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The PSEA promoter element of the *Drosophila* U1 snRNA gene is sufficient to bring DmSNAPc into contact with 20 base pairs of downstream DNA

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ABSTRACT

Most of the major spliceosomal small nuclear RNAs (snRNAs) (i.e. U1, U2, U4 and U5) are synthesized by RNA polymerase II (pol II). In *Drosophila melanogaster*, the 5′-flanking DNA of these genes contains two conserved elements: the proximal sequence element A (PSEA) and the proximal sequence element B (PSEB). The PSEA is essential for transcription and is recognized by DmSNAPc, a multi-subunit protein complex. Previous site-specific protein–DNA photo-cross-linking assays demonstrated that one of the subunits of DmSNAPc, DmSNAP43, remains in close contact with the DNA for 20 bp beyond the 3′ end of the PSEA, a region that contains the PSEB. The current work demonstrates that mutation of the PSEB does not abolish the cross-linking of DmSNAP43 to the PSEB. Thus the U1 PSEA alone is capable of bringing DmSNAP43 into close contact with this downstream DNA. However, mutation of the PSEB perturbs the cross-linking pattern. In concordance with these findings, PSEB mutations result in a 2- to 4-fold reduction in U1 promoter activity when assayed by transient transfection.

INTRODUCTION

The genes that code for the spliceosomal small nuclear RNAs (snRNAs) are unusual transcription units in that most of the snRNA genes (U1, U2, U4 and U5 genes) are transcribed by RNA polymerase II (Pol II), but U6 genes are transcribed by RNA polymerase III (Pol III). However, the promoters for both classes of genes are similar and both utilize a unique multi-subunit transcription factor that has variously been termed the snRNA activating protein complex (SNAPc) (1,2), PTF (3,4) or PBP (5,6). In vertebrates, this protein complex recognizes a proximal sequence element, or PSE, centered ~50–55 bp upstream of the transcription start site of both classes of snRNA genes (7–10). U6 gene promoters additionally contain a TATA-box that is required for the Pol III specificity of the vertebrate U6 genes (11,12). The occurrence of a TATA-box 25–30 bp upstream of the transcription start site of U6 genes appears to be a conserved feature that is maintained throughout all branches of metazoan evolution. The vertebrate snRNA genes that are transcribed by Pol II, on the other hand, lack TATA-boxes or any other recognizable conserved element in the −25 to −30 region. However, TATA-binding protein (TBP) is still required for the transcription of these genes (1,13).

Our lab is studying the expression of the snRNA genes of the fruit fly *Drosophila melanogaster*. Interestingly, the *D.melanogaster* snRNA genes transcribed by Pol II contain two conserved elements in their 5′-flanking DNAs that are termed proximal sequence element A (PSEA) and proximal sequence element B (PSEB) separated by 8 bp of non-conserved sequence (Figure 1A). The three *D.melanogaster* U6 genes on the other hand each contain a PSEA separated by 12 bp from a downstream TATA-box.

The upstream PSEAs of both classes of fruit fly genes are recognized by DmSNAPc, a multi-subunit protein complex formerly called DmPBP (14–16). It contains three distinct subunits that are homologous to subunits of human SNAPc: DmSNAP43, DmSNAP50 and DmSNAP190. Site-specific protein–DNA photo-cross-linking studies have established the architectural arrangement of these three subunits on the U1 and U6 PSEAs (15,16). This is shown schematically for the

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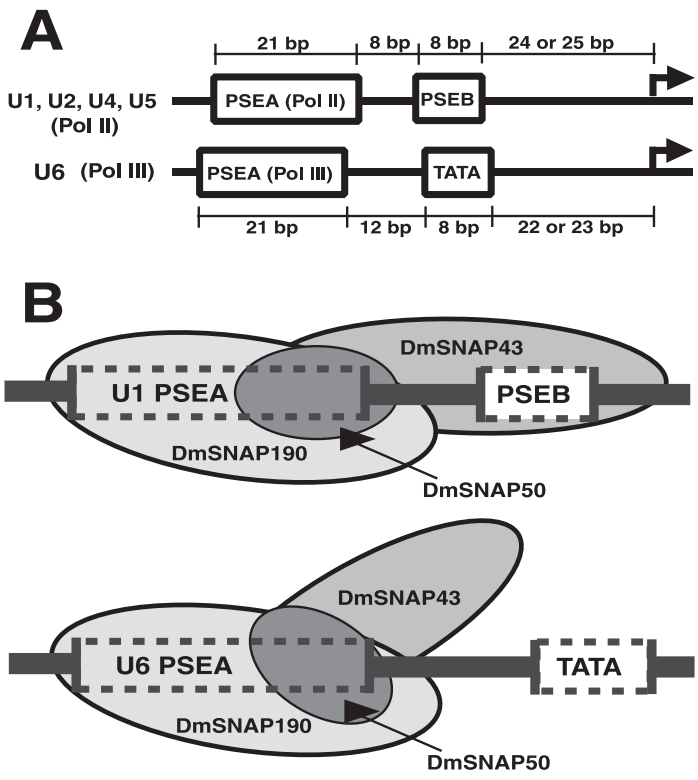

