

Regulation of CTnDOT Conjugative Transfer Is a Complex and Highly Coordinated Series of Events

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ABSTRACT CTnDOT is a 65-kb conjugative transposon that is found in *Bacteroides* spp., which are one of the more abundant members within the lower human gastrointestinal tract. CTnDOT encodes resistance to the antibiotics erythromycin and tetracycline (Tc). An interesting feature of CTnDOT is that exposure to low levels of Tc induces a cascade of events that ultimately results in CTnDOT conjugative transfer. However, Tc is apparently not a switch that activates transfer but rather a signal that appears to override a series of negative regulators that inhibit premature excision and transfer of CTnDOT. In this minireview, we summarize over 20 years of research that focused on elucidating the highly coordinated regulation of excision, mobilization, and transfer of CTnDOT.

IMPORTANCE *Bacteroides* spp. are abundant commensals in the human colon, but they are also considered opportunistic pathogens, as they can cause life-threatening infections if they should escape the colon. *Bacteroides* spp. are the most common cause of anaerobic infections and are rather difficult to treat due to the prevalence of antibiotic resistance within this genus. Today over 80% of *Bacteroides* are resistant to tetracycline (Tc), and a study looking at both clinical and community isolates demonstrated that this resistance was specifically due to the conjugative transposon CTnDOT.

Bacteroides has recently garnered much recognition due to their abundance in the complex assemblage of microbes that inhabit the colon. This community within the gut may contain up to 1,500 species, present at a density of approximately 3×10^{11} bacterial cells per gram of feces (1). Although the microbiota is quite diverse, *Bacteroidetes* and *Firmicutes* are the two most numerically abundant phyla within the human colon. Early cultivation-based studies had identified *Bacteroides* as abundant and suggested that they comprise roughly 25 to 30% of the colonic microbiota (2–4). However, more-recent sequence-based studies have demonstrated that the members of the *Bacteroidetes* phylum represent upwards of 40% of the human gut microbiota (5, 6).

ROLES OF BACTEROIDES SPP. BENEFICIAL TO HUMAN HEALTH

The abundance of *Bacteroides* within the gut community appears to be vital to human health, as a decrease in the proportion of *Bacteroidetes* within the colon has been implicated in diseases such as obesity (7–9) and type 1 diabetes (10). *Bacteroides* bacteria are important for the acquisition of nutrients by the host by virtue of their role in the biosynthesis of vitamins and breakdown of complex polysaccharides that would otherwise go undigested (11, 12). This capacity for carbohydrate utilization is due to over 200 encoded gene products involved in the hydrolysis of glycosidic bonds, resulting in *Bacteroides* having one of the largest glyco-biomes among any of the bacteria sequenced (13, 14). The short-chain fatty acids produced by *Bacteroides* as a result of this carbohydrate metabolism are also a significant source of caloric energy for the host (15). The role of *Bacteroides* with respect to nutrient acquisition is so paramount that malnutrition can result if there is a deficit of *Bacteroides* in the colon (16). In addition to providing important vitamins, sugars, and fatty acids to the host, *Bacteroides* spp. also protect the host from colonization by pathogens such as *Clostridium difficile* and *Helicobacter hepaticus* (15, 17).

Detrimental effects of *Bacteroides* on human health. Despite the health benefits conferred by *Bacteroides* in the lower gastrointestinal tract, *Bacteroides* can have significant deleterious effects on human health if they escape the confines of the colon through disease or most commonly, when the lining of the colon has been compromised due to surgical trauma (15). When *Bacteroides* escapes the colon, opportunistic infections result and some can be life-threatening. The most common problem is intra-abdominal sepsis, but other complications include appendicitis and necrotizing soft tissue infections. *Bacteroides* spp. are the most commonly isolated organism from anaerobic infections. Although rare, *Bacteroides* can also cause endocarditis, meningitis, and septic arthritis (15). *Bacteroides* infections are becoming increasingly difficult to treat due to the prevalence of antibiotic resistance among members of this genus, which makes *Bacteroides* infections a serious public health threat (18). The problem of increasing antibiotic resistance in this genus is illustrated by tetracycline (Tc), which used to be a frontline antibiotic used for the treatment of *Bacteroides* infections due to its broad spectrum, ease of use, low incidence of side effects, and cost (19). After the debut of Tc in the 1950s, few isolates were resistant. By the 1970s, 20 to 30% of clinical and community isolates were Tc resistant, and by the 1990s, over 80% of *Bacteroides* isolates from clinical and community sources were resistant to Tc (20–23). Resistance to Tc has become so commonplace that *Bacteroides* strains are often not tested for susceptibility and are assumed to be resistant (24, 25). In many

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cases, Tc-resistant strains carried a conjugative transposon, also referred to as an integrated conjugative element, and most of those strains harbored the conjugative transposon CTnDOT (22, 26, 27).

