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Repression of IS*200* transposase synthesis by RNA secondary structures

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ABSTRACT

The IS*200* transposase, a 16 kDa polypeptide encoded by the single open reading frame (ORF) of the insertion element, has been identified using an expression system based on T7 RNA polymerase. In wild-type IS*200*, two sets of internal inverted repeats that generate RNA secondary structures provide two independent mechanisms for repression of transposase synthesis. The inverted repeat located near the left end of IS*200* is a transcriptional terminator that terminates read-through transcripts before they reach the IS*200* ORF. The terminator is functional in both directions and may terminate >80% of transcripts. Another control operates at the translational level: transposase synthesis is inhibited by occlusion of the ribosome-binding site (RBS) of the IS*200* ORF. The RBS (5'-AGGGG-3') is occluded by formation of a mRNA stem–loop structure whose 3' end is located only 3 nt upstream of the start codon. This mechanism reduces transposase synthesis ~10-fold. Primer extension experiments with AMV reverse transcriptase have provided evidence that this stem–loop RNA structure is actually formed. Tight repression of transposase synthesis, achieved through synergistic mechanisms of negative control, may explain the unusually low transposition frequency of IS*200*.

INTRODUCTION

IS*200* is an insertion sequence of 707–710 bp, originally found in the genome of *Salmonella typhimurium* LT2 (1). IS*200* elements are abundant in the genus *Salmonella*, both on the chomosome and on plasmids (1–6). Insertion elements with various degrees of relatedness to IS*200* have also been described in *Shigella* (3), *Escherichia coli* (4), *Yersinia* (7,8), *Vibrio* (9) and *Clostridium* (10). Furthermore, a recent survey of IS structure and organisation has suggested that IS*200* may be related to the IS*605* element of *Helicobacter pylori* (11).

A long-known and enigmatic trait of IS*200* behaviour is its poor contribution to spontaneous mutagenesis (12,13), which can be correlated with the extremely low transposition frequency of the element (14,15). In fact, only a few IS*200* insertion mutations have been characterised in *S.typhimurium*: the *hisD984* mutation whose analysis led to the discovery of IS*200* (1), an insertion in the *gpt* gene (13), and an insertion in the *pef* operon of the pSLT virulence plasmid (6). Hunts for IS*200*-induced mutants in *S.typhimurium*, sometimes involving positive selection strategies, have confirmed that transposition is rare (12–15). Furthermore, surveys carried out in field isolates have indicated that IS*200* transposition is likewise infrequent in natural populations of *Salmonella* (5).

Previous studies have shown that IS*200* elements from *Salmonella* have a highly conserved structure (6,16). Relevant traits shared by all IS*200* copies are the absence of terminal repeats, direct or inverse (6), and the presence of an open reading frame (ORF) encoding a putative peptide of 152 amino acids (6,16,17). This ORF is driven by a weak promoter located between nucleotides 1–41 (15). Structural analysis has also shown that the 'left' end of IS*200* contains two sets of inverted repeats, which are highly conserved among IS*200* copies. Upon transcription, the repeats can be expected to form a hairpin and a stem–loop (6).

This study shows that the internal inverted repeats of IS*200* behave as regulatory signals which repress synthesis of the single IS*200*-encoded protein, the putative transposase. The hairpin located near the IS*200* left end acts as a transcriptional terminator which terminates impinging transcripts, while the stem–loop element located near the beginning of the IS*200* ORF occludes the ribosome-binding site of the IS*200* transposase gene. The existence of signals for both transcriptional termination and translational repression, combined with the weakness of the IS*200* transposase promoter (15), seem to bring an explanation for the extremely low transposition frequency of IS*200*.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Escherichia coli DH5α (18) was used for cloning purposes. LT2 was used as the wild-type strain of *S.typhimurium*. *Salmonella abortusovis* SAO44 (19) was provided by S. Rubino, Istituto di Microbiologia, Università degli Studi di Sassari, Sassari, Italy. Unless indicated otherwise, bacteria were grown in LB at 37°C. When necessary, antibiotics were used at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 20 μg/ml.

