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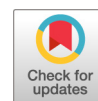
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# Incompatibility Group I1 (Incl1) Plasmids: Their Genetics, Biology, and Public Health Relevance

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**SUMMARY** Bacterial plasmids are extrachromosomal genetic elements that often carry antimicrobial resistance (AMR) genes and genes encoding increased virulence and can be transmissible among bacteria by conjugation. One key group of plasmids is the incompatibility group I1 (Incl1) plasmids, which have been isolated from multiple *Enterobacteriaceae* of food animal origin and clinically ill human patients. The Incl group of plasmids were initially characterized due to their sensitivity to the filamentous bacteriophage  $\lambda$ f1. Two prototypical Incl1 plasmids, R64 and pCollb-P9, have been extensively studied, and the plasmids consist of unique regions associated with plasmid replication, plasmid stability/maintenance, transfer machinery apparatus, single-stranded DNA transfer, and antimicrobial resistance. Incl1 plasmids are somewhat unique in that they encode two types of sex pili, a thick, rigid pilus necessary for mating and a thin, flexible pilus that helps stabilize bacteria for plasmid transfer in liquid environments. A key public health concern with Incl1 plasmids is their ability to carry antimicrobial resistance genes, including those associated with critically important antimicrobials used to treat severe cases of enteric infections, including the third-generation cephalosporins. Because of the potential importance of these plasmids, this review focuses on the distribution of the plasmids, their phenotypic characteristics associated with antimicrobial resistance and virulence, and their replication, maintenance, and transfer.

**KEYWORDS** incompatibility group I1 plasmids, plasmid replication, plasmid maintenance, plasmid transfer, virulence, antimicrobial resistance, plasmid biology, plasmid genetics, public health

## INTRODUCTION

Bacterial plasmids are extrachromosomal genetic elements that are linear or circular DNA molecules that exist independently of the host chromosome in microbial cells and can replicate autonomously (1). Plasmids are seen most often in bacteria but have

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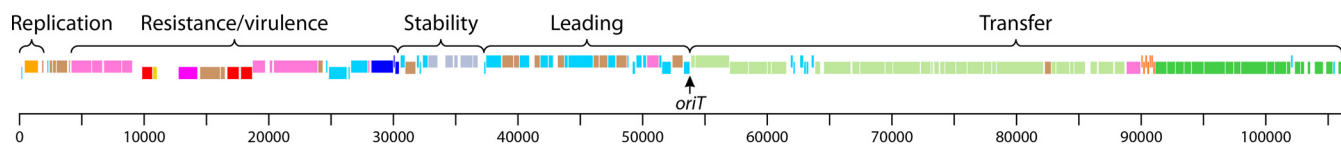
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also been detected in archaea and eukaryotic organisms, where they are typically associated with the mitochondria (2). Plasmids have their own replication origin and can be stably inherited. Despite some similarities with chromosomal elements, plasmids do differ from bacterial chromosomes in several key characteristics (3, 4). Compared to bacterial chromosomes, plasmids typically contain fewer genes, are not essential for host survival, and most of the time have multiple copies in a cell (4). Many plasmids, including several that carry antimicrobial resistance (AMR) genes and genes encoding increased virulence, are transmissible by conjugation (5). While most infections caused by pathogens such as *Salmonella enterica*, *Escherichia coli*, and related organisms are self-limiting, some are more severe due to a variety of factors, including the infectious dose, route of inoculation, host immunity, and virulence characteristics of the infecting organisms (6). Severe manifestations of disease often require the use of antimicrobial agents to manage the infection. One set of challenges that has arisen is that many bacteria have developed resistance to antimicrobial agents used to control them.

Historically, plasmids were classified based on their compatibility for coexistence with one another in a single strain (7). With this typing approach, plasmids are assigned to different incompatibility (Inc) groups based on their incompatibility to coexist in the same cell (8–10). These incompatibility typing methods have been used to study the dissemination of plasmid-mediated antimicrobial resistance and the corresponding evolution of plasmids, with some of the more common examples among enteric bacteria being the IncF, IncI1, IncA/C, and IncX groups (11). Among these groups, the IncI1 plasmids are isolated from bacteria from human patients and food animals and are often associated with clinically relevant strains, although their host range appears to be relatively limited to a few enteric species, including *E. coli* and *S. enterica* (12). Several representative plasmids have been identified for their potential to carry and disseminate antimicrobial resistance among enteric pathogens (9, 13). Along with IncA/C plasmids, the IncI1 plasmids are the most common plasmid types associated with the dissemination of genes encoding resistance to extended-spectrum cephalosporins, which are the antimicrobial agents used in management of severe *Salmonella* infections (14).

IncI1 plasmids are characterized by the following distinguishing traits. They encode two types of sex pili, thin, flexible pili to aid in liquid matrix mating and thick, rigid pili needed for mating in both liquid and surface environments (15). In addition, IncI1 plasmids carry *sog*, which encodes a DNA primase that is vital for the establishment of plasmids following DNA transfer into recipient cells (16, 17). IncI1 plasmids can also carry genes responsible for antimicrobial resistance, attachment, and virulence and those that contribute to stable inheritance during cell division and plasmid maintenance (14, 18). Some IncI1 plasmids are bacteriocinogenic plasmids, which are capable of synthesizing bacteriocins, i.e., toxic compounds produced by host bacteria to antagonize other bacteria (19–21).

The IncI group of plasmids were initially characterized due to their sensitivity to the filamentous bacteriophage If1 (22). The receptors for the phage were determined to be on the tips of the thin flexible pili encoded on the IncI1 plasmid (23). There are two prototypical IncI1 plasmids that have been extensively studied, namely, R64 and pCollb-P9, that share functional similarity in plasmid replication, stability, and conjugal transfer mechanisms (24–26). More recently, isolated IncI1 plasmids have been characterized that share many of these characteristics; however, there are often differences in genes that encode antimicrobial resistance and/or increased virulence which are highlighted throughout the review (27, 28). The sequence of the R64 plasmid can be broken down into 5 different regions associated with replication, antimicrobial resistance, plasmid stability/maintenance, leading (first sequence transferred during conjugation to establish stable plasmid in the recipient), and transfer sequences (25). More detailed discussion of the regions will be presented in the following sections and are highlighted in Fig. 1. This review will focus on IncI1 plasmids, their phenotypic characteristics associated with antimicrobial resistance and virulence, and their transfer and genetics.



**FIG 1** Overview of the major regions that generally make up IncI1-type plasmids as described by Sampei et al. (25). These regions include the plasmid replication and control regions, variable regions encoding antimicrobial resistance and/or virulence-associated genes, genes associated with plasmid stability and partitioning, the leading region, which may play a role in conjugal transfer, and regions associated with conjugal transfer (25). The plasmid represented is pSH1148\_107, GenBank accession number [JN983049](#) (43).

## DISTRIBUTION

With the enhanced capabilities of DNA sequencing, an increasing number of whole plasmid sequences are available to researchers. When GenBank was searched using microbial nucleotide BLAST by querying the reference replicon sequences for IncI1 plasmid types using a reference sequence (29) against the “complete plasmids” genome database, a total of 133 IncI1 complete plasmid sequences were identified. When the resultant plasmid GenBank information was extracted using FeatureExtract 1.2 program (Danish Technical University), a total of 16,636 IncI1 loci were cataloged. In an attempt to develop a nonredundant data set, extracted duplicate sequences were removed in Excel and then manually reviewed to generate a draft nonredundant data set, which contained approximately 1,400 unique IncI1 coding sequences, including variants of genes. Half of these sequences were identified as encoding “hypothetical proteins,” while other genes were predicted to be associated with antimicrobial resistance, biocide/heavy metal resistance, virulence, horizontal gene transfer elements, and plasmid transfer (Table 1). IncI1 plasmid sequences were detected in isolates from the following bacterial species in GenBank: *Escherichia albertii*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Shigella dysenteriae*, *Shigella flexneri*, and *Shigella sonnei*, and thus the host range appears to be limited to the *Enterobacteriaceae*.

A factor in the persistence of IncI1 plasmids in the *Enterobacteriaceae* is that they contribute traits such as antimicrobial resistance and colicin production yet appear to convey minimal metabolic burden on the host strains. Johnson et al. demonstrated that the acquisition of an IncI1 plasmid did not significantly affect the fitness of the host bacterium, and in some cases the fitness cost associated with the acquisition of an IncI1 plasmid was negative (i.e., beneficial) (30). For example, in *E. coli* strains carrying IncA/C plasmids, the fitness cost of the acquisition of an IncI1 was no greater than that of carrying the IncA/C plasmid alone or, in some cases, was a negative cost (30). Similarly, Kaldhone et al. found that many IncI1-positive *Salmonella* isolates with the greatest ability to multiply and persist in intestinal epithelial cells carried additional large plasmids, including IncA/C, IncHI2, IncFIB, and IncB/O, which in theory should have high metabolic costs (31). Freire Martín et al. cured an IncI1 plasmid to evaluate mechanical burden of carrying the plasmid and determined that there was not a burden associated with IncI1 plasmids in *S. enterica* serotype 4,5,12:i:- (32). Conversely, an IncI1 CTX-M1 plasmid imparted a growth disadvantage upon *K. pneumoniae* (33). Potential reasons for this disparity in findings across studies are that the *K. pneumoniae* plasmid may have been recently acquired and not gone through coevolution far enough to compensate for the growth and fitness costs or that there were differences in the plasmids that led to variable costs, since the IncI1 plasmid multilocus sequence typing (pMLST) classifications of the plasmids in *K. pneumoniae* and *S. enterica* 4,5,12:i:- were different (32).

