The proximal sequence element (PSE) plays a major role in establishing the RNA polymerase specificity of Drosophila U-snRNA genes

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ABSTRACT

Most small nuclear RNA (snRNA) genes are transcribed by RNA polymerase II, but some (e.g., U6) are transcribed by RNA polymerase III. In vertebrates a TATA box at a fixed distance downstream of the proximal sequence element (PSE) acts as a dominant determinant for recruiting RNA polymerase III to U6 gene promoters. In contrast, vertebrate snRNA genes that contain a PSE but lack a TATA box are transcribed by RNA polymerase II. In plants, transcription of both classes of snRNA genes requires a TATA box in addition to an upstream sequence element (USE), and polymerase specificity is determined by the spacing between these two core promoter elements. In these examples, the PSE (or USE) is interchangeable between the two classes of snRNA genes. Here we report the surprising finding that the Drosophila U1 and U6 PSEs cannot functionally substitute for each other; rather, determination of RNA polymerase specificity is an intrinsic property of the PSE sequence itself. The alteration of two or three base pairs near the 3′-end of the U1 and U6 PSEs was sufficient to switch the RNA polymerase specificity of Drosophila snRNA promoters in vitro. These findings reveal a novel mechanism for achieving RNA polymerase specificity at insect snRNA promoters.

INTRODUCTION

The small nuclear RNAs (snRNAs) are a metabolically stable class of RNA molecules that reside in eukaryotic nuclei. The best-known snRNAs (U1, U2, U4, U5 and U6) are involved in pre-mRNA splicing. In most eukaryotes, the U1, U2, U4 and U5 snRNAs are synthesized by RNA polymerase (RNAP) II, but U6 snRNA is synthesized by RNAP III (1–5). Despite this difference in polymerase specificity, U6 snRNA genes have promoters that are closely related in structure to those transcribed by RNAP II. Among the higher eukaryotes, the promoter elements of snRNA genes have been best characterized in vertebrates, sea urchins, insects and plants. In all of these organisms, transcription of snRNA genes by RNAP II or RNAP III requires a unique and essential proximal sequence element, or PSE (called the USE in plants), which is located upstream of position –40 relative to the transcription start site (4–12).

In vertebrates, the most obvious difference between RNAP II-transcribed and RNAP III-transcribed snRNA genes is that U6 gene promoters contain a TATA box, whereas the RNAP II snRNA promoters lack a TATA box. Experimentally, the TATA box acts as a dominant element in determining the polymerase specificity of vertebrate snRNA promoters recognized by RNAP III (13,14). Further studies revealed that the vertebrate U2 and U6 PSEs could be exchanged into each other’s promoter context without affecting RNAP specificity (14,15). Similarly, in the sea urchin system the U1, U2 and U6 gene PSEs are functionally interchangeable despite being highly divergent in sequence (16).

In plants, transcription of snRNA genes by either RNAP II or RNAP III requires two basal promoter elements: the USE and a TATA box (11,12), and RNAP specificity is determined by differences in the spacing between the two core promoter elements (32–36 bp spacing between the USE and TATA box for RNAP II and 23–26 bp for RNAP III) (17–19). In all of the above examples, the interchangeability of the PSE (or USE) among U1, U2 and U6 genes suggested that the sequence of the PSE itself does not contribute directly to the selection of RNAP. Rather, RNAP specificity is determined by the presence or absence of a canonical TATA box, or by differences in spacing between two core promoter elements.

In Drosophila, the promoter elements of the snRNA genes are exceptionally well-conserved with respect to both sequence and location (9,20). Figure 1A shows a structural comparison of Drosophila snRNA promoters recognized by RNAP II and RNAP III. Both classes of genes contain an essential 21 bp PSEA, which is similar in location to the PSE of vertebrate snRNA genes. It was named PSEA to distinguish it from PSEB (10). The PSEB is a well-conserved 8 bp element present in the 5′-flanking DNA of 13 Drosophila U1, U2 and U4 genes that have been sequenced (20,21). The PSEB is found at the position of a TATA box (Fig. 1A), but it has the 8 bp consensus sequence CATGGAg/aA. Mutation of PSEB to a GC-rich sequence decreased transcriptional efficiency of a U1 gene at least 8-fold in vitro (10). Instead of a PSEB sequence, the U6 genes of Drosophila contain canonical TATA boxes (Fig. 1A). Of the three
U6 genes that have been cloned and published, two contain the sequence TATATATA and one has the sequence TTATATATA (9).

