDOT/ERL family of CTns [22]. CTn*12256* does not belong to the CTnDOT/ERL family because its transfer is not tetracycline regulated. Instead, transfer is regulated by the outer element that does not cross-hybridize to CTnDOT/ERL probes. Another *Bacteroides* conjugative transposon, CTn*7853,* is also unrelated to the CTnDOT/ERL family of conjugative transposons except that it contains a copy of *tetQ* and a truncated *rteA* gene. DNA from this conjugative transposon does not cross-hybridize with that from CTn*12256*. CTn*7853* was the first *Bacteroides* element identified that contained the *ermG* resistance determinant, previously only found in Gram-positive organisms [15]. More recently other self-transmissible elements also carrying *ermG* (CTnGERM1) or *ermB* (CTnBst) but no *tetQ* gene have been identified in *Bacteroides* clinical and community isolates [3].

In contrast to the CTnDOT/ERL family of conjugative transposons, other *Bacteroides* conjugative transposons do not require tetracycline induction in order to transfer, and instead appear to transfer constitutively at a level equivalent to that of the CTnDOT/ERL family of elements after induction (10^{-6} transconjugants per recipient) [14, 22, 46, 47]. Also, elements such as CTn*12256* and CTn*7853* have been observed to transfer from *Bacteroides* to other genera such as *Prevotella*, whereas the CTnDOT elements do not appear to transfer into these hosts [14, 15, 47]. Consequently, these other elements have the potential to be even more pervasive than the CTnDOT/ERL family of conjugative transposons, because antibiotic stimulation is not required for transfer and they appear to have a broader host range than CTnDOT/ERL elements.

It should be noted, however, that *Bacteroides* spp. are very diverse, having between 0 and 80% DNA homology between different species, as determined by DNA-DNA hybridization studies, and consequently the CTnDOT/ERL elements still have a broad host range [48, 49].

Resistance genes carried by *Bacteroides* conjugative transposons

In order for *Bacteroides* spp. to serve as a reservoir for antibiotic resistance genes, *Bacteroides* have to be able to ac-

quire and pass genes into other bacteria transiting the same site. *Bacteroides* are in a good position to do this as intestinal bacteria that come into contact with microbes associated with food and other sites of the human body including the mouth, respiratory and intestinal tracts. Also, the high numbers of bacteria and the presence of plant and mucin particles in the intestinal tract encourage biofilm formation and provide a solid surface that is required by most conjugation systems for conjugal transfer. To estimate how often transfer occurs between *Bacteroides* and other bacteria in nature, one can look for evidence of natural gene transfer events. One way to do this is to detect the presence of a virtually identical gene in distantly related bacteria.

Many of the *Bacteroides* conjugative transposons characterized so far have been found to carry a gene, *tetQ*, which encodes a ribosome protection type of tetracycline resistance that renders the host bacterium resistant to all of the clinically used tetracyclines [8]. So far in *Bacteroides*, *tetQ* has been associated exclusively with conjugative transposons. The sequence identity between *tetQ* determinants present in different *Bacteroides* strains and other genera in which it is found, ranges from 96 to 100%, which is consistent with a horizontal mode of acquisition [3]. The CTnDOT/ERL family of conjugative transposons are the elements primarily responsible for the spread of the *tetQ* gene among *Bacteroides* spp., since *tetQ* is associated with DNA that hybridizes to CTnDOT/ERL ends in almost all *Bacteroides* strains [3].

The *tetQ* gene has also been found in oral *Prevotella* and *Porphorymonas* species. In these strains it also appears to be associated with conjugative transposons that transfer in the absence of any tetracycline induction, such as *Bacteroides* elements CTn*12256* and CTn*7853* [13, 50–52]. The presence of *tetQ* is significant because it provides evidence for the existence of horizontal transfer between human intestinal *Bacteroides* spp. and oral *Prevotella* spp. and *Porphorymonas* spp. [13]. Where this transfer occurred is unclear. Possibly, swallowed oral bacteria interacted in the colon with colonic *Bacteroides* spp. and then reentered the mouth via the fecal-oral route. This horizontal transfer is of concern because increased resistance to tetracycline could detrimentally affect the usefulness of tetracycline in treating peridontal disease.

A *tetQ* with 95% nucleotide sequence identity to the *tetQ* from CTnDOT has also been identified in a bovine isolate of *Prevotella ruminicola*, in which the resistance gene was found on a plasmid and not as part of an integrated conjugative transposon [14, 53]. The presence of *tetQ* in a bacterium of bovine origin provides evidence for transfer between human and animal isolates of bacteria. Subsequently, *Bacteroides* conjugative transposons have been utilized to introduce cloned DNA into *Prevotella ruminicola* [14, 54].

Many *Bacteroides* strains also contain *erm* type MLS (macrolide-lincosamide-streptogramin B) resistance genes that confer high-level resistance to clinically important antibiotics such as erythromycin and clindamycin [55, 56]. In a recent survey, ~30% of *Bacteroides* strains were shown to be MLS resistant, and the majority of this resistance (72%) was attributable to the presence of three *erm*-type resistance genes, *ermF*, *ermG* and *ermB*. The identity of the genes responsible for the remaining macrolide resistance (28%) has not yet been determined [3].

