IS902, an insertion element of the chronic-enteritis-causing *Mycobacterium avium* subsp. *silvaticum*

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An insertion sequence element of *Mycobacterium avium* subsp. *silvaticum* was isolated and its complete nucleotide sequence determined. IS902 is 1470 bp in size and is repeated 10–12 times per genome. An open reading frame of 1200 bp was identified, encoding a protein product of *M*, 43932. This protein is highly similar to the predicted proteins of IS900 of *Mycobacterium paratuberculosis*, IS116 of *Streptomyces clavuligerus* and IS110 of *Streptomyces coelicolor*. IS902 lacks terminal inverted repeats and flanking direct repeats but displays insertion site specificity.

Introduction

IS900, identified in a Crohn's disease isolate of *Mycobacterium paratuberculosis*, was the first mycobacterial insertion sequence (IS) element to be reported and characterized (Green *et al.*, 1989). IS900 showed homology to the previously identified IS110 of *Streptomyces coelicolor* (Bruton & Chater, 1987) and more recently to IS116 of *Streptomyces clavuligerus* (Leskiw *et al.*, 1990). Together, these elements appear to constitute a family of related IS elements and are distinct from the IS elements and IS-like repetitive DNA sequences identified in *Mycobacterium tuberculosis* (Eisenach *et al.*, 1988; Hermans *et al.*, 1990; Thierry *et al.*, 1990), *Mycobacterium leprae* (Clark-Curtiss & Docherty, 1989) and *Mycobacterium fortuitum* (Martin *et al.*, 1990).

When used as a probe, IS900 identified restriction fragment length polymorphisms (RFLPs) able to distinguish between mycobacterial species (McFadden *et al.*, 1987b; Hampson *et al.*, 1989). On the basis of their respective RFLP patterns, *M. paratuberculosis* and the closely related *Mycobacterium avium* could be differentiated with considerable ease. When hybridized with IS900, a woodpigeon strain of *M. avium* [recently designated *M. avium* subsp. *silvaticum* by Thorel *et al.* (1990)] produced a unique multi-banded RFLP pattern

(McFadden *et al.*, 1988), suggesting the presence of an IS900-related IS element within the genome of this organism.

Mycobacterium paratuberculosis causes chronic enteritis in cattle and other animals (Chiodini *et al.*, 1984a) and growing evidence points to the involvement of this organism in Crohn's disease in humans (Chiodini *et al.*, 1984b, c; McFadden *et al.*, 1987a; Sanderson *et al.*, 1991). *M. avium* subsp. *silvaticum* can also cause chronic enteritis in animals (Matthews & McDiarmid, 1979) and preliminary work (M. T. Moss, unpublished results) suggests that this organism may also be involved in Crohn's disease.

In this paper we report that IS902, the IS900-related element from *M. avium* subsp. *silvaticum*, has the characteristic features of the previously proposed family of unique IS elements (Green *et al.*, 1989; Leskiw *et al.*, 1990). Target sites for IS902 insertion have been identified and their comparison with the insertion sites of IS900 and IS116 revealed conserved motifs. The amino acid sequences encoded by these elements were similarly observed to show a high degree of homology.

Methods

Mycobacterial isolates. Table 1 lists the isolates examined in this study, together with details of their respective sources.

Preparation of mycobacterial DNA. Mycobacterial cells were grown in Dubos medium containing 2 µg mycobactin J ml⁻¹ (Rhône-Mérieux) for 3–4 weeks at 37 °C. Cells were harvested by centrifugation at 5000 r.p.m. for 15 min, then lysed according to the method described

Abbreviation: RFLP, restriction fragment length polymorphism.

The nucleotide sequence data reported in this paper have been submitted to EMBL and have been assigned the accession number X58030.

