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Genetics

# Maize *Spm* transposable element has an enhancer-insensitive promoter

(plant promoters/transient assay/particle bombardment/luciferase gene/transcription regulation)

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ABSTRACT **We have used a transient assay system to investigate the promoter region of the maize *Suppressor-mutator* (*Spm*) transposable element. All of the sequence required for constitutive promoter activity is confined to the 0.2-kb sequence upstream from the transcription start site of the element at nt 209 and designated the upstream control region. The element's promoter is weak, lacks a conventional TATA box, and depends on the presence of multiple, short repetitive sequence elements. The *Spm* promoter is quite insensitive to the enhancer sequence of the cauliflower mosaic virus 35S promoter. Enhancer sensitivity can be restored by providing a −30 TATA sequence and removing the G+C-rich sequence encoding the untranslated leader of the element, designated the downstream control region. Although the downstream control region is without effect on *Spm* promoter activity, it completely inhibits the 35S core promoter and markedly inhibits activity of the complete 35S promoter. The properties of the *Spm* element's promoter buffer it from both mutational and position-dependent changes in activity. We suggest that the inherent characteristics of the promoter are part of the genetic mechanism that controls the element's transposition frequency, ensuring it remains low and insertion-site independent.**

Paradoxically, transposition endangers the survival of a mobile element. Transposable-element insertions can disrupt essential genes and cause deleterious chromosomal rearrangements. An element can also insert near a strong promoter or enhancer that could potentially increase its expression and thereby its probability of causing damage. Transposable elements have a hierarchy of mechanisms that contribute to minimizing transpositional activity, including both conventional repressor proteins and a number of unusual properties of promoters, transcripts, and transposases (for review, see ref. 1). Although less is known about the control mechanisms of eukaryotic than prokaryotic transposable elements, it has been reported that the *P* element of *Drosophila melanogaster* is regulated by both alternative splicing and transcriptional repression (2, 3). The transpositional activity of the maize *Suppressor-mutator* (*Spm*) element is controlled by several mechanisms (4). The mRNAs coding for the two element-encoded proteins required for transposition arise by alternative splicing of the single primary transcript of the element (5–7). These mRNAs are produced in vastly different amounts and the protein encoded by the least abundant transcript may be rate-limiting for transposition. *Spm* elements are also subject to an epigenetic inactivation mechanism that results in transcriptional inactivation and extensive methylation of sequences near the element's transcription start site (8, 9). Moreover, fully methylated elements are poorly mobilizable *in trans*, indicating that methylation interferes with transposition, as well as with transcription (8).

*Spm* encodes a positive regulatory gene product that can transiently reactivate an inactive element, as well as promote its heritable reactivation (10, 11). TnpA, which is one of the two element-encoded proteins required for transposition, has recently been identified as the positive regulator (12). TnpA is a DNA-binding protein, and there are multiple copies of its 12-bp binding site located at the 5′ end of the element, just upstream from the transcription start site, as well as at the 3′ end of the element, adjacent to its 13-bp terminal inverted repeat (TIR) sequence (13). To gain further insight into molecular mechanisms regulating *Spm* transposition at the transcriptional level, we have analyzed the promoter of the element using transcriptional fusions to a firefly luciferase (LUC) gene. We report here that the *Spm* promoter has unusual properties that contribute not only to minimizing expression but which also render it resistant to the influence of a nearby enhancer.

