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Genetics

IS*5*: A mobile enhancer of transcription in *Escherichia coli*

(mobile DNA element/DNA topology/promoter activation/transactivator/catabolite gene activator protein)

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ABSTRACT **The cryptic *bgl* operon of *Escherichia coli* is activated by the spontaneous insertion of mobile DNA elements. Screening of a collection of such mutations revealed insertion of the 1195-base-pair element IS*5* into various positions both upstream and downstream of the *bgl* promoter P_0. Activation of the operon was in all cases attributable to enhancement of P_0 activity. Introduction of internal deletions into IS*5* almost completely abolished P_0 enhancement, demonstrating that enhancement is not simply the result of mutational inactivation of some inhibitory sequences. Intact copies of IS*5* in trans restored the enhancing activity of the deletion derivatives. The trans-activator is encoded by IS*5* gene *ins5A*, an essential transposition function. Activation of gene expression by means of interaction of a defective mobile element in cis with functions encoded by a nondefective element in trans has so far been described only for a maize controlling element.**

Mobile DNA elements were first discovered in maize by Barbara McClintock, who named them "controlling elements" (see refs. 1–3 for reviews). Much later such elements were found and analyzed in bacteria (see ref. 4 for a recent review), which triggered their discovery in a wide range of species (5) and also led to "rediscovery" of the maize controlling elements.

The inactivation of genes by insertion of mobile DNA elements into coding and control regions and the activation of gene expression by promoter activity provided by mobile elements are well-known phenomena in both eukaryotic and prokaryotic organisms (5). Modulation of expression of adjacent genes due to the presence of enhancers or silencers within mobile elements, however, has thus far been detected only in eukaryotes (1–3, 6–11). In all cases in which mobile elements interfere with the expression of adjacent genes, this interference leads to disturbance of their normal regulatory regime.

The *bgl* operon of *Escherichia coli* (responsible for β-glucoside utilization) is unique in that it depends on integration of mobile elements to be active: the operon is silent in the wild-type state and is activated by spontaneous transposition of the mobile insertion elements IS*1* or IS*5* into a proximal region termed *bglR* (12). In independent isolates of activated mutants both elements were found to be integrated in both orientations at different sites within a region of 49 base pairs (bp) (13, 14). Once activated, the operon is coordinately regulated by its substrate and the catabolite gene activator protein (CAP)–cAMP complex (12, 15). The basis for activation is a drastic enhancement of activity (up to 60-fold; ref. 14) of the major *bgl* promoter (P_0) (14–16). The scattered and orientation-independent occurrence of activating mutations made it conceivable that in the wild-type state, activity of promoter P_0 may be somehow inhibited by its upstream sequences and that activation by IS elements is based on the uncoupling of these sequences from the promoter (13) or on their insertional inactivation. However, upon systematic examination of our collection of spontaneously derived activated mutants (Fig. 1), we found two cases in which IS*5* was integrated downstream of promoter P_0, within the leader of the operon. This finding seemingly contradicts the above concept and prompted us to undertake a more detailed analysis of the molecular basis of activation. We demonstrate that integration of IS*5* either upstream of P_0 or into the leader between P_0 and the first structural gene leads to 40- to 60-fold enhancement of P_0 activity. This enhancement is the cause of activation of the operon in all cases studied. Various internal deletions in IS*5* reduce promoter P_0 activity in an IS*5*-free background to near the wild-type level. However, P_0 activity can be restored in such deletion derivatives when an IS*5* is introduced in trans. Thus IS*5* provides cis-activating sequences which respond to a trans-activating function encoded by IS*5*. This trans-activator is a product of IS*5* gene *ins5A*, a gene essential for transposition of the element. Our results extend the previously reported target range for transpositional activation to 223 bp.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* K-12 strain R1360 is *ara* Δ*(lac–pro) strA thi* Δ*(bglR–bglB)::tet*. This strain is a derivative of R1243 (a Bgl^+ mutant of CSH50; ref. 17) in which the *bgl* operon encompassing *bglR* to *bglB* was deleted by gene displacement. *Salmonella typhimurium* LT2 strain SL5235 (= R1403), *metA metD trpD leu rpsL hsdL*(r^-m^+) *hsdSA*(r^-m^+), obtained from B. Stocker (Stanford University), has been described (16).

