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# Evolution of the IS*CR3* Group of IS*CR* Elements[▽]

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**The IS*CR* elements IS*CR3*, IS*CR4*, IS*CR5*, IS*CR14*, and IS*CR16* all share a percent G+C of 68 to 69%. They also share between 75% and 97% identity in their transposase open reading frames. Furthermore, with the exception of IS*CR5*, they are all found adjacent to sections of *groEL* that display the highest identity to the same gene from *Xanthomonas* spp. The combined information is consistent with the descent from an ancestral IS*CR* element in a *Xanthomonas*-like organism.**

IS*CR* elements are an unusual group of insertion sequences that have similarities to the IS*91* family in both structure and function (7, 8). At present there are 16 members of the IS*CR* family (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html), and all are found adjacent to genes that are not the normal complement of the host cell, the vast majority being antibiotic resistance genes (8). They are thus implicated in the acquisition of these genes by the host bacterium via plasmids. Previously we noted that IS*CR* elements vary in percent G+C from 54 to 69%, indicating different origins (8). We also noted that both IS*CR4* and IS*CR3* are found adjacent to partial *groEL* genes (8). Here we extend this analysis to new members of the IS*CR* family, i.e., IS*CR14* and IS*CR16*, and provide an explanation of the evolution of the IS*CR3* group of IS*CR* elements.

Searches of the EMBL databases at EMBL-EBI (using the FASTA protein similarity search at http://www.ebi.ac.uk/fasta33) with IS*CR* elements IS*CR5* and IS*CR3* revealed high identities with several recent additions to the database. These included identical sequences (Fig. 1a) found in the following two separate plasmids: pSN254 from *Salmonella enterica* serovar Newport (GenBank accession number CP000604) and pAPEC-01-R from avian pathogenic *Escherichia coli* (GenBank accession number DQ517526). This new IS*CR* element has been named IS*CR16*. A further sequence, that of IS*CR14* (Fig. 1b), has been found both in a panresistant *Pseudomonas aeruginosa* isolate from Brazil (GenBank accession number DQ914960) (2) and in a *Klebsiella pneumoniae* isolate (GenBank accession number EU269034). The genetic loci of these new IS*CR* elements together with the genetic loci of the closely related IS*CR* elements IS*CR3* and IS*CR4* are drawn for comparison, shown in Fig. 1.

Interestingly, *groEL* gene sections of various lengths have now been found upstream of four different IS*CR* elements (Fig. 1). While ISCR*16* has complete copies of *groEL* and *groES* immediately upstream, IS*CR14*, IS*CR4*, and IS*CR3* have 5′ truncated versions of the *groEL* gene which are missing 189 bp, 1,266 bp, and 1,287 bp, respectively. Furthermore, all *groEL* genes have the highest identity with the following various *Xanthomonas groEL* genes: 86.6% and 85.9% identity to *groEL* and *groES* genes over 1,700 bp from *Xanthomonas axonopodis* pv. citri and *Xanthomonas campestris* pv. citri for IS*CR16*, respectively; 86.9% and 86.7% identity over 1,450 bp to *groEL* genes from *Xanthomonas axonopodis* pv. citri and *Xanthomonas campestris* pv. vesicatoria for IS*CR14*, respectively; 92.6% and 92% identity over 160 bp to *groEL* from *Xanthomonas axonopodis* pv. citri and *Xanthomonas campestris* pv. citri for IS*CR4*, respectively; and 83.1% and 79.3% identity over 640 bp to *groEL* genes from *Xanthomonas campestris* pv. vesicatoria and *Xanthomonas campestris* pv. citri for IS*CR3*, respectively. Importantly, *groEL* genes are found only upstream of the IS*CR* transposase gene, adjacent to the terminus of each IS*CR* element. The other end of the IS*CR* element includes the *ori*IS sequence, and therefore, replicative transposition of each IS*CR* element, as shown in Fig. 1, proceeds from the right-hand (*ori*IS) end to the left-hand (*ter*IS) end of each element. IS*CR* elements function by initially transposing next to a target gene or section of DNA. In a second or subsequent transposition event(s), the adjacent gene or genes are cotransposed. For the related element IS*1294*, this cotransposition of adjacent DNA happens at approximately 10% of each transposition event (6) and the sequence cotransposed is always adjacent to the *ter*IS of the element. Where subsequent replicative transposition events mobilize larger sections at each movement event, they have the effect of accumulating a sequence of a different origin at the *ter*IS end of the element. Therefore, analysis of this sequence can provide a history of the movement of the IS*CR* element. For example, immediately upstream of IS*CR16* are complete *groES* and *groEL* genes that share most identity with the same genes from various *Xanthomonas* species. Further upstream is an *aacC* gene that is not in the form of a gene cassette, i.e., it is not of integron origin, as well as a small section of *qacEΔ1* (110 bp) and then an *aadA1* gene cassette, followed by an integrase gene (Fig. 1a). Therefore, the likely history of this element is that once it was in a position adjacent to *groEL* and *groES* genes in a *Xanthomonas*-like organism, a second transposition event moved the *groEL* and *groES* genes next to an *aacC* gene, a *qacEΔ1* gene, and subsequently into another integron adjacent to an *aadA1* gene, with each transposition event having the effect of accumulating additional DNA sequences. The final insertion adjacent to the *aadA1* gene is consistent both with replicative