When examined from a host source range perspective, IncI1 plasmids have been isolated from environmental sources and several different animal species, including cats, cattle, chickens, dog, fish, goats, horses, rabbits, sheep, swine, and turkeys (31, 34–36). When factors such as the host, geographical origin of isolation of the IncI1 plasmids present in GenBank (described above), and the literature were assessed, isolates were found to originate from South America, North America, Europe, Asia, Australia, and Africa (28, 37–42). The sequenced strains were isolated from as far back as 1969

**TABLE 1** Summary of gene products associated with antimicrobial resistance, metal/biocide resistance, virulence, partition/maintenance, conjugal transfer, and gene transfer detected in fully sequenced IncI1 plasmids

Function	Product	
Antimicrobial resistance	Aminoglycoside <i>N</i> -acetyltransferase AAC(3)-I	
	Aminoglycoside <i>N</i> -acetyltransferase AAC(3)-II	
	Aminoglycoside acetyltransferase AacC	
	Aminoglycoside adenylyltransferase AadA1	
	Aminoglycoside adenylyltransferase AadA2	
	Aminoglycoside 3'-phosphotransferase APH(3')-I	
	Aminoglycoside <i>N</i> -acetyltransferase III ACC(3)-III	
	Aminoglycoside <i>N</i> -acetyltransferase AAC(3)-VIa	
	Aminoglycoside <i>O</i> -phosphotransferase APH(3')-II	
	Aminoglycoside <i>O</i> -phosphotransferase APH(6)-Ic	
	Hygromycin resistance protein Hrp	
	Streptomycin phosphotransferase protein StrA	
	Streptomycin phosphotransferase protein StrB	
	Chloramphenicol efflux MFS transporter CmlA	
	Chloramphenicol/florfenicol efflux MFS transporter FloR	
	Extended-spectrum beta-lactamase SHV-12	
	Extended-spectrum beta-lactamase CTX-M-1	
	Extended-spectrum beta-lactamase CTX-M-3	
	Extended-spectrum beta-lactamase CTX-M-8	
	Extended-spectrum beta-lactamase CTX-M-14	
	Extended-spectrum beta-lactamase CTX-M-15	
	Extended-spectrum beta-lactamase CTX-M-55	
	Extended-spectrum beta-lactamase CTX-M-123	
	Class C beta-lactamase CMY-2	
	Class C beta-lactamase CMY-4	
	Class C beta-lactamase CMY-42	
	Class C beta-lactamase CMY-111	
	Beta-lactamase, TEM-1	
	Beta-lactamase, TEM-20	
	Beta-lactamase, TEM-52	
	Beta-lactamase, TEM-57	
	Beta-lactamase, TEM-210	
	Oxacillin-hydrolyzing class D beta-lactamase OXA-2	
	Dihydrofolate reductase DhfrA1	
	Dihydrofolate reductase DfrA17	
	Dihydropteroate synthase type-1 Sul1	
	Dihydropteroate synthase type-2 Sul2	
	Fosfomycin resistance glutathione transferase FosA3	
	Macrolide ABC transporter permease/ATP-binding protein MacB	
	Macrolide 2'-phosphotransferase MphB	
	Tetracycline resistance MFS efflux pump TetA	
	Tetracycline efflux MFS transporter TetC	
	Tetracycline resistance ribosomal protection protein TetM	
	Tetracycline resistance transcriptional regulator TetR	
	Metal/biocide resistance	Arsenical pump-driving ATPase ArsA
		Arsenical efflux pump membrane protein ArsB
		Arsenate reductase ArsC
		Arsenical resistance operon transcriptional repressor ArsD
		Arsenical resistance operon transcriptional regulator ArsR
		Mercuric ion reductase MerA
		Organomercurial lyase MerB
		Mercuric transport protein MerC
		Mercuric resistance operon coregulator MerD
Mercuric transporter protein MerE		
Mercuric transcriptional regulator MerR		
Quaternary ammonium compound efflux SMR transporter QacE		
Quaternary ammonium compound resistance protein QacH		
Quaternary ammonium compound resistance protein SugE		
Silver- or copper-binding protein SilE		

(Continued on next page)

**TABLE 1** (Continued)

Function	Product
Virulence	Colicin 1B Cib
	Colicin 1B immunity protein Cbi
	Colicin Ia immunity protein Cia
	Colicin Ia protein Cai
	Colicin M activity protein Cma
	Colicin M immunity protein Cmi
	CS1 fimbrial subunit A CfaA
	CS1 fimbrial subunit B CfaB
Plasmid-encoded fimbriae Pef	
Partition/maintenance	Stable plasmid inheritance protein A ParA
	Stable plasmid inheritance protein B ParB
	Plasmid maintenance protein CcdA
	Plasmid maintenance protein CcdB
	Plasmid maintenance protein VagD
	Plasmid maintenance protein VagC
	Plasmid maintenance protein PndA
	Plasmid maintenance protein PndB
	Plasmid maintenance protein PndC
	Plasmid maintenance protein RelB
Plasmid maintenance protein RelE	
Conjugal transfer	Conjugal transfer protein TraA
	Conjugal transfer transcription antiterminator TraB
	Conjugal transfer protein TraC
	Conjugal transfer system coupling protein TraD
	Conjugal transfer protein PilI
	Conjugal transfer protein PilJ
	Conjugal transfer protein PilK
	Conjugal transfer outer membrane protein PilL
	Conjugal transfer protein PilM
	Conjugal transfer protein PilN
	Conjugal transfer protein PilO
	Conjugal transfer pilus biogenesis protein PilP
	Conjugal transfer protein PilQ
	Conjugal transfer pilus biogenesis protein PilR
	Conjugal transfer pilus biogenesis protein PilS
	Conjugal transfer lytic transglycosylase PilT
	Conjugal transfer peptidase PilU
	Conjugal transfer pilus-tip adhesin protein PilV
	Conjugal transfer pilus assembly protein PilX
	Conjugal transfer pilus assembly protein TraE
	Conjugal transfer protein TraF
	Conjugal transfer protein TraG
	Conjugal transfer pilus assembly protein TraH
	Conjugal transfer lipoprotein TraI
	Conjugal transfer protein TraJ
	Conjugal transfer protein TraK
	Conjugal transfer pilus assembly protein TraL
	Conjugal transfer protein TraM
	Conjugal transfer protein TraN
	Conjugal transfer protein TraO
	Conjugal transfer protein TraP
	Conjugal transfer protein TraQ
Conjugal transfer protein TraR	
Conjugal transfer protein TraS	
Conjugal transfer protein TraT	
Conjugal transfer pilus assembly protein TraU	
Conjugal transfer protein TraV	
Conjugal transfer pilus assembly protein TraW	
Conjugal transfer pilus acetylation protein TraX	
Conjugal transfer integral membrane protein TraY	
Conjugal transfer protein TrbA	

(Continued on next page)