## MATERIALS AND METHODS

***Spm* Promoter Fusion Constructs.** The firefly LUC gene served as the reporter gene in the present experiments. The LUC-encoding region in pDO432 is between the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase polyadenylylation signal (14). The *Bam*HI site at position 3476 of pDO432 was eliminated by fill-in to give pDC107, which was used to make promoter fusions. The *Spm* promoter [nt 1–576; upstream control region (UCR) plus downstream control region (DCR) from pdSpmΔRV (15)] was filled in and inserted into the *Sma* I site of Bluescript II KS(+) to give pDC105 (sense orientation, *Spm* 5′-end at the *Hin*dIII site) and pDC103 (antisense orientation, *Spm* 5′-end at the *Bam*HI site). The *Hin*dIII–*Bam*HI promoter fragments from pDC105 and pDC103 were transferred to pDC107 to yield pDC120 and pDC117, respectively. The *Hin*dIII–*Bam*HI promoter fragment from pDC105 was cut with *Mbo* II, and both the 220-bp UCR and the 356-bp DCR fragments were filled in and cloned into the *Sma* I site of Bluescript II KS(+), giving pDC121 and pDC124 (UCR in the sense and antisense orientations, respectively), as well as pDC131 and pDC130 (DCR in the sense and antisense orientations, respectively). The *Hin*dIII–*Bam*HI promoter fragments from pDC121 and pDC124 were transferred to pDC107, giving pDC141 and pDC146, respectively. The 3′-end of *Spm* from p*dSpm*ΔRV was transferred to the filled-in *Nde* I site of pDC120 and pDC141 as a filled-in 350-bp *Hpa* II–*Xho* I fragment.

Abbreviations: *Spm*, *Suppressor-mutator*; UCR, upstream control region; DCR, downstream control region; TIR, terminal inverted repeat; CaMV, cauliflower mosaic virus; LUC, luciferase; CAT, chloramphenicol acetyltransferase.

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**5′-Terminal Deletions in the *Spm* Promoter.** The 5′-end of the *Spm* promoter in pDC105 was progressively deleted using exonuclease III and S1 nuclease (New England Biolabs), as described in Sambrook *et al.* (16), then filled in, and religated to a *Sma* I linker. Deletion end points were identified by sequencing (United States Biochemical Sequenase kit). Deleted promoter fragments were transferred to pDC107 as *Bam*HI–*Hin*dIII fragments, giving the following plasmids and deletion endpoints: pDC223 (−195); pDC225 (−146); pDC227 (−95); pDC229 (−50); pDC241 (−41); pDC233 (+65).

**CaMV 35S Enhancer Constructs.** The core CaMV 35S promoter includes 46 bp upstream of the initiation site, and the enhancer of the promoter extends from −46 to −343 (17). A *Fok* I site at −58 in the 35S promoter of pDC107 was converted to a *Hin*dIII site by partial *Fok* I digestion, filling in, and addition of a *Hin*dIII linker to give pRR421, from which the 35S enhancer was removed as a *Hin*dIII fragment to give a plasmid having only the CaMV 35S core promoter driving the LUC gene. The *Hin*dIII site at position 1 of pRR421 was removed by filling in to yield pRR425, which has the CaMV 35S core promoter (nt −1 to −58) between the *Hin*dIII and *Bam*HI sites. The core promoter of pRR425 was replaced by *Hin*dIII–*Bam*HI fragments containing the complete *Spm* promoter (UCR plus DCR) or UCR only or a deletion derivative (−41UCR plus DCR), yielding plasmids pRR428, pRR429, and pRR436, respectively. The chloramphenicol acetyltransferase gene (CAT) and nopaline synthase 3′ termination sequence were transferred from pUC8CAT (18) to pFF19G (19) as an *Eco*RI–*Bam*HI fragment to yield the CAT reference plasmid pDC155.

**Site-Directed Mutagenesis.** Mutations were introduced in the putative TATA box of the *Spm* promoter (−30 TATGAAT, ref. 20) by site-directed mutagenesis (Muta-gene phagemid *in vitro* mutagenesis kit, version 2, Bio-Rad) of pDC105 (UCR plus DCR), pDC121 (UCR), and pDC221 (−41UCR plus DCR). The oligonucleotide 5′-TTAGGGTAACTAAGTATAGAGTGTC-3′ was used to completely change the putative TATA box. The following oligonucleotides were used to introduce 1- and 3-bp changes (indicated in boldface type) in the putative TATA box: 5′-TTAGGGTAACATTTATAAGAGTGTC-3′, and 5′-TTAGGGTAACTTATATAAGAGTGTC-3′. The changes were verified by sequencing, and inserts from the plasmids carrying these changes were transferred as *Hin*dIII–*Bam*HI fragments to pDC107 and pRR425, which lack and contain the 35S enhancer, respectively.