The highly conserved nature of the snRNA promoters in Drosophila make them an attractive system in which to study the mechanisms of RNA polymerase specificity. First, the 8 bp spacing between the PSEA and PSEB is strictly conserved in Drosophila snRNA genes transcribed by RNAP II (20,21). Second, the spacing between the PSEA and the TATA box of the three Drosophila U6 genes is also strictly conserved, but in this case at a distance of 12 bp (9). Third, whereas the PSEs of other organisms tend to be very degenerate in sequence, the Drosophila PSEAs are well conserved over their entire length of 21 bp (see Fig. 1B for a comparison of the PSEA sequences from 16 Drosophila snRNA genes). We have now studied the contribution of each of these structural elements to the determination of RNAP specificity in a Drosophila in vitro transcription system. To our surprise, we found that RNAP specificity in Drosophila is determined primarily by relatively minor sequence differences within the PSEAs themselves, and not by the presence of a PSEB or TATA element, nor by the differences in the conserved spacing (8 versus 12 bp) that exist between the promoter elements.

**MATERIALS AND METHODS**

**Plasmid templates for in vitro transcription**

The plasmid constructs prepared for in vitro transcription studies contained either a ‘wild-type’ or ‘hybrid’ U6 PSEA box separated 8 or 12 bp from either a TATA or PSEB box (Fig. 2A). The constructs were similar in design to in vitro transcription templates described in two earlier publications (22,23). In essence, the promoter elements and a transcription initiation site were cloned into the polylinker region of pUC18 by making use of synthetic oligonucleotides (oligos). The various PSEA sequences were inserted into the constructs by cloning oligos of appropriate sequence between the EcoRI and KpnI sites of the pUC18 vector. Once the space between the PSEA and downstream element was controlled by synthesizing the oligos with or without the sequence TGA T between the PSEA and downstream element, it was expressed by synthesizing the oligos with or without the sequence TGA T between the PSEA and downstream element, which is a more recent addition to the nucleotide sequence database (21). The U6 sequences are from Das et al. (9). The U6-2 sequence as shown has been corrected from the sequence reported in the original publication (9). Our sequencing data indicate that a T residue originally reported between positions 13 and 14 is in actuality not present in the U6-2 PSEA.
In vitro transcription

Transcription reactions, purification of transcription products and analysis by primer extension were as described previously (22), except that 15 µl of soluble nuclear fraction (SNF) was added (instead of 10 µl), and the amount of buffer was correspondingly reduced. To determine the polymerase preference of each template, the RNAP II inhibitor α-amanitin and/or the RNAP III inhibitor tagetitoxin (24) (Tagetin, Epicentre Technologies, Madison, WI) were included in selected reactions as indicated in the figures. For primer extension analysis, the 32P-labeled 1211z oligo (similar to New England Biolabs 1211, but extended by 7 nt at its 5′-end) was used as previously described (22). Primer extension products were separated in 10% denaturing polyacrylamide gels that were dried and subjected to autoradiography at −70°C. Transcription initiation sites were mapped for selected templates by running the primer extension products alongside a sequencing ladder generated by using the 32P-labeled 1211z primer (data not shown).

RESULTS

The PSEA plays a major role in determining the RNA polymerase specificity of Drosophila snRNA gene promoters

To study the RNAP specificity of Drosophila snRNA gene promoters in vitro, we utilized a series of transcription templates in which the promoter elements and the spacing between them could be readily manipulated while leaving other parameters constant (Fig. 2A). Figure 3 shows the results of in vitro transcription experiments using all possible combinations of U1 or U6 PSEA, 8 or 12 bp spacing and PSEB or TATA box. The construct assayed in Figure 3A, lanes 2–5, has a wild-type U1 structure with respect to the sequence and spacing of the promoter elements. It behaved as a strong promoter and was transcribed exclusively by RNAP III (not inhibited by α-amanitin but inhibited by tagetitoxin, lanes 7 and 8). Thus, in the U1 promoter context, the U6 PSEA acted as a dominant element promoting RNAP III transcription. Of further interest, the major transcription start site was displaced 3 nt downstream, with a minor start site 5 nt downstream (mapping data not shown). A reasonable interpretation is that when the U6
PSEA was inserted 4 bp downstream of its normal position (due to the 8 bp rather than 12 bp spacing), it acted as a dominant element and ‘pushed’ the transcription start site further in the 3’ direction, resulting in shorter primer extension products.

