Plant Molecular Biology **30**: 255–268, 1996.

Structure and characterization of a putative drought-inducible H1 histone gene

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Received 7 June 1995; accepted in revised form 10 October 1995

Key words: abscisic acid, diurnal cycling, *Lycopersicon* spp.

Abstract

A drought- and abscisic acid (ABA)-inducible gene, *His1*, was isolated from *Lycopersicon pennellii*, a drought-resistant relative of cultivated tomato, and the gene structure was defined experimentally. The nucleotide sequence of *His1* predicts a protein of 202 amino acid residues, with a significant sequence homology to plant H1 histones. Consensus sequences for both H1 histone-specific promoter elements as well as an ABA-responsive element were identified in the 5′-flanking region of *His1*. Transcripts of this gene accumulate in leaf tissue in response to drought in three tomato species including cultivated tomato (*L. esculentum*), *L. pennellii*, and *L. chilense*, as well as in tobacco. Transcripts for *His1* are constitutively expressed in roots; transcript abundances in tomato root tips were equivalent to transcript abundances in more mature regions of the seedling root. The accumulation in leaves of transcripts for *His1* preceded visible symptoms of drought stress in the plants. Transcript accumulation was detected in both drought-sensitive and drought-resistant species at similar leaf water potentials, Ψ_w −1.3 to −1.4 MPa.

Introduction

One of the responses of plants to abiotic stresses such as drought is an alteration in gene expression as evidenced by changes in abundances of specific mRNAs and newly synthesized proteins [reviewed in 8, 8, 36]. In addition to the induction of transcription of specific genes, drought stress also results in the repression of transcription of specific genes, such as *rbcS* and *cab* [4]. While some of the steps regulating the induction of transcription, often via the hormone ABA, are well characterized, virtually nothing is known about the equally important repression of transcription of genes.

In eukaryotic cells, transcription takes place in an environment in which the genome is packaged into hierarchial structures of chromatin. The fundamental repeating unit of chromatin is the nucleosome, each consisting of a histone core par-

The nucleotide sequence data reported will appear in the GenBank Nucleotide Sequence Database under the accession number U01890.

ticle around which two 80 bp turns of DNA are wrapped [2]. The core particle consists of two copies each of histones H2A, H2B, H3 and H4. These nucleosome cores are linked together with H1 histone. H1 histones from animals and plants have a tripartite structure that is characterized by an evolutionarily conserved central globular domain, about 80 amino acids in length [18]. All higher eukaryotes have several H1 subtypes which differ in amino acid sequence and in the ability to mediate the formation of higher order chromatin structure [14]. For example, in *Xenopus* there are three variants of linker histone: an early embryonic form, called B4, a normal somatic histone H1, and histone H1°, which is only expressed in terminally differentiated cells [40]. The association of histone H1 with nucleosome arrays is believed to be primarily responsible for the formation of higher orders of chromatin compaction, thus inactivating the transcription of associated genes. Croston *et al.* [15] purified a general repressor of transcription by polymerase II which was identified as histone H1. There may be some selectivity at the DNA sequence level for the H1 histone-DNA interaction [21]. Further, the observation that there are developmentally controlled H1 histone variants suggests important roles of different H1 subtypes in plant and animal development [14].

There are only a few examples of DNA and or amino acid sequences for plant H1 histones. In *Arabidopsis*, the H1 histone gene family appears to have three members, two of which have been cloned and sequenced [18]. Outside of the central globular domain, these two H1 proteins do not share homology with one another or other H1 proteins. A genomic sequence for a tomato H1 histone has been reported [24] as well as several cDNA sequences: tobacco [37] pea [17] and maize [32].

Several groups have characterized alterations in gene expression in response to drought in tomato (*Lycopersicon esculentum*) and other members of the genus [7, 10, 13, 23, 31]. In particular we have been interested in comparing the expression of drought-responsive sequences between tomato species which differ in drought resistance. There are at least two members of the *Lycopersicon* genus which are drought-resistant, *L. pennellii* Corr. and *L. chilense* Dun. [33]. Alterations in the abundance of four drought- and abscisic acid (ABA)-inducible cDNAs have been compared between tomato and *L. pennellii*. These studies demonstrated a similar but not identical pattern of expression for all four genes in both species [25]. One of these cDNAs represented a gene which was also differentially regulated in a developmental manner: transcripts accumulated in response to ABA or drought in leaf tissue, but were expressed constitutively in roots. Here we describe the structure of this gene, *His1*, and present evidence that the gene product is an H1 histone variant.

Materials and methods

Plant material, drought-stress treatments and plant water status

Lycopersicon esculentum Mill. cv. UC82, *L. pennellii* Corr. LA716, and *L. chilense* Dun. LA1959 were grown from seed in the greenhouse. Unless otherwise described, plants were kept well watered, and fertilized with Miracle Gro. For the isolation of RNA from different sections of roots, seeds were germinated in Seed-Pack Growth Pouches (Vaughan's Seed Co., Downers Grove, IL). When the primary roots were ca. 8 cm long, the root tips were collected (1–2 cm) as well as the most basal 1 cm section of root. Plants to be used in a drought treatment were grown in a sandy soil mix (1:1 v/v) in the greenhouse with daily watering. A drought cycle was initiated by withholding water until the plants demonstrated visible symptoms of stress, wilting in the case of cultivated tomato and *L. pennellii*, and severe leaf curling in the case of *L. chilense*. Leaf water potential (Ψ_W) was determined on size-matched plants of equivalent maturity using a Scholander pressure bomb. In any one drought cycle, three to four plants of each accession or cultivar were measured at each time point in the cycle. Morning readings were taken between 09:00 and 10:00,

afternoon readings between 16:30 and 18:00. Leaf tissues were collected for RNA isolation at the same time that the plant water status was determined during the time course experiments. Root tissues were destructively harvested for RNA isolation from either well watered plants or from visibly wilted plants.