Plasmids and DNA Manipulations. Plasmids were constructed by standard recombinant techniques (18). Plasmid pFDX733 (16) contains the wild-type *bgl* operon on plasmid vector pACYC177 (19). Bgl^+ mutant derivatives of pFDX733 were isolated as described (16). Plasmids pFDX733-H3 (14), pFDX733-S7 (16), pFDX733-S9, pFDX733-S6, and pFDX733-517 are spontaneous IS*5* insertion mutants of plasmid pFDX733 (see Fig. 1 for integration sites). Insertion mutants S7, S9, and S6 carry a "tagged" copy of IS*5* with an 8-bp *Sal* I linker (GGTCGACC) inserted in the single *Xmn* I site of IS*5* (16, 20–22). Bgl^+ plasmids pFDX733-C234 and pFDX733-BC75 are spontaneous mutants of plasmid pFDX733 with point mutations in the CAP binding site (see Fig. 1). For construction of pFDX733-S7-Δ, a *Hae* III fragment carrying the polylinker region of pUC13 (23) was first ligated to a *Xho* I linker (CCTCGAGG). The ligation product was digested with *Xho* I, and the protruding ends were made blunt by Klenow polymerase. The DNA was subsequently cut with *Sal* I and the smaller fragment carrying the pUC13 polylinker was isolated. This fragment was used for substitution of IS*5*-internal DNA of the "tagged" copy of IS*5* in plasmid pFDX733-S7 (see above) from the *Sal* I linker insertion 25 bp away from the left terminus of IS*5* to the *Sau*3A1 site 32 bp away from the right terminus. Plasmid

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Abbreviation: CAP, catabolite gene activator protein.

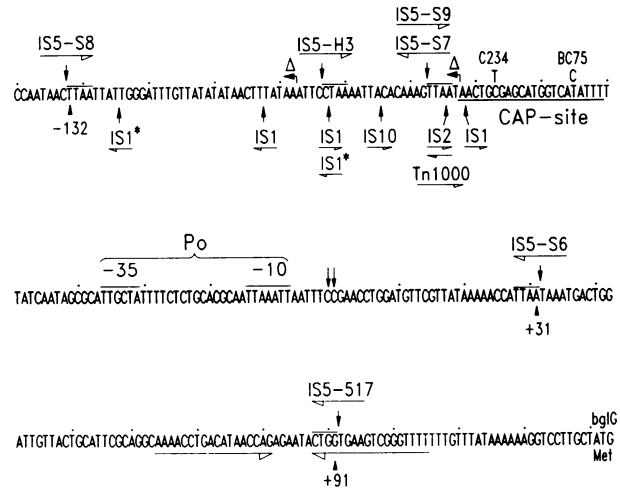

FIG. 1. DNA sequence of promoter and leader region of the wild-type *bgl* operon (16). Sequenced Bgl$^+$ alleles with insertions of IS*1*, IS2, IS*5*, IS*10*, and Tn*1000* are indicated, and the positions of their integration sites (coordinates relative to the transcription start) are marked with arrowheads below the sequence. For IS*5*, the 4-bp target-site duplications are also given. Horizontal arrows give the relative orientations of the insertion elements according to their defined left and right ends. Positions of elements marked with an asterisk were taken from ref. 13. The CAP binding site is underlined and base exchanges within the CAP site are given above the sequence. The proximal deletion endpoints are indicated by right-angled arrows marked with Δ. Promoter P_0 is marked by a brace and the transcriptional start site as revealed by S1 analysis (see Fig. 2) is indicated by vertical arrows above the sequence. The inverted repeats of terminator *t1* (14, 16) are underlined by horizontal arrows below the sequence. The start codon of *bglG* (ATG) marks the 3′ end of the sequence.

