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The role of conjugative transposons in the *Enterobacteriaceae*

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Abstract. Although widely studied in Gram-positive *Streptococci* and in the Gram-negative *Bacteroides*, there is a scarcity of information on the occurrence and nature of conjugative transposon-like elements in the well-studied *Enterobacteriacea*e. In fact, some of the major reviews on conjugative transposons prior to 1996 failed to mention their occurrence in this group. Recently, their presence has been reported in *Salmonella*, *Vibrio* and *Proteus* species, and in some cases such as the SXT element in *Vibrio* and the IncJ group element CTnR391, there has been some molecular characterization. The elements thus far examined appear to be larger than the common Gram-positive conjugative transposons and to be mosaic in structure, with genes derived from several sources. Recent evidence suggests that in the *Enterobacteriaceae* the elements may be related to enteric pathogenicity islands. The evolution, distribution and role of these elements in the *Enterobacteriaceae* is discussed.

Key words. CTnR391; SXT; CTn*scr-94*; enterobacterial conjugative transposons.

Introduction

Traditionally, plasmids have been observed as the major means of conjugative transfer in Gram-negative bacteria. Although conjugative transposons were initially discovered in gram-positive cocci in the late 1970s [1, 2], and although widely studied from both a genetic and clinical perspective, the first report of conjugative transposon-like elements in the *Enterobacteriaceae* only occurred relatively recently [3, 4]. There is, however, some debate as to whether the term 'conjugative transposon' should indeed be used when referring to such elements in the *Enterobacteriaceae*. The elements are mosaic in structure, possessing phage-like integrases and plasmid-like transfer sequences and could indeed be termed 'phagemids' [Lengeler, personal communication], although this would be to confuse them with genetically engineered cloning vectors. Those discovered thus far resemble Gram-positive conjugative transposons in certain properties; however, their preference for unique integration sites makes their integration more phage-like and unlike illegitimate transposon insertion. Some of these features have prompted the use of a new name to describe the elements, particularly in the case of the SXT element [6], which has been termed a CONSTIN (conjugative self-transmissible, integrating element). However, the designation CTn (conjugative transposon) will be used here as it is currently most widely accepted. Only three groups of conjugative transposons have thus far been identified in *Enterobacteriaceae*. Elements belonging to the incompatibility J group (the prototype IncJ element is CTnR391, formally R391) and thought to be unusual extrachromosomal plasmids [5] were in fact shown to possess all of the properties of conjugative transposons when transferred to *Escherichia coli* [3]. Since then two other types of elements, the CTn*scr94* element from *Salmonella senftenberg* [4] and the SXT element from *Vibrio cholera* 0139 [6], both capable of transfer to a range of Gram-negative bacteria, have been reported. Although the pool of conjugative transposon-like elements found in *Enterobacteriaceae* is currently small, there have been structural similarities observed between them and enteric pathogenicity islands. This review will concentrate on the known properties, structure and roles of these elements in *Enterobacteriaceae*.

The IncJ conjugative transposons

Discovery and origin

The global distribution of IncJ-like elements appears to be quite restricted, with possibly only four separate examples

being reported. They were initially discovered when 12 resistance factors from strains of *Proteus rettgeri,* which had previously been isolated from human faeces in South Africa during 1967, were transferred to *Escherichia coli* K12 [5]. Among these, three kanamycin resistance factors, designated R391, R392 and R397, coexisted stably with standard R-factors of all known plasmid incompatibilility groups. It was proposed to designate R391 as the prototype for a new group, IncJ.

Of 61 other South African strains [7] later examined, two contained transmissible kanamycin resistance factors and were designated R748 and R749, and classified as also belonging to the IncJ group. In a follow-up study of elements from *Proteus rettgeri* and *Providence* species originating from South Africa, Hedges discovered R705 and R706; but again, these were found to be identical to R391 in all testable properties. Incompatibilility among the prototypes R391, R392, R397, R748, R749, R705 and R706 could not be tested as they were identical in all testable properties such as kanamycin and mercury resistance, low transfer frequency [5], ultraviolet (UV) sensitization [8], *umuCD* complementation and pulsed field gel electrophoresis restriction patterns [9]. A Tn7 mutant of R391, designated R391-3 b-1, was used to confirm anomalous incompatibility where although some elimination occurred, inter-element recombination was also observed [10, 11]. Since all these isolates originated in the same hospital in South Africa, it was possible that all were in fact clones.

Other than the South African isolates, only three further examples of the group have been reported worldwide. A strain of *Vibrio cholera* encoding resistance to chloramphenicol, streptomycin and sulphonamide, which originated in the Philippines in 1977, was reported to harbour a conjugative element, pJY1, with IncJ properties [12]. However, this element has not been characterized further. In 1979, Matthew et al. in a study of types of β-lactamases associated with plasmids reported an R-factor, R997 from *Proteus mirabilis*, with IncJ properties [13]. The only other report of an IncJ element was pMERPH from *Pseudomonas putrefaciens* isolated from the River Mersey, Great Britain, and transferred to *Escherichia coli* [14] (table 1). Transfer of IncJ elements has been demonstrated to a number of enterobacterial species including *Escherichia coli* K12, *Salmonella typhimurium*, *Serratia marcessens*, *Proteus mirabilis* 5006, *Vibrio cholera* (El-Tor) and *Pseudomonas* [3, 5, 15–17].