Conjugative transposons. Conjugative transposons (CTNs) are mobile elements that normally reside in a single copy on the bacterial chromosome. These elements account for the majority of antibiotic resistance transfer within *Bacteroides* (28). CTNs range in size from 18 to 500 kb with the majority of CTNs averaging roughly 50 to 80 kb. The large size of most CTNs is due to the need to encode the entire suite of gene products necessary to facilitate conjugative transfer, such as relaxases, coupling proteins, and mating bridge proteins (29, 30). Interestingly, these elements contain genes reminiscent of transposons, plasmids, and bacteriophages and thus share characteristics with these other mobile DNA elements. CTNs possess the transposon-like property of maintenance within the chromosome, yet they can excise from the chromosome and integrate elsewhere. However, molecular mechanisms of excision and integration more closely resemble that of bacteriophage rather than transposition. The CTnDOT integrase and excision proteins themselves are quite similar to those from bacteriophage. Last, conjugative transposons are plasmid-like in their ability to form a covalently closed circular intermediate, while their lack of autonomous replication distinguishes CTNs from plasmids (26).

Many conjugative transposons are able to mobilize other elements. For example, many coresident plasmids are mobilized by a conjugative transposon *in trans*. This occurs when a plasmid containing an *oriT* utilizes the CTn-provided mating pore proteins for transfer to a recipient cell. The *Bacteroides* CTNs have also been shown to mobilize elements when *in cis*, a feature that is not typical for CTNs. For example, if CTnDOT excises from the chromosome and integrates on a plasmid, it can provide the mating pore, an *oriT*, and the mobilization (relaxase/coupling) proteins, allowing it to transfer the entire plasmid by acting “*in cis*.” This ability to use both *trans* and *cis* mechanisms of mobilization is unusual and suggests that the *Bacteroides* CTNs have a greater capacity to mobilize other elements (31).

In order for transfer of the conjugative transposon to occur, there are three main steps that must take place (Fig. 1). The first step is excision from the chromosome to form a covalently closed circular intermediate. Second, a single-stranded copy is then transferred through the mating pore to a recipient cell, after which the copy becomes double stranded. Third, the intact double-stranded CTn integrates into the chromosome of the recipient (30). Conjugative transposition is replicative, as a copy of the CTn is retained in the donor cell. Because the element resides within the chromosome, it is also transferred vertically to progeny cells. This is important because when antibiotic resistance determinants are present on CTNs, they are not only transferred readily within the population, but they are also very stably maintained from generation to generation. Further, it is believed that *Bacteroides* may serve as a reservoir of antibiotic resistance determinants which disseminates these genes to other organisms outside the *Bacteroides* genus, possibly even transferring these elements to organisms that are transiently passing through the gut (28, 32, 33). The vast spread of these conjugative transposons may thus pose a serious threat to the future ability to treat infections with antibiotics. The remainder of this minireview will focus on the *Bacteroides*

conjugative transposon CTnDOT, which is a model CTn and was the first CTn identified in Gram-negative bacteria (34).