**TABLE 1** (Continued)

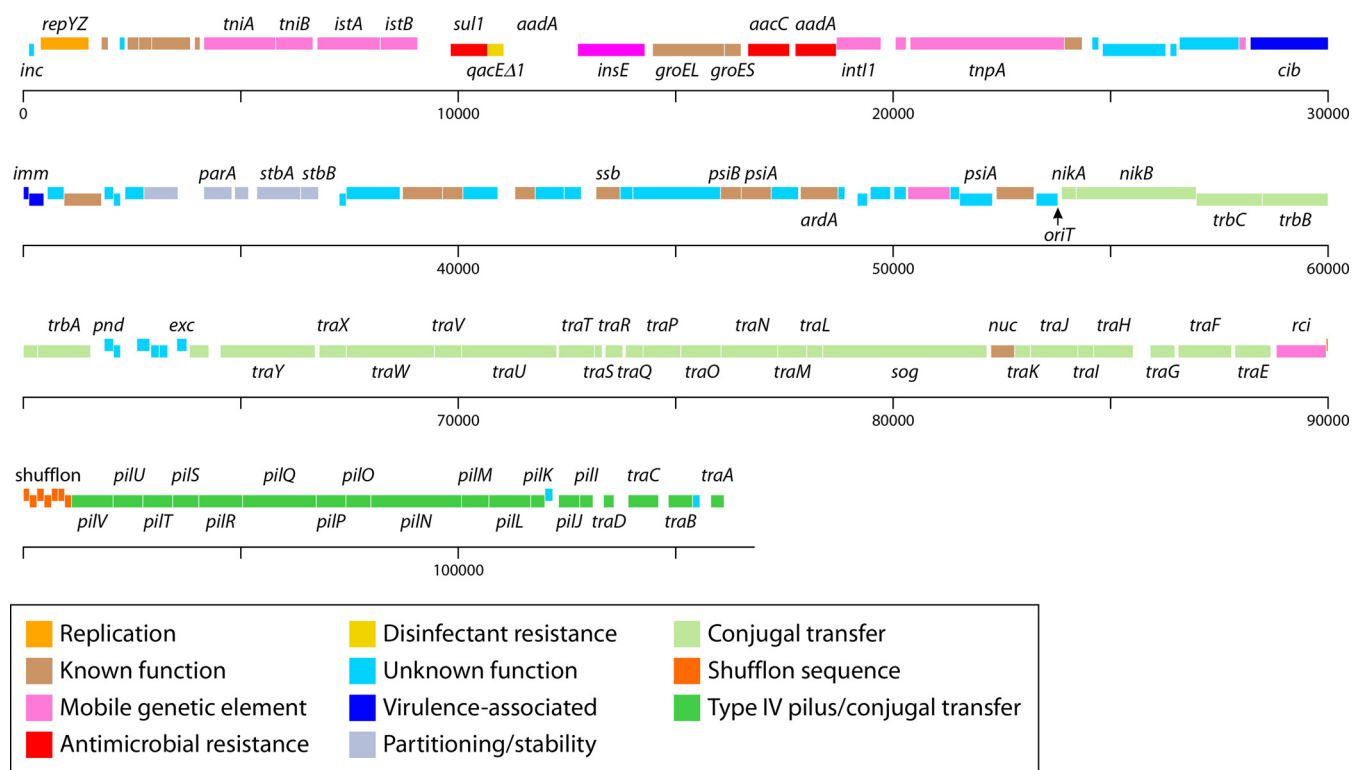
Function	Product
	Conjugal transfer protein TrbB
	Conjugal transfer protein TrbC
	Conjugal transfer oriT-specific DNA-binding protein NikA
	Conjugal transfer relaxase protein NikB
	Conjugal transfer protein FinQ
Gene transfer	Insertion element IS1 protein InsA
	Insertion element IS1 protein InsB
	IS629 element
	IS100 element
	IS4321 element
	IS200 element
	IS256 element
	IS26 element
	IS3 element
	IS4 element
	IS481 element
	ISEcp1 element
	IS1294 element
	IS5 element
	IS6 element
	IS630 element
	IS66 element
	IS91 element
	ISKra4 element
	ISL3 element
	Class 1 integron integrase IntI1
	Tn21 protein Urf2
	Tn3 transposase

(GenBank accession: [NZ\\_CP018638](#)) and came from a wide range of animal species (as noted above), retail foods (retail beef, chicken, pork, and turkey products), and forest soil (GenBank accession: [NZ\\_CP010233](#)). Inc11 plasmids have been detected in isolates collected from several human patients. Among the sequenced plasmids present in GenBank, *E. coli*, *S. enterica*, *S. sonnei*, and *K. pneumoniae* were isolated from a variety of specimens, including stool, blood, urine, wound sites, and peritoneal fluid; these findings correspond well to the published literature (14, 21, 38, 43–45). Inc11 plasmids have also been isolated from the stools of mothers during pregnancy and lead to subsequent transmission to their newborns (46, 47).

### PLASMID REPLICATION

The process of plasmid replication and partitioning during bacterial cell division is a multistep process which is often encoded by a *rep* operon (48, 49). Because replication of plasmids can be metabolically costly to bacteria, there are regulatory mechanisms that limit the copy numbers of plasmids in strains to allow enough copies to ensure that daughter cells retain plasmids and avoid postsegregation killing yet not too many copies to be overly metabolically taxing (49, 50). The Inc11 plasmids are low copy number plasmids, whose replication is tightly controlled by negative regulation of replication initiation (51).

The control of Inc11 plasmid replication is likely best studied in the plasmid Collb-P9 (52, 53). Collb-P9 and other Inc11 plasmids, such as R64, have a 3-kb replication control region that encodes the initiation, control, and termination of unidirectional replication of the Inc11 plasmids (upper left portion of Fig. 2) (25, 51). This replication control region is generally conserved across sequenced Inc11 plasmids (31, 45, 54). The main replication initiation protein is the 39-kDa RepZ protein. RepZ interacts with the origin of replication (*ori*) which is near *repZ* to initiate replication of the plasmid sequence. Termination of plasmid replication occurs at *CIS*, which is located between *repZ* and *ori* (Fig. 3A). Control of *repZ* expression and



**FIG 2** Genetic map of IncI1 plasmid pSH1148\_107, GenBank accession number [JN983049](#) (43). The gene names of known genes are included along with color coding of the predicted functions of each of the genes (25).

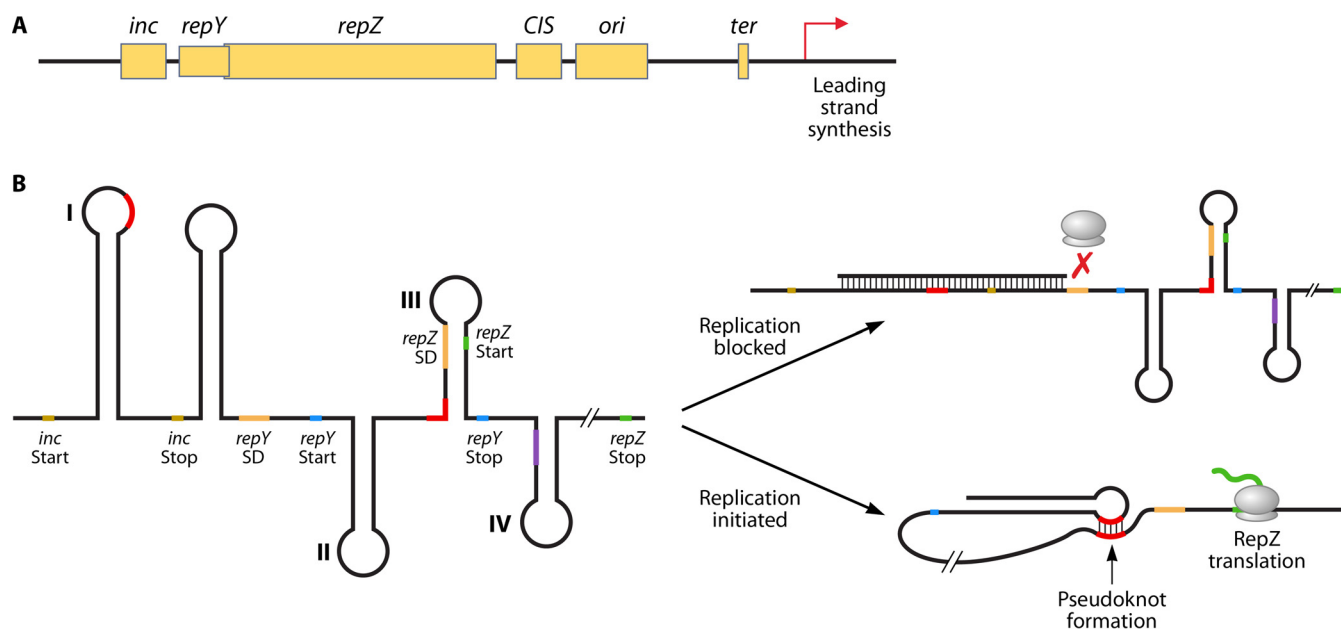
translation, and subsequently control of plasmid replication and copy number, is associated with the negative regulator *inc* and positive regulator *repY* (55). The *inc* gene encodes an antisense RNA of approximately 70 bp in length, which contains nucleotides (5'-rGCCA-3') that bind to the conserved 5'-rUUGGCG-3' motif in the "structure I" stem-loop configuration of the Rep mRNA repressing translation of RepZ (56) (Fig. 3B). The interaction of the Inc RNA and Rep mRNA prevents the formation of a pseudoknot structure that leads to RepZ translation (57).

For RepZ mRNA to be translated, RepY, a short protein of 29 amino acids, must first be translated. The 3' of *repY* overlaps the 5' *repZ* and is located adjacent to the *repZ* start codon. RepY expression is also under the control of *inc*, such that the binding of the Inc RNA to structure I causes steric hinderance of the ribosomal binding site (RBS) for translation of *repY* (55) (Fig. 3B). Translation of *repY* leads to the formation of the activated pseudoknot, which is initiated during the termination step of *repY* translation, opening access to the *repZ* RBS and facilitating base pairing between nucleotides in structure III and structure I of the RepZ mRNA (55) (Fig. 3B). The pseudoknot structure allows for translation of RepZ that is needed for plasmid replication. Following initiation of RepZ translation, the Inc RNA rapidly binds to structure I of the RepZ mRNA, inhibiting further translation of RepZ, thus keeping the plasmid replication in check (55).