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Plasmids

Plasmid pIZ44 is a pUC9 derivative bearing a *Hinc*II fragment of the *S.typhimurium* chromosome which includes the insertion *hisD984*::IS*200* (3). pIZ50 is a derivative of pBluescript II SK(+) carrying a 1 kb *Stu*I–*Ssp*I fragment of pIZ44 cloned in the *Sma*I site (15). The cloned fragment includes the whole IS*200* element and flanking *his* regions. pIZ894 carries a *Sal*I–*Sac*I fragment of pIZ50 cloned in the multiple cloning site (MCS) of pT7-6 (20). The construction places the single IS*200* ORF, including the stem–loop-generating structure, under the control of the T7 promoter. To obtain a mutated version of IS*200* unable to form the mRNA stem–loop, a 256 bp *Hin*dIII–*Eco*RI fragment which included the stem-forming repeats and 154 bp of the IS*200* ORF was cloned onto pBluescript II SK(+) digested with the same enzymes. The resulting plasmid, pIZ92, was used in site-directed mutagenesis using the single-strand procedure of Bio-Rad (Richmond, CA) to generate a 70 bp deletion in the stem–loop region. The primer used was 5'-CGACGAATC-GATCTGCA-AAAGTTCA-3'. The resulting plasmid was designated pIZ94. The deletion was transferred to the original *IS200* insertion by cloning a *Sal*I–*Eco*RI fragment of pIZ94 onto pIZ50 digested with the same enzymes; this was the origin of pIZ803. Plasmid pIZ895 is a pT7-6 derivative that carries the ORF of IS*200* but only one of the stem-forming repeats. This plasmid was generated by cloning the *Sal*I–*Sac*I fragment of pIZ803 onto pT7-6. For expression experiments, plasmids were electroporated into a derivative of *S.typhimurium* LT2 carrying pGP1-2, a plasmid which bears the T7 RNA polymerase gene under the control of the P_L promoter of bacteriophage λ, and the *cI* gene transcribed from p_{tac} (20). When grown in LB with IPTG at 30°C, the lambda repressor prevents T7 RNA polymerase synthesis. To induce the system, the strains are grown at 42°C without IPTG (20).

The terminator-probe vector pIC551 carries the P_R promoter of bacteriophage λ driving transcription of a *lac*Z gene in which the native signals for translation initiation have been replaced by those of *galK* (21). pIC551 carries a MCS between the promoter and the reporter *lacZ* gene. pIZ882 and pIZ883 are derivatives of pIZ551 which carry the 194 bp *Ssp*I–*Hin*dIII fragment of pIZ44 (3), made blunt with Klenow DNA polymerase and cloned onto the *Sma*I site in either orientation.

pIC552 is a promoter-probe vector that carries a *galK'::'lacZ* fusion (as above) located downstream of a MCS (21). The MCS is preceded by elements which prevent read-through transcription, the Ω interposon, and stop codons in every frame. Plasmid pIZ1500 carries the *Sal*I–*Eco*RV fragment of pIZ44 cloned onto pIC552, previously digested with *Xho*I and *Sma*I (15).

pIZ888 is a vector for translational fusions created by replacing the *Eco*RI–*Bam*HI fragment of pIC551 with a 95 bp *Eco*RI–*Bam*HI fragment from pRS229 (22). The replacement eliminates the P_R promoter of bacteriophage λ and introduces the *lac* UV5 promoter. pIZ891 carries an in-frame translational fusion between the first 154 bp of the ORF of IS*200* (which include the stem–loop-generating repeats) and a fragment of the *lacZ* gene. This plasmid was constructed by cloning a *Sma*I–*Xho*I fragment of pIZ92 onto pIZ888 digested with the same enzymes. pIZ892 was constructed by replacing the *Sma*I–*Xho*I fragment of pIZ888 with the *Sma*I–*Xho*I fragment of pIZ94. pIZ892 carries the same translational fusion as pIZ891 but contains only one of the inverted repeats which give rise to the stem–loop structure. β-galactosidase assays were performed in strains which carried either pIZ891 or pIZ892 and the *lacI*^{q}-carrying plasmid pIZ227 (23).