CTnDOT OVERVIEW

CTnDOT is a 65-kb conjugative transposon that carries genes encoding resistance to the antibiotics erythromycin (*ermF*) and tetracycline (*tetQ*). Other elements within the CTnDOT-like family include CTnERL and CTn341, although these elements differ from CTnDOT, as they lack the 13-kb *ermF*-encoding region (35–37). A notable feature of CTnDOT is that exposure to low levels of Tc stimulates its excision and conjugative transfer (38). Upon Tc induction (Fig. 2), a regulatory cascade is initiated, leading to translation of the *tetQ-rteA-rteB* operon. RteB then activates the transcription of *rteC*, and RteC in turn activates transcription of the excision operon, *xis2c-xis2d-orf3-exc* (*xis2c* and *xis2d* were referred to as *orf2c* and *orf2d*, respectively, in publications prior to 2012). As the names of genes in this operon imply, the encoded proteins (with the exception of Orf3) are needed to excise CTnDOT from the chromosome. Deletions of *orf3* reveal no phenotype (38). Even more interesting is that these proteins have a regulatory function, as Xis2c, Xis2d, and Exc also collectively activate transcription of the *tra* and *mob* operons, which encode other products required for transfer and mobilization, and ultimately allow CTnDOT conjugative transfer (Fig. 2). When Tc is absent, there is no detectable excision or transfer of CTnDOT (38–41). This is due largely to a repressor of conjugative transfer, the small RNA RteR. In addition, we recently showed that the CTnDOT-encoded integrase, IntDOT, may also inhibit conjugative transfer (42, 43). An overview of the CTnDOT transcriptional cascade is shown in Fig. 2, and each of the gene products and their regulatory mechanism(s) are described in further detail in the following sections. Table 1 provides a summary of each of the key regulators described, in addition to their functional roles.

The *tetQ-rteA-rteB* operon. Upon tetracycline (Tc) induction, the first step in the CTnDOT regulatory cascade occurs through an operon containing *tetQ*, *rteA*, and *rteB*. This operon is regulated via translational attenuation. Although the operon is transcribed constitutively, it is only upon exposure to Tc that translation occurs (19, 44–46). Similar mechanisms of inducible antibiotic resistance have also been reported for Tc induction in the *Enterococcus* Tn916 and related elements, as well as vancomycin induction of the Gram-positive Tn1546 (47).

CTnDOT *tetQ* encodes resistance to Tc via ribosomal protection, which appears to be one of the most common forms of Tc resistance, along with tetracycline efflux, in comparison to either modification or other rarer mechanisms (48). The two most heavily investigated genes encoding ribosomal protection in Gram-negative and Gram-positive bacteria are *tetO* and *tetM*, respectively (49).

Sequence homology suggests that RteA and RteB comprise a two-component regulator where RteA recognizes a signal and RteB is a response regulator. It is not yet clear what signal RteA is sensing, but findings suggest that it is not Tc (26). Earlier studies of RteA and RteB demonstrated that these two proteins were quite important; insertional disruptions of *rteA-rteB* eliminated conjugative transfer of CTnDOT. Additionally, mobilization of coresident plasmids and the *Bacteroides* nonreplicating *Bacteroides* units (NBUs) was also abolished (50, 51). Later studies from that laboratory demonstrated that the primary role for RteB is transcriptional activation of the downstream-encoded *rteC*. RteA and RteB

