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An upstream U-snRNA gene-like promoter is required for transcription of the *Arabidopsis thaliana* 7SL RNA gene

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ABSTRACT

The genes transcribed by RNA polymerase (pol) III can be placed into four distinct groups based on the nature and position of their promoter elements. In the higher eukaryotes equivalent genes usually belong to the same sub-type of pol III promoters and there are few examples of genes which have changed promoter type during evolution. In this work we demonstrate that the promoter of the *Arabidopsis thaliana* 7SL RNA gene is located upstream of the coding region and is identical to the promoters of pol III-specific plant U-small nuclear RNA (U-snRNA) genes. Sequence analysis of two different 7SL genes from *A.thaliana* revealed that both genes contain two sequence elements in their 5′ flanking regions identical in sequence and position to the highly conserved USE and TATA elements of the pol III-transcribed plant U-snRNA genes. Mutational analysis of these elements in the At7SL-2 gene indicates that the USE and TATA elements are both necessary and account for ≥90% of the transcriptional activity of this gene in transfected plant protoplasts. Within the coding region of both genes there is a sequence element which is a 10/11 nt match to the consensus B-box element of tRNA genes, however, this element is not important for gene activity. These findings distinguish the plant genes from the human 7SL gene, which has both internal and upstream promoter elements and its upstream elements are different from those found in the human U-snRNA genes.

INTRODUCTION

The genes transcribed by RNA polymerase (pol) III display a surprising diversity in terms of promoter structure and can be divided into four groups, depending on the nature and position of their promoter elements (for recent reviews see 1–3). The first group (type 1) is restricted to the 5S ribosomal RNA (rRNA) genes, characterized in many different organisms, which are unique among pol III genes in that they have a tripartite intragenic promoter which spans ~50 base pairs (bp) and consists of an A block at about +45, a downstream C block and an intermediate (I) element between A and C (3,4). Type 2 genes are characterized by a bipartite intragenic promoter consisting of an A block positioned 10–20 bp downstream of the transcription start site and a B block located further downstream. Most of the genes encoding tRNAs are members of this group, as well as the adenovirus VA RNA genes (3,5). The third group (type 3) of pol III genes have promoter elements which reside entirely upstream of the coding region of the gene. The *cis*-acting elements in these genes are identical to those found in the promoters of many pol II-transcribed genes. This group includes genes for small RNAs generally localized in the nucleus, including U6, 7SK and 7-2/MRP RNA genes in all higher eukaryotes, as well as the U3 gene in plants (6–8). A fourth type of pol III genes consists of genes with mixed promoters, having both intragenic and upstream elements. This rather diverse group includes the *Xenopus* selenocysteine tRNA gene, the gene for human (h) 7SL RNA and the EBER genes of Epstein–Barr virus (3). The promoters of the type 4 genes share some elements with both the type 2 and type 3 genes (described above) and also contain pol II-like elements different from those found in the type 3 genes and, in the case of the h7SL RNA gene, a novel intragenic element (see below).

If one compares the promoters of pol III-specific genes from different organisms, it appears that the equivalent genes belong to the same type of pol III promoter. The conservation of promoter structure is most apparent in the 5S rRNA (type 1) and tRNA (type 2) genes, as the promoter organization and also the sequences of the *cis*-acting elements are nearly identical in diverse organisms. In the type 3 pol III-transcribed genes of higher eukaryotes this conservation of promoter structure is also apparent. For example, in all higher eukaryotes, but not in yeast (3), the pol III-transcribed U-snRNA gene promoters belong to the type 3 class of promoters, although the number and sequences of the upstream elements may vary between different organisms (reviewed in 6–8). Indeed, in the higher eukaryotes there are only a few known cases of the same genes belonging to a different type

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of pol III-specific promoter in different organisms and to date these are restricted to the tRNA genes. Unlike other tRNA genes, the *Xenopus* selenocysteine tRNA gene has a mixed promoter, which is located predominantly upstream of the coding region, but expression is modulated by an internal B block (9–11). In silkworm, apart from a requirement for intragenic A- and B-boxes, the gene coding for alanine $tRNA_C$ is absolutely dependent on two AT-rich elements upstream of the transcription start site, as mutation of either element reduces transcription to undetectable levels (12).

While cDNAs encoding 7SL RNA, the RNA component of the signal recognition particle (SRP), have been characterized in many organisms from the yeasts to man, little is known about the elements controlling transcription of these genes in eukaryotes. In the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* the genes encoding 7SL RNA contain sequences analogous to the internal A and B blocks typical of tRNA genes (13; reviewed in 3), however, it has not yet been demonstrated whether these elements actually comprise the promoter of the gene. In protozoans of the family Trypanosomatidae the promoter elements of the pol III-transcribed 7SL RNA genes, as well as those coding for the homologues of U2, U6 and U3 snRNAs, appear to consist of the A- and B-boxes of companion tRNA genes positioned 100 bp upstream of the transcription start sites and in the opposite orientation (14). To our knowledge the only 7SL gene promoter which has been characterized to date in higher eukaryotes belongs to one of the four transcriptionally active 7SL genes found in the human genome (15). Deletion analyses have indicated that the h7SL gene belongs to the type 4 pol III promoters, as it is comprised of both upstream and gene internal elements (15–18). Homologies to the A- and B-boxes of tRNA promoters can be found within the coding region of h7SL (5,16). That these elements are active in h7SL gene expression was inferred from experiments in which the upstream sequences were deleted and the 7SL gene was still expressed weakly both *in vitro* and in microinjected frog oocytes (15). More recently it was demonstrated that while most mutations made within the A-box homology present in the h7SL coding region have little effect on transcription *in vitro*, a CG dinucleotide at position +15/+16 appears to be absolutely required for expression, as mutation of either nucleotide completely inactivates the gene in HeLa cell extracts (16). In the upstream flanking sequences of the h7SL gene a binding site for the transcriptional activator ATF, a factor shown previously to be involved in pol III-specific expression of the EBER genes (19), has been found centered ~50 bp upstream of the h7SL transcription start site (18). Mutation of this sequence resulted in a 50% reduction in 7SL gene expression both *in vitro* and *in vivo* and it appears that this site is occupied *in vitro* by a cellular factor (18). There may also be an enhancer element further upstream of the ATF binding site, which is required for full activity of the gene *in vivo* (16), although this element has not yet been characterized. The h7SL gene, therefore, appears to have a mixed promoter with both pol II-like upstream elements and a novel intragenic sequence element.