So far *ermF* has only been found in *Bacteroides* spp. and related genera such as *Porphorymonas* spp. and *Prevotella* spp. [51, 57, 58]. In *Bacteroides* spp., in 87% of cases, *ermF* is linked to the presence of CTnDOT, but *ermF* (or *ermFS*) has also been found on three *Bacteroides* plasmids, on which *ermF* was part of a compound transposon which was flanked by IS*4351* (table 1) [59–61]. However, IS*4351* is rare, being present in only 2.6% of *Bacteroides* strains surveyed, and is linked to only 13% of *ermF* genes present in the *Bacteroides* population [3]. This observation suggests that conjugal plasmids are making only a minor contribution to the *erm* gene transfer, compared with conjugative transposons.

ermG from CTn*7853* has 99% nucleotide identity to an *ermG* from *Bacillus sphaericus*, evidence of horizontal transfer between a Gram-positive soil bacterium and a Gram-negative bacterium of human intestinal origin [15]. Similarly, *ermB* is widespread in Gram-positive bacteria including *Clostridium*, *Streptococcus* and *Enterococcus*, suggesting that there is gene transfer occurring in the colon between *Bacteroides* and Gram-positive bacteria [3, 55, 62].

It is also notable that many *Bacteroides* conjugative transposons carry both tetracycline and erythromycin resistance genes, which means that the use of tetracycline selects for erythromycin resistant strains and vice versa, adding to the antibiotic resistance problem.

It also interesting that the distribution of *tetQ* and *erm* genes in community and clinical isolates of *Bacteroides* was similar, suggesting that resistance genes are maintained in the absence of selection [3]. Further evidence for this hypothesis is that some *Bacteroides* compound transposons and CTnDOT carry a tetracycline resistance gene, *tetX*, and a streptomycin resistance protein, *aadS*, that do not appear to confer any selective advantage to *Bacteroides* (table 1). *tetX* does not appear to be functional under the anaerobic conditions required for growth of *Bacteroides* spp., but under aerobic conditions this gene confers tetracycline resistance to *E. coli* host cells [38, 63, 64]. Similarly, *aadS*, a gene found on *Bacteroides* compound transposons and on CTnDOT (table 1), has significant homology to Gram-positive streptomycin-dependent adenyltransferases, but is phenotypically silent in *Bacteroides* [65]. Even if *aadS* were expressed in a *Bac-*

teroides host, its expression would be irrelevant because *Bacteroides* spp. are inherently resistant to high concentrations of streptomycin, and so acquisition of a streptomycin resistance gene is unlikely to provide a selective advantage [66]. Why *Bacteroides* conjugative transposons have retained genes such as *tetX* and *aadS* intact has remained a mystery. However, the retention of such resistance genes may be advantageous in terms of horizontal transfer if they provide a selective advantage for the conjugative transposon in a non-*Bacteroides* host.

Some might think that the exchange of resistance genes between distantly related bacteria might not pose a serious problem because the genes expressed in one host may not be expressed properly in another. However, in *Bacteroides* spp. there are several antibiotic resistance genes, including those for metronidazole, erythromycin and cefoxitin resistance, that are expressed due to the insertion of insertion sequence (IS) elements upstream of the resistance gene, hence overcoming expression problems [67–70]. Therefore, it is conceivable that other bacteria might also utilize such a strategy to overcome expression problems.

Conclusions

The ability of *Bacteroides* conjugative transposons to self-transfer, mobilize coresident plasmids in trans or in cis, and mobilizable transposons, gives them many different means by which to transfer resistance genes to other bacteria. Not only do *Bacteroides* spp. have the means to spread resistance, but laboratory experiments have demonstrated that *Bacteroides* conjugative transposons are able to acquire resistance genes and transfer themselves and other elements into distantly related bacteria such as *E. coli*. Perhaps more significantly, the presence of almost identical resistance genes in human intestinal *Bacteroides* isolates and in isolates of oral *Prevotella* and *Porphorymonas* spp., animal isolates of *Prevotella* spp., and in Gram-positive organisms, indicates that in nature *Bacteroides* spp. can and do exchange resistance genes with other bacteria transiently colonizing the same ecological niche. This has serious implications in terms of the spread of antibiotic resistance, as we are already beginning to see. In the last three decades the prevalence of tetracycline and MLS-type resistances has rapidly and dramatically increased in *Bacteroides* spp., and this rise in resistance is directly related to the spread of *Bacteroides* conjugative transposons, particularly those of the CTnDOT/ERL family [3]. The fact that these *Bacteroides* conjugative transposons are so prevalent in current clinical and community *Bacteroides* isolates surveyed suggests that the probability of resistance gene transfer between *Bacteroides* spp. and other bacteria has also increased. Thus, in the future, we can probably expect to see many more examples of acquisition and transfer of antibiotic resistance driven by conjugative transposons, and the significant clinical implications of these horizontal transfer events.

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