pFDX733-S6-Δ was derived from pFDX733-S6 and carries an IS*5*-internal deletion from the *Sal* I site to the rightmost *Sau*3A1 site. For construction of plasmid pFDX733-H3-Δ, the IS*5*-internal DNA in plasmid pFDX733-H3, from the *Xmn* I site 25 bp away from the left terminus to the *Eco*RI site 103 bp away from the right terminus, was removed and the DNA was ligated after filling in with Klenow polymerase. Plasmid pFD51 has been described (24). pFDX52 is similar to pFD53 (24) but carries the IS*5*-containing *Hin*dIII fragment in the inverse orientation. Plasmid pFDX52-insA$^-$ was derived from pFDX52 by filling in the *Eco*RI site, which generates a +1 frameshift within *ins5A* at codon 12 (H.-J. Ronecker and B.R., unpublished work). Plasmid pFDX52-insBC$^-$ carries a derivative of IS*5* (generated by site-directed mutagenesis) with a mutation at the beginning of the *ins5C* frame that abolishes the presumptive translational start codon, and in addition contains two tandem stop codons at the beginning of the *ins5B* frame. All three mutations are silent for the overlapping, oppositely arranged *ins5A* frame (F. Brombacher and B.R., unpublished work).

RNA Isolation, Slot Blot Hybridization, and S1 Nuclease Mapping. For isolation of RNA, cells were grown in synthetic M9 medium (17) containing glycerol as carbon source with the necessary supplements, Casamino acids (0.66%), methyl β-D-glucoside (5 mM) as inducer of the *bgl* operon, and kanamycin (40 μg/ml) or ampicillin (50 μg/ml) where necessary. RNA was isolated by a "hot phenol" method (14). RNA for slot blot hybridization was isolated from transformants of *S. typhimurium* LT2 (strain SL5235), which is devoid of IS*5* sequences (ref. 25, and unpublished observations). Quantification of RNA by slot blot hybridization and densitometric scanning was performed as described (14, 26). The values were corrected for the relative copy number of the template plasmids (14). S1 mapping of transcripts initiated at promoter P_0 was performed according to Aldea *et al.* (27). For generation of the probe used for mapping RNA initiated at the wild-type promoter and alleles *H3, S7, S9, C234*, and *BC75*, a 753-bp *Ssp* I–*Eco*RV fragment of pFDX733 encompassing the promoter region was first cloned into the *Sma* I site of M13mp9 (23), resulting in M13fdx954. The S1 probe was subsequently generated by priming of single-stranded DNA of M13fdx954 with an oligonucleotide complementary to the noncoding strand and thus to the mRNA from position +147 to +122. For S1 mapping of alleles *S6* and *517*, 1165-bp and 1225-bp *Mlu* I–*Pvu* II fragments, respectively, encompassing the *bgl* promoter and part of the IS*5* element were ligated into the *Sma* I site of M13mp9, resulting in M13fdy99 and M13fdy101. In both cases an oligonucleotide hybridizing to sequences 164 bp inside of IS*5* was used for priming. Labeled probes were generated with T7 DNA polymerase as for sequencing (Pharmacia; T7 sequencing kit) but with the omission of dideoxynucleotides in the "termination" mix and with [α-^{32}P]dATP (3000 Ci/mmol; 1 Ci = 37 GBq). Labeled DNA was extracted once with phenol and three times with chloroform/isoamyl alcohol (24:1, vol/vol) and was precipitated with ethanol. Subsequently, 100 μg of RNA was lyophilized together with the labeled probe, resuspended in 40 μl of hybridization buffer (3 M sodium trichloroacetate/50 mM Pipes, pH 7.0/5 mM EDTA), and incubated for 5 min at 60°C and for 4 hr at 45°C. For S1 nuclease digestion 190 μl of S1 buffer (250 mM NaCl/40 mM sodium acetate, pH 5.5/1 mM $ZnCl_2$ with denatured calf thymus DNA at 20 μg/ml) containing S1 nuclease at 250 units/ml was added and the mixture was incubated for 30 min at 37°C. The reaction was stopped on ice. DNA was precipitated with ethanol and resuspended in 7 μl of sequencing loading buffer. Samples were loaded on a sequencing gel [6% acrylamide/*N,N'*-methylenebisacrylamide with 7 M urea, 100 mM Tris borate (pH 8.3), and 2 mM EDTA] beside a sequencing ladder generated with the same primer/templates as for labeling of the probes.

