Discovery of an insertion sequence, IS116, from *Streptomyces clavuligerus* and its relatedness to other transposable elements from actinomycetes

BRENDA K. LESKIW,^{1*}† MOSHE MEVARECH,^{1‡} LORINDA S. BARRITT,¹ SUSAN E. JENSEN,¹ DUNCAN J. HENDERSON,^{2§} DAVID A. HOPWOOD,² CELIA J. BRUTON² and KEITH F. CHATER²

¹ Department of Microbiology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

² John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

(Received 5 February 1990; revised 20 March 1990; accepted 2 April 1990)

We have identified an insertion sequence, IS116, present in *Streptomyces clavuligerus* at one copy per genome. The element was discovered as a 1·4 kb insertion into the multicopy plasmid pIJ702 after propagation in *S. clavuligerus*. The nucleotide sequence of IS116 and the flanking sequences from pIJ702 have been determined. The junctions with pIJ702 show no target site duplication and there are no inverted repeats at the ends of the element. One putative coding open reading frame of 1197 bp was identified which would code for a protein product of 399 amino acids. This protein resembles deduced integrase/transposase proteins specified by three other transposable elements of actinomycetes: IS110 and the mini-circle from *Streptomyces coelicolor* A3(2), and – most particularly – IS900 of *Mycobacterium paratuberculosis*. Two regions that are relatively conserved among these gene products show features found in similar positions in many reverse transcriptases. IS116 and IS900 are also closely similar in their general organization and (apparently) in their insertion site specificity, whereas IS110 and the mini-circle are quite different in these features.

Introduction

Members of the genus *Streptomyces* are noted amongst bacteria for their high level of spontaneous genetic variability, which can affect many characters, including their two most notable properties: the production of secondary metabolites and their sporulation. In recent years, with the availability of an increasingly wide variety of molecular genetic techniques for use in *Streptomyces*, some of the basis for this genome flux is being revealed, with the description of deletion and amplification cycles in the chromosomal DNA of these organisms (reviewed by Hütter & Eckhardt, 1988). Several mobile genetic elements, including insertion sequences and transposons, which might play a role in

genome flux in streptomycetes have also been described, most notably in *Streptomyces coelicolor* A3(2), which is genetically the most studied member of the genus (reviewed by Chater *et al.*, 1988).

Among the few elements sequenced so far, relatedness to other known prokaryotic or eukaryotic sequences in the major protein databases has not been revealed. However, IS110 (Chater *et al.*, 1985; Bruton & Chater, 1987) and the 2·6 kb mini-circle (Lydiat *et al.*, 1986; Henderson *et al.*, 1989) from *S. coelicolor* and the recently discovered IS900 from *Mycobacterium paratuberculosis* (Green *et al.*, 1989) have been found to form a 'family' of related elements based on similarity between amino acid sequences of their deduced gene products, despite the diversity of structure and host strains of these elements. The use of both IS110 and the mini-circle sequences to probe the DNA of a series of taxonomically varied streptomycetes revealed related elements in a minority of strains (Chater *et al.*, 1985; Lydiat *et al.*, 1986), but no hybridization to *S. clavuligerus* DNA. However, as we report here, an element (IS116) containing a major open reading frame resembling those of IS110, the mini-circle and, most particularly, IS900 has now been isolated from *S. clavuligerus*.

† Present address: John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, UK.

‡ Present address: Department of Microbiology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

§ Present address: Fisons plc, Pharmaceutical Division, Bakewell Road, Loughborough LE11 0RH, UK.

Abbreviation: ORF, open reading frame.

The nucleotide sequence data reported in this paper have been submitted to GenBank (accession number M31716).

Methods

Bacterial strains, culture conditions, plasmid and phage vectors. *Streptomyces clavuligerus* NRRL 3585 and other *Streptomyces* cultures were maintained on sporulation medium as described previously (Jensen *et al.*, 1982). For mutagenesis, *S. clavuligerus* spores were pregerminated for 5 h in seed medium [O'Sullivan *et al.*, 1979, except that phytone (Difco) replaced soy flour and sucrose was omitted] and harvested by filtration (0.45 µm pore size, Millipore). For the preparation of protoplasts, *S. clavuligerus* was cultured as described by Bailey & Winstanley (1986). Protoplasts of *S. clavuligerus* were prepared and the protoplasts were transformed and regenerated as described previously (Leskiw *et al.*, 1988). For the preparation of genomic DNA, *S. clavuligerus* was cultivated as described by Jensen *et al.* (1986), and all other *Streptomyces* strains were cultivated in YEME as described by Hopwood *et al.* (1985). *S. lividans* 1326 containing the high-copy-number plasmid pIJ702 (Katz *et al.*, 1983) was cultured as described by Hopwood *et al.* (1985).