## HOST ADDICTION SYSTEMS

Many plasmids encode host addiction systems consisting of long-acting toxins and shorter-acting antitoxins, which, if the plasmids were lost (cured), would be lethal to the bacterium through a process known as postsegregation killing (58, 59). The following toxin/antitoxin (TA) systems have been identified in IncI1 plasmids: *ccdAB*, *relBE*, and *pndBCA* (59, 60). PndCA is part of the Hok/Sok TA family, in which there is the stable mRNA encoding a toxin (e.g., Hok) and the more unstable antisense RNA (e.g., Sok) that limits toxin translation (61). Pnd is named due to its promotion of nucleic acid

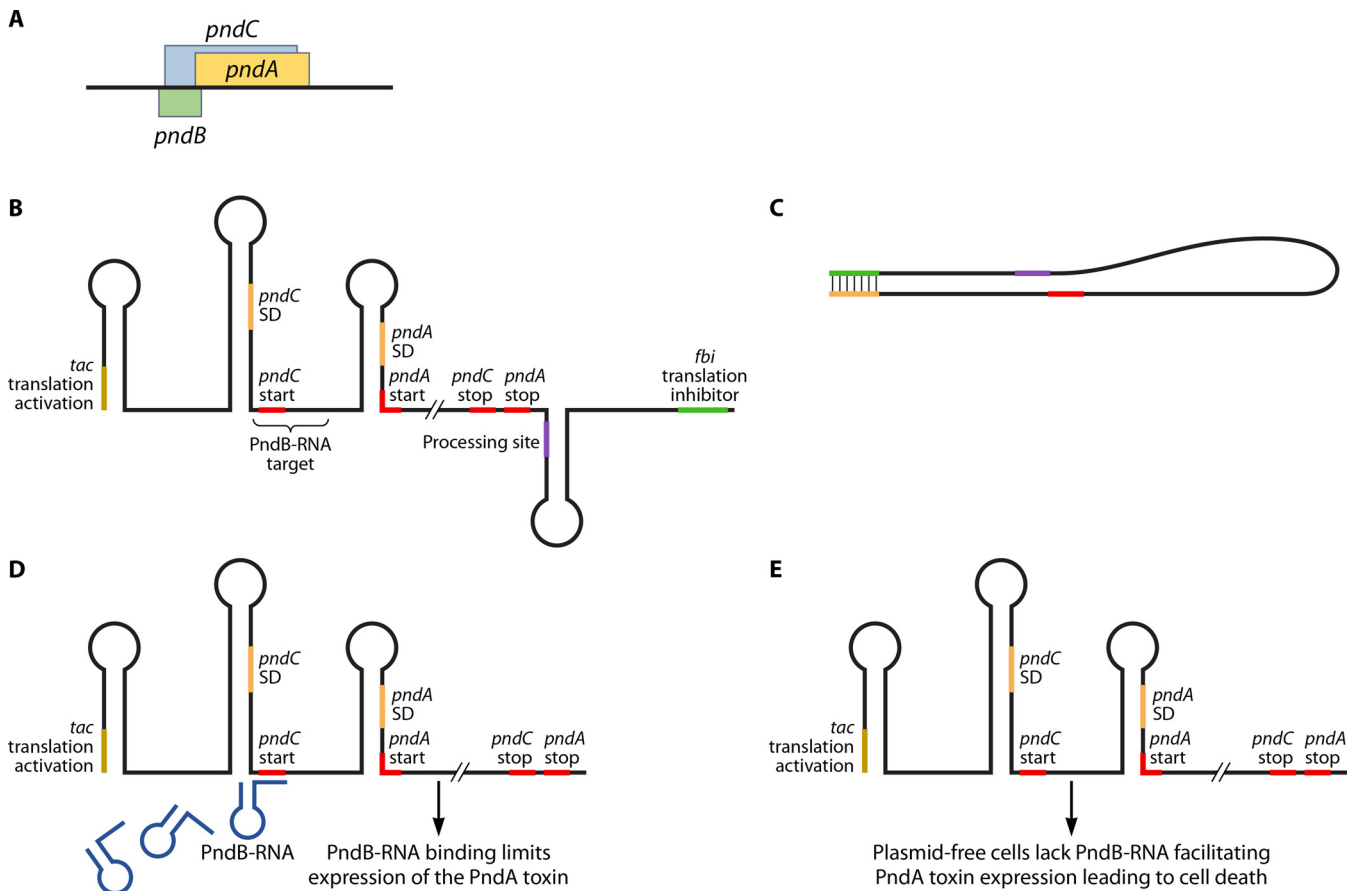




**FIG 3** (A) Diagram of the replication control region for Inc1 plasmids. RepZ is the main replication initiation protein and interacts with the origin of replication (*ori*), which is near *repZ*, to initiate replication of the plasmid sequence. Termination of plasmid replication occurs at *CIS*, which is located between *repZ* and *ori* (57). (B) Predicted RNA structure of the replication control (Rep) region of the Inc1 plasmid and predicted mechanisms of replication control. Control of *repZ* translation, and subsequently control of plasmid replication and copy number, is associated with the negative regulator *inc* and the positive regulator *repY*. To control replication, *inc* mRNA binds to the *inc* sequence and blocks the ribosomal binding site to inhibit RepY translation. To activate replication, *inc* mRNA is unbound from *inc*, allowing translation of RepY, which facilitates pseudoknot formation (binding of structure I to structure III at the binding sites indicated in red) that opens the ribosomal binding site to facilitate RepZ expression (based on data from reference 55).

degradation (62). The *pnd* TA genes are located within the transfer region of plasmid R64 and pSH1148\_107 (43, 63) (Fig. 2). *pndC* overlaps *pndA* in the plasmid sequence, while *pndB* is located on the opposite DNA strand (Fig. 4). Studies indicate that the presence of the Pnd toxin leads to degradation of RNA following the addition of the antibiotic rifampin to *pnd*-positive *E. coli* (64, 65). Rifampin is a drug that targets and inactivates DNA-dependent bacterial RNA polymerase (66). This inhibition of RNA synthesis can lead to a reduction of the *pndB* transcription and subsequent degradation of the inhibitory RNA molecules. This PndB RNA degradation subsequently leads to an increase in PndCA translation (65, 67). PndA serves as a toxin that functions by damaging the bacterial cell membrane, while PndC acts by promoting the translation of *pndA*. The *pndCA* mRNA is very stable in bacteria; however, its levels are negatively regulated through an unstable complementary *pndB*-encoded RNA molecule that prevents the toxin translation (61, 63). In cells that lose plasmids carrying the TA system, the unstable RNA degrades, allowing for translation of the residual *pndCA* mRNA, thereby killing the plasmid-free host cells (63).

The regulation of the expression of the Pnd toxin is due to the binding of the small *pndB*-encoded RNA molecule that binds to the more stable PndCA mRNA (68). The antisense PndB RNA is complementary to both the translation initiation sequence of *pndC* and the leader region of PndA mRNA, thus regulating PndA translation (61, 69). Based on the model Hok/Sok TA system, the translation of *pndC* would be tightly controlled by the antisense PndB RNA (Fig. 4). The expression of *pndA* is coupled to the expression of *pndC*; thus, the regulation of PndA levels by *pndB* likely occurs indirectly through *pndC* (69). For translation of PndA to occur, the PndA mRNA is cleaved at its 3' end, which converts the inactive form of the mRNA to the form that is translated. The presence of the PndB RNA binding likely limits this cleavage and keeps the translation in check when present at adequate levels. In strains where the plasmids are lost, the antisense RNA is degraded and the expression of the PndA is able to be initiated from the more stable mRNA molecules, leading to cell death of the plasmid cured bacteria (61).



**FIG 4** Diagram of the Pnd toxin-antitoxin system. The system can encode the PndA toxin that causes lethal damage to the bacterial cell membrane when expressed. (A) The Pnd operon is made up of *pndC* that overlaps *pndA* in the plasmid sequence, and *pndB* is located on the opposite DNA strand. *pndC* modulates *pndA* expression, and *pndB* encodes an RNA molecule that suppresses expression of *pndA* and toxin formation. (B) Transcription of the *pnd* operon leads to the formation of a complex mRNA molecule whose translation is regulated by multiple mechanisms. The 5' end of the inactive RNA molecule contains a translational activation element (*tac*), and the 3' end contains a fold-back inhibition element (*fbi*). Between these elements are the nucleotides for the translation of PndC and PndA and a processing site for the formation of the functional mRNA molecules. (C) The *fbi* and *tac* sites are complementary to one another and bind to prevent translation of the unprocessed RNA molecule. (D) Following processing (cleavage at the processing site and removal of the *fbi* element), the regulation of the translation of the Pnd toxin in the activated mRNA is due to the binding of the small, very short-lived PndB RNA molecules that bind to the more stable PndCA mRNA overlapping the translation start site for PndC. (E) In cases where the plasmids are lost, all of the short-lived PndB are degraded, allowing the translation of the long-lived PndA toxin leading to cell membrane damage and cell death.