RESULTS

Enhancement of Promoter P_0 by Insertion of IS*5* Upstream or Downstream of P_0 Is Responsible for *bgl* Activation. We have previously shown that activation of the *bgl* operon by integration of IS*5* upstream of promoter P_0 is caused by a 60-fold enhancement of P_0 activity (14). To find out how the operon may be activated in mutants in which IS*5* is integrated downstream, into the leader region between P_0 and the *bgl* structural genes we first made a deletion in allele *bglR*::IS*5-S6* (which maps within the leader; Fig. 1) encompassing promoter P_0 and the CAP binding site (positions −73 to +31 relative to the transcriptional start site). This deletion rendered the operon inactive, demonstrating that integrity of the promoter region is essential for activity of the operon not only when IS*5* is integrated upstream of P_0 but also when IS*5* is integrated downstream. Moreover, the finding excludes simple models in which integration of IS*5* into the leader may activate a promoter located further downstream or may itself furnish promoter activity.

The results obtained with the deletion derivative encouraged us to use different alleles activated by IS*5* transposition (Fig. 1) for a comparative S1 analysis of RNA initiated at P_0. Fig. 2*A* gives data obtained with RNA expressed from the operon with IS*5*-induced mutations mapping upstream of P_0 and from the wild-type operon. Also depicted are results obtained with two spontaneously obtained point mutations that map within the CAP binding site. These data will be discussed later. Because alleles *bglR*::IS*5-S6* and *bglR*::IS*5-517* have IS*5* sequences in the leader, we had to employ probes derived from these individual alleles, and the results are given in Fig. 2 *B* and *C*.

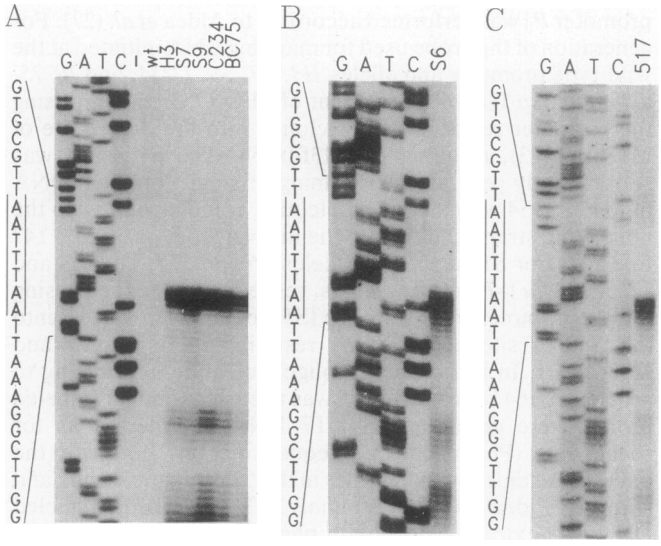

FIG. 2. S1 nuclease mapping of 5′ RNA termini at promoter P_0. Products were separated by electrophoresis beside a sequencing ladder (lanes G, A, T, and C). (*A*) Control with RNA isolated from the untransformed strain R1360 (−); transformants harboring plasmid pFDX733 with the wild-type operon (wt) or plasmid derivatives with Bgl$^+$ alleles: pFDX733-H3 (H3), pFDX733-S7 (S7), pFDX733-S9 (S9), pFDX733-C234 (C234), and pFDX733-BC75 (BC75). (*B*) Plasmid pFDX733-S6 (S6). (*C*) Plasmid pFDX733-517 (517).