Figure 3A, lanes 10–13, shows the effect of increasing the normal 8 bp spacing between the U1 PSEA and the PSEB to 12 bp. This template promoted transcription exclusively by RNAP II, but the efficiency of transcription was reduced ~20-fold. This is consistent with earlier data indicating that transcription of a Drosophila U1 RNA gene was severely reduced by changing the spacing between the PSEA and the PSEB (10).

In clear contrast, the U6 PSEA at a 12 bp spacing from the PSEB was able to promote a reasonably high level of RNAP III transcription but no detectable RNAP II transcription (Fig. 3A, lanes 14–17). Moreover, the major transcription start site occurred at the normal position, presumably because the 12 bp spacing shifted the U6 PSEA 4 bp farther upstream relative to its location in the construct that was used in lanes 6–9.

In summary, results presented in Figure 3A demonstrated: (i) RNAP specificity was determined by the sequence of the PSEA itself and (ii) the PSEB did not act as a dominant element to specify RNAP II transcription. Data from analogous constructs, but with a TATA box (TA) replacing the PSEB element (PB), are presented in Figure 3B. It is very important to note that in these constructs the TATA box promotes a basal level of both RNAP II and RNAP III transcription that is independent of the PSEA and the PSEA-binding protein (22, 23). Because the template is in excess, the SNF always assembles a given fraction of the TATA-containing templates into active transcription complexes due to the independent binding of TFIIH or TFIIIB to the TATA box (23). Earlier studies indicated that transcription from a construct containing the TATA sequence TTATATA, but no other promoter elements, was ~2/3 due to RNAP III and 1/3 to RNAP II (23). Results shown in Figure 3B, lanes 17–20, reiterate those earlier findings. In interpreting the data of Figure 3B, it is, therefore, important to consider only the PSEA-dependent level of transcription that exists over and above that mediated by the TATA box alone.

When the U1 PSEA was placed 8 bp upstream of the TATA box, transcription by RNAP II was strongly stimulated in comparison to the construct that lacked a PSEA (Fig. 3B, compare lanes 3 and 19). This indicates that the U1 PSEA at an 8 bp spacing cooperated with the TATA box to stimulate RNAP II transcription. In contrast, the U1 PSEA did not increase levels of RNAP III transcription above that obtained with the TATA box alone (compare lanes 2 and 18). On the other hand, the U6 PSEA specifically stimulated RNAP III but not RNAP II transcription (lanes 5–8). Moreover, the ‘new’ PSEA-dependent RNAP III transcripts (lanes 5 and 6) were shorter, indicating that their 5’-ends were displaced several nucleotides downstream of the normal start site. This is the same phenomenon seen with the PSEB element in Figure 3A, lanes 6–9. On the other hand, the full-length transcripts in Figure 3B, lanes 5–7, were similar in intensity and pattern to those observed in lanes 17–20 and represent PSEA-independent transcription products that arose from the autonomous binding of TFIIH or TFIIIB to the TATA box.

Even when the U1 PSEA and TATA box were separated by 12 bp, the TATA box did not act as a dominant element to specify PSEA-dependent RNAP III transcription (Fig. 3B, lanes 9–12). Compared to a template that lacked a PSEA, the transcription level by RNAP III was relatively unchanged by the addition of the U1 PSEA at a 12 bp spacing from the TATA box (Fig. 3B, compare lanes 10 and 18). Furthermore, there was a detectable but only marginal increase in RNAP II transcription (compare lanes 11 and 19). At the 12 bp spacing, the U1 PSEA was, therefore, relatively ineffective at promoting transcription by either polymerase. The U6 PSEA on the other hand measurably stimulated RNAP III transcription when separated from the TATA box by a 12 bp spacing (Fig. 3B, compare lanes 14 and 18) but conversely had no significant impact on RNAP II transcription (compare lanes 15 and 19).

In summary, the experiments shown in Figure 3 demonstrate that the U1 and U6 PSEAs have intrinsically different properties. The RNA polymerase specificity was controlled by the type of PSEA sequence in the construct, not by the presence of a PSEB versus TATA box, nor by the differences in spacing between the two core promoter elements.

