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Cell-Specific Expression of Plant Histone H2A Genes

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Histone H2A is a component of eukaryotic chromatin whose expression has not been studied in plants. We isolated and characterized a tomato and a pea cDNA encoding histone H2A. We found that in tomato H2A is encoded by a small gene family and that both the pea and the tomato mRNAs are polyadenylated. Tomato H2A has 82% amino acid residue identity to pea H2A, 83% to wheat, and 65% to human and yeast H2A. Plant H2As differ from fungal and animal H2As in their amino-terminal and carboxy-terminal regions. Carboxy-terminal plant H2A regions contain the motif SPKK, a peptide implicated in binding of A/T-rich DNA regions. By using RNA gel blot analysis, we determined that the steady-state mRNA level of these genes was abundant in apices and early developing fruit and very low in mature tissues. In situ RNA hybridization showed strong spatial regulation because the mRNA was abundant in some cells and not detectable in others. In tomato shoot tips, H2A-expressing cells were distributed irregularly in or near meristems. In tomato or pea root tips, expressing cells were concentrated near the apex, and their distribution was consistent with that expected of cycling cells. Other H2A transcripts were found in nondividing cortical cells that are known to undergo endoduplication during the late maturation phase of primary development.

INTRODUCTION

Histones are a major component of eukaryotic chromatin in association with DNA and nonhistone chromosomal proteins. With the exception of a few specialized cell types, such as mammalian erythrocytes and vascular plant sieve tube members, chromatin components are present in all living cells. The synthesis of chromatin is not a continuous process but occurs in the late G1 and S phases of the cell cycle or during DNA endoduplication. The core histones H2A, H2B, H3, and H4 are among the most abundant and highly conserved proteins in eukaryotic organisms (Stein et al., 1984). These proteins and their mRNAs are usually synthesized in a cell cycle-dependent fashion at the beginning of S phase (Old and Woodland, 1984; Schumperli, 1986; Marzluff and Pandey, 1988). This mode of synthesis is necessary to allow nucleosome formation on the duplicated DNA. Some histones are expressed in a non-cell cycle-dependent manner. For example, chicken histone $H2A_F$ transcription is not coupled to DNA synthesis, and a variant form of H2A (hv1) in *Tetrahymena* is transcribed during all stages of the cell cycle (White and Gorovsky, 1988; Dalton et al., 1989). This mode of synthesis is characteristic of replacement histone genes, which, in response to natural histone turnover, are expressed at a low constitutive level outside the S phase.

Histone H3 and H4 genes have been characterized in several plants (Tabata et al., 1983, 1984; Chaubet et al., 1987; Gigot, 1988; Wu et al., 1988). Raghavan and Olmedilla (1989) localized the expression of H3 in rice embryos and germinating seedlings, reporting that it was absent in the shoot apex, leaf primordia, and root quiescent center but present in the scutellum and in other tissues. The amino acid sequence of wheat histone H2A (Rodrigues et al., 1985) and the cDNA sequence of parsley H2A (Spiker et al., 1990) have been determined. Thomas and Padayatty (1983) reported that the H2A and H2B genes of rice were closely linked and transcribed in the same direction. However, the H2A and H2B genes were not characterized. Thus, there is information neither on these genes nor on the temporal and spatial pattern of accumulation of their mRNA in plants. In fact, expression of H2A and other histones proposes interesting hypotheses regarding how the plant cells accommodate the acute need for histones during chromatin synthesis. For example, H2A mRNA accumulation could be cell cycle dependent, and, therefore, only certain cells would be expressing H2A at a given time. In contrast, H2A mRNA may be present throughout the cell cycle and its translation could be regulated. In this case, the mRNA would be present uniformly in all cells. As a tissue reaches maturity,

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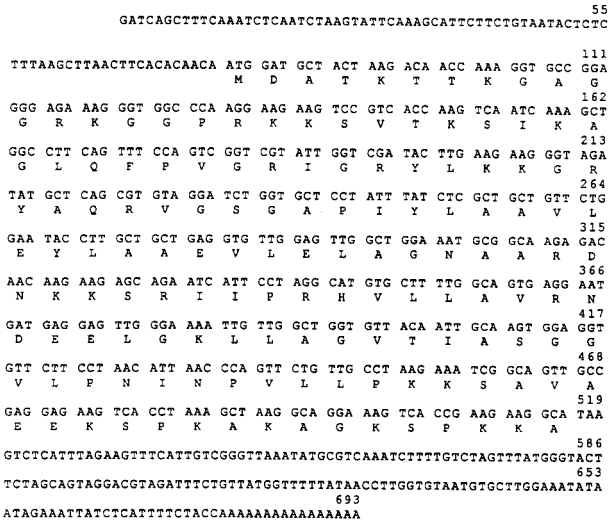

Figure 1. Sequence Analysis of the Tomato H2A-1 cDNA Clone.