**Transient Expression Assay.** The promoter activity of the constructs was assayed in tobacco NT1 suspension cells, grown as described in Russell *et al.* (21). Midlogarithmic-phase NT1 cells were collected on filter paper discs (Whatman, no. 1001042) by vacuum filtration, and the filters were placed on agar plates supplemented with 2,4-dichlorophenoxyacetic acid (0.2 mg/liter) for bombardment (21). Test plasmids were purified by banding in CsCl-ethidium bromide density gradients, and the concentration was determined spectrophotometrically. Tungsten particles (95 mg, 1.1-μm diameter; M-17, Bio-Rad) were washed in 1 ml of absolute ethanol and resuspended in 1 ml of 50% glycerol; 22.5 μl of the particle suspension was mixed with 22.5 μl of a DNA solution containing 2.5 μg of the test plasmid and 1 μg of the reference CAT plasmid in a microcentrifuge tube with continuous mixing. This procedure was followed by addition of 37.5 μl of 2.5 M $CaCl_2$ and 15 μl of 0.1 M spermidine (free base, Sigma). Mixing was continued for 5 min, and the tubes were spun briefly to pellet the tungsten particles. The supernatant was removed, and the particles were washed once with 70% (vol/vol) ethanol and then resuspended in 25 μl of absolute ethanol. Aliquots of 6 μl each were spotted onto the macrocarriers and dried *in vacuo*. The NT1 cells were bombarded by these particles under 28 inches of Hg (1 in = 2.54 cm) vacuum with 1100 psi (1 psi = 6.9 kPa) rupture discs using the particle gun (Biolistic PDS-1000/He System, Bio-Rad). Each sample was bombarded in triplicate. The cells were incubated at room temperature for ≈16 hr.

**Enzyme Assays.** The NT1 cells were transferred from the filter to a microcentrifuge tube with a spatula. Cell extracts were prepared by grinding the cells in 0.4 ml of buffer (0.1 M Tris, pH 7.8/1 mM dithiothreitol/bovine serum albumin at 0.1 mg/ml), followed by centrifugation at 12,000 rpm for 3 min. LUC activity was determined twice for each extract by mixing 50 μl of supernatant with 200 μl of assay buffer (25 mM Tricine, pH 7.8/15 mM ATP/bovine serum albumin at 0.5 mg/ml) and initiating the reaction by injecting 100 μl of 0.5 mM luciferin (Sigma) into the mixture. Peak light intensity was measured in a luminometer (Monolight 1500; Analytical Luminescence Laboratory, San Diego). The remaining cell extract was incubated at 60°C for 10 min and centrifuged at 12,000 rpm for 3 min. To assay CAT activity, 20 μl of the supernatant was transferred to a fresh tube, and to this 100 μl of CAT assay buffer containing 0.1 M Tris, pH 7.8, 5 μl of 1-butyryl CoA (5 mg/ml, Sigma), 1.75 μl of [$^{14}$C]chloramphenicol (50 mCi/ml, NEN; 1 Ci = 37 GBq) was added. Samples were incubated at 37°C for 1 hr, and the reaction was terminated by extraction with 300 μl of mixed xylene. The organic phase was transferred to a fresh tube and back-extracted with 300 μl of 0.1 M Tris, pH 7.8. Fifty microliters of the organic phase was mixed with 5 ml of scintillation mix, and radioactivity was counted in a liquid scintillation counter.