In this work we describe the analysis of the promoter organization of two *Arabidopsis thaliana* 7SL genes (At7SL-1 and -2). Mutational analysis indicates that the *Arabidopsis* 7SL genes, unlike their human counterparts, can be placed within the type 3 subset of pol III-specific genes. Furthermore, the plant 7SL RNA genes differ from the h7SL gene in that the upstream promoter elements are identical to those of the U-snRNA gene family. This demonstrates that the 7SL genes are one of the few examples of pol III genes which have changed promoter type during evolution.

MATERIALS AND METHODS

Cloning and sequence of the 7SL genes

Unless otherwise stated, all DNA manipulations were done by standard techniques (20). Two genes coding for the *A.thaliana* 7SL RNA (At7SL-1 and At7SL-2) were isolated from an *A.thaliana* genomic library cloned in the lambda vector EMBL4 (21). Restriction fragments containing the genes and flanking sequences were subcloned and sequenced by the dideoxy-nucleotide termination method using an ALF automatic sequencer (Pharmacia). The corresponding sequences were submitted to the EMBL data library under accession nos X72228 and X72229.

At7SL-2 mutagenesis

For transient expression studies and site-directed mutagenesis of the At7SL-2 gene the wild-type sequence corresponding to nucleotides –72 to +346 of the gene sequence was amplified by the polymerase chain reaction (PCR) using the 5′ primer O1 (GCGGGATCCTCTTTTGTCCCACATCGG) and 3′ primer O2 (GCGAATTCGCGTAGAAAGTTTTGG). A 2 bp mutation in the USE element (USE-Dn) was also obtained by PCR amplification using oligonucleotide O3 (GCGGGATCCTCTTTTGGCC-AACATCGG), rather than O1, as the 5′ primer. PCR amplification of At7SL-2 DNA with these primers results in the incorporation of *Bam*HI and *Eco*RI sites at the 5′- and 3′-ends of the gene respectively. The PCR-derived DNA fragments were cloned into the *Bam*HI–*Eco*RI sites of the vector pBluescript SK– (Stratagene), creating plasmids pAt7SL-2WT and pAt7SL-2USE-Dn. All other constructs were prepared by site-directed mutagenesis, performed by the Kunkel method (as described in 20). The following mutagenic primers were used:
TATA-Dn, CATTTTGTGTTTGGTGCACGATTACACGAACCG;
B-Dn1, GTGGAACACTGAATTGGGTCCACGGGCCG;
B-Dn2, GTGGAACACTGAATTGGGTTTACGGGCCG;
B-Up, CACTGGTTCGAGTCCACGGGC;
A-Up, TACGTCGAGCTTAGCAAAGTGGGCTTGTAACCC.

Transient expression in transfected protoplasts and RNase A/T_1 mapping

Transient expression was tested in protoplasts of *Nicotiana plumbaginifolia* transfected by the polyethylene glycol method (22). In each case 10 μg of the 7SL gene construct, either wild-type or mutant, was co-transfected into 6×10^5 protoplasts, along with 5 μg of a construct expressing the *Arabidopsis* U2syn gene (23) as an internal reference for transfection efficiency. Protoplasts were incubated for 24 h and total RNA was then prepared from the protoplasts by the guanidinium thiocyanate method (24). Levels of steady-state RNA arising from the transfected genes were determined by RNase A/T_1 mapping (22,24) using ^{32}P-labeled antisense RNA probes specific for the U2 reference gene and either the wild-type or mutant 7SL genes. For the 7SL constructs probes were generated by *in vitro* transcription with T7 RNA polymerase using templates linearized by *Bam*HI and therefore they protect the entire coding region of the gene, as well as upstream and downstream flanking regions