The South African IncJ elements have been shown to be repressed for pilus synthesis, while in *E. coli,* CTnR997 has been shown to synthesise conjugative pili constitutively [15, 18], possibly accounting for its higher transfer frequency. Pilus-specific phage such as phage J (φJ), capable of forming hazy plaques on CTnR997-containing hosts, have been isolated. However they did not form plaques on CTnR391-containing hosts but formed hazy plaques on *E. coli* strains harbouring some IncC and IncD plasmids [18]. Indeed, pili of IncC and IncJ plasmids have been found to be serologically related [15].

Table 1. Properties of the IncJ elements.

IncJ element	Phenotype	Transfer frequency	Original host	Ref.
CTnR391	Hg^r Km^r	10^{-5}–10^{-7}	*Proteus rettgeri*	5
	RumAB, UV^s	10^{-2}–10^{-3}	*Proteus*	13
CTnR997	Ap^r *RumAB*, UV^s		*mirabilis*	
CTnpMERPH	Hg^r *RumAB*, UV^s	10^{-6}	*Pseudomonas putrefaciens*	14
pJY1	Cm^r Sm^r Su^r	10^{-5}	*Vibrio cholera*	12

Hg^r, mercury resistance; Km^r, kanamycin resistance; Ap^r, ampicillin resistance; Cm^r, chloramphenicol resistance; Sm^r, streptomycin resistance; Su^r, sulphonamide resistance Uv^s, UV sensitization.

There have been numerous reports of the inability to isolate extrachromosomal DNA from IncJ-containing hosts [7, 19, 20]. It was initially speculated that the difficulty in isolating CTnR391-specific DNA might be because of a DNase encoded by the element itself; however, there was little evidence to support this contention, and later a number of workers investigated the possibility that CTnR391 existed integrated into the host chromosome [19, 20, 21]. Using Hfr mapping techniques, it was demonstrated that CTnR391 inserted at a specific location on the chromosome mapping between the *uxuAB* and *serB* loci [11]. Furthermore, CTnR391 transfer and establishment in the recipient was shown to be independent of a functional homologous recombination system [3], as transconjugants to a range of *recA* mutants could be recovered at transfer frequencies comparable to wild-type strains, in the absence of detectable extrachromosomal DNA. Based on this evidence it was argued that CTnR391 encodes a site-specific recombination system and exhibits site-specific conjugative transposition in *E. coli* hosts [3], the first example of conjugative transposition by elements indigenous to the *Enterobacteriaceae*. The exact integration site of CTnR391 has been shown to occur within the 5′ end of the *prfC* gene, encoding a peptide release factor [22, 23, 35]. Inverse PCR analysis utilizing primers to the *prfC* gene has demonstrated that CTnR997 and CTnpMERPH also integrate into this gene [B. McGrath and J. T. Pembroke, unpublished]. Sequence analysis of the ends of CTnR391 in the integrated form indicated that integration restored a functional copy of the *prfC* gene, indicating that the gene or a partial copy is encoded within CTnR391 [22, 23, 35].

Evolutionary Divergence

The IncJ elements CTnR391 and CTnpMERPH encode mercury resistance determinants shown to be homolo-

gous by sequence analysis [24, 25]. Both CTnR391 and CTnpMERPH encode four putative mer genes (*merT, merP, merC* and *merA*) on the basis of homology to published *mer* sequences. Although CTnR391 contains a *merR* regulatory element, in the case of CTnpMERPH no putative regulatory genes could be identified adjacent to the structural genes, as with Tn*21*, even though genetic analysis of CTnpMERPH indicated that it must encode regulatory genes, suggesting their presence at another location on the element [24]. Phylogenetic analysis of the *merA* sequence indicated that the CTnpMERPH, and indeed its close relative CTnR391, were the most divergent Gram-negative *mer* determinants characterised. Because of the occurrence of all four structural genes, however, it was hypothesised that the CTnpMERPH *mer* determinant was a derivative of an ancestral Tn*21*-like determinant but considerably diverged, indicating an ancient history [24].

