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Negative Regulation of IS*2* Transposition by the Cyclic AMP (cAMP)-cAMP Receptor Protein Complex

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Three sequences similar to that of the consensus binding sequence of the cyclic AMP (cAMP)-cAMP receptor protein (CRP) complex were found in the major IS*2* promoter region. Experiments were performed to determine whether the cAMP-CRP complex plays a role in the regulation of IS*2* transposition. In the gel retardation assay, the cAMP-CRP complex was found to be able to bind the major IS*2* promoter. A DNA footprinting assay confirmed that the cAMP-CRP complex binds to the sequences mentioned above. With an IS*2* promoter-luciferase gene fusion construct, the cAMP-CRP complex was shown to inhibit transcription from the major IS*2* promoter. IS*2* was found to transpose at a frequency approximately 200-fold higher in an *Escherichia coli* host defective for CRP or adenyl cyclase than in a wild-type host. These results suggest that the cAMP-CRP complex is a negative regulator of IS*2* transposition.

The insertion sequence IS*2* is a member of the IS*3* family (56, 65, 66). It is 1,331 bp in length, with a pair of 42-bp imperfect inverted repeats (18, 26, 64). The IS*2* genome contains five open reading frames (ORF1 to -5) of greater than 50 amino acids; however, only two IS*2*-encoded proteins, of 14 and 46 kDa, have been detected (27, 28). The 14-kDa protein, referred to as the InsA protein (27), is encoded by ORF1. The 46-kDa protein is designated InsAB′. It is encoded by ORF1 and ORF2 via a −1 frameshift mechanism (28) at the frameshift signal AAAAAAG, which is located between the 3′ end of ORF1 and the 5′ end of ORF2 (8, 28, 56). The mRNAs encoding both proteins are transcribed from the promoter located within the left inverted repeat (LIR) of IS*2*. This promoter has been shown to be the major promoter of IS*2* (27). The production of InsAB′ by a −1 frameshift mechanism appears to be a general phenomenon in members of the IS*3* family, because it also occurs in IS*150* (56, 78), IS*911* (55, 56), and IS*3* (69).

The 14-kDa InsA is a DNA binding protein. It binds to the sequence 5′-TATCACTTAAATAAGTGATA-3′ (27), which is located around the −10 sequences of the major IS*2* promoter (Fig. 1). Since this promoter is responsible for the expression of both InsA and InsAB′, binding of InsA to this sequence may affect transcription. This notion is supported by the observation of a decrease in IS*2* transposition when InsA is overexpressed (27). InsA functions as a homodimer. Dimerization of InsA takes place at the C-terminal end of the molecule, whereas the DNA binding domain of InsA is located at its N terminus (38).

The 46-kDa InsAB′ protein has typical transposase motifs WxxD (36), N3 (61), and C1 (41, 60), collectively known as the DDE motif (20, 36, 54), located at its C terminus (28) and a helix-turn-helix DNA binding motif, TVSLVARQHGVAA SQLFLWR, located at its N terminus (amino acid positions 31 to 50) (38). InsAB′ has the ability to bind both terminal repeats of IS*2* (28). Overexpression of InsAB′ has been shown to increase IS*2* transpositional recombination and formation of two transpositional products, IS*2* minicircles and figure eight molecules (39). These observations suggest that InsAB′ is a transposase of IS*2*.

It is possible that host factors may affect IS*2* transposition. Examination of the nucleotide sequence of the IS*2* promoter revealed three putative cyclic AMP (cAMP)-cAMP receptor protein (CRP) complex binding sequences located in the major IS*2* promoter (Fig. 1). Since cAMP-CRP is a global transcriptional regulator which may activate or inactivate gene expression (34, 50, 62, 63), we investigated the possible role of cAMP-CRP in the regulation of IS*2* transposition.

MATERIALS AND METHODS

PCR. PCRs were performed in a 100-μl mixture containing 10 ng of template DNA, PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100), 20 pmol of each PCR primer, 0.2 mM (each) deoxynucleoside triphosphate, and 2 U of *Taq*I DNA polymerase. Temperature cycling for PCR included 1 cycle of 95°C for 5 min; 25 cycles of 95°C for 1 min, 48°C for 1 min, and 72.5°C for 2 min; and a 10-min extension at 72.5°C. The PCR products were electrophoresed on a 1.0% agarose gel to determine the sizes of the amplified products.

