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Characterization of the U3 and U6 snRNA genes from wheat: U3 snRNA genes in monocot plants are transcribed by RNA polymerase III

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Abstract

We have demonstrated recently that the genes encoding the U3 small nuclear RNA (snRNA) in dicot plants are transcribed by RNA polymerase III (pol III), and not RNA polymerase II (pol II) as in all other organisms studied to date. The U3 gene was the first example of a gene transcribed by different polymerases in different organisms. Based on phylogenetic arguments we proposed that a polymerase specificity change of the U3 snRNA gene promoter occurred during plant evolution. To map such an event we are examining the U3 gene polymerase specificity in other plant species. We report here the characterization of a U3 gene from wheat, a monocot plant. This gene contains the conserved promoter elements, USE and TATA, in a pol III-specific spacing seen also in a wheat U6 snRNA gene characterized in this report. Both the U3 and the U6 genes possess typical pol III termination signals but lack the *cis* element, responsible for 3′-end formation, found in all plant pol II-specific snRNA genes. In addition, expression of the U3 gene in transfected maize protoplasts is less sensitive to α-amanitin than a pol II-transcribed U2 gene. Based on these data we conclude that the wheat U3 gene is transcribed by pol III. This observation suggests that the postulated RNA polymerase specificity switch of the U3 gene took place prior to the divergence of angiosperm plants into monocots and dicots.

Introduction

Transcription of the genes encoding small nuclear RNAs (snRNAs), the RNA components of small nuclear ribonucleoprotein particles (snRNPs), has many features which distinguish it from the transcription of other gene classes (reviewed in [3, 4, 18]). Although some of the genes for the abundant spliceosomal U-snRNAs are transcribed by pol II (U1, U2, U4 and U5 snRNA genes) and some (U6 snRNA genes) by pol III, their promoters are strikingly similar. At the same time, the promoters of these genes differ considerably from the promoters of other pol II- and pol III- transcribed genes. The plant snRNA gene promoters are clearly distinct from their yeast and mammalian counterparts, in both the essential *cis* elements and the determinants of RNA polymerase specificity (reviewed in [3, 4, 18]). All higher-plant U-snRNA genes characterized to

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X63065 and X63066.

date contain two upstream promoter elements which are essential and sufficient for transcription initiation *in vivo*: upstream sequence element (USE, consensus TCCCACATCG) and the TATA box (reviewed in [4]). The USE is a plant snRNA gene-specific element; it is not found in other gene classes [25]. The TATA box is structurally and functionally indistinguishable from the TATA elements of mRNA encoding genes [25]. In pol II-specific U-snRNA genes the USE and TATA box are centred approximately four DNA helical turns apart while in pol III-specific U6 genes they are positioned one helical turn closer together (reviewed in [4]). Experiments carried out with the U2 and U6 genes of *Arabidopsis* have demonstrated that it is this difference in promoter element spacing that determines the choice of RNA polymerase [27].

The U3 snRNA is the RNA component of the nucleolar U3 snRNP which functions in an early step of pre-ribosomal RNA processing [11]. In lower eukaryotes, such as yeast and *Dictyostelium discoideum*, and vertebrates, the U3 snRNA is synthesized by pol II. It contains the pol II-specific 2,2,7-trimethylguanosine (m_3G) cap and its synthesis is inhibited by α-amanitin and dichlorobenzidazole riboside (DBR) in a manner expected for pol II transcripts (reviewed in [3, 4]). In marked contrast, the U3 genes in dicotyledonous plants were recently proved to be transcribed by pol III and not by pol II [13]. The plant U3 gene polymerase specificity assignment was based upon the observations that synthesis of plant U3 snRNA is resistant to α-amanitin and terminates in a stretch of T residues, a typical pol III termination signal, and that plant U3 snRNA contains a 5′-terminal cap different from the methylguanosine caps characteristic of the pol II transcripts. Furthermore, the USE and TATA promoter element spacing is pol III-specific in U3 genes [13], and we have shown that it is possible to convert the U3 gene into a functional pol II gene by increasing the spacing between the promoter elements and inserting the pol II-specific 3′-end formation signal downstream of the coding region [13].

The similarities between pol II and pol III U-snRNA gene promoters in plants and the demonstration that RNA polymerase specificity of the U3 gene has changed during evolution offered additional support to the notion that pol II and pol III enzymes evolved from a common ancestor [16], as has been proposed also for pol II- and pol III-specific U-snRNA gene promoters [3, 4, 18, 20]. In addition, these results suggest that one mechanism of promoter diversification during the evolution of eukaryotes involved alterations in the spacing of promoter regulatory elements [13, 27]. Plant U-snRNA gene promoters closely resemble eubacterial promoters, which also contain two upstream elements, the −35 box and the −10 TATA-like sequence. Appropriate spacing between these two elements is crucial for optimal promoter function [22].

