MOLECULAR AND CELLULAR BIOLOGY, Feb. 1992, p. 650–660
0270-7306/92/020650-11$02.00/0

Vol. 12, No. 2

Characterization of Two Developmentally Regulated Sea Urchin U2 Small Nuclear RNA Promoters: A Common Required TATA Sequence and Independent Proximal and Distal Elements†

BRANKO STEFANOVIC AND WILLIAM F. MARZLUFF‡*

Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306

Received 17 September 1991/Accepted 18 November 1991

The promoters of two U2 small nuclear RNA genes isolated from the sea urchin *Lytechinus variegatus* were mapped by microinjection of genes into sea urchin zygotes. One gene, LvU2E, is expressed only in oocytes and embryos and is found in a tandemly repeated gene set, while the other gene, LvU2L, is a single-copy gene and is expressed in embryos and somatic cells. The promoters each contain a TATA sequence at −25 which is required for expression, a proximal sequence element (PSE) centered at −55 required for expression, a sequence at −100 which couples the core promoter (PSE plus TATA box) to the upstream element, and an upstream sequence which stimulates expression fourfold. The PSE together with the TATA sequence is sufficient to determine the transcription start site. There is no sequence similarity between the −100 and PSE sequences of the two genes. The −100 sequences can be interchanged between the two genes. The LvU2E PSE functions in the context of the LvU2L gene, but the LvU2L PSE functions poorly in the context of the LvU2E gene.

The U series of small nuclear RNAs (snRNAs) are among the genes most rapidly transcribed by RNA polymerase II. The vertebrate snRNA genes are also unique among genes transcribed by RNA polymerase II in that initiation from an snRNA promoter is necessary for formation of snRNA 3′ ends (7, 21). Most of the snRNA genes are transcribed by RNA polymerase II, although U6 snRNA is transcribed by RNA polymerase III (8, 24), as is U3 snRNA in plants (36). Surprisingly, there are common promoter elements for the snRNA genes transcribed by RNA polymerases II and III (3, 9). The promoters have been best characterized in the cases of the vertebrate U1 (18, 21), U2 (1, 23, 32), and U6 (5, 27) genes as well as plant snRNA genes (35, 37). There are two major *cis*-acting elements, the distal sequence element (DSE) and the proximal sequence element (PSE), which are necessary and sufficient to accurately drive the transcription of vertebrate snRNA genes (23). The DSE is located around positions −200 to −250 and is often composed of a perfect octamer motif combined with one or more transcription factor binding sites (22). The PSE, located at about −60, is absolutely required for transcription, defines the selection of the initiation site (23, 33), and, by interaction with the DSE, directs the assembly of a unique transcription complex capable of recognizing the snRNA gene-specific 3′ end formation signal (6, 23). The U6 gene, in addition to the DSE and PSE, has a TATA box at about 30 nucleotides (nt) from the start site (5). The 7SK small RNA gene, which has an internal promoter and is transcribed by RNA polymerase III, also contains a TATA box.

The promoters of invertebrate snRNA genes have not been as well studied. The sea urchin U7 snRNA promoter (29) has an upstream sequence element which enhances transcription only fivefold. There is a proximal element, mapped as an AT-rich sequence extending from −25 to −57, which is absolutely required for expression (29). The sea urchin U1 promoter has two major elements, one at about −300 and one at about −50, which are absolutely required for expression (37a), although neither of these elements is similar to the vertebrate sequence elements. Several *Caenorhabditis elegans* snRNA genes (34) have been cloned, and, on the basis of sequence conservation, a consensus PSE was proposed. *Drosophila* snRNA gene promoters have not been mapped, but a consensus sequence has been proposed for the PSE and DSE (2, 25). None of these putative elements have been functionally tested. There is no sequence conservation between the *cis*-acting elements of vertebrate and invertebrate genes, and hence the failure of invertebrate snRNAs to be expressed in *Xenopus* oocytes is not surprising (31).

In contrast, plant snRNA promoters have a completely different structure (35). They contain only two required elements, the −30 TATA box that selects initiation site and the −70 upstream sequence element that is indispensable for transcription. It is interesting that the spacing between these two elements determines the polymerase specificity of a promoter (36).

Recently, we have described the cloning of tandemly repeated U2 genes from *Lytechinus variegatus* and *Strongylocentrotus purpuratus*, LvU2E and SpU2E, respectively, and of an isolated *L. variegatus* U2 gene, LvU2L (30). The tandemly repeated U2E genes are expressed early in development and are not expressed in somatic cells, while the LvU2L gene is expressed constitutively. Here we describe experiments to characterize the promoters of both the LvU2E and LvU2L U2 snRNA genes from the sea urchin *L. variegatus*. Surprisingly, each promoter contains an essential TATA sequence at about −25, as well as an essential PSE between −50 and −60, an important element at about −100, and distal upstream sequences which have a lesser effect on expression. Other than the TATA box, the pro-

* Corresponding author.

† This report is publication no. 1065 from the Florida State University Marine Laboratory.

‡ Permanent address: Program in Molecular Biology and Biotechnology, 132 MBBRL, CB no. 7100, University of North Carolina, Chapel Hill, NC 27599.

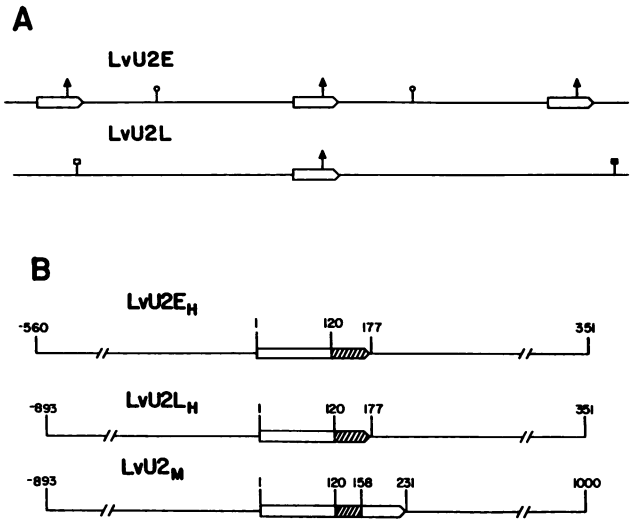


FIG. 1. *L. variegatus* U2 snRNA genes. (A) The structures of the U2 snRNA genes from *L. variegatus*, the tandemly repeated LvU2E gene and the LvU2L gene, are shown. The restriction enzyme sites used in constructing the modified U2 genes are shown and are as follows: *Cla*I (⚲), *Hin*cII (⚲), *Nco*I (⚲), and *Sal*I (⚲). (B) The modified U2 genes used in the microinjection experiments are shown. The U2E$_H$ gene contains the promoter of the LvU2E gene and the first 120 nt of the U2 coding region which were fused to the 3′ end of the *L. variegatus* U1.2 gene by using a synthetic linker. The U2L$_H$ gene is identical except that it contains the promoter of the LvU2L gene. The LvU2$_M$ gene contains an insertion in the coding region of the LvU2L gene. The inserted sequence contains the same synthetic linker used to join the U2 and U1 genes to make the hybrid genes. The deletion and linker-scanning experiments were done with the hybrid genes, while the LvU2$_M$ gene served as an internal control. For the riboprobe assay, an antisense riboprobe complementary to the LvU2L$_H$ gene was synthesized (see Fig. 2B).

moter elements of the constitutive and embryonic U2 snRNA genes are different.

MATERIALS AND METHODS

Construction of the test genes and the internal standard gene. We have previously described the isolation, characterization, and temporal expression of two U2 snRNA genes, the embryonic LvU2E gene and the constitutive LvU2L gene from *L. variegatus* (30). The LvU2E$_H$ and LvU2L$_H$ hybrid genes were constructed by fusing the 5′ portion of the appropriate U2 gene at the *Nco*I site at nt 120 to the 3′ portion of the LvU1.2 gene (38) by using a synthetic polylinker containing an *Nco*I site (Fig. 1). Deletions of the 5′ flanking regions of the hybrid genes were constructed by the exonuclease III-mung bean nuclease method. Site-directed mutagenesis was performed by the method of Kunkel (10), which was modified for phagemid vectors. The identity of each of the mutants was confirmed by dideoxy sequencing. The internal standard gene (LvU2$_M$) was constructed by inserting 30 nt including the same synthetic polylinker used in construction of the LvU2$_H$ genes and 20 nt of the U1.2 gene coding sequence (nt 120 to 140) into the *Nco*I site of the LvU2L gene.