A hybrid U1/U6 PSEA can promote both RNAP II and RNAP III transcription

The sequences of the wild-type U1 and U6 PSEAs used in this study differed from each other at only 5 of 21 nt positions (Fig. 2B). It follows that some or all of these five base differences must be responsible for the RNAP specificity exhibited by the PSEA. Inspection of the PSEA sequences from 16 Drosophila snRNA genes revealed two, but only two, positions at which consistent differences exist between the PSEAs from the RNAP II- and RNAP III-transcribed snRNA genes (Fig. 1B, bold letters). At position 19, the U1, U2 and U4 genes contain either an A or a G, whereas all three U6 genes have a T at this position. At position 20, all of the RNAP II-transcribed genes contain a G, but the U6 genes have a conserved C. We reasoned that these two positions might be involved in determining the RNAP specificity of the PSEA.

To test this hypothesis, we synthesized the construct 11166-8-PB. (The nomenclature indicates that the PSEA contains the U1-specific nucleotides at positions 7, 14 and 16 and the U6-specific nucleotides at positions 19 and 20.) This construct contains an entirely U1-like PSEA except for the 2 nt which are U6-specific. The PSEB was used to avoid the background levels of RNAP II and RNAP III transcription that occur in constructs that contain a TATA box.

Figure 4, lanes 1–12, shows results obtained using either the wild-type U6 PSEA, the wild-type U1 PSEA or the hybrid PSEA sequence. As previously shown in Figure 3, the U6 PSEA and the U1 PSEA overwhelmingly supported only RNAP III or RNAP II transcription respectively (Fig. 4, lanes 1–8). Interestingly, the 11166 hybrid PSEA gave a result intermediate between the wild-type U1 and U6 PSEAs in that RNAP III and RNAP II transcription both occurred at similar levels (Fig. 4, lanes 9–12). The two-base substitution in the U1 PSEA was, therefore, sufficient to activate RNAP III transcription (lane 10). A significant amount of RNAP II transcription nevertheless remained (lane 11), but its level was reduced in comparison to that obtained with the wild-type U1 PSEA (lane 7). It furthermore should be noted that the RNAP II transcripts were primarily full-length and, therefore, were initiated at the ‘normal’ transcription start site, whereas the RNAP III transcripts were shorter and, therefore, had their 5’-ends displaced in the 3’ direction. These data demonstrate that the identities of the nucleotides at positions 19 and 20 of the PSEA indeed play an important role in determining the RNAP specificity of Drosophila snRNA promoters. However, when the two U6 nucleotides were placed into a promoter context that was otherwise entirely U1-like, suppression of RNAP II transcription was not complete.
Figure 4. Role of specific nucleotides within the PSEA in contributing to RNA polymerase specificity. Templates with various combinations of U1- and U6-specific nucleotides at positions 7, 14, 16, 19 and 20 were transcribed in vitro and their polymerase specificities examined using the inhibitors α-amanitin and tagetitoxin. (A larger amount of recovery standard than usual was used in the experiment shown in lanes 30–43.)

Successive point mutations reveal the relative contributions to RNAP specificity of the 5 nt that differ between the U1 and U6 PSEAs

A number of additional constructs were synthesized to more fully map out the contributions toward RNAP specificity of the nucleotides that differ between the U1-95.1 and U6-2 PSEAs. These constructs were named 11116-8-PB, 11161-8-PB, 11666-8-PB, 16166-8-PB and 61166-8-PB. The numbers ‘1’ or ‘6’ designate, in order, whether the base at position 7, 14, 16, 19 or 20 corresponds to the base found in the U1 PSEA or the U6 PSEA (Fig. 2B).

When the single nucleotide at position 20 in the U1 PSEA was changed to C to match the U6 sequence, the RNAP II specificity was essentially equal to that obtained with the wild-type U1 configuration (Fig. 4, compare lanes 18–21 with lanes 14–17). Substitution of a T (U6) for the A (U1) at position 19 activated a very low level of RNAP III transcription and caused a slight reduction in transcription by RNAP II (lanes 22–25). However, when the nucleotides at the 19th and 20th positions were both changed in the same construct, RNAP III transcription was synergistically activated and RNAP II transcription was further reduced (lanes 26–29). (Results from the same construct in a different transcription reaction are shown in lanes 9–12.)