Phylogenetic trees derived for eukaryotes suggest that *D. discoideum* and probably yeast branched off prior to the divergence of eukaryotes into green plants and metazoa [6, 8]. Since the U3 genes in *D. discoideum*, yeast and metazoa are all pol II-specific, it is likely that a U3 gene promoter specificity change, from pol II to pol III, occurred during the evolution of green plants [13]. The proposal that the primordial U3 gene was pol III-specific would require two independent pol III to pol II conversion events, one taking place in lower eukaryotic and one in metazoan evolution. We are interested in mapping the proposed U3 gene polymerase specificity change in plant evolution and in monitoring the conservation of the plant snRNA gene promoter elements, the configuration of which was shown to be essential in establishing RNA polymerase specificity of the promoter. To date only one monocot U-snRNA gene, a pol II-transcribed U2 gene from maize, has been studied [2]. We report here the characterization of wheat U3 and U6 genes. Both genes contain the conversed USE and TATA box promoter elements positioned approximately three DNA helical turns apart, a feature characteristic of pol III-specific genes of dicot plants. We provide evidence that the wheat U3 gene is indeed transcribed by pol III. These results are consistent with a U3 gene promoter specificity switch occurring prior to the

divergence of higher plants into monocots and dicots.

Materials and methods

Isolation and sequencing of the wheat U6 gene

Unless otherwise stated, all techniques for DNA manipulation were as described [23]. Wheat (*Triticum aestivum*) total genomic DNA was isolated from four-week-old seedlings as described [15]. This DNA was digested to completion with *Eco* RI and then cloned into the vector λ-ZAPII (Stratagene) and packaged into phage particles according to the manufacturer's protocol. 50000 recombinant plaques were screened with a 5′-^{32}P-labelled oligonucleotide, complementary to nt 1 to 35 of the *Arabidopsis thaliana* U6 snRNA [26], as probe. Hybridization was performed in a solution containing 6 × SSC, 10 mM NaH_2PO_4, 1% SDS, 1 mM EDTA, 5 × Denhardt's solution, 0.1 mg/ml salmon sperm DNA for 36 h at 42 °C. Washes were performed three times in 6 × SSC, 1% SDS at 50 °C for 30 min. A single hybridizing recombinant plaque was purified and cloned into pBSt(+) (Stratagene) to give the clone pWU6. This clone was partially sequenced using Sequenase (USB) protocols. The wheat U6 subclone, pWU6△, used for antisense probe preparation, was constructed by cloning a 363 bp long *Sal* I-*Rsa* I fragment, containing 124 bp of upstream sequences and 137 bp of downstream sequences, into pBSt(+) cut with *Eco* RV and *Sal* I.

Isolation and sequencing of the wheat U3 gene

A wheat (*T. aestivum*) size-fractionated partial *Sau* 3A genomic DNA library in the vector λ-EMBL3 (obtained from Clontech) was screened using a 230 bp *Eco* RI-*Hin*d III DNA fragment, encoding the *A. thaliana* U3C gene [15], ^{32}P-labelled using the random priming method, as a probe. Hybridization conditions were as above and filters were washed twice in 1 × SSC, 1% SDS and twice in 0.5 × SSC, 1% SDS at 65 °C for 30 min. Five positive phage were isolated after screening 125000 recombinant phage, representing approximately one genomic equivalent. One λ-EMBL3 recombinant, encoding a single wheat U3 gene, was subjected to restriction analysis and subcloned as a 3.5 kb *Eco* RI fragment and a 1.3 kb *Eco* RI-*Pst* I subfragment into pBSt(+) to produce pWU3-3.5K and pWU3-1.3K, respectively. pWU3-1.3K was sequenced using Sequenase (USB) protocols. pWU3-64△, the construct used for antisense probe preparation, was obtained using PCR amplification, as described [15], of sequences between −64 and +233 of the pWU3-1.3K (Fig. 3B). The oligonucleotide primers used introduced a *Bam* HI site upstream of the USE sequence and a *Xba* I site downstream of the termination signal which were used for cloning into pBSt(+).

Plant tissues and preparation of RNAs

RNA was isolated from wheat seedlings (*T. aestivum*), cultured cells and wheat germ, as described [5]. Wheat (*Triticum monococcum*) cells, kindly provided by Dr H. Lörz, University of Hamburg, were cultured as described [14].

Determination of the wheat U3 snRNA 5′-terminus using primer extension

Primer extension was carried out as described [15]. An 18-mer oligonucleotide, complementary to the wheat U3 snRNA sequence at positions 27–47 (Fig. 3B), was 5′-^{32}P-labelled using polynucleotide kinase and used as a primer for reverse transcription of 5 μg of RNA isolated from wheat germ as reported [15]. Primer extension products were then analyzed by 8 M urea/polyacrylamide gel electrophoresis alongside pWU3-1.3K DNA sequencing reactions carried out with the same primer.