Nucleic acid isolation and blot hybridization

Plant tissues to be extracted for RNA were collected directly into liquid N_2. Total RNA and genomic DNA were prepared as described [25]; poly(A)$^+$ mRNA was purified from total RNA preparations using oligo(dT)-cellulose (Promega). RNA blots were prepared after electrophoresis of ethidium bromide-stained RNA samples in 1% high-EEO agarose (Sigma) gels run in 10 mM sodium phosphate pH 6.8. Equal RNA loads in different samples on each gel were confirmed following electrophoresis by inspection of the ribosomal NRA bands under UV light. Prior to transfer the gels were soaked in 7% formaldehyde for 10 min. RNA was transferred from the gels to nylon membranes using 10 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate pH 7.0).

DNA sequencing and analysis

The region of the phage clone WS-1 containing the hybridization signal to pLE20 was subcloned into the plasmid vector pBluescript KS (Stratagene). Overlapping deletions of the inserts were generated by using ExoIII/Mungbean Nuclease System (Stratagene). Both strands of the set of deletion clones were sequenced using the Sequenase 2.0 DNA sequencing kit (US Biochemicals). DNA and the predicted amino acid sequences were searched against DNA and protein databases using the Blast algorithm [1]. The secondary structure predictions were deduced from the derived amino acid sequence of *His1* using the nnpredict mail server [26].

Primer extension analysis

The transcript initiation point was mapped using primer extension analysis as described by Ausubel *et al.* [3]. An oligonucleotide primer, complementary to nucleotides 69–100, was 5′ end-labeled with [γ^{32}P]dATP and T4 polynucleotide kinase. The labeled oligonucleotide was annealed at 40 °C to 5 μg poly(A)$^+$ mRNA, isolated from leaves of drought-stressed *L. pennellii*, and extended with AMV reverse transcriptase (RT) (Promega). Products from the reaction were resolved by electrophoresis through DNA sequencing gels, 5% LongRanger, along with the sequencing reaction generated using the same primer.

S1 nuclease mapping

S1 analysis was carried out to map the 3′ splicing junction of the intron as described by Ausubel *et al.* [3]. An oligonucleotide primer, complementary to nucleotides 463–487, was labeled at its 5′ end with [γ-^{32}P]dATP using T4 polynucleotide kinase. The radiolabeled oligonucleotide was annealed to the denatured recombinant plasmid, pBL1.3. pBL1.3 contains the genomic DNA corresponding to the entire coding sequence, from nucleotide −3 to 1382 (Fig. 1). The sample was annealed at 40 °C for 15 min, and elongated by Klenow fragment. The elongated products were digested with SpeI (restriction site at nucleotide −215, Fig. 1), the DNA was ethanol-precipitated and loaded onto alkaline low-melting agarose gels [3]. After electrophoresis, the end-labeled single-stranded probe was purified by phenol extraction. The probe was annealed at 30 °C for 18 h to 10 μg poly(A)$^+$ mRNA isolated from leaves of drought-stressed *L. pennellii*. The RNA-DNA hybrids were then subjected to S1 digestion at 30 °C for 1 h. The products were precipitated, resuspended and resolved on 5% LongRanger sequencing gels next to sequencing reactions from the same primer for size determination.

Reverse transcriptase-PCR (RT-PCR)

An RT-PCR assay was used to map the 5′ splice site of the intron. A primer complementary to nucleotides 734–754 was used to prime first-strand cDNA synthesis using AMV RT and 5 μg poly(A)$^+$ mRNA isolated from leaves of drought-stressed *L. pennellii*. PCR amplification of this cDNA was performed using primers corresponding to nucleotides 58–78 and complementary to nucleotides 502–525. The genomic clone, pBL1.3 was used as a control template in a separate PCR amplification. PCR conditions were: final volume 50 μl, 0.1 μg each primer, 2.5 U *Taq* polymerase, 0.2 mM each dNTP, 3 mM MgCl, 50 mM KCl, 20 mM Tris-Cl pH 8.4, 30 cycles of 95 °C/1 min, 63 °C/1 min, 72 °C/1 min. The sizes of the products of the PCR reaction were determined following electrophoresis in a 2% agarose gel and visualization of ethidium bromide-stained bands.

Results

Structure of a drought- and ABA-inducible gene in L. pennellii*: a putative H1 histone gene*

Genomic Southern analyses of tomato and *L. pennellii* using the tomato cDNA pLE20 as a probe demonstrated that this sequence is present in only one copy in either species [25]. pLE20 was isolated from a library of tomato leaf transcripts based on differential screening for drought- and ABA-induced elevations of transcript levels [7, 8]. A genomic library was constructed from *Sau* 3A partially digested nuclear DNA from *L. pennellii* cloned into the *Bam* HI site in the phage vector EMBL-3. One and a half genomic equivalents (3.3×10^5 pfu) were plated out and lifts of the library were probed with the cDNA clone pLE20. The single positive plaque was purified to homogeneity and characterized. The 15 kb genomic fragment was mapped with restriction endonucleases and the region containing the gene located. The gene identified by hybridization with the tomato cDNA probe will be referred to as *His1* [19]. About 2 kb of genomic DNA sequence was determined (Fig. 1) (GenBank accession number U01890). A sequence alignment between the sequence of the partial cDNA, pLE20 (Bray, Z11842) and *His1* demonstrated that the region of homology extended over the entire pLE20 sequence, suggesting that *His1* is the counterpart of the tomato gene. A search of DNA and protein databases with the *His1* sequence was performed. Most of the identified entries with one or more homologous regions were H1 histones, the best matches were with H1 histone sequences from plants [6, 17, 18, 32].