C

GENE	PSEB
U1:21D	CATGGAAA
U1:82Eb	CATGGAAA
U1:95Ca	CATGGAAA
U1:95Cb	TATGGAGA
U1:95Cc	CAAGGAGA
U2:14B	CATCAAGT
U2:34ABa	CATGGAGA
U2:34ABb	CATCGAGA
U2:34ABc	CATCGAGA
U2:38ABa	CATGAAGT
U2:38ABb	CATGAAGT
U4:25F	CATAGAGA
U4:38AB	AATGGAAT
U4:39B	CATGGTAT
U5:23D	AATCTAGG
U5:34A	AATGATCG
U5:35D	AGTGGTAC
U5:63BC	AATAGAGT
U5var:14B	ATCCTAGA
U5var:38ABa	AGTATGGA
U5var:38ABb	ATCCTAGA
Consensus:	$^{C}_{A}$ATGGA$^{GA}_{AT}$

Figure 1. (**A**) Organization of promoter sequences in the 5′-flanking DNA of *D.melanogaster* spliceosomal snRNA genes transcribed by RNA polymerase II (U1, U2, U4 and U5) or RNA polymerase III (U6). (**B**) Schematic representations of the interaction of the subunits of DmSNAPc with the U1 (upper) and U6 (lower) promoters as determined by site-specific protein–DNA photo-cross-linking (15,16). Each subunit (DmSNAP190, DmSNAP50 and DmSNAP43) is drawn to indicate the approximate region of the DNA with which it makes contact. (**C**) A listing of the sequences of the PSEBs of the known U1, U2, U4 and U5 genes of *D.melanogaster*. A consensus sequence is shown at the bottom.

U1 and U6 gene promoters in Figure 1B. On both promoters DmSNAPc contacts the PSEA, but on the U1 promoter the DmSNAP43 subunit further contacts nucleotides for 20 bp beyond the PSEA. This finding raised the possibility that the PSEB might provide some sequence-specificity for the binding of DmSNAP43 to DNA sequences downstream of the U1 PSEA. The PSEB sequences of the *D.melanogaster* U1, U2, U4 and U5 genes are compared in Figure 1C. A consensus sequence is shown at the bottom of the column.

The DNA contacts that DmSNAP43 makes near and within the PSEB seem to be important because substitution of a U6 PSEA for the U1 PSEA in the U1 promoter both (i) abolished the contacts of DmSNAP43 with the PSEB and (ii) suppressed transcription from the U1 promoter by Pol II (16,17). Photo-cross-linking and partial proteolysis assays indicate that DmSNAPc binds in altered conformations to the U1 and U6 PSEAs (15,16,18). In our working model (Figure 1B), the different sequences of the U1 and U6 PSEAs act as differential allosteric effectors of DmSNAPc, which then adopts conformations compatible with the formation of only Pol II or Pol III transcription initiation complexes.

Considerable work has been done on the function of the U1 and U6 PSEAs, but the function of the PSEB in transcription of fruit fly snRNA genes by Pol II is not understood. In an earlier study, we found that site-specific mutation of the PSEB resulted in an 8-fold decrease in promoter activity when assayed by transcription *in vitro* (19). However, its effect on transcription in living cells had not been investigated. Moreover, it seemed important to determine whether the PSEB sequence itself was required for DmSNAP43 to make contact to the DNA downstream of the U1 PSEA (Figure 1B). Here we report the results of such experiments.

MATERIALS AND METHODS

Site-specific protein–DNA photo-cross-linking assays

Forty-four different probes, each containing cross-linking reagent at a unique position, were used to scan for DmSNAPc interactions with the DNA downstream of the U1 PSEA on the template and non-template strands. Two series of probes were prepared: one having a U1 PSEA with a wild-type PSEB (CATGGAAA) downstream, and an analogous series with an 8 bp mutation in the PSEB (AGGCCTCT) (19). Cross-linker was incorporated at every second phosphate position as indicated by the asterisks in Figure 2. Probes were prepared as described previously (15,16), based upon earlier methods as described by Yang and Nash (20) and Lagrange *et al*. (21). Briefly, DNA oligonucleotides 23 to 38 bases long were synthesized with phosphorothioate incorporated 5′ of the third nucleotide from the 5′ end. The phosphorothioate-incorporated oligonucleotides were derivatized with azidophenacyl bromide and then radiolabeled at the 5′ end by using [γ-^{32}P]ATP and T4 polynucleotide kinase.