Transient expression and RNase A/T_1 mapping

Protoplasts (6×10^5), prepared from leaves of *Nicotiana plumbaginifolia* or maize seedlings, were

transfected by the polyethylene glycol method [5], using 10 μg of plasmid per transfection. RNA was isolated after 24 h as described [5]. Incubations with α-amanitin were carried out as described [13].

Probes for RNase protection analysis were made from appropriately linearized plasmids pWU3-64Δ, pZMU2 (clone U2-27 [2]), or pWU6Δ, by *in vitro* transcription with T3, SP6 or T7 polymerase using [α-^{32}P]CTP, sp. act.800 Ci/mmol, as a label. Approximately 1×10^5 cpm of gel-purified antisense probe was hybridized to 5 μg of RNA isolated from transfected protoplasts or wheat tissues. Samples were subjected to RNase A/T_1 digestion and protected RNAs were analysed by polyacrylamide gel electrophoresis. Protected fragments were quantitated by scintillation counting [5].

Results

Isolation and sequence of a wheat U6 gene

The only characterized monocot U-snRNA gene, a pol II-transcribed U2 gene from maize, contains in its promoter region the USE and TATA elements centred approximately four DNA helical turns apart, similarly as in the pol II-specific U-snRNA genes from dicot plants [2]. To establish that the difference between pol II- and pol III-specific promoters in monocot plant snRNA genes is the same as in dicots, i.e. that they differ in promoter element spacing, we have cloned a wheat U6 gene. The U6 gene in all organisms studied to date, including dicot plants, is transcribed by pol III [3, 4, 18, 20, 26]. A ^{32}P-labelled oligonucleotide complementary to nucleotides 1–35 of the *A. thaliana* U6 snRNA [26], was used as a probe to isolate a U6 gene from a wheat genomic library. The sequence of the gene is shown in Fig. 1. The upstream region of the wheat U6 gene contains two conserved promoter elements, USE and TATA, and their spacing is the same as in pol III-specific dicot snRNA genes [13, 15, 26]. A stretch of T residues, known to act as a pol III termination signal [1], is positioned downstream of the coding region. The high conservation of the U6 RNA in different organisms allowed the probable 5′ and 3′ termini to be assigned by comparison to other plant U6 snRNA sequences [7] (Fig. 1). The wheat U6 gene has a potential to encode a 102 nt long RNA which displays 97–98% and 70–87% homology to plant and metazoan U6 snRNA sequences respectively [7, 26]. The potential RNA encoded by the wheat

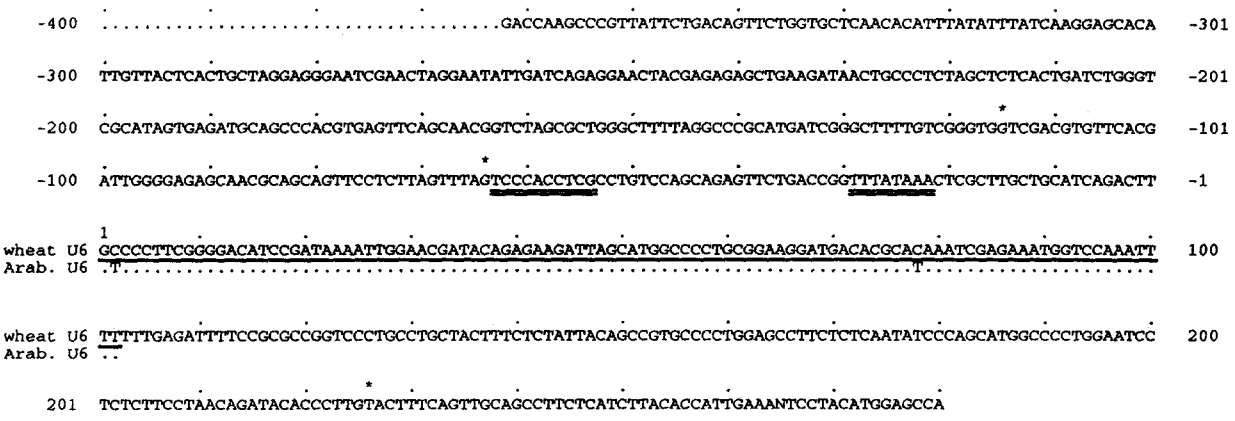


Fig. 1. Sequence of the cloned wheat U6 gene and alignment of the wheat U6 coding region with the *A. thaliana* U6 snRNA sequence [26]. Sequence identity is indicated with dots. The wheat U6 coding region is underlined. Conserved promoter elements, USE and TATA box, are double-underlined. Limits of the sequences retained in the pWU6Δ construct, used for antisense probe synthesis, are indicated with asterisks.

gene differs at two positions from *A. thaliana* U6 RNA (Fig. 1). One nucleotide substitution, $C_{79} \rightarrow T_{79}$, is also found in U6 snRNAs of other plants [7]. The second, $C_2 \rightarrow T_2$, is only present in the wheat sequence; it could potentially destabilize the 5′-terminal stem-loop which constitutes part of the signal required for U6 RNA capping in mammalian cells [24].

