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Vector for IS element entrapment and functional characterization based on turning on expression of distal promoterless genes

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Abstract

We constructed and characterized a novel trap vector for rapid isolation of insertion sequences. The strategy used for the isolation of IS elements is based on the ability of many IS elements to turn on the expression of otherwise silent genes distal to some sites of insertion. The simple transposition of an IS element can sometimes cause the constitutive expression of promoterless antibiotic resistance genes resulting in selectable phenotypes. The trap vector pAW1326 is based on a pBR322 replicon, it carries ampicillin and streptomycin resistance genes, and also silenced genes that confer chloramphenicol and kanamycin resistance once activated. The trap vector pAW1326 proved to be efficient and 85 percent of all isolated mutations were insertions. The majority of IS elements resident in the studied *Escherichia coli* strains tested became trapped, namely IS2, IS3, IS5, IS150, IS186 and Tn1000. We also encountered an insertion sequence, called IS10L/R-2, which is a hybrid of the two IS variants IS10L and IS10R. IS10L/R-2 is absent from most *E. coli* strains, but it is detectable in some strains such as JM109 which had been submitted to Tn10 mutagenesis. The distribution of the insertion sequences within the trap region was not random. Rather, the integration of chromosomal mobile genetic elements into the offered target sequence occurred in element-specific clusters. This is explained both by the target specificity and by the specific requirements for the activation of gene transcription by the DNA rearrangement. The employed trap vector pAW1326 proved to be useful for the isolation of mobile genetic elements, for a demonstration of their transposition activity as well as for the further characterization of some of the functional parameters of transposition.

Keywords: Insertion sequences; *Escherichia coli*; Spontaneous mutation; Polar effect; Transposition; IS2; IS3; IS5; IS150; IS186; IS10; Tn1000

1. Introduction

Insertion of IS elements into an operon usually causes the mutation of the target gene. In addition these mutations frequently exert a polar effect, which may result in the inactivation of downstream genes belonging to the same operon (Shapiro, 1969). However, the polar effect of insertion can also bring about a constitutive expression of downstream genes. This latter phenomenon was detected with several insertion sequences (for example, IS1, Prentki et al., 1986; IS2, Saedler et al., 1974; IS10, Ciampi et al., 1982; IS30, Dalrymple and Arber, 1985). In some cases the inserted IS element was shown to carry an outward directed promoter, in other cases a new fusion promoter was formed by the insertion (for review, see Galas and Chandler, 1989).

Positive selection systems had been developed to entrap functional transposable elements (Gay et al., 1985; Raabe et al., 1988; Simon et al., 1991). The common idea of these selection systems is the following: if the insertion of a transposable element (or another kind of spontaneous mutation) destroys a gene (cI, lambda phage repressor; *rpsL*, mediating streptomycin sensitivity; *sacB*, coding for levansucrase, which is lethal when bacteria grow on sucrose) then this can result in a selectable phenotype.

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Abbreviations: A, *Acc*I; Ap^R, ampicillin-resistant; B, *Bam*HI; Bg, *Bgl*II; bp, base pair(s); $Cm^{R/S}$, promoterless chloramphenicol resistance determinant; Cm^R, chloramphenicol-resistant; Cm^S, chloramphenicol-sensitive; E, *Eco*RI; H, *Hin*dIII; kb, kilobase(s) or 1000 bp; $Km^{R/S}$, promoterless kanamycin resistance determinant; Km^S, kanamycin-sensitive; N, *Nco*I; ORF, open reading frame; P, *Pst*I; pBSK, Bluescript SK; Pv, *Pvu*II; RV, *Eco*RV; S, *Sma*I; Sa, *Sal*I; Sm^R, streptomycin-resistant; Sp, *Sph*I; Tc^S, tetracycline-sensitive; Tc^R, tetracycline-resistant; T, *Taq*I