DNA manipulations

Plasmids were extracted by the alkaline lysis method (24). Enzymes were purchased from Boehringer Mannheim. DNA transformation and electroporation were performed using standard methods (25).

β-galactosidase assays

Cells were grown in LB broth until mid-exponential phase. Assays were performed by the method of Miller (26).

RNA manipulations

RNA was extracted with a modification of the guanidium isothiocyanate–phenol method (27). The oligonucleotide 5'-CTGC-CTACTGCCCTACGCTTCT-3', complementary to nucleotides 235–256 of IS*200* (6; EMBL accession number X56834), was end-labelled with [γ-^{32}P]ATP, and annealed to 100 µg of total RNA prepared from *S.typhimurium* LT2 bearing plasmid pIZ1500. The oligonucleotide used is complementary to an internal region of the single IS*200* ORF (6). For annealing, 10^5 c.p.m. of the oligonucleotide were used. cDNA was synthesised using AMV reverse transcriptase, as described elsewhere (28). The products of reverse transcription were analysed in urea–polyacrylamide sequencing gels. For auto-radiography, gels were exposed to a Kodak Biomax MR film.

In vivo radioactive labelling

For protein expression experiments, strains carrying plasmid pGP1-2 and either of the plasmids pT7-6 and pIZ895 were grown up to an OD_{600} = 0.4 in LB with IPTG (1 mM) at 30°C. The cultures were washed twice in E minimal medium with the corresponding antibiotics and incubated at 42°C for 90 min. Rifampicin (200 µg/ml) was then added to the cultures and incubation at 42°C was continued for 30 min. Ten mCi of ^{35}S-labelled methionine and cysteine (labelling grade L, Amersham) were added to each culture, and the preparations were incubated during 5 min at 37°C. The cells were then collected by centrifugation and lysed with 4% SDS.

Protein electrophoresis

Cellular extracts (5–10 µl) were loaded on polyacrylamide–SDS gels (29) and run at 15–20 mA in the stacking gel and 30–35 mA in the separating gel. Gels were dehydrated with 1% glycerol and 10% acetic acid, and dried in a Slab Gel Dryer, model SE-1160 (Hoeffer, San Francisco, CA) during 1 h at 80°C. Radioactively labelled material was detected by exposure to a Kodak Biomax X-ray film.

RESULTS

Transcription termination upstream of the IS*200* ORF

A highly conserved structure found in all IS*200* elements is an inverted repeat near the 'left' end (Fig. 1). If transcribed, the inverted repeat can be expected to give rise to the typical hairpin structure of a Rho-independent transcriptional terminator, with a predicted ΔG_0 = –14.1 kcal/mol (6). In fact, it is well known

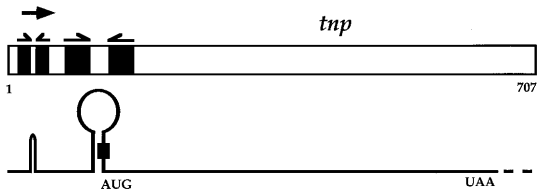

Figure 1. A diagram of IS*200*. The element is 707–710 bp long and the single ORF encompasses three-fifths of its length (456 bp). The transcriptional terminator located upstream of the transposase gene (nucleotides 9–32) is shown as a hairpin, and the translational repressor (nucleotides 66–85 and 120–139) is shown as a stem–loop that occludes the ribosome-binding site (indicated as a black box in the right strand of the stem). The coordinates given are for the IS*200* copy inserted in the *hisD* gene of *S.typhimurium* (*hisD984*::IS*200*) and may vary slightly among different copies of IS*200* (6). The arrow indicates the location of the promoter of the transposase gene.