Gel retardation assay. Cell lysates of *Escherichia coli* JM109(DE3) (73) containing pSK^-CRP (Fig. 2A), pT7-7 (74), or pT7insA (27) were used as the sources of DNA binding proteins in the gel retardation assay. These cell lysates were prepared by repeated freezing-thawing and sonication of cells from rifampin-treated cultures as described previously (27, 38). Aliquots of clarified cell lysates containing various concentrations of protein were used. The gel retardation reaction mixture contained the cell lysate, a ^{32}P-labeled DNA fragment, 50 mM Tris-HCl (pH 7.4), 70 mM KCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, 7 mM $MgCl_2$, 3 mM $CaCl_2$, 10% glycerol, 25 μg of herring sperm DNA, and 200 μg of bovine serum albumin/ml in a total volume of 25 μl. The reaction mixture was incubated at room temperature for 25 min. After the addition of 5 μl of a DNA electrophoresis loading buffer (1 μg of bovine serum albumin/ml, 50% glycerol, 0.01% xylene cyanol) to each reaction mixture, the mixtures were electrophoresed on a 5% native polyacrylamide gel (16). Retarded protein-DNA complex bands were visualized by autoradiography of the gel.

In situ DNA footprinting. The DNA-protein reaction mixtures described above were electrophoresed on a 5% native acrylamide gel. After being washed with 200 ml of 50 mM Tris-HCl (pH 8.0) solution, the whole gel was soaked at room temperature for 8 min in an in situ DNA footprinting solution containing 1 mM 1,10-phenanthroline, 0.225 mM $CuSO_4$, and 29 mM 3-mercaptopropionic acid as described previously (27, 37). The gel was washed with water and then exposed to an X-ray film to detect protein-bound DNA bands. The gel was then aligned with the autoradiogram, and the portions of the gel containing the bands were isolated. The DNA present in the gel slices was eluted by soaking the gel in 0.5 ml of a solution of 0.5 M ammonium acetate and 1 mM EDTA overnight. The eluted DNA was precipitated with ethanol and then electrophoresed on a 6% DNA sequencing gel.

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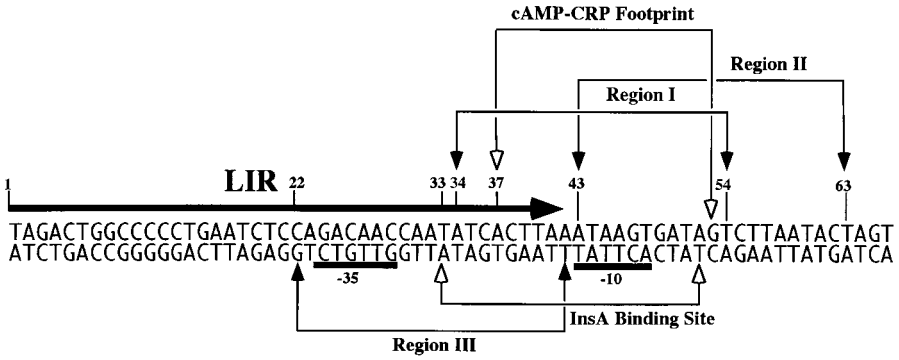

		Matches to consensus
Region I	ATCACTTAAATAAGTGATAGT	7/13
Region II	ATAAGTGATAGTCTTAATACT	8/13
Region III	TTAAGTGATATTGGTTGTCTG	6/13
CRP consensus	AAnTGTGAnnTnnnnCAnATT	

FIG. 1. Locations of binding sequences for the cAMP-CRP complex in the major IS*2* promoter. The LIR of IS*2* is indicated by a large arrow above the sequence. The solid bars below the sequence indicate the locations of the −10 and −35 sequences of the major IS*2* promoter. At the bottom, three putative cAMP-CRP complex binding sequences, region I (IS*2* nucleotide positions 34 to 54), region II (positions 43 to 63), and region III (positions 42 to 22), present in the promoter area are aligned with the consensus cAMP-CRP complex binding sequence (matching bases are underlined). The cAMP-CRP complex binding sequence (positions 37 to 53) determined by the DNA footprinting experiment is bracketed above the sequence, and the InsA binding site (positions 33 to 52) is indicated below the sequence.

Luciferase assay. One hundred microliters of 1% (vol/vol) *n*-decyl-aldehyde (in ethanol) was added to 500 μl of a late-log-phase (A_{600} = 0.8) culture of *E. coli* containing appropriate plasmids. The reaction mixture was incubated at room temperature for 10 s, and then the luciferase activity was measured with a luminometer (AutoLumat LB 593; EG & G, BERTHOLD, Bad Wildbad, Germany). The bioluminescence generated from each culture was shown as relative light units (RLU).