## CONJUGAL TRANSFER

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection (pilus) between two cells. Conjugation facilitates the horizontal transfer of different genes among different bacteria (70) and potentially allows for the rapid adaptation and evolution observed among bacteria to respond to varied stresses, such as antibiotic exposure, and an increase in their ability to colonize hosts and cause disease (71). The ability of IncI1 plasmids to disseminate among enteric bacteria has been well studied to develop an understanding of their plasmid conjugation strategies. One of the best-studied conjugative transfer systems is that of R64, which, like many other IncI1 plasmids, is a relatively complex system compared to most other plasmid types (15). The transfer region of IncI1 plasmids is larger than those of most other plasmid types at approximately 54 kb in size (Fig. 1); in comparison, the transfer regions of most IncF plasmids are generally around 33 kb (72). The large IncI1 transfer region typically contains 48 open reading frames (ORFs) (Fig. 2), which encode both a thick, rigid conjugal pilus and a thin, flexible pilus (15).

Much of the early work elucidating the mechanisms of IncI1-associated conjugation

mechanisms originated from studies by Komano and colleagues in Japan (for examples see references 15, 25, and 73–76). The transfer region typically consists of genes for the thick conjugal pilus encoded by the *tra/trb* genes and a thin flexible pilus encoded by *pil* genes that plays a key role in facilitating conjugation in liquid environments (15, 77) (Fig. 2). Based on the analyses by Sampei et al. (25), the transfer region of the prototypical IncI1 plasmid R64 could be separated into four major functional groupings, including those with regulatory functions (*traABCD*), the relaxation complex for conjugation initiation (*oriT* and *nikAB*), the type IV pilus (T4P) formation (*pil* gene cluster), and the *tra/trb* general conjugal apparatus. The IncI1 Tra and Trb proteins share amino acid sequence similarity with the Dot/Icm virulence plasmids from *Legionella pneumophila* and the tumor-inducing (Ti) VirB/D4 T4SS of *Agrobacterium tumefaciens*, rather than with the more widely studied IncF plasmids (76, 78, 79). Likewise, the IncI1 T4P is ancestrally related to the toxin co-regulated pilus (TCP) from *Vibrio cholerae* and the bundle-forming pilus (BFP) of enteropathogenic *E. coli* (EPEC) and is classified as a type IVb pilus (T4bP) based on its physical structures (80–83).

In the sequence of R64 and similar plasmids, the transfer region is adjacent to the replication initiation sequence, with the *traABCD* just upstream of the *inc* replication regulatory sequence and downstream of the *pil* operon (25). Both *traB* and *traC* encode proteins that are essential for conjugative transfer of IncI1 plasmids in both liquid and solid media (73). TraB is homologous to NusG, a protein that interacts with RNA polymerase, increases the rate of transcription, and affects transcription termination (84, 85). TraC is also predicted to be a positive regulator for expression of transfer-associated genes (73). TraA is predicted to have a helix-turn-helix domain that is characteristic of a DNA-binding domain and may affect the regulation of transcription (86). In the IncI1 plasmid pESBL-EA11, when the region adjacent to *traA* was disrupted by a transposon, it led to an elevated (>10-fold) transfer efficiency; thus, this region has been termed the high frequency of transfer (Hft) region (87, 88). This disruption of the Hft region led to overexpression of TraA, which led to the observed increased rate of the conjugal transfer. The expression of TraA likely facilitates the activation of TraBC in pESBL-EA11, which had previously been reported to be key to the transcription of the downstream transfer-associated genes (87). The function of TraD is currently unknown, and several sequenced IncI1 plasmids appear to lack the *traD* gene annotated in R64 (25, 84, 88).

The *tra* and *trb* genes encode the thick, rigid conjugal pilus for both solid and liquid media mating (76). As noted above, the *tra* and *trb* genes have been sequenced and their proteins possess predicted structural similarities to the Dot/Icm and Ti T4SS (Table 2 and Fig. 5). Early studies mapping the plasmid gene function in R64 indicated that the *traEFG* genes likely form an operon that is not essential for conjugation to occur (74). TraF and TraG do not appear to have protein homologs within the Dot/Icm and Ti T4SS (76, 78, 79, 89–92). The functions of these gene products are not well understood; TraG likely functions as a histidine phosphatase (76), and more recent studies have indicated that the expression of *traF* is significantly upregulated in *E. coli* strains carrying plasmid pTF2. These strains demonstrated increased conjugation rates following exposure to cephalosporin antibiotics (93). TraE is predicted to be homologous to the VirE1 of the Ti T4SS (76). VirE1 functions in a chaperone-like fashion to facilitate the export of VirE2, a nonspecific, single-stranded DNA-binding protein that was shown to transfer tumor DNA from *A. tumefaciens* to plant cells (94, 95).

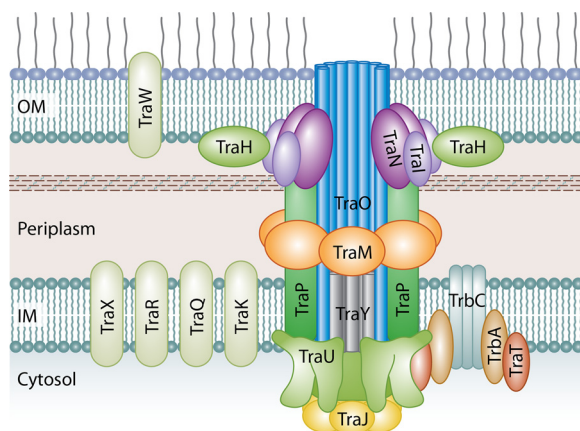
The gene products of *traH* through *traY* likely form a distinct operon from that of *traEFG* (76). When Komano et al. (76) disrupted the various genes in the *tra* region, they found that *traMNPQRUVWY* were essential for conjugal transfer of R64, whereas disruption of the *traJKLOTX* genes significantly affected plasmid transfer efficiency but did not completely eliminate it (76). The predicted homologs for several of the genes are shown in Table 2; many of the genes are associated with the formation of the pilus structure (Fig. 5). Even though there are overlapping homologies with many of the proteins in the more extensively studied Dot/Icm and VirB/D4 T4SSs, there are some

**TABLE 2** Genes in the IncI1 type 4 secretion system (T4SS) and homologs of the proteins from other better-characterized T4SS

IncI1 gene	Predicted function/product	Protein homolog(s) <sup>a</sup>	Reference(s)
<i>trbC</i>	Type 4 coupling protein	DotL (VirD4)	76, 103
<i>trbB</i>	Protein disulfide isomerase	TrbC(F)	25, 91
<i>trbA</i>	T4SS coupling complex protein	DotM	76, 103
<i>traY</i>	Integral membrane protein	DotA	76, 79
<i>traX</i>	Inner membrane protein		25, 76
<i>traW</i>	Outer membrane lipoprotein		25, 76
<i>traV</i>	Cytoplasmic transfer protein		25, 76
<i>traU</i>	ATPase, nucleotide binding protein	IcmB/DotO (VirB4)	76, 79, 91
<i>traT</i>	T4SS coupling complex protein	IcmJ/DotN	102, 103
<i>traS</i>	Cytoplasmic transfer protein		25, 76
<i>traR</i>	Inner membrane protein	IcmD/DotP, IcmC/DotE, DotV	102
<i>traQ</i>	Inner membrane protein	IcmD/DotP, IcmC/DotE, DotV	25, 102
<i>traP</i>	Inner membrane protein-T4SS core complex	DotF (VirB3)	25, 79
<i>traO</i>	Inner membrane protein-T4SS core complex	DotG (VirB10)	76, 79, 91
<i>traN</i>	Outer membrane lipoprotein-T4SS core complex	DotH (VirB9)	76, 91
<i>traM</i>	Inner membrane protein-T4SS core complex	DotI (VirB8)	76, 78, 79, 89
<i>traL</i>	Outer membrane/periplasmic protein-signal peptide		25, 76
<i>traK</i>	Inner membrane protein	IcmT	76
<i>traJ</i>	Outer membrane lipoprotein-T4SS core complex	DotB (VirB11)	76, 101
<i>tral</i>	Outer membrane lipoprotein-T4SS core complex	DotC (VirB7)	76, 92
<i>traH</i>	Outer membrane lipoprotein	DotD	76, 102
<i>traG</i>	Outer membrane/periplasmic protein-signal peptide		25, 76
<i>traF</i>	Outer membrane/periplasmic protein-signal peptide		25, 76
<i>traE</i>	Cytoplasmic transfer protein	(VirE1)	25, 76

<sup>a</sup>Proteins from the Dot/ICM T4SS and the VirB/D4 Ti T4SS (in parentheses).

protein homologs that are not apparent in the IncI1 Tra/Trb T4SS, which may affect extrapolation of the IncI1 T4SS structure. The inner membrane portion of the T4SS is predicted to be made up of TraJ, TraM, TraO, TraP, TraU, and TraY proteins, which are homologous to VirB11 (DotB), VirB8 (DotI), VirB10 (DotG), VirB3 (DotF), VirB4 (DotO), and DotA, respectively (76, 78, 79, 96, 97). The IncI1 T4SS lacks a homologous protein to DotJ of *Legionella* Dot/Icm T4SS membrane complex (92). DotJ forms a heteroocto-