To make the photo-cross-linking probes double-stranded, a derivatized oligonucleotide and an unmodified upstream

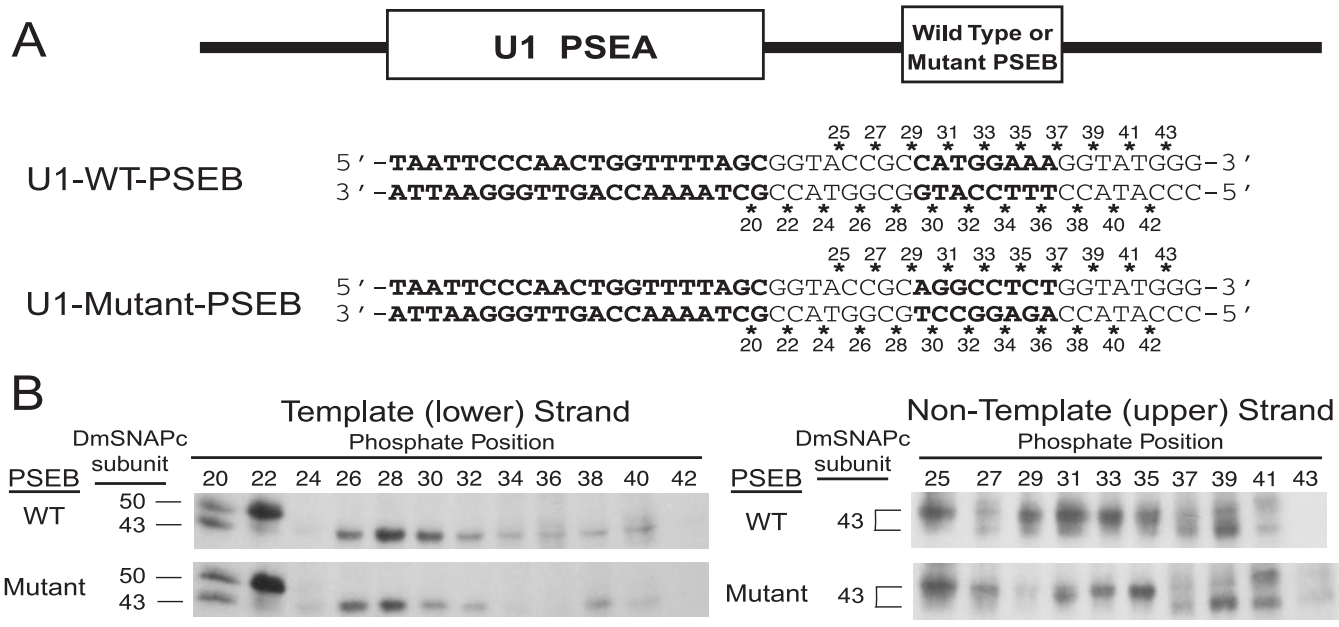

Figure 2. Site-specific protein–DNA photo-cross-linking of DmSNAPc subunits to phosphate positions downstream of the U1 PSEA in probes that contain either the wild-type or a mutant PSEB. (**A**) Sequences of the relevant areas of the U1 photo-cross-linking probes. The sequences of the 21 bp U1 PSEA, of the PSEB and of the mutant PSEB are shown in boldface type. Except in the region of the PSEB, the probes were identical to ensure that any differences in the cross-linking pattern were due solely to the substitutions in the PSEB. The individual phosphate positions at which a cross-linker was incorporated are indicated by the asterisks and numbers above and below the sequences. (**B**) Forty-four different radiolabeled site-specific probes were incubated in separate reaction mixtures with DmSNAPc. Following UV irradiation and nuclease digestion, polypeptides that cross-linked to the DNA were detected by SDS–PAGE and autoradiography. Only the regions of the gels that correspond to DmSNAP43 and DmSNAP50 are shown. The left panels show the results of cross-linking to the template strand, and the right panels show cross-linking to the non-template strand. In each case, the upper panel illustrates the pattern for the wild-type PSEB and the lower panel illustrates the pattern for the mutant PSEB. When cross-linked to positions 37–41 on the non-template strand, a portion of DmSNAP43 runs with an anomalous mobility, creating a doublet. (Evidence that the lower band is due to DmSNAP43 comes from photo-cross-linking experiments performed with DmSNAPc that contains a higher molecular weight tagged DmSNAP43. The tagged DmSNAP43 shifts the lower band (as well as the upper band) upward in the gel [data not shown]. The anomalous mobility is possibly due to the position of nucleotide linkage to the polypeptide chain.)