Construction of the genes with portions of the promoters exchanged. The LE$_p$L gene was made by truncating the LvU2L$_H$ gene starting with a deletion mutation extending to nt −21 and inserting the LvU2E promoter fragment extending from nt −26 to −118 5′ of this deletion. The LvU2L gene fragment from nt −893 to −151 was then fused to the 5′ end of this construct, yielding the LE$_p$L gene. The EL$_{60}$E and LE$_{60}$L genes were made by creating unique *Pst*I and *Sal*I sites at positions −43 and −80 of the early gene promoter and at positions −39 and −83 of the late gene promoter by site-directed mutagenesis. These sites are contained within the linker-scanning mutants LS 38-47,74-83 and LS 34-43,76-85. Then the *Pst*I and *Sal*I fragments were exchanged between the genes. Similarly, the EL$_{100}$E and LE$_{100}$L genes were made by exchanging the *Sst*II-*Sal*I fragments between the genes by using the *Sst*II site created by site-directed mutagenesis at position −124 of the early and −132 of the late gene promoter. These sites are present in the mutants LS 74-83,118-127 and LS 76-85,126-135.

Microinjection of DNA into sea urchin embryos. Microinjection of DNA was performed essentially as described by McMahon and coworkers (17), as modified by Colin et al. (4). Briefly, the test gene and internal standard gene were linearized at the 3′ end, the concentration of DNA was adjusted to 50 μg/ml, and equal amounts of the test DNA and standard DNA solutions were mixed together. *Lytechinus pictus* eggs were dejellied by shaking in seawater, pH 4.5, and layered on a petri dish coated with protamine sulfate. Eggs were fertilized and injected immediately after formation of the fertilization membrane. Ten to 20 pl of DNA solution was injected per embryo, and about 100 embryos were injected per test gene. Embryos were grown at 15°C to the hatching blastula stage (20 h), and the embryos which developed normally were collected and the total cellular RNA was prepared.

RNA analysis. Embryos were washed in 0.5 M NaCl–50 mM EDTA–10 mM Tris HCl (pH 7.4) and dissolved in 200 μl of 0.1 M NaCl–1 mM EDTA–10 mM ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)–10 mM Tris HCl (pH 7.4)–0.5% sodium dodecyl sulfate. The solution was extracted with water-saturated phenol-chloroform, and the RNA was recovered by precipitation with ethanol. A 449-nt antisense LvU2L$_H$ riboprobe, extending from nt +121 to −151 and uniformly labeled with [α-^{32}P]UTP, was synthesized with Sp6 RNA polymerase. The RNA samples were hybridized to the antisense LvU2L$_H$ probe in 80% formamide–50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)–0.4 M NaCl at 50°C for 10 to 15 h. The reaction was digested with RNase A, and the RNase-resistant fragments were resolved on an 8% polyacrylamide gel in 7 M urea as previously described (30). This riboprobe simultaneously protected the following: transcripts from the hybrid gene giving a fragment of 177 nt, transcripts from the LvU2$_M$ internal standard gene giving a fragment of 158 nt, and transcripts from the endogenous U2 snRNA genes giving a fragment of 120 nt (Fig. 2). The intensity of protected bands was estimated by densitometry or directly by using a Betascope, and the expression of the test clone was calculated relative to the coinjected internal standard gene.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences in this report are M58447 and M58448 for LvU2E and LvU2L, respectively.

RESULTS

We have previously reported the isolation of two U2 snRNA genes from the sea urchin *L. variegatus* (30). One of these genes, LvU2E, is repeated about 50 times in a 1-kb tandemly repeated unit, while the other gene, LvU2L, is an

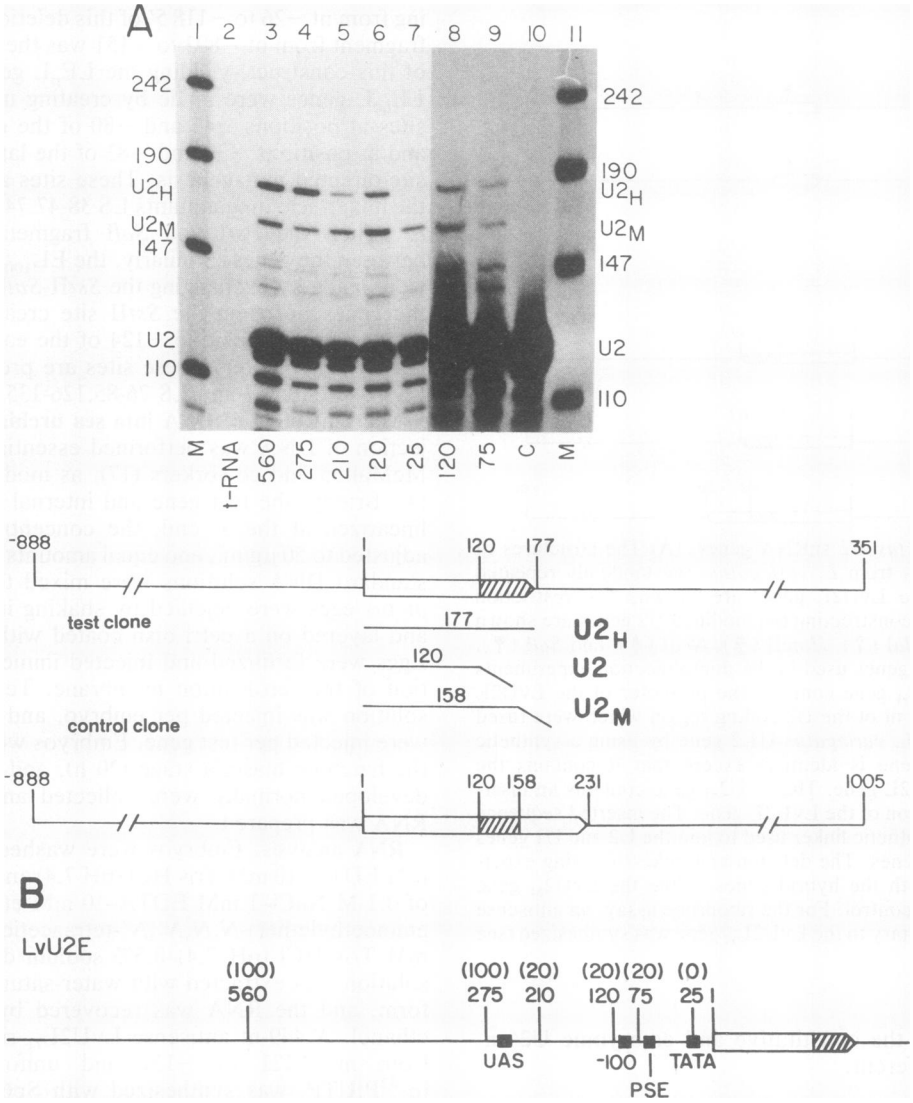


FIG. 2. Expression of deletions of the LvU2E gene. (A) 5′ deletions of the $U2E_H$ gene were injected into sea urchin zygotes together with the $LvU2_M$ gene. The embryos were harvested at the blastula stage, and the RNA was analyzed by using a riboprobe from the $LvU2L_H$ gene. The assay is diagrammed below the figure. Lanes 1 and 11 are marker pUC18 digested with *Hpa*II, lane 2 is analysis of 10 μg of *Saccharomyces cerevisiae* tRNA, and lane 10 is RNA from an equal number of control embryos. Lanes 3 to 9 are analyses of RNA from embryos injected with the 5′ deletion clones. The numbers underneath each lane indicate the 5′ boundary of the deletion. The protected fragments are as follows: for $U2_H$, 177 nt, protection of the transcripts from the $U2E_H$ gene; for $U2_M$, 158 nt, protection of the fragments from the $LvU2_M$ gene; for U2, 120 nt, protection of the endogenous U2 snRNA. M, marker; C, control. (B) Summary of deletion analysis of the LvU2E gene promoter. The relative expression of various deletion mutants is given as a percentage of the maximal expression and plotted against the lengths of the 5′ flanking sequences. The positions of the critical *cis*-acting elements described in the text are indicated.

isolated single-copy gene. On the basis of the results of microinjection experiments, we found that the tandemly repeated LvU2E gene was expressed at the same rate as the LvU2L gene in early development (up until the blastula stage) and fourfold less than the LvU2E gene at the gastrula stage (30). We have studied the promoter elements of these two differentially expressed U2 snRNA genes.

Construction of hybrid and maxigenes. To assess the expression of the sea urchin U2 snRNA genes, we injected modified U2 genes into sea urchin zygotes and determined the amount of RNA accumulated from the injected genes just after hatching. The parent LvU2L and LvU2E genes are shown in Fig. 1A. The hybrid genes were made by fusing the promoter and first 120 nt of the coding region of the LvU2E and LvU2L genes with the 3′ end of the U1 snRNA gene from *L. variegatus*, to give the U2-U1 hybrid genes $U2E_H$ and $U2L_H$, which were described previously (30). These hybrid genes encode the same RNA. A maxigene, the $LvU2_M$ gene, constructed by inserting a portion of the U1 snRNA gene into the U2 gene by using an oligonucleotide linker, was injected as an internal control. The $LvU2_M$ gene contains the LvU2L promoter. The structures of the hybrid genes and the maxigene are shown in Fig. 1B. All mutations tested were in the 5′ flanking regions, and the mutant genes were microinjected together with the $LvU2_M$ gene as an internal control into *L. pictus* one-cell embryos. The *L. variegatus* genes are expressed well in *L. pictus* embryos since these two species are closely related.