**FIG 5** Predicted structure of the Tra/Trb T4SS of IncI1 plasmids based on homologs from the better-characterized Dot/Icm and Ti T4SSs. The inner membrane portion of the T4SS is predicted to be made up of TraJ, TraM, TraO, TraP, TraU, and TraY proteins. TraP forms a multimer with TraO and TraY through the inner membrane and into the periplasmic space where the complex appears to interact with the outer membrane complex. TraU has homology to DotO of the Dot/Icm T4SS, which forms a hexamer that sits at the base of the main pore channel of the secretion system where it interacts with the TraJ multimer. TraJ homologs are predicted to form hexamers that are on the cytoplasmic side of the secretion system and form a transitory complex with the TraU multimer in line with the core complex. TrbC is a T4CP that forms a complex with TrbA and TraT and functions to help deliver macromolecules from the cytoplasm to the T4SS secretion apparatus to traverse the cell membranes. The outer membrane complex made up of 13 subunits comprised of TraL, TraH, and TraN surrounds the TraO secretion channel consisting of 18 subunits. OM, outer membrane; IM, inner membrane.

mer complex with DotI, while TraM, the DotI homolog, forms a homohexamer, which is similar to the VirB8 structure of the *Agrobacterium* Ti T4SS (92). Based on homology to DotG, TraO is predicted to form an 18-mer that serves as a periplasmic channel for the secretion system (98). The TraO multimer interacts with an outer membrane complex that is made up of 13 subunits comprised of TraI, TraH, and TraN (98–100) (Fig. 5). The TraO multimer appears to extend from the inner membrane complex to, or through, the outer membrane complex, forming the channel for the plasmid DNA to be transferred out of the cytoplasm during conjugation (101). Interestingly, inactivation of *traH*, *nuc*, and *traS* did not inhibit conjugation in R64, and thus they may not be required for conjugation (76). The TraR and TraQ proteins were identified as having indirect homology with VirB2, which forms a multiprotein complex serving as the pilus extending from the bacterial cells of the VirB/D4 T4SSs (79). In the Dot/Icm T4SS, there does not appear to be an analogous protein polymer to VirB2 (78, 99, 101). Because of the lack of direct homology to VirB2 and the lack of a homolog in the Dot/Icm T4SS, it is not clear whether or not TraR and/or TraQ forms the pilus appendage. Other investigators have predicted that TraR and TraQ are likely inner membrane proteins (76, 102). TraP forms a multimer with TraO through the inner membrane and into the periplasmic space where the complex appears to interact with the TraM, TraI, and TraH multimer (99, 101). TraY was shown to encode transmembrane helices that are homologous to those of DotA of the Dot/Icm T4SS (76, 102) and analogous to VirB6 of the VirB/D4 T4SS (79). These proteins have been predicted to be part of the inner membrane complex and interact with the homologs of TraO and TraP in the secretion system (100). DotA, the TraY homolog, was also shown to be essential for *Legionella* pathogenicity (78, 102, 103).

Several other *tra* genes encode proteins that are predicted to be associated with the inner membrane based on their homology to the Dot/Icm T4SS; these include TraK, TraQ, TraR, and TraX (76, 79, 102). TraK is predicted to be an ortholog of IcmT, an integral membrane protein that was shown to be essential for the T4SS function; however, its specific role in the process is currently not known (102). One of the membrane proteins for which little is known about its function is TraW, an outer membrane lipoprotein that has been shown to be essential for conjugal transfer in R64 (76).

T4SSs are characterized by the presence of ATPases that energize the secretion functions. In the Ti T4SS, these ATPases are VirB4, VirB11, and VirD4, while in the Dot/Icm T4SS the corresponding proteins are DotO (IcmB), DotB, and DotL, respectively (104). In R64 and most other IncI1 plasmids, the corresponding ATPases are TraU, TraJ, and TrbC, respectively (76, 79, 102). Each of these ATPases is predicted to form hexamers that help drive the molecules being secreted across the T4SS (79). TrbC is a type 4 coupling protein (T4CP) that forms a complex with TrbA and TraT, which are DotM and DotN (IcmJ) homologs, respectively (76, 103, 105, 106). A predicted structure of the T4CP based on recent findings for the Dot/ICM system shows that the TrbC hexamer forms a central channel and each monomer interacts with a TrbA subunit that is bound to a TraT monomer (105, 106) (Fig. 5). The Dot/ICM system has additional cytoplasmic components (IcmS, IcmW, and LygA) likely involved in substrate recognition that appear to be absent in the IncI1 T4CP complex (106). T4CP complexes function by helping deliver macromolecules from the bacterial cytoplasm to the T4SS section apparatus to traverse the cell membranes (100–103). TraU has homology to DotO (IcmB) and VirB4 and plays key roles in type 4 secretion (70, 102). The related proteins, such as DotO, form hexamers that sit at the base of the main pore channel of the secretion system (Fig. 5) and likely lead to conformation changes in the T4SS core complex facilitating transfer of macromolecules across the cell membranes (96, 97, 107). TraJ homologs, such as DotB, are predicted to form hexamers that are on the cytoplasmic side of the secretion system and form a transitory complex with the DotO (TraU) in line with the core complex (96, 97). In this model, the TraJ hexamer docks with the TraU complex to facilitate

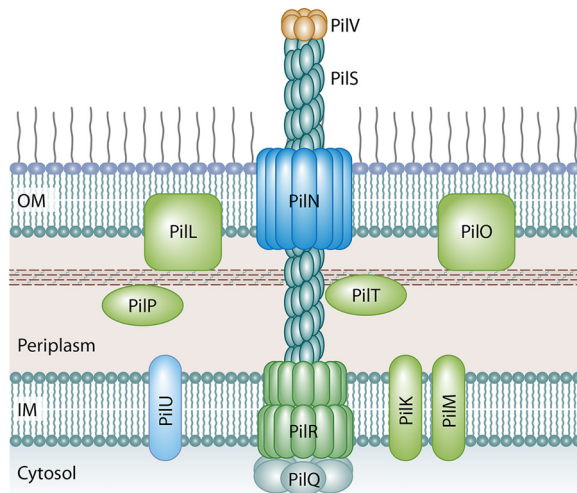
the transfer of macromolecules from the T4CPs on the cytoplasm to the base of the T4SS channel, where the TraJ hexamer facilitates the loading of the macromolecules into the core complex for secretion across the membranes (107, 108).

Upstream of *traY* are *excAB* and *pndCA*, which encode surface exclusion and host addiction, respectively (25, 109). Adjacent to *pndCA* is the *trbABC* operon, where the *trbA* and *trbC* genes were found to be essential for conjugal transfer of IncI1 plasmids (63). As noted above, TrbC and TrbA are key elements of the T4CP complex that likely plays a key role in the delivery of macromolecules to the T4SS (78, 89). *trbB* deletion mutants in R64, while significantly less efficient in conjugal transfer, maintain the ability to transfer plasmids (63); TrbB is a homolog to the IncF pilus protein TrbC<sub>F</sub>, where TrbC<sub>F</sub> resides in the periplasm and appears to function in pilus stabilization and pore formation (91).

During the conjugal transfer of DNA across the pili, the processing of plasmid DNA transfer is initiated through a relaxation complex or relaxosome encoded by an operon that includes *nikA* and *nikB*, along with *oriT* (110, 111) (Fig. 2). The NikA and NikB proteins of the relaxosome function as a nickase that recognizes a short DNA motif (5'-YATCCTG\*Y-3') in *oriT* where the double-stranded DNA is nicked (\* marks the nick site) (111). The nicked DNA strand is transferred to the recipient cell during conjugation. *oriT* also contains two inverted repeats, one that is 8 bases and another that is 17 bases. The 17-bp inverted repeats have a single nucleotide difference between the repeat sequences. To form the active relaxosome, NikA binds specifically to one of the 17-bp repeat sequences, leading to a change in the bending of the *oriT* DNA (112). The three-dimensional structure of NikA is similar to that of known transcriptional repressors and when bound to *oriT* interacts with the relaxase protein NikB to form the active relaxosome. The bending of the *oriT* DNA orients the nick site and NikB, which in turn introduces a nick in *oriT* to initiate transfer (111, 112). Following nicking, the single-stranded DNA molecule is transferred along with NikB, which remains attached to the single-stranded DNA (ssDNA), into the recipient cells. The second 8-bp inverted repeat in *oriT* plays a role in termination of DNA transfer into the recipient cell, after which the transferred ssDNA molecules are recircularized and a complementary strand synthesized (25). The *sog* gene, located between *traL* and *nuc* in the *tra* region (Fig. 2), encodes two proteins, SogS and SogL. SogL functions both as a primase to help initiate synthesis of the complementary strand of the newly transferred plasmid and for suppression of *E. coli dnaG* mutations which can negatively affect DNA synthesis and, subsequently, conjugation (16, 113, 114). Both SogL and SogS are transferred into the recipient cell as part of the conjugal transfer of Collb-P9; however, SogS lacks the primase activity of SogL, and its function is less well understood (16, 113).

