

NOTE

Transposition of IntAs into the Conserved Regions of IS3 Family Elements

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Together with the previous reports, my computer survey revealed that several bacteria contain six copies of the type group II intron IntA. The sequence analysis of IntAs showed the high level of homology in the nucleotide sequence (91.9~99.8%). The consensus sequence, 2,270 base pair long, was derived from the nucleotide sequences of all IntA members. The size of the open reading frame *intA* was 502 amino acids long, that is homologous to reverse transcriptase-like proteins encoded within the group II introns. It was reported that EPEC.IntA and Sf.IntA were inserted into IS911 and IS629, respectively. The sequence of the flanking region IntA was analyzed here. The data show the insertion of EC.IntA into IS629, the insertion of EHEC.IntA into IS3, the insertion of Yp.IntA into IS904-like sequence, and the insertion of EK12.IntA into IS911. Interestingly, these IS elements nested by IntAs were the members of IS3 family elements. The sequences of the IS3 members correspond to the OrfB with the DDE motif conserved in retroviral integrases. Alignment of the flanking sequences of IntAs revealed that the flanking regions -25 to +10 of insertion sites, that are generally believed to be required for the retrohoming, were not strongly conserved. The data presented here suggests that the retrohoming pathway of IntA seems to differ from those of other group II introns.

Key words: group II intron, IntAs, IS3 family, target-site specificity

Group II introns are mobile genetic elements in fungi, plant, and bacteria (Belfort *et al.*, 1995; Michel and Ferat, 1995). Bacterial group II introns have been found in proteobacteria (*Azotovacter vinelandii*, *Escherichia coli*, *Sinorhizobium meliloti*, *Shigella flexneri*, and *Yersinia pestis*), cyanobacteria (*Calothrix* sp.), and gram-positive bacteria (*Bacillus halodurans* C-125, *Lactococcus lactis*, and *Clostridium difficile*) (Ferat and Michel, 1993; Ferat *et al.*, 1994; Knoop and Brennicke, 1994; Amemura *et al.*, 1995; Mills *et al.*, 1996; Mullany *et al.*, 1996; Shearman *et al.*, 1996; Rajakumar *et al.*, 1997; Hu *et al.*, 1998; Lindler *et al.*, 1998; Martinez-Abarca *et al.*, 1998; Tobe *et al.*, 1999; Takami *et al.*, 2001). Bacterial group II introns are believed to be disseminated by mobile genetic elements (Ferat *et al.*, 1994). IntA and IntB are inserted at two distinct sites in IS677 (=H-repeat) and IntD is located within IS629 (=IS3411) (Ferat *et al.*, 1994). RmInt1 is inserted within ISRm2011-2 (Martinez-Abarca *et al.*, 1998). IntA and EPEC.IntA are inserted into IS629 and IS911, respectively (Rajakumar *et al.*, 1997; Tobe *et al.*, 1999). IntC is

inserted into IS679 (Han *et al.*, 2001). The group II intron found in *C. difficile* was found in the conjugative transposon, Tn5397 (Mullany *et al.*, 1996). Similarly, the lactococcal intron, LI.LtrB, was detected within a transfer gene of conjugative elements (Shearman *et al.*, 1995; Mills *et al.*, 1996). This study focused on a type of group II intron that appears to be present in several bacteria by collecting the members and analyzing their flanking sequences.

The programs, FASTA (Pearson and Lipman, 1988) and BLAST (Altschul *et al.*, 1990), were used for the homology search of the nucleotide sequences in the DDBJ/GenBank/EMBL databases. Multiple sequences were aligned using the program CLUSTAL W version 1.7 (Thompson *et al.*, 1994). The nucleotide sequences were analyzed with the HarrPlot 2.0 and GENETYX-Mac 10.1 system (Software Development Co, Japan).

A family of the group II intron seems to be present in several bacteria (Table 1). Sf.IntA is 2,272 bp in length identified from *S. flexneri*. The *sfiA* gene encodes a putative protein exhibiting the high level of similarity to reverse transcriptase-like proteins encoded within the introns of fungi (GenBank accession no. X55026, U41288, and X57546), plants (GenBank accession no.

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Table 1. Target IS elements of IntAs

IntAs	Co-ordinates to referred accession no.	Target IS (IS3 family)	Organism	Referred accession no.
EC.IntA	1~1590	IS629	<i>E. coli</i> C	D37918
EHEC.IntA	65789~65864	IS3	EHEC plasmid pO157	AB011549
EK12.IntA	7985~9748	IS911	<i>E. coli</i> K-12	AE000133
EPEC.IntA	48555~50824	IS911	EPEC EAF plasmid	AB024946
Sf.IntA	C 519~2787	IS629	<i>S. flexneri</i> SBA1336	U97489
Yp.IntA	54417~55559	IS904-like sequece	<i>Y. pestis</i> plasmid pMT-1	AF074611

Position begin with C, represents Sf.IntA in minus orientation.