Transcripts corresponding in sequence to the wheat U6 gene are detected in wheat tissues but not in transfected plant protoplasts

In order to analyse the expression of the wheat U6 gene, a ^{32}P-labelled antisense RNA probe specific for the cloned wheat U6 gene was hybridized to RNA isolated from wheat tissues or from transfected protoplasts and subjected to RNase A/T_1 protection analysis. Transcripts corresponding in sequence to the isolated U6 gene were detected in RNA preparations obtained from cultured wheat cells and wheat germ but not in wheat seedling RNA (Fig. 2). Transcripts of the cloned wheat U6 gene were also not detected in transfected maize or *N. plumbaginifolia* protoplasts (data not shown). Therefore, it is possible that the cloned U6 gene is a pseudogene or its transcript is unstable due to the observed sequence difference within the 5′-terminal stem-loop (see above). It cannot be excluded that U6 RNA transcripts detected in wheat cells and wheat germ originate from other gene(s) encoding different U6 RNAs which are indistinguishable from the cloned wheat U6 sequence due to the limitations of the RNase A/T_1 protection assay. Alternatively, it is possible that expression of the cloned U6 gene is under some developmental control and the gene is not transcribed in transfected plant protoplasts.

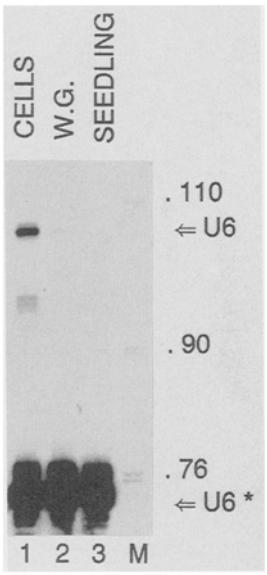
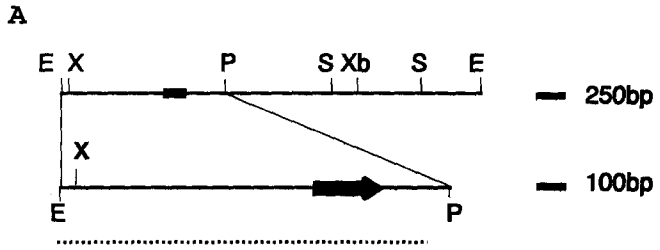


Fig. 2. Detection of U6 snRNAs corresponding in sequence with the cloned wheat U6 gene in wheat tissues. Lanes 1, 2 and 3, RNase A/T_1 mapping of cultured wheat cell (Cells), wheat germ (WG) or wheat seedling (Seedling) total RNA. U6 = wheat U6 gene-specific protected fragments; U6* = partially protected fragments of other U6 snRNA sequence variants which are likely to have related sequences. Lane M, size markers in bp (*Hpa* II-digested pBR322).

Isolation and sequence of a wheat U3 gene

Using a ^{32}P-labelled DNA fragment containing an *A. thaliana* U3 gene [15] as probe, a wheat U3 gene was isolated from a wheat genomic DNA library constructed in λ-EMBL3. A 1.3 kb *Eco* RI-*Pst* I subfragment containing the U3 gene was subcloned and partially sequenced (Fig. 3A). The gene contains two upstream promoter elements, USE and TATA, at the pol III-specific spacing seen in dicot snRNA genes and in the wheat U6 gene described above. Consistent with transcription by pol III, the coding region of the gene is devoid of clusters of T residues longer than three nucleotides. However, a long stretch of T residues is positioned eight nucleotides downstream of the coding region. The 5′ terminus of the wheat U3 RNA was determined by primer extension of an oligonucleotide annealed to wheat germ RNA (Fig. 4). The 3′ terminus of the U3 snRNA (see Fig. 3B) was assigned by sequence comparison with other plant U3 snRNAs: tomato, *A. thaliana* and broad bean U3 snRNAs share 12 out of 13 terminal nucleotides with the wheat U3 snRNA ([7, 12, 15], C. Marshallsay, unpublished data).