Transposition assay. Transposition assays and the determination of transposition frequencies were performed as described previously (26). A kanamycin resistance (Km^r) gene was inserted into IS*2* so that transposition of IS*2* could be detected by determining kanamycin resistance. pMIS2K (Fig. 2D), which carries this kanamycin gene-marked IS*2*, was introduced into the isogenic *E. coli* strains TP7811 (*xyl araH1 his*), TP7839 (*xyl araH1 his* Δ*crp39*), and TP7860 (*xyl araH1 his* Δ*cya*) (4) containing an F-derived plasmid, pCJ105, which served as the target for IS*2* transposition. These three isogenic *E. coli* strains also harbor IS*2*, which provides the IS*2* transposase in the transposition assay. Since pCJ105 carries a chloramphenicol resistance gene, transposition of IS*2* onto pCJ105 will render pCJ105 able to confer on an *E. coli* host both kanamycin- and chloramphenicol-resistant phenotypes. To determine the transposition frequency, pCJ105::IS*2* was mated out from TP7811, TP7839, or TP7860 to HB101 (5) by conjugation. The transconjugants were selected on Luria-Bertani agar containing chloramphenicol (50 μg/ml), kanamycin (50 μg/ml), and streptomycin (50 μg/ml), since HB101 is resistant to streptomycin. The transposition frequency was calculated by dividing the number of HB101 cells that were resistant to kanamycin, chloramphenicol, and streptomycin by those that were resistant to only chloramphenicol and streptomycin.

RESULTS

Binding of cAMP-CRP to the major IS*2* promoter. To determine whether the cAMP-CRP complex has the ability to bind the major IS2 promoter, the gel retardation assay was performed. A cell lysate containing overexpressed CRP was used as the source of CRP for this experiment. To overexpress CRP, a 0.7-kb DNA fragment containing the *crp* gene was amplified by PCR with genomic DNA isolated from *E. coli* XL1-Blue (7) as the template and primers CRP-N (5′-TTAT CTGGCTCTGGAGAAAGCTT-3′), which has a *Hin*dIII site at its 3′ end, and CRP-C (5′-TCGAAGTGCATAGTTGATA TCGG-3′). The PCR product was digested with *Hin*dIII and then cloned between the *Hin*dIII and *Sma*I sites of pBluescript II SK(−), generating pSK$^-$CRP (Fig. 2A). The nucleotide sequence of the cloned *crp* gene was verified by sequencing. pSK$^-$CRP was then introduced into *E. coli* JM109(DE3), and the lysate of JM109(DE3) cells containing pSK$^-$CRP was used for the gel retardation assay.

A DNA fragment referred to as P*insA*, which is the 89-bp *Eco*RI-*Spe*I fragment containing the IS*2* LIR, was isolated from pKS$^+$IS2 (28), labeled at its 3′ end with [α-^{32}P]dATP, and then incubated with cell lysate of JM109(DE3) containing pSK$^-$CRP in the presence or absence of cAMP. The reaction products were then electrophoresed on a polyacrylamide gel to detect bands that migrated more slowly than those in control reactions which lacked CRP or cAMP. The same labeled fragment was reacted with the InsA protein to serve as a positive DNA binding control, since InsA is known to bind the LIR. Binding of cAMP-CRP to the *lacZ* gene promoter was also performed to serve as an additional positive DNA binding control, because the cAMP-CRP complex binds to the *lacZ* gene promoter. Reaction of cAMP-CRP with a 170-bp *Eco*RI-*Pvu*II fragment of pBluescript II SK(+), which does not contain a cAMP-CRP binding site, was done to serve as a negative control.

The results of this experiment are shown in Fig. 3. A retarded band which migrated more slowly than the naked P*insA* (Fig. 3, lane 1) was seen when P*insA* was reacted with the cell lysate (30 μg of total protein) containing InsA (Fig. 3, lane 2), indicating that P*insA* has the InsA binding sequence. In the presence of 20 mM cAMP, both 30 and 15 μg of total protein of the cell lysate of JM109(DE3) cells containing pSK$^-$CRP generated a retarded band when incubated with P*insA* (Fig. 3, lanes 5 and 6). This retarded band was not seen when cAMP was omitted in the DNA binding reaction (Fig. 3, lane 4). Similarly, no retarded band was seen when a cell lysate of JM109(DE3) without pSK$^-$CRP was used (Fig. 3, lane 3).