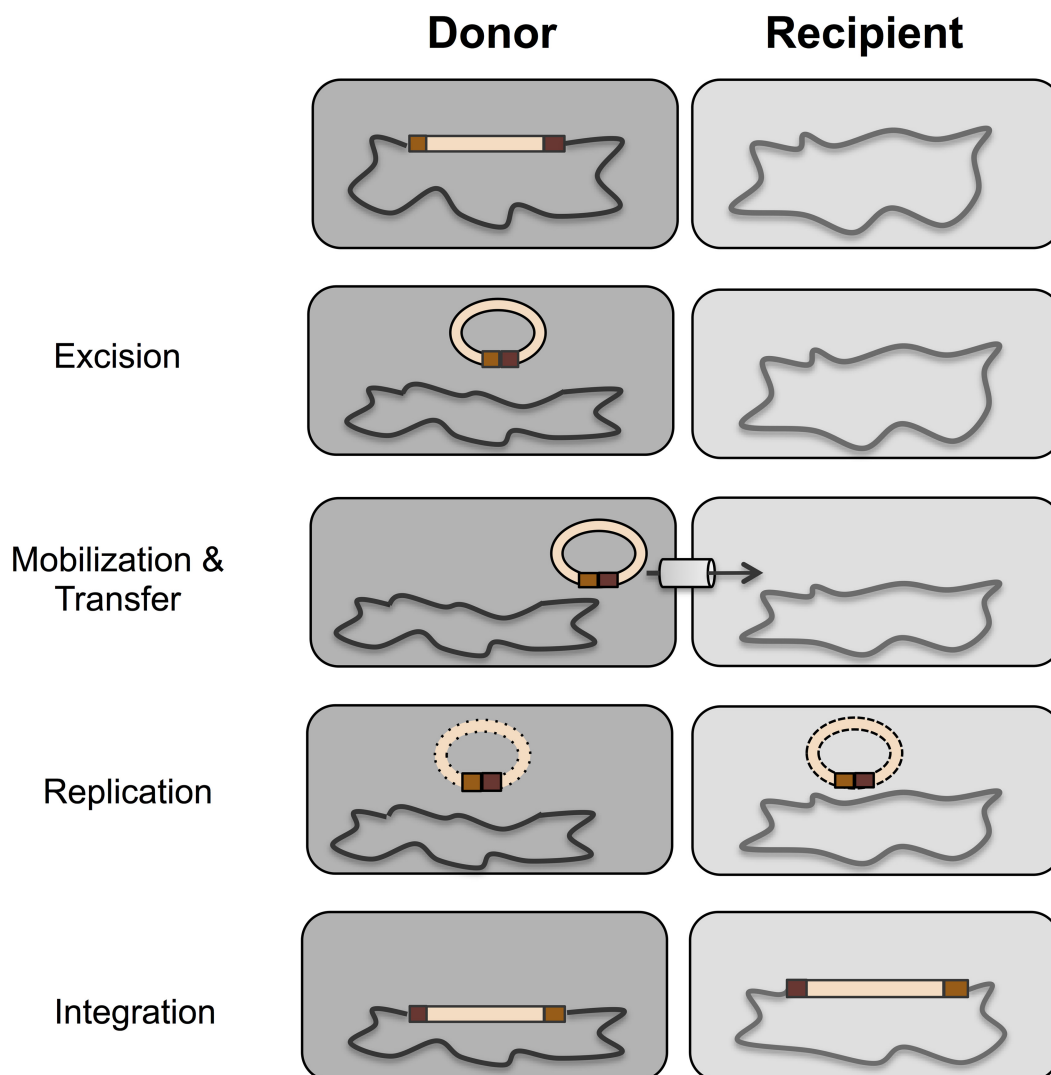


FIG 1 Overview of the CTnDOT life cycle. CTnDOT is a 65-kb conjugative transposon present in *Bacteroides* spp. that encodes resistance to the antibiotics erythromycin and tetracycline. The conjugative transfer of CTnDOT is stimulated by tetracycline induction. The first step in conjugative transfer is that CTnDOT must first excise from the host chromosome to form a circular intermediate. CTnDOT is then nicked at the *oriT* after which CTnDOT is then replicated and made double-stranded before integrating into the chromosomes of both the donor and recipient.

are not involved in the regulation of the *tetQ-rteA-rteB* operon itself.

Although activating transcription within CTnDOT appears to be the primary function of RteA and RteB, these two proteins may have a more widespread role within the cell. RteB has demonstrated an ability to also regulate the expression of genes carried on chromosomes (52). The first class of affected genes was not unexpected; a microarray-based approach revealed four cryptic CTns that had not yet been identified in the *Bacteroides thetaiotaomicron* chromosome. More surprising was finding that chromosomal genes unlinked to such elements were also affected. For example, an *ompA* homologue, a polysaccharide export protein, an aminotransferase, and the chaperone protein *groE* were all upregulated by at least 7-fold. Although most of the genes that were downregulated were identified as hypothetical proteins of unknown function, an ATP-dependent helicase, glycosyltransferase, and a type 1 restriction enzyme were included in these regulated proteins (52).

Although most genes identified in this study were regulated by RteA and RteB, two genes were regulated by RteC, the regulatory protein that is encoded downstream of *rteB*. Many questions remain as to why genes not involved with conjugative transfer were affected. Nonetheless, these observations further demonstrate the complexity of CTnDOT.