The transcript initiation site was confirmed experimentally by primer extension. The major product from this reaction was 98 nucleotides in length with two minor products, 96 and 101 nucleotides (data not shown). By comparing the product with the sequencing ladder derived from the same primer, the start of transcription was mapped to the A and numbered position 1 in Fig. 1. Potential TATA and CAAT boxes were located at positions −32 and −80, respectively. The first ATG downstream of position 1 was assumed to be the translational initiation codon. The cDNA sequence at the 3′ end of pLE20 was aligned with *His1* to identify the 3′ end of the mature transcript at nucleotide 982 (Fig. 2). The 5′- and 3′-untranslated regions of the *His1* transcripts are 39 and 206 nucleotides long respectively.

An intron was expected in *His1* based on the Blastx homology searches. S1 nuclease was used to map the 3′ end of the intron. Two bands were observed corresponding to the A at position 330 and to the G at position 328 (data not shown). These results demonstrated there is an intron in this gene with the 3′ splicing junction in the region predicted. The most probable placement for the 3′ splice site is the G/A at position 331/332, based on the consensus 5′GT--AG3′.

RT-PCR was used to map the 5′ end of the intron. Both first strand cDNA and genomic DNA were used as templates in separate PCR reactions. Only one amplification product was observed for each template, a 468 bp fragment in the genomic sample and a 338 bp fragment in the cDNA sample (data not shown). These results

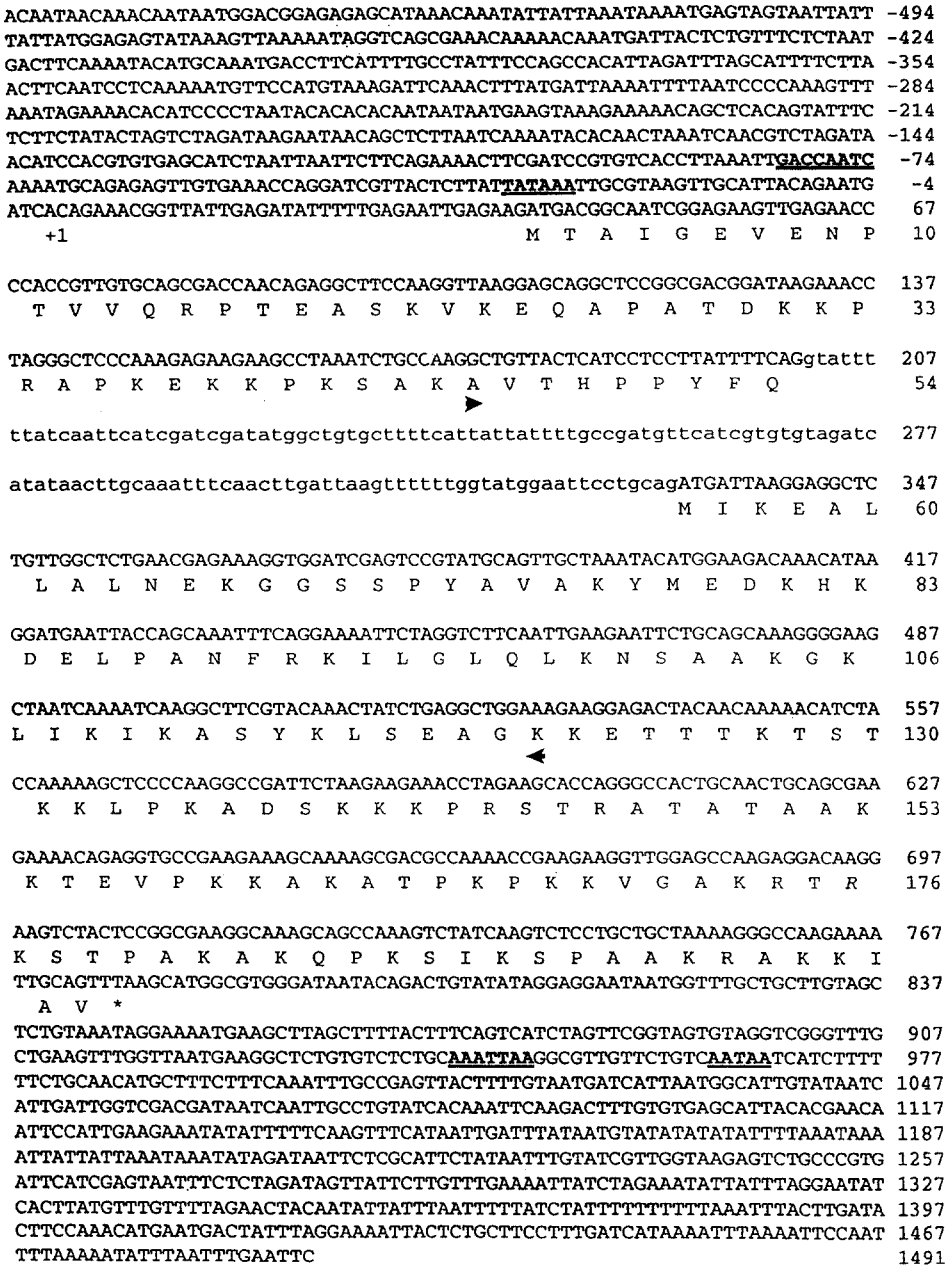

Fig. 1. Nucleotide and deduced amino acid sequence of *His1*. The deduced amino acid sequence is shown below the nucleotide sequence. The transcription start site is indicated as + 1, the TATA box and CAAT box are in bold and underlined. Two putative polyadenylation signals are in bold and underlined. The conserved central globular domain of the gene product is delineated by arrowheads.

demonstrated the presence of an intron of the predicted size, 130 bp long. Based on the size of the intron and the mapped position of the 3′ end of the intron, the 5′ end of the intron was determined to be nucleotide 210.