RNA was prepared from embryos collected after hatching (20 h), and the transcripts were mapped by using an RNase protection assay. As shown in Fig. 2, transcripts from both the hybrid genes ($U2_H$, 177 nt) and the maxigene ($U2_M$, 158 nt) as well as the endogenous U2 snRNA (U2, 120 nt) were all mapped as distinct fragments by using the riboprobe from the $U2L_H$ gene. Thus the relative expression of different genes can be assessed by comparing the expression of the hybrid gene and that of the maxigene. We have previously shown that the ratio of expression of the control and test genes reflects the relative expression of these two genes over a range of DNA concentrations (30).

We show below that there are four major *cis*-acting elements in each of the sea urchin U2 snRNA genes: an upstream activating sequence(s) (UAS) located more than 200 nt 5′ of the start site, a sequence at −100 which is required for maximal expression, a PSE at about −55 required for expression, and the TATA box, which is also required for expression.

Expression of deletion mutations of the LvU2E gene. A series of deletion mutants was constructed, and the results with deletions of the LvU2E gene are shown in Fig. 2A. The LvU2E deletion mutants were coinjected with the $LvU2_M$ gene. At least two separate injections into different batches of eggs were performed for each gene. The longest construct contains 560 nt of 5′ flanking region, which we assume contains all the necessary *cis*-acting elements, and this gene was assigned 100% expression. Deletion of the early promoter from −560 to −275 did not affect the expression of the hybrid gene (Fig. 2A, lanes 3 and 4). However, deletion of the region between −275 and −210 resulted in a fivefold decrease in promoter activity (Fig. 2A, lanes 4 and 5), indicating that these 65 nt contain a sequence that has a moderate effect on expression. We refer to this as a UAS to distinguish it from the DSE of the vertebrate promoters since it exhibits a quantitatively weaker effect and it is not known whether it is an enhancer. This element was not further characterized.

Additional deletions were created to define the minimal promoter. Two additional deletions, −120 and −75, had no affect on the residual activity (Fig. 2A, lanes 6, 8, and 9). Thus a promoter containing only 75 nt of 5′ sequence has about 20% of the activity of the complete promoter. Further deletion to −25 abolished expression (Fig. 2A, lane 7). Note that the results of two separate injections of the deletion mutant −120 are shown (Fig. 2A, lanes 6 and 8) and that this gene was expressed identically relative to the $LvU2_M$ gene in each experiment. The microinjection experiments are highly reproducible when an internal standard is included.

From the above analysis, we conclude that the LvU2E promoter has a basal promoter which contains at most 75 nt of 5′ flanking sequence. In addition, there is a sequence between nt 210 and 275 which has a fivefold effect on expression. These results are summarized in Fig. 2B. The positions of the TATA sequence and the UAS are indicated, as are the PSE and −100 sequences, which are defined below (see Fig. 3B).

Linker scanning of the LvU2E promoter. To identify essential *cis*-acting elements within the basal promoter defined by the deletion analysis, we constructed linker-scanning mutations in the proximal region of the LvU2E promoter. Some double mutants were made to create the unique restriction sites later used for swapping critical elements of the promoters.

Figure 3A shows the sequence of the LvU2E promoter together with the positions and sequences of the mutations.

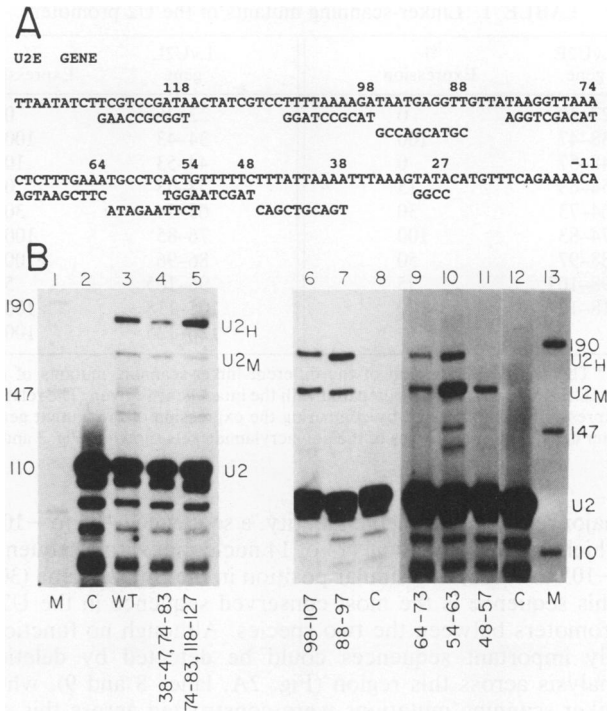


FIG. 3. Elements in the LvU2E promoter identified by linker scanning. (A) Sequence of the LvU2E gene promoter and the nucleotides which were substituted to create the linker-scanning mutants. (B) The linker-scanning mutations shown in panel A were injected together with the $LvU2_M$ gene into sea urchin zygotes, and the embryos were harvested at the blastula stage and analyzed as for Fig. 2A. The protected fragments are labeled as for Fig. 2A. Lanes 1 and 13 are pUC18 digested with *Hpa*II; lanes 2, 8, and 12 are RNA from uninjected control embryos; and lane 3 is analysis of from embryos injected with the complete $U2E_H$ gene. Lanes 4 to 11 are analyses of RNA from embryos injected with the linker-scanning mutants. Beneath each lane the nucleotides changed are indicated. Where two regions are given, both regions were changed in the same mutant. C, control; M, marker; WT, wild-type.

The results of microinjection experiments are displayed in Fig. 3B. The linker-scanning mutations revealed two separate elements in the LvU2E gene promoter. There is an essential sequence located between nt 48 and 57 (Fig. 3B, lane 11), and expression is also greatly reduced when nt 54 to 63 are altered (Fig. 3B, lane 10). By analogy with the vertebrate snRNA promoters, which also have an essential element located in this position, we have named this element the PSE. The PSE is probably quite large since the mutant LS 64-73 (Fig. 3B, lane 9) is also expressed at reduced levels. Nucleotides 48 to 53, at the 3′ end of the PSE, may be particularly important since mutation of this region completely abolished expression (Fig. 3B, lane 11) while the other mutations (Fig. 3B, lanes 9 and 10) in this region only reduced expression. The double mutant in which nt 38 to 47 and nt 74 to 83 were changed was expressed at about 50% of the wild-type level (Fig. 3B, cf. lanes 3 and 4). Similar results were obtained with single mutations in these regions (data not shown). These two mutations flank the PSE and thus show that the maximal size of the PSE is 25 nt, from −48 to −73.

Comparing the tandemly repeated LvU2E gene with the tandemly repeated U2 gene from *S. purpuratus*, which is presumably the homolog of the LvU2E gene, there is a single

TABLE 1. Linker-scanning mutants of the U2 promoter[a]

LvU2E gene	% Expression	LvU2L gene	% Expression
27–30	0	22–26	0
38–47	100	34–43	100
48–57	0	44–53	10
54–63	15	54–63	0
64–73	30	65–74	30
74–83	100	76–85	100
88–97	50	86–96	>100
98–107	25	96–105	5
118–127	>100	105–118	>100
		126–135	100

[a] The relative expression of the different linker-scanning mutants of the LvU2E and LvU2L genes compared with the intact gene is given. The relative expression was calculated by comparing the expression of the mutant genes after densitometric analyses of the polyacrylamide gels shown in Fig. 3 and 5.

major region of extensive identity, a sequence (−96 to −109) which is nearly identical (13 of 14 nucleotides) to a sequence (−105 to −118) at a similar position in the SpU2E gene (30). This sequence is the most conserved sequence in the U2E promoters between the two species. Although no functionally important sequences could be detected by deletion analysis across this region (Fig. 2A, lanes 8 and 9), when linker-scanning mutations were constructed across this sequence, an element which stimulated expression was detected. Alteration of sequences between −74 and −97 (Fig. 3B, lanes 4 and 7) did not significantly affect expression. However, altering the sequences from −98 to 107, the precise region conserved between the two species, reduced expression about fourfold (Fig. 3B, lane 6), indicating the presence of an element in this region required for maximal expression. We term this element the −100 box, since there is an important element in the LvU2L gene located at a similar position. Alterations of the sequence from nt −118 to −127 resulted in a slight increase (about twofold) in expression compared with that of the intact LvU2E gene (Fig. 3B, lanes 3 and 5).