Table 1. *Isolates of Mycobacterium avium subsp. silvaticum used in this study*

Isolate	Animal source	RFLP type†
0016*	Deer	1
0109*	Goat	2
0011*	Woodpigeon	2
0013*	Woodpigeon	2
0009†	Woodpigeon	3
0010†	Woodpigeon	3
0012†	Woodpigeon	3
0015†	Woodpigeon	3
0020†	Woodpigeon	3
0108†	Woodpigeon	3

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‡ This study.

by Visuvanathan *et al.* (1989). Following two phenol/chloroform extractions, genomic DNA was recovered by ethanol precipitation.

Genomic library production. *M. avium* subsp. *silvaticum* genomic DNA (isolate 0016) was digested for 1 h at 37 °C with 1 unit of *Bam*HI per µg of total DNA. DNA fragments were fractionated by electrophoresis on a 1% (w/v) agarose gel. The 1–5 kb fragments were isolated from the gel using a GeneClean kit as described by the supplier (Bio 101), then ligated into the *Bam*HI site of pUC18. Recombinant plasmids were cloned into the *Escherichia coli* DH5 (Gibco BRL) using standard techniques.

DNA probes and genomic library screening. A 1.5 kb *Nru*I fragment of pMB22 (Green *et al.*, 1989) containing the entire IS900 element was labelled with [α -³²P]dCTP (ICN Biomedicals) by the random hexanucleotide priming technique of Feinberg & Vogelstein (1984). Colony lifts and Southern blots (Southern, 1975) were hybridized with this radiolabelled probe. Hybridizations were carried out at 65 °C overnight, as described previously (Moss *et al.*, 1991). Membranes were washed at 65 °C in 3 × SSC, 0.1% SDS for 2 × 30 min, prior to autoradiography (1 × SSC is 0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0).

Sequencing strategy. Two clones, pZM22 and pZM25, were subjected to double-stranded DNA sequencing by the chain-terminating method (Sanger *et al.*, 1977) using Sequenase (USB), and 10% (v/v) DMSO. In each sequencing reaction, 10 ng of primer was used with 1 µg of linearized plasmid DNA. The reaction mixes were electrophoresed through 6% and 8% (w/v) acrylamide gels, in the presence or absence of 40% (v/v) formamide. Sequencing data were initiated from sequencing primers F and R, which flank the multi-cloning region of pUC18. Primer F (5'-AACAGCTATGACCATG) is equivalent to the M13 reverse primer and primer R (5'-GTAAAACGACGGCCAGT) is the M13 'universal' primer. New sequencing primers were selected from the newly determined sequences, chemically synthesized (Oswel DNA Services) and used to extend the DNA sequences in the direction 5' to 3'. Using this strategy, overlapping sequences were generated, extending along both strands of the two clones.

Computer analysis of nucleotide and predicted amino acid sequences. Sequence data were assembled and analysed on a PC AT using Staden-Plus software (Amersham). Codon usage analysis (Staden & McLachlan, 1982) and comparison with IS900 was used to determine the open reading frame (ORF) within IS902. The CLUSTAL program (Higgins &

Sharp, 1988) [running via SEQNET at SERC Daresbury] was employed for the comparison and alignment of the nucleotide sequence and predicted protein sequence of IS902 with the ORFs of IS900 (*M. paratuberculosis*), IS116 (*Streptomyces clavuligerus*) and IS110 (*Streptomyces coelicolor* A3(2)).

Results

Southern blots of *Bam*HI-digested *M. avium* subsp. *silvaticum* isolates (Table 1) were hybridized with radiolabelled IS900 (see Methods). Multiple banding patterns were observed (data not shown), confirming the existence of multiple copies of an IS900-related insertion sequence (IS) element within the genome of the *M. avium* subsp. *silvaticum* isolates. This element has been designated IS902. The multiple banding patterns differentiated the 10 isolates into three RFLP types. One isolate exhibited the RFLP type 1 pattern, three isolates exhibited the RFLP type 2 pattern and six isolates the RFLP type 3 pattern (Table 1). Fig. 1(a) shows one example of each of the three RFLP type patterns. The varying intensities of the hybridizing bands are due to the presence of doublets and/or bands carrying more than one copy of the element.