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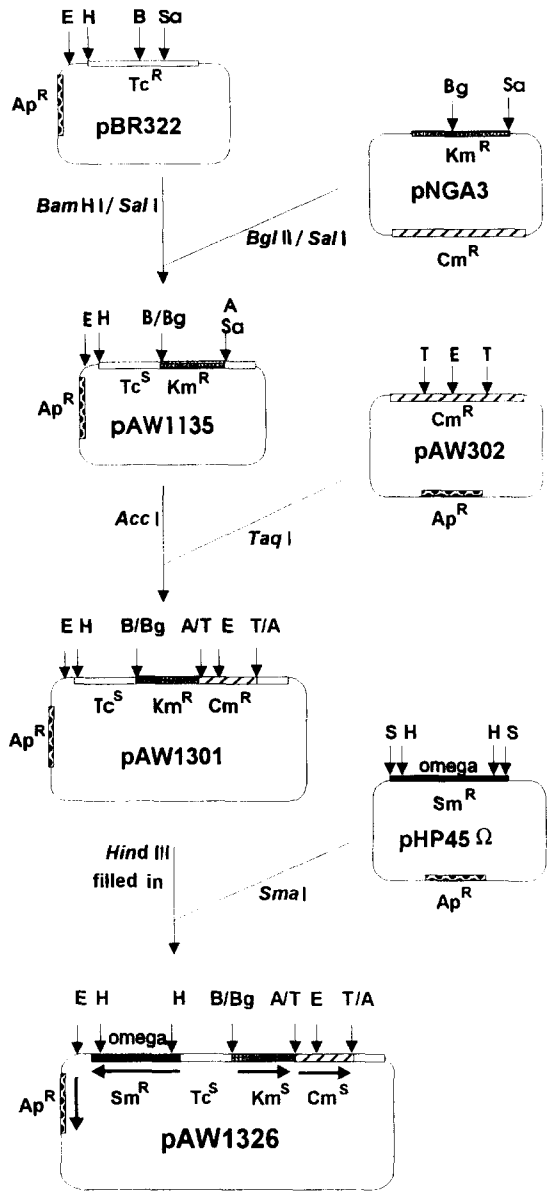

Fig. 1. The pAW1326 plasmid is a pBR322 derivative (Bolivar et al., 1977), and it was constructed from plasmids pNGA3 (kindly provided by N. Garamszegi and L. Orosz) and pAW302 (Stalder and Arber, 1989) by inserting the promoterless *neo* (1169 bp long) and *cat* (762 bp) genes, respectively, and further the 2 kb long functional Sm^R gene from plasmid pHP45Ω (Prentki and Krisch, 1984). The resulting plasmid, called pAW1326 (accession No. U46780), is the trap vector, used throughout in our investigations. The arrows in pAW1326 indicate the direction of possible expression of antibiotic resistance genes. The plasmids are not drawn to scale. **Methods**: The commonly used DNA techniques were performed as described in the handbook of Sambrook et al. (1989) with minor modifications. The following procedure was used by isolation of pAW1326 mutants. Competent cells from *E. coli* strains JM101 (Messing, 1979) or JM109 (Yanisch-Perron et al., 1985) were transformed with pAW1326. The transformants were plated onto solid LB medium supplemented with antibiotics Ap and Sm. After overnight (O/N) growth at 37°C, the transformed colonies were inoculated into 3 ml of liquid LB medium, containing the same antibiotics and incubated O/N at 37°C. These cultures were stored at room temperature (RT) for 10 days. Aliquots of these cultures were then plated in parallel onto solid LB medium containing either Cm or Ap, and the plates were incubated O/N at 37°C. The frequency of mutation to Cm^R was calculated as the ratio of Cm^R versus Ap^R colonies. A representative portion of Cm-resistant colonies (1–20) from each culture) was tested for resistance against the other antibiotics (Km, Ap, Sm). Antibiotics were obtained from Sigma and used at the following final concentrations (micrograms per milliliter): ampicillin (Ap) 150; chloramphenicol (Cm) and kanamycin (Km) 20; streptomycin (Sm) 50 and tetracycline (Tc) 10. Bacteria were grown in LB medium (Miller, 1972).