The essential difference between substitution analysis and deletion analysis is that in the former the rest of the promoter is intact while in the latter the upstream sequences are missing. The relative expression of the mutant LS 98-107 is comparable to that of the deletion mutants lacking the UAS (Fig. 2A, lanes 5, 6, 8, and 9). Thus substitution of nt 98 to 107 reduced expression of the LvU2E gene (Fig. 3B, lane 5) by the same amount as removal of the UAS. This result suggests that in the presence of the UAS, the −100 sequence is functionally important. However, it is not essential for promoter activity since deletion of this sequence did not cause any further reduction in activity from that observed after deletion of the UAS (Fig. 2). Additional evidence that a *cis*-acting element resides between nt −83 and −118 is presented later (see Fig. 7). The linker-scanning data obtained with the mutants of the LvU2E gene are summarized in Table 1.

The LvU2L promoter is more complex than the early LvU2 promoter. A similar deletion analysis was performed with the LvU2L gene promoter. To ensure inclusion of all promoter elements in our analysis, we first tested the clones having 2.5 kb and 893 nt of 5′ flanking sequence. These genes were expressed identically (data not shown) and are defined as having wild-type expression. Figure 4A shows the relative expression of various mutants. There is no change in activity if the promoter is truncated up to nt −685 (Fig. 4A, lanes 1 to 3). However, there is a fourfold decrease in expression if the region between nt −685 and −352 is removed (Fig. 4A, cf. lanes 3 and 4) and an additional twofold decrease upon deletion of the sequence between nt −352 and −256 (Fig. 4A, lanes 4 and 5). The residual activity of the −256 deletion is about 10% of the wild type.

We made additional deletion mutations within the −685 and −352 boundaries. A threefold loss of activity results from removal of nt −516 to −445 (Fig. 4A, lanes 7 and 9). This result suggests that the LvU2L gene has an element which is functionally equivalent to the LvU2E gene UAS and which has a relatively small effect on expression.

To delineate the minimal promoter requirements, additional deletions were made extending closer to the gene. There was at most a twofold decrease in expression upon removal of the region between −256 and −134 (Fig. 4A, compare lanes 5 and 10), and this low level of expression declined only slightly when the deleted region was extended to −73 (Fig. 4A, lane 11). However, activity is abolished when an additional 8 nt are deleted (Fig. 4A, lane 12). The minimal promoter of the LvU2L gene is about 75 nt, like the minimal promoter of the LvU2E gene. The results of the deletion analysis of the LvU2L gene are summarized in Fig. 4B.

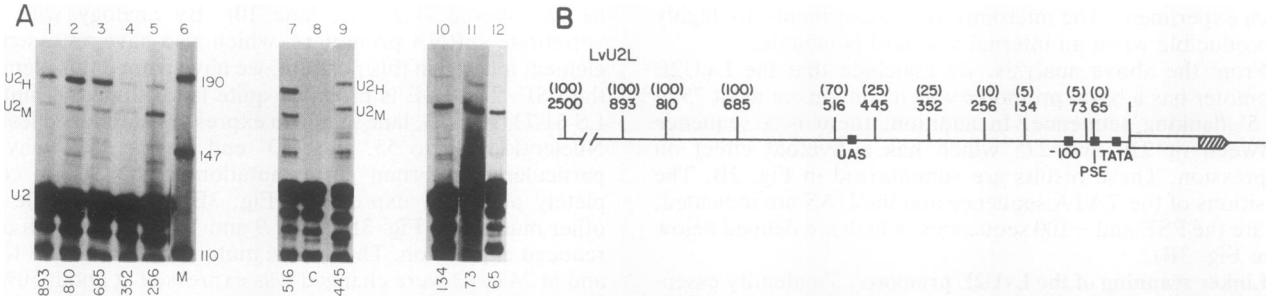


FIG. 4. Expression of deletions of the LvU2L gene. (A) 5′ deletions of the $U2L_H$ gene were injected into sea urchin zygotes together with the $LvU2_M$ gene. The embryos were harvested at the blastula stage, and the RNA was analyzed by using the riboprobe assay described in the legend to Fig. 2A. Lane 6 is pUC18 digested with *Hpa*II and lane 8 is analysis of RNA from an equal number of control embryos. The other lanes are analyses of the 5′ deletion mutants. The numbers underneath each lane indicate the amount of 5′ sequence present. The protected fragments are as follows: for $U2_H$, 177 nt, protection of the transcripts from the $U2L_H$ gene; for $U2_M$, 158 nt, protection of the fragments from the $LvU2_M$ gene; for U2, 120 nt, protection of the endogenous U2 snRNA. C, control; M, marker. (B) Summary of deletion analysis of the LvU2L gene promoters. The relative expression of various deletion mutants is given as a percentage of the maximal expression and plotted against the lengths of the 5′ flanking sequences. The important *cis*-acting elements described in the text are indicated.

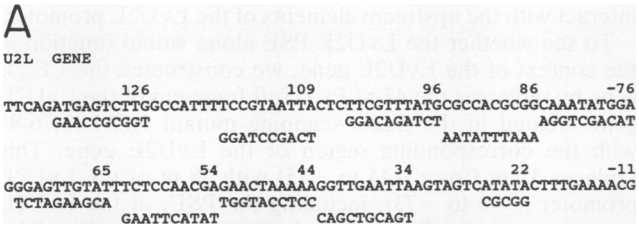
Linker scanning of the LvU2L promoter. The wild-type sequence and the substitutions introduced into the LvU2L gene are shown in Fig. 5A. Figure 5B presents the results of the analyses. Two important elements between nt −34 and −135 were defined by the linker-scanning analyses and were located in positions similar to those of the PSE and the −100 box defined in the LvU2E gene. Alteration of nt 54 to 63 (Fig. 5B, lane 9) abolished expression, while alteration of the nucleotides flanking this sequence, nt 44 to 53 and nt 65 to 74 (Fig. 5B, lanes 12 and 13), greatly reduced expression. Alteration of sequences on either side of the PSE (nt 34 to 43 and nt 76 to 85) did not alter expression whether the two regions were altered simultaneously (Fig. 5B, lane 3) or separately (Fig. 5B, lane 8 and data not shown). Thus the PSE of the LvU2L gene is located between 44 and 74 nt 5′ of the gene, with the most critical region located between 54 and 63 nt 5′ of the gene.

A second important element is located between nt 96 and 105. Alteration of this sequence reduced expression at least 10-fold (Fig. 5B, lane 6), although expression was not abolished. Alteration of the sequences directly flanking this region did not significantly affect expression (Fig. 5B, lanes 4, 7, and 15), defining the −100 box as a much smaller element than the PSE. Like the −100 element in the LvU2E gene, the −100 element in the LvU2L gene functions only in the presence of the upstream promoter sequences. Substitution of the −100 sequence in the LvU2L gene reduced expression about the same amount as deletion of the distal elements of the promoter (Fig. 4A, lanes 10 and 11, and Fig. 5B, lane 6). Each of these mutants was expressed at about 5% of the wild type. These results suggest that the −100 box interacts with upstream elements of the promoter rather than with the PSE. The similarity of the structural organizations of the LvU2L and LvU2E promoters, with important elements located at similar distances from the start site, is striking.

A TATA sequence is required for expression of both LvU2 genes. There is a TATA-like sequence located around position −25 in both the LvU2E and LvU2L genes as well as in the tandemly repeated SpU2E gene isolated from the sea urchin *S. purpuratus* (30). Although TATA boxes are not found in vertebrate snRNA genes, we decided to investigate their possible role in the sea urchin U2 genes because the sequence was conserved in all three sea urchin U2 genes we have isolated. We replaced the TATA sequence of both the LvU2E and LvU2L genes with a GC-rich sequence by site-directed mutagenesis, creating the mutants LvU2E-TATA and LvU2L-TATA (the sequences are shown in Fig. 4A and 5A, respectively).

Neither of these mutations in the TATA sequence was expressed when the genes were injected into sea urchin embryos, even though the PSE, the −100 box, and the upstream sequences were intact (Fig. 6, lanes 4 and 6). Not only were there no normal-size transcripts, but we could detect no incorrectly initiated transcripts. In particular, any transcripts from the LvU2E gene starting upstream of nt −5 would have been detected as a single protected fragment 5 nt longer than the normal transcript, since the riboprobe used (derived from the LvU2L gene) diverged from the LvU2E gene 5 nt 5′ to the start of the gene. The results show that the TATA box, like the PSE, is necessary but not sufficient for expression of the LvU2 genes.