The *Escherichia coli* plasmid pUC119 (Vieira & Messing, 1987) was a gift from J. Vieira, Waksman Institute of Microbiology, Rutgers University. *E. coli* JM109 (Yanisch-Perron *et al.*, 1985) competent cells and plating cultures for use as a host for pUC119 and M13 phages were purchased from Stratagene Cloning Systems.

DNA isolation and handling. *E. coli* and *Streptomyces* plasmids were isolated using alkaline lysis procedures. For *E. coli* this was a modification of the method of Birnboim & Doly (1979) as described by Maniatis *et al.* (1982) and for *Streptomyces* the method of Kieser (1984) as described by Hopwood *et al.* (1985). Total DNA was isolated from all the *Streptomyces* strains using procedure 1 of Hopwood *et al.* (1985).

Southern hybridization and probe preparation. Southern blots of *Bam*HI-digested total DNA were prepared as described by Maniatis *et al.* (1982). The 1.0 kb *Sal*I fragment of IS116 was sub-cloned into M13mp19, labelled with α -[³²P]dATP using the M13 hybridization probe primer (Pharmacia) and used to probe the Southern blots. Prehybridization and hybridization were performed at 65 °C; the blots were then washed twice in 2 × SSC and twice in 0.2 × SSC solutions containing 0.1% SDS for 30 min at 65 °C (Maniatis *et al.*, 1982).

Mapping of the insertion site in pIJ702, preparation of sub-clones and DNA sequence determination. The insertion of IS116 into pIJ702 was located by restriction mapping to a position between the *Apa*I site at 1320 bp and the *Bcl*I site at position 2255 bp on the pIJ702 circular map (Hopwood *et al.*, 1985). One internal *Bcl*I fragment from IS116 and two *Bcl*I fragments containing IS116 and pIJ702 flanking DNA, as well as the pIJ702 *Bcl*I fragments containing the unoccupied IS116 insertion site, were sub-cloned into pUC119. The fragment containing the right-hand end of IS116 DNA was further sub-cloned as smaller fragments into pUC119 and then all sub-clones in pUC119 were directionally cloned into the M13 vectors mp18 and mp19 (Yanisch-Perron *et al.*, 1985; Fig. 1). DNA was sequenced by the chain-termination procedure (Sanger *et al.*, 1977) using the 'universal' primer and two specially synthesized oligonucleotide primers. The special primers permitted sequencing of two regions without the need to obtain small sub-clones. Both α -[³²P]dATP (Amersham) and α -[³⁵S]dATP (New England Nuclear) were used as radioactive tracer in different experiments. Aberrations in the banding pattern were relieved by using 'sequenase' (United States Biochemicals) reactions with either dITP (Mills & Kramer, 1979) or 7-deaza-dGTP (Mizusawa *et al.*, 1986) in place of dGTP.

Analysis of sequences. The primary sequence data of IS116 were compiled using MacGene (Applied Genetic Technology, Inc.) and analysis of the nucleotide sequence for open reading frames (ORFs) was performed using FRAME (Bibb *et al.*, 1984).

The FastP (Lipman & Pearson, 1985) and Sweep programs were used

Fig. 1. Restriction map and strategy for DNA sequencing of IS116 and flanking pIJ702 DNA. The thin line indicates pIJ702 DNA and the bold line indicates IS116 DNA. The numbers in parentheses refer to the base positions of the restriction sites, where 1 is the first base of IS116 and 1421 is the last base. Vertical bars on arrows denote that a specially prepared synthetic oligonucleotide was used to obtain the sequence. Arrowheads show the length of sequence read from each sub-clone.

to screen the PIR protein sequence database (Release 10, W. C. Barker *et al.*, National Biomedical Research Foundation, Georgetown University Medical Center, Washington, DC 20007, USA) and the Owl database (Version 1.1) for homology to the IS116 ORF1197, IS110 ORF1215 and the mini-circle ORF1. (The Sweep program is an extension of FastP, and the Owl database is a composite database compiled from several currently available protein and DNA sequence databases by A. J. Bleasby, University of Leeds, Leeds, UK).