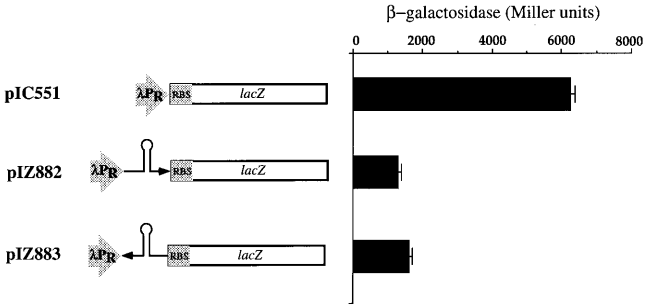

Figure 2. Ability of the IS*200* hairpin to terminate transcription. β-galactosidase activities of plasmids pIZ882 and pIZ883, compared with a control which lacks the hairpin-forming fragment (the vector pIC551). Data are averages of three independent experiments.

that insertions of IS*200* are polar at least in one orientation, and this polarity depends on a DNA stretch which includes the hairpin-forming structure (30).

To examine whether the leftmost inverted repeat of IS*200* had the properties of a transcriptional terminator, a 194 bp DNA fragment including the hairpin-forming structure was probed in the terminator-test vector pIC551 (21). This expression system relies on the P_R promoter of bacteriophage λ, located upstream of a reporter *lacZ* gene preceded by an MCS. A linker containing a Rho-independent transcription terminator is present upstream of the P_R promoter to stop transcription from external (plasmid) promoters (21). This design permits unequivocal detection of transcriptional terminators: if a DNA stretch containing a transcriptional terminator is cloned in the MCS, the presence of the terminator between the promoter and the *lac*Z gene will prevent RNA polymerase access into *lac*Z; hence a decrease in β-galactosidase activity will be observed (21). Plasmids pIZ882 and pIZ883 bear the 23 bp hairpin-forming sequence cloned in the *Sma*I site between P_R and *lac*Z (each plasmid in one orientation). Figure 2 shows the β-galactosidase activities of these constructs compared with a control that lacks the hairpin-forming fragment. While the latter yielded high levels of β-galactosidase, plasmids pIZ882 and pIZ883 showed an 80% reduction of β-galactosidase activity. Thus the DNA sequence tested has a strong terminator activity in both orientations. We therefore expect that, in its natural location, this structure will act as a transcriptional terminator, avoiding read-through transcription from genes located upstream of the IS*200* ORF. To our knowledge, this is the first description of a transcriptional terminator located upstream of a transposase gene. RNA structures that regulate transposase synthesis by a different mechanism have been described for IS*10* (31) and IS*50* (32), and will be discussed below. Hindrance of ingoing transcription may also operate in other transposable elements (11). The fact that the IS*200* hairpin acts as a terminator in both directions (5'-3' and 3'-5') explains the observed polarity of the insertion *hisD984*::IS*200* (30). It is worth noting that the termination of transcription detected by these authors (30) corresponds to the 'unnatural' situation exemplified by plasmid pIZ883, because transcription of *hisD* in the *hisD984*::IS*200* insertion runs opposite to the direction of transcription of the IS*200* ORF (6).

Repression of IS*200* mRNA translation

Downstream of the hairpin structure discussed above, two additional, larger inverted repeats are found (Fig. 1). When transcribed, these repeats can give rise to a stem–loop structure with a predicted ΔG_0 = –24.8 kcal/mol (6). The rightmost repeat of this structure finishes 3 bp upstream of the start codon of the IS*200* ORF. If formed, the stem of the structure can be expected to occlude the predicted ribosome-binding site of the ORF (6).