primer were annealed to one of four long oligonucleotides (see 81 and 82mer sequences below) that contained either the desired template or non-template strand sequences. The primers were extended by using T4 DNA polymerase and the four deoxynucleoside triphosphates. The nick that remained at the 5′ end of the derivatized oligonucleotide was sealed by using T4 DNA ligase. Wild-type probes with cross-linking reagent in the template strand were prepared by using the following 82mer in the reactions described above: 5′-ACGAATTCATTCTTA**TAATTCCCAACTGGTTTTAGC**GGTACCGCCATGGAAAGGTATGGGATCCTCAATACTTCGGCATGCA-3′. To generate wild-type probes with the cross-linking reagent in the non-template strand, the following 81mer was used: 3′-GCTTAAGTAAGAAT**ATTAAGGGTTGACCAAAATCG**CCATGGCGGTACCTTTCCATACCCTAGGAGTTATGAAGCCGTACGA-5′. (The letters in boldface correspond to the U1 PSEA and the underlined nucleotides represent the PSEB.) To prepare cross-linking probes that contained the mutated PSEB sequence, analogous oligonucleotides were used that contained the sequence 5′-AGGCCTCT-3′ instead of CATGGAAA in the underlined region of the 82mer (or its complement in the 81mer).

Photo-cross-linking reactions and analysis were carried out as described previously (15,16). Briefly, derivatized DNA fragments were incubated with partially purified DmSNAPc (HA300 fraction) from fruit fly embryo nuclear extracts (14). After 30 min at 25°C in the dark, reaction mixtures were irradiated with UV light for 5.5 min followed by digestions with DNase I and S1 nuclease. Samples were electrophoresed through SDS–PAGE, and the cross-linked proteins were detected by autoradiography.

Wild-type and mutant reporter constructs

Constructs that contained the promoter of the U1:95Ca gene (formerly referred to as the U1-95.1 gene) fused to the firefly luciferase gene have been described previously (17). Each of these constructs contained 381 bp of 5′-flanking DNA plus 32 bp of the U1 coding region. Constructs that contained the U6-2 maxi-gene together with 409 bp of 5′-flanking DNA have also been described (17). The U6 maxi-gene contained a 10 bp insertion between 66 and 67 nt that allowed for the annealing of a complementary maxi-gene specific oligonucleotide for primer extension analysis without interference from endogenous U6 snRNA. Mutations were introduced either by cloning synthetic oligonucleotides between pre-existing restriction sites in either template or by using Stratagene's Quik-Change Site-Directed Mutagenesis kit. The wild-type U1 PSEA was 5′-TAATTCCCAACTGGTTTTAGC-3′; the U6 PSEA substitution was 5′-TAATTCTCAACTGCTCTTTCC-3′; the mutant PSEA sequence was 5′-CCTGATAGGTGACCAGGACTA-3′; the wild-type PSEB was 5′-CATGGAAA-3′; the mutant PSEB sequence was 5′-AGGCCTCT-3′; and the TATA substitution that replaced the PSEB was 5′-TTTATATA-3′.

The U6 PSEA and TATA sequences used for these substitutions were derived from the *D.melanogaster* U6-2 gene, one of three U6 genes in the *D.melanogaster* genome (22,23).

Transient expression assays

D.melanogaster S2 tissue culture cells were grown and transfected as described previously (17). All transfections were performed in triplicate, and each trial was carried out as a distinct experiment on a different day. In experiments that measured luciferase activity, the U1-(firefly)luciferase constructs were co-transfected with a *Renilla* luciferase expression plasmid as an internal control for transfection efficiency. Assays were carried out by using Promega's Dual-Luciferase Reporter Assay System and a Turner TD-20/20 luminometer.

For primer extensions to measure wild-type and mutant U1 and U6 promoter activities, total RNA was isolated from transfected cells as described previously (17). RNA aliquots (25 μg) each were co-precipitated with a ^{32}P-labeled 54mer oligonucleotide recovery standard before the extension reaction. Extensions made use of Promega's Primer Extension System with Invitrogen's SuperScript II reverse transcriptase substituted for Promega's AMV reverse transcriptase. The oligonucleotide primers used for the extension assays have been described previously (17). Briefly, the U1-luciferase primer was complementary to a region near the 5′ end of the luciferase gene and yielded an 84 nt extension product; the U6 primer was partially complementary to the U6 maxi-gene insert and yielded an 89 nt product. Following gel electrophoresis, the intensities of the extension products were quantified by phosphorimager analysis.