A genomic library was constructed with DNA from isolate 0016. Colony lifts of about 4000 recombinant clones were screened with radiolabelled IS900 (see Methods) and two clones, pZM22 and pZM25, hybridizing strongly with this probe were recovered. Characterization by restriction mapping and Southern blotting (data not shown) confirmed that the two clones were unique isolates, each containing a complete copy of IS902. The restriction site profile of pZM22 is shown in Fig. 2. The restriction site profiles of both clones only differed within the genomic DNA flanking their respective copies of IS902.

The 2.3 kb insert of clone pZM22 and the 2.5 kb insert of clone pZM25 produced identical multiple banding patterns when used to probe Southern blots of the 10 *M. avium* subsp. *silvaticum* isolates (data not shown). The three RFLP patterns identified with these two copies of IS902 were virtually identical (a solitary band did not hybridize) to the patterns obtained when the isolates were probed with radiolabelled IS900 (Fig. 1a). The missing band (identified by arrowheads in Fig. 1a) may represent a single copy of a second IS element present in the genome of these isolates which shows more homology to IS900 than to IS902. This element was identified in all 10 *M. avium* subsp. *silvaticum* isolates (data not shown). Fig. 1(b) records the results of probing the Southern blot shown in Fig. 1(a) with the radiolabelled insert of clone pZM22. With IS902 containing no internal *Bam*HI sites the hybridization results suggest the presence of 10–12 copies of the IS902 element within the *M. avium* subsp.

Fig. 1. Southern blots of *Bam*HI-digested *M. avium* subsp. *silvaticum* isolates. One isolate was chosen to represent each of the three RFLP patterns identified. (a) The three isolates hybridized with radiolabelled IS900; (b) the blot was reprobbed with the radiolabelled 2.3 kb *Bam*HI insert of pZM22. Lanes: 1, *Hind*III digest of λ DNA; 2, isolate 0016 (RFLP type 1); 3, isolate 0013 (RFLP type 2); 4, isolate 0009 (RFLP type 3). The arrowheads in (a) indicate a second IS900-related IS element.

silvaticum genome. IS900 is repeated 15–20 times in the *M. paratuberculosis* genome.

Clones pZM22 and pZM25 were each sequenced in both directions and in a similar manner. Fig. 2 outlines the strategy used to sequence IS902 in clone pZM22. The complete nucleotide sequence of IS902 is shown in Fig. 3. The element is 1470 bp in length, with an overall G + C content of 62 mol%, the same as the G + C composition of the host genomic DNA which flanks the element. Homologies were observed with IS900 of *M. paratuberculosis* (Green *et al.*, 1989), IS116 of *S. clavuligerus* (Leskiw *et al.*, 1990) and IS110 of *S. coelicolor* (Bruton & Chater, 1987). DIAGON plots comparing these sequences identified the 3' end as the region showing the greatest homology between the four IS elements (data not shown). Weak homology was also observed with IS1000 from *Thermus thermophilus* (Ashby & Bergquist, 1990). Comparison (1) of the sequence of the two copies of IS902 for their points of divergence and (2) with the endpoints and putative insertion site sequence of IS900 allowed identification of the 5' and 3' termini of IS902.

Analysis of the sequences adjacent to the 5' and 3' termini revealed that, in common with IS900 and IS116, IS902 contained neither flanking direct repeats nor terminal inverted repeats. However, analysis of the sequences flanking IS902 in clones pZM22 and pZM25 identified sequences (CATN₈CCTTC and CATN₈CCTTC, respectively) similar to the putative insertion site sequences described for IS900 and IS116. Fig. 4(a) shows the putative insertion site sequences for two copies of IS902, five copies of IS900 and a single copy of IS116. Regions conserved between these eight sequences are marked. A consensus insertion site sequence for this family of related IS elements is shown in Fig. 4(b).