Possible regulatory elements in 5′- and 3′-flanking regions of His1

His1 transcripts have been shown to accumulate in leaves in response to ABA [25]. A putative

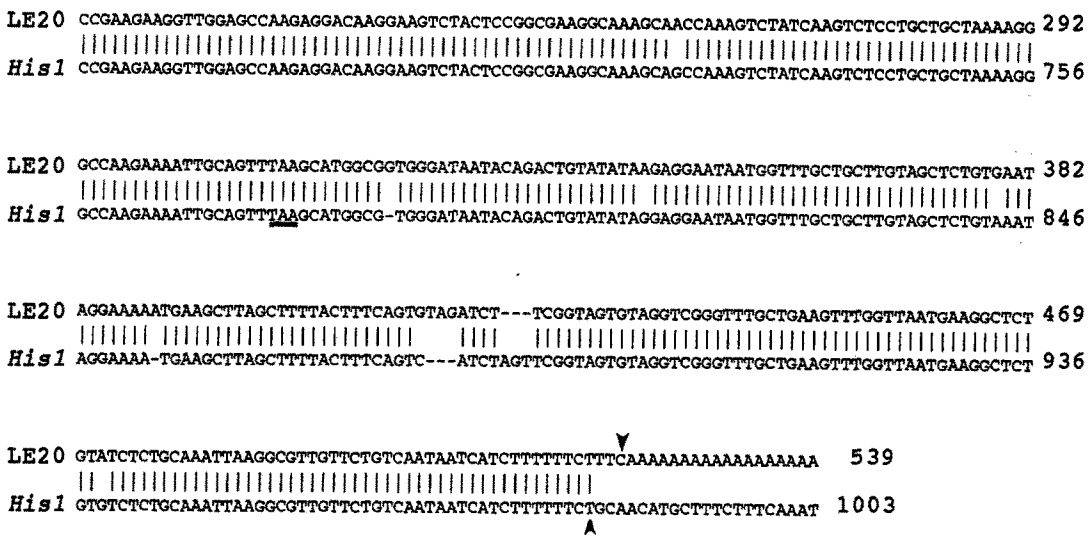

Fig. 2. Alignment of 3′ UTRs of *His1* and pLE20. The DNA sequence between 665 and 1003 in *His1* and the sequence between 202 and 539 of the tomato cDNA clone pLE20 were aligned. The position of the translational stop codon is underlined, the postions of transcript termination are marked with arrowheads for pLE20 and *His1*.

ABRE (ABA-responsive element), CCACGTGT (consensus sequence CCACGTGG [30]), is present at a conserved position, −140 bp from the transcription initiation site in the 5′-flanking region. A coupling element (CE1-like) [35] was also found at position −92 (Table 1). Both of the components necessary for ABA responsiveness, a G-box type ABRE and a CE1 element, were identified in the DNA sequence of the promoter region of *His1*.

Sequences of several regulatory elements common to histone genes were found at conserved positions in the 5′ flank of *His1* (Table 1): a histone family-specific element, HEX box [29]; a CGC box found in nearly all plant histone genes [18]; a sequence, GATCC, usually found in histone gene promoters about 10 bp upstream from the TATA box [22]; and a ubiquitous AC box found in both plant and animal H1 histone promoters [16]. A sequence similar to the AS-1 element, described by Benfey *et al.* [5] to confer root-specific expression, was also found in the distal 5′ flank of *His1*. This element was contained within the HEX box. The AS-1 element has also been identified in a root-specific ABA-responsive gene in *Arabidopsis* [42].

Table 1. Identification of consensus sequences for 5′ *cis*-elements in *His1*. The map location in nucleotides relative to transcription initiation is given within parentheses.

cis element	Consensus sequence (position)	*His1* sequence (position)
ABRE [30]	CCACGTGG	CCACGTGT (−140)
CE1-like [35]	CACC	CACC (−92)
AS-1 [5]	TGACGTCA	TGACTTCA (−424)
HEX box [29]	GACTTC	GACTTC (−423)
CGC box [18]	GATCCGCG (−70 to −180)	GATCCGTG (−100)
GATCC [22]	GATCC (−42)	GATCG (−48)
AC box [16]	AAACACA (−200)	AAACACA (−276)

There are two striking features about the 3′-flanking region of *His1*. First is the high degree of interspecific conservation in the sequence of the 3′-untranslated region (UTR) (Fig. 2). For at least 200 nucleotides beyond the translation stop signal, the sequence of the tomato cDNA clone is virtually identical (95% sequence identity), to the 3′ UTR of the *L. pennellii* form of this gene. This degree of sequence conservation is similar to that within the coding region of *His1* and pLE20. A second significant characteristic of the 3′ flank of *His1* is an A + T-rich region of 500 bp in length

just beyond the 3′ UTR (Fig. 1). This region is 76% A + T and contains four repeats of the motif ATATATTT. These features are typical of nuclear scaffold attachment regions [12, 28].

Predicted characteristics of the His1 *gene product H1-D*

DNA sequence predicts that *His1* codes for a H1 histone protein composed of 202 amino acid residues with the calculated molecular weight of 22 kDa and a predicted pI of 10.6. We will identify the gene product of *His1* as H1-D, (drought-responsive H1 histone). Typical of H1 histones, H1-D is very lysine-rich (23%), has a high lysine/arginine ratio (41:8) and is extremely hydrophilic. Also typical of H1 histones is the absence of tryptophan or cysteine. There are nine consensus sites for phosphorylation by protein kinase C, S/T-X-R/K [41] and two sites for phosphorylation by cAMP- or cGMP-dependent protein kinases, R/K-R/K-X-S/T [20] on the deduced polypeptide. The protein encoded by *His1* is essentially the same as the protein product predicted for the tomato cDNA clone pLE20; only one amino acid residue difference was found, serine versus alanine at position 149. One other tomato H1 histone has been described, tomH1B [24]. TomH1B, a cDNA isolated in a screen for meiotin-related genes, is quite different from *His1*. This gene is only 70% identical to *His* 1 and only over a 95 nucleotide stretch in the conserved central globular domain.