The regulatory protein, RteC. RteC is a regulatory protein that is expressed only after Tc induction, due to transcriptional activation from the upstream-encoded RteB. The primary function of RteC is to activate transcription of the operon containing *xis2c*, *xis2d*, and *exc*, which are required for excision from the chromosome as well as activation of the transfer region (the excision operon is discussed in further detail below). A structural homologue search revealed that RteC contains a winged-helix motif, which is common among DNA-binding proteins (53). Later studies demonstrated that putting *rteC* under the control of a heterologous promoter, and thus bypassing the need for RteB, resulted in

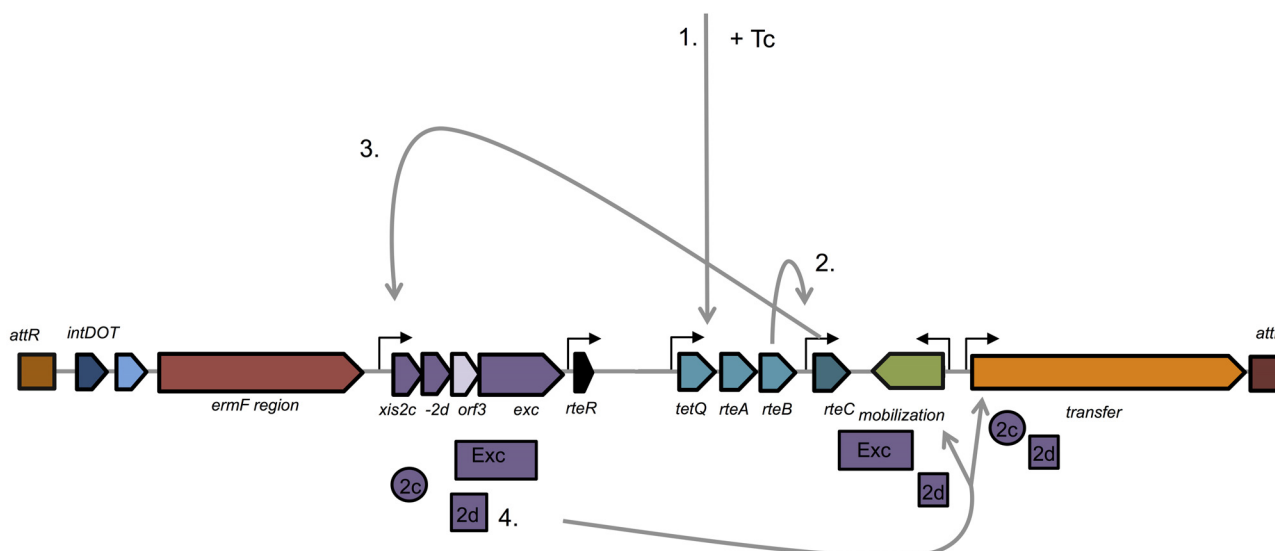


FIG 2 Positive regulation of CTnDOT upon exposure to tetracycline. In step 1, the *tetQ-rteA-rteB* operon is regulated by a translational attenuation mechanism where after exposure to tetracycline (+ Tc), translation of this region resumes; translation of this region ultimately allows for RteB to activate the transcription of RteC (step 2). RteC then activates transcription of the excision operon (step 3). Proteins encoded by the excision operon not only excise CTnDOT from the chromosome but are also involved in transcriptional regulation (step 4). Xis2c and Xis2d are involved in regulation of the *tra* region, whereas Xis2d and Exc are involved in enhancement of *mob* transcription. We have yet to identify any functional role for Orf3.

transcriptional activation of the excision operon (41). This further confirmed that the primary roles of the *tetQ-rteA-rteB* operon are resistance to Tc and activation of *rteC* transcription for downstream gene regulation (39, 41). RteC-mediated transcriptional activation of the excision operon occurs by the binding of RteC to the region located 50 to 70 nucleotides (nt) upstream of the excision promoter region, which was defined using both site-directed mutagenesis and electrophoretic mobility shift assay (EMSA) analysis (53).

The excision operon. The excision operon is located downstream of the *ermF* region of CTnDOT and contains *xis2c*, *xis2d*, *orf3*, and *exc*. As the name implies, proteins encoded within this region are involved in excising CTnDOT from the chromosome in order to form the circular transfer intermediate. We have not yet identified any structural or regulatory function for Orf3, as *orf3* deletions yield no phenotype, as excision and conjugative transfer are unaffected (38, 40). In addition to Xis2c, Xis2d, and Exc, the chromosomally encoded host factor Bhfa and the CTnDOT-encoded integrase, IntDOT, are also required for excision (54).