Other evidence of evolutionary divergence of resistance determinants associated with the IncJ group comes from a study of the β-lactamases encoded by CTnR997. Designated HMS-1, it was distinguished from other mobile-element-mediated β-lactamases by isoelectric focusing and its inability to cross-react with antiserum raised to TEM-1, the most common plasmid-mediated class of β-lactamase. Unlike the TEM class, it was susceptible to inhibition by *para*-chloromercuribenzoate and cloxacillin [13]. It was also reported that the level of enzymatic activity encoded by CTnR997 was less than chromosomally mediated β-lactamases (unlike other plasmid-mediated β-lactamases), yet the minimum inhibitory concentration for ampicillin or carbenicillin for CTnR997-containing strains was in excess of 1000 µg ml^{-1}, compared with 10 µg ml^{-1} for an R^- strain. It was suggested that the resistance associated with CTnR997 might also be due to a reduced uptake mechanism [13]. Sequence analysis of the kanamycin resistance determinant associated with CTnR391 and, indeed, the other kanamycin-resistant South African isolates has revealed the presence of an aminoglycoside-3-phosphotransferase with 100% homology to a previously characterised *Klebsiella* determinant. The determinant is surrounded by three copies of IS26 with 100% homology to IS26 isolated from *Proteus* [25, 26]. The lack of any sequence divergence is suggestive of a recent acquisition of this resistance determinant.

DNA repair functions

All IncJ group elements thus far examined encode an unusual UV-sensitising function in *E.coli* [8] that appears to be associated with the elements encoding functions that interfere with DNA repair. The sensitisation was found to be independent of photoreactivation and excision repair processes but dependent on recombination repair, as the sensitising function was eliminated in recombination deficient (*recA*) derivatives [8]. The exact mechanism appears to be the result of an interaction with the *ruv*C-mediated mechanism of Holliday junction resolution during recombination [27]. CTnR391 was also found to reduce recombination levels in crosses by Hfr strains of *E. coli* to F^- R391-containing strains, a phenomenon not due to entry exclusion [8]. Even though it appeared that the recombination reduction ability and the UV-sensitising functions were related, they were demonstrated to be separate functions by cloning [19, 21].

In addition to the unusual UV-sensitising function, like many mobile elements [28] CTnR391, CTnR997 and CTnpMERPH have been shown to encode a UV-protection and mutagenesis-enhancing function [16]. In the case of CTnR391 this function is associated with the presence of two genes, *rumA* and *rumB*, which encode functional homologues of the *E. coli UmuDC* mutagenesis-enhancing proteins [21] and the well-studied *MucAB* system associated with pKM101 used in the Ames mutagen tester strains [29]. *UmuDC* has been shown to encode a new type of polymerase termed DNA polymerase V that polymerizes in an error-prone manner past DNA damage without template assistance [30]. The CTnR391 *RumAB* proteins have also been shown to possess DNA polymerase V activity, although with different specificity of error-prone base insertion than the chromosomal homologue [31]. The ability of mobile elements to enhance mutagenesis and repair in stressed environments obviously has evolutionary and survival advantages and is quite widely distributed amongst mobile elements. Quite what the role of possessing two separate functions (one UV protecting and one UV sensitizing) might be remains to be elucidated; however, one possibility has emerged from sequencing of the CTnR391 element. Downstream from the *rumAB* genes we have located a cluster of genes with homology to DNA polymerase III subunits, one a homologue of an ε subunit that confers putative proofreading activity. Interaction of such a subunit with the *RumAB* polymerase and possibly the host's *UmuDC* polymerase could alter their error-prone repair capabilities, effectively sensitising a host expressing the activities [C. MacMahon and J. T. Pembroke, unpublished]. Indeed, this observed UV sensitisation might only be an artifact in *E.coli* since it evolved elsewhere where CTnR391-encoded subunits might interfere with the host's *umuDC*-encoded DNA polymerase V in an aberrant way to reduce its protection effect. Alternatively, possessing a function to modify mutagenesis capabilities could have the effect in certain highly DNA damaging environments, such as surfaces exposed to UV, of modifying the error-prone repair and mutation-generating system to prevent too much mutation from occurring and hence prevent the host from mutating itself away.

Extrachromosomal behaviour and structure of IncJ elements

Strains harbouring one IncJ element, such as CTnR997, in a *recA* background contain the element in an integrated form. When CTnR391 was transferred to such a strain it was observed that the incoming element existed in an extrachromosomal form, allowing the element to be isolated for the first time [9] and amenable to molecular characterization. It is at present unclear whether this extrachromosomal form is replicative or possibly a transfer intermediate generated by the higher quantities of integrase present in the strain when two IncJ elements are present. IncJ elements have been shown to undergo extensive inter-element recombination when they come in contact in recombination-proficient hosts [9]. This feature, which is associated with the occupation of a single integration site, may play an important role in spreading acquired sequences between elements. It has been noted that CTnR391 can mobilize chromosomal genes close to its integration site [20]. Transfer to a host already containing an IncJ element could easily lead to evolution of new elements by inter-element recombination and subsequent spread to new hosts. The presence of several repair-and-recombination-like genes associated with the elements may aid such recombination. In addition, the elements are extremely stable [3], such that the IncJ elements can survive thousands of generations without apparent loss of detectable markers or gross observable rearrangement as determined by PFGE [J. T. Pembroke and B. McGrath, unpublished]. Recently the IncJ element CTnR391 was completely sequenced [25]. Preliminary examination (fig. 1) of the sequence has revealed the element to be a mosaic with a phage λ-like integrase and a transfer system resembling that of the *Salmonella* plasmid R27. It contains some 96 ORFs (open reading frames) and modules associated with transfer, maintenance, integration, resistance determinants and a wider selection of DNA repair-associated homologues than previously thought [25].