RESULTS

Mutation of the PSEB does not abolish the cross-linking of DmSNAP43 to DNA downstream of the U1 PSEA

When DmSNAPc binds to DNA that contains a U1 PSEA and a PSEB, the DmSNAP43 subunit not only contacts the 3′ end of the PSEA but also contacts the DNA near and within the PSEB (Figure 1B) (16). This conclusion was based upon results of high resolution site-specific protein–DNA photo-cross-linking experiments (15,16). The fact that DmSNAP43 contacts the PSEB in the U1 promoter raised the question of whether the sequence of the PSEB is necessary for these contacts to occur. Alternatively, it is possible that the U1 PSEA (but not the U6 PSEA) provides all the necessary information to place DmSNAP43 in contact with the DNA in the vicinity of the PSEB.

To examine this question, site-specific protein–DNA photo-cross-linking assays were carried out with DmSNAPc and radiolabeled DNA probes that contained either a wild-type or mutant PSEB downstream of the wild-type U1 PSEA (Figure 2A). Forty-four individual probes were prepared. Each probe contained cross-linker at an unique phosphate position either on the lower (template) strand or on the upper (non-template) strand at the individual positions indicated by asterisks in Figure 2A. A ^{32}P-radiolabel was incorporated at the adjacent phosphate in each case. Each probe was incubated individually with DmSNAPc from *D.melanogaster* embryos. The reaction mixtures were then irradiated with UV light to activate the cross-linking agent, subjected to extensive nuclease digestion and run on SDS–PAGE. Protein bands that contained cross-linked radiolabel were detected by autoradiography.

Figure 2B (left side) shows the results of cross-linking DmSNAPc to the template strand at positions 20 through 42 with probes that contain the wild-type PSEB (upper panel) or mutant PSEB (lower panel). The subunit known as DmSNAP50 cross-linked to both types of probes at positions 20 and 22, but this subunit did not cross-link to the DNA further beyond that point. In contrast, DmSNAP43, as seen previously (16), cross-linked to the wild-type probe as far as position 40, two turns of the DNA helix beyond the PSEA. The strongest cross-links were at positions 28 and 30.

Interestingly, probes that contained the mutant PSEB substitution also cross-linked to DmSNAP43 at positions downstream of the PSEA (Figure 2B, lower left panel). Some differences in the pattern of relative cross-linking intensities could be noted relative to the wild-type probes. For example, when the cross-linking at positions 20 and 22 were used for purposes of normalization, the wild-type probes cross-linked more strongly at positions 28 and 30 than did probes that contained the PSEB mutation. Differences were sometimes observed at positions 34, 36 and 40, but results with these weaker bands were less consistent from experiment to experiment.

When cross-linking experiments were carried out with the template strand, a similar set of results was obtained (Figure 2B, right side). SNAP43 cross-linked downstream of the U1 PSEA regardless of whether the probe contained the wild-type or mutant PSEB. At some positions, differences in the cross-linking intensity could be observed. For example, positions 29, 31 and 33 cross-linked more intensely in the wild-type probe than they did in the probe with the mutated PSEB. Conversely, positions 27 and 35 cross-linked with relatively greater intensity in the mutant PSEB probe.

In summary, the results of the photo-cross-linking experiments indicate that the U1 PSEA by itself is capable of bringing DmSNAP43 into close proximity to the DNA that encompasses the PSEB. Although the PSEB is not required for these contacts, the PSEB appears to subtly affect the way in which DmSNAP43 interacts with this region of the DNA.

The PSEB contributes to U1 promoter efficiency *in vivo*

Because mutation of the PSEB did not abolish the interaction of DmSNAP43 with the DNA downstream of the U1 PSEA, we wished to determine the extent to which the PSEB contributes to transcriptional efficiency in living cells. To do this, we carried out transient transfections in triplicate of *D.melanogaster* S2 tissue culture cells using U1 promoter constructs that contained substitutions in either the PSEB or the PSEA (Figure 3). We used luciferase as a reporter gene because previous work indicated that the *D.melanogaster* U1 promoter efficiently drives the synthesis of functional mRNAs (17). The results of luciferase assays are shown in the column at the right side of Figure 3. As noted previously (17), mutation of the PSEA either to an unrelated sequence (mutant PSEA) or to a U6 PSEA (which normally promotes efficient transcription by Pol III in the context of the U6 promoter) resulted in a reduction in reporter gene expression to 1–2% of the wild-type level (constructs B and C).