B

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-900  .....GAATTCATCCTCACGTTCAACACCACATAGGGGGCATACCTCAGTATCCTCGAGGTG  -801
-800  CCGTGAAGCCTTGTTGGCCCAAGTAGCAAGTGAATTTGTGACAACCCTTCATGCATATACGGTAGTTTTATTTATTTTACTCTCTACATTGTTCTCATGA  -701
-700  TATTCATCCATGCAGCCTCTCTTTCCTGGGGAAAATTCAGTTGATCAACTGGTTGAGATTATCAAGGTGAAATCTTGTAGTATGTACGACATTTGGAACT  -601
-600  GCTTTCTCCTGCGACATGCATTCTTTACAATGACATGTTCAGCCATAGTACTCCAAGAAGGGAAGAGACCAAGTGCATGAATCCAAACCACACGGAGTTC  -501
-500  AAATTCCCACAGATTAAGGCTCGTCCGTCGCACAAGGTAATGTGTGAATATTATATCTGTCGTGCAAAATTGCCTGGCCTGCACAATTGCTGTTATAGTT  -401
-400  GGCGGCAGGGAGAGTTTTAACATTGACTAGCGTGCTGATAATTTGTGAGAAATAATAATTGACAAGTAGATACTGACATTTGAGAAGAGCTTCTGAACTG  -301
-300  TTATTAGTAACAAAAATGGAAAGCTGATGCACGGAAAAAGGAAAGAAAAAGCCATACTTTTTTTTAGGTAGGAAAAGAAAAAGCCATACGAGACTGATGT  -201
-200  CTCTCAGATGGGCCGGGATCTGTCTATCTAGCAGGCAGCAGCCCTACCAACCTCACGGGCCAGCAATTACGAGTCCTTCTAAAACGTCCCGCCGAGGGCG  -101
-100  CGTGGCCGTGCTGTGCAGCAGCACGTCTAACATTAGTCCCACCTCGCCAGTTTACAGGGAGCAGAACCAGCTTATAAGCGGAGGCGCGGCACCAAGAAGC  -1

   1  ACGACCTTACTTGAACAGGATCTGTTCTATAGGCTCGTACCGCTGCATCCTTTACCAATAAGGAGGCAAGCACTTCAGTCTGGTTGATGCATCTGACCTC  100
 101  TGGGCCAGAGCGTGATTAACGGCCAGGATTCCCTTATGGGGGCAATCCACGGCTGTAGAGGATCGTTCCTGCCTTGCTCATGCTTGTGCAGGGTGGCCCA  200
 201  GAGGTTGTCTGACAGA

                    CTACAACATTTTTTTTGTCCTTCTGTTTTTTTAGTCAGTCTCTTTTTTCAGAAGTACAACATCTTTTTTTTGTCCTTCTGTTTT  300
 301  TTTAGTCAGTCTCTTTTTTCAGAAGTACTCTATGATGTATCGTTCTGGGAAATGTCTGTCTGTCTACAACCCATAATCTATATTTGCAATCACACATCTA  400
 401  ATATCTCTGTGACAAGACAGCCGAACAAA
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Fig. 3. A. Restriction map of the 3.5 kb and 1.3 kb wheat U3 genomic clones. E, X, P, Xb, S, are *Eco* RI, *Xho* I, *Pst* I, *Xba* I, and *Spe* I respectively. The U3 gene is represented by an arrow. The sequenced region is indicated with a dotted line. B. Sequence of the isolated wheat U3 gene. The coding region is underlined. The USE, TATA box and pol III termination signal are double-underlined. Limits of the sequences retained in pWU3-64△, which was used for making the antisense probe for RNase protection, are indicated with asterisks.

Expression of the wheat U3 gene in transfected plant protoplasts and wheat tissues

Functionality of the cloned wheat U3 gene was established by two methods.

1. Expression of the wheat U3 genomic clone, pWU3-1.3K, was detected in transfected maize (Fig. 5, lanes 4 and 5) or *N. plumbaginifolia* (data not shown) protoplasts. Protected fragments, corresponding in length to both the mature U3 snRNA and a putative U3 snRNA precursor were detected by RNase A/T_1 protection analysis. The U3 snRNA precursor would arise from pol III transcription termination occurring at a stretch of

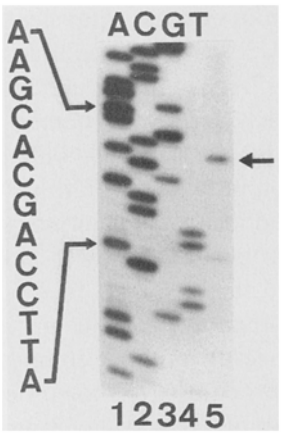


Fig. 4. Primer extension analysis of the wheat U3 snRNA 5′ terminus. The products of reverse transcription of wheat germ RNA are in lane 5. The sequencing reactions, with pWU3-1.3K DNA as a template, are shown in lanes 1–4.