We describe here a different positive selection system, which we have developed and which is especially useful for rapid detection of insertions mediated by transposable elements. The system is based on the ability of many IS elements to turn on the expression of otherwise silent genes distal to some sites of insertion. Some IS elements have outward directed promoters which are responsible for polar gene activation. Alternatively, IS elements contain relatively often an outward directed −35 promoter region in their ends. The insertion of these elements into different target sequences may sometimes yield a new complete promoter region, provided that an appropriate −10 region is present in the target sequence and that the fusion of the two regions occurs in an appropriate distance. These conditions are surprisingly often fulfilled, as was revealed by the functional analysis of the constructed direct selection trap vector pAW1326 carrying promoterless reporter genes. With this trap vector we were able to catch a majority of IS elements resident in *E. coli* K12.

2. Results and discussion

2.1. The structure and use of the trap vector pAW1326

The construction of the trap vector is summarized in Fig. 1. The pAW1326 contains two promoterless, 'silent' reporter genes: aminoglycoside 3′-phosphotransferase (*neo*, $Km^{R/S}$), and chloramphenicol acetyl-transferase (*cat*, $Cm^{R/S}$). The two reporter genes are located behind the omega fragment which contains the functional streptomycin-spectinomycin acetyltransferase (*aadA*, Sm^R) gene flanked by stop codons in all three frames and by a terminator region. These sequences serve to inhibit the spontaneous expression of the reporter genes (Prentki and Krisch, 1984). The trap region extends over 1627 bp and contains a 345 bp long pBR322 derived sequence (part of the Tc^R gene, positions 2067–2412) and the $Km^{R/S}$ gene (positions 2413–3582) originally derived from transposon Tn5 as well as a small part of the omega segment (positions 1955–2066) in front of the open reading frame of the Sm^R gene. The numbering of the positions of pAW1326 begins with the first base of the omega segment and it reads clockwise. The full sequence of pAW1326 is submitted to the GenBank database (accession No. U46780).

The strategy on which the use of the trap vector is based relies on the expectation that the insertion of an IS element into the trap region might provide a promoter for the transcription of the $Cm^{R/S}$ and in some cases also of the $Km^{R/S}$ genes. This can happen if the inserted IS element carries an outward directed promoter in the correct orientation or also if the insertion of an IS element gives rise to a fusion promoter, in which the −35 region is provided by the end of the IS element and the −10 region by sequences of the trap region. Such insertion of an IS element in front of the promoterless $Km^{R/S}$ gene results in Km^RCm^R mutants, while insertion into the $Km^{R/S}$ gene itself produces the Km^SCm^R phenotype. A computer search showed that the entire trap region [positions 1955–3582; from the ORF of the Sm^R gene through the Tc^S region to the end of the $Km^{R/S}$ region (Fig. 2)] is quite rich in potential −10 boxes in the orientation required for the resistance genes used for the selection (Cm^R and for the insertions into the 5′ part of the trap region also Km^R). Indeed this DNA segment has 43 potential −10 boxes if two mismatches are allowed and 173 if three mismatches to the consensus TATAAT are allowed. In view of this abundance it is feasible to assume that opportunities exist for some IS elements (Galas and Chandler, 1989) to insert at an appropriate distance from a −10 box to give rise to a functional fusion promoter. The presence of two promoterless reporter genes (*neo*, *cat*) results in the following advantages: possibility of double selection and better identification of the location of target sites.

2.2. Isolation of mutants with the trap vector

In five sets of experiments 83 independent *E. coli* cultures of JM101 (pAW1326) and 40 cultures of JM109 (pAW1326) were tested for spontaneous Cm^R mutants. After growth to saturation and storage at room temperature for 10 days there was no culture without Cm^R mutation in the trap vector. The variable number of mutants per culture pointed to a clonal distribution (Luria and Delbrück, 1943) and the average number of mutants was slightly below 10 mutants/ml of culture. A total of 1073 mutants were isolated: 835 from the JM101 (pAW1326) cultures and 238 from JM109 (pAW1326)