Leskiw *et al.* (1990) drew attention to the sequences CAT and CCT, occurring to the left and right

Fig. 2. The restriction map and strategy used to sequence IS902 in pZM22. IS902 (■) and flanking genomic DNA (□) were sequenced using a series of overlapping primers (●→). Sequence data were initiated from primers F (■→; 5'-GTAAAACGACGGCCAGT) and R (□→; 5'-AACAGCTATGACCATG) situated within the multi-cloning region of pUC18 (■). B, *Bam*HI; C, *Bcl*I; E, *Eco*RI; H, *Hin*I; M, *Sma*I; P, *Pvu*I; S, *Sal*I; V, *Pvu*II; X, *Xho*II.

TCCTTAGGGTTGAAGGGCTCTGGGATTGGATGCTCTGGATGCCAGGACGGTGGGGTGTG
 10 20 30 40 50 60
 GCTAATGTGTGTGTGCAACGGTGTGTGCTGAAAGGAATGGCCGCTGCGGTTTTGGAC
 70 80 90 100 110 120
 GGTCTGGCCACTGATTGAGATCTGACGCGTACTCGATGACGCTGCTTAAGGACCTGTT
 130 140 150 160 170 180
 SD M A E P D R
 GCGGGGGTGTGCTCGCGGGGACTGCGACGACAGGAGTAGCGGTATGGCCGAACCCGACCGA
 190 200 210 220 230 240
 V W V G I D V G K S T H H A C A I D D T
 GTGTGGGTGGTATCGACGTCGGTAAGTCCACTCATCATGCGTGGCGGATCGATGACACC
 250 260 270 280 290 300
 G K V V W S K K I P N E Q A A I E D L I
 GGAAAGGTGGTGTGGTGAAGAAAATCCCGAACGAACAGGCGCGATCGAAGACCTGATC
 310 320 330 340 350 360
 A Q G G R I A N H V V W A I D L T S P P
 GCCCAGGGCGGCGGATTGCTAACACGCTGGTGTGGCGGATCGATTTGACCTCGCCGCG
 370 380 390 400 410 420
 A A L L I A V L L S A K R E V V Y V P G
 GCGGCGCTGTGATCGCCGTACTGCTGAGCGGAAACGCGAGGTGGTGTATGTGCGGGC
 430 440 450 460 470 480
 R T V N T M S H A F R G E G K T D A K D
 CGCAGGGTAAACAGATGAGTATCGGTTCCGCGGCAAGGCAAGACCGCAAGAC
 490 500 510 520 530 540
 A R V I A E T A R H R R D L S P V V P G
 GCGCGGGTAAATCGCCGAACCGCTCGGACGACGAGATCTGCCCCGGTCTGACCCGCG
 550 560 570 580 590 600
 E D L V A E L R S L T R Y R S D L M A D
 GAAGACCTGGTGTGCGAATGCGGTGCGTACCGGATACCGGTGCGATCTGATGGCTGAC
 610 620 630 640 650 660
 W V R G V N R L R S M L T A I F P A L E
 TGGGTGCGAGGCGTGAACCGGCTGCGCTGCTGCTACCGCATCTTCCCTGCTCTGGA
 670 680 690 700 710 720
 A A F D Y S T R A P L I L V S A M C T P
 GCTGCGTTGCTACTCCACCCGCGCGCGCTGATCCTGGTATCCGCTATGTGCACTCCG
 730 740 750 760 770 780
 G E I R S A K R A G V I K H L R K N R A
 GGCGAAATCCGGTCGGCAAAAGAGCTGGCGTGATCAAGCACTTCGGAAACCCGGGCA
 790 800 810 820 830 840
 W P N N I D T I A D K A L A A A A G Q I
 TGGCCCAACACATCGACGATCGCCGCAAGCGCTCGCCGCGGCGAGGCGAGATA
 850 860 870 880 890 900
 I T L P G E E A A A L I K Q L A A R L L
 ATCACCTTCCCGGCGAAGACCGCGCGCTCATCAAGCACTCGAGCACGGCTGCTG
 910 920 930 940 950 960
 D L D R Q I K D I D K Q I T N K F G E H
 GACTGGATCGGACATCAAGGACATCGATAAGCAATACCAACAAATTTGGTAGCAT
 970 980 990 1000 1010 1020
 P S A A I I E S M P G M G P H L G A E F
 CCCAGCGCGCCATCATCGATCGATGCGCGGATGGGGCCACCTGGGCGGTGAGTTC
 1030 1040 1050 1060 1070 1080
 L V I T G G N M A A F T N P G R L A S F
 CTCGTAATCACCGGCGCAACATGGCGCGCTTACCAACCCCGGCGGACTGGCATCGTTC
 1090 1100 1110 1120 1130 1140
 A G L V P V P R D S G R I T G N L H R P
 GCCGATTGGTACCCGTCACCGGATTCGCGCGGTATCACCGGCAATCTGCATCGGCCC
 1150 1160 1170 1180 1190 1200
 K R Y A N R R L R R V F Y L A A L S S L K
 AAGCGCTACAACCGCGCTGCGCGGTGTTCTACCTCGCGCGCTGTCCAGCCTCAAG
 1210 1220 1230 1240 1250 1260
 I E G S S R A F Y D R K R S E N H I H T
 ATCGAAGTTCTCGCGGGCTTTCTACGACCGCAACGATCCGAGAACCATATCCACACC
 1270 1280 1290 1300 1310 1320
 Q A L L A L A R R H V D V L W A L L R D
 CAGGCGCTGCTGCGCTGGACGCGCGCTCGAGCTCTGTGGGCACTGCTGCGCGAC
 1330 1340 1350 1360 1370 1380
 N R T W Q P Q Q P T V A A A *
 AACAGAACTGGCAACCCAGCAACCAACCGTGGCAGCTGCCTGACGCACTCACCGGGCG
 1390 1400 1410 1420 1430 1440
 TCCTCACCGCTTGACACGCTCATTTGAGATT
 1450 1460 1470