Although associated with transfer of antibiotic resistance, it would appear that IncJ elements might not be as important in the dissemination of antibiotic resistance as their relatives in Gram-positive cocci. CTnpMERPH does not encode any antibiotic resistance determinants, while the determinant associated with CTnR391 would appear to have been recently acquired by transposition, possibly from *Klebsiella* or *Proteus*. The determinant on CTnR997 appears to be rather ancient, as judged from its mechanism, and not widely distributed. The elements other than CTnR997 have a low transfer frequency which would limit spread and hence fitness of such elements as transfer agents. The nature and specificity of the integration site of the IncJ elements and that of SXT into *prfC* [22, 23] may be a key factor in limiting host range, for although the gene is highly conserved, the sequence specificity of the integration would naturally limit their degree of spread. Sequence analysis suggests that the CTnR391 transfer genes are related to other Gram-negative enterobacterial sequences, a factor again limiting host range unlike the Tn*916* family in *Enterococci*.

CTn*scr-94*

In 1975 there was a report of transmissible sucrose-metabolizing ability associated with the so-called *scr-94* element from *Salmonella senftenberg* 5494-57 [32]. Unlike other such transmissible elements, no extrachromosomal DNA could be isolated from strains harboring *scr-94*. The element has been shown to transfer to *E. coli* and integrate in a *recA*-independent manner. Recent analysis of the *scr* gene, encoding a phosphoenolpyruvate-dependent sucrose:phosphotransferase system and a sucrose metabolic pathway, revealed that the genes were located on a conjugative transposon of 100 kb renamed CTn*scr-94* [4]. Field inversion gel electrophoresis (FIGE) analysis of *E. coli* strains containing CTn*scr-94* revealed integration in an orientation-specific manner at two preferential sites identified as the two copies of the *E. coli* phenylalanine-specific transfer RNA (tRNA) [4]. *pheV* maps at 67 mins, while *pheU*, which is identical, maps at 94 mins. Sequencing of both ends of the CTn*scr-94* element revealed

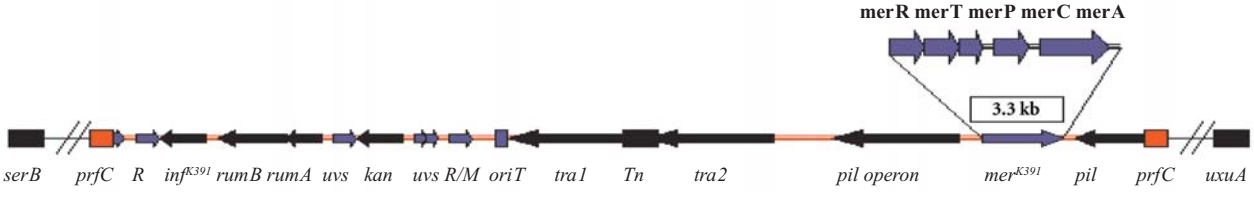

Figure 1. Outline structure of the conjugative transposon CTnR391. The element integrates into the *prfC* gene of *E. coli* located near the *serB uxuA* region at 92 min. Integration restores a functional copy of *prfC*. The element contains a phage-like integrase with a repressor located close by followed by a number of DNA repair-related genes including *rumAB* (homologues of *umuDC*) and several uncharacterized genes. The element contains a restriction modification system (R/M) and three clusters of transfer and pilus assembly genes interrupted by the mercury resistance operon (*mer*), which is highly homologous to that of CTnpMERPH. For convenience, not all of the genes identified are shown.