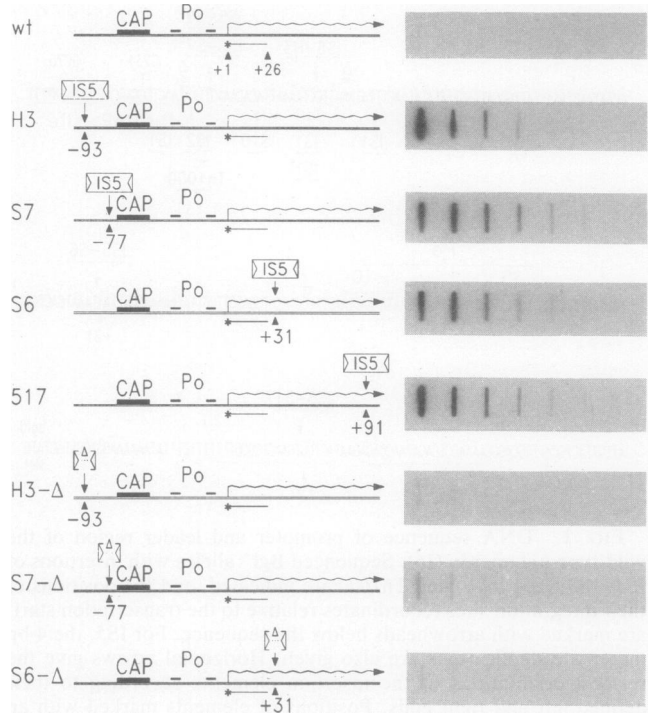

FIG. 3. Quantitation of steady-state levels of RNA initiated at promoter P_0. Relevant plasmid structures used to direct *in vivo* RNA synthesis are diagrammed at left. CAP binding site is marked by a thick bar. Promoter P_0 with −10 and −35 sequence motifs (thin bars) is indicated. Asterisk represents the 3′ label of the synthetic oligonucleotide probe. Transcripts are shown as wavy lines. Autoradiograms of slot blot hybridizations (1:2 dilutions of the RNA) are shown at right. Alleles *bglR*::IS*5-H3* (H3), *bglR*::IS*5-S7* (S7), *bglR*::IS*5-S6* (S6) and *bglR*::IS*5-517* (517) are described in Fig. 1. Alleles *bglR*::IS*5-H3*-Δ (H3-Δ), *bglR*::IS*5-S7*-Δ (S7-Δ), and *bglR*::IS*5-S6*-Δ (S6-Δ) carry IS*5*-internal deletions. For other details see text.

While no S1 signal was detectable with the wild-type operon (pFDX733 in Fig. 2*A*) for the exposure time used, all mutants analyzed gave prominent signals at the position previously determined as the transcriptional start site of P_0 (14, 15). This demonstrates that activity of promoter P_0 is greatly enhanced not only when IS*5* is integrated upstream of P_0 but also when it is integrated downstream of P_0. In addition, the results obtained with the downstream alleles (Fig. 2 *B* and *C*) exclude the possibility (28) that the IS*5* sequences are posttranscriptionally eliminated as intron sequences.

The S1 analyses with the various probes were not suitable for quantification of the individual transcripts. To obtain a quantitative estimate of the rate of enhancement of P_0 activity, we performed slot blot hybridizations with RNA isolated from transformants of *Salmonella typhimurium* LT2 [which is devoid of IS*5* sequences (ref. 25 and unpublished observation) and a *bgl* operon (16)] harboring plasmids that carry the *bgl* operon in the wild-type state or activated by various IS*5* insertions. A synthetic oligonucleotide complementary to nucleotides 1–26 of P_0-initiated RNA was used as a uniformly radioactive probe. Comparison of the autoradiogram obtained for wild-type RNA with those obtained for RNAs derived from four mutants (Fig. 3, top five lines) clearly demonstrates that integration of IS*5* upstream of promoter P_0 and in both possible orientations (alleles *bglR*::IS*5-H3* and *bglR*::IS*5-S7*) or downstream of P_0 and at different positions (alleles *bglR*::IS*5-S6* and *bglR*::IS*5-517*) leads to a considerable enhancement of P_0 activity (40- to 60-fold, as shown by densitometric scanning).

Taken together the data strongly suggest that enhancement of P_0 is responsible for operon activation not only in the IS*5*-induced mutations mapping upstream but also in those mapping downstream of P_0, within the leader of the operon. Consequently, expression of the operon in the latter class of mutants would require transcription to proceed through the entire 1195-bp IS*5* sequence before it reaches the *bgl* structural genes. Indeed, analysis of the IS*5* element cloned into a terminator test vector (pFDX104; ref. 14) revealed that IS*5* is only marginally transcriptionally polar in that orientation in which it was found integrated into the leader of the *bgl* operon (unpublished work).