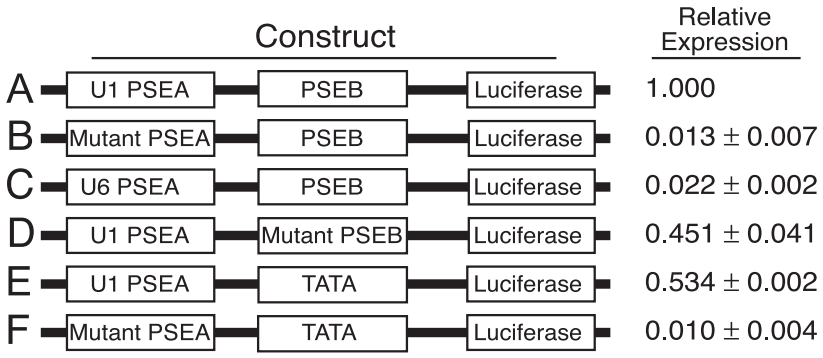

Figure 3. PSEB mutations reduce luciferase reporter gene expression ~2-fold. S2 cells were transfected with constructs that used the U1 promoter to drive luciferase gene expression. Construct A represents the wild-type configuration. The additional constructs (B–F) were identical to the wild-type except for substitutions in the PSEA and/or PSEB. The relative luciferase activities in extracts from cells transiently transfected with each of the individual constructs are given at the right. Errors are the standard deviation of the mean for three separate transfection experiments.

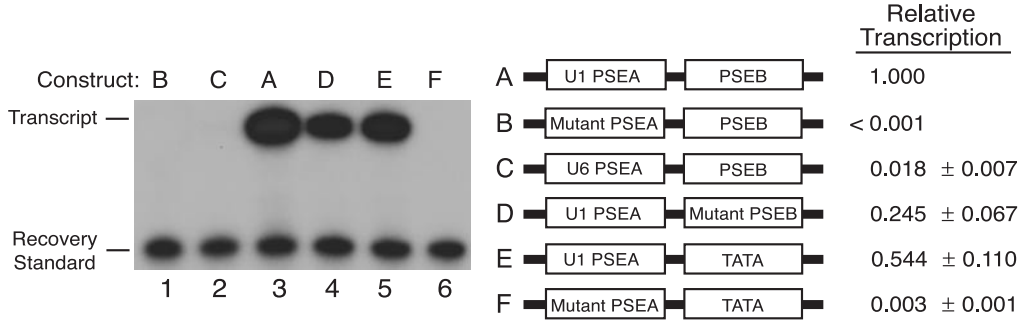

Figure 4. Primer extension assays indicate that mutations in the PSEB reduce U1 promoter activity ~2- to 4-fold. The autoradiogram at the left shows the results of primer extension assays with RNA isolated from S2 cells transiently transfected with the U1 promoter constructs shown in the middle of the figure. The relative promoter activities determined by phosphorimaging are shown in the column at the right. Errors are the standard deviation of the mean for three separate transfection experiments.

These results re-affirmed the critical importance of the U1 PSEA in promoting transcription by Pol II.

Mutation of the PSEB, on the other hand, to a GC-rich sequence (Construct D) reduced luciferase activity only by a factor of ~2. Interestingly, the replacement of the PSEB by a TATA-box (Construct E) similarly reduced expression ~2-fold. It is notable that the TATA-box worked only slightly better as a promoter element than the random GC-rich sequence (53 versus 45% relative expression efficiency). Although the contribution of the PSEB to U1 promoter activity was not dramatic, these results indicate that the PSEB represents a preferred sequence for U1 promoter activity.

The results with the final construct in Figure 3, which contains a TATA-box together with a mutant PSEA, indicates that the TATA-box alone, in the context of the U1 promoter, is insufficient for transcription *in vivo*. Thus, a TATA-box cannot compensate *in vivo* for inactivation of the PSEA. This contrasts with *in vitro* data in which substitution of a TATA-box for the PSEB increased transcription to 4.5-fold and made the high level of transcription independent of the PSEA (19).

To confirm the results of the luciferase assays, primer extension reactions were subsequently carried out. An autoradiogram of typical results is shown in Figure 4. Lane 3 illustrates the level of transcription obtained from the wild-type U1 gene promoter. In agreement with the results of the luciferase assays, an extensive mutation of the PSEA, as well as the conversion of the U1 PSEA to a U6 PSEA, reduced transcription from the U1 promoter to nearly undetectable levels (lanes 1 and 2). On the other hand, when the PSEB was changed either to a GC-rich sequence or to a TATA-box, a more modest reduction in transcription was observed (lanes 4 and 5). Furthermore, when the TATA substitution was combined with an inactive PSEA, transcription was almost negligible (lane 6). Thus, as observed with the luciferase assays, the PSEA was still required for transcription *in vivo* even when the PSEB was converted to a TATA-box.