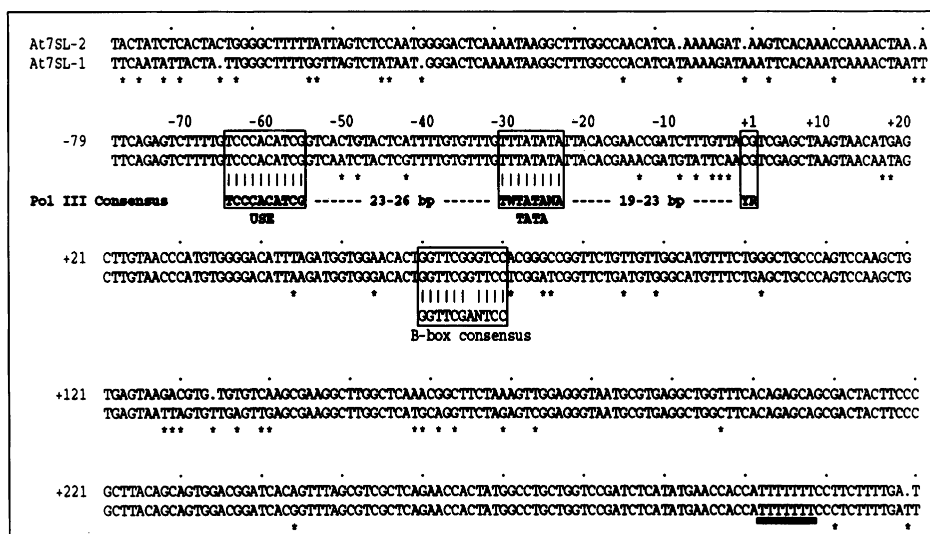

Figure 1. Alignment of the At7SL-1 and At7SL-2 genes and comparison with the pol III U-snRNA consensus promoter. Numbering corresponds to the At7SL-2 gene. Mismatches between the two genes are indicated by asterisks. The putative transcription start (+1) and termination (underlined T residues) sites predicted from alignments with other known plant 7SL sequences (21) are indicated. The dicotyledenous plant pol III U-snRNA gene consensus USE and TATA elements, the pyrimidine/purine (YR) sequence at the transcription start site and the spacing between these elements in base pairs (bp) is indicated in bold (W = T or A). The B-box consensus sequence is also shown. A run of T residues which would act as a pol III termination signal is underlined.

(see Fig. 2A). Protected fragments were seperated by denaturing PAGE and bands quantitated by means of a PhosphorImager (Molecular Dynamics). The level of expression of each 7SL gene was always normalized to the expression of the reference gene in that transfection.

RESULTS

Sequence analysis

The sequences of both *Arabidopsis* 7SL RNA genes, At7SL-1 and -2, are indicated in Figure 1. Analysis of the sequences of the two *Arabidopsis* genes has revealed that the upstream regions of each contain two sequence elements identical to the highly conserved USE element and TATA box sequences present in all plant U-snRNA gene promoters and that these elements are in positions typical of pol III-specific genes (reviewed in 6). This suggests that the *Arabidopsis* 7SL genes are transcribed by pol III from a promoter which resembles the promoters of plant U-snRNA genes. However, unlike the known U-snRNA genes of plants, both At7SL RNA genes contain an intragenic sequence element centered at position +65, which is a 10/11 match to the consensus B-box promoter element characteristic of type 2 pol III promoters (Fig. 1). This raises the possibility that, unlike the pol III-specific U-snRNA genes, a gene internal element may be involved in *Arabidopsis* 7SL RNA gene expression.

7SL gene expression in plants is controlled by elements upstream of the coding region

In order to determine which of the putative promoter elements in the *Arabidopsis* 7SL-2 gene are active *in vivo*, mutations were introduced into each of these elements by site-directed mutagenesis (as indicated in Fig. 2A) and constructs were tested in transfected protoplasts of *N.plumbaginifolia*. RNase protection analysis of total RNA isolated from protoplasts transfected with the wild-type At7SL-2 gene, using an antisense RNA probe homologous to the full-length gene, including flanking sequences, resulted in protection of a fragment of the expected size, indicating that transcription started at the proposed +1 and terminated in the T-stretch at the 3′-end of the gene (Fig. 2B).

When point mutations shown previously to essentially completely inactivate the *Arabidopsis* U2 gene (25) were introduced into the USE element of the 7SL-2 gene (USE-Dn), a 20-fold decrease in the level of 7SL transcripts was observed (Fig. 2B, lane 2 and Fig. 2C). Mutations in the TATA box element shown previously to inactivate U6 gene expression in plant protoplasts (26,27) also caused 7SL gene activity to drop to virtually undetectable levels (TATA-Dn; Fig 2B, lane 3). Mutation of the USE and TATA elements simultaneously produced a similar effect to the TATA mutant alone (USE+TATA-Dn; Fig. 2B, lane 4). In contrast to mutations in the upstream promoter elements, two different mutations in the B-box homology (B-Dn1 and B-Dn2) had little effect on transcription from this gene, both allowing 90% of wild-type gene activity (Fig. 2B, lanes 5 and 6). To determine whether the B-box element contributes to the residual activity observed with the USE down mutation, the two B-box mutations were also analysed in the context of the USE-Dn mutant. Interestingly, in this promoter context the B-Dn1 mutation resulted in a level of expression comparable with the USE-Dn mutant, whereas the more severe B-Dn2 mutation reduced 7SL expression a further 2-fold, to levels comparable with the TATA-Dn mutant (Fig 2B, lanes 7 and 8; see also Fig. 2C). This suggests that the B-box-like sequence may contribute to the residual 7SL gene activity observed with the USE down mutant.