EPEC, Enteropathogenic *Escherichia coli*; EAF, adherent factor; EHEC, Enterohemorrhagic *Escherichia coli*; Y., *Yersinia*; S., *Shigella*.

M68929), and bacteria (GenBank accession no. U7795, U50902, X98606, and X71404) (Rajakumar *et al.*, 1997). EPEC.IntA, 2,270 bp in length, is identified from enteropathogenic *E. coli* (EPEC) (Tobe *et al.*, 1999). *E. coli* K-12 chromosome contains a group II intron truncated at the right end region (Rajakumar *et al.*, 1997; unpublished data). This was termed as group II intron EK12.IntA. EK12.IntA is truncated by IS5-mediated deletion (unpublished data). Two group II introns were identified: *S. flexneri* reverse transcriptase (RT) and a RT-like protein. The former was identified from *E. coli* C. The latter was identified from *Y. pestis* (Amemura *et al.*, 1995; Hu *et al.*, 1998; Lindler *et al.*, 1998). These elements were termed as EC.IntA and Yp.IntA, respectively. Using these group II intron-sequences as queries, a new copy was identified from enterohemorrhagic *E. coli* (EHEC) O157:H7 by the computer-aided homology search. Thus, this element was termed EHEC.IntA which was identified from EHEC O157:H7 (accession no. AB011549 and AF 074613).

Although these group II introns were present at several bacteria, the sequences of group II introns had the high level of nucleotide identity (91.9~99.8%) over their entire length to one another. I described these group II introns as IntAs. With the exception of EPEC.IntA and Sf.IntA, other IntAs were truncated at the either side of the end region.

Sf.IntA is 2,272 bp in length and has 431 amino acids (Rajakumar *et al.*, 1997). However, the aligned sequences generated the 2,270-bp consensus sequence from IntAs (data not shown). This consensus sequence contains the open reading frame (ORF) homologous to reverse transcriptase. The size of ORF named *intA* was 502 amino acids. Compared with the 2,270-bp consensus sequence, I found two base insertions in the entire Sf.IntA. One in the ORF of Sf.IntA resulted in the frameshifting that introduces a stop codon. Thus, the *sfiA* (431 amino acids) of Sf.IntA was shorter than *intA* (502 amino acids) of the consensus sequences of IntAs (data not shown). Yp.IntA showed the 92.0% homology to the consensus sequence of IntAs. Comparing with the consensus sequence of IntAs, Yp.IntA contained the deletion of a 16-bp sequence

in the ORF and showed 8% nt substitution (data not shown).

The flanking sequences of three members of IntAs have previously been reported. Sf.IntA has been reported to be inserted into the intact IS629 element (Rajakumar *et al.*, 1997). Either EK12.IntA or EPEC.IntA has been reported to be inserted into IS911 (Tobe *et al.*, 1999; unpublished data). In addition, I identified and characterized both flanking sequence of other IntAs. Computer survey revealed that EHEC.IntA and EC.IntA were inserted into IS3 and IS629, respectively. In the case of Yp.IntA, the flanking sequence of Yp.IntA was found to be the IS-like sequence. IS904 (Rauch *et al.*, 1990) was most homologous to the IS-like sequence nested by Yp.IntA. The end region of both sides, however, can not be characterized because the IS-like sequence was severely truncated. Thus, I called this IS-like sequence IS904-like sequence.

Unlike other types of group II introns, the result of the analysis of the flanking sequences of IntAs revealed that IntAs were present in several bacterial and inserted into various IS elements such as IS3, IS629, and IS911 (Timmerman and Tu, 1985; Matsutani and Ohtsubo, 1990; Prère *et al.*, 1990). Interestingly, these IS elements nested by IntAs belong to the IS3 family. The IS3 family is the largest group characterized so far, which includes 28 IS elements such as IS3, IS629, IS904, and IS911 (Ohtsubo and Sekine, 1996). They are present not only in gram-negative bacteria but also in gram-positive bacteria (Ohtsubo and Sekine, 1996). They greatly differ one another in the nucleotide sequence, but almost all members of this group have two open reading frames corresponding to those in IS3 (Ohtsubo and Sekine, 1996). Although the predicted amino acid sequences of the ORFAs show little homology, those of ORFBs are significantly related (Schwartz *et al.*, 1988; Fayet *et al.*, 1990; Prère *et al.*, 1990). Comparison of the nucleotide sequences of the IS elements nested by IntAs revealed that IntAs was inserted into the ORFB of IS3 family. Moreover, the regions of IS elements flanking IntAs were found to correspond to the conserved region of the ORFB of IS3 family (Fig. 1A), which correspond to the integrase domain shared by ret-