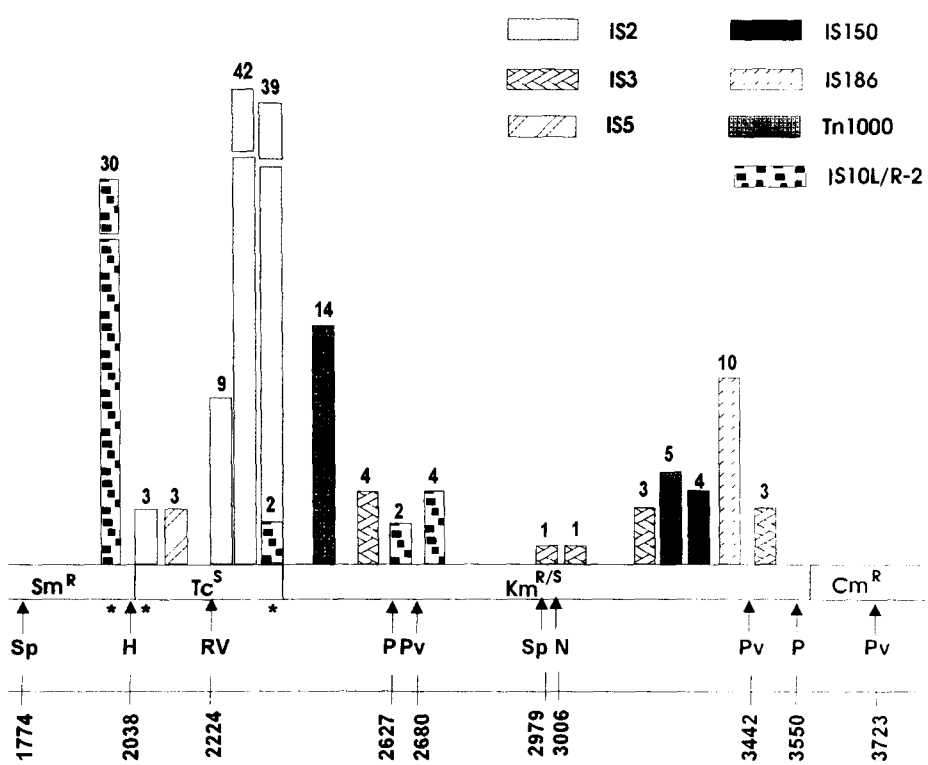

Fig. 2. The distribution of insertion sites of IS elements in the trap region of pAW1326. The locations of insertions of the IS elements listed in Table 1 were determined by restriction mapping with a precision of ±50 bp. Individual isolates falling within this narrow region were represented as a group by a vertical bar. The number of isolates forming such a group is given on the top of the bar. Bars representing large numbers of isolates are drawn interrupted. Different textures of the bars relate to the different IS elements. Some of the IS2 isolates occurred in the form of tandem repeats (Szeverényi et al., 1996). The numbers at the bottom mark the positions of the respective restriction sites. Asterisks at the bottom identify the three groups of isolates of IS2 and IS10L/R-2 from which junction sequences were determined. The IS10L/R-2 isolates located at position 2034 are not sensitive to Sm, as this segment falls outside of the resistance gene.

cultures. The frequency of mutants in individual cultures varied from 10^{-6} to 10^{-9} per viable cell.

2.3. Analysis of mutations in the trap region on the basis of resistance against Km and Cm and by restriction mapping

The distribution of the analyzed mutants according to their resistance against the two antibiotics Cm and Km was as follows: 77.2% of them were Cm^RKm^S (site of mutation within the $Km^{R/S}$ segment), 22.2% Km^RCm^R (site of mutation within the pBR322 sequence or omega segment) and there were a few (0.6%) derivatives with Cm^S phenotypes (these subclones must have lost upon propagation the Cm^R phenotype, for which primary selection had been made, but these have not been further characterized). The ratio Cm^RKm^R/Cm^RKm^S is about 1:3 and corresponds roughly to the ratio between the lengths of the two parts of the trap region (456 versus 1169 bp).

Between one and four mutant plasmids from each culture were subjected to restriction cleavage in order to determine the major type of mutation observed with the trap vector. By analyzing a total of 211 mutants from 1073 isolates various forms of DNA rearrangements were found. Eighty five percent of the mutants turned out to carry an insertion and 5% a deletion, while 7% had a point mutation or a small deletion, and 3% were other types of DNA rearrangement (this category represents illegitimate recombination and multiple rearrangements, for example substitution or insertion/inversion). The presence of a majority of insertion mutants shows that the pAW1326 vector can be used quite efficiently to trap insertions such as IS elements.