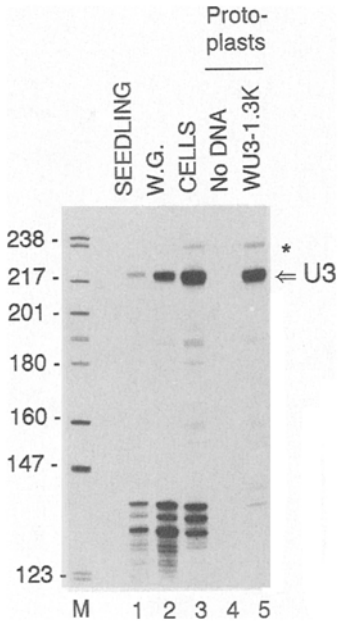


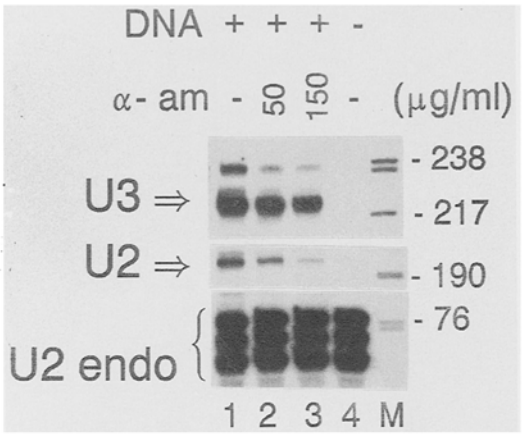
Fig. 5. Expression of the wheat U3 gene in wheat tissues and in transfected protoplasts. RNase A/T_1 mapping with a wheat U3-specific antisense probe was used to detect U3 gene transcripts in wheat tissues or maize protoplasts transfected with the wheat U3 gene. Lanes 1–3, mapping of wheat seedling (Seedling), wheat germ (WG) or cultured cells (Cells) total RNA. Lanes 4 and 5, mapping of RNAs isolated from non-transfected control (No DNA) and pWU3-1.3K-transfected (WU3-1.3K) maize protoplasts. The gene-specific protected fragments are indicated with an arrow (mature U3 snRNA) and asterisk (putative U3 snRNA precursor). Lane M, size markers.

T residues positioned 8 bp downstream of the mature U3 RNA 3′ end. In the tomato U3 gene the T-rich sequence is also present 8 bp downstream of the gene and the formation of a U3 snRNA precursor terminated at this region has been directly demonstrated [13].

2. Transcripts of the cloned wheat U3 gene also were detected in RNA preparations from wheat germ, wheat seedlings and cultured wheat cells using the same RNase A/T_1 protection assay (Fig. 5, lanes 1–3).

Effects of α-amanitin on wheat U3 and maize U2 gene expression in transfected maize protoplasts

The wheat U3 gene has the same promoter element spacing as other pol III-transcribed plant snRNA genes, and the size of the putative U3 snRNA precursor is consistent with pol III transcription terminating at a downstream stretch of T residues. To obtain additional evidence that the wheat U3 gene is pol III-specific, we studied the effect of α-amanitin on transcription. A concentration of 50–100 μg of the toxin per ml of protoplast culture medium is known to inhibit transcription by pol II but to have little effect on transcription by pol III [13, 17, 26]. Maize protoplasts where cotransfected with the wheat U3 gene and a maize U2 gene. Relative expression levels of these genes, in the presence of increasing concentrations of the toxin, were obtained by quantitation of the gene-specific protected fragments in a RNase A/T_1 protection assay (Fig. 6). Transcription of the U2 gene was sensitive to even low (50 μg/ml) toxin concentrations, whereas wheat U3 gene expression, and also pol III-specific *A. thaliana* U6 gene expression (data not shown), were largely resistant to threefold higher toxin concentrations (Fig. 6). The observed small inhibition of wheat U3 gene or *A. thaliana* U6 gene (data not shown) expression in transfected plant protoplasts is similar to the effect of α-amanitin noted previously with other pol III-specific genes [13, 17, 26].



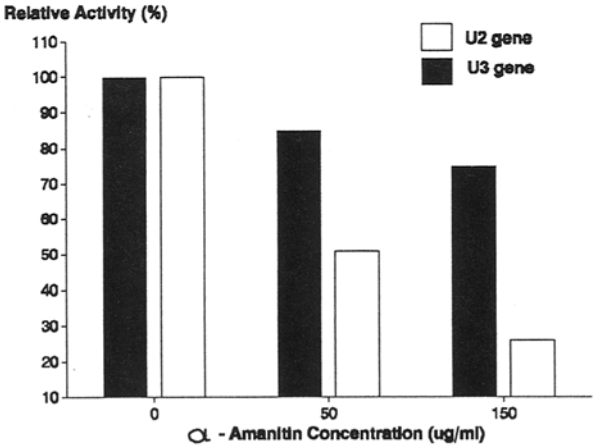


Fig. 6. Effect of α-amanitin on transcription of the wheat U3 gene. Maize protoplasts were co-transfected with equimolar amounts of wheat U3 and maize U2 genes. A (top). RNase A/T_1 mapping with U3 (upper panel) or U2 probes (lower panel). Protoplasts were incubated in the absence (lane 1) or in the presence (lanes 2–3) of α-amanitin. Lane M, size markers. The positions of U3- and U2-specific protected fragments are indicated with arrows. Fragments protected by the endogenous maize U2 snRNA (U2 endo) are shown to demonstrate similar RNA recoveries in the same RNase A/T_1 mapping experiment. B (bottom). Quantitation of the experiment shown in A. The experiment was performed two times with a similar result.