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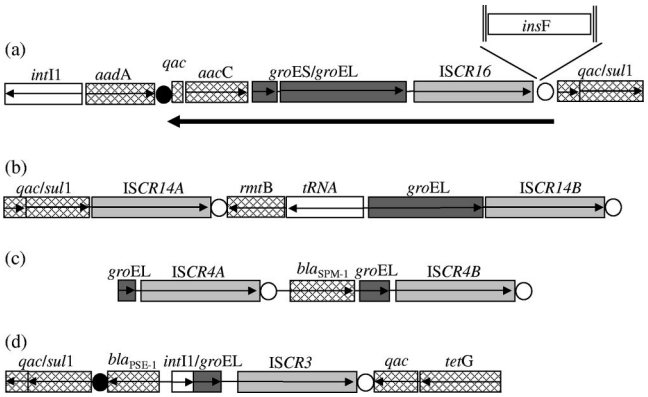



FIG. 1. Schematic of the genetic loci of IS*CR* elements found adjacent to *groEL* sequences: (a) IS*CR16*; (b) IS*CR14*; (c) IS*CR4*; (d) IS*CR3*. Open reading frames are depicted as open boxes with arrows indicating the direction of transcription. Open reading frames encoding resistance are hatched. *groEL* genes are dark gray and IS*CR* elements are light gray. Filled circles depict the 59 base elements of the various gene cassettes. The origin of replication of the various IS*CR* elements are shown as open circles and the inverted repeats found at the ends of the insertion sequence *ins*F are shown as vertical parallel lines. The large arrow underneath panel a shows the amount of DNA accumulated upstream of IS*CR16* due to subsequent replicative transposition events. The sequences of IS*CR14A* and IS*CR14B* are identical, as are the sequences of IS*CR4A* and IS*CR4B*.

transposition events as described above or homologous recombination as suggested previously (4). However, these elements can also transpose just their own DNA, as seen in Fig. 1b for IS*CR14A*, or a smaller section of DNA found adjacent to them, as can be seen in Fig. 1b, c, and d where truncated sections of *groEL* have been mobilized.

A phylogenetic tree based on an alignment of these IS*CR* transposase sequences with other known IS*CR* transposases using DNAStar software reveals some more-interesting observations regarding the possible evolution of these IS*CR* elements. The alignment shows that IS*CR3*, IS*CR4*, IS*CR5A&B*, IS*CR14*, and IS*CR16* are closely related, with identities ranging

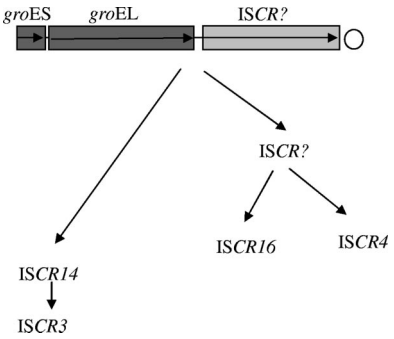
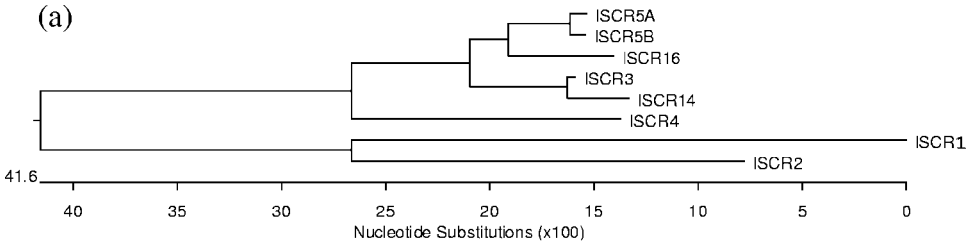



FIG. 3. Schematic of the hypothetical evolution of IS*CR3* group elements.

from 76% to 97%, while IS*CR1* and IS*CR2* are significantly different (Fig. 2a and b). Furthermore, while IS*CR3-5*, IS*CR14*, and IS*CR16* all share a percent G+C of 68% to 69%, IS*CR1* and IS*CR2* have a percent G+C of 54% and 59%, respectively (Fig. 2).