Table 1 presents the summary of the results of the expression of the linker-scanning mutations of the LvU2L promoter and compares them with the results reported above



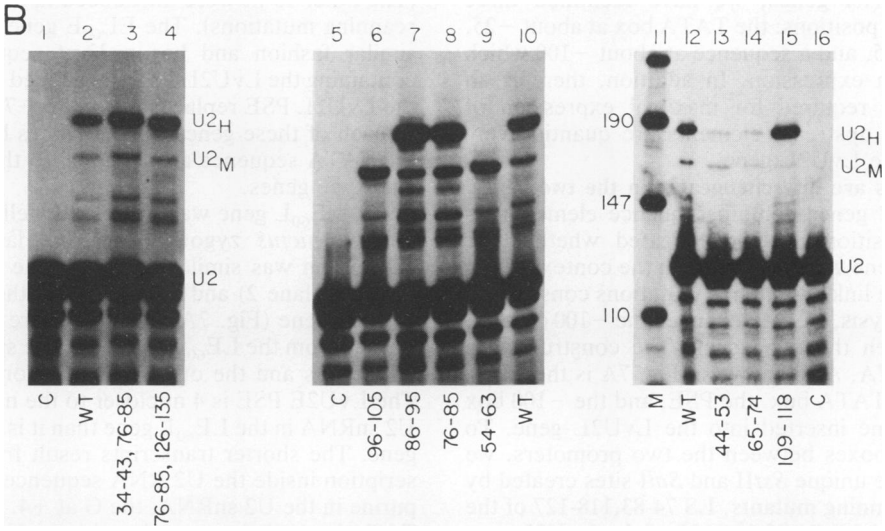


FIG. 5. Elements in the LvU2L promoter identified by linker scanning. (A) Sequence of the LvU2L promoter and the nucleotides substituted in creating the linker-scanning mutations. (B) The linker-scanning mutations shown in panel A were injected together with the $LvU2_M$ gene into sea urchin zygotes, and the embryos were harvested at the blastula stage and analyzed as for Fig. 2. Lanes 1, 5, and 16 are analyses of RNA from uninjected control embryos. Lane 11 is pUC18 digested with *Hpa*II. Lanes 2, 10, and 12 are analyses of RNA from embryos injected with the intact LvU2L gene. The other lanes are analyses of RNA from embryos injected with the linker-scanning mutants. Beneath each lane the nucleotides changed are indicated. Where two regions are given, both regions were changed in the same mutant. The protected fragments are labeled as in Fig. 2A. C, control; WT, wild type; M, marker.

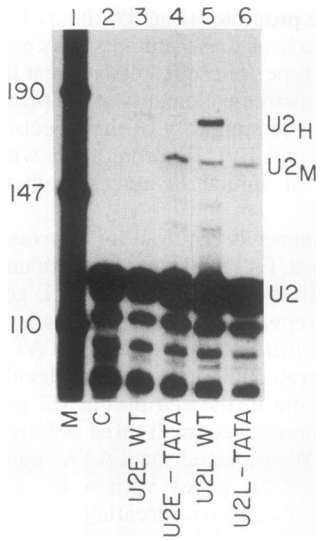


FIG. 6. An essential TATA sequence in the LvU2 snRNA genes. The sequence of the wild-type LvU2E promoter and start of the U2 RNA is shown in Fig. 1C. The mutations introduced into the TATA sequence of the LvU2E (nt 27-31) and LvU2L (nt 22-27) genes are shown in Fig. 4A and 5A, respectively. The U2E-TATA and U2L-TATA genes have a GC sequence substituted for the TATA sequence. The genes were injected together with the $LvU2_M$ gene into sea urchin zygotes, the embryos were harvested at the blastula stage, and RNA was prepared. The expression of the injected genes was assayed by using the riboprobe assay described for Fig. 2A. Lane 1 is pUC18 digested with *Hpa*II. Lane 2 is analysis of RNA from uninjected embryos. Lane 3 and 5 are analyses of RNA from embryos injected with the intact LvU2E and LvU2L genes, respectively. Lanes 4 and 6 are analyses of RNA from embryos injected with the U2E-TATA and U2L-TATA genes, respectively. The protected fragments are labeled as in Fig. 2A. M, marker; C, control; WT, wild type.

for the LvU2E promoter. For both the LvU2E and LvU2L sea urchin U2 snRNA genes, we have identified three elements at the same positions: the TATA box at about −25, the PSE at about −55, and a sequence at about −100 which play a major role in expression. In addition, there is an upstream element(s) required for maximal expression of both genes, and the upstream elements are quantitatively more important in the LvU2L gene.

The −100 elements are interchangeable in the two genes. Since the two LvU2 genes contain sequence elements located in similar positions, we investigated whether the elements from one gene would function in the context of the other gene. Using the linker-scanning mutations constructed for the promoter analysis, we interchanged the −100 element and the PSE between the two genes. The constructs are diagrammed in Fig. 7A. Also shown in Fig. 7A is the LE_pL gene, which has the TATA box, the PSE, and the −100 box from the LvU2E gene inserted into the LvU2L gene. To exchange the −100 boxes between the two promoters, we took advantage of the unique *Sst*II and *Sal*I sites created by the double linker-scanning mutants, LS 74-83,118-127 of the LvU2E promoter and LS 76-85,126-135 of the LvU2L promoter. We exchanged the 34-nt fragment including the −100 element of the LvU2E gene with a 40-nt fragment containing the same region of the late gene, creating the $EL_{100}E$ and the $LE_{100}L$ genes (Fig. 7A). These genes were coinjected with the $LvU2_M$ gene in parallel with the control genes (LvU2E: LS 74-83,118-127; LvU2L: LS 76-85,126-135) which had the same changes introduced in the sequences flanking the −100 element, and the relative expression of each gene was estimated.

Both the $LE_{100}L$ and the $EL_{100}E$ genes were expressed well. The $LE_{100}L$ gene was expressed at a level similar to that of the reference gene, LvU2L (LS 76-85,126-135) (Fig. 7B, lanes 3 and 5), and the $EL_{100}E$ gene was expressed at about 50% of the level of the reference gene LvU2E (LS 74-83,118-127) (Fig. 7B, lanes 2 and 4). Thus, the −100 sequences function well in the context of either gene, presumably interacting with the upstream sequences of each gene. Note that random alteration of the LvU2L −100 sequence reduced expression of the LvU2L gene by at least 90% (Fig. 5B, lane 6), although substitution of the LvU2E −100 sequence allowed normal expression (Fig. 7B, lane 5). This convincingly shows that the LvU2E −100 sequence is an important *cis*-acting element which can replace the LvU2L −100 element.

The LvU2E PSE can function in the LvU2L gene. The LvU2E promoter, including all the sequences from the TATA box to the −100 box, functions with the LvU2L upstream sequences. In the LE_pL gene, the LvU2E TATA box, PSE, and −100 sequence were introduced into the LvU2L promoter but 16 nt 5′ of the first nucleotide of the U2 RNA. A single transcript which was about 16 nt longer than the normal U2 RNA was formed from this gene (Fig. 7C, lane 9), and the LE_pL gene was expressed at about the same level as the LvU2L gene (Fig. 7C, lanes 8 and 9). Thus, the LvU2E proximal promoter elements (−20 to −120) are sufficient to determine the start site of transcription and interact with the upstream elements of the LvU2L promoter.

To see whether the LvU2E PSE alone would function in the context of the LvU2L gene, we constructed the $LE_{60}L$ gene by replacing the 47-nt *Pst*I-*Sal*I fragment of the LvU2L gene created in the linker-scanning mutant LS34-43,76-85 with the corresponding region of the LvU2E gene. This replaces 32 nt (from −44 to −75) with 28 nt of the LvU2E promoter (−49 to −73), including the PSE, of the LvU2L promoter with the PSE of the LvU2E promoter (Fig. 7A). (The other 15 nt were introduced in constructing the linker-scanning mutations). The $EL_{60}E$ gene was constructed in a similar fashion and has a 32-nt sequence (−44 to −75) containing the LvU2L PSE introduced into the LvU2E gene; the LvU2L PSE replaces nt −48 to −73 of the LvU2E PSE. In each of these genes, the distances between the PSE and the TATA sequence are identical to those in the respective wild-type genes.

The $LE_{60}L$ gene was expressed well when it was injected into *L. pictus* zygotes (Fig. 7C, lane 5). The level of expression was similar to that of the LvU2E control gene (Fig. 7C, lane 2) and about 25% of the level of the control LvU2L gene (Fig. 7A, lane 4). There were two transcripts formed from the $LE_{60}L$ gene, one the size of the normal $U2_H$ transcripts and the other slightly shorter (Fig. 7C, lane 5). The LvU2E PSE is 4 nt closer to the normal start site of the U2 snRNA in the $LE_{60}L$ gene than it is in the natural LvU2E gene. The shorter transcripts result from initiation of transcription inside the U2 RNA sequence, probably at the first purine in the U2 snRNA, the G at +4. Note that the LvU2L TATA box is in its normal position relative to the start site of the U2 RNA in the $LE_{60}L$ gene. The shorter transcript probably is a result of the PSE sequence being 4 nt closer to the start site and playing a role in the determination of the start site.