## RESULTS

**The Reporter Gene Transient Assay for *Spm* Promoter Activity.** We constructed transcriptional fusions containing sequences from the 5′ end of the *Spm* element fused to a complete firefly LUC cDNA and a nopaline synthase transcription termination sequence (14). Initially we used a 5′-terminal 576-bp *Dde* I fragment of *Spm* containing the transcription initiation site (nt 209) and the untranslated first exon (20). The choice was based on the results of earlier experiments showing that all of this 5′-terminal sequence is unmethylated in transcriptionally active *Spm* elements and methylated in transcriptionally silent ones (8). We had further subdivided this region based on differential methylation into a 0.2-kb UCR extending from the TIR of the element to its transcription initiation site and an 0.4-kb DCR comprising most of the first untranslated exon sequence (Fig. 1; ref. 8). The UCR contains nine repeats of a sequence that has at least 83% identity to the 12-bp consensus TnpA-binding site (CCGACACTCTTA). The DCR sequence is highly G+C-rich and contains 11 direct repeats of 17-bp sequences with homology to the consensus sequence CGGGCGGGCGGCCTCGC (8, 22). To assess the contribution of these sequences to promoter activity, the UCR and DCR were separated at an *Mbo* II site located 220 bp from the 5′ end of the element (Fig. 1). Plasmids in which the LUC gene was expressed from an intact, deleted, or altered *Spm* promoter sequence were introduced into tobacco suspension cells together with a reference 35S-CAT plasmid by microprojectile bombardment. CAT and LUC activities were measured after 16 hr, and the results are reported as the ratio of LUC

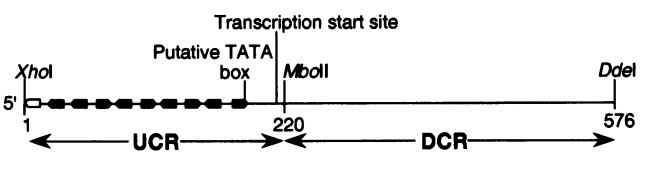


FIG. 1. The 5′ end of *Spm* element. The open arrowhead represents the 5′-TIR, and filled arrowheads represent TnpA-binding sites.

activity to the CAT activity obtained with the reference plasmid (see *Materials and Methods*).

***Spm*'s Weak Promoter Is Contained Within the UCR.** When compared with the widely used viral promoter that drives expression of the CaMV 35S transcript, the 5′ terminal 576-bp sequence of the *Spm* element displays weak promoter activity (Table 1). The promoter activity of the *Spm* element's 5′ end comprising both UCR and DCR is generally 1–2% of that observed using the complete, enhancer-containing CaMV 35S promoter and is comparable to that seen with the CaMV core promoter lacking enhancer sequences (see below). The 5′ terminal sequence of the *Spm* element nonetheless permits expression of the LUC gene at 8–10 times the background level seen in the absence of a promoter (Fig. 2*a*). Virtually identical LUC/CAT values were obtained using either the UCR alone or both UCR and DCR sequences. Plasmids having the UCR or the UCR plus DCR in reverse orientation gave LUC/CAT values no higher than those seen with control plasmids lacking a promoter sequence. Thus, expression of the LUC gene depends on the orientation of the promoter, and all promoter activity detected by this assay resides in the 5′-terminal 220-bp UCR fragment.

To further localize the sequences responsible for *Spm* promoter activity, the UCR sequence was deleted progressively from the 5′ TIR into the DCR, as shown in Fig. 2*b*. Elimination of the TIR results in a small, but reproducible enhancement of promoter activity. Deletion of the region from −195 to −41 causes the progressive loss of 90% of promoter activity; half of the loss is attributable to elimination of the sequence between −195 and −146. Approximately 10% of the activity of the complete promoter was detected with the −41 deletion, whereas the +65 deletion supported no LUC expression over background. The DCR, therefore, has no independent promoter function, reinforcing the earlier conclusion that only the UCR has promoter activity (Fig. 2*a*).