The IS116 ORF1197, ORF1215 of IS110, ORF1197 of IS900 and the mini-circle ORF1 sequences were aligned using the CLUSTAL programs (Higgins & Sharp, 1988) for multiple sequence alignments. Pairwise comparisons of the putative polypeptide sequences were done using the COMPARE and DOTPLOT programs in the package supplied by the University of Wisconsin Genetics Computer Group, Version 5.3 (Devereux *et al.*, 1984) with window and stringency parameters set at 40 and 19.0 respectively.

Results

Isolation and distribution of IS116 and closely related sequences

The insertion sequence IS116 was first observed by chance after introduction of the *Streptomyces* plasmid pIJ702 (isolated from *S. lividans* 1326) into protoplasts prepared from NTG-mutagenized *S. clavuligerus* spores. Because of restriction of the foreign DNA, only three thiostrepton-resistant transformants were obtained, one of which was found to contain pIJ702 bearing a 1.4 kb insertion about 300 bp anticlockwise of the *Bcl*I site at position 2255 bp on the pIJ702 circular map. The insertion was named IS116. A 1.0 kb *Sal*I fragment from within IS116 (Fig. 1) was used to probe Southern blots of *Bam*HI-digested total DNA from *S. clavuligerus* and nine other *Streptomyces* species using the stringency conditions described in Methods: *S. lividans* 1326, *S. cattleya*

NRRL 8057, *S. fradiae* NCIB 8233, *S. griseus* NCIB 8237, *S. lipmanii* NRRL 3584, *S. phaeochromogenes* NCIB 8505 and *S. rimosus* NCIB 8229 showed little or no hybridization, but *S. venezuelae* NCIB 823 and *S. violaceoruber* SANK 95570 showed strong hybridization, with many bands being visible (data not shown). The probe hybridized to a single band of approximately 10 kb from *S. clavuligerus*, suggesting the presence of only one copy of IS116 in the genome.

IS116 contains one major gene, which is homologous to genes in otherwise disparate actinomycete transposable elements

The nucleotide sequence of IS116 is shown in Fig. 2. The FRAME program was used to search the sequence for putative protein-coding ORFs. The plot identified a single ORF ending at a UGA triplet located only 15 bp from the end of IS116. Consideration of the FRAME analysis and of the availability of potential ribosome-binding sites tended to exclude all the ATG or GTG triplets as likely translational start codons, but a TTG at position 207 is preceded by a good potential ribosome-binding site (Fig. 2). Moreover, a protein starting at this codon would show convincing end-to-end sequence alignment with the putative products of ORFs from other actinomycete mobile genetic elements (see below). This TTG codon was therefore chosen as the most likely start for the IS116 ORF, which would have a length of 1197 bp.

The putative protein product of ORF1197 showed no significant relatedness to any proteins in the major databases. However, pairwise comparisons to ORF1215 of the *S. coelicolor* IS110 and to ORF1 of the *S. coelicolor* 2.6 kb mini-circle revealed a high degree of similarity between the protein products. The putative polypeptides from the two insertion sequences are more similar to each other than to the mini-circle ORF1 polypeptide, resembling each other throughout their length. An even closer relatedness was subsequently seen to the recently published (Green *et al.*, 1989) sequence of the major ORF of IS900 from *Mycobacterium paratuberculosis*. The most conserved regions of the four polypeptides are towards the ends, particularly the C-terminus. No extensive similarities between the DNA sequences of the four ORFs were revealed by DOTPLOT analysis.

In order to highlight any regions of the polypeptide sequences which, by virtue of their conservation, might represent structural or functional domains, they were aligned using the CLUSTAL programs for multiple sequence alignments (Fig. 3). Regions highly conserved among related proteins are potentially of functional significance, so short peptide sequences corresponding to these (Fig. 3) were used in a further search of the Leeds

University protein database (using the SCANDAL program developed by A. Bleasby, Department of Biophysics, University of Leeds: T. K. Attwood, personal communication). No significant relatedness to any proteins in the database was uncovered by this computer-assisted procedure, but examination of conserved regions by eye led to the recognition of some possible similarities to reverse transcriptase (see Discussion).