Addition of consensus A- and B-boxes to the *Arabidopsis* 7SL-2 gene

To further test whether an efficient internal promoter could be constructed in a plant 7SL gene, consensus A- and B-boxes were incorporated into the coding region of the 7SL-2 sequence by

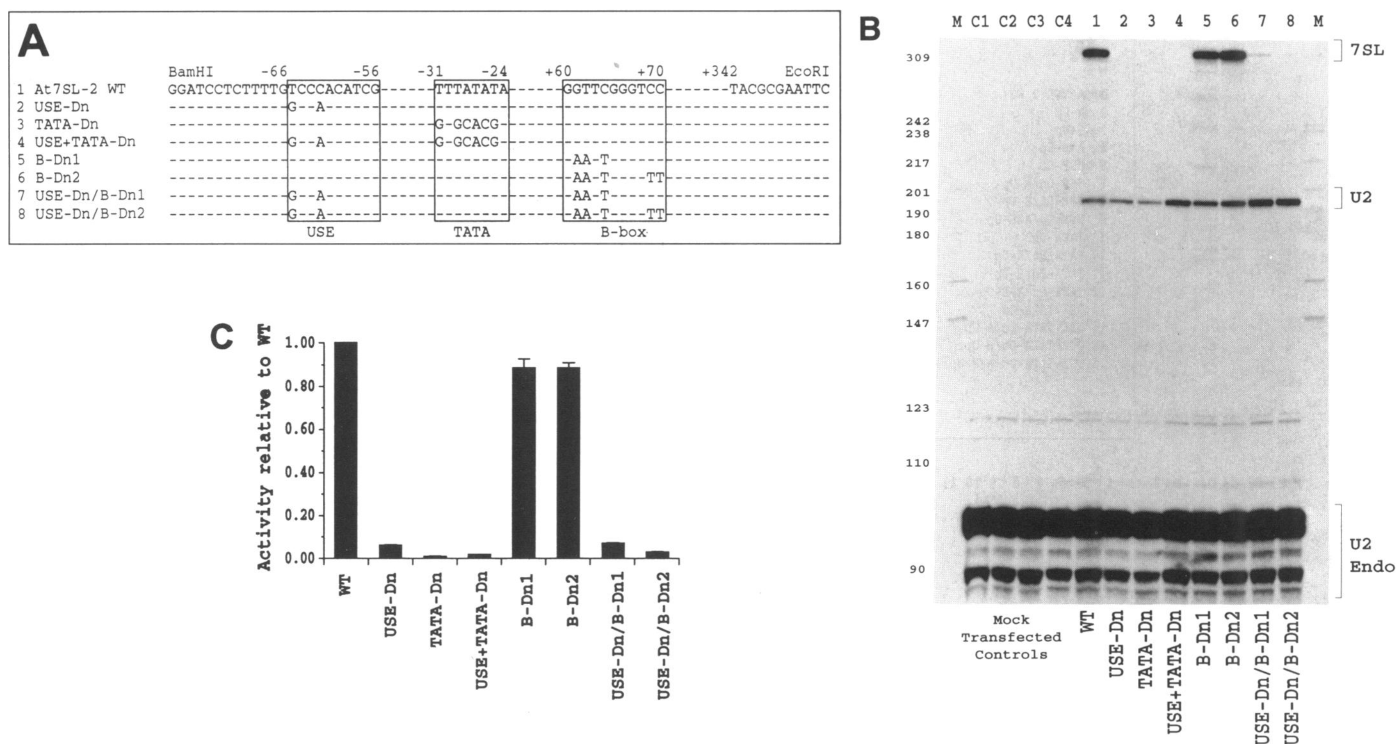

Figure 2. Expression of the At7SL-2 wild-type gene and various promoter element mutants in transfected *N.plumbaginifolia* protoplasts. (**A**) Sequences of the putative At7SL-2 promoter elements and positions of the mutations introduced are shown. Numbering is as in Figure 1. *Bam*HI and *Eco*RI sites introduced by PCR are also indicated. (**B**) RNase A/T_1 mapping of total RNA isolated from protoplasts co-transfected with the 7SL constructs shown in (A) and the *Arabidopsis* U2 gene as an internal reference of transfection efficiency. Lanes M, *Hpa*II-digested pBR322 size markers. Lanes C1–C4; RNase mapping of total RNA from mock-transfected protoplasts protected with the U2 probe alone (C1) or the U2 probe and probes for different 7SL coding regions: wild-type (C2), B-Dn1 (C3) and B-Dn2 (C4). Lanes 1–8, mapping of RNA from protoplasts transfected with the various 7SL constructs; lane numbers correspond to the construct numbers indicated in (A). Protected RNA fragments arising from the transfected 7SL and U2 genes are indicated on the right. The U2 probe also partially protects endogenous *N.plumbaginifolia* U2 transcripts (indicated as U2 Endo). The size of the protected 7SL RNA fragment is slightly larger than the coding region due to the fact that the enzymes used in RNase A/T_1 mapping do not cut A residues in the probe corresponding to the T stretch at the 3′-end of the RNA, resulting in a product of about 308 nt. (**C**) Quantitation of the results from independent transient expression experiments. Values represent the means ± SEM from at least three independent transfections (errors less than ±0.02 are not visible on this graph). Radioactivity of protected probe RNA fragments was measured using a PhosphoImager and, after correction for transfection efficiency, the level of expression of each 7SL construct is compared with wild-type At7SL-2 gene expression.

site-directed mutagenesis. While the B-box homology could be made into a consensus sequence by a single G→A nucleotide change at position +66, a 5 nt mutation was required to introduce a consensus A-box into the coding region (Fig. 3A). The region mutated to introduce an A-box consensus was chosen such that the mutations should have little effect on the secondary structure, rather than to optimize A-box position relative to the start site (see Fig. 4). All combinations of internal and external promoter elements were then tested by transfection into plant protoplasts.