2.4. Identification of IS elements carried by insertion mutants of the trap vector and location of the insertions in the trap region

The insertion mutants have been further analyzed by determining the site and size of the insertion using restriction mapping. Identification of inserted sequences was achieved by Southern hybridization using digoxigenin-labeled probes of known IS elements of *E. coli*. The results of this hybridization in which we encountered no unexpected IS element, except for IS10L/R-2 (see below) are summarized in Table 1.

Five of the IS elements resident in the genome of *E. coli* strain JM101 were identified as a result of their insertion into the trap vector: IS2 was most frequently found and IS3, IS5, IS186 and Tn1000 less often (Table 1). With strain JM109, most of the identified insertions were IS10L/R-2, while IS3, IS150 and Tn1000 were encountered at lower frequencies. Absence of mutants caused by insertion of IS4 or IS30 might be explained by their strong target specificity and relatively low transposition rates (Mayaux et al., 1984; Caspers et al., 1984; Olasz et al., 1993). We have indeed not found significant similarity between the consensus target sequences of IS4 or IS30 and the trap region of pAW1326. The target specificity of IS1 which is present in the genome of both JM101 and JM109 in 4 or more copies (data not shown) does not give an explanation for the lack of IS1 insertions. This might perhaps rather be related to the room temperature used for the 10 days storage of the cultures, since at low temperature IS1 mainly produces adjacent deletions rather than simple transposition (Reif and Saedler, 1975). For IS5, IS150 and IS186 the statistical relevance of the data shown in Table 1 is low. However, most of these insertions were independent. Indeed, all three IS5 studied originated from independent cultures, and seven of the ten IS186 isolates were also independent.

Different IS elements showed different preferences with regard to their target regions (Fig. 2). A pronounced target specificity was observed for IS5, IS150 and IS186. The three studied independent IS5 isolates inserted into the same DNA region of the fragment of pBR322 origin, while all four IS150 inserted into a specific site in the Km^R gene. The ten isolates of IS186 were found at another single position of the Km^R gene. Three quarters of the Tn1000 insertions were carried at one site, the rest at another site of the Km^R gene. All IS2 insertions were carried in the DNA segment originating from pBR322. This can correspond to the regional specificity of IS2 in transposition (Sengstag and Arber, 1983; Sengstag and Arber, 1987; Sengstag et al., 1983). Eighty-one out of 93 IS2 isolates (87%) inserted into the same narrow region between positions 2300–2400 of the trap vector. A majority of the IS10L/R-2 elements were found in the Sm^R segment, but a few others were scattered at three other locations. All of the IS3 elements inserted into the $Km^{R/S}$ gene, but into five different regions. It was surprising that all isolated IS elements were inserted in one orientation only. This finding presumably reflects the selection constraint for either the presence or the generation of an outward directed promoter (see below).

2.5. Analysis of the *IS*10L/R-2 element isolated with the trap vector

An insertion was isolated many times independently from the JM109 strain of *E. coli*. Restriction analysis of these mutants resulted in a map different from the restriction maps of all IS elements known so far. Furthermore no DNA hybridization was detected with known residential IS elements of *E. coli*. The insertion is about 1.3 kb long and contains two *Eco*RV sites flanking a 0.6-kb fragment. This fragment was used as a template for producing DIG-labeled probes. The copy number of the unknown IS element in various strains of *E. coli* was determined by hybridization with these DIG-

Table 1
Identification of IS elements[a]

Bacterialstrain	IS2	IS3	IS5	IS150[b]	IS186	Tn1000[b]	IS10L/R-2
JM 101	11	3	0	0	5	3	0
JM 101	60	4	0	0	0	5	0
JM 101	22	4	3	0	5	5	0
JM 109	0	1	0	4	0	6	14
JM 109	0	0	0	0	0	0	24
Total	93	12	3	4	10	19	38