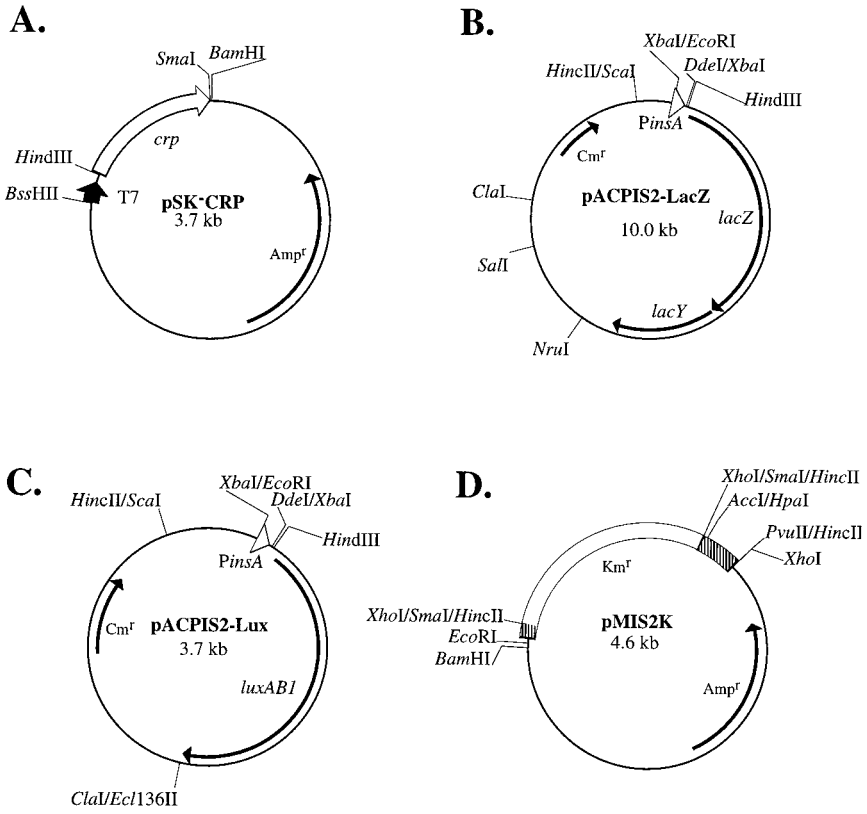

FIG. 2. Plasmids used in this study. The shaded regions in panel D are IS*2* sequences which remained. Abbreviations: *crp*, CRP gene; Amp^r, ampicillin resistance gene; Cm^r, chloramphenicol resistance gene; Km^r, kanamycin resistance gene; *luxAB1*, luciferase gene from *Vibrio harveyi* (45); P*insA*, the *insA* gene promoter, which is also the major IS*2* promoter.

Although the molecular mass of CRP is approximately 1.5 times (22 versus 14 kDa) that of InsA (2, 27), it generated a retarded band which migrated to the same level as that generated by InsA (Fig. 3, lanes 2, 5, and 6). A possible reason is that InsA may have the ability to bend DNA as CRP does, which would make the migration of DNA bands disproportional to the size of the bound protein. The same cell lysate containing CRP also generated retarded bands (Fig. 3, lane 10) in the presence of 20 mM cAMP when reacted with P*lacZ*, which is a 277-bp *Eco*RI-*Pvu*II fragment containing the *lacZ* gene promoter, but did not generate any retarded bands with the 170-bp *Eco*RI-*Pvu*II fragment of pBluescript II SK(+) which did not contain a CRP binding site (Fig. 3, lane 8).

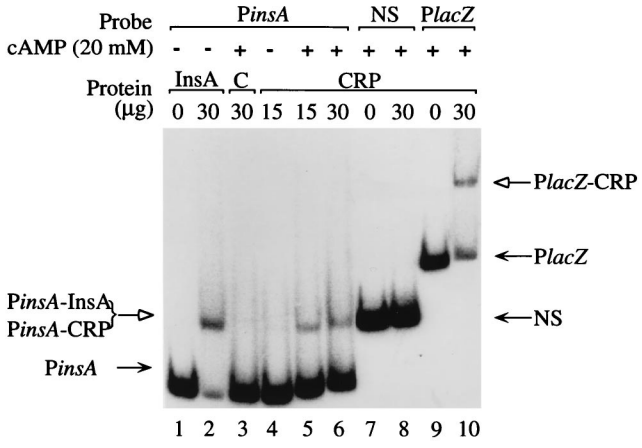

FIG. 3. Binding of the cAMP-CRP complex to the major IS*2* promoter. P*insA* is an 89-bp *Eco*RI-*Spe*I DNA fragment containing the major IS*2* promoter, P*lacZ* is a 277-bp *Eco*RI-*Pvu*II fragment containing the *lac* promoter, and NS is the 170-bp *Eco*RI-*Pvu*II fragment of pBluescript II SK(+) which does not contain a CRP binding site. Three kinds of cell lysate were used: CRP, *E. coli* JM109(DE3) containing pSK$^-$CRP; C, JM109(DE3) containing pT7-7; and InsA, JM109(DE3) containing pT7insA. The amounts of cell lysates used for each reaction with (+) or without (−) cAMP are as indicated. Solid arrows indicate bands of free DNA fragments, and open arrows indicate those of protein-bound DNA fragments.