The LvU2L PSE does not function well in the LvU2E gene. The $EL_{60}E$ gene (Fig. 7A) has the LvU2L PSE substituted

for the LvU2E PSE, with the distance between the PSE and the TATA box constant. As a result of the different distances from the TATA box to the start site in the LvU2E and LvU2L genes, the LvU2L PSE is 4 nt farther 5′ of the start site in the $EL_{60}E$ gene than it is in the intact LvU2L gene. The $EL_{60}E$ gene was expressed at very low levels (<10%) compared with the control gene (Fig. 7C, lanes 2 and 3). Since it is possible that the alteration in distance between the PSE and the start site affected expression, we deleted 4 nt to obtain the $EL_{60}E^*$ gene, which has the PSE in its proper location relative to the start site. The $EL_{60}E^*$ gene was coinjected with the LE_pL gene (Fig. 7C, lane 10). The $EL_{60}E^*$ gene is clearly expressed but still at a very low level. From these data we conclude that while the LvU2E PSE functions well in the context of the LvU2L gene, the LvU2L PSE does not function efficiently in the context of the LvU2E promoter. The expression of the $EL_{60}E$ gene is similar to the expression of the LvU2L gene, with all but 75 nt of the promoter deleted (Fig. 4A, lane 9). This suggests that the factor(s) which interacts with the PSE sequence of the LvU2L gene does not interact with the factors which bind the distal sequences from the LvU2E gene.

In summary, the two differentially expressed U2 snRNA genes contain four different promoter elements, the TATA box, the PSE, the −100 box, and a UAS. The two genes have similar overall promoter structures, but different factors probably interact with the PSE and the −100 element in the two promoters. It is likely that one or both of these factors is developmentally regulated, accounting for the developmental regulation of expression of these genes.

DISCUSSION

The isolation of two U2 genes from the same sea urchin species provided us with a unique opportunity to analyze the promoter elements of genes encoding identical RNAs that show different temporal patterns of expression. snRNA promoters are of particular interest since they differ significantly from the promoters of genes encoding mRNAs (22). Although many of the snRNAs are transcribed by RNA polymerase II, it is relatively easy to change the polymerase specificity of the promoters of both the vertebrate (12, 16) and the plant (36) snRNA genes. In addition, common factors are normally involved in transcription of the snRNAs by RNA polymerases II and III (11, 20, 27).

We can draw several major conclusions about the structure of the promoter of the sea urchin U2 snRNA genes from this study.

(i) There are four *cis*-acting elements involved in expression: a UAS which has a fourfold effect on expression; a sequence located at −100 nt 5′ to the gene, which interacts with the UAS to stimulate the expression of both genes but is not absolutely required for expression; a PSE sequence at about −55 which is absolutely required for expression of both genes; and a TATA sequence at −25 which is essential for expression of both genes.

(ii) Either of the sequences at −100 functions well in the context of the LvU2E or the LvU2L promoter.

(iii) The LvU2E PSE functions well in the context of the late promoter, but the LvU2L PSE functions only weakly in the context of the early promoter.

Deletion analysis of the sea urchin U2 snRNA genes. Deletion analysis allows detection of upstream elements which play a role in the expression of the genes. Both the LvU2E and LvU2L genes contain an upstream element (termed the UAS) which has a modest stimulatory effect on expression of these genes. The UAS in each gene enhances expression only three- to fourfold, much less than the vertebrate DSE, the deletion of which greatly reduces expression of the vertebrate U1 and U2 genes (1, 18, 28). The UAS of the LvU2E gene is located between nt −275 and −210. The UAS of the LvU2L gene is located between the −516 and −445, farther 5′ than any known stimulatory elements in other snRNA genes (22). Within the LvU2L gene promoter, there are also other weak upstream elements located between nt −445 and −134. There is residual expression of both sea urchin U2 snRNA genes from promoters with only 75 nt of 5′ flanking sequence, indicating that the minimal requirements for expression include the PSE. A similar amount of sequence 5′ of the gene is required for expression of the sea urchin U7 gene (29).

An essential TATA sequence. Each gene contains an essential TATA element at about −25, a PSE at about −55, and a stimulatory element at about −100. The TATA sequence is located in the normal position for a TATA box, which determines the start site and is required for efficient expression of many genes transcribed by RNA polymerase II. However, TATA sequences are generally not found in snRNA genes transcribed by RNA polymerase II. This is true for both the sea urchin U1 (39) and U7 snRNA genes (29). TATA sequences are present in the promoters of some vertebrate snRNA genes transcribed by RNA polymerase III, including the 7SK snRNA (19) and U6 snRNA (5, 13, 27). In the U6 snRNA, transcription factor IID is involved in transcription of the gene by RNA polymerase III (14, 15, 26). TATA sequences are also present in plant snRNA genes, and the distance from the TATA sequence to the upstream element determines whether RNA polymerase II or III transcribes the plant snRNA gene (35–37). We speculate that the LvU2 TATA box, by binding the ubiquitous polymerase II transcription factor IID, contributes to formation of the transcription initiation complex. Alternatively, it is possible that there is a unique factor for U2 snRNA transcription which interacts with the TATA sequence. Mutation of the TATA sequence might lead to incomplete or unstable initiation complex assembly, resulting in inactivation of the promoter. The exact sequence of the TATA element is not conserved among the different sea urchin U2 genes (30). However, while the TATA sequence is essential for expression of the sea urchin U2 snRNA genes, it is not the sole determinant of the transcription start site.

The position of the PSE affects the choice of the transcription start site. Characteristic of snRNA genes from vertebrates is a conserved essential sequence at about −50 to −60 termed the PSE (22), and this element determines the position of the transcription start site. The PSE has a central role in expression of the LvU2 genes. We have shown that the LvU2 PSE is absolutely required for expression and may also play a role in selecting the U2 start site. In both the LvU2E and LvU2L genes, the critical portion of the PSE is at the 3′ end, located at about −50. Substitutions 5′ to the core sequence out as far as −75 nt reduce expression but do not completely inactivate the promoter, suggesting that the entire PSE spans about 25 nt. The nucleotides close to the 3′ end of the PSE appear to be most critical for its function. Interestingly, Lobo and coworkers have found that the 3′ end of human U2 PSE is primarily involved in selection of the transcription start site (13).

Neither the PSE nor the TATA box alone is sufficient to unambiguously determine the start site of transcription. About 50% of the transcripts from the hybrid $LE_{60}L$ promoter initiated 4 nt 3′ of the normal start site. In the $LE_{60}L$