[a] Insertional mutants were analyzed by Southern hybridization and restriction mapping. **Methods**: Six different plasmids, each containing one known IS element of *E. coli* were kindly provided by Thierry Naas. The following inner fragments, containing solely IS-derived sequences were isolated from the plasmids: pRAB1::IS1 (*Pvu*II and *Tth*111I, 630 bp); pRAB2::IS2 (*Hin*fI, 1070 bp); pRAB2::IS3 (*Hin*dIII, 916 bp); pRAB2::IS5 (*Bgl*II and *Eco*RI, 939 bp); Raabe et al., 1988), pAW304 containing IS30 (*Bgl*II and *Hin*dIII, 656 bp; Stalder and Arber, 1989), pAW40::IS186 (*Sma*I and *Hin*dIII, 915 bp; Sengstag et al., 1986) and pAW1326::IS10L/R-2 (*Eco*RV, 600 bp; described in this paper). These fragments were used as templates in random priming reactions for producing digoxigenin-labeled probes according to the protocol of the DIG DNA Labeling and Detection Kit of Boehringer-Mannheim. DNA was purified from the Cm^R colonies, and it was bound to Hybond-N membrane (Amersham). Prehybridization, hybridization and immunological detection with IS probes were performed according to the protocol of the manufacturer.
[b] These IS elements were identified by restriction mapping.

labeled probes. The element was found in strains TG2 (Sambrook et al., 1989) (one copy) and JM109 (Yanisch-Perron et al., 1985) (1–2 copies), while it was absent from the following strains: HB101 (Boyer and Roulland-Dussoix, 1969; Bolivar and Backman, 1979), TG1 (Gibson, 1984), JM101 (Messing, 1979) and W3110 (Bachmann, 1987). The hybridization patterns of various JM109 strains obtained from different laboratories differed from each other (Fig. 3).

The partial determination of the sequence of this insertion revealed that we had isolated a hybrid of IS10L and IS10R insertion sequences (for review, see Kleckner, 1989). We thus called the element IS10L/R-2. IS10L and IS10R are parts of the composite transposon Tn10. IS10R is active in transposition processes, while IS10L is not. The outer end (o) of IS10L/R-2 is identical to the 'o' end of IS10L, and the inner end (i) of the hybrid element is identical to the 'i' end of IS10R. The sequenced

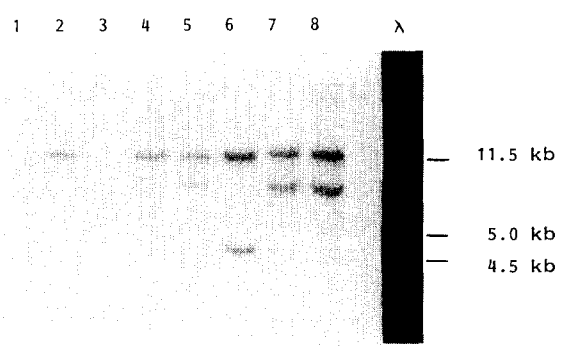

Fig. 3. Hybridization of the DIG-labeled *Eco*RV fragment from IS10L/R-2 to genomic DNA isolated from various *E. coli* strains. All DNA samples were digested with *Pvu*II before electrophoresis and blotting. Lane 1, TG1; lane 2, TG2; lane 3, JM101; lanes 4–8, JM109 from different sources: 4, from József Attila University of Szeged; 5, from Biological Research Center Hungarian Academy of Sciences; 6–8 subclones of the strain provided by Promega. λ, DNA molecular weight standard (electrophoresis pattern of λ DNA digested with *Pst*I).

part of the 0.6-kb *Eco*RV fragment of the IS10L/R-2 exhibits full sequence homology with IS10R (the corresponding sequence of IS10L is not available in data banks). The partial sequences available of IS10L/R-2 are identical to those of the corresponding segments of IS10L/R-1 (Bogosian et al., 1993), but we called our isolate IS10L/R-2, since its full identity with IS10L/R-1 was not yet investigated.