To examine whether this stem–loop structure affected translation of the IS*200* ORF, translational *lac* fusions were constructed on plasmid pIZ888. This vector carries a *lac*Z gene (without its own transcriptional and translational initiation signals) under the control of the *lac* UV5 promoter, and an MCS located between the promoter and the *lacZ* gene. Use of the *lac* UV5 promoter instead of the IS*200* native promoter is justified because the latter yields β-galactosidase levels which are too low to permit easy detection of differences in expression (15). For cloning, a 256 bp *Eco*RI–*Hin*dIII fragment carrying the stem–loop structure of IS*200* and the initial 154 bp of the IS*200* ORF was introduced in the MCS of pIZ888, to generate pIZ891. Using site-directed mutagenesis, the left arm of the stem and half of the loop sequence were deleted, and the resulting DNA fragment was cloned in the same site of pIZ888 to obtain pIZ892. Plasmids pIZ888, pIZ891 and pIZ892 were examined for their ability to direct synthesis of β-galactosidase in the presence of IPTG. The host strain was an LT2 derivative bearing a *lacI*^{q} allele on plasmid pIZ227 (23). Relevant results, summarised in Figure 3, were as follows: (i) plasmid pIZ888 (the vector without cloned DNA) gave nearly undetectable levels of β-galactosidase activity. (ii) pIZ891, in which the *lacZ* gene is preceded by the stem–loop-generating repeats of IS*200*, yielded low levels of β-galactosidase. (iii) pIZ892, which contains the ribosome binding site and the start codon of the IS*200* ORF but cannot form the stem–loop structure, yielded a β-galactosidase activity 10-fold higher than pIZ891.

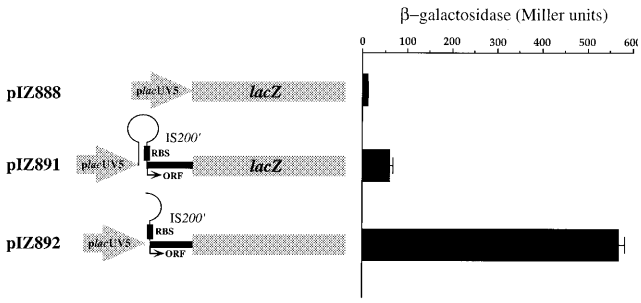

Figure 3. Repression of translation by the IS*200* stem–loop. Removal of one of the stem-forming repeats by site-directed mutagenesis causes a 10-fold increase in the expression of a IS*200* ORF::*lacZ* fusion (compare plasmids pIZ891 and pIZ892). Data are averages of three independent experiments.