The binding sequence of the cAMP-CRP complex on the major IS*2* promoter. The DNA footprinting experiment was performed to determine the binding sequence of the cAMP-CRP complex on the major IS*2* promoter. The same DNA fragment, P*insA*, used for the gel retardation assay was used. This 89-bp *Eco*RI-*Bam*HI fragment containing the IS*2* LIR region was labeled at the 5′ *Eco*RI end and then incubated with the cAMP-CRP complex before footprinting. The same fragment was also subjected to Maxam-Gilbert sequencing reactions (44). All the reaction mixtures were electrophoresed on a 5% DNA sequencing gel. A footprint was seen on DNA reacted with the cAMP-CRP complex when the gel was autoradiographed (Fig. 4). The binding sequence of the cAMP-CRP complex was deduced to be 5′-CTATCACTTATTTAA GT-3′ (Fig. 4) by comparing the footprinting patterns of P*insA* incubated with (Fig. 4, lane 4) or without (Fig. 4, lane 3) cAMP-CRP complex with the banding patterns of the Maxam-Gilbert G (Fig. 4, lane 1) and G+A (Fig. 4, lane 2) sequencing reactions. This sequence is complementary to the sequence 5′-ACTTAAATAAGTGATAG-3′ (IS*2* nucleotide positions 37 to 53), which is located on the upper strand of the sequence shown in Fig. 1.

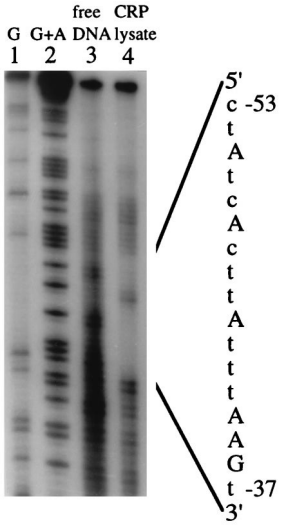

FIG. 4. Determination of the cAMP-CRP complex binding sequence in the major IS*2* promoter. The 89-bp *Eco*RI-*Spe*I DNA fragment containing the cAMP-CRP binding site was labeled by the Klenow enzyme with [α-^{32}P]dATP at the *Eco*RI end, incubated with the cell lysate containing cAMP-CRP, and subjected to in situ DNA footprinting. Lanes 1 and 2, Maxam-Gilbert (44) G and G+A reactions, respectively, of the DNA fragment; lane 3, footprinting reaction of unbound DNA; lane 4, footprinting reaction of the cAMP-CRP-bound DNA. The binding sequence of the cAMP-CRP complex is deduced from the footprint as shown. The uppercase letters on the right represent sequences actually determined from the gel; the lowercase letters were filled in on the basis of known sequences.

Effect of cAMP-CRP on the transcription of the major IS*2* promoter. To determine whether the cAMP-CRP complex has any effect on transcription from the major IS*2* promoter, the major IS*2* promoter was fused with a promoterless luciferase gene and assayed for transcription in the presence or absence of cAMP-CRP. The 6.8-kb *Sal*I-*Sca*I fragment containing the IS*2* promoter fused with the *lacZ* structural gene was isolated from pInsApLacZ (27) and then ligated with the 3.2-kb *Sal*I-*Hin*cII fragment containing the p15A replication origin and the chloramphenicol resistance gene of pACYC184, resulting in pACPIS2-LacZ (Fig. 2B). To replace the *lacZ* gene with the luciferase gene *luxAB1* (45), the plasmid pACPIS2-LacZ was digested with *Cla*I. The *Cla*I ends were filled in with the Klenow enzyme, and the fragment was further digested with *Hin*dIII to delete the *lacZ* gene, producing a 3.2-kb DNA fragment. This 3.2-kb fragment was then ligated with the 2.3-kb *Hin*dIII-*Ecl*136II DNA fragment containing the *luxAB1* (45) gene from pUCD1752 (a gift from C. I. Kado), generating pACPIS2-Lux (Fig. 2C).