An operon encoding the T4P is located just upstream of *traD* in R64 (*traC* in pESBL-EA11 and R621a) and is made up of 14 genes (*pill* to *pilV*) (115). The T4P is a thin, flexible pilus that early studies indicated was required for conjugal transfer of R64 and Collb-P9 in liquid media but not on solid media (116). More recent studies have indicated that this observation is not universal among IncI1 plasmids, in that *pilRSTUV* are also required for transfer of pESBL-EA11 on solid media (88). Of the 14 *pil* genes, 12 appear to be required for the formation of the conjugal pilus (*pilK* to *pilV*) in R64 and Collb-P9, while the functions of *pill* and *pilJ* remain unknown (15). The T4P encoded on the pSERB1 plasmid of enteroaggregative *E. coli* was also shown to contribute to conjugal transfer of the IncI1 plasmid and to aid adherence of the bacteria to epithelial cells and surfaces, facilitating biofilm formation (77). Similar contributions of T4P to biofilm formation have also been noted in several other species (77, 83). The function of the T4P in conjugation in liquid media appears to be stabilization of the mating bacteria through the formation of aggregates of the donor and recipient strains (116).

The *pil* genes that encode the T4P structure extending from the surface of the bacterial cell are *pilS*, which encodes the major prepilin, and *pilV*, which encodes the minor prepilin. Other structural genes include *pilR*, which encodes the inner membrane



**FIG 6** Predicted structure of the T4P of IncI1 plasmids based on homologs from the better-characterized TCP and BFG pili. PiIS forms the major prepilin polymer complex that extends from the cell to form the T4P and is capped by PiIV subunits that make up the minor prepilin multimer that interacts with specific oligosaccharide in LPS on a recipient cell. Other structural elements include PiIR, which is the integral inner membrane spanning protein, and PiIN, which forms the outer membrane secretin through which the PiIS polymer extends. PiIT is predicted to be a lytic transglycosidase that may function to create a pore through the peptidoglycan layer to allow elongation of the pilus structure. PiIR proteins are predicted to be platform proteins that transfer energy from the system ATPases, such as PiIQ, to the T4P structure.

spanning protein, and *pilN*, which encodes the outer membrane secretin through which the PiIS polymer extends (76, 84, 115) (Fig. 6). The PiIS prepilin is initially translated as a 22-kDa precursor that is cleaved by the PiIU prepilin peptidase to form a 19-kDa monomer that is assembled into a polymer that is secreted to form the extending T4P structure (117) (Fig. 6). At the terminal tip of the pilus is the PiIV adhesin, which has been shown to interact with lipopolysaccharide (LPS) on a recipient cell. Through these interactions, PiIV functions in recognition of the recipient that facilitates conjugation in liquid environments. Different PiIV variants recognize specific oligosaccharide moieties in the LPS core on the surface of recipient cells, and this affects the range of recipients that the donor strains can conjugate with in liquid media (118–120). Control of recipient recognition is mediated by a shufflon, which is a multi-inversion system that is located at the 3' end of *pilV* and functions as a biological switch that mediates variable expression of the PiIV protein (118, 121, 122). Shufflons are generally composed of four DNA segments, three of which (segments A, B, and C) are divided into two open reading frames that are subject to inversion and are separated by seven recombination sites. The combination of the DNA segments and recombination sites allows for the potential formation of 7 different PiIV adhesion variants (118). The recombination of the shufflon elements is mediated by Rci, which is a site-specific recombinase whose gene is located just upstream of the shufflon region (25, 123). Recent next-generation sequencing experiments have identified that the shufflon region may display even greater variability due to deletions of *pilV* segments or insertion sequence (IS) elements inserted at recombination sites (124, 125). Because of the location of the shufflon sequences at the C-terminal region of *pilV*, the proteins formed have a conserved N-terminal region of approximately 361 amino acids and variable C-terminal regions that can vary in size between 69 and 113 amino acids (118).

PiIT is predicted to be a lytic transglycosidase that is localized to the periplasm, and it has been suggested that it functions to create a pore through the peptidoglycan layer to allow elongation of the pilus structure (126). PiIR is predicted to be an inner membrane protein whose amino acid sequence has similarity to those of BfgE and TcpE of the BFP and TCP T4bP (115, 126). These proteins are predicted to be platform

proteins that transfer energy from the system ATPases, such as PilQ, to the T4P structure (127). The other proteins identified as essential for facilitating R64 and Collb-P9 transfer include PilK and PilM, which are inner membrane-associated pilus biogenesis proteins, PilL outer membrane lipoprotein, PilN outer membrane pilus secretin protein, PilO outer membrane-associated pilus biogenesis protein, PilP periplasmic-associated pilus biogenesis protein, and PilQ cytoplasmic ATPase (25, 115, 128). The PilN outer membrane monomers form a ring structure that is predicted to serve as the channel for passage of extending T4P structure across the outer membrane of R64 during elongation, and the PilQ complex is a cytoplasmic ATPase that powers the assembly of the pilus structure (115, 126–128).

## ANTIMICROBIAL RESISTANCE

A key reason that IncI1 plasmids have drawn attention by the public health community is their ability to carry antimicrobial resistance genes, including those associated with crucial antimicrobials used to treat severe cases of enteric infections such as the third-generation cephalosporins, fluoroquinolones, and macrolides (129–131). IncI1 plasmids are known to carry a variety of different resistance genes (Table 1), and hence they possess the potential to encode multidrug resistance (MDR) in bacterial pathogens (37, 132, 133).

The best-studied antimicrobial resistance associated with the IncI1 plasmids is that of those genes that encode  $\beta$ -lactam resistance.  $\beta$ -Lactamases are enzymes that cause hydrolysis of oxyimino- $\beta$ -lactam antimicrobial agents (134). There are multiple generations of  $\beta$ -lactam antimicrobial compounds that exhibit a spectrum of activity levels, and they are widely used in clinical practice (129, 134). Extended-spectrum  $\beta$ -lactamases (ESBLs) are enzymes that can inhibit a broader range of  $\beta$ -lactam antibiotics (135, 136). Numerous researchers have investigated the prevalence of these ESBL genes in IncI1 plasmids (9, 18, 27, 38, 137–157). Many variants of ESBL enzymes have evolved over a period of time, largely due to mutations with the genes encoding the enzymes (158). There are some broad classes of ESBLs, including the TEM and SHV families of  $\beta$ -lactamases that were prevalent in the 1980s and 1990s, respectively, and CTX-M  $\beta$ -lactamases that have been prominent since the early 2000s (159–161). The *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-20</sub>, *bla*<sub>TEM-52</sub>, and *bla*<sub>SHV-12</sub> genes are those most commonly associated with IncI1 plasmids (14, 37, 133, 151, 162–165).

The *bla*<sub>CTX-M</sub> variants are unique from *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> enzyme types (166). The *bla*<sub>CTX-M</sub> family contains multiple subtypes, and many have been reported to be associated with IncI1 plasmids (37). The *bla*<sub>CTX-M</sub> variants are present in enteric organisms, including *E. coli*, *Salmonella* spp., and *Klebsiella* spp. from around the world, and have been detected in both nosocomial and community settings (37, 160). The global presence of *bla*<sub>CTX-M</sub> variants led to in-depth studies to understand them (143, 167–172). Some *bla*<sub>CTX-M</sub> variants are associated with insertion sequences (IS) present on the plasmids. The *ISEcp1* is associated with *bla*<sub>CTX-M-5</sub> and *bla*<sub>CTX-M-15</sub>, while *ISCR1* is linked to *bla*<sub>CTX-M-2</sub> and *bla*<sub>CTX-M-9</sub> (27, 146). These IS elements are hypothesized to carry outward reading promoters that confer high level expression of *bla*<sub>CTX-M</sub>. The *bla*<sub>CTX-M-15</sub> is the most universal ESBL among *E. coli* strains carrying *bla*<sub>CTX-M</sub>. These strains often belong to ST131 and are resistant to quinolones in addition to cephalosporins (173). The increasing prevalence of *E. coli* in community-associated and nosocomial infections make this broadening of antimicrobial resistance a public health concern.

Additionally, IncI1 plasmids have been identified that carry the *bla*<sub>CMY</sub> gene that encodes AmpC  $\beta$ -lactamase (141, 162, 174–179) leading to resistance to several  $\beta$ -lactam antibiotics, including ampicillin, cefoxitin, ceftriaxone, and amoxicillin clavulanate (180). The primary *bla*<sub>CMY</sub> variant associated with IncI1 plasmids is CMY-2. The *bla*<sub>CMY-2</sub> gene has been found in a diverse range of IncI1 plasmids based on pMLST, with the gene being detected in plasmids representing a variety of sequence types and clonal clusters (37). Within the IncI1 plasmids, the *bla*<sub>CMY-2</sub> genes are typically found in IS elements, including *ISEsp1* and



IS1294, that facilitate their mobilization and insertion to different regions of the plasmids (14, 181, 182). Other *bla*<sub>CMY</sub> genes that have been identified in IncI1 plasmids include *bla*<sub>CMY-4</sub> (183), *bla*<sub>CMY-42</sub> (164, 176, 184), and *bla*<sub>CMY-111</sub> (183).