The amino acid sequence alignment of H1-D with the central globular domain of several plant H1 histones is presented in Fig. 3A. In the central globular domain, the amino acid sequence of H1-D is between 50 and 60% identical with other plant H1 histones. If conservative substitutions are allowed, and the sequence of H1-D is compared with a composite amino acid sequence of the central globular domain of all other plant H1 histones (plus signs in Fig. 3A), then H1-D ap-

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A
                 ++++++     ++++++  +     ++ +++ +++      +  +++++      ++ ++++++
  H1-D        AVTHPPYFQ    MIKEALLALN    EKGGSSPYAV    AKYMEDKHKD    ELPANFRKIL
tomH1B        TPTHPPYFE    MIKDAIVTLK    ERTGSSQHAI    TKFIEEKQKS    -LPSNFKKLL
 tobH1        PPTHPSYFE    MIKDAIVTLK    DKTGSSQHAI    TKFIEDKQKN    -LPSNFRKLL
   pea        PASHPTYEE    MIKDAIVSLK    EKNGSSQYAI    AKFIEEKQKQ    -LPANFKKLL
AralH1        VSSHPTYEE    MIKDAIVTLK    ERTGSSQYAI    QKFIEEKRKE    -LPPTFRKLL
Ara2H1        TSSHPTYEE    MIKDAIVTLK    ERTGSSQYAI    QKFIEEKHKS    -LPPTFRKLL
 maize        SPTHLPYAE    MVSEAITSLK    ERTGSSSYAI    AKFVEDKHKA    KLPPNFRKLL
 wheat          AHPSYAE    MVSEAIAALK    ERSGSSTIAI    GKFIEDKHEA    HLPANFRKIL

                +++   +     +++++ ++++     ++  + +      %I           position
  H1-D        GLQLKNSAAK    GKLIKIKASY    KLSEAGK                    #46-121
tomH1B        LTQLKKFVAS    EKLVKVKNSY    KLPSGSK          52%       #53-127
 tobH1        LVQLKKLVAS    GKLVKVKSSY    KLPAARS          55%       #56-130
   pea        LQNLKKNVAS    GKLIKVKGSF    KLSAAAK          56%       #59-133
AralH1        LLNLKRLVAS    GKLVKVKASF    KLPSASA          49%
Ara2H1        LVNLKRLVAS    EKLVKVKASF    KIPSARS          48%       #59-133
 maize        NVQLKKLVAG    GKLTKVKNSY    KLSSATK          59%
 wheat        LTQIKKLVAA    GKLTKVKGSY    KLAKAPA          53%

B
  H1-D   AVTHPPYFQMIKEALLALNEKGGSSPYAVAKYMEDKHKDELP
   GH5   SASHPTYSEMIAAAIRAELSRGGSSRQSIQKYIKSHYKVGHN
                    I                  II