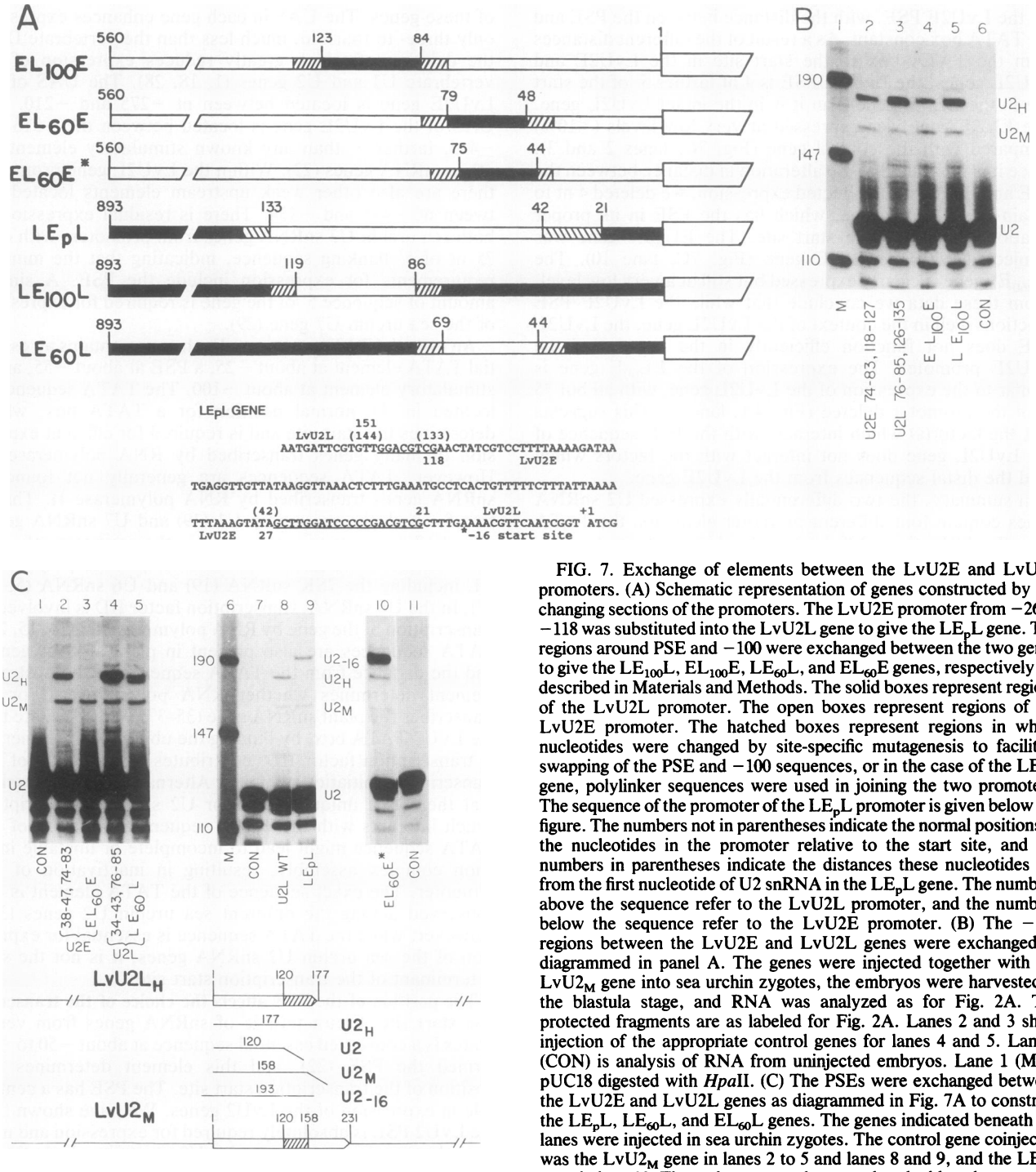


FIG. 7. Exchange of elements between the LvU2E and LvU2L promoters. (A) Schematic representation of genes constructed by exchanging sections of the promoters. The LvU2E promoter from −26 to −118 was substituted into the LvU2L gene to give the LE_pL gene. The regions around PSE and −100 were exchanged between the two genes to give the $LE_{100}L$, $EL_{100}E$, $LE_{60}L$, and $EL_{60}E$ genes, respectively, as described in Materials and Methods. The solid boxes represent regions of the LvU2L promoter. The open boxes represent regions of the LvU2E promoter. The hatched boxes represent regions in which nucleotides were changed by site-specific mutagenesis to facilitate swapping of the PSE and −100 sequences, or in the case of the LE_pL gene, polylinker sequences were used in joining the two promoters. The sequence of the promoter of the LE_pL promoter is given below the figure. The numbers not in parentheses indicate the normal positions of the nucleotides in the promoter relative to the start site, and the numbers in parentheses indicate the distances these nucleotides are from the first nucleotide of U2 snRNA in the LE_pL gene. The numbers above the sequence refer to the LvU2L promoter, and the numbers below the sequence refer to the LvU2E promoter. (B) The −100 regions between the LvU2E and LvU2L genes were exchanged as diagrammed in panel A. The genes were injected together with the $LvU2_M$ gene into sea urchin zygotes, the embryos were harvested at the blastula stage, and RNA was analyzed as for Fig. 2A. The protected fragments are as labeled for Fig. 2A. Lanes 2 and 3 show injection of the appropriate control genes for lanes 4 and 5. Lane 6 (CON) is analysis of RNA from uninjected embryos. Lane 1 (M) is pUC18 digested with *Hpa*II. (C) The PSEs were exchanged between the LvU2E and LvU2L genes as diagrammed in Fig. 7A to construct the LE_pL, $LE_{60}L$, and $EL_{60}L$ genes. The genes indicated beneath the lanes were injected in sea urchin zygotes. The control gene coinjected was the $LvU2_M$ gene in lanes 2 to 5 and lanes 8 and 9, and the LE_pL gene in lane 10. The embryos were harvested at the blastula stage and the RNA was analyzed as described by using the $LvU2L_H$ riboprobe. The riboprobe protection assay is diagrammed below the figure. Lanes 1, 7, and 11 are analyses of RNA from uninjected control embryos. Lane 6 is marker pUC18. Lanes 2 and 4 are analyses of RNA from embryos injected with the LvU2E and LvU2L control genes, respectively, and lane 8 is analysis of RNA from embryos injected with the intact LvU2L gene. Lanes 3 and 5 are analyses of RNA from embryos injected with the $EL_{60}E$ and $LE_{60}L$ genes, respectively; lane 9 is analysis of RNA from embryos injected with the LE_pL gene; and lane 10 is analysis of RNA from embryos injected with the $EL_{60}E$* gene. The protected fragments are labeled as for Fig. 2A. $U2_{-16}$ represents protection of the transcripts from the LE_pL gene. CON, control; M, marker; WT, wild type.

promoter, the TATA box is in the normal position with respect to the first nucleotide of U2 snRNA but the PSE was inserted 4 nt closer to the normal start site than in the wild-type gene. Placing the LvU2E PSE in the LvU2L gene at the proper distance from the start site restores proper initiation, consistent with a role for the PSE in selecting the start site (unpublished results). Clearly, the PSE alone cannot determine the start site since 50% of the transcripts from the $LE_{60}L$ gene started at the normal start site. Moreover, in the analogous $EL_{60}L$ gene, the transcripts

initiated at the normal start site, albeit very inefficiently, even when the PSE was displaced 4 nt. Thus, it is likely that proper initiation requires interaction of the PSE and the TATA box.

The −100 sequence is required for interaction with distal elements. The third element adjacent to the start of the gene involved in expression is a sequence located at about −100 nt from the start of the U2 snRNA. Deletion of this sequence did not have a major effect on transcription when the 5′ deletion mutants were analyzed. The −100 sequence is not a part of the core promoter but rather is necessary for coupling the upstream sequences to the core promoter (PSE plus TATA sequence). The sequence of the LvU2E −100 sequence differs completely from that of the LvU2L late gene −100 sequence and interacts with different factors (unpublished results).

Some of the U2 snRNA promoter elements are interchangeable. Despite the fact that the −100 sequences of the LvU2E and LvU2L genes are completely different and interact with different proteins (unpublished results), these two elements are interchangeable. The factors which interact with the two −100 sequences are each capable of coupling the UASs to the core promoter.

The two PSEs do not share any sequences, suggesting that these two elements probably interact with different factors. The LvU2E PSE functions well when it is placed in the LvU2L gene, either with or without other elements of the LvU2E promoter (Fig. 7C). However, the LvU2L PSE functions very weakly when placed in the LvU2E gene (Fig. 7C). It is not clear why the LvU2L PSE does not function well in the context of the LvU2E promoter. The LvU2L PSE is able to function together with the LvU2E −100 sequence in the $LE_{100}L$ gene (Fig. 7B). We can only speculate that the LvU2L PSE does not interact well with the LvU2E −100 sequence and upstream elements in the context of the complete LvU2E promoter. An alternative possibility is that the LvU2L PSE requires its cognate TATA box to function.

Sequence comparison of the PSEs of various sea urchin snRNA genes. The sea urchin PSE may be analogous to its vertebrate counterpart with respect to the function but not with respect to conservation of the PSE sequence among different snRNA genes. Inspection of the sequence around position −60 in cloned sea urchin snRNA genes revealed a limited degree of conservation among the tandemly repeated U1 and U2 snRNA genes of different sea urchin species. Preliminary results indicate that different protein factors interact with the LvU1 PSE and the LvU2E PSE (37a). The LvU2L gene PSE differs completely from the other sea urchin snRNA gene sequences. It is likely that the LvU2L PSE interacts with factors different from those with which the LvU2E PSE interacts (unpublished results). There are also no obvious similarities between the sea urchin U2 and the sea urchin U7 promoters (29), while the mammalian U7 sequences show conserved proximal and distal elements like those of other mammalian snRNA genes. Thus, it seems possible that each of the sea urchin snRNA genes characterized thus far (the U1, U2, and U7 genes) has a different PSE sequence interacting with different protein factors. This is different from the situation in vertebrates, which seem to have a common PSE sequence (although the factors which interact with this sequence have not yet been characterized) and from the nematode sequences, in which there is a strongly conserved consensus sequence in the PSEs of all the spliceosomal snRNAs (34).

ACKNOWLEDGMENTS

We thank Brian Wendelburg for many helpful discussions and for the unpublished data on the sea urchin U1 snRNA genes.

This work was supported by grant GM 27789 from the NIH to W.F.M.