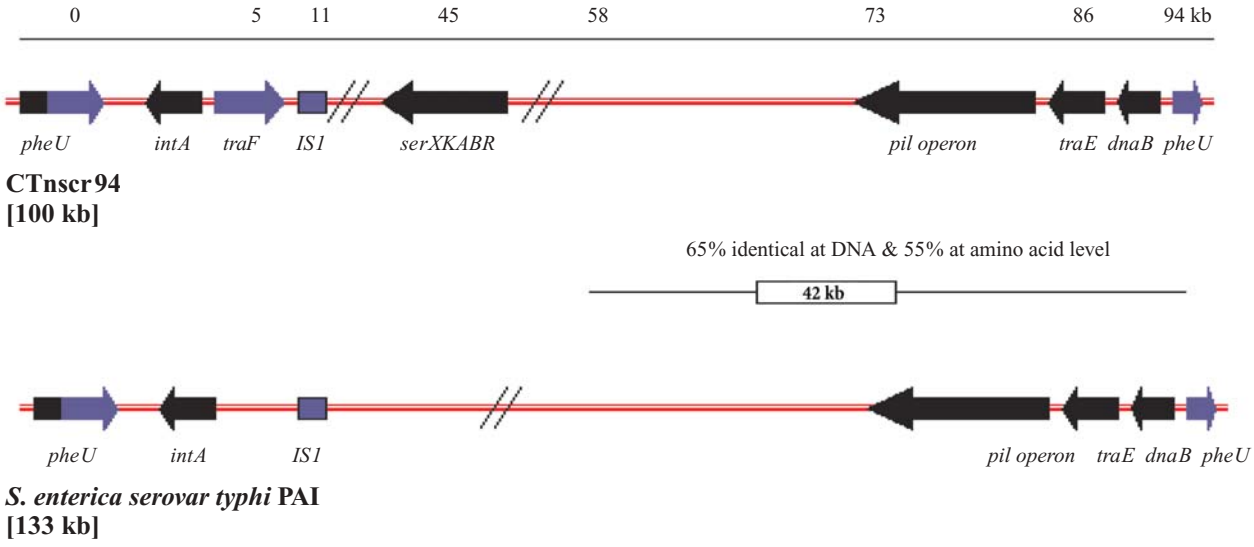

Figure 2. Structural comparison of the conjugative transposon CTn*scr-94* [4] and the 118-kb PAI island integrated into the *pheU* gene of *Salmonella enterica* serovar *typhi* [61]. Large portions of both elements are similar, both contain a type IVB *pil* operon [62, 63], *pilV* and a site specific recombinases *rci*. The analysis outlined above is based on data kindly provided by Joseph Lengeler, University of Osnabrück.

the presence of the 3′ portion of *pheV* on one end such that after integration, a complete *pheV* gene is retained; hence the orientation-specific integration is important in restoration of gene function. Although the element has not been completely sequenced, partial sequencing has revealed the presence of a phage λ-type integrase and plasmid transfer genes [J. Lengeler, personal communication]. Phenotypic analysis of CTn*scr-94* has failed to reveal antibiotic resistance determinants or pathogenic properties [4].

The ability to ferment sucrose is a variable phenotype, and hence it has been suggested that the *scr* genes belong to a collective gene pool called the collective chromosome [33]. Very few strains of *E. coli* or *Salmonella* can utilize sucrose; however, under favourable conditions, cells of these genera can acquire such genes from the collective pool by means such as the transfer of mobile elements carrying genes associated with the pool. Indeed, in support of this proposal plasmids such as pUR400 [34] and the conjugative transposon CTn*scr-94* have been isolated. It is an interesting concept that mobile genetic elements such as CTn*scr-94* evolved as a mechanism to shuttle the collective chromosome [4].

The SXT element of *Vibrio*

The SXT element was discovered as a conjugative transposon-like element encoding antibiotic resistance in *Vibrio cholera* 0139, the first non-01 serogroup to give rise to epidemic cholera [6]. The 62-kb element encoding resistance to sulphamethoxazole, trimethoprim, chloramphenicol and streptomycin was self-transmissible to *E. coli* and *Salmonella enterica*, where it integrated in a *recA* independent, site-specific manner [6, 22, 35]. Like the IncJ elements, no extrachromosomal replicative form could be detected. Sequence analysis of recombinant SXT-specific clones has revealed it to encode a λ-like integrase required for excision from the chromosome and circularisation by recombination between the right and left ends of the integrated element [22, 35]. The integration site in *Vibrio* has been shown to occur into the 5′ end of the *prfC* gene [22], which also encodes a protein chain release factor in *Vibrio*. Integration of SXT restores a functional *prfC* gene by possessing sequences that restore its N-terminus [22]. This mechanism appears to be identical to the integration of CTnR391 and other IncJ elements in *E. coli* [23, 35]. The gene transfer capacity of the SXT element goes beyond its self-transfer. In *E. coli* it has been shown to mobilise plasmids in trans and transfer chromosomal DNA in cis. Mobilisation of RSF1010 has been shown to occur in an *oriT*-independent manner in the absence of integrase [36]. This would appear to be similar to the F-integrating conjugative system, but unlike Tn*916* where the expression of the SXT transfer system is not dependent on its excision [37].

Since SXT integration resembles phage integration rather than transposition, it was felt that the term ‘conjugative transposon’ was not altogether informative in relation to the classification of SXT. It was proposed to call the element a CONSTIN (an acronym for conjugative, self-transmissible and integrating element) [22]. Polymerase chain reaction (PCR) primer pairs capable of amplifying *traC*, *B* and *F* orthologues of SXT were also capable of amplifying identical fragments in CTnR391, while Southern hybridisation revealed a similar organisation [35]. Sequence analysis of the SXT and CTnR391 integrase genes revealed 96% nucleotide sequence identity and 99.5% at the