The nucleotide sequence of the tomato H2A-1 cDNA described in Results is shown. The deduced amino acid sequence is given in single-letter code.

DNA synthesis subsides and the need for histone synthesis should be very low and limited to protein turnover. We would expect the mRNA level of cell cycle-dependent histones to be very low in these cells.

To address these hypotheses, we characterized the expression of a tomato and a pea cDNA encoding H2A. We report that histone H2A mRNA is differentially expressed during development and that it exhibits a distinct cell-specific pattern of expression.

RESULTS

Isolation and Characterization of a Histone H2A cDNA

We constructed a cDNA library from poly(A^+) mRNA isolated from tomato shoot tips and screened it with cDNA probes made from shoot tip, root, and mature leaf mRNA. This screen identified several cDNAs that were preferentially expressed in the shoot and root tips. Some of these cDNA clones were sequenced, and the putative polypeptides were compared with known protein sequences in the Swiss Protein Data Bank. Clone pCGN 7108 encoded a protein very similar to plant and animal histone H2A. We concluded that we had isolated a tomato cDNA encoding histone H2A and called this locus H2A-1. The DNA sequence of this gene has been deposited in the EMBL and GenBank data banks and was given the accession number X54109. To obtain a homologous H2A clone for in situ hybridization to pea root apex (see below), we also isolated the H2A gene of pea by screening a cDNA library prepared from pea root apex with the tomato H2A probe (E. Tanimoto, unpublished results).

Figure 1 shows the sequence of the 693-bp tomato H2A cDNA. A reading frame of 438 bp encoding a putative protein of 146 amino acids starts from the first ATG at position +79 to a stop codon at position +517. This putative protein, named tomato histone H2A-1, has a molecular mass of 15,000 and in Figure 2 is compared with the H2A of pea, parsley, wheat, yeast, and humans. The DNA sequence for pea is not shown here but was deposited in GenBank and given the accession number M64838. These proteins share a basic "core region" typical of all H2A histones, but they vary in the amino-terminal and carboxy-terminal regions. Plant H2As form a distinct group: they have conserved amino-terminal and carboxy-terminal regions. In addition, the carboxy termini of plant H2As are the longest of any described (Wells and McBride, 1989), and they contain the "SPKK" motif, a peptide sequence that binds the minor groove of A/T-rich double-stranded DNA (Churchill and Suzuki, 1989). Wheat H2A differs from tomato, pea, and parsley H2As by several additional amino acid residues on the amino-terminal end (Figure 2); however, because the wheat H2A sequence was obtained by amino acid sequencing of purified histone, this difference may be due to post-translational processing. The carboxy terminus of wheat H2A has 5 additional amino acids, making it the longest of the plant carboxy-terminal

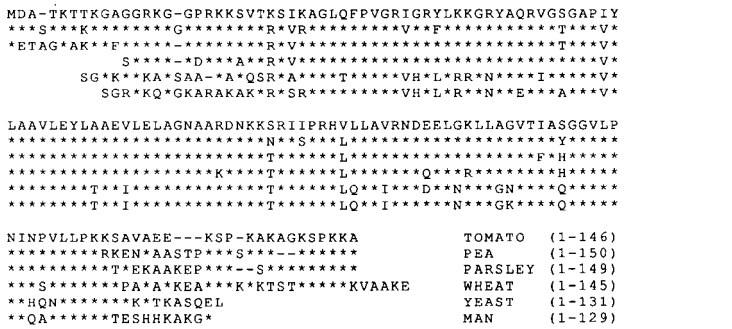

Figure 2. Amino Acid Sequence Comparison of H2A-1 with Other Histone H2As.

The figure compares the amino acid sequences of tomato and pea H2As with several other histone H2As including parsley (Spiker et al., 1990), wheat (Rodrigues et al., 1985), yeast H2A-1 (Choe et al., 1982), and human H2A 1-1 (Hayashi et al., 1980). Sequences are aligned to maximize identities. Hyphens designate absent residues. Asterisks designate identities to the tomato sequence in the top line. For an inclusive comparison and alignment of other H2A sequences, see Wells and McBride (1989).

regions. Tomato H2A has 82% amino acid residue identity to pea H2A, 83% to wheat, and 65% to human and yeast H2A. The pea protein is slightly closer to a generic H2A consensus in having 85% identity to wheat, 68% to yeast, and 70% to human H2A. The presence in the tomato cDNA of the entire putative coding region and of 78 additional nucleotides suggests that this clone is full length or nearly full length. We confirmed that it was a full-length clone by primer extension and RNA sequencing (data not shown). It was transcribed in a polyadenylated mRNA because sequencing revealed an A/T-rich region resembling a polyadenylation consensus signal and a polyadenylated tract 20 bases to its 3′ (Figure 1).