  H1-D   ANFR-KILGLQLKNSAAKGKLILILASYKLSEAGK
   GH5   ADLQIKLSIRRLLAAGVLKQTKGVGASGSFRLAKSDK
               III
```

Fig. 3. Amino acid alignments of the central globular domain of H1-D. **A.** Alignment of H1-D and H1 histones from tomato [24], tobacco [37], pea [17], *Arabidopsis* [18], maize [32] and wheat [6]. Percent identical amino acid were compared between each entry and H1-D and are reported as %I. Conserved amino acids in H1-D and at least two other plant H1s are marked with a plus sign; gaps introduced to maximize alignments are indicated by dashes. The boundary of the central globular domain in the H1 histones is listed as amino acid position. **B.** Alignment of H1-D with GH5 [11]. The mapped positions of the α-helices, I, II, and III in GH5 are underlined; the predicted positions of three α-helices in H1-D are underlined.

proaches 75% similarity to other plant H1 histones.

The secondary structure of the central globular domain of H1-D was predicted using the nnpredict mail server. The results suggested that there are three α-helices in the central globular domain. The secondary structure of the central globular domain of chicken histone subtype GH5 was determined by NMR [11, 43]. Figure 3B indicates that the sizes and the relative positions of the three α-helices predicted in H1-D are similar to those of helix I, II and III in the chicken protein. Like helix I in the chicken H1 histone, helix I predicted in H1-D is rich in hydrophobic residues. The amino acid sequences of the central globular domain of H1-D and GH5 are not identical yet the secondary structure of this region of the two proteins appears to be conserved.

A significant difference between H1-D and other plant H1 histones examined so far is that H1-D is much smaller, 202 residues versus 250–280 residues [18, 24]. Both the central globular domain and the positively charged amino terminal domain of H1-D is similar in size to other plant proteins, 76 and 45 residues respectively. However, the carboxy terminal domain is shorter in H1-D, 81 residues in the *L. pennellii* protein versus 125–148 residues in other plant H1 histones [18]. As the carboxy terminal domain is also highly positively charged, this results in a decrease in the net charge of H1-D relative to other plant H1 histones. The average net charge based on the amino acid sequence of plant H1 histones is 57, the predicted net charge based on the amino acid sequence of H1-D is 39.

Drought-responsive putative H1 histone genes are common and expressed early in the drought response

Previously, we demonstrated that transcripts for *His1* accumulated in leaves in response to drought stress in cultivated tomato and the drought-resistant species *L. pennellii* [25]. To determine if this was a general response, *His1* transcript accumulation was measured in a second drought-resistant tomato species, *L. chilense* [33], as well as tobacco (Fig. 4). All three tomato species as well as tobacco showed an accumulation of transcripts for *His1* sequences in response to drought.

The two drought resistant tomato species, *L. pennellii* and *L. chilense*, take a much longer time to demonstrate symptoms of drought stress than the relatively drought-sensitive cultivated tomato. In a previous study we demonstrated that the accumulation of transcripts for *His1* occurred before levels of other drought-responsive genes increased but at unknown leaf water potentials (Ψ_w) [25]. To compare the time course of *His1* drought induction in tomato and a drought-resistant tomato species, we needed to normalize the response. Ψ_w of tomato and *L. chilense* leaves was determined at the time of harvest for RNA isolation during a drought cycle. The transcript accumulations for three genes, *His1*, pLE16 and *rbcS* are shown in Fig. 5A as well as Ψ_w at each time point (Fig. 5B). Tomato plants started to appear wilted at 48 h ($\Psi_w = -1.6$ MPa) and were considered approaching permanent wilting point at 56 h ($\Psi_w = -2.0$ MPa). *L. chilense* plants started to appear drought-stressed at 224 h ($\Psi_w = -1.4$ MPa) and were considered drought-stressed at 248 h ($\Psi_w = -1.45$ MPa). Transcript accumulation for *His1* in cultivated tomato was maximal at 30 h corresponding to a Ψ_w of −1.4 MPa. A similar level of accumulation of *His1* transcripts was observed in *L. chilense* at 104 h corresponding to a Ψ_w of −1.35 MPa. This

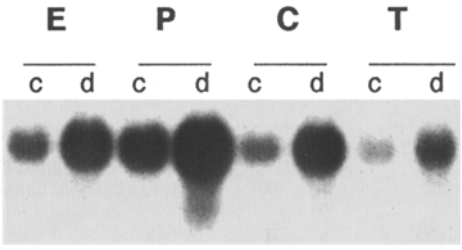

Fig. 4. Comparison of *His1* transcript accumulation in leaves of drought-stressed *L. esculentum* (E), *L. pennellii* (P), *L. chilense* (C) and *Nicotiana tabacum* (T). A northern blot was prepared containing 20 μg of total RNA isolated from leaves of control (c) or drought-stressed (d) plants. The blot was probed with oligolabelled *His1* and exposed to X-ray film. Equal abundances of ribosomal RNA were used to confirm equal RNA loads in each lane.

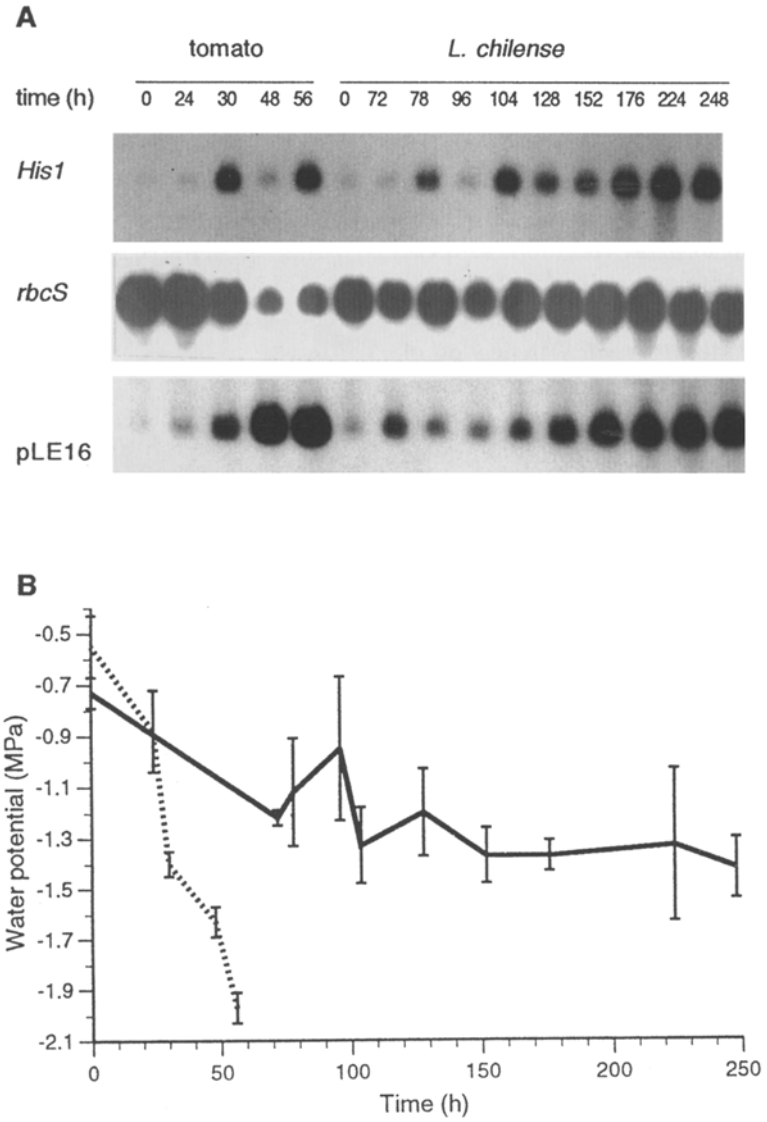

Fig. 5. Accumulation of *His1* and *rbcS* transcripts as a function of Ψ_w. **A.** Northern blots were prepared containing total RNA (20 μg) isolated from leaves of tomato or *L. chilense* LA1959 at the indicated times following the imposition of a drought cycle. Replicate blots were probed with oligolabeled *His1*, pLE16 or *rbcS*. Equal abundances of ribosomal RNA were used to confirm equal RNA loads in each lane. **B.** Ψ_w was determined on leaves immediately prior to RNA isolation in A; dotted line, tomato cv. UC82; solid line, *L. chilense* LA1959. Error bars represent the SD of readings from 3–4 different plants.

was prior to the appearance of drought-stress symptoms in either species. Transcript abundances for *His1* continued to increase throughout the drought cycle in *L. chilense*.

The transcript abundance of *His1* appears to fluctuate diurnally. All lanes in Fig. 5A had identical RNA loads as judged by the abundance of ribosomal RNA (data not shown) and good quality mRNA as judged by the hybridization pattern of pLE16 (see below). The time of day for time points 24–56 h was alternatively 09:30 and 16:30. So at 48 h (09:30), even with a Ψ_w of -1.6 MPa, tomato leaves did not have an appreciable accumulation of *His1* transcripts. The influence of Ψ_w on *His1* transcript accumulation can be seen easily in the *L. chilense* samples. With the exception