Enhancement of P_0 Activity by IS*5* Transposition Is Not Merely Due to Insertional Inactivation of Inhibitory Sequences. To find out whether activation was due to the presence of specific IS*5* sequences or to the insertional inactivation of inhibitory sequences, we made deletions of various lengths in resident IS*5* elements and tested the deletion derivatives by slot blot hybridization under the conditions used above. In deletion derivative *bglR*::IS*5-H3*-Δ all but 25 bp of the defined left end (20–22) of IS*5* and 103 bp of its right end were deleted. Deletion derivatives *bglR*::IS*5-S7*-Δ and *bglR*::IS*5-S6*-Δ contained 32 bp of the right end and 25 bp of the left end of IS*5* connected in the case of *bglR*::IS*5-S7*-Δ by a 50-bp linker and in the case of *bglR*::IS*5-S6*-Δ by a 6-bp linker. Enhancement of P_0 activity is severely reduced by all of these deletion mutations (Fig. 3, bottom three lines), but not to the level of wild-type activity. Densitometry revealed for the various deletion derivatives a 3- to 6-fold activity above the wild-type level.

IS*5* Encodes a Trans-Activator That Interacts with the Termini of IS*5* in Cis. The above findings demonstrate that enhancement of promoter P_0 activity cannot be due solely to insertional inactivation of some inhibitory sequences. We therefore tested the possibility that an IS*5*-encoded function may participate in activation. For these experiments we employed allele *bglR*::IS*5-S7*, which had brought about the strongest enhancement of promoter P_0 (60-fold), and its deletion derivative *bglR*::IS*5-S7*-Δ. Plasmids carrying *bglR*::IS*5-S7*, *bglR*::IS*5-S7*-Δ, or the wild-type operon were used to transform *S. typhimurium* containing a compatible

plasmid with or without an intact IS5. RNA was isolated from these transformants and subjected to slot blot analysis of P_0-initiated RNA as described above (Fig. 4). In this experiment IS5 in trans had no influence on P_0 activity in the wild-type state or in allele *bglR*::IS5-*S7*, carrying an intact copy of IS5. However, P_0 activity was increased about 20-fold above the wild-type level when deletion derivative *bglR*::IS5-*S7*-Δ, carrying only the termini of IS5, was provided with an intact IS5 in trans (Fig. 4). Thus IS5 must express some function that is capable of interacting in trans with a defective element. This interaction leads to stimulation of activity of the neighboring promoter P_0.

The Trans-Activator Is Encoded by IS5 Gene *ins5A*. IS5 contains three genes (24, 29), which are candidates for the source of the trans-activating function. Gene *ins5A* covers almost the entire length of the element, while genes *ins5B* and *ins5C* are arranged in tandem and on the opposite strand (Fig. 4). To test these genes for their trans-activation potential, we repeated the slot blot experiment with mutant alleles of IS5 in trans.

One mutant IS5 employed carried a frameshift mutation in the 5′ region of *ins5A*, leaving genes *ins5B* and *ins5C* intact, while the other mutant IS5 carried two translational stop codons within the 5′ region of *ins5B* and in addition a GTG → GTA mutation within the presumptive translational start codon of *ins5C*. These mutations were silent for *ins5A*; i.e., they did not alter the amino acid sequence of the *ins5A* gene product. While the *ins5A*-negative element could not restore enhancer function of the defective element, the *ins5B*$^-$, *ins5C*$^-$ double mutant was capable of trans-activation (Fig. 4). Thus the trans-activator must be encoded by *ins5A*.

DISCUSSION

IS5 is an 1195-bp mobile DNA element that is present in a variable number of copies in the genomes of various *E. coli* K-12 strains (25, 29–31). It contains three genes. Gene *ins5A* covers almost the entire length of the element, whereas genes *ins5B* and *ins5C* are arranged in tandem on the opposite strand within the boundaries of *ins5A* (refs. 24 and 29; see Fig. 4). Of these genes only *ins5A* is essential for transposition and thus encodes the IS5 transposase (K. Fuchs, F. Brombacher and B.R., unpublished data).