Wheat U3 snRNA secondary structure and phylogenetic comparisons

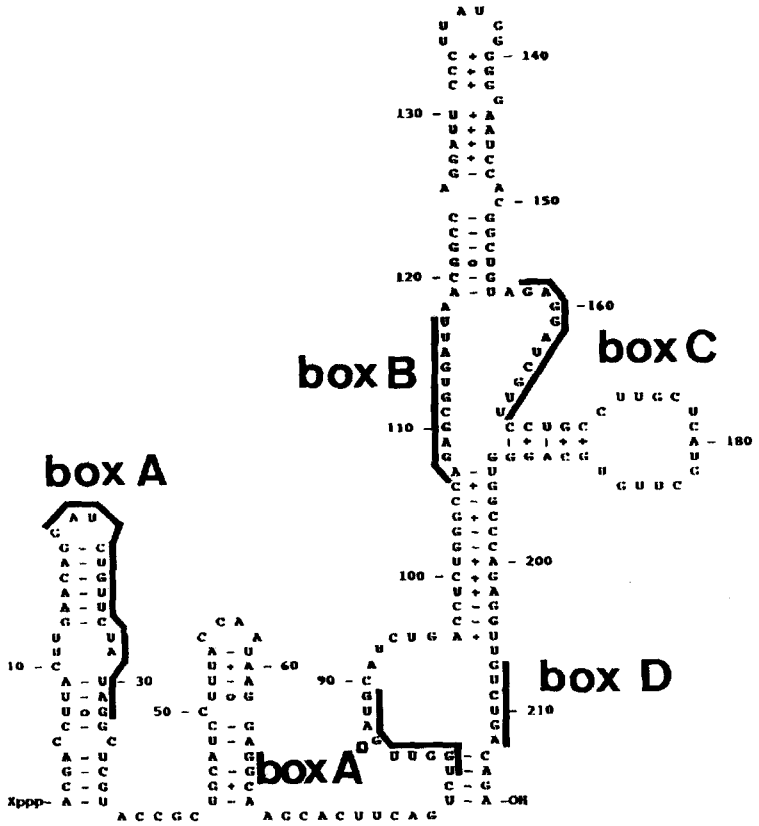
The wheat U3 snRNA is 216 nt long and shows 69–72% sequence similarity to U3 snRNA from other plants, but less than 50% similarity with either the human or yeast equivalents [7]. A possible wheat U3 snRNA secondary structure is shown in Fig. 7A. This structure, although based upon the models derived for human and *Xenopus laevis* U3 snRNAs [10, 19], differs at the 5′-terminal region in that two hairpin structures are present instead of a single hairpin; similar structures were proposed for lower eukaryotic and dicot plant U3 snRNAs [12, 15, 21]. Features of the major 3′-terminal hairpin are substantiated by the presence of multiple compensatory base changes when compared to other plant U3 snRNA sequences (e.g., 18 compensatory base changes when compared to the tomato U3 snRNA) ([12, 15], C. Marshallsay, unpublished data).

The wheat U3 snRNA sequence contains the sequence motifs A, B, C, and D which are found in all reported U3 snRNAs [7, 9]. In addition, all plant U3 snRNAs share identical 5′-terminal 42 nucleotides and possess an additional motif, referred to as A°, located between nucleotides 81 and 89. Motif A° is also conserved in yeasts, *D. discoideum* and *Bombyx mori* [7] (Fig. 7B), while in vertebrates a related sequence is conserved at this location (Fig. 7B). Motif A° co-localizes with a region of the metazoan U3 snRNA presumed to be relatively exposed in the intact snRNP as based on the accessibility of the U3 snRNA to chemical modification agents and to RNase cleavage [10, 11, 19]. It is possible that this motif plays a role in a U3 snRNA/pre-rRNA interaction [11].

Discussion

We have isolated wheat U3 and U6 snRNA genes and studied their expression in wheat tissues and transfected maize and *N. plumbaginifolia* protoplasts. RNA products corresponding to both genes were identified in wheat tissues by RNase A/T1 mapping, and the wheat U3 gene, but not the U6 gene, was shown to be actively transcribed in transfected protoplasts. The apparent inactivity of the wheat U6 gene may be the result of sequence alterations within the gene or, alterna-

A



B

All plants (9 sequences)	TGGTTGATG
S.cerevisiae (2 sequences)	TGGTTGATG
S.pombe	CGGATGATG
D.discoideum	AGGATGATG
B.mori	ACGATGAGG
X.laevis	ACGAGGAAG
X.borealis	ACGAGGAAG
Human	ACGAGGAAG
Rat (2 sequences)	ACGAGGAGG
Mouse	ACGAGGACG
Vertebrate motif A°cons.	ACGAGGANG
Plant/lower eukaryote motif A° cons.	YGGATGATG

Fig. 7. Secondary structure and conserved motifs of the wheat U3 snRNA. A. A possible secondary structure model for the wheat U3 snRNA. Compensatory base changes as compared to the tomato U3 snRNA sequence [12] are indicated as +. The conserved boxes A, A°, B, C, D are indicated. The plant U3 snRNA cap structure is marked with X. B. Conservation of motif A° in reported U3 snRNA sequences [7, 12, 15, and C. Marshallsay, unpublished data]. Cons. = consensus.

tively, reflect regulated gene expression (reviewed in [3, 4]).