In view of the similarity of the major ORFs of IS116 and IS110, we examined the sequence of IS116 to see if two minor features of IS110 noted by Bruton & Chater (1987) were also present. A possible ORF of 330 nucleotides (ORF330) reading from the opposite strand of IS110 ORF1215 had a smaller counterpart (of 141 nucleotides) in IS116, and a conspicuous inverted repeat in ORF330 of IS110 was echoed by a somewhat shorter inverted repeat at the same position (nucleotide 1034) in IS116. Although an inverted repeat was not present at that location in the mini-circle ORF1 or IS900, potential ORFs (of 186 and a maximum length of 408 nucleotides, respectively) were found. However, as was the case for the ORF in IS116, these ORFs are preceded by weak ribosome-binding sites and the codon usage is not as expected for high-G+C DNA.

Features of the junctions of IS116 with its target site in pIJ702

IS116 was inserted between bases 1922 and 1923 (Kendall & Cohen, 1988) of pIJ101 (from which pIJ702 was derived), with no duplication of the target site (in contrast with the common situation with transposition of both prokaryotic and eukaryotic elements) (Fig. 4). Furthermore, the termini of IS116 did not contain inverted repeat sequences, which usually characterize the ends of IS elements (Iida *et al.*, 1983). Similarities of the IS116::pIJ702 junction sequences with those of several IS900 insertions sequenced by Green *et al.* (1989) were noticed (see Discussion).

Discussion

A family of transposable elements in actinomycetes

A 1.4 kb insertion in pIJ702, isolated from *S. clavuligerus* and named IS116, is a previously undescribed insertion element that is probably present as a single copy in the chromosome of *S. clavuligerus*. The element is 1421 bp long and contains an ORF which spans most of its length. Use of the most likely start codon (a TTG) would allow this ORF (ORF1197) to encode a protein product of M_r 44 766 (399 amino acids). This product is related to deduced gene products specified by IS900 from

Mini-circle	-----MWEDSLTVFCGIDWAERHHDVAI	23
IS110	-----MFDTEVGVFGLDVGKTAHHGHG	24
IS116	-----LSTRHDIWVGIDAGKGHHWAVA	23
IS900	-----VAQPVWAGVDAGKADHYCMV	20
	* *	
Mini-circle	VDDTGTLAKARITDDV----AGYNKLLDLLAEHGDSSATPIPVAIETSHGLLVAALRTG	79
IS110	LTPAGKKVLDKQLPNSEPRRLRAVFDKLAAKFGT-----VLVIVDQPASIGALPLTVARD	78
IS116	VDADGETLFSTKVINDE----AQVLTLLIE---TAREREEVRWAVDISGRASTLLLALLVA	76
IS900	INDDAQRLLSQRVANDE----AALLELIAAVTTLADGGEVTAIDLNAGGAALLIALLIA	76
	↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	
Mini-circle	SRKVFAINPLAAARYDRHGVSRRKSDPGDALVLANILRTDMHAHRPLPADSELAQAITV	139
IS110	AGCKVAYLPGLAMRRIADLYPGEAKTDAKDAAVIADAARTMAHTLRSLELTDEITAELSV	138
IS116	HGQNVVYVPGRVTNRMMSGAYKGEGKTDADKARVIADQAR-MRRDFAPLDRPPELVTTLRL	135
IS900	AGQRLLYIPGRVTVHHAAGSYRGEKTDADKAAI IADQAR-MRHDLPQLRAGDDIAVELRI	135
	* * * * *	
HTLV-I	YTGPGNNPVFPVKKANGTW HLQTIQLRDAFFQIPL	
	56 . o o 107 . . o . . o	
Mini-circle	LARAQDDAVWNROQVANQVRSLLREYYPAALHAFQSKDGGLTRPDARVILTMAPTAKAA	199
IS110	LVGFDQDLAAEATRTSNRIRGLLTQFHPSLERVLPRLDHQ-----AVTW	183
IS116	LTNHRADLIADRVRLINRLDLLTGICPALERAFDYSAAG-----PVVM	180
IS900	LTSRRSDLVADRTRAIEPNARPAAGILSALERAFDYNKSRA-----ALIL	180
	* *	
Mini-circle	KLTLAQLRAGLKRSGRTRAFNTEIERLRGIFRSEYARQLPAVEDAF----GHQLLALLRQ	255
IS110	LLERYGSPAALRKAGRRRLVELVRPKAPRMAQRLIDDIFDALDEQTVVVPGTGTLDIVVP	243
IS116	-LTEYQTPAALRRTGVKRLTTWLGRKVRDADTVAAKAIEAARTQQVVLPGEKRA TKLVC	239
IS900	-LTGYQTPDALRSAGGARVA AFLRKRKARNADTVAATALQAANAQHSIVPGQQLAATVVA	239
	* * * * *	
Mini-circle	LDATCLAADDLAKAVEDA-----FREHADSEILLSFPGLGPLLGARVLAIEIGDDRSRFTD	310
IS110	SLASSLTAVHEQRRALEAQINALLEAHPLSPVLTSMPGVGVRTAAVLL-VTVGDGTSFPT	302
IS116	DLAHQLLALDERIKDNDREIRETFRTDDRAEIIESMPGMGPVLGAEFV-AIVGDLSGYKD	298
IS900	RLAKEVMALDTEIGD TDAMIEERFRHRHAEIILSMPGFGVILGAEF LAATGGDMAAFAS	299
	* * * * *	
Mini-circle	ARALKSYAGSAPITRASGRKH FVGRRFVK-NNRLMNAGFLWAF AALQASPGANAHYRRRR	369
IS110	AAHLASYAGLAPTTKSSGTSIHGEHAPRGGNRQLKRAMFLSAFACMNADPASRTYYDRQR	362
IS116	AGRLASHAGLAPVPRDSGRRTGNYHRPQRYNRRLRWLFYMSAQ TAMMRPGPSRDYYLKRR	358
IS900	ADRLAGVAGLAPVPRDSGRISGNLKRPRRYDRRLLRACYLSALVSIRTDPSSRTYYDRKR	359
	* * * * *	
Mini-circle	EHGDWHAAAQRHLLNRF LGQLHHCLOTQHFDEQRAFA PLLQAAA-	414
IS110	ARGKTHTQALLRLARQISVLFAMLRDGTIFYESRMPAGVELAA---	405
IS116	GEGLLHTQALLSLARRVDVLWAMLRDKRLFT---PAPPVTQTA--	399
IS900	TEGKRHTQAVLALARRRLNVLWAMLRDHAVYH---PA---TTTAAA	399
	* * * * *	