When the upstream promoter elements are wild-type, improvement of the B-box homology to a consensus sequence resulted in a small but significant increase in 7SL RNA accumulation, to ~115% of the level observed with the wild-type gene (Fig. 3B, compare lanes 1 and 3, and Fig. 3C). When the USE is mutated and the TATA box is left wild-type, a consensus B-box also appears to increase 7SL RNA accumulation ~2-fold, as compared with the construct with a mutant USE and wild-type coding sequence (Fig. 3B, lanes 5 and 7). In contrast, when the TATA box is mutated, 7SL RNA accumulation drops to almost undetectable levels and no effect of a consensus B-box is observed, regardless of the condition of the USE element (Fig 3B). Introduction of an A-box consensus sequence into the coding region of the otherwise wild-type 7SL-2 gene resulted in a 5- to 6-fold reduction in 7SL RNA accumulation relative to the wild-type gene (Fig. 3B, lane 2). A similar reduction was observed when the A- and B-box mutations were made simultaneously (lane 4). No rescue of 7SL RNA accumulation was observed in the presence of consensus A- and B-box sequences when the USE and/or the TATA box were mutated, indicating that this reduction in 7SL gene expression was not due to interference between the gene internal and external promoter elements (Fig 3B, lanes 13–16). These results suggest that the mutations creating the A-box consensus may cause a dramatic decrease in 7SL RNA stability. Two of the nucleotides mutated in the A-box up constructs (U_{12} and C_{15}, Fig. 4), which, in comparison with the tomato 7SL structure (28) should be present in the loop region of a 5′ proximal hairpin (Fig. 4), are conserved in all known higher eukaryotic 7SL RNAs (29). It is possible that these mutated nucleotides are responsible for the decrease in the level of 7SL RNA arising from the A-box up constructs, either by changing the RNA structure or preventing the correct assembly of protein components of the signal recognition particle (see 29). It should be noted that, since we are measuring steady-state levels of 7SL RNA, the results obtained with the A-box up mutants do not eliminate the possiblity that the introduction of the A-box consensus has some positive effect on transcription which may not be observable due to a concurrent loss of RNA stability.