We also examined several additional combinations to determine which nucleotides were required to fully support RNAP III specificity in the context of the U1 PSEB and 8 bp promoter spacing. In combination with the two U6-derived nucleotides at positions 19 and 20, the U6 nucleotide at either position 16 or 14 resulted in nearly complete RNAP III specificity (constructs 11666-8-PB and 16166-8-PB, Fig. 4, lanes 30–37). The construct 61166-8-PB also supported primarily RNAP III transcription (Fig. 4, lanes 38–41), but a residual amount of RNAP II transcription remained (lane 40). It thus appears that all five bases in the U6 PSEA that differ from the U1 PSEA may play some role in contributing to the RNAP III specificity of the U6 PSEA. However, the pre-eminent role of the nucleotides at the 19th and 20th positions was confirmed by the experiment described in the following section.

A ‘U6’ PSEA with an A and a G in the 19th and 20th positions, respectively, has properties similar to a U1 PSEA

The experiments shown in Figure 4 did not formally exclude the possibility that one or more of the U6 nucleotides at positions 7, 14 or 16 might play a dominant role in specifying RNAP III transcription independently of the U6 nucleotides at positions 19 and 20 (although an examination of the PSEA sequences in Fig. 1B makes such an interpretation less likely). To experimentally confirm that the nucleotides at the 19th and 20th positions are pre-eminent in determining RNAP specificity, we generated the reciprocal construct 66611-8-PB, which has a PSEA sequence identical to the wild-type U6 PSEA except that it has the U1 nucleotides substituted at positions 19 and 20. Significantly, this construct was transcribed solely by RNAP II (Fig. 5, lanes 1–4), although the efficiency was somewhat less than that obtained from the wild-type U1 PSEA (lane 5). Thus, at least in the context of an 8 bp spacing from the PSEB, two base changes at positions 19 and 20 were sufficient to alter the polymerase specificity of the U6 PSEA from RNAP III to RNAP II.

We also examined the effect of this two base substitution in the context of a 12 bp spacing from the PSEB. As previously shown, the U6 PSEA cooperated with the PSEB at this spacing to specifically promote RNAP III transcription, whereas the U1 PSEA promoted RNAP II transcription, but very inefficiently (Fig. 3A, lanes 10–17). These results are reiterated in Figure 5, lanes 7 and 8. Consistent with the predominant role of the
nucleotides at the 19th and 20th positions, the 66611-12-PB template was transcribed no better than the template with the wild-type U1 PSEA separated 12 bp from the PSEB (Fig. 5, compare lanes 8 and 9). Because of the very low level at which this template is transcribed, it has been difficult to obtain an autoradiography film clearly demonstrating its RNAP specificity. However, the data indicate that it has both an RNAP II component and an RNAP III component (not shown). Thus, the 12 bp spacing between the 66611 PSEA and the PSEB allows some RNAP III transcription to occur from this template. The more general conclusion, however, is that the 66611 PSEA at the 12 bp spacing functions very poorly and promotes a level of transcription that is more in line with the U1 PSEA than with the U6 PSEA.

**DISCUSSION**

**A novel role of the PSEA sequence in determining the RNAP specificity of Drosophila snRNA genes**

The results presented above reveal that the major determinant of RNA polymerase specificity at snRNA promoters in *Drosophila* is different from previously described systems. In vertebrates, RNAP specificity is determined by the presence or absence of a TATA box at a conserved distance downstream of the PSE. Mutation of the TATA box to an unrelated sequence was found to change the U6 promoter specificity from RNAP III to RNAP II (13,14). Conversely, the introduction of a TATA box into the U1 or U2 promoters altered their specificity to RNAP III (13,14). Further studies revealed that the vertebrate U2 and U6 PSEs could be exchanged with each other without affecting RNAP specificity (14,15). For efficient RNAP III transcription, however, it was necessary to maintain the conserved spacing between the PSE and the TATA box (25–27). Overall, those studies indicated that the PSEs of vertebrate RNAP II- and RNAP III-transcribed snRNA genes have similar properties and that the PSE sequence itself does not contribute directly to the selection of RNA polymerase.

In plants, the USE and the TATA box together are both essential for snRNA gene transcription by either RNAP II or RNAP III, and it is a 10 bp spacing difference between the USE and the TATA box that determines the RNAP specificity of the plant snRNA genes (17–19). Spacing considerations, and perhaps the presence or absence of a canonical TATA box, likewise seem to play a role in determining the RNAP specificity of sea urchin snRNA genes (16). As with vertebrates, the PSE (in sea urchins) or USE (in plants) could be exchanged between U1, U2 and U6 snRNA genes without affecting the choice of RNA polymerase, suggesting that the sequence of the PSE or USE itself is irrelevant with respect to determining RNAP specificity.