2.6. Target sites of IS elements and possible expression of reporter genes

The strategy of the use of the trap vector is based on the polar effect sometimes obtained with insertion sequences to cause expression of adjacent genes. It was thus relevant to examine the DNA sequences of junctions between inserted IS elements and the trap vector in order to determine whether new hybrid promoters were formed by the insertion of an IS element. For this analysis we chose IS2 which was most active in our studies and which was known to form hybrid promoters. On the basis of determined junction sequences we searched for the presence of hybrid promoters by computer analysis. The junctions formed between the trap vector pAW1326 and each of four independent IS2 insertions around position 2385 (see Fig. 2) turned out to be absolutely identical (Fig. 4A). This sequence displays a hybrid promoter in which the −35 sequence is provided by IS2 and the −10 sequence by the plasmid pAW1326. This putative promoter region has two mismatches in the −35 and two mismatches in the −10 promoter boxes as compared to the consensus. The spacing region between the two boxes is 17 bp long and corresponds very well to the spacing of an optimal prokaryotic promoter. Another hybrid promoter was found with an IS2 insertion carried at position 2079 (Fig. 2). It has two mismatches to the consensus in its

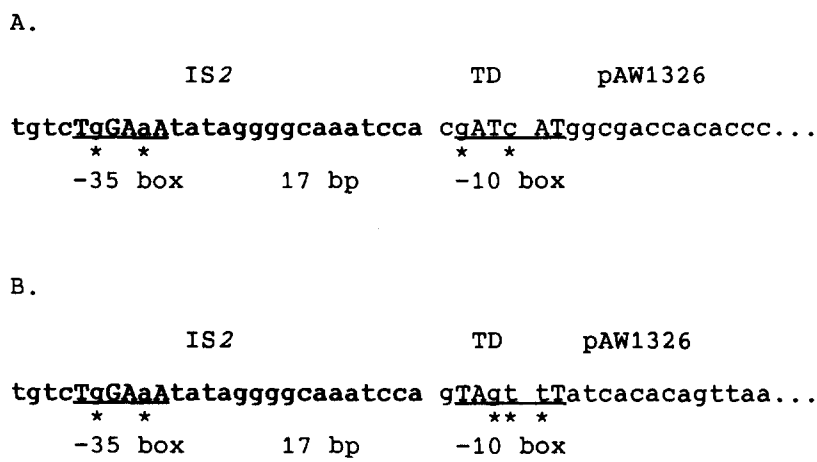

Fig. 4. Putative promoter sequences at junctions of IS2 insertions with the trap vector pAW1326. (**A**) Site of four independent IS2 insertions in the trap vector at position 2385. (**B**) Site of one IS2 insertion in the trap vector at position 2079. Bold letters correspond to IS2 sequences and normal letters to sequences of the trap vector. The junctions positions marked TD identify the 5-bp target sequences which had been duplicated by the insertion. Putative promoter boxes are underlined. Asterisks indicate mismatches to the *E. coli* promoter consensus sequence (TTGACA N_{16-18} TATAAT). Capital letters show the matches with the consensus. The criteria for identifying promoters were as follows: 1, the sequences of the −35 and −10 promoter boxes are similar to the consensus; 2, three mismatches are allowed per box, but the two boxes together should not contain more than 5 mismatches; 3, the spacing between these boxes should be 16–18 bp. *Methods*: The junctions between IS2 and plasmid DNA of five IS2 isolates were determined by sequencing. The relevant fragments were isolated and cloned into pBSK (Short et al., 1988). The IS2-plasmid junctions on the side of the Sm^R resistance marker were isolated from the first four mutants (IS2 insertion at position 2385) as *Eco*RV and *Hin*dIII fragments of ca. 660 bp length. For cloning the corresponding junction from the fifth mutant (IS2 insertion at position 2079) a 470-bp *Hin*dIII fragment was isolated. The other junctions facing the Km^R gene were cloned from the first four mutants as *Hin*cII and *Pst*I fragments of 350 bp length, and from the fifth mutant as *Hin*cII and *Eco*RV fragment of ca. 330 bp length. ^{35}S alpha dATP for sequencing was obtained from Amersham. Double stranded DNA was sequenced using the chain termination method of Sanger et al. (1977) and the Sequenase version 2.0 kit of USB. Computer analysis was performed with the GCG software-package (Devereux et al., 1984).

−35 box and three mismatches in its −10 box and the spacing between these boxes is also 17 bp (Fig. 4B).