A

IS3	ACCAGAAGTGGGCGGGAGACATCACGTATT	EHEC.IntA	////////////////
IS629	////////////////	EC.IntA	TCAGCACATGGCGGGGCTTC
IS629	ACCAGCTGTGGGTGGCTGATTTTACTTACG	Sf.IntA	TCAGCACATGGCAGGGCTTC
IS904-like seq	ACCAGGTGTGGGTGAGCGATATCACCTATC	Yp.IntA	////////////////
IS911	ATCAGGTGTGGTGCGGTGATGTGACCTATA	EK12.IntA	////////////////
IS911	ATCAGGTGTGGTGCGGTGATGTGACCTATA	EPEC.IntA	TCTGGACGGGTAAGCGCTGG

B

	-30	-20	-10	-1		+1	+10	+20
IS3	ACCAGAAGTGGGCGGGAGACATCACGTACT	EHEC.IntA	TACGTACAGATGAAGGCTGG					
IS629	ACCAGCTGTGGGTGGCTGATTTTACTTACG	EC. & Sf.IntA	TCAGCACATGGCAGGGCTTC					
IS904-like seq	ACCAGGTGTGGGTGAGCGATATCACCTATC	Yp.IntA	////////////////					
IS911	ATCAGGTGTGGTGCGGTGATGTGACCTATA	EK12. & EPEC.IntA	TCTGGACGGGTAAGCGCTGG					
Consensus seq	A CAG GTGG	GA T AC TA	T G AC A GCT					

Fig. 1. DNA sequences of IS elements corresponding to the 5' and 3' junctions of IntAs. (A) Target regions of IS elements flanking IntAs, which were identified from databases. (B) Original regions of IS elements flanking IntAs. Both IS elements and IntAs were in plus orientation. The truncated regions were indicated by slashes. The sequences of IS3 family members flanking IntAs correspond to *OrfB* that has a DDE (Aspartic acid, Aspartic acid, Glutamic acid) motif conserved in retroviral integrases. An acidic amino acid triad is involved in catalysis, and its role is presumably in coordinating divalent metal cations. Nucleotides shown in bold type correspond to the first D of the DDE motif. The first nucleotides from 5' and 3' junctions indicate -1 and +1 positions, respectively. The positions of flanking sequences of IntAs are shown at the top in Arabic numerals. The underlined nucleotide corresponds to the position at the top.

roelements (Fayet *et al.*, 1990; Khan *et al.*, 1991; Kulko-sky *et al.*, 1992; Rezsöházy *et al.*, 1993).

Although the regions of IS3 family where six IntAs were inserted, were conserved, the sequences of the target regions differed. Alignment of flanking regions -13 to +1 of the target sites of IntAs showed eight positions (-13, -12, -9, -7, -6, -4, -3, and +1) with the common nucleotide sequence (Fig. 1B). This region was considered intron binding site 1 (IBS1) and intron binding site 2 (IBS2) that are complementary to the intron sequence exon binding site 1 (EBS1) and exon binding site 2 (EBS2) located in the region of domain I of the intron RNA (Michel *et al.*, 1989; Ferat and Michel, 1993; Michel and Ferat, 1995). Recently, three group II introns have been investigated extensively to characterize the mechanism of a group II intron retrohoming. In the case of a bacterial group II intron which is identified from a conjugative relaxase gene, *ltrB* (Mills *et al.*, 1996), L1.ltrB, intron RNA base pairs with positions -13 to +1 of the DNA homing site (Mills *et al.*, 1996; Matsuura *et al.*, 1997). The flanking region -25 to -13 of the insertion site and +2 to +10 of the insertion site were not strongly conserved, indicating seven positions (-23, -22, -21, -20, +4, +6, and +7) with common nucleotide sequences (Fig. 1B). In the case of L1.ltrB, the flanking regions, -25 to -13 of the insertion site and +2 to +10 of the insertion site, are recognized by the LtrA protein (Cousineau *et al.*, 1998). A single nucleotide change in IBS1 of the target DNA was sufficient to almost completely block the DNA endonuclease and reverse splicing activity (Matsuura *et al.*, 1997). The RNP recognition span from -25 to +10 corresponds to that need for retrohoming (Cousineau *et al.*, 1998). My work suggests that the retrohoming pathway of IntAs differs from

those of other group II introns.

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