A quantitation of primer extension data from three independent transfections are shown in the column at the right of Figure 4. The quantitative results of the primer extension assays were in close agreement with the results of the luciferase assays (Figure 3). The greatest discrepancy was with Construct D, but even in this case only a 2-fold difference was observed between the two types of assays. Though the effects of mutating the PSEB were relatively modest, the PSEB was reproducibly a 2- to 4-fold better U1 promoter element *in vivo* than either a mutant PSEB or a TATA sequence.

Can the PSEB substitute for a TATA-box in the U6 promoter?

Since mutation of the PSEB to a TATA-box had only a modest effect on U1 promoter activity *in vivo*, it became of interest to

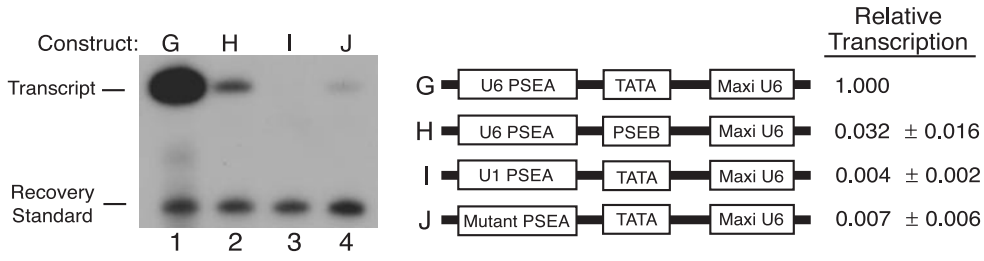

Figure 5. The PSEB cannot effectively substitute for the TATA-box in the U6 promoter. The U6 promoter was used to drive expression of a reporter U6 maxi-gene. The wild-type U6 promoter construct (G) and three promoter substitution constructs (H, I and J) were used to transiently transfect S2 cells. RNA was isolated and used for primer extension assays. An autoradiogram of typical results is shown in the panel at the left. The relative promoter activities determined by phosphorimaging are shown in the column at the right. Errors are the standard deviation of the mean for three separate transfection experiments.

determine whether a PSEB might substitute effectively for the TATA-box of the U6 snRNA gene promoter. To examine this possibility, the U6 maxi-gene reporter constructs diagrammed in Figure 5 were prepared and used for transfection of S2 cells. RNA was prepared and U6 promoter activity was measured by primer extension assays using a DNA primer specific for RNA transcribed from the maxi-gene constructs (17).

The results of a typical primer extension assay are shown in the panel at the left side of Figure 5. Lane 1 depicts the level of transcription obtained from the wild-type U6 promoter. When the 8 bp TATA sequence was changed to the sequence of the PSEB (CATGGAAA), transcription was still evident upon a long exposure but was dramatically reduced (Lane 2). As a baseline for comparison, mutation of the U6 PSEA either to a U1 PSEA or to an unrelated sequence reduced transcription nearly to undetectable levels (lanes 3 and 4).

The transfections were performed in triplicate and the results of the primer extensions were quantified by phosphorimaging. These data are shown in the column at the right side of Figure 5. Either a complete mutation of the U6 PSEA or its substitution with a U1 PSEA reduced transcription more than 100-fold. Mutation of the TATA-box to the PSEB reduced transcription ~30-fold. Thus, although mutations of the TATA-box to a PSEB may not be quite as detrimental as mutations in the U6 PSEA, the PSEB is a very poor substitute for a TATA sequence. Consequently, the TATA-box appears to play a more significant role in expression of the *D.melanogaster* U6 gene than the PSEB does in expression of the U1 gene.

DISCUSSION

Earlier photo-cross-linking studies indicated that the U1 PSEA, but not the U6 PSEA, was able to bring the DmSNAP43 subunit of DmSNAPc into contact with the downstream PSEB. However, those studies did not address whether the PSEB played a role in those protein–DNA interactions. A mutation that changes every nucleotide in the PSEB indicates that the U1 PSEA alone contains sufficient information to bring DmSNAP43 into close contact with the downstream DNA. However, the PSEB mutation did produce more or less subtle changes in the cross-linking pattern. This suggests that the PSEB contributes some measure of specificity to the interactions that take place.