The 0.3-kb sequence adjacent to the *Spm* elements 3′ TIR is similar in organization to the UCR and contains 14 12-bp repeats with at least 75% sequence identity to the consensus TnpA-binding site. Because of the sequence resemblance, we assessed its effect on *Spm* promoter activity by introducing the 3′ end of the element downstream of the LUC gene (7, 22, 23). The presence of the 3′ end of the element had no effect in either orientation on the activity of the promoter consisting of either UCR alone or both UCR and DCR (Fig. 3).

**The *Spm* Promoter Is Unresponsive to an Adjacent Enhancer Sequence.** To determine whether the weakness of the *Spm* promoter can be explained simply by the absence of enhancer sequences, we introduced the strong enhancer of the CaMV 35S promoter just upstream of the *Spm* promoter. The 35S enhancer stimulates expression from its own core promoter by 100- to 200-fold but enhances expression from the *Spm* promoter by a factor of only 2–4 (Fig. 4*a*). Removal of either the DCR or deletion of the UCR to −41 had little effect on the ability of the *Spm* promoter to respond to the enhancer, although the latter decreases its basal activity markedly. The enhancer insensitivity of the *Spm* promoter must, therefore, be attributable either to some feature of the sequence between −41 and the transcription start site or a combination of several factors.

Table 1. LUC expression from CaMV 35S and *Spm* promoters

| Promoter | Replicate | LUC* | CAT* | LUC/CAT |
|---|---|---|---|---|
| CaMV 35S | 1 | 650,281 | 4089 | 159 |
| | 2 | 726,892 | 4013 | 151 |
| | 3 | 693,269 | 4227 | 164 |
| Mean | | | | 158 ± 6.6 |
| *Spm* | 1 | 7,384 | 4923 | 1.5 |
| | 2 | 6,932 | 5332 | 1.3 |
| | 3 | 7,023 | 4869 | 1.4 |
| Mean | | | | 1.4 ± 0.1 |

*Extracts from each replicate were assayed in duplicate as described in *Materials and Methods*, and the average of the two values obtained is shown.

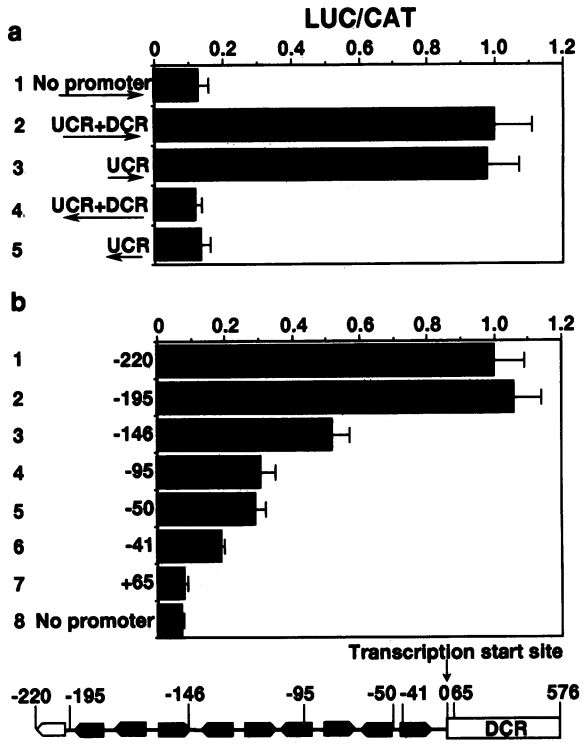


FIG. 2. (*a*) Promoter activity of the 5′ end of the *Spm* element. The *Spm* sequence tested for promoter activity is shown, and orientation of the promoter with respect to the LUC gene is indicated by the arrow (left to right indicates sense orientation). (*b*) Effect of 5′-terminal promoter deletions. Numbers on the *y* axis refer to deletion end points shown in diagram at the bottom. Results are represented as the ratio of LUC to CAT activity.