of samples collected at 72 h and 96 h (each at 09:30), all the other samples were collected in the late afternoon. There is a steady increase in the abundance of *His1* transcripts in these samples as the drought stress persists, time points 104 through 248 h. The slight reduction in transcript accumulation at 128 and 152 h versus 104 h is probably due to plant to plant variation, as these RNA samples were obtained randomly from different individuals undergoing the drought cycle.

Transcript abundances for *rbcS* in leaves of tomato and *L. chilense* during a drought cycle are also presented in Fig. 5A. In tomato, the levels of *rbcS* transcripts were decreased in RNA samples isolated from drought-stressed tomato leaves (Fig. 5A). While there was a general reduction in the abundance of *rbcS* transcripts in *L. chilense* leaves relative to tomato, the reduction in Ψ_W in *L. chilense* leaves did not appear to repress the transcription of *rbcS*. Similar results for repression of *rbcS* transcription in response to drought in cultivated tomato have been reported [4].

The pattern of transcript accumulation for pLE16 is included in Fig. 5A as a control on the quality of the RNA characterized in the northern blots. pLE16 encodes a drought- and ABA-inducible leaf specific gene with significant sequence homology to non-specific lipid transfer proteins [25, 31]. There is a low level of expression of this gene under non-stress conditions, and then a steady and continuous increase in transcript accumulation for pLE16 as the drought-stress progresses in both tomato and *L. chilense*. The reduction in *His1* and *rbcS* transcripts at 48 h is not due to poor quality mRNA but rather due to diurnal and drought effects on the specific transcription of *His1* and *rbcS* respectively.

His1 *expression in roots*

His1 is constitutively expressed in roots of tomato, *L. pennellii* [25] and *L. chilense* (data not shown). To test whether *His1* transcripts accumulated only in regions of rapid cell division or in both growing and mature regions of the root, RNA was isolated from two different regions of growing roots of tomato seedlings: the 1–2 cm section at the root tip, and a 1 cm section immediately below the hypocotyl. The abundance of *His1* transcripts in these RNAs was compared with RNAs isolated from the entire root of either control or drought-stressed tomato plants (Fig. 6). Transcripts for *His1* were present at similar levels in all four preparations of root RNA. Exactly the same pattern of *His1* expression was observed in root RNA preparations from *L. pennellii* (data not shown). The constitutive expression of *His1* in the root was therefore not based on the activity of the root tip, a region of the root with high mitotic activity, but due to a widespread expression of the gene in mature regions of the root as well.

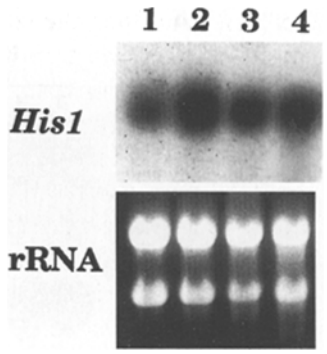

Fig. 6. Expression of *His1* in tomato roots. Total RNA was isolated from root tips (lane 1), or the most basal 1 cm section of the seedling root (lane 2) or from all of the roots of a mature control (lane 3) or drought stressed (lane 4) tomato plant. A northern blot was prepared using 15 μg RNA of each sample. The blot was then probed with oligolabeled *His1*. Equal loading of RNA was confirmed by inspection of the rRNA bands stained with ethidium bromide.

Discussion

A drought- and ABA-responsive gene, *His1*, was isolated from an *L. pennellii* genomic library; the gene was designated *His1* based on its deduced amino acid sequence. Since only a partial cDNA clone was available, the gene structure could not be determined simply by aligning the cDNA and genomic DNA sequences. Computer-aided analysis of the genomic sequence was used to predict

the structure of *His1*, and then the structure was confirmed experimentally. The transcription initiation site was mapped experimentally, and several classes of consensus sequences for 5′ *cis* elements were located (Table 1). The presence of a single intron in *His1* was also confirmed experimentally. To date, only three complete genomic clones for plant H1 histones have been described, one from *Arabidopsis* [18] and two from tomato species [this report, 24]. All three of these genes have introns and all are polyadenylated. The introns are all located at the same position in the central globular domain of the coding region. The derived amino acid sequence of *His1* predicts the gene product to be an H1 histone, and based on the pattern of expression, *His1* encodes an H1 histone subtype. The amino acid sequence and composition, the predicted domain structure, the predicted secondary structure of the central globular domain, and the presence of histone-specific promoter elements, all support the identification of *His1* as a gene for an H1 histone.

One especially intriguing feature about the sequence of *His1* is the very high conservation of DNA sequence in the 3′ UTRs of the *L. esculentum* and *L. pennellii* sources of the gene (Fig. 2). Two possible functions for this sequence can be proposed: the 3′ UTR is involved in a post-transcriptional regulation of *His1* expression or the 3′ UTR contains an open reading frame accessed following alternative splicing of *His1* transcripts. No suggestion of alternative splicing has been observed however in any of the dozens of northern hybridizations performed with various probes derived from *His1*. Gantt and Lenvik [18] described a highly conserved region in the 3′ UTR of two members of an H1 histone gene family in *Arabidopsis*. This region bears no sequence similarity to the conserved region in the two *His1* alleles.

H1s are the least conserved at the level of their primary sequence among the five major classes of histones, H1, H2A, H2B, H3 and H4. In many organisms there are several H1 subtypes differing in size, sequence, modification and ability to condense chromatin [14]. Despite differences in the amino and carboxy terminal domains, all H1 histones have a moderately conserved central globular domain. H1-D, the drought inducible H1 histone, shares a significant sequence similarity in this domain with other plant H1s (Fig. 3A). Furthermore, computer analysis suggested that the central globular domain of H1-D has three α-helices (Fig. 3B), which are similar in size and relative position as compared with those of chicken GH5 [11], in spite of differences in amino acid sequence.