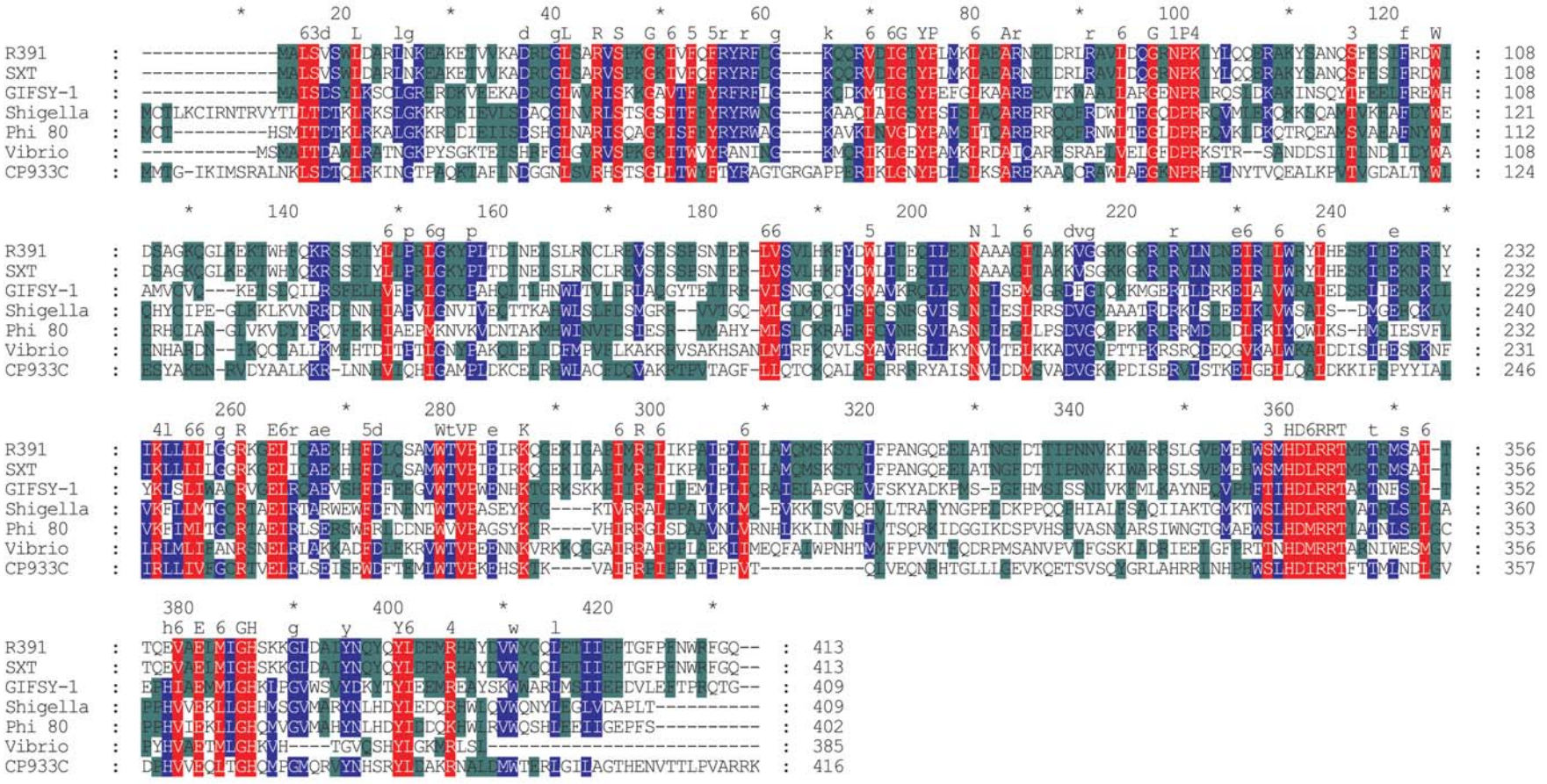

Figure 3. A Clustal alignment of the CTnR391 integrase compared with integrases from the *Vibrio* conjugative transposon SXT (Q9ZFJ7) Gifsy-1, prophage from *Salmonella* (O84888); *Shigella*, a defective *Shigella* prophage (9LA43); Phi 80, ϕ 80 integrase; *Vibrio*, a *V. cholera* integrase (Q9KMP2) located close to a *thr* tRNA gene; CP933C, prophage found in *E. coli* O157 (AAG55933). The alignment is displayed in GENEDOC using red for 100%, blue for 70% and green for 50% conservation. The SXT and R391 integrases are very similar, differing in only 3 *amino* acids out of 413. Based on previous analysis [60] there are two conserved λ integrase boxes with a conserved β-sheet intervening sequence between the boxes. The conserved H-R-Y sequence between 360 and 400 a is present in both CTnR391 and SXT integrases.

deduced amino acid level [35 and fig. 3]. When integrated into the chromosome, CTnR391 is flanked by a 17-bp repeat sequence associated with the core *att* site. This 17-bp *attR* sequence differs from the corresponding SXT *attR* by only one nucleotide, suggesting that the mechanism of integration is similar for both elements. Although incompatibility has not been tested, it may be that SXT is in fact an IncJ element.