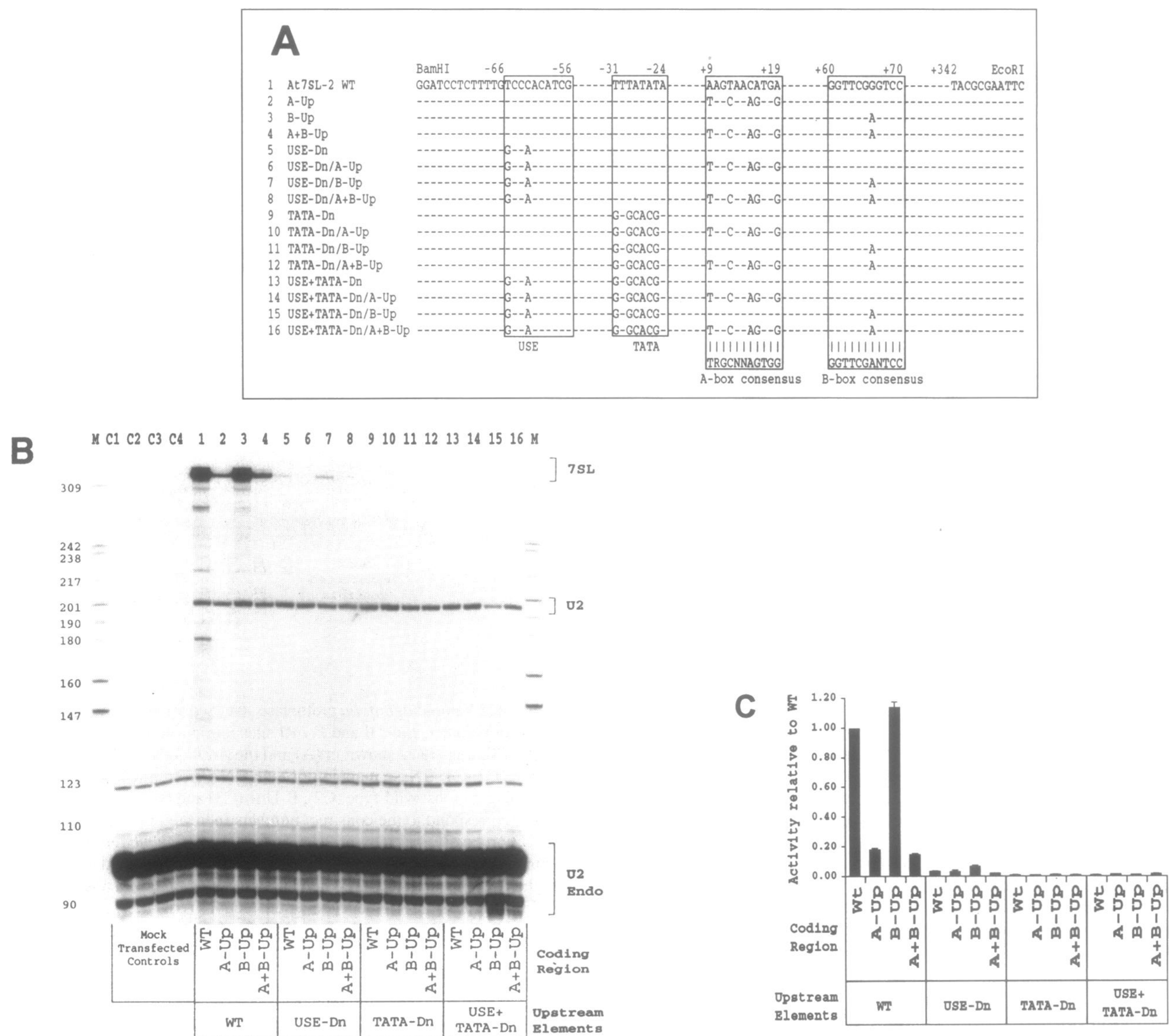

Figure 3. Effect of consensus A- and B-box sequences on expression of At7SL-2 constructs with either wild-type or mutant upstream promoter elements. **(A)** Relevant sequences of wild-type and mutant At7SL-2 constructs showing mutations made in upstream elements and those which introduce consensus A- and B-box elements into the coding region. Consensus A- and B-boxes of tRNA genes (5) are shown. **(B)** RNase A/T_1 mapping of total RNA from protoplasts transfected with the constructs shown in (A). Lane M, *Hpa*II-digested pBR322 size markers. Lanes C1–C4, RNase mapping of total RNA from mock-transfected protoplasts protected with the U2 probe and probes for the different 7SL coding regions: wild-type (C1), A-up (C2), B-up (C3) and A+B-up (C4). Lanes 1–16, mapping of RNA from protoplasts transfected with the various 7SL constructs, lane numbers correspond to the construct numbers indicated in (A). **(C)** Average results from the quantitation of at least three independent experiments. Values represent the means ± SEM. Other details as in the legend to Figure 2.

DISCUSSION

Sequence analysis of genomic copies of the *Arabidopsis* 7SL genes has revealed the presence of promoter elements typical of pol III-transcribed plant U-snRNA genes (reviewed in 6), namely a TATA box at position –25 and a consensus upstream sequence element (USE) centered at position –60 relative to the transcription start site. Although we have not tested the polymerase specificity of this gene directly, three lines of evidence strongly suggest that the At7SL-2 gene is transcribed by pol III. First, the USE and TATA elements in 7SL genes are centred approximately three helical turns apart, typical of the pol III-transcribed U6 and U3 snRNA genes in plants. It has been rigorously demonstrated that the USE–TATA spacing determines the RNA polymerase specificity of the U-snRNA genes (23,30). Secondly, RNase A/T_1 analysis of total RNA from transfected protoplasts indicates that the size of the At7SL-2 transcripts detected is consistent with transcription terminating at the run of T residues around position +305, as would be expected if the gene were transcribed by pol III (for example, see Fig. 2B). Lastly, RNAs arising from transcription by pol II are capped co-transcriptionally by a 7-methyl-guanosine (m^7G) cap structure which, in the case of all pol II-transcribed U-snRNAs, becomes modified in the cytoplasm to a trimethylguanosine (m_3G) structure (31). No transcripts of the size expected for 7SL RNA could be detected by immunoprecipition with anti-m^7G or anti-m_3G antibodies, (see 32; T. Kiss and W. Filipowicz unpublished results).

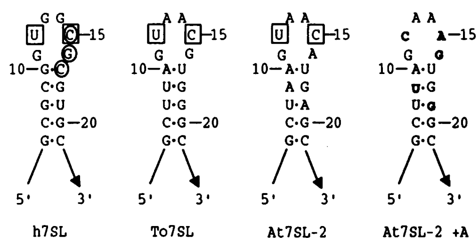

Figure 4. Comparison of the experimentally determined structures for human (h7SL; 42) and tomato (To) 7SL RNA (28) with predicted structures of At7SL-2 wild-type and +A mutants (mutated bases are shown in bold). Residues conserved in all higher eukaryotic 7SL RNAs (29) are boxed. Nucleotides in h7SL which have been proposed to interact with the 9 kDa/14 kDa heterodimeric SRP proteins (29) are circled. Numbers correspond to actual positions from the transcription start site for each RNA. Dots represent paired bases.

Both the USE and TATA sequences present in the 5′ flanking region of the *Arabidopsis* 7SL-2 gene are necessary for At7SL gene transcription (Fig. 2). Mutation of the TATA box results in complete inactivation of the gene in transfected protoplasts. The same mutation was previously shown to abolish *Arabidopsis* U6 gene transcription *in vivo* (26,27). While the point mutations made in the USE sequence of At7SL-2 were not previously tested in pol III-specific U-snRNA genes, it has been observed that a different point mutation in the USE had similar effects on both pol II and pol III U-snRNA transcription in plant protoplasts (33). In agreement with this finding, the 20-fold decrease in At7SL-2 gene expression resulting from the USE-Dn mutation is similar to that reported for the pol II-specific *Arabidopsis* U2 snRNA gene (25).