Phosphorylation and dephosphorylation of lysine residues of H1 histone have been implicated in condensation and decondensation of eukaryotic chromatin [review 34]. Like all other H1s, H1-D has multiple consensus sites for phosphorylation [20, 41]. The functional significance of the shortened carboxy terminal domain, with its concomitant reduction in the net charge and number of phosphorylation sites in H1-D relative to other plant H1 histones is not known.

Several conserved histone family-specific and H1-specific promoter elements have been identified [29] and functionally tested [16]. Both histone family-specific and H1-specific *cis*-acting elements are present in the *His1* promoter (Table 1). However, the appearance of the H1-specific promoter element (AC box) in the *His1* promoter is intriguing. In an animal system, the AC box was demonstrated to have a role in cell-cycle-dependent transcription. Deletion of this element from a chicken H1 promoter or 4 bp substitution mutations in the same motif reduced the overall level of H1 transcription and specifically abolished S-phase activation [16]. If *His1* is not expressed in a cell-cycle-dependent manner why is such a conserved *cis*-acting element present in its promoter? The AC box found in most histone H1 promoters is very similar to the AC-rich sequence (consensus AAACACA) in the promoter of plant seed storage protein genes. This element has been shown to interact with a nuclear protein and drive the expression of seed storage protein genes during seed development [39].

There are two classes of histone genes in most eukaryotes. The expression of the first class is replication-dependent, i.e., expressing only in the S phase of cell cycle. These genes are the most

abundant in animals, and are characterized by a lack of introns and no polyadenylation of their transcripts. The expression of the second class of histone genes is replication-independent; these genes are expressed at low but quite constant levels throughout the cell cycle [29]. This second class of histone genes have introns and their transcripts are polyadenylated. *His1* was found in the poly(A)$^+$ mRNA fraction and a small intron was identified by S1 mapping and RT-PCR techniques. Therefore *His1* can be categorized in the second class of histones genes based on gene structure. While a dependence on S phase for transcription was not tested directly, transcript abundances for *His1* were equivalent in both the mature region of the root and in the root tip (Fig. 6). If *His1* encoded a cell-cycle-dependent H1, then transcript abundances should have been greater in the RNA from the root tip. We propose that *His1* encodes a cell-cycle-independent H1 histone subtype, constitutively expressed in roots and up-regulated in the aerial portions of the plant by drought or ABA.

The transcript abundance of *His1* appears to fluctuate diurnally in leaf tissue. A diurnal cycling of *His1* transcripts was suggested in our preliminary characterization of the expression of this gene in tomato and *L. pennellii* [25]. Thompson and Corlett [38] have also used *His1* cDNA sequences as probes and demonstrated a diurnal influence on the drought induction of *His1* transcripts. in tomato leaves. Transcripts for a tobacco H1 histone have also been observed to fluctuate diurnally [37]; The phase of minimal transcript accumulation for the tobacco gene was 06:00 to 10:00. The time point with minimal *His1* transcript accumulation in the two tomato species was also early in the morning 09:30.

Transcript accumulation for *His1* is ABA-inducible [25] as well as drought-inducible. Many but not all of the alterations in gene expression induced by drought can be mimicked by exogenous applications of ABA [8, 9]. The *cis* elements and corresponding *trans*-acting factors for ABA responsiveness have been described in detail [9, 30, 35]. Consensus sequences for the necessary *cis* elements in the 5′-proximal region of *His1* were identified (Table 1). Modeling the function for *His1* in the signal transduction pathway for the drought response needs to consider the ABA responsiveness of *His1*.

Formation of nucleosomes presents many impediments to the recognition of DNA by transcription factors [40]. The binding of histone H1 to linker DNA further prevents access to DNA. The association of histone H1 with nucleosome arrays is believed to be primarily responsible for the formation of higher orders of chromatin compaction, thus inactivating the transcription of associated genes [15, 27]. Further, the observation that there are developmentally controlled H1 histone variants suggests important roles of different H1 subtypes in plant and animal development [14]. A tobacco cDNA clone for H1 histone was isolated in a screen for DNA binding proteins [37]. The *cab* 5′ promoter region was used as the target DNA.

The interesting question is, how is an H1 histone involved in the response to drought. This presumes that the gene product of *His1* is in fact an H1 histone, and that the expression of *His1* in response to drought stress in leaves is positively adaptive. In tomato, as in other plants, gene expression is altered in response to drought [7]. Perhaps, *His1* is involved in the alteration of gene expression by changing chromatin structure. The repression of *rbcS* transcript accumulation in tomato leaves was preceded by accumulation of *His* 1 transcripts (Fig. 5). In contrast, *L. chilense* leaves demonstrated no reduction in *rbcS* transcript levels. We are interested in determining if the persistence of *rbcS* transcription in *L. chilense* during drought stress is a component of its drought resistance and if this is related to allelic differences in *His1*. The role of H1 histones in the regulation of gene expression is far from understood. Since expression of *His1* is developmentally regulated and responsive to environmental signals this histone gene will be an excellent system to study the function of H1 histone both in plant development and in plant responses to abiotic stresses.

Acknowledgements

We would like to thank Beth Bray for providing us with the cDNA clone, pLE20, and preliminary sequence information; Owen White and Marcela Treviño for the isolation of the genomic clone from the library, and Sue Fender for the preparation of RNA. This work was supported in part by the New Mexico Agricultural Experiment Station and USDA Southwest Consortium on Plant Genetics and Water Resources.

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