REFERENCES

1. **Ares, M., Jr., M. Mangin, and A. M. Weiner.** 1985. Orientation-dependent transcriptional activator upstream of a human U2 snRNA gene. Mol. Cell. Biol. **5**:1560–1570.
2. **Beck, E., J. L. Jorcano, and A. Alonso.** 1984. Drosophila melanogaster U1 and U2 small nuclear RNA genes contain common flanking sequences. J. Mol. Biol. **173**:539–542.
3. **Carbon, P., S. Murgo, J. P. Ebel, A. Krol, G. Tebb, and I. W. Mattaj.** 1987. A common octamer motif binding protein is involved in the transcription of U6 snRNA by RNA polymerase III and U2 snRNA by RNA polymerase II. Cell **51**:71–79.
4. **Colin, A. M., T. L. Catlin, S. H. Kidson, and R. Maxson.** 1988. Closely linked early and late histone H2B genes are differentially expressed after microinjection into sea urchin zygotes. Proc. Natl. Acad. Sci. USA **85**:507–510.
5. **Das, G., D. Henning, D. Wright, and R. Reddy.** 1988. Upstream regulatory elements are necessary and sufficient for transcription of a U6 RNA gene by RNA polymerase III. EMBO J. **7**:503–512.
6. **Hernandez, N., and R. Lucito.** 1988. Elements required for transcription initiation of the human U2 snRNA gene coincide with elements required for snRNA 3′ end formation. EMBO J. **7**:3125–3134.
7. **Hernandez, N., and A. M. Weiner.** 1986. Formation of the 3′ end of U1 snRNA requires compatible snRNA promoter elements. Cell **47**:249–258.
8. **Kunkel, G. R., R. L. Maser, J. P. Calvet, and T. Pederson.** 1986. U6 small nuclear RNA is transcribed by RNA polymerase III. Proc. Natl. Acad. Sci. USA **83**:8575–8579.
9. **Kunkel, G. R., and T. Pederson.** 1988. Upstream elements required for efficient transcription of a human U6 RNA gene resemble those of U1 and U2 genes even though a different polymerase is used. Genes Dev. **2**:196–204.
10. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
11. **Lescure, A., P. Carbon, and A. Krol.** 1991. The different positioning of the proximal sequence element in the Xenopus RNA polymerase II and III snRNA promoters is a key determinant which confers RNA polymerase III specificity. Nucleic Acids Res. **19**:435–441.
12. **Lobo, S. M., and N. Hernandez.** 1989. A 7 bp mutation converts a human RNA polymerase II snRNA promoter into an RNA polymerase III promoter. Cell **58**:55–67.
13. **Lobo, S. M., S. Ifill, and N. Hernandez.** 1990. *cis*-acting elements required for RNA polymerase II and III transcription in the human U2 and U6 snRNA promoters. Nucleic Acids Res. **18**:2891–2899.
14. **Lobo, S. M., J. Lister, M. L. Sullivan, and N. Hernandez.** 1991. The cloned RNA polymerase II transcription factor IID selects RNA polymerase III to transcribe the human U6 gene in vitro. Genes Dev. **5**:1477–1489.
15. **Margottin, F., G. Dujardin, M. Gerard, J.-M. Egly, J. Huet, and A. Sentenac.** 1991. Participation of the TATA factor in transcription of the yeast U6 gene by RNA polymerase C. Science **251**:424–426.
16. **Mattaj, I. W., N. A. Dathan, H. D. Parry, P. Carbon, and A. Krol.** 1988. Changing the RNA polymerase specificity of U snRNA gene promoters. Cell **55**:435–442.
17. **McMahon, A. P., C. N. Flytzanis, B. R. Hough-Evans, K. S. Katula, R. J. Britten, and E. H. Davidson.** 1985. Introduction of cloned DNA into sea urchin egg cytoplasm: replication and persistance during embryogenesis. Dev. Biol. **108**:420–430.
18. **Murphy, J. T., J. T. Skuzeski, E. Lund, T. H. Steinberg, R. R. Burgess, and J. E. Dahlberg.** 1987. Functional elements of the human U1 RNA promoter. Identification of five separate regions required for efficient transcription and template competi-

tion. J. Biol. Chem. **262**:1795–1803.
19. **Murphy, S., C. Di Liegro, and M. Melli.** 1987. The in vitro transcription of the 7SK RNA gene by RNA polymerase III is dependent only on the presence of an upstream promoter. Cell **51**:81–87.
20. **Murphy, S., A. Pierani, C. Scheidereit, M. Melli, and R. G. Roeder.** 1989. Purified octamer binding transcription factors stimulate RNA polymerase III-mediated transcription of the 7SK RNA gene. Cell **59**:1071–1080.
21. **Neuman de Vegvar, H. E., and J. E. Dahlberg.** 1989. Initiation and termination of human U1 RNA transcription requires the concerted action of multiple flanking elements. Nucleic Acids Res. **17**:9305–9318.
22. **Parry, H. D., D. Scherly, and I. W. Mattaj.** 1989. 'Snurpogenesis'; the transcription and assembly of U snRNP components. Trends Biochem. Sci. **14**:15–19.
23. **Parry, H. D., G. Tebb, and I. W. Mattaj.** 1989. The *Xenopus* U2 gene PSE is a single, compact, element required for transcription initiation and 3′ end formation. Nucleic Acids Res. **17**: 3633–3644.
24. **Reddy, R., D. Henning, G. Das, M. Harless, and D. Wright.** 1987. The capped U6 small nuclear RNA is transcribed by RNA polymerase III. J. Biol. Chem. **262**:75–81.
25. **Saba, J. A., H. Busch, D. Wright, and R. Reddy.** 1986. Isolation and characterization of two putative full-length Drosophila U4 small nuclear RNA genes. J. Biol. Chem. **261**:8750–8753.
26. **Simmen, K. A., J. Bernues, H. D. Parry, H. G. Stunnenberg, A. Berkenstam, B. Cavallini, J.-M. Egly, and I. W. Mattaj.** 1991. TFIID is required for *in vitro* transcription of the human U6 gene by RNA polymerase III. EMBO J. **10**:1853–1862.
27. **Simmen, K. A., and I. W. Mattaj.** 1990. Complex requirements for RNA polymerase III transcription of the *Xenopus* U6 promoter. Nucleic Acids Res. **18**:5649–5657.
28. **Skuzeski, J. M., E. Lund, J. T. Murphy, T. H. Steinberg, R. R. Burgess, and J. E. Dahlberg.** 1984. Synthesis of human U1 RNA. J. Biol. Chem. **259**:8345–8352.
29. **Southgate, C., and M. Busslinger.** 1989. *In vivo* and *in vitro* expression of U7 snRNA genes: *cis*- and *trans*-acting elements required for RNA polymerase II-directed transcription. EMBO J. **8**:539–549.
30. **Stefanovic, B., J.-M. Li, S. Sakallah, and W. F. Marzluff.** 1991. Isolation and characterization of developmentally regulated sea urchin U2 snRNA genes. Dev. Biol. **148**:284–294.
31. **Strub, K., and M. L. Birnstiel.** 1986. Genetic complementation in the Xenopus oocyte: co-expression of sea urchin histone and U7 RNAs restores 3′ processing of H3 pre-mRNA in the oocyte. EMBO J. **5**:1675–1682.
32. **Tanaka, M., U. Grossniklaus, W. Herr, and N. Hernandez.** 1988. Activation of the U2 snRNA promoter by the octamer motif defines a new class of RNA polymerase II enhancer elements. Genes Dev. **2**:1764–1778.
33. **Tebb, G., and I. W. Mattaj.** 1988. Positionally exact initiation is required for the formation of a stable RNA polymerase II transcription complex *in vivo*. EMBO J. **7**:3785–3792.
34. **Thomas, J., K. Lea, E. Zucker-Aprison, and T. Blumenthal.** 1990. The spliceosomal snRNAs of *Caenorhabditis elegans*. Nucleic Acids Res. **18**:2633–2642.
35. **Vankan, P., and W. Filipowicz.** 1989. A U-snRNA gene-specific upstream element and a −30 'TATA box' are required for transcription of the U2 snRNA gene of *Arabidopsis thaliana*. EMBO J. **8**:3875–3882.
36. **Waibel, F., and W. Filipowicz.** 1990. RNA-polymerase specificity of transcription of *Arabidopsis* U snRNA genes determined by promoter element spacing. Nature (London) **346**:199–202.
37. **Waibel, F., and W. Filipowicz.** 1990. U6 snRNA genes of *Arabidopsis* are transcribed by RNA polymerase III but contain the same two upstream promoter elements as RNA polymerase II-transcribed U-snRNA genes. Nucleic Acids Res. **18**:3451–3458.

37a. **Wendelburg, B., and W. F. Marzluff.** Unpublished data.

38. **Yu, J. C., M. A. Nash, C. Santiago, and W. F. Marzluff.** 1986. Structure and expression of a second sea urchin U1 RNA gene repeat. Nucleic Acids Res. **14**:9977–9988.
39. **Yu, J.-C., B. Wendelburg, S. Sakallah, and W. F. Marzluff.** 1991. The U1 snRNA gene repeat from the sea urchin (*Strongylocentrotus purpuratus*): the 70 kilobase tandem repeat ends directly 3′ to a U1 gene. Nucleic Acids Res. **19**:1093–1098.