The 5′-flanking regions of the wheat U3 and U6 genes contain the conserved USE and TATA elements identified previously in snRNA genes of dicot plants (reviewed in [4]; see Fig. 8). The

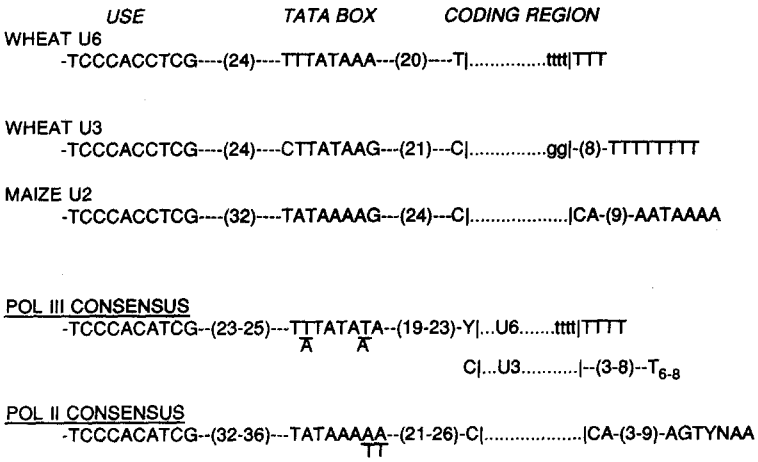


Fig. 8. Structure of plant snRNA gene promoters and 3′-adjacent regions. Pol II and pol III consensus is based on the sequences of 21 pol II-transcribed snRNA genes and 8 pol III-transcribed genes ([4, 13], and C. Marshallsay, unpublished data). Structure of the maize U2 snRNA gene was as described [2]. The length of the spacers separating different transcription signals are indicated. Vertical lines delineate borders of the coding regions. Nucleotides of the coding region are shown as lower-case letters.

USE in both genes differs at one position, A_7 to C_7, relative to the USE consensus, TCCCACATCG, established for dicot plants [4]. The USE and TATA elements, in the wheat U6 and U3 genes, and also in a maize U3 snRNA gene (D. Leader; J.W.S. Brown, personal communication), are spaced approximately three DNA helical turns apart, which is one DNA helical turn closer together than in a pol II-transcribed maize U2 snRNA gene [2]. In three pol II-specific U5 snRNA genes of maize characterized recently (D. Leader, S. Connelly, R. Waugh, W. Filipowicz, J.W.S. Brown, manuscript in preparation) the spacing between the elements is similar to that found in maize U2 gene. These observations suggest that, as in dicot snRNA genes [13, 27], promoter element spacing is the primary determinant of polymerase specificity in monocot snRNA genes.

Apart from the pol III-characteristic promoter element configuration, the following data support the conclusion that synthesis of U3 snRNA in wheat is catalysed by pol III:

1. Transcription of the wheat U3 gene, in transfected maize protoplasts, is largely resistant to α-amanitin, in contrast to the expression of a co-transfected pol II-specific U2 gene.
2. The wheat U3 gene lacks stretches of T residues, potential pol III terminators, within the coding regions. This property is characteristic of U3 snRNA genes in plants, but not in other organisms [7].
3. The length of a putative U3 precursor, seen in RNase A/T_1 protection assays using RNAs from transfected protoplasts or wheat tissues (Fig. 5), is consistent with U3 gene transcription termination occurring at the first T-stretch after the coding region, as described for tomato U3 gene transcription [13].
4. The 3′-end formation signal found in all pol II-specific plant snRNA genes is absent.
5. Wheat U3 snRNAs, similarly to U3 snRNAs from dicot plants [12, 13], is not precipitable with anti-m_3G cap antibodies (our unpublished results).

Phylogenetic data (see Introduction) have suggested that the U3 gene polymerase specificity conversion was from pol II to pol III and that it occurred after the divergence of metazoa and green plants, which took place approximately one billion years ago [6, 8]. The observation that U3 genes in both the monocot (wheat) and dicot (tomato, tobacco, *A. thaliana*) sub-divisions of angiosperm plants are transcribed by pol III in-

dicates that the proposed U3 gene promoter specificity switch must have occurred prior to the divergence of monocots and dicots, hence more than approximately 200 million years ago [28].

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