The *Drosophila* system on the other hand exhibits distinctive properties. RNAP specificity in this organism is an intrinsic property of the PSEA. A U6 PSEA sequence was sufficient to specify RNAP III transcription *in vitro* even when placed into the heterologous promoter context, and a U1 PSEA sequence was capable of supporting only RNAP II transcription. Interestingly, the U1 PSEA could promote transcription efficiently only when spaced 8 bp from a downstream PSEB or TATA box. The importance of the PSEA sequence was further demonstrated by using “hybrid” PSEAs that combined nucleotides from the U1 and U6 gene PSEAs into the same construct. In certain instances, these hybrid PSEAs promoted transcription by both RNAP II and RNAP III. In general, however, the more U6-like the PSEA sequence, the greater was the percentage of RNAP III transcription and vice versa (Fig. 4).

There are 2 nt in the PSEA (positions 19 and 20, Fig. 1B) that are conserved as a TC in the U6 genes but are a conserved a/gG in the U1, U2 and U4 genes. This evolutionary distinction indeed plays an important role in establishing the RNAP specificity of *Drosophila* snRNA promoters. Whereas the U6-8-PB construct (which could alternatively be designated 66666-8-PB) was transcribed exclusively by RNAP III, the 66611-8-PB template was transcribed exclusively by RNAP II. The converse construct (11166-8-PB), on the other hand, was transcribed by both RNAP II and RNAP III. Thus, the polymerase specificity of the 11166 hybrid PSEA appears to be somewhat ambiguous. In this instance, the promoter context (i.e., the 8 bp spacing between the PSEA and downstream PSEB element) may make a secondary contribution toward polymerase specificity.

**Role of the PSEB, TATA box and inter-element spacing**

In order for the U1 PSEA to function as a strong promoter element, an 8 bp separation from the downstream element (either PSEB or TATA) was required. At a 12 bp spacing or, as previously shown, at 3, 13, 18 or 23 bp spacings (10), the U1 PSEA functioned only as a very weak promoter element. The activity of the U6 PSEA, on the other hand, was not strongly affected by changes in spacing. Interestingly, when the U6 PSEA was placed 8 bp from either the PSEB or TATA box, the transcription start sites were displaced 3–5 nt downstream. This suggests that the *Drosophila* U6 PSEA probably acts dominantly to position TBP on a sequence that begins 12 bp downstream of the U6 PSEA, even if the downstream sequence is not a good TATA box. If this is true, the downstream displacement of the site of TBP interaction presumably displaces the RNAP III initiation site a similar distance in the 3′ direction.

Although the TATA box supports a basal level of transcription by both RNAP II and RNAP III that is independent of the PSEA, the PSEB element alone does not. Interestingly, we were unable to detect any fundamental qualitative difference in the activities of these elements in combination with a PSEA box. At an 8 bp spacing, the U1 PSEA cooperated very effectively with either a PSEB or a TATA element to promote RNAP II transcription. Similarly, the U6 PSEA cooperated with either a TATA box or a PSEB at a 12 bp spacing to promote RNAP III transcription. These findings are consistent with our earlier suggestion that PSEB may be a site of contact between the DNA and TBP in the complete RNAP II pre-initiation complex formed on *Drosophila* snRNA genes (10). Indeed, the PSEB may be more permissive than random sequence DNA for TBP binding since a three base transition
(CATGGAAA to TATAAAAA) can generate a canonical TATA box that functions as a strong promoter in vitro (10,22,23).

How do sequence differences in the PSEA translate into differences in RNAP specificity? Two potential mechanisms are worth mentioning. First, the sequence differences at the 3'-end of the PSEA may comprise distinct binding sites that are differentially recognized by possible polymerase-specific subunits of the protein complex that binds to the PSEA. On the other hand, it is conceivable that the different DNA sequences may allosterically induce alternative conformations in the protein–DNA complex which are individually compatible with the recruitment of only one type of RNA polymerase. To distinguish between these and other possibilities, a better understanding of the structure of the Drosophila PSEA-binding protein and its interaction with the DNA will be required. These matters are currently under investigation.

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