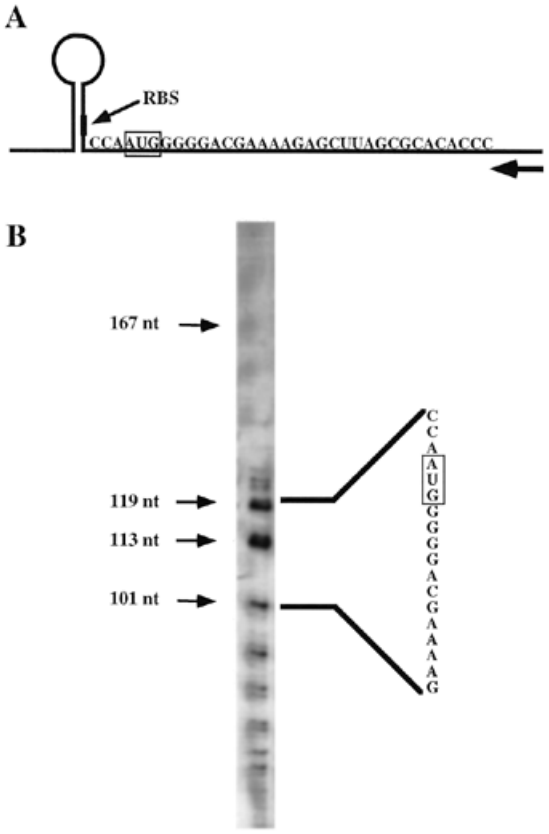

Figure 4. Reverse transcription analysis of IS*200* mRNA. (**A**) A diagram of the 5' end of IS*200* mRNA, showing the predicted stem–loop structure that occludes the ribosome-binding site (RBS). The first AUG of the coding sequence is shown inside a box. The arrow indicates the relative position of the labelled primer used in the AMV reverse transcriptase assay. (**B**) Total RNA isolated from *E.coli* DH5α/pIZ1500 was annealed with a 5'-labelled oligonucleotide complementary to the coding sequence of IS*200* and extended with AMV–RT. Plasmid pIZ1500 carries a IS*200*::*lacZ* fusion which includes the leftmost 382 nt of the IS. The extension temperature was 44°C. The longest extension product obtained (167 nt) mapped 2 nt downstream of the stem–loop. The bands were accurately mapped using the molecular weight marker VIII from Boehringer, as well as sequencing reactions run in parallel. The arrows indicate the sizes of some of the extension products obtained.

These data suggest that the stem–loop structure is actually formed *in vivo*, and that it represses translation of any protein-coding sequence starting at the ATG located immediately downstream of the stem.

Formation of the RNA stem–loop

Results presented in the former section suggested that the predicted stem–loop structure that precedes the start codon of the IS*200* ORF was actually formed *in vivo*: removal of the structure by site-directed mutagenesis caused a 10-fold increase in β-galactosidase synthesis. Direct evidence for the formation of such a structure was provided by RNA analysis. It is known that RNA regions with highly stable secondary structure, if used as templates for reverse transcription, can cause premature termination of RNA synthesis by AMV reverse transcriptase (33,34). In these cases, the 5' end of the shorter, prematurely terminated cDNA molecules maps at the bottom of the structure (34). To investigate whether the IS*200* RNA stem–loop terminated AMV–RT synthesis, total RNA was isolated from *S.typhimurium* cells bearing plasmid pIZ1500 and reverse primer extension of 100 μg of total RNA was carried out, using an end-labelled oligonucleotide complementary to the IS*200* ORF. Figure 4 shows that >90% of the extended products had their 3' end downstream of the predicted structure, and that the longest of them finished exactly 2 nt downstream of the 3' end of the stem. Smaller products with 5' ends at 6, 12 and 20 nt downstream of the start codon were also detected. No other band of similar intensity was observed with normal exposure times. No band was observed after primer extension of the same amount of total RNA isolated from a strain lacking plasmid pIZ1500 (not shown). In an attempt to destabilise the double-stranded RNA structures formed, the extension was repeated at three different temperatures, 44, 50 and 60°C (the highest temperature compatible with AMV–RT activity), but no differences in the band pattern were observed (data not shown). This was not unexpected, since Zucker's algorithm (35) predicts a T_m of 80°C for the RNA stem, far above the highest temperature used in our assays. Similar results were obtained when the reverse primer extension assays were carried out on RNA templates from *S.typhimurium* LT2 and *S.abortusovis* SAO44, although the intensity of the bands was lower (data not shown). We thus conclude that the predicted mRNA stem–loop is actually formed, and that it represses translation of the IS*200* ORF. Confirmation of this model will require further experiments of site-directed mutagenesis, to introduce mutations that perturb formation of the stem–loop structure but do not remove large amounts of genetic material.

Cloning and expression of the IS*200* ORF

To ascertain whether the IS*200* ORF was indeed capable of directing protein synthesis, we cloned an IS*200* fragment containing the ORF in the MCS of the expression vector pT7-6 (20). The ORF had been previously mutagenised to delete one of the stem-forming repeats located upstream of the ORF. In