Fig. 3. Alignment of the IS116 ORF1197, IS900 ORF1197, IS110 ORF1215 and the mini-circle ORF1 polypeptide sequences generated using CLUSTAL programs for multiple sequence alignments. The set parameters were as follows: gap penalty 4, cut-off 10, window 10, and filtering 2.5. Pads added to maintain the alignment are indicated by (-). Amino acid residue numbers are given at the ends of the lines. The short polypeptide sequences, derived from conserved regions, which were used to search databases are indicated by horizontal lines above the sequences. Symbols above and below the sequences denote the following: *, conserved residue among the four transposable elements; ↓, regions showing similarity to reverse transcriptase motifs; ., conserved residue type among reverse transcriptases; o, very highly conserved residue among reverse transcriptases; □, shown to be significant by site-directed mutagenesis of HTLV-I.

(a) Element-target site junctions

Mini-circle :: chromosome

GCGGCGTACCGGTGCCCGGATACACATCTCAGGGGGCTAAGAAGTCA---/---ACGAGCTGCTTACACCCCTGGATITAGGTTCGGGGACCGTGGCGAACAG

IS110 :: ϕ C31

CGGTAACGCATAGGGCACCCCCCCCCTTATGTTTTCGCGGTCC---/---CGAAGGACATAGAGGCACCCCCCCCCTTCCGGGACACCCCC

IS116 :: pIJ702

GTCGAAGTCGGTCATGGTCGCTCCTAAGAAATGTGAGGCGGTCCGG---/---ACGGCTTGACACGATCATTGAGATCTCCTGGTGGGTGGGGCGGGGC

IS900 :: chromosome

TGGTCATGTGGTGTCTCTT---/---CATTCAGAAATCTCCTTCGCG

(b) Insertion sites

Mini-circle

GAGCGCGGTACCGGTGCCCGGATAGGGTCCGGGACCGTGGCGAACAGAATC

IS110

CACCGGTTACGCATAGGGCACCCCCCTTCCGGGACACCCCCCTATCCGTGAC

IS116

TCCGTGAAGTCGGTCAATGGTCCGTCTCTTGGTGGGTGGGGCGGGCGCCACG

IS900

TGGTCATGTGGTGTCTCTCTTCGCG

Fig. 4. Target site junctions (a) and insertion sites (b) of the four actinomycete transposable elements (the insertion site shown for IS900 was isolated in clone pMB22; Green *et al.*, 1989). Direct and inverted repeats are indicated by arrows and the junction points are boxed. Symbols > < in (b) show the limits within which the element-target site recombination is deduced to have occurred.