The B-box homology present in the At7SL-2 gene has the sequence GGTTCGGGTCC, only 1 nt different from the consensus B-box of tRNA genes (GGTTCGANTCC, invariant nucleotides indicated in bold). However, this mismatch is at a position which is 100% conserved in all tRNA B-boxes (5). Two different down mutations in the At7SL-2 B-box homology, which targeted at least two other invariant positions (see Fig. 2), still allowed ≥90% of wild-type 7SL RNA accumulation in the presence of wild-type upstream promoter elements. Both of the B-box down mutations result in a similar level of 7SL RNA accumulation when the upstream elements were wild-type, indicating that any effects the two mutations may have on the stability of the RNA product must be similar. Interestingly, in the context of the USE down mutation, the more severe of the B-box down mutations, B-Dn2, has a stronger effect on the residual 7SL gene activity (see Fig. 2C). These results indicate that, while the B-box element may contribute weakly to *Arabidopsis* 7SL gene expression, it is not essential for promoter activity in transfected plant protoplasts. In the U6 snRNA gene of *S.cerevisiae* it was found that a B-box sequence located downstream of the coding region of the gene is not required for gene activity in a purified *in vitro* system, but is required for transcription from reconstituted chromatin or *in vivo* (34 and references therein). Therefore, we cannot rule out the possibility that the B-box homology might contribute to expression of the *Arabidopsis* 7SL-2 gene in its natural chromatin environment.

Additional evidence that the B-box is not likely to be essential for plant 7SL gene expression comes from analysis of other plant 7SL sequences. Comparison of known plant 7SL RNA sequences from tomato (28), wheat (35) and maize (36) revealed poor sequence homology to the consensus B-box within the tomato and maize 7SL RNAs and one of three wheat RNA sequences. No B-box homologies were detected in the two other wheat 7SL RNAs (results not shown). All these plant 7SL RNA sequences were obtained from cDNA cloning and therefore must arise from actively transcribed genes, further indicating that internal tRNA-like promoter elements are not required for expression of these plant 7SL RNAs. However, this does not exclude a requirement for other, as yet uncharacterized, internal sequence elements. Previous work has demonstrated that internal sequences are not required for efficient transcription of the pol III-specific *Arabidopsis* U6 snRNA gene (26) and that the promoters of the *Arabidopsis* U6 and tomato U3 genes alone can drive the transcription of completely unrelated sequences (33; D. J. Heard and W. Filipowicz, unpublished results). Since the *Arabidopsis* 7SL genes have upstream promoter elements identical to the U-snRNA genes, it is likely that internal sequence elements do not play a significant role in plant 7SL RNA transcription.

Taken together, these findings suggest that plant 7SL genes belong to the type 3 pol III-transcribed genes, which have their promoters situated upstream of the transcribed region. Furthermore, the 7SL upstream promoter is identical to the promoters present in the U-snRNA genes in plants (26,30,37–39). In humans the 7SL RNA gene contains a type 4 promoter, with essential elements positioned both upstream and within the coding region (15–18). None of the promoter elements in the h7SL RNA gene are typical of those found in the promoters of vertebrate U-snRNA genes (reviewed in 3). Therefore, the 7SL RNA gene is one of the few examples of a pol III-transcribed gene which has changed promoter type during evolution.

The 7SL RNA gene of yeast appears to have an internal tRNA-like promoter (13). The plant 7SL genes may have evolved from these type 2 promoter-containing genes, perhaps explaining the internal B-box homology in the coding region of the *Arabidopsis* 7SL genes. To test whether transcription of the *Arabidopsis* 7SL-2 gene can be driven by an engineered internal tRNA-like promoter, consensus A- and B-box elements were placed within the coding region of the gene. Improvement of the B-box homology to the consensus resulted in a weak stimulation of 7SL RNA accumulation when the USE element was functional, as well as a 2-fold increase in the amount of 7SL RNA when the USE was mutated (Fig. 3). This suggests that the effect of the B-box down mutations on 7SL-2 RNA levels, as described above, could be due to the loss of a weak contribution to 7SL transcription by factors recognizing this element. Interestingly, the effect of the B-box mutations was apparent only in the presence of a functional TATA element, suggesting that factors binding to the B-box also work via the TATA element. Similar effects on transcription were observed previously by Parry and Mattaj (40) when a consensus B-box was inserted into the *Xenopus* U6 gene. Together, these findings suggest that the transcription factors binding to the *cis*-acting upstream elements of type 3 pol III genes and those binding to the intragenic B-box element of type 2 genes may essentially perform a similar task, of positioning TFIIIB upstream of the transcription start site (reviewed in 41), thereby contributing additively to the transcription of these genes in the presence of a TATA box element. Unlike the *Arabidopsis* 7SL RNA genes, the human gene lacks a TATA element in its upstream region (15). Perhaps the h7SL RNA gene requires its internal element for correct positioning of TFIIIB in

the absence of the TATA box. It is noteworthy that the intragenic element in h7SL overlaps the region in which A-box elements are situated in the tRNA genes (5).

In contrast to the B-box up mutations, introduction of a consensus A-box sequence (A-box up) into the At7SL-2 gene or its mutants resulted in a dramatic decrease in 7SL RNA accumulation, likely to be caused by decreased stability of the RNA (see Results). An alternative explanation, that the A-box up mutations may directly affect 7SL transcription, either by destroying an unknown internal promoter element similar to that found in the h7SL gene (16) or by interfering with normal transcription from the upstream elements, is rather unlikely (see above).

To date, genes having type 3 and type 4 pol III promoters have not been described in yeast, suggesting that these promoters may have evolved since the divergence of the higher eukaryotes and fungi (3). The B-box elements found in At7SL genes and the A-box-like sequence present in plant and vertebrate U6snRNA genes (reviewed in 6,8) may therefore represent the remnants of the internal tRNA-like promoters found in their yeast counterparts. It would be interesting to determine the promoter organization of U-snRNA and 7SL genes from other, more primitive, plants and animals to learn more about the pathway of pol III gene evolution.