Xis2c and Xis2d, formerly known as Orf2c and Orf2d, respectively, are both small basic proteins that contain helix-turn-helix DNA binding domains. Xis2c shares similarities with lambda Xis, and Xis2d is homologous to the *Escherichia coli* excisionase TorI

and the Tn916 excisionase (54–57). Exc contains a topoisomerase III domain and is capable of relaxing DNA *in vitro* (58). Not surprisingly, a mutation in Exc that changes the catalytic tyrosine residue to phenylalanine in the topoisomerase domain can no longer relax supercoiled DNA and can no longer catalyze the *in vitro* excision reaction. However, there is no noticeable defect in the *in vivo* excision reaction (58). These observations suggest that although the topoisomerase activity is relevant for the Exc function, it is likely not critical.

Xis2c, Xis2d, and Exc play an additional role, as they are required for positively regulating two very important regions of CTnDOT upon Tc induction, the transfer and mobilization regions.

The transfer operon. The transfer (*tra*) genes are assembled in a 13-kb operon containing 17 protein-encoding genes, *traA* through *traQ* (59). These Tra proteins are required to assemble the mating bridge, a type IV secretion-like apparatus that is required for conjugative transfer. The *tra* genes have a much higher GC content (48 to 60%) than the *Bacteroides* chromosome (approximately 42%), which suggests that the *tra* genes originated from another genus of bacteria (60).

Evidence for a regulatory role of the CTnDOT excision operon was provided by studies in which this operon was sufficient to increase expression of a plasmid, pLYL72, that contains an 18-kb

TABLE 1 Summary of CTnDOT regulatory proteins and RNAs

| Regulator | Function(s) | Reference(s) |
|-----------|---|--------------|
| RteB | Activates transcription of <i>rteC</i> | 40 |
| RteC | Activates transcription of the excision operon (<i>xis2c</i> , <i>xis2d</i> , <i>orf3</i> , and <i>exc</i>) | 42, 54 |
| Xis2c | Required for excision. Activates transcription of the <i>tra</i> operon. | 62, 71 |
| Xis2d | Required for excision. Activates transcription of both the <i>tra</i> and <i>mob</i> operons. | 44, 72 |
| Exc | Required for excision <i>in vivo</i> and enhances <i>in vitro</i> excision. Required for transcriptional activation of the <i>mob</i> operon. | 44, 55 |
| IntDOT | Required for integration and excision. Putative negative regulator. | 44, 55, 71 |
| RteR | Small RNA that negatively regulates the <i>tra</i> operon | 43 |

region of CTnDOT that is sufficient for self-transfer. Due to the limitations of *Bacteroides* genetics, we have extensively used pLYL72 to study regulation of CTnDOT conjugative transfer. While TraG, TraN, and TraP were translated from pLYL72 with or without Tc induction, if CTnDOT was integrated in the chromosome, no Tra gene products were detected without Tc and elevated levels of Tra protein were observed in the presence of Tc. Not only were levels of the Tra proteins affected, but transfer of pLYL72 was affected in parallel (38). A plasmid containing the excision operon and RteR was sufficient to mediate this effect. In this section, we will focus on positive regulation by the proteins of the excision operon; RteR-mediated repression is discussed later in this minireview.

Subcloning analysis suggested that the excision operon (*xis2c-xis2d-orf3-exc*) was mediating regulation of the transfer operon. The excision operon was placed under the control of a heterologous inducible promoter (P_{susA} , which is induced with maltose) to liberate expression of this operon from the requirement for RteC. Jeters et al. demonstrated that expressing the excision genes under control of the heterologous promoter activated a *traA::uidA* fusion, similar to that seen when the excision genes were dependent on RteC. This observation confirmed that the excision proteins are mediating activation of the *tra* operon and that *rteC* is required in this context only for activation of the excision operon (59).

A recent study confirmed more specifically that the excision proteins Xis2c and Xis2d are necessary and sufficient for transcriptional activation of the transfer operon. A deletion of either *orf3* or *exc*, contained in this same operon, revealed no observable defect in activation of the *tra* operon. Xis2c and Xis2d appear to mediate transcriptional activation by binding upstream of the *tra* promoter (Fig. 2) as indicated by EMSA analysis, demonstrating that Xis2c and Xis2d DNA binding is sequence specific. Mutational analysis within the upstream *tra* promoter region showed a loss of Xis2d binding, further supporting the idea that binding upstream of the *tra* promoter results in regulation of the transfer operon (61).