Mycobacterium paratuberculosis (50% identity, three gaps in the alignment) and by IS110 (ORF1215; 35% identity with six gaps) and the mini-circle (ORF1; 25% identity with nine gaps), both from *S. coelicolor* A3(2). The latter two polypeptides show 22% identity (seven gaps). Thus the mini-circle ORF1 appears to be more divergent than the other three. Since ORF1 of the mini-circle is essential for integration of the circular form of the element into the *S. lividans* chromosome (D. J. Henderson, unpublished), it probably encodes an integrase or transposase. The

resemblance of this protein to those specified by IS110, IS116 and IS900 suggests that they have a comparable function.

Similarities and differences between the ends and target sites of members of the family of elements

The similarities of their major gene products lead one to anticipate that IS110, IS116, IS900 and the mini-circle should show similar features at their ends. In fact,

however, IS110 and the mini-circle possess imperfect inverted repeats at their ends, but IS116 and IS900 do not (with the caveat that only one example of IS116 has been sequenced; however, the possibility that it might have suffered deletion of some terminal sequences seems unlikely since the sequences flanking the element are not deleted). Unusually, the insertion of IS116, IS900 or the mini-circle does not cause target site duplications (Fig. 4; Green *et al.*, 1989; Henderson *et al.*, 1989) [the situation for IS110 is unclear because in the particular insertion event that was sequenced by Bruton & Chater (1987) the element had inserted into a run of seven C residues and was then flanked by runs of more than seven C residues]. The site into which IS116 is inserted in pIJ702 (CATGGTCGGTCTCCTGGT) corresponds closely to the consensus sequence CATG(N)₄₋₆CPyCCTT proposed as the target site for IS900 insertion, based on analysis of three insertion events (Fig. 4; Green *et al.*, 1989). Moreover, the leftmost 6 and rightmost 17 residues of IS900 (Green *et al.*, 1989) are closely similar to the corresponding segments of IS116.

IS116 shows little homology to its target site in pIJ702, with the caveat mentioned above, although the sequences CAT, repeated a few bases to the left of both junctions of the inserted element, and CCT, a few bases to the right of these junctions, merit emphasis because they are also found in all three of the IS900 insertion events in *Mycobacterium* analysed by Green *et al.* (1989). These short sequences might conceivably guide the alignment of a circular transposition intermediate and its target site as has been proposed for short regions of similarity between both the mini-circle and IS110 and their respective target sites (Henderson *et al.*, 1989; Bruton & Chater, 1987). Such alignments between short related sequences have been strongly implicated in integration of Tn7 in *E. coli* (Gringauz *et al.*, 1988) and Tn554 in *Staphylococcus aureus* (Murphy, 1988).

A weak similarity between proteins specified by the elements and reverse transcriptases

Database searches failed to reveal other proteins containing sequences similar to the regions most highly conserved between the putative transposases (integrases) of IS116, IS110, IS900 and the mini-circle. Nevertheless, it is interesting to note that one of the most highly conserved segments (corresponding to IS116 residues 103–107; Fig. 3) contains an invariant motif D--DA that is also conserved in retroviral reverse transcriptases (Patarca & Haseltine, 1984; Johnson *et al.*, 1986; Xiong & Eickbush, 1988) and which has been shown there, by site-directed mutagenesis, to be functionally significant (Fig. 3; Larder *et al.*, 1987). On the other hand, the actinomycete sequences lack two other sequence motifs

that are very highly conserved among retroviral reverse transcriptases: LPQG and Y-DD, the latter being conserved also among other nucleic acid polymerases (Johnson *et al.*, 1986; Argos, 1988). However it is worth noting that a region upstream of the D--DA motif (starting in IS116 at P₈₅) shows some degree of similarity to a second moderately well conserved region in the retroviral enzymes (Johnson *et al.*, 1986; Xiong & Eickbush, 1988) (Fig. 3). Comparison with the recently discovered reverse transcriptases of Gram-negative bacteria reinforces these tentative alignments (Lampson *et al.*, 1989; Inouye *et al.*, 1989; Lim & Maas, 1989). Thus, although these sequence comparisons do not imply that some or all of these actinomycete elements use reverse transcription during their transposition, it seems plausible that their principal gene products may have some properties in common with reverse transcriptases. In addition, reverse transcriptases are a very highly diverged but undoubtedly homologous family of enzymes and no other transposition-related enzyme family is encoded by, and crucial to, such a wide variety of transposing elements. These two features are also shown by the gene products of the actinomycete elements discussed here.