**Sequence Features Responsible for the Enhancer-Insensitivity of the *Spm* Promoter.** Because the sequence postulated to be the TATA box of the *Spm* element (−30 TATGAAT) differs substantially from that of the 35S promoter (TATATAA), we first asked whether this sequence is necessary for element transcription (20). When this putative TATA sequence was completely changed so that each base was replaced by its complement, there was no diminution of promoter activity (Fig. 4*b*). Similarly, when the sequence was replaced by an optimal TATA sequence, there was no increase in *Spm* promoter activity. Thus, the −30 TATGAAT is not essential to the function of the promoter of the *Spm*

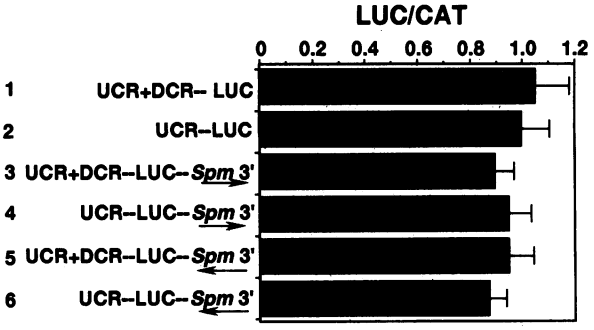


FIG. 3. Effect of the 3′ terminus of the element on *Spm* promoter activity. The 3′ terminus of the *Spm* element is downstream from the LUC gene. The arrow from left to right represents the normal orientation of the 3′ end with respect to the 5′ end of the element.

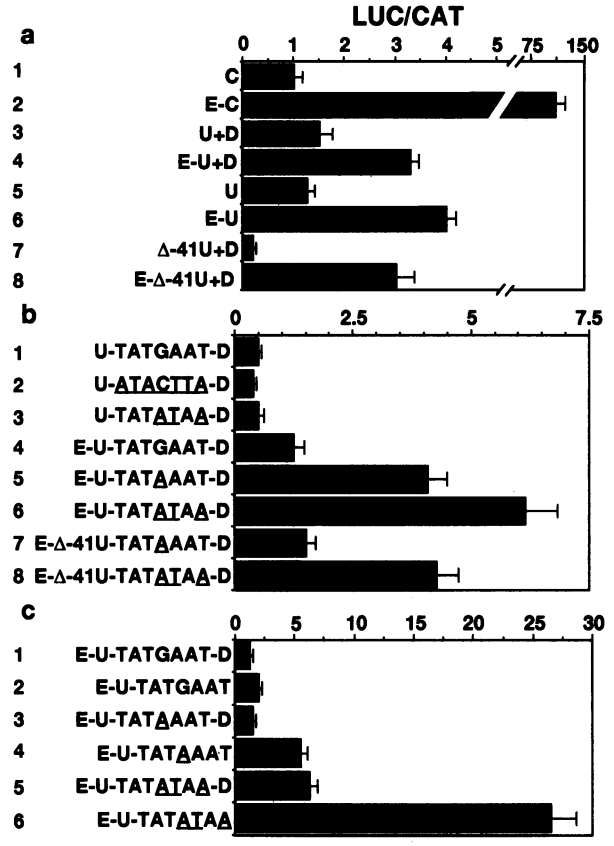


FIG. 4. Effect of changing the −30 sequence and deleting the DCR on *Spm* promoter activity. (*a*) Effect of the CaMV 35S enhancer on intact and deleted *Spm* promoters. (*b*) Effect of changing the −30 TATGAAT sequence on *Spm* promoter activity with and without the 35S enhancer. (*c*) Effect of 35S enhancer on activity of *Spm* promoter with an altered −30 sequence with and without the DCR. Altered bases in the −24 to −30 sequence are underlined. C, 35S core promoter; E, 35S enhancer; U, UCR; D, DCR.

element. However, when the sequence was changed so that it more closely resembled an optimal TATA box, the *Spm* promoter became more responsive to the 35S enhancer (Fig. 4*b*). This was true even when only a single base was changed from guanine to adenine at position −27. But the poor response of the *Spm* promoter to the enhancer cannot be entirely attributed to the suboptimal TATA sequence because complete conversion of the *Spm* −30 sequence to the optimal TATA sequence of the 35S promoter results in only a modest further enhancement.