Fig. 3. Nucleotide sequence of IS902 and inferred amino acid sequence of ORF1200. A potential Shine–Dalgarno (SD) sequence, preceding the predicted initiation codon (ATG), is underlined. *, Stop codon.

(a) Element/clone	Putative insertion site sequence
IS902/pZM22 ⁽¹⁾	CATGATCAATT <u>CCT</u> TTTC
IS902/pZM25 ⁽¹⁾	CATTTACAGT <u>CCT</u> TTTC
IS900/pMB22 ⁽²⁾	CATGTGGTGT <u>CCT</u> TTTC
IS900/pMB15 ⁽²⁾	CATGTGTT <u>CCC</u> TTTA
IS900/pMB55 ⁽²⁾	CATGGTGG <u>CCC</u> TTTG
IS900/pMBJ2 ⁽³⁾	CATGACGACT <u>CCT</u> TTG
IS900/pMBJ3 ⁽³⁾	CATGTGGT <u>CCT</u> TTTC
IS116/pIJ702 ⁽⁴⁾	CATGGTCGGT <u>CCT</u> CTGGT
(b) Consensus:	CAT(N) _{6–9} CCT

Fig. 4. (a) Comparison of the putative insertion sequences of IS902, IS900 and IS116. (b) Consensus insertion sequence for this family of related IS elements. Underlined sequences are conserved between each of the eight insertion sequences. ▼, Insertion position of each of the respective IS elements. (1), This study; (2), Green *et al.* (1989); (3), unpublished data; (4), Leskiw *et al.* (1990).

respectively of both junctions of the IS116 and IS900 elements, and suggested their involvement in the alignment of a circular transposition intermediate and its insertion site sequence. These sequences were similarly found at the 5' and 3' element-insertion site junctions of IS902. Our analysis of two copies of IS902, five copies of IS900 and a single copy of IS116 revealed that the spacing between these two sequences is invariably 5–9 nucleotides for the 5' CAT/CCT pairing and 7–9 nucleotides for the 3' CAT/CCT pairing (Fig. 5a). Consensus sequences for the 5' and 3' element-insertion site junctions of this family of related IS elements are shown in Fig. 5(b).