We thank Teresa Attwood and John Wooton of the University of Leeds for assistance with database searches. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (B.K.L., S.E.J.), Alberta Heritage Foundation for Medical Research (B.K.L., S.E.J., M.M.) and the John Innes Foundation (D.J.H.).

References

- ARGOS, P. (1988). A sequence motif in many polymerases. *Nucleic Acids Research* **16**, 9909–9916.
- BAILEY, C. R. & WINSTANLEY, D. J. (1986). Inhibition of restriction in *Streptomyces clavuligerus* by heat treatment. *Journal of General Microbiology* **132**, 2945–2947.
- BIBB, M. J., FINDLAY, P. R. & JOHNSON, M. W. (1984). The relationship between base composition and codon usage in bacterial genes and its use in the simple and reliable identification of protein coding sequences. *Gene* **30**, 157–166.
- BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513–1523.
- BRUTON, C. J. & CHATER, K. F. (1987). Nucleotide sequence of IS110, an insertion sequence of *Streptomyces coelicolor* A3(2). *Nucleic Acids Research* **15**, 7053–7065.
- CHATER, K. F., BRUTON, C. J., FOSTER, S. G. & TOBEK, I. (1985). Physical and genetic analysis of IS110, a transposable element of *Streptomyces coelicolor* A3(2). *Molecular and General Genetics* **200**, 235–239.
- CHATER, K. F., HENDERSON, D. J., BIBB, M. J. & HOPWOOD, D. A. (1988). Genome flux in *Streptomyces coelicolor* and other streptomycetes and its possible relevance to the evolution of mobile antibiotic resistance determinants. In *Transposition*, pp. 7–42. Edited by A. J. Kingsman, K. F. Chater & S. M. Kingsman. Cambridge: Cambridge University Press.
- DEVEREUX, J., HAEGERLI, P. & SMITHIES, O. (1984). A comprehensive set of sequence analysis programs for the Vax. *Nucleic Acids Research* **12**, 387–395.