Deletion of the UCR sequence to −41 reduces the responsiveness of the TATA-containing *Spm* promoter to the 35S enhancer (Fig. 4*b*). However, when the DCR region is removed from an *Spm* promoter with an optimal TATA box at −30, it becomes highly responsive to the nearby enhancer (Fig. 4*c*). Although the combination of removing the DCR and introducing a single-base change that increases resemblance of the −30 sequence to a TATA box has some effect, the largest increase is seen when the −30 sequence is replaced by the TATA sequence of the CaMV 35S promoter. Thus, an *Spm* promoter comprising the UCR with an optimal TATA sequence at −24 to −30 is ≈50 times more active when adjacent to the enhancer than is the complete *Spm* promoter without the enhancer, regardless of its TATA sequence (Fig. 4 *b* and *c*; note the scale difference). This level of enhancement is within a factor of 3 of that seen when the 35S enhancer is combined with its own core promoter, suggesting that the major factors accounting for the enhancer-insensitivity of the *Spm* promoter are the presence of the DCR sequence and the absence of a conventional TATA box.

**The DCR Inhibits the 35S Promoter but Does Not Inhibit the *Spm* Promoter.** The DCR sequence decreased the activity of the complete 35S promoter by 10-fold when placed downstream from it (Fig. 5*a*). When placed in the same relative position, the DCR had no effect on the activity of the *Spm* promoter but reduced the activity of the 35S core promoter to a virtually undetectable level (Fig. 5*b*). Thus, the DCR sequence is without effect on the activity of its own promoter but is highly inhibitory to expression from the 35S promoter.

## DISCUSSION

The promoter of the *Spm* element is unusual in several ways that contribute to the transcriptional control of transposition frequency. Not surprisingly, the basal constitutive activity of the *Spm* promoter is low. When compared with a strong plant viral promoter, that of the CaMV 35S transcription unit, the *Spm* promoter was found to be weaker by about two orders of magnitude. Promoter activity is confined to the 0.2-kb UCR sequence between the 5′-TIR and the transcription start site and is lost progressively as the sequence is deleted. The most striking feature of this sequence is the presence of nine repeats, in both orientations, of sequences homologous to or identical with the 12-bp TnpA-binding site. Indeed, promoter strength is reduced by 50% upon deletion of the most 5′ distal tail-to-tail TnpA-binding site dimer, previously identified as the strongest TnpA-binding site sequence (23). Elimination of successive TnpA-binding sites causes a further reduction in activity, although 10% of the activity of the entire promoter is retained by a deletion derivative containing only a single, monomeric TnpA-binding site. The *Spm* promoter, therefore, appears to require multiple sequence elements present within the UCR. Because TnpA-binding sites comprise the repetitive elements of the UCR, the sequence elements required for promoter activity may either overlap or coincide with the TnpA-binding sequences.

The promoter sequence of the *Spm* element is coextensive with the 5′-terminal sequence required for optimal transposition frequency (4, 22, 24). TnpA is one of two element-encoded proteins required for *Spm* transposition, and TnpA-binding sites are present at both element ends (7, 12, 13). Thus, the interactions between TnpA and its target binding sequences are clearly central to both regulation of transcription and transposition of the *Spm* element. There is evidence that an inactive, methylated *Spm* promoter is reactivated by TnpA, either directly or indirectly (8, 12). Conversely, TnpA inhibits expression of the unmethylated promoter (ref. 25; R.R. and N.F., unpublished work). Hence, TnpA exerts both positive and a negative regulatory effects on its own pro-