Differences in the behaviour of SXT and R391

While undoubtedly highly related, there are differences between the SXT and CTnR391 elements. The elements are different in size, SXT reported as being 62 kb [6] (although this has recently been revised to 99.5 kb [Beaber et al., this issue], while CTnR391 is approximately 90 kb [11]. The elements encode different antibiotic resistances; however, this may only be a reflection of different selective pressures in different backgrounds and environments. There also appears to be a difference in the behaviour in the interaction of the elements. When the IncJ elements CTnR391 and CTnR997 are present in the same cell with CTnR997 integrated in a *recA* background, it has been demonstrated that a circular form of CTnR391 can be isolated [9]. At first sight these data might indicate that the CTnR391 element is capable of forming a replicative form; however, recent sequence analysis of the entire CTnR391 element has failed to reveal a *rep*-like function [25]. In contrast, when the SXT element and CTnR391 are present in the same *recA* background, chromosomal tandem arrays of SXT and CTnR391 are observed rather than an extrachromosomal form of either [35]. The observed ability to isolate an extrachromosomal form in a CTnR997/CTnR391 but not in an SXT/CTnR391 background could reflect differences in the sequence of the restored CTnR997 *prfC* gene, such that a site for CTnR391 integration is not restored and the element is capable of autonomous replication. The transfer rate of CTnR997 is relatively high in comparison with CTnR391 or SXT, which suggests yet another possibility. This increased rate of transfer may be associated with elevated expression of the CTnR997 integrase since transfer requires excision, such that in CTnR997/CTnR391 doubles (but not in SXT/CTnR391 doubles) the increased integrase expression may account for the ability to isolate an extrachromosomal, although nonreplicative form, of CTnR391. In support of this latter possibility it has been observed that overexpression of the CTn*scr-94* integrase results in enhanced recovery of an extrachromosomal form of this element [J. Lengeler, personal communication]. The integrases, which are key features of conjugative transposition, of SXT and CTnR391 when compared with other λ-like integrases show all the signatures of enterobacterial phage-like integrases (fig. 3).

Pathogenicity Islands, Virulence regions and the evolution of Conjugative Transposons

Like CTn*scr-94*, many genetic elements, such as pathogenicity islands (PAIs), appear to integrate into tRNA

Table 2. Some characteristics of the major pathogenicity islands found in the *Enterobacteriaceae* indicating their insertion locus where known, and phage or plasmid related sequences possessed.

PAI	Insertion site	Characteristics	Ref.
LEE *E. coli* strain RW1374 (O103:H2)	*pheV* tRNA gene at 67 min	80-kb, contains prophage sequences	45
E. coli O91:H-island	*selC* tRNA	33-kb, serine protease, *espI* and *iha* like adherence loci	46
Shigella resistance locus (SRL)	*serX* tRNA gene	66-kb, 22 prophage-related open reading feames, resistance to Sm, Ap, Cm, Tc.	47
Shigella She PAI	*pheV* or a *phe* tRNA gene		48
VPI PAI *V. cholera*		Embedded in CTX prophage	49
SHI-3 iron transport island *Shigella boydii*	between *lysU* and *pheU* tRNA gene	21-kb, aerobactin operon	50
E. coli PAI I-AL862,	tRNA (*pheR*)	63-kb, integrase and genes for sugar metabolism	51
Yersinia pestis (Yps) HPI	*asn* tRNA locus	35–45-kb P4-type integrase, siderophore yersiniabactin	52
Pseudomonas syringae HRP PAI	*leu* tRNA		53
Bacteroides fragilis BsPAI		contains two plasmid-like metaloprotease toxin and mobilization genes (*bfmA* and *bfmB* like *traD*)	54
E. coli (EPEC) adherence factor EAP PAI		69-kb EAF plasmid pB171	55
P. syringe PAI		154-kb plasmid borne contains IS100 from *Yersinia*	56
S. enterica SPI-3 PAI	*selC* tRNA	17-kb contains ToxR like regulatory protein of *V. cholerae*	57