Although no studies have been performed on the CTnDOT Tra proteins to confirm specific roles of each protein in conjugative transfer, some work has defined which of the Tra proteins are required for conjugative transfer. Insertion deletion analysis was performed in CTnERL, a *Bacteroides* conjugative transposon that for the most part is similar to CTnDOT but lacks the *ermF* region, thereby allowing the use of erythromycin as a selectable marker (35).

Insertion deletions in *traG*, *traI*, *traJ*, and *traM* were sufficient to abolish CTnERL transfer, which suggested that these proteins were essential for conjugative transfer. Mutations in *traH* and *traN* resulted in a 100-fold decrease in CTnERL transfer, measured as transconjugants per recipient. A surprising finding from this study was that some insertions actually resulted in increased conjugative transfer. Disruption of *traO* resulted in a 10-fold increase in transfer, while insertions in *traP* and *traQ* resulted in a 100-fold increase. No insertions were generated in *traABCD* because this region was too poorly conserved between CTnERL and CTnDOT to make reliable interpretations. No insertions were made in *traF* or *traL*, because the open reading frame was too small to construct a reliable insertion, and Bonheyo et al. were unsuccessful in the attempt to construct an insertion in *traK* (62).

Thus far, we have reported that the *tra* promoter is independently always in the “on” state (i.e., when detected from pLYL72),

but when the excision operon is present, Xis2c and Xis2d bind upstream to further activate transcription through this operon. While the focus has been on transcription initiation, there appears to be post-initiation effects. A deletion of the 5' *tra* leader region results in a 10-fold activation of a *tra::uidA* fusion (Rob Jeters, unpublished results). An earlier hypothesis was that this region contained the RteR binding site, but we later ruled this out, as similar levels of *traA* are detected whether or not *rteR* is present. This observation warrants further investigation into the regulation of the *tra* leader region.

The mobilization operon. The mobilization region, which is divergently transcribed from the transfer region, is an approximately 4-kb operon containing *mobA*, *mobB*, and *mobC*. Recently, a study of a CTnDOT-like element, CTn341, revealed that MobA and MobB function as the relaxase proteins that nick the *oriT* to initiate transfer. MobC is the coupling protein that shuttles the circular transfer intermediate to the mating pore. A null mutation made in either *mobA* or *mobB* abolished transfer of CTn341, yet coresident elements were still able to transfer to recipient cells. An unforeseen finding was that a null mutation of *mobC* still resulted in transfer of CTn341. This was unexpected because in other systems, the coupling protein is absolutely required for mobilization. Further insight revealed the presence of four *mobC* homologues in the chromosome, so it is likely then that at least one of these chromosomally encoded proteins could compensate for the deletion of *mobC* which ultimately resulted in wild-type transfer of CTn341 (63).

We have recently shown that the *mob* operon is actually transcribed constitutively with respect to Tc induction when detected from the self-transmissible plasmid pLYL72, which contains an 18-kb region of CTnDOT containing the mobilization and transfer regions as well as an *oriT* (64). However, when the *mob* genes are detected from a chromosomal copy of CTnDOT or CTnERL, a transcript is detectable only upon Tc induction.

When the intact excision operon is provided in *trans* to pLYL72, an approximately 10-fold increase in *mob* transcript is detected relative to the constitutive level of transcription. Sequential deletion analysis of the excision operon demonstrates that an in-frame deletion of either *xis2d* or *exc* is sufficient to diminish *mob* transcription to the constitutive levels, while a deletion of either *xis2c* or *orf3* has no effect (43). While both the *mob* operon and *tra* operon share a requirement for *xis2d* to enhance transcription, they differ in that the *mob* operon also requires *exc*, and the *tra* operon requires *xis2c* in addition to *xis2d* (Fig. 2).

Similar to regulation of the transfer region, transcription of the *mob* promoter is constitutive from pLYL72, yet detectable only from CTnDOT upon Tc induction. This observation suggests that a negative *mob* regulator is present on CTnDOT. At this time, however, we do not know what is preventing *mob* transcription in the absence of Tc, but we have ruled out RteR.