An ORF of 1200 nucleotides was identified within the 1470 bp of IS902. Occurring between positions 225 and 1418, ORF1200 encodes a protein of 400 amino acids (*M*_r 43932). When the amino acid sequence of ORF1200 was aligned with those of the ORFs of IS900, IS116 and IS110 using the CLUSTAL program, values were obtained for the degree of homology existing at the amino acid level between the protein products of these four elements. IS902 showed 50% identity with both IS900 and IS116 and 35% identity with IS110 (data not shown). DIAGON plots comparing the amino acid sequence of ORF1200 of IS902 with the ORFs of IS900, IS116 and IS110 were prepared (Fig. 6). The highly conserved regions existing between the protein products of these four IS elements are identified as a broken diagonal.

(a) Element/clone	5' element-insertion site junction	3' element-insertion site junction
IS902/pZM22 ⁽¹⁾	<u>CATGATCAATT</u> ■ <u>TCCTT</u> <u>CATTGAGATT</u> ■ CCT	
IS902/pZM25 ⁽¹⁾	<u>CATTACAGT</u> ■ <u>TCCTT</u> <u>CATTGAGATT</u> ■ CCT	
IS900/pMB22 ⁽²⁾	<u>CATGTGGTGT</u> ■ <u>TCCTT</u> <u>CATTGAGAAT</u> ■ CTCCT	
IS900/pMB15 ⁽²⁾	<u>CATGTGTT</u> ■ <u>TCCTT</u> <u>CATTGAGAAT</u> ■ CCCCT	
IS900/pMB55 ⁽²⁾	<u>CATGGTGG</u> ■ <u>TCCTT</u> <u>CATTGAGAAT</u> ■ CCCCT	
IS900/pMBJ2 ⁽³⁾	<u>CATGACGA</u> ■ <u>TCCTT</u> <u>CATTGAGAAT</u> ■ CTCCT	
IS900/pMBJ3 ⁽³⁾	<u>CATGTGG</u> ■ <u>TCCTT</u> <u>CATTGAGAAT</u> ■ CTCCT	
IS116/pIJ702 ⁽⁴⁾	<u>CATGGTCGG</u> ■ <u>TCTCCT</u> <u>CATTGAGAT</u> ■ TCTCCT	
(b) Consensus:	CAT (N) ₅₋₉ CCT	CAT (N) ₇₋₉ CCT

Fig. 5. (a) Comparison of the 5' and 3' element-insertion site junctions of IS902, IS900 and IS116. (b) Consensus sequences for the 5' and 3' element-insertion site junctions for this family of related IS elements. Underlined sequences are conserved between both the 5' and 3' element-insertion site junctions. ■, Endpoints of the IS elements. (1), This study; (2), Green *et al.* (1989); (3), unpublished data; (4), Leskiw *et al.* (1990).

Fig. 6. DIAGON comparison of the predicted protein of ORF1200 of IS902 (vertical) with (a), the predicted protein of ORF1197 of IS900 (horizontal); (b), the predicted protein of ORF1197 of IS116 (horizontal); (c), the predicted protein of ORF1215 of IS110 (horizontal). DIAGON plots were prepared by proportional matching using the Dayhoff MDM78 matrix. Span length 11 amino acids.

Discussion

A previously uncharacterized insertion element has been identified and isolated from the genome of a deer isolate of *M. avium* subsp. *silvaticum*. This element has been designated IS902. Analysis of Southern blots hybridized with IS902-derived probes suggests that there are 10–12 copies of the element dispersed throughout the genome. The presence of different numbers of copies of IS902 in these isolates raises the possibility that this element is mobile. In 10 independent disease isolates of *M. avium* subsp. *silvaticum* examined to date, three RFLP patterns have been identified. IS902 will be as useful in the precise identification of strains of *M. avium* subsp. *silvaticum* as IS900 has proven to be in the identification of *M. paratuberculosis* strains (Collins *et al.*, 1990; Whipple *et al.*, 1990).