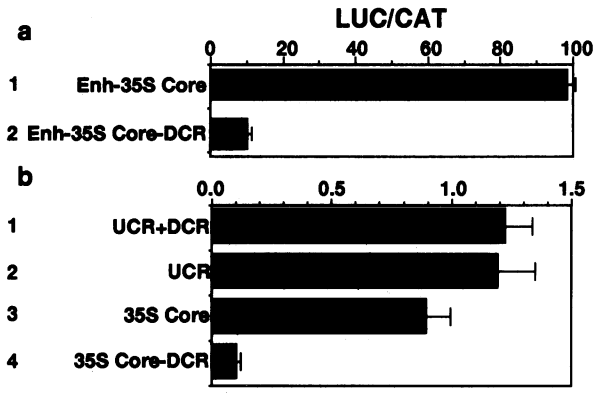


FIG. 5. Effect of DCR on activity of the complete CaMV 35S promoter (*a*) and the *Spm* promoter and CaMV 35S core promoter (*b*).

moter, contributing to the transcriptional regulation of transposition by activating the inactive, methylated form and inhibiting the constitutive, unmethylated form of the *Spm* promoter.

The *Spm* promoter is unusual, although not unique, in lacking a conventional TATA sequence (26–28). There are several similar A+T-rich stretches of 9–10 bp having the composition $T_{2\text{-}3}A_2T_{2\text{-}3}A_{2\text{-}3}$ repeated upstream from the transcription initiation site. Although these overlap the TnpA-binding sites and may prove important for promoter function, the −30 TATGAAT sequence previously suggested to be its TATA box is not essential for *Spm* promoter activity (20). Moreover, the *Spm* promoter is strikingly insensitive to the effect of a nearby strong enhancer sequence. The 35S enhancer sequence, which boosts the activity of its own core promoter by 100- to 200-fold, enhances *Spm* promoter activity by only 2- to 4-fold, a relatively minor effect. The enhancer-insensitivity of the *Spm* promoter is primarily attributable to two sequence features: the absence of a TATA box and the presence of the G+C-rich DCR. When the −30 TATGAAT sequence was replaced by an optimal TATA box and the DCR was removed, the ability of the *Spm* promoter to respond to the adjacent enhancer sequence increased by more than an order of magnitude. *Spm* promoter activity is unaffected by the presence of the DCR, implying that the leader sequence does not significantly affect the stability or translatability of the LUC mRNA. By contrast, it completely inhibits the 35S core promoter and markedly inhibits the complete CaMV 35S promoter when placed in an analogous position. Thus, the DCR may also interfere with read-through transcription from an external promoter, as reported for both the UCR and complete internally deleted *Spm* elements (23).

The results of the present experiments indicate that the *Spm* promoter has several features that serve to ensure low transcription activity, independent of the genomic position of the element. The promoter is weak, and the sequence elements required for promoter activity appear to be distributed within the internally repetitive UCR. The redundancy of weak promoter elements may serve to protect the *Spm* promoter from the potentially large effects of single-base changes. The UCR contains binding sites for the element-encoded TnpA protein, which exerts an inhibitory effect on constitutive expression of the promoter (25). Perhaps the most striking feature of the *Spm* promoter is its inability to respond to an external enhancer. A major determinant of this enhancer-insensitivity is the DCR sequence that encodes the untranslated leader of all of the element's transcripts (5). The DCR likewise appears to interfere with transcription from a promoter other than its own. Thus, the DCR is or contains a silencer sequence somewhat analogous to those identified in yeast and rat (29, 30). But because the DCR does not interfere with expression of the *Spm* promoter, its primary effect may be to buffer the element from the influence of an external enhancer or promoter near which it might insert. We conclude that these several inherent properties of the *Spm* promoter ensure minimal element transcription, contributing to the maintenance of a low transposition frequency, independent of the site of insertion of the element.

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