The plasmid pACPIS2-Lux was introduced into the isogenic *E. coli* strains TP7811, TP7839, and TP7860, and the transformed cells were then assayed for the production of luciferase. TP7811 is wild type for cAMP-CRP, TP7839 is defective for CRP, and TP7860 is unable to produce cAMP. The major IS*2* promoter was found to be able to drive the expression of the luciferase gene in TP7811 and produced 2.7×10^5 RLU of luciferin (Table 1). However, a fourfold increase (10.8×10^5 RLU) in the production of luciferase was seen when the same plasmid was introduced into the CRP$^-$ TP7839. A more profound (17.1-fold) increase in the production of luciferase was observed when pACPIS2-Lux was introduced into the Cya$^-$ TP7860. To ensure that the difference in the expression of the luciferase gene was not due to a difference in the copy number of pACPIS2-Lux in different hosts, plasmid DNA was isolated from the same numbers of TP7811, TP7839, and TP7860 cells and then quantitated. No difference in the copy number of pACPIS2-Lux in TP7811, TP7839, or TP7860 was observed. These results indicate that the major IS*2* promoter is more active in the absence of CRP or cAMP and suggest that the cAMP-CRP complex is a negative regulator of the major IS*2* promoter.

TABLE 1. Effect of CRP and cAMP on transcription from the major IS*2* promoter

Bacterial strain	Luciferase activity (RLU)[a]	Relative fold increase[b]
TP7811 (WT)[c]	$(2.7 \pm 0.5) \times 10^5$	1.0
TP7839 (Δ*crp*)	$(10.8 \pm 0.6) \times 10^5$	4.0
TP7860 (Δ*cya*)	$(46.3 \pm 3.0) \times 10^5$	17.1

[a] Each value represents the mean of four independent assays ± standard deviation.
[b] Fold increase in RLU is relative to that of TP7811(pACPIS2-Lux), which is set as 1.
[c] WT, wild type.

Effect of cAMP-CRP on IS*2* transposition. Since the major IS*2* promoter is responsible for the transcription of the IS*2* transposase InsAB′, the effect of cAMP-CRP on IS*2* transposition was examined. A plasmid containing a mini-IS*2* with a kanamycin resistance marker was constructed as follows. The plasmid pKS$^+$ISF (27) was digested with *Acc*I and *Hpa*I to delete the IS*2* sequence from nucleotide 578 to 1173, resulting in the plasmid pKS$^+$ISFd1. IS*2* nucleotides 103 to 440 were then removed by deleting the 337-bp *Xho*I-*Sma*I fragment of pKS$^+$ISFd1. The 1.3-kb *Hin*cII fragment containing a kanamycin resistance gene from pUC4K (Pharmacia Biotech, Uppsala, Sweden) was then inserted into the blunt-ended *Xho*I and *Sma*I sites of pKS$^+$ISFd1, generating pMIS2K (Fig. 2D). Since a total of 937 bp of internal IS*2* sequence in pMIS2K were deleted, all functional genes of IS*2* were destroyed and the mini-IS2 in this plasmid could transpose only in hosts that harbor IS*2*.

To ensure that strains TP7811, TP7839, and TP7860 contained IS*2*, PCR was performed with primers $IS_{700–720}$ (5′-ATGCGCCAGAATGCGCTGTTG-3′) and $IS_{1294–1273}$ (5′-TTAACCCATTACAAGCCCGCTG-3′), which amplify IS*2* from nucleotide 700 to 1294. An expected 595-bp fragment was amplified from all three hosts. This fragment was sequenced, and the sequence was verified to be derived from IS*2*. pMIS2K was then introduced into TP7811, TP7839, and TP7860 containing pCJ105. The frequency of IS*2* transposition onto pCJ105 in each host was then determined by mating pCJ105 out to another host. The results of this experiment are summarized in Table 2. In the wild-type host (TP7811), IS*2* transposed at a frequency of 10^{-5}. A 130-fold increase in transposition frequency was observed in the CRP$^-$ host, TP7839. A much higher transposition frequency (290-fold increase) was

TABLE 2. Effect of the cAMP-CRP complex on IS*2* transposition

Bacterial strain	Transposition frequency[a]	Relative fold increase[b]
TP7811 (WT)[c]	$(1.0 \pm 0.3) \times 10^{-5}$	1.0
TP7839 (Δ*crp*)	$(1.3 \pm 0.5) \times 10^{-3}$	130
TP7860 (Δ*cya*)	$(2.9 \pm 0.1) \times 10^{-3}$	290

[a] Numbers represent transposition frequencies of IS*2* in different hosts and are the means (± standard deviations) of four independent experiments.
[b] Fold increase in transposition frequency is relative to that of the mini-IS*2* in TP7811, which is set as 1.
[c] WT, wild type.