genes. PAIs are chromosomal clusters of pathogen-specific virulence genes often found at tRNA loci (table 2). Their DNA sequences, particularly their GC content, often differ from the surrounding chromosomal DNA sequences, a fact that has been interpreted as being due to their mobility between genomes by horizontal gene transfer [38]. The use of tRNA genes as integration sites for a variety of phage and plasmids may favour interspecies gene transfer, as tRNA genes are particularly well conserved in bacteria. *E. coli* and other *Enterobacteriaceae* are highly adaptive bacterial species that are both members of the commensal intestinal flora and versatile pathogens associated with numerous types of intestinal and systemic infections in humans and other animals. The spectrum of diseases caused by *E. coli* in particular is due to acquisition of specific virulence genes harboured on mobile elements, or within PAIs that are absent from the genomes of commensal *E. coli* strains. PAIs are likely to have been transferred horizontally and may have integrated into the *E. coli* chromosome through bacteriophage, plasmid integration or conjugative transposition. The origin of conjugative transposition to tRNA genes may initially have evolved as bacteriophage picking up tRNA genes (or genes such as *prfC*) by transduction to aid rapid protein synthesis in infected hosts. Indeed, some bacteriophage such as T5 or the cholera phage ϕ149 carry their own tRNA genes [39, 40]. Transfer of such phage to diverse hosts would be aided by recombination with highly conserved tRNA genes catalysed by phage integrases. Many conjugative transposons carry integrase genes highly related to bacteriophage integrase genes. In fact, the IncJ, SXT and CTn*scr-94* elements all contain related λ-like integrases [41 and fig. 3], indicating a common evolution. The ability to spread not just by a phage-mediated mechanism but also by conjugation would have evolutionary advantage and could evolve by one of several mechanisms: integration of bacteriophage and plasmid at adjacent sites and acquisition of transfer genes by transduction, transfer of genes from plasmid to bacteriophage by insertion sequences or by bacteriophage integration into plasmids. Loss of function by bacteriophage could result in maintenance as a plasmid, as in the case of P4 [42]. The mosaic-like structure of the enteric conjugative transposons thus far characterised indicates that they may be considered as 'natural phagemids', while the structure of the PAIs thus far characterised in Gram negatives suggests an evolutionary history related to conjugative transposons.

The high-pathogenicity island (HPI) of virulent *Yersiniae* consists of a functional core encoding for biosynthesis and uptake of the siderophore, yersiniabactin. This *Yersinia* HPI has been shown to be widely distributed among different pathotypes of *E. coli*. The enteroaggregative *E. coli* (EAggEC) strain 17–2, the uropathogenic (UPEC) *E. coli* strain 536 and the probiotic *E. coli* DSM6601 contain the HPI associated with the *asn* tRNA gene [43]. Distribution of the HPI amongst the *Enterobacteriaceae* has been investigated [44]. Among some 67 isolates pertaining to 18 genera and 52 species tested, 9 (13.4%) harboured the island. These isolates were 3 *E. coli*, 1 *Citrobacter diversus* and 5 *Klebsiella* species (*Klebsiella pneumoniae, Klebsiella rhinoscleromatis, Klebsiella ozaenae, Klebsiella planticola and Klebsiella oxytoca*). As in *Yersinia* sp., all 9 isolates synthesized the HPI-encoded iron-repressible proteins HMWP1 and HMWP2. In the *K. oxytoca* strain, the right-end portion of the HPI was deleted, whereas the entire core region of the island was present in the 8 other enterobacterial strains analysed. In most of these isolates, the HPI was bordered by an *asn* tRNA locus, as in *Yersinia* spp. Thus, although HPI has not been shown to be mobile, its distribution is indicative of recent and widespread horizontal transmission.

Because of the small number of conjugative transposons characterised in the *Enterobacteriaceae*, direct comparison between them and PAIs has not been possible. Recently, however, nucleotide sequence comparisons [Lengeler, personal communication] between the 100-kb conjugative transposon CTn*scr-94* and the 118-kb *Salmonella enterica* serovar *typhimurium* PAI has revealed some striking homology (fig. 2). Both elements integrate into *pheV* tRNA genes, share structural homology and gene organisation, contain related integrase genes and possess very high homology amongst pilin and transfer genes.

There is considerable evidence that virulence determinants carried as PAIs may be transferred horizontally between bacteria of different genera and such movement could be mediated by bacteriophage, plasmids carrying bacteriophage genes or conjugative transposons, which insert into tRNA genes. In uropathogenic *E. coli* the virulence determinants are associated with a PAI which is inserted in tRNA genes and excised at high frequency [58, 59]. In *Dichelobacter nodosis*, the causative agent of ovine footrot, the virulence determinants are also housed on a PAI inserted into *ser* tRNA genes; it also contains an integrase gene with homology to *Shigella* Sf6 and *E. coli* P4 bacteriophage, while containing plasmid genes related to the *E. coli* F-plasmid [42]. Clearly, there are similarities in the structure and evolution of such elements and the conjugative transposons.

The complex and mosaic structure demonstrated by the small number of conjugative transposons thus far characterised in the *Enterobacteriaceae* suggests that we are perhaps just looking at the tip of the iceberg in terms of their abundance and distribution, their lack of discovery or citation perhaps being due to lack of rigorous searching or knowing what to look for. Many researchers working on antibiotic resistance determinants undoubtedly possess isolates which have been overlooked as containing 'integrating plasmids' or 'chromosomally transposed' resistance determinants which have not merited further inves-

tigation. Many of the elements may in fact be cryptic and form part of the collective chromosome [4]. The sequence data already available for CTnR391, CTn*scr-94* and SXT should allow the design of probes to investigate their abundance and distribution further. Recently, the nucleotide sequence of CTnR391 was completed and is available at the NCBI accession No. AY090559 [64].

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