The junction fragments containing the putative promoter and the *neo* gene were cloned in front of the *lacZ* gene in plasmids pNM480, pNM481 and pNM482 (Minton, 1984) in all three frames. The activity of the putative promoters was verified by quantitatively analyzing the beta-galactosidase activity of the NEO-βGAL fusion protein produced (data not shown).

We were not able to detect a hybrid promoter in the junction sequence of a selected IS10L/R-2 insertion at position 2034. This finding is not surprising, since it was described (Ciampi et al., 1982; for review, see Kleckner, 1989) that a promoter – called pOUT – allows efficient expression of genes located adjacent to the insertion of Tn10. The same orientation of all IS10L/R-2 insertions studied supports this explanation, and the direction of the pOUT promoter corresponds to the direction of the promoterless antibiotic resistance reporter genes.

3. Conclusions

We describe in this paper the construction and the use of a multicopy plasmid, which can serve to entrap IS elements:

(1) The strategy for the isolation of IS elements is based on a positive polar effect exerted by their insertion and bringing about the constitutive expression of promoterless antibiotic resistance genes. As compared with other types of mutation, simple transposition of IS elements is by far the most abundant (85%) cause of spontaneous mutation towards expression of the selected Cm^R marker. This together with the rapidity and simplicity of the experimental procedure renders this selection strategy useful for the isolation of IS elements, although the overall mutation rate to the selected phenotype is relatively low (10^{-6} to 10^{-9} antibiotic resistant mutants per viable cell).

(2) The positive polar effect of an IS element on which selection is based can depend on two factors: the target specificity of the insertion sequence and the ability to promote constitutive expression of genetic determinants located downstream of the site of insertion. The first condition is fulfilled by IS elements with low or regional target specificity. The second condition can be satisfied by IS elements, which have either a complete promoter region facing outward from the element (like IS10, Ciampi et al., 1982 and IS10L/R-2) or the ability to form a fusion promoter upon insertion like IS2 (Saedler et al., 1974). These factors define the possibility for the isolation of an individual insertion sequence. In our vector-system 6 of 9 IS elements of *E. coli* fulfilled the two conditions, namely IS2, IS3, IS5, IS150,

IS186 and Tn1000 (Fig. 2). The lack of detection of IS4 and IS30 can be explained by their characteristic high target specificity, that of IS1 by its low rate of simple transposition at room temperature.

(3) The IS element IS10L/R-2 was the most frequently isolated insertion in strain JM109. It is a hybrid derivative of the IS10L and IS10R elements and it is similar or identical to the IS10L/R-1 element described previously (Bogosian et al., 1993). It was found in strains TG2 and JM109, which had been submitted to Tn10 mutagenesis (Tn10 is a composite transposon of IS10L and IS10R). The different RFLP patterns of IS10L/R-2 in various subclones of JM109 (Fig. 3) together with the relatively frequent simple transposition into pAW1326 indicate that this IS10L/R-2 element is quite active in *E. coli*.

(4) A significant difference was detected in the occurrence of IS elements isolated from JM101 and JM109 strains (Table 1). Most strikingly a high number of IS2 were isolated from strain JM101 (93 isolates out of 130), but none from strain JM109 (total 49 IS isolates), despite of the fact that IS2 is present at least in four copies in JM109. This significant difference could be interpreted as probably the genetic background of the strain JM109 did not allow the simple transposition of IS2. It was published earlier that some IS elements [Tn5, for review, see Berg (1989), and IS30 (Olasz et al., 1993)] prefer a *recA*$^+$ genetic background for their efficient transposition. In fact the strain JM109 is *recA*$^-$, while the strain JM101 is *recA*$^+$. We can not exclude the possibility that other differences between the strains JM101 and JM109 (*endA1*, *hsdR17*, *gyrA96* and *relA1*) could play a dominant role in the transposition of IS2. The use of the pAW1326 direct selection system could be very helpful for studies on the role of the genetic background and/or interactions between IS elements in the transposition of insertion sequences.

All of our data suggest that pAW1326 can serve as an efficient trap vector for the isolation of IS elements. The construction of analogous vectors applicable to both Gram-negative and Gram-positive bacteria is in progress.

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