TABLE 3. Putative cAMP-CRP binding sequences present in members of the IS*3* family

TE[a]	Promoter	Sequence[b]	Nucleotide positions	Match[c]	Reference(s)
IS*3411*	*orfV*	AATCGTGAACTGCGCCGCAGT	292–312	10	29
IS*51*	*orf1*	ACCGGTGGAATATGACCGATT	104–124	9	81
IS*476*	*orf1*	AGTTGTGCCGTCGGCATGGTT	141–161	8	32
IS*3*	*orfA*	TCATGTGAGTCACCTCTGACT	69–49	8	75
IS*911*	*orfA*	CACACTGAATTTGGCCACCTG	9–29	8	56
IS*150*	*orfA*	AAATGGAATAGCCCCTAATAT	29–49	7	66
IS*186*	*orf1*	GGTTGTGGTATTACGCCTGAT	18–38	7	9, 70
IS*600*	*orfA*	CCTTCTGATGCCATTCTATTT	56–36	7	43
IS*861*	*orf1*	ATAACTTAATTTCATCAGAAA	128–108	7	65

[a] TE, transposable element. Transposable elements examined include IS*3411*, IS*51*, IS*476*, IS*3*, IS*911*, IS*150*, IS*186*, IS*600*, IS*861*, IS*R1* (57), and IS*629* (43).
[b] Underlined bases match the consensus sequence AAnTGTGAnnTnnnnCAnATT (11).
[c] Number of bases which match the 13-bp consensus sequence.

seen in the Cya$^-$ host, TP7860. These results indicate that IS*2* transposes more efficiently in hosts defective in CRP or adenyl cyclase, suggesting that IS*2* transposition is negatively regulated by the cAMP-CRP complex.

DISCUSSION

The consensus binding sequence for the cAMP-CRP complex is 5′-AAnTGTGAnnTnnnnCAnATT-3′ (11). This sequence is found in the major IS*2* promoter at three regions: IS*2* nucleotide positions 34 to 54, 43 to 63, and 42 to 22 (Fig. 1). The sequence of region III (IS*2* nucleotides 42 to 22) is located on the lower strand of the IS*2* LIR, whereas those of region I (IS*2* nucleotides 34 to 54) and region II (IS*2* nucleotides 43 to 63) are on the upper strand. Seven residues in region I, eight in region II, and six in region III conform to this 13-residue consensus cAMP-CRP binding sequence. This finding suggests that IS*2* transposition may be subject to cAMP-CRP regulation. This hypothesis is supported by the demonstration that the cAMP-CRP complex binds to the major IS*2* promoter (Fig. 3 and 4) and that binding of the cAMP-CRP complex to the IS*2* promoter has a negative effect on transcription from this promoter (Table 1). As a consequence, the production of transposase is decreased and IS*2* transposition frequency is reduced. This supposition was demonstrated in this study by the observation that IS*2* transposition frequency is higher in *E. coli* mutants defective in CRP or adenyl cyclase than in a wild-type host (Table 2). It is also possible that the binding of the cAMP-CRP complex to the LIR interferes with the binding of the IS*2* transposase to the same region to initiate transposition. This possibility remains to be investigated.

The cAMP-CRP complex was determined to bind the sequence 5′-ACTTAAATAAGTGATAG-3′ located at IS*2* nucleotides 37 to 53 (Fig. 1). This sequence is located within region I of the three putative cAMP-CRP binding sites mentioned above. This cAMP-CRP binding sequence overlaps almost entirely with that of the InsA binding sequence, which is located at IS*2* nucleotides 33 to 52 (Fig. 1). This area covers the entire −10 sequence and its flanking regions of the major IS*2* promoter. We have previously shown that the binding of InsA to this region also suppresses transcription from this promoter and thus decreases IS*2* transposition frequency (27). In this study, we have demonstrated that the cAMP-CRP complex binds to the same region and has the same suppressive effect as the InsA on IS*2* transposition. InsA is a native IS*2* protein, whereas CRP is a host protein. It is conceivable that the host has a mechanism to limit IS*2* transposition, since overtransposition could be detrimental to the host, but it is quite intriguing to find that IS*2* produces a protein to suppress its own transposition. It remains to be investigated whether InsA and the cAMP-CRP complex compete with each other for binding to the same site. It appears that InsA and the cAMP-CRP complex do not bind concomitantly, since a cell lysate containing both proteins did not produce a band that migrated more slowly than the one produced by either InsA or the cAMP-CRP complex alone in the gel retardation assay (data not