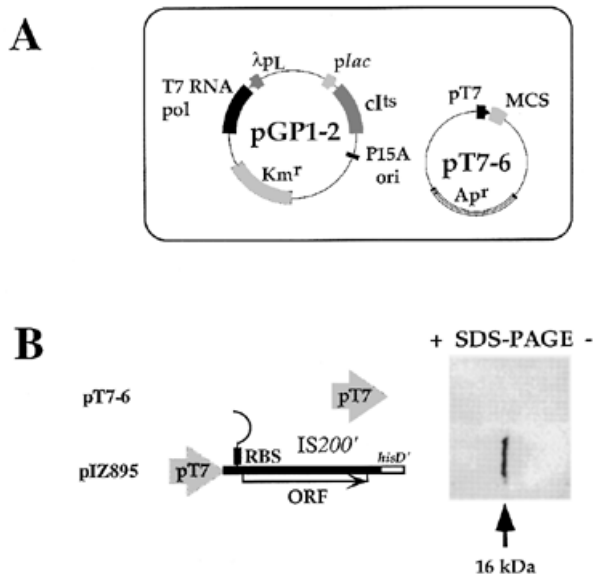

Figure 5. Detection of the product of the IS*200* ORF. The two-plasmid expression system used for detection is depicted in (**A**) (see Materials and Methods for a detailed description). (**B**) pIZ895 is a pT7-6 derivative which expresses the IS*200* ORF without the stem–loop structure. The ^{35}S-labelled 16 kDa IS*200* product was separated by SDS-PAGE and detected by autoradiography. The size of the protein product was inferred from a protein size marker run in parallel (not shown). Protein expression from plasmid pT7-6 without insert was used as a negative control.

the resulting plasmid, pIZ895, the ORF was placed under the control of the strong T7 promoter present in pT7-6 (20). When supplied with T7 RNA polymerase (provided in *trans* by plasmid pGP1-2), pIZ895 was able to direct rifampicin-resistant synthesis of a major, 16 kDa polypeptide (Fig. 5). The autoradiography shows the only radiolabelled protein detected upon a pulse–chase with a mixture of methionine and cysteine labelled with ^{35}S after incubation in the presence of rifampicin. The size of the protein detected (16 kDa) agrees with the size of the IS*200* ORF, which predicts a peptide of 152 amino acids. Therefore, we conclude that the ORF present in IS*200* does encode the predicted 16 kDa protein. We have designated this protein TnpA (transposase), on the following grounds: (i) overproduction of this protein increases IS*200*-associated rearrangements including transposition in stab cultures (14,15); (ii) relevant homology is found between the IS*200* ORF and the transposase of insertion element IS*1005* (9). Unfortunately, the rarity of IS*200* transposition has so far hampered a direct survey of TnpA function (e.g. mutations in *tnpA* should render a non-transposing element). Parallel experiments using pIZ894, a pT7-6 derivative in which the IS*200 tnpA* gene is also transcribed from a T7 promoter but is preceded by its original translational signals (including the stem–loop structure), yielded lower levels of the 16 kDa product. Densitometric analysis (not shown) indicated that the difference was at least 10-fold.

DISCUSSION

Most, if not all, prokaryotic insertion elements undergo a tight control of transposase synthesis, which can be viewed as part of a self-restraint strategy and may provide evolutionary advantages (36). Control of transposase synthesis is achieved through a variety of mechanisms: negative control of the transposase promoter by *trans*-acting factors encoded by the insertion element (37,38) or by the host (39); repression of the transposase promoter by DNA methylation (40); inhibition of transposase mRNA translation by antisense RNA (41); programmed translational frameshifting (42); and assembly of a transposase promoter upon programmed DNA rearrangements (43). Mechanisms that avoid uncontrolled transposase synthesis resulting from impinging transcription are also known (31,32), and will be discussed below.