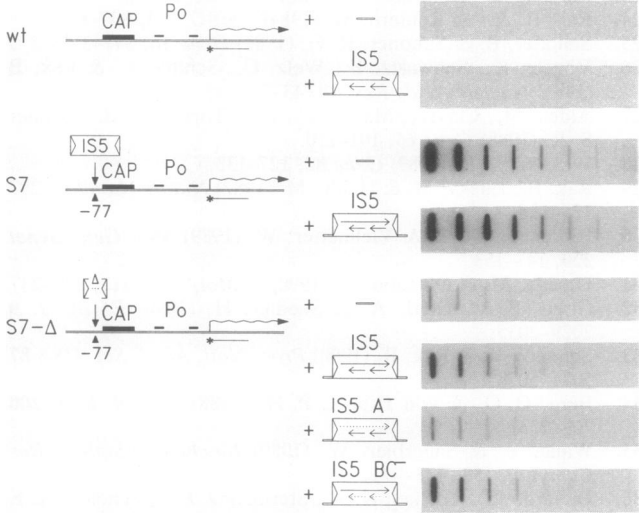

FIG. 4. Slot blot experiment demonstrating trans-activation of promoter P_0 activity by IS5 gene *ins5A* product in conjunction with the IS5 termini in cis. RNA was isolated from double transformants carrying on one plasmid the wild-type *bgl* operon (wt), alleles *bglR*::IS5-*S7* (S7), or *bglR*::IS5-*S7*-Δ (S7-Δ) and on a second, compatible plasmid the wild-type IS5 element (IS5), mutant IS5 elements (*ins5A*$^-$; *ins5B*$^-$*C*$^-$), or no IS5 (bars). For other symbols and conditions see Fig. 3.

We have provided evidence that IS5 enhances *bgl* promoter P_0 activity 40- to 60-fold when integrated upstream or downstream of P_0 and over variable distances. The most distal integration site found for IS5 is separated by 223 bp from the most proximal integration site (Fig. 1). Promoter P_0 enhancement is the basis for transpositional activation of the *bgl* operon. Analysis of P_0 activity in IS5-internal deletions demonstrated that enhancement is not simply due to insertional inactivation of sites inhibitory to P_0 but is an activity brought about by DNA sequences and the coding capacity of IS5. At least part of the enhancer function of IS5 is attributable to the interaction of the *ins5A* gene product with the termini of the element. This interaction operates in trans and is remarkably efficient. Likewise, a DNA segment flanked by only the termini of IS5 efficiently transposes when complemented by an intact copy of IS5 in trans (K. Fuchs and B.R., unpublished work). Whether the presence of both termini of IS5 is necessary for enhancement of P_0 activity is not known.

The only other known case in which activity of a genetic function is controlled by interaction of a defective element in cis with a function encoded by a nondefective element in trans is that of the maize mobile element *Spm/En* (suppressor-mutator/enhancer). In addition to reactivation of the locus by excision of the defective element (mutator function), both suppression and activation of the locus in cis by an intact element in trans were observed. This phenomenon was observed and accurately described by Barbara McClintock nearly 40 years ago and is now being studied at the molecular level (reviewed in refs. 1–3). Suppressor and activator functions in trans are exerted by protein TnpA, the major gene product of *Spm/En*. The responsive sequences in cis are subterminal repetitive sequence motifs of the element (7, 8, 32). The molecular basis for the activation is not known.

How IS5 sequences act in conjunction with functions expressed by IS5 to modulate promoter P_0 activity needs further investigation. The following observations may, however, give us some clues as to possible mechanisms. Promoter P_0 is under positive control by the CAP–cAMP complex (12, 15, 33). The only point mutations that we found in our collection of spontaneous mutations activating the *bgl* operon were base exchanges at two positions within the CAP binding site (alleles *C234* and *BC75* in Fig. 1). The same exchanges have previously been found after chemical mutagenesis as the only activating point mutations (13, 15). Both exchanges lead to a sequence that is more similar to the CAP consensus site (34), and it has been shown that the exchange corresponding to allele *C234* leads to increased CAP–cAMP binding (15). On the other hand, the wild-type sequence of the CAP binding site, when compared with other known CAP binding sites (34), appears not to be extraordinarily inefficient. The distances from the centers of the CAP sites to the transcriptional start sites are identical in the *bgl* and *lac* operon. In fact, substitution of the CAP binding site of the *lac* operon for that of the *bgl* operon demonstrated that the *bgl* CAP site was fully functional in this context (unpublished results). The sequences upstream of promoter P_0 are notably A+T-rich (>80%). A third type of spontaneously derived activating mutation carries deletions of these upstream sequences that have one endpoint close to the CAP binding site (unpublished data; see also Fig. 1). We speculate that the regulatory region of the *bgl* operon is in an unfavorable topological state for which the A+T-rich upstream region is in part responsible. The A+T-rich region may be in a partially unwound state and thereby may interfere with CAP–cAMP and/or RNA polymerase binding. This effect may be overcome by an increased affinity brought about by the mutant CAP sites. A tightly bound CAP–cAMP complex may work like a clip stabilizing the double strand. Lopilato and Wright