Figure 3 shows a DNA gel blot analysis of tomato genomic DNA. Using tomato H2A-1 as probe under low- or moderately low-stringency conditions (see Methods for details), we saw one predominant band and three to four minor bands in each digest (Figure 3, lanes 2 to 5). Under highly stringent conditions, we saw only one intense band that corresponded to the major band in the low-stringency hybridization (data not shown). Copy reconstruction standards suggested the presence of one to two copies per genome (Figure 3, lanes 6 to 9).

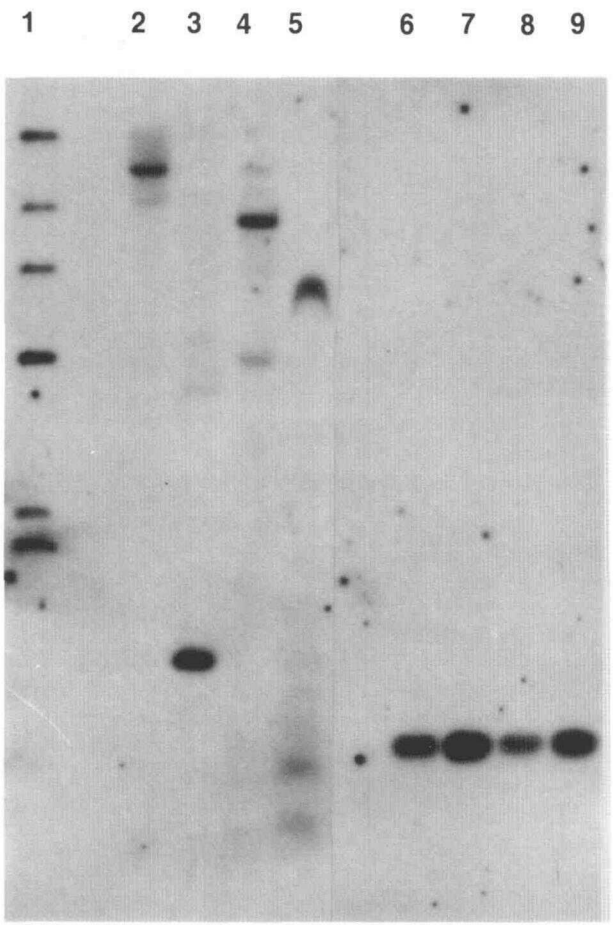

Figure 3. DNA Gel Blot Analysis of Tomato DNA Probed with H2A-1 cDNA.

Lane 1 contains λ HindIII molecular weight standards 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kb. Lanes 2 through 5 contain 10 μg of tomato DNA digested with BamHI (lane 2), HindIII (lane 3), BclI (lane 4), or TaqI (lane 5). Lanes 6 through 9 contain gene copy number reconstructions at 1.5 copies per haploid genome (lane 6), 3 copies (lane 7), 1 copy (lane 8), or 2 copies (lane 9).

RNA Gel Blot Analysis of H2A-1 Expression

We probed equal amounts of total RNA from tomato shoot tips, roots, and mature leaves with histone H2A-1 cDNA to define its expression pattern, as shown in Figure 4. As expected from the cDNA library screening, it was highly expressed in shoot apices and was much less abundant in mature leaves. Intermediate levels were seen in root RNA prepared from both the mature portion of the root and the apices.

We wanted to determine whether the less homologous loci identified in the DNA gel blot analysis contributed to H2A expression in the shoot tip. We screened the cDNA library with the H2A-1 probe under low-stringency conditions and identified all hybridizing clones by autoradiography. When washed under more stringent conditions, we could still detect 43 of the 45 low-stringency-detectable clones. This suggested that H2A-1 was the predominant clone in the cDNA library and reflected its high level of expression in shoot apices.

We investigated H2A-1 expression in different stages of developing fruit by RNA gel blot analysis, as shown in Figure 5. As in typical berries, tomato fruit consists mainly of fleshy pericarp with seeds. Tomato H2A-1 gene expression increased as the fruit developed, showing a maximum at 4 to 10 days postanthesis and then dropped off gradually. Ovary enlargement began, under our growth conditions, around day 7.