IS*200* is a transposable element with extreme self-restraint, as indicated by the scarcity of IS*200*-induced mutations (12,13), the low frequency of IS*200*-induced rearrangements (14,15) and the constancy of IS*200* fingerprints in natural populations (5). This behaviour correlates with the low expression of the single IS*200* ORF. One cause of this low expression is the existence of a weak promoter (15), a feature common to most transposase genes. In addition, this study shows the existence of two additional mechanisms which contribute to keep IS*200* transposase synthesis low: the termination of read-through transcripts and the repression of transposase synthesis by an mRNA secondary structure. The DNA sequence elements that embody these structural motifs are as follows: (i) the inverted repeats located near the 'left' end of IS*200* (nucleotides 9–32 in the *hisD984*::IS*200* copy: see ref. 6) encode a transcriptional terminator active in both orientations. Its structure is reminiscent of that of a Rho-independent terminator (44), but its independence from Rho has not been tested. This terminator is not located at the end of any gene but upstream of the IS*200* transposase gene, and terminates >80% of impinging transcripts. Interestingly, this region overlaps with the IS*200 tnpA* promoter, but the significance of this fact (if any) has not been analysed. (ii) The larger inverted repeats located downstream of the IS*200 tnpA* promoter (nucleotides 66–85 and 120–139 in the *hisD984*::IS*200* copy: see ref. 6) give rise to a stem–loop structure which represses translation of the *tnpA* gene. The stability of the stem–loop is such that the structure remains double-stranded at 60°C *in vitro*.

If transcriptional termination prevents >80% of read-through transcripts from reaching the transposase gene and translational repression decreases 10-fold the efficiency of translational initiation, any impinging transcript which escapes the terminator will encounter the translational inhibitor, and the combination of two separate hurdles will secure repression. Of course, transcripts initiated at the *IS200 tnpA* promoter will encounter only one hurdle, the mRNA stem–loop, but the combination of low transcription levels and efficient translational repression can be expected to allow transposase synthesis only rarely. Interestingly, a mechanism to inhibit transposase synthesis from impinging transcripts has been previously described in both IS*10* and IS*50* (31,32). The mechanism is based upon formation of an RNA stem–loop structure which sequesters translation initiation signals. Shorter transcripts initiated at the transposase promoter lack one of the repeats required to make the stem, which is thus not formed (31,32). Therefore these

insertion elements make use of translational inhibition to counter the effects of inward transcription, while IS*200* has two separate mechanisms for transcription termination and repression of translation. This design appears efficient both for avoiding uncontrolled transposase synthesis and for keeping transposase levels low even when transcripts are initiated at the IS*200* promoter. In fact, unequivocal detection of the 16 kDa polypeptide predicted by sequence analysis required both the use of a heterologous promoter and the removal of the stem–loop mRNA structure by site-directed mutagenesis.

A question unanswered in this study is whether the translational repression exerted by the mRNA stem–loop can be lifted under specific circumstances (in other words, whether cellular or environmental signals can trigger IS*200* transposition by disrupting the stem–loop). The possibility that IS*200* transposition is regulated receives support from the old and enigmatic observation that IS*200* undergoes 'bursts' of transposition in stab cultures kept at room temperature for months or years (1,15). These bursts are not a consequence of IS*200* 'activation' by mutational events (6). Mechanisms for disruption or removal of RNA secondary structures have been discussed in the literature (45–47), and the possibility that they operate in IS*200* cannot be ruled out. However, trials using a *lac* reporter gene fused to the IS*200* ORF have so far failed to reveal any laboratory conditions in which expression is increased (C.R.Beuzón and J.Casadesús, unpublished results). A caveat is that laboratory conditions may not reproduce the natural situations which could induce IS*200* transposition. An alternative view, that IS*200* is a transposable element with intrinsically low activity, is supported by the observation that IS*200* transposition is infrequent not only in the laboratory but also in natural populations of *Salmonella* (5). According to Doolittle *et al.* (36), a strategy of this kind will minimise the risk of causing mutations detrimental to the host, thereby favouring the maintenance of the element in natural populations. IS*200* may fit in this scenario because sequence comparisons indicate that the element is extremely old (4,6,16). The possibility that the evolutionary endurance of IS*200* has been favoured by the possession of tight mechanisms of transposase control is an attractive speculation.

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