A further alignment of the sequence found between the stop codon of *groEL* and the start codon of the various IS*CR* transposases reveals that the IS*CR3*- and IS*CR14*-associated sequences are identical. Their transposases also share 96.7% identity, which suggests that IS*CR3* is a direct descendant of IS*CR14* or that they share a recent common ancestor. The sequences found between *groEL* and IS*CR4* and between *groEL* and IS*CR16* are both approximately 35 bp shorter than those of the respective sequences from IS*CR3* and IS*CR14* and are only 75% identical (Fig. 3). This suggests that IS*CR16* and IS*CR4* also have a common ancestor but that it is not as recent as IS*CR3* and IS*CR14*. The data therefore indicate that the IS*CR3* group of IS*CR* elements has originated from an ancestral IS*CR* element that was at one time found adjacent to a *groES-groEL* operon in a *Xanthomonas*-like organism. A hypothetical model of the evolution of the IS*CR3* group of IS*CR* elements is shown in Fig. 3.




(b)

| ISCR2 | ISCR3 | ISCR4 | ISCR5A | ISCR5B | ISCR14 | ISCR16 | |
|---|---|---|---|---|---|---|---|
| 64.6 | 53.1 | 51.5 | 52.8 | 52.8 | 52.7 | 53.2 | ISCR1 |
| | 56.3 | 56.9 | 56.3 | 56.5 | 56.7 | 56.7 | ISCR2 |
| | | 77.5 | 91.6 | 91.6 | 96.7 | 87.6 | ISCR3 |
| | | | 75.9 | 76.1 | 76.3 | 78.3 | ISCR4 |
| | | | | 98.4 | 88.8 | 91.9 | ISCR5A |
| | | | | | 89.0 | 91.6 | ISCR5B |
| | | | | | | 85.9 | ISCR14 |

(c)

| | G+C% |
|---|---|
| ISCR1 | 54% |
| ISCR2 | 59.6% |
| ISCR3 | 68.7% |
| ISCR4 | 69% |
| ISCR5A | 69.3% |
| ISCR5B | 69.2% |
| ISCR14 | 68.4 |
| ISCR16 | 69.2 |

FIG. 2. Comparison of IS*CR* elements. (a) Phylogenetic tree of IS*CR3* group elements with IS*CR1* and IS*CR2*. (b) Sequence identity comparisons, based on a Clustal alignment with the PAM250 matrix prepared using Lasergene DNAStar software. Alignments were undertaken on the available complete IS*CR* transposase sequences. (c) Percent G+C comparisons of the DNA sequences of various complete IS*CR* elements.

The sequences of the IS*CR3* group of IS*CR* elements have diverged nearly 25% since their original ancestor adjacent to *groEL*. This is also mirrored by a similar divergence in sequence of the associated *groEL* genes of between 74 and 98% (data not shown). The fact that IS*CR14* and IS*CR4* were discovered in the same *Pseudomonas* isolate (3, 9) suggests that homologous recombination plays a role in the divergence of IS*CR* and *groEL* sequences; this is especially due to the fact that *groEL* sequences are well conserved.

Finally, it is interesting to note that *groEL* sequences show the highest identity to *Xanthomonas* spp. often of the pathovar citri, a fruit pathogen. IS*CR16* was found on plasmids in an avian pathogenic *E. coli* isolate from a turkey in Iowa (5) as well as a similar plasmid in *Salmonella enterica* serovar Newport (10). IS*CR3* is found in *Salmonella enterica* phage type DT104 and several other *Salmonella enterica* pathovars. IS*CR14* and IS*CR4* have been found in a panresistant strain of *P. aeruginosa* causing serious infection control problems in Brazil (1, 3). Thus, it appears that the IS*CR3* group of IS*CR* elements is mobilizing genes from environmental organisms to clinically relevant pathogens. One possible route could be small birds feeding both on fruit and in turkey sheds and then from *Salmonella* strains of avian origin into prevalent human-pathogenic *Salmonella* species such as *Salmonella enterica* phage type DT104.

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