(13) recently inserted linker sequences upstream of the A+T-rich region and subsequently screened the resulting clones for their Bgl phenotype. Interestingly, all Bgl^+ clones that they obtained were found to contain multiple tandem linker inserts. Since each linker was identical and palindromic, these inserts could potentially form extended, complex cruciform structures. Cruciform formation is known to be induced by neighboring A+T-rich sequences and would absorb negative supercoiling, thereby allowing the A+T-rich region to base-pair more stably (35).

That the *bgl* operon in its wild-type state is also active in mutant strains with an overall reduced superhelical density (36) supports the general concept of an unfavorable topological state of the control region caused by the A+T-rich region and induced by negative superhelicity. In this context and in view of the data presented here, we have to assume that a resident IS*5* alters the topological state of the DNA in its vicinity and that this alteration can be induced, at least in part, by the interaction of the termini of the element with a product expressed from its transposase gene and active in trans. This adds to the long list of activities attributable to mobile DNA elements. The wild-type *bgl* operon is not only active in host mutants with an overall decrease in negative superhelicity (36) but, paradoxically, is also active in host mutants with an overall increase (37). This observation makes us hesitant to speculate that identical mechanisms operate in P_0 activation when IS*5* is integrated upstream or downstream from promoter P_0.

The upstream A+T-rich region appears to be a hot spot for transposition of IS*1* and IS*5*. Integration of IS*1* and IS*5* into the upstream region occurs with remarkably high rates (12), while mutants containing IS*5* within the leader are rare (<1 in 1000 mutants analyzed). High-frequency transposition of IS*1* and IS*5* into a certain region has also been observed for a derivative of plasmid pBR322 with enhanced negative superhelicity (38). This could be taken as circumstantial evidence that the attractiveness of the A+T-rich upstream region may in part be due to enhanced negative superhelicity of this region. It has been suggested that inactivity of the *bgl* operon in the wild type provides a selective advantage due to the abundance of toxic β-glucosidic compounds (12). We speculate that the upstream region has evolved not only to silence the operon but additionally to attract integration of the insertion elements IS*1* and IS*5*. Consequently, transpositional activation could be viewed as a switching mechanism on the population level rather than as a mutational activation. A switch needs to be turned both ways, and it is well known that insertion elements can be lost by precise excision (4). In fact, such excision has been observed in the case of the *bgl* operon (unpublished results).

About 98% of all spontaneous Bgl^+ mutants that we have analyzed so far were due to transposition of IS*5* or IS*1* (14). We have, however, also found rare Bgl^+ mutants in which IS*2*, IS*10*, and Tn*1000* were integrated into the *bgl* upstream region (unpublished results; see Fig. 1). Thus, the potential of insertion elements to alter DNA topology in their neighborhood may be a more general one.

Site-specific mutants of IS*5* were constructed by Frank Brombacher. Hans-Jörg Ronecker constructed the *ins5A* frameshift mutant and selected mutant alleles *C243* and *BC75*. Gudrun Hoeksma and Elge Koalick contributed with skillful technical assistance. Oligonucleotides were synthesized by Gabor Igloi. Edward Schwartz carefully read the manuscript. Many thanks to all of them. This work was financed by the Deutsche Forschungsgemeinschaft, Grants SFB31 and Ra276/3-7.

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