In addition to the  $\beta$ -lactam resistance genes, IncI1 plasmids have also been characterized that carry resistance genes for several other antimicrobial agents, including sulfonamides, trimethoprim, chloramphenicol, aminoglycosides, and tetracyclines (28, 43, 185) (Table 1). For chloramphenicol resistance, the genes *cmlA* and *floR* have been identified and are typically associated with integrons and IS elements, such as IS26 and ISCR2 (18, 28, 186). Many of the chloramphenicol resistance genes are colocated with the sulfonamide resistance gene *sul2* (28, 186, 187). In addition, *sul1* has also been detected in several IncI1 plasmids of organisms collected from a variety of animal sources and diverse geographical locations (43, 187, 188). Trimethoprim resistance genes, including *dfrA1* and *dfrA17*, have been identified in plasmids that were isolated from *E. coli* in human patients, food animals, and wild birds in Europe (162, 187). The most commonly detected tetracycline resistance gene in IncI1 plasmids appears to be *tetA* (21, 188, 189). There is greater diversity of aminoglycoside resistance genes that have been detected on the plasmids, including those associated with gentamicin [*aacC* and *aac(3)-IV*] (28, 43), kanamycin [*aph(4)-1a*] (18), and streptomycin (*aadA1*, *aadA2*, *aadA5*, *strA*, and *strB*) (28, 43, 188). This wide variety of genes encoding resistance to clinically relevant antimicrobials is concerning, especially in light of the ability of several IncI1 plasmids to conjugally transfer among different bacteria (21, 43). Many of the resistance genes are associated with IS elements, integrons, and transposons which may further facilitate their transmission among plasmids that are coresident within bacteria and potentially the host chromosome (21, 43, 190). Therefore, the distribution of antimicrobial resistance genes to and from IncI1 plasmids is an important area for public health surveillance.

## VIRULENCE AND COLICIN PRODUCTION

To cause infection, *Salmonella* must traverse the upper gastrointestinal tract, compete with commensal intestinal bacteria, and invade and persist within the intestinal epithelia (6). The potential contribution of IncI1 plasmids to virulence has not been well understood, as much of the research has focused on their contributions to antimicrobial resistance (21, 31). There have been several efforts to sequence IncI1-positive bacteria to examine the genetics of IncI1 plasmids (25, 45, 54, 84, 88), and several have identified genes that may be associated with virulence (191). Virulence factors allow the bacterium to have an increased ability to colonize a host niche, provide entry into, survive within, and exit from a host, evade or suppress the host's immune response, or obtain required nutrients that are limited in the host environment (192, 193). Among these factors are biofilm formation that increases colonization ability, bacteriocin production which limits niche competition, nutrient acquisition, such as for iron, bacterial uptake systems that facilitate invasion and improve intracellular survival in host cells, and regulatory factors that mediate the expression of virulence genes (192).

Many of the sequenced IncI1 plasmids carry genes, such as *cib*, that encode the production of bacteriocins which can provide a competitive advantage for the host against members of the microbiota that they may be competing against for niche colonization (19, 194). The *cib* gene, which encodes colicin Ib, has been reported to be commonly carried on IncI1 plasmids (21, 195). Most of these strains positive for *cib* also carry a colicin immunity gene (*imm*) that protects the strain from the toxin (14, 21). The spectrum of inhibition of colicin Ib appears to be quite narrow; in characterization studies by Kaldhone et al., strains expressing the colicin were able to inhibit a limited number of *E. coli* strains and none of the non-*E. coli* species tested, including *S. enterica*, *K. pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (31).

In functional studies examining the role of colicin in pathogenesis, Nedialkova et al.

demonstrated that colicin Ib produced by *Salmonella enterica* serotype Typhimurium strains could inhibit the growth of other enteric organisms in a murine model system, especially when there was inflammation in the gastrointestinal (GI) tract, while colicin-negative strains lacked the competitive colonization advantage (196). The expression of colicins appears to be dependent on external factors, including during periods of iron limitation, as is observed during the GI inflammatory response, where *cib* expression becomes altered (197). The impact of intestinal inflammation and iron limitation on bacteriocin activity is likely due to enterobactin siderophore receptors on the surface of bacterial cells that can also serve as colicin Ib receptors (19, 198). Periods of iron limitation can lead to increased expression of siderophore receptors (also known as colicin receptors) by commensal organisms and to increased susceptibility to colicin Ib. In *Salmonella*, this competitive colonization advantage coupled with an increased ability to acquire iron from the environment helps facilitate *Salmonella* uptake into the intestinal epithelial cells (194). These potential contributions to virulence coupled with the apparent minimal metabolic costs of IncI1 plasmid carriage, especially in the presence of other plasmids, may explain why a high percentage of IncI1 plasmid-carrying isolates contain multiple large plasmids, including those of the IncA/C, IncHI2, IncFIB, and IncX replicon types (31, 45, 54).

Several IncI1 plasmids carry genes that encode DNA repair mechanisms following DNA damage due to UV light exposure and DNA-damaging compounds (199–201). The *imp* group mutation and protection (*imp*) operon contains three genes, *impA*, *impB*, and *impC*, that are functionally similar to the chromosomally encoded *umuCD* genes of the error-prone DNA repair system (201). DNA damage can initiate an SOS response in bacteria. Expression SOS response in genes is generally held in check by a LexA repressor binding to the SOS box in the *umuCD* promoter region (202, 203). During the SOS response, single-stranded (damaged) DNA initially interacts with and activates RecA, which then leads to autoproteolysis of the LexA repressor and expression of a cascade of genes, including *umuC* and *umuD*, due to derepression of the LexA repressor (202). Additionally, activated RecA triggers the autocleavage of UmuD to its active form associated with its error-prone DNA repair capabilities (204). The *imp* system has been best characterized in the IncI1 plasmid TP110 and is found in both R64 and Coll1-P9 (25, 199–201). When the IncI1 positive *Salmonella* strains characterized by Kaldhone et al. were assessed, 24/43 (56%) of the isolates contained the full *impCAB* operon, while 4 (9%) additional isolates carried all but the 3' end of the *impB* sequence, which was a similar phenomenon to that reported previously in *Shigella* (25, 31, 202, 205).

In the *impCAB* operon, *impC* overlaps the *impA* start codon by 2 nucleotides and *impA* overlaps the *impB* translation initiation sequence by 1 nucleotide (202). The *imp* promoter region, which is upstream of *impC*, contains the sequence of an SOS box, where a LexA repressor could potentially bind. Based on homology to UmuCD, ImpA and ImpB likely serve as an error-prone DNA polymerase (206). ImpC may function in a regulatory role for the expression of *impA* and *impB*, as it has homology to regulatory proteins, including DinI, which can inhibit the LexA and UmuD cleavage functions of RecA (207). *Shigella* strains that lost the *impCAB*-containing plasmid or had a mutated *impB* gene exhibited reduced ability to survive following UV irradiation compared to that of the wild-type strains, indicating the importance of these genes for UV resistance (202, 208–210). The process of error-prone DNA repair leads to increased rates of mutagenesis in the strains, potentially facilitating compensatory mutations associated with survival during high-stress periods (208). Some of the mutations may manifest as increased levels of resistance to certain clinically relevant antimicrobials, including the fluoroquinolones (208, 211, 212), and the ability to survive in the GI tract following exposure to bile salts, such as sodium deoxycholate, that can damage bacterial DNA (208, 210).

## CONCLUSIONS

This review focused on the genetic and phenotypic characterization of IncI1 plasmids.

Incl1 plasmids have garnered significant attention due to the carriage and dissemination of a wide range of antimicrobial resistance genes, including those encoding resistance to critically important antimicrobials such as the third-generation cephalosporins (37, 132, 133). The widespread carriage of ESBL- and AmpC  $\beta$ -lactamase-encoding genes is very problematic, as these genes in Incl1 plasmids have been isolated globally from a wide range of animal species and patients (37, 160). Also concerning is the fact that these plasmids have been shown to carry genes encoding resistance for several other antimicrobial agents, including aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and tetracyclines (28, 43, 185). This carriage of the wide variety of resistance genes is critical, especially in light of the ability of several Incl1 plasmids to conjugally transfer among different bacteria (21, 43). Additionally, many of the resistance genes are associated with IS elements, integrons, and transposons that contribute to their transmission among plasmids and the chromosome and other plasmids that are coresident within bacteria, leading to a further potential to spread. Because of the multiple transfer mechanisms carried on the plasmids, their host addiction systems, and their relatively low fitness costs, the Incl1 plasmids will likely remain a concern for the maintenance and dissemination of antimicrobial resistance transfer in the future.

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