The element is 1470 bp in length with an ORF of 1200 nucleotides, coding for a protein of 400 amino acids (*M*_r 43932). The ORF of IS902 and its predicted protein product are virtually identical in length and size to those of IS900 (Green *et al.*, 1989), IS116 (Leskiw *et al.*, 1990) and IS110 (Bruton & Chater, 1987).

The overall homology at the nucleotide level between IS902, IS900, IS116 and IS110 is low. Greatest homology exists between these four elements in their 3' regions. IS902 shows no homology with the recently identified IS6110, an IS3-like element from *M. tuberculosis* (Thierry *et al.*, 1990) and IS6100, an IS6-like element from *M. fortuitum* (Martin *et al.*, 1990). Weak homology was observed with IS1000 from *Thermus thermophilus* (Ashby & Bergquist, 1990).

CLUSTAL analysis and DIAGON plots revealed significant homology between the amino acid sequence of the protein product of IS902 and those of IS900, IS116 and IS110. The degree of conservation existing between the predicted protein products of these four elements presumably reflects important structural domains and is highly suggestive that these proteins will exhibit comparable functions. The homologies found with IS900, IS116 and IS110 support the proposal that IS902 belongs to the family of IS elements identified by ourselves (Green *et al.*, 1989) and by Leskiw *et al.* (1990). No significant homology was observed between IS902 and the putative mycobacterial transposase of IS6100 or the putative mycobacterial integrase of Tn610 (Martin *et al.*, 1990).

An insertion site sequence sharing sequence homology with the previously described target sites of IS900 and IS116 was identified for IS902 insertion into the *M. avium* subsp. *silvaticum* genome. Although each of these IS elements has its own unique target site sequence, the sequences CAT and CCT are constant features of each of them. The spacing between the CAT and CCT sequences is most probably an important feature of the target sites. Examination of eight insertion site sequences for IS902, IS900 and IS116 (Fig. 4) revealed that the CAT and CCT sequences were invariably separated by 6–9 nucleotides. It was also observed that insertion of the appropriate IS element resulted in positioning an internal CCT sequence within 5–9 nucleotides of the insertion site CAT and positioning an internal CAT within 7–9 nucleotides of the insertion site CCT sequence (Fig. 5). Successful insertion of the appropriate element would appear to recreate CAT/CCT sequence spacing comparable to the original insertion site. Analysis of the nucleotide sequences adjacent to the IS902 termini revealed an absence of flanking direct repeats and terminal inverted repeats, features characteristic of most IS elements previously characterized. In this respect, IS902 again resembles both IS900 and IS116, which also lack these features.

M. paratuberculosis and *M. avium* subsp. *silvaticum* cause chronic enteritis (Johne's disease) in animals (Chiodini *et al.*, 1984a; Matthews & McDiarmid, 1979) and evidence is gathering for their involvement in human Crohn's disease (Chiodini *et al.*, 1984b, c; McFadden *et al.*, 1987a; Sanderson *et al.*, 1991). On the basis of the IS902 nucleotide sequence, we are currently developing PCR for the specific and sensitive detection of *M. avium* subsp. *silvaticum* to complement the previously described PCR for the detection of *M. paratuberculosis* (Moss *et al.*, 1991). Together these PCR assays will prove useful for the detection of these enteritis-associated mycobacteria both in the veterinary field and in human Crohn's disease.

Note added in proof. Since submitting this manuscript we have discovered that an insertion sequence from *Mycobacterium avium* has been independently characterized by Kunze *et al.* (1991), *Molecular Microbiology* 5, 2265–2272. This shows 98% DNA homology to IS902 and has been designated IS901.

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