- GREEN, E. P., TIZARD, M. L. V., MOSS, M. T., THOMPSON, J., WINTERBOURNE, D. J., MCFADDEN, J. J. & HERMON-TAYLOR, J. (1989). Sequence and characteristics of IS900, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. *Nucleic Acids Research* **17**, 9063-9073.
- GRINGAUZ, E., ORLE, K. A., WADDELL, C. S. & CRAIG, N. L. (1988). Recognition of *Escherichia coli* attTn7 by transposon Tn7: lack of specific sequence requirements at the point of Tn7 insertion. *Journal of Bacteriology* **170**, 2832-2840.
- HENDERSON, D. J., LYDIATE, D. J. & HOPWOOD, D. A. (1989). Structural and functional analysis of the mini-circle, a transposable element of *Streptomyces coelicolor* A3(2). *Molecular Microbiology* **3**, 1307-1318.
- HIGGINS, D. G. & SHARP, P. M. (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* **73**, 237-244.
- HÜTTER, R. & ECKHARDT, T. (1988). Genetic manipulation. In *Actinomycetes in Biotechnology*, pp. 89-184. Edited by M. Goodfellow, S. T. Williams & M. Mordarski. London: Academic Press.
- HOPWOOD, D. A., BIBB, M. J., CHATER, K. F., KIESER, T., BRUTON, C. J., KIESER, H. M., LYDIATE, D. J., SMITH, C. P., WARD, J. M. & SCHREMPF, H. (1985). *Genetic Manipulation of Streptomyces - a Laboratory Manual*. Norwich: John Innes Foundation.
- IIDA, S., MEYER, J. & ARBER, W. (1983). Prokaryotic IS elements. In *Mobile Genetic Elements*, pp. 159-221. Edited by J. A. Shapiro. New York: Academic Press.
- INOUE, S., HSU, M.-Y., EAGLE, S. & INOUE, M. (1989). Reverse transcriptase associated with the biosynthesis of the branched RNA-linked msDNA in *Myxococcus xanthus*. *Cell* **56**, 709-717.
- JENSEN, S. E., WESTLAKE, D. W. S. & WOLFE, S. (1982). Cyclization of δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine to penicillins by cell free extracts of *Streptomyces clavuligerus*. *Journal of Antibiotics* **35**, 483-490.
- JENSEN, S. E., LESKIW, B. K., VINING, L. C., AHARONOWITZ, Y., WESTLAKE, D. W. S. & WOLFE, S. (1986). Purification of isopenicillin N synthetase from *Streptomyces clavuligerus*. *Canadian Journal of Microbiology* **32**, 953-958.
- JOHNSON, M. S., MCCLURE, M. A., FENG, D.-F., GRAY, J. & DOOLITTLE, R. F. (1986). Computer analysis of retroviral *pol* genes: assignment of enzymatic function to specific sequences and homologies with non-viral enzymes. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 7648-7652.
- KATZ, E., THOMPSON, C. J. & HOPWOOD, D. A. (1983). Cloning and expression of the tyrosinase gene from *Streptomyces antibioticus* in *Streptomyces lividans*. *Journal of General Microbiology* **129**, 2703-2714.
- KENDALL, K. J. & COHEN, S. N. (1988). Complete nucleotide sequence of the *Streptomyces lividans* plasmid pIJ101 and correlation of the sequence with genetic properties. *Journal of Bacteriology* **170**, 4634-4651.
- KIESER, T. (1984). Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* **12**, 19-36.
- LAMPSON, B. C., SUN, J., HSU, M.-Y., VALLEJO-RAMIREZ, J., INOUE, S. & INOUE, M. (1989). Reverse transcriptase in a clinical strain of *Escherichia coli*: production of branched RNA-linked msDNA. *Science* **243**, 1033-1038.
- LARDER, B. A., PURIFOY, D. J. M., POWELL, K. L. & DARBY, G. (1987). Site-specific mutagenesis of AIDS virus reverse transcriptase. *Nature, London* **327**, 716-712.
- LESKIW, B. K., AHARONOWITZ, Y., MEVARECH, M., WOLFE, S., VINING, L. C., WESTLAKE, D. W. S. & JENSEN, S. E. (1988). Cloning and nucleotide sequence determination of the isopenicillin N synthetase gene from *Streptomyces clavuligerus*. *Gene* **62**, 187-196.
- LIM, D. & MAAS, W. K. (1989). Reverse transcriptase-dependent synthesis of a covalently linked branched DNA-RNA compound in *Escherichia coli* B. *Cell* **56**, 891-904.
- LIPMAN, D. J. & PEARSON, W. R. (1985). Rapid and sensitive protein similarity searches. *Science* **227**, 1435-1441.
- LYDIATE, D. J., IKEDA, H. & HOPWOOD, D. A. (1986). A 2.6 kb DNA sequence of *Streptomyces coelicolor* A3(2) which functions as a transposable element. *Molecular and General Genetics* **203**, 79-88.
- LYDIATE, D. J., ASHBY, A. M., HENDERSON, D. J., KIESER, H. M. & HOPWOOD, D. A. (1989). Physical and genetic characterization of linear copies of the *Streptomyces coelicolor* mini-circle. *Journal of General Microbiology* **135**, 941-955.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning - a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MILLS, D. R. & KRAMER, F. R. (1979). Structure-independent nucleotide sequence analysis. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 2232-2235.
- MIZUSAWA, S., NISHIMURA, S. & SEELA, F. (1986). Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. *Nucleic Acids Research* **14**, 1319-1324.
- MURPHY, E. (1988). Transposable elements in *Staphylococcus*. In *Transposition*, pp. 59-89. Edited by A. J. Kingsman, K. F. Chater & S. M. Kingsman. Cambridge: Cambridge University Press.
- O'SULLIVAN, J., APLIN, R. T., STEVENS, C. M. & ABRAHAM, E. P. (1979). Biosynthesis of a 7- α -methoxycephalosporin. *Biochemical Journal* **179**, 47-52.
- PATARCA, R. & HASTELTINE, W. A. (1984). Sequence similarity among retroviruses - erratum. *Nature, London* **309**, 728.
- SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5463-5467.
- VIEIRA, J. & MESSING, J. (1987). Production of single-stranded plasmid DNA. *Methods in Enzymology* **153**, 3-11.
- XIONG, Y. & EICKBUSH, T. H. (1988). Similarity of reverse transcriptase-like sequences of viruses, transposable elements, and mitochondrial introns. *Molecular Biology of Evolution* **5**, 675-690.
- YANISCH-PERRON, C., VIEIRA, J. & MESSING, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103-119.