The results of the expression assays are in accord with this concept. The PSEB is not an essential element for transcription; rather it seems to act as a modifier of transcriptional activity. Although not essential, the wild-type PSEB provides a 2- to 4-fold higher transcription efficiency than a random GC-rich sequence or a TATA-box. The interaction of DmSNAP43 (as part of DmSNAPc) with the PSEB may in some ways be analogous to the interaction of TFIIB with sequences just upstream of the TATA-box of mRNA genes. TFIIB has a weak but non-essential preference for a consensus sequence (the BRE) adjacent to the 5′ edge of the TATA-box; the more optimal BRE sequences result in stronger promoter activities (24).

To our knowledge, conserved elements analogous to the PSEB have not been identified in the Pol II-transcribed snRNA genes of other metazoans in which functional studies have been carried out. However, we have noted that snRNA genes of other insects contain conserved nucleotides in this location (unpublished data). It is possible that fruit flies (and other insects) have taken advantage of utilizing the specificity of the PSEB to modulate the strength of snRNA promoters over an evolutionary time scale. For example, three variant U5 genes, which are probably expressed at low levels, have PSEBs that are among the most divergent from the consensus PSEB (Figure 1C).

Interestingly, substitution mutations in the −33 to −20 region of a human U2 gene have been found to have minor effects on the transcription start site (25). It is therefore possible that the *D.melanogaster* PSEB, which is located within this region, may play a role in helping to establish the correct start site. Earlier work from our lab (19) indicated that transcription of the *D.melanogaster* U1 gene requires the TBP. Due to the location of the PSEB (−25 to −32) and its 8 bp length, it seems possible that the PSEB may be a site of DNA interaction with TBP. The PSEB may represent a 'compromise' sequence that allows it to be co-occupied simultaneously both by DmSNAP43 and TBP.

To see if this might be possible, we modeled TBP bound to DNA as if the PSEB were a TATA-box (Figure 6) (26,27). Then, taking into consideration that the PSEA is separated from the PSEB by exactly 8 bp, we identified the sites in red color where DmSNAP43 would cross-link with the DNA. Two different orientations are shown in the figure to fully reveal the sites of DmSNAP43 cross-linking relative to the bound TBP. The modeling illustrates that the phosphates that cross-link to DmSNAP43 are not occluded by TBP, and further suggests that DmSNAP43 could interact with the DNA both 'behind' and 'beneath' TBP. Further experiments will be required to examine the validity of this working model.

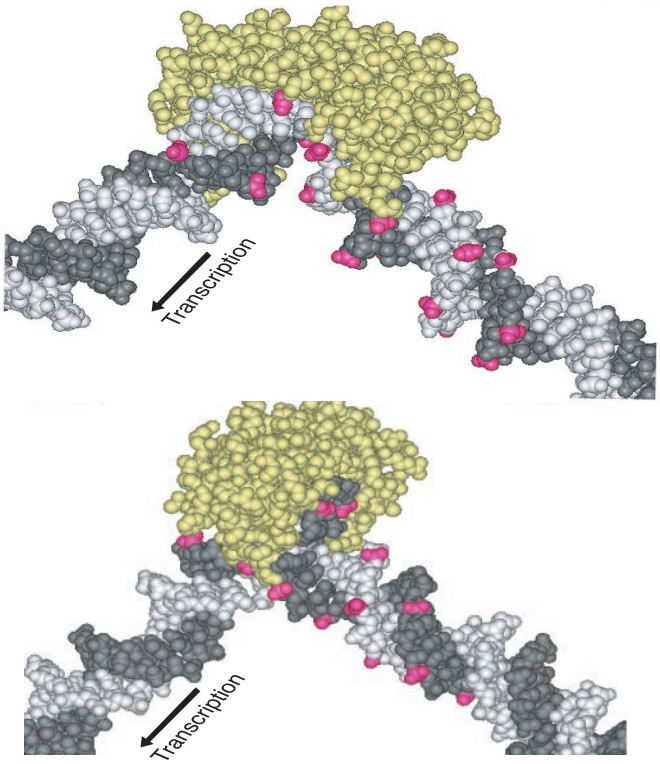

Figure 6. A speculative model of TBP and DmSNAP43 interacting simultaneously with the PSEB. TBP (yellow) is modeled as binding to the 8 bp PSEB as if the PSEB were a TATA sequence, bent by ~90° (26,27). Taking into consideration the conserved 8 bp spacing between the PSEA and the PSEB, the phosphate positions where DmSNAP43 can be cross-linked to the DNA are marked in red. Two views of the complex are shown to reveal that DmSNAP43 appears to bind both 'beneath' TBP (upper illustration) as well as 'behind' TBP (lower illustration). The template strand is represented by the darker gray strand. (Since only every other phosphate position was assayed in the photo-cross-linking assay (Figure 2), additional phosphates at the un-assayed positions likely could cross-link to DmSNAP43, but these are not indicated due to the absence of data.)

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