TABLE 4. Putative cAMP-CRP binding sequences present in non-IS*3* family transposable elements

TE[a]	Promoter	Sequence[b]	Nucleotide positions	Match[c]	Reference(s)
Tn*1000*	*tnpR*	AAATGTATCCTAAATCAAATA	2779–2799	10	6
Tn*1000*	*tnpA*	ATGTGTGCGATAATTTATAAT	2865–2885	9	6
IS*4*	*tnp*	ACAAGTGAGCGTTTCCGGATT	70–50	9	33, 77
IS*30*	*orfA*	AACTGTTGCGTTGACCAATTG	29–9	9	10
Tn*3*	*tnpR*	AAATGTACCTTAAATCGAATA	3207–3187	9	24
Tn*3*	*tnpA*	CTATGTCTGATAATTTATAAT	3121–3101	7	24
IS*15*	*orfI*	GATGGTGGCGTAAGCCGTCTT	409–429	8	76
IS*50*	*tnp*	TATCATGAACGTTACCATGTT	98–78	8	35
Tn*7*	*tnsA*	CAGTATGCTTTTTCACAGCAT	124–104	8	14
IS*102*	*orfI*	TCTGGTGATTAAACGCGTATT	254–274	8	3
IS*21*	*istA*	GATTGTCCGCTCCACCCAACA	40–60	7	59
IS*10*	pIN	AGATGTGTATCCACCTTAACT	58–38	7	22, 71
IS*10*	pOUT	AGATGTGCGAACTCGATATTT	106–126	7	22, 71
IS*903*	*tnp*	ATGCGTGATCTGATCCTTCAA	1005–1025	7	21

[a] TE, transposable element. Transposable elements examined include Tn*1000*, IS*4*, IS*30*, Tn*3*, IS*15*, IS*50*, Tn*7*, IS*102*, IS*21*, IS*10*, IS*903*, IS*1* (68), and IS*5* (12).
[b] Underlined bases match the consensus sequence AAnTGTGAnnTnnnnCAnATT (11).
[c] Number of bases which match the 13-bp consensus sequence.

shown). It is unknown whether there is a mechanism to coordinate the binding of these two negative regulators. How IS*2* derepresses the suppression by InsA or the cAMP-CRP complex also remains to be studied.

The cAMP-CRP complex of *E. coli* is involved in the activation or repression (34, 50, 62, 63) of many genes. For example, the cAMP-CRP complex alone activates the transcription of $lacp_1$ (42), $galp_1$ (48, 49), *malTp* (67), P_C and P_{BAD} of the AraCBAD operon (40), *papp* (15), and *gyrA* (19). In the absence of the CytR repressor, it also activates $deop_2$ (46, 53, 72), *tsx*-p_2 (17), *nupG* (47), and *cdd* (25). The promoters *malKp* and *malEp* (62, 67) require both the MalT and the cAMP-CRP complexes, and that of the *ansB* gene (31) requires both the FNR and the cAMP-CRP complexes for transcriptional activation.

In this study, the binding of the cAMP-CRP complex was found to have a negative effect on transcription from the major IS*2* promoter. Negative regulation by the cAMP-CRP complex has also been demonstrated to occur on $lacp_2/p_3$ (13, 42, 79), $galp_2$ (48), $proPp_1$ (80), and *crp* (1, 30). In addition, the cAMP-CRP complex together with the CytR repressor inhibits the transcription of $deop_2$ (46, 53, 58, 72), *cytRp* (23, 52), *tsx*-p_2 (17), *nupG* (51), and *cdd* (25).

The effect of cAMP-CRP on IS*2* transposition described in this report is the first example of regulation of the transposition of transposable elements by the cAMP-CRP complex. It is not known whether the cAMP-CRP complex has a similar effect on other members of the IS*3* family. We have searched for the presence of the cAMP-CRP binding sequence on the transposable elements of the IS*3* family (56, 65, 66) and found that 9 of 11 members of the IS*3* family have a putative CRP binding sequence on the promoter region (Table 3) which has 7 or more bp matched with the 13-bp consensus sequence, suggesting that the cAMP-CRP complex also regulates their transpositions. We also found that 11 of 13 non-IS*3* transposable elements have 7 or more bp that matched with the putative CRP binding sequence (Table 4). Whether the cAMP-CRP complex has any effect on the transposition of these transposable elements remains to be determined.

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