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REFERENCES

1 Gabrielson,O.S. and Sentenac,A. (1991) *Trends Biochem. Sci.*, **16**, 412–416.
2 Geiduschek,E.P. and Kassavetis,G.A. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation*, Vol. 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 247–280.
3 Willis,I.M. (1993) *Eur. J. Biochem.*, **212**, 1–11.
4 Geiduschek,E.P and Tocchini-Valentini,G.P. (1988) *Annu. Rev. Biochem.*, **57**, 873–914.
5 Sharp,S.J., Schaack,J., Cooley,L., Burke,D.J. and Söll,D. (1985) *CRC Crit. Rev. Biochem.*, **19**, 107–144.
6 Goodall,G.J., Kiss,T. and Filipowicz,W. (1991) *Oxford Surv. Plant Mol. Cell Biol.*, **7**, 255–296.
7 Kunkel,G.R. (1991) *Bichim. Biophys. Acta*, **1088**, 1–9.
8 Hernandez,N. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation*, Vol. 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 281–313.
9 Lee,B.J., Kang,S.G. and Hatfield,D. (1989) *J. Biol. Chem.*, **264**, 9696–9702.
10 Carbon,P. and Krol,A. (1991) *EMBO J.*, **10**, 599–606.
11 Myslinski,E., Schuster,C., Krol,A. and Carbon,P. (1993) *J. Mol. Biol.*, **234**, 311–318.
12 Palida,F.A., Hale,C. and Sprague,K.U. (1993) *Nucleic Acids Res.*, **21**, 5875–5881.
13 Ribes,V., Dehoux,P. and Tollervey,D. (1988) *EMBO J.*, **7**, 231–237.
14 Nakaar,V., Dare,A.O., Hong,D., Ullu,E. and Tschudi,C. (1994) *Mol. Cell. Biol.*, **14**, 6736–6742.
15 Ullu,E. and Weiner,A.M. (1985) *Nature*, **318**, 371–374.
16 Bredow,S., Kleinart,H. and Benecke,B.-J. (1990) *Gene*, **86**, 217–225.
17 Kleinert,H., Gladen,A., Geisler,M. and Benecke,B.-J. (1988) *J. Biol. Chem.*, **263**, 11511–11515.
18 Bredow,S., Sürig,D., Müller,J., Kleinert,H. and Benecke,B.-J. (1990) *Nucleic Acids Res.*, **18**, 6779–6784.
19 Howe,J.G. and Shu,M.-D. (1989) *Cell*, **57**, 825–834.
20 Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21 Marques,J.P, Gualberto,J.M. and Palme,K. (1993) *Nucleic Acids Res.*, **21**, 3581.
22 Goodall,G.J., Wiebauer,K. and Filipowicz,W. (1990) *Methods Enzymol.*, **181**, 148–161.
23 Waibel,F. and Filipowicz,W. (1990) *Nature*, **346**, 199–202.
24 Goodall,G.J. and Filipowicz,W. (1989) *Cell*, **58**, 473–483.
25 Vankan,P. and Filipowicz,W. (1989) *EMBO J.*, **8**, 3875–3882.
26 Waibel,F. and Filipowicz,W. (1990) *Nucleic Acids Res.*, **18**, 3451–3458.
27 Heard,D.J., Kiss,T. and Filipowicz,W. (1993) *EMBO J.*, **12**, 3519–3528.
28 Haas,G., Klanner,A., Ramm,K. and Sänger,H.L. (1988) *EMBO J.*, **7**, 4063–4074.
29 Strub,K., Moss,J. and Walter,P. (1991) *Mol. Cell. Biol.*, **11**, 3949–3959.
30 Kiss,T., Marshallsay,C. and Filipowicz,W. (1991) *Cell*, **65**, 517–526.
31 Reddy,R. and Busch,H. (1988) In Birnstiel,M.L. (ed), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, Germany, pp 1–37.
32 Krol,A., Ebel,J.-P., Rinke,J. and Lührmann,R. (1983) *Nucleic Acids Res.*, **16**, 8583–8594.
33 Connelly,S. and Filipowicz,W. (1993) *Mol. Cell. Biol.*, **13**, 6403–6415.
34 Burnol,A.-F., Margottin,F., Schultz,P., Marsolier,M.-C., Oudet,P. and Sentenac,A. (1993) *J. Mol. Biol.*, **233**, 644–658.
35 Marshallsay,C., Prehn,S. and Zwieb,C. (1989) *Nucleic Acids Res.*, **17**, 1771.
36 Campos,N., Palau,J. and Zwieb,C. (1989) *Nucleic Acids Res.*, **17**, 1573–1588.
37 Marshallsay,C., Kiss,T. and Filipowicz,W. (1990) *Nucleic Acids Res.*, **18**, 3459–3466.
38 Kiss,T. and Solymosy,F. (1990) *Nucleic Acids Res.*, **18**, 1941–1949.
39 Kiss,T., Marshallsay,C. and Filipowicz,W. (1992) *EMBO J.*, **11**, 3737–3746.
40 Parry,H.D. and Mattaj,I.W. (1990) *EMBO J.*, **9**, 1097–1104.
41 Hernandez,N. (1993) *Genes Dev.*, **7**, 1291–1308.
42 Zwieb,C. (1985) *Nucleic Acids Res.*, **13**, 6105–6124.

NOTE ADDED IN PROOF

The sequence of the *Arabidopsis* 7SL-1 gene has also been reported by Shimomura *et al.* [*Plant Cell Physiol.* **34**, 633–637 (1993)].