NEGATIVE REGULATION OF CTnDOT

Thus far, we have described a multilayered regulatory cascade that initiates upon exposure of cells to Tc, but it is important to clarify that regulation of transfer is not this simple. Tc does not simply act like a switch, triggering a domino-like effect resulting in CTnDOT transfer. Instead, this induction is overriding a series of negative regulators that serve to prevent premature transfer of CTnDOT.

The small RNA, RteR. The most extensively characterized of the CTnDOT negative regulators is the regulatory noncoding

RNA, RteR. This small RNA was first identified in an attempt to identify the positive regulators of CTnDOT transfer and is the first small RNA to be characterized in the *Bacteroidetes* phylum. When an approximately 7-kb region containing the excision operon and *rteR* was provided in *trans* to pLYL72, this was sufficient to not only enhance transfer upon Tc induction 100- to 1,000-fold but also entirely prevent pLYL72 transfer in the absence of Tc. A 500-bp region downstream of *exc* was sufficient for inhibition of pLYL72 conjugative transfer whether or not Tc was present in the growth medium, suggesting that the CTnDOT negative regulator was localized to this fragment (38).

This 500-bp region encodes the small RNA, RteR, which is a 90-nt transcript that is transcribed from an independent promoter that is constitutive with respect to Tc induction. Previous studies demonstrated that no TraG protein (using TraG-specific antibodies) or *traG* mRNA was detected from pLYL72 when a fragment containing RteR was in *trans* (38, 59). We later demonstrated that similar levels of *traA* are detected when *rteR* is in *trans* to pLYL72, yet the downstream *tra* genes are barely detectable. Further, the *tra* half-life is the same whether or not *rteR* was present. Taken together, these observations suggested that RteR may be initiating premature transcription termination within the *tra* operon (42).

IntDOT. The CTnDOT-encoded integrase IntDOT is a member of the tyrosine recombinase family, similar to the integrase on the conjugative transposon Tn916. IntDOT is required for integration of CTnDOT into the chromosome, a reaction that also requires the *Bacteroides* host factor Bhfa (65, 66). Although structurally similar, the Tn916 integrase and IntDOT differ with respect to integration. Tn916 integration appears to be random, whereas CTnDOT integrates into specific sites, although there are multiple sites within the *Bacteroides* chromosome (67). Another interesting feature of IntDOT is that while most other tyrosine recombinases require perfect homology in the overlap sequences they bind in order for recombination to occur, IntDOT can still catalyze integration even with a large mismatch of this 7-bp sequence. Further, the frequency of integration is the same whether the overlap sequence is homologous or whether mismatches are present, as long as the 2 bp adjacent to the catalytic site are homologous (68, 69). IntDOT is also required for the excision of CTnDOT from the chromosome, and interestingly, the homology of the overlap sites matters for excision. The excision reaction is more efficient when there is perfect homology compared to a mismatched overlap sequence (70, 71).

Although the CTnDOT integrase IntDOT has been well studied, we recently made the serendipitous discovery that when *intDOT* is provided in *trans* to the self-transmissible plasmid pLYL72, there is no detectable transfer, with or without Tc induction. A possible role for IntDOT as a negative regulator of CTnDOT transfer was first investigated when trying to elucidate the negative regulator of the *mob* operon. The first suspect was RteR, which was ruled out as the *mob* genes are transcribed constitutively with or without *rteR* in *trans* (42). The integrase of Tn916 can bind the Tn916 *oriT* region to prevent premature conjugative transfer, which prompted us to investigate whether the CTnDOT integrase could perform a similar function (72). Although the *mob* genes were transcribed whether or not IntDOT was in *trans*, pLYL72 transfer was not detectable from this same strain. This additional role of IntDOT remains to be further explored.

CONCLUSIONS

CTnDOT has established itself as a fascinating element, as the regulation of self-transfer is highly coordinated so as to promote conjugative transfer promptly upon ideal conditions—in this case exposure to the antibiotic Tc. Further, this prevents the unnecessary transfer until it is vital to the community to harbor CTnDOT, as conjugative transfer bears a metabolic cost to the donor (73). While studies of CTnDOT have offered further insight as to the complex nature of conjugative elements, we still have much to learn about this element.

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