The pea histone clone was used to analyze expression in pea root apices. Pea has large, well-characterized roots where, for most tissues, the apical 1.5-mm section is composed mostly of dividing cells and the root cap, the 1.5- to 5.5-mm section, is enriched for enlarging cells, and the 5.5- to 11-mm section contains mainly maturing cells. We prepared mRNA from each of these root sections, from young pea shoots (10 cm, including all the parts above ground), and from mature leaves. Equal amounts of these mRNAs were electrophoresed, blotted, and probed with the pea H2A cDNA. Figure 4B, lanes 1 to 3 show that H2A gene expression is strongest in the dividing cell region but that substantial expression is found in enlarging cells and detectable expression in maturing cells. Very low but

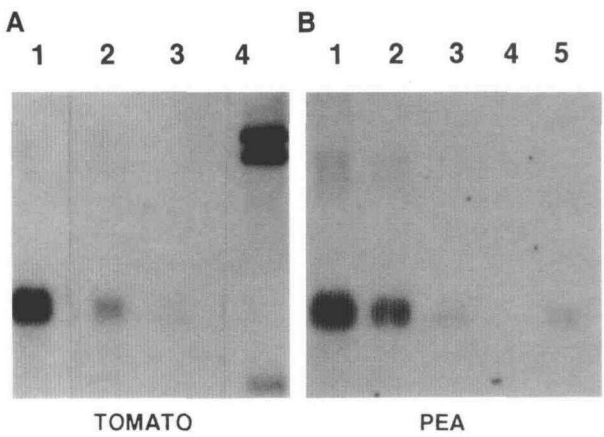

Figure 4. RNA Gel Blot Analysis.

(A) Total tomato RNA. The blot was probed with tomato H2A-1 cDNA. Lane 1, shoot apices (0.5-mm apical plumule); lane 2, total roots; lane 3, mature leaves. Lane 4 represents DNA molecular weight standards: 2.3, 2.0, and 0.6 kb. Equal amounts of RNA were loaded in each lane.
(B) Pea mRNA. The blot was probed with pea H2A clone. Lane 1, 0- to 1.5-mm region of root apex; lane 2, 1.5- to 5.5-mm region of root apex; lane 3, 5.5- to 11-mm region of root apex; lane 4, mature leaves; lane 5, 2-week-old shoots of the stem, leaves, and apex.

still detectable expression is found in mature leaves, and intermediate expression is found in shoots.

Position of Cells Expressing H2A-1 mRNA

Tomato shoot tips and root tips and pea root tips were sectioned and examined for H2A-1 expression by in situ hybridization. The H2A-1 message was found distributed irregularly in cells in and near shoot apices. Figures 6A and 6B show hybridization in cells of a tomato shoot axillary bud apex (arrow) and in cells in its immediate vicinity. The sense probe (Figures 6C and 6D) does not show any label. Hybridization signal was also observed in internode segments of young tomato shoots, mostly in cortical cells, as shown in Figure 7. This is not surprising because these cells commonly undergo endoduplication of their DNA. Flowering shoots (not shown) were also hybridized and contained H2A-1-labeled cells scattered near the floral apex. Label was also found in tight clusters at the location of new primordia.

Root tips of both species were examined; however, only pea root tips are shown in Figures 8A and 8B. A median longitudinal section shows label distributed in cells of the root meristem in a scattered cell-specific pattern that increases in density acropetally and mimics the expected distribution of cells undergoing DNA synthesis (Figure 8A). Label was not found in root cap cells, and its distribution becomes more dispersed basipetally in the cortex (Figure 8B). Cells of the pericycle, however, continue to cycle some distance from the root tip and continue to show hybridization with H2A-1. Eventually, only pericycle cells adjacent to the protoxylem poles continue to cycle. Cross-sectional analysis of the in situ hybridizations confirmed that sectors of pericycle accumulate H2A-1 mRNA (data not shown). Tomato root apices showed a similar expression pattern.

DISCUSSION

We have characterized cDNAs encoding tomato and pea H2A. The deduced amino acid sequence of H2A shows conservation of residues in the core region with more variable amino and carboxy termini. Pea H2A has greater identity (70%) to human H2A than human has to tomato H2A (65%). These similarities suggest a paradoxical phylogeny; however, Figure 3 shows that the residues responsible are interspersed in extremely conserved regions, where strong evolutionary constraints are most likely. Because these changes are conservative, i.e., Arg to Lys, Ile to Val, etc., they probably represent iterative fluctuations in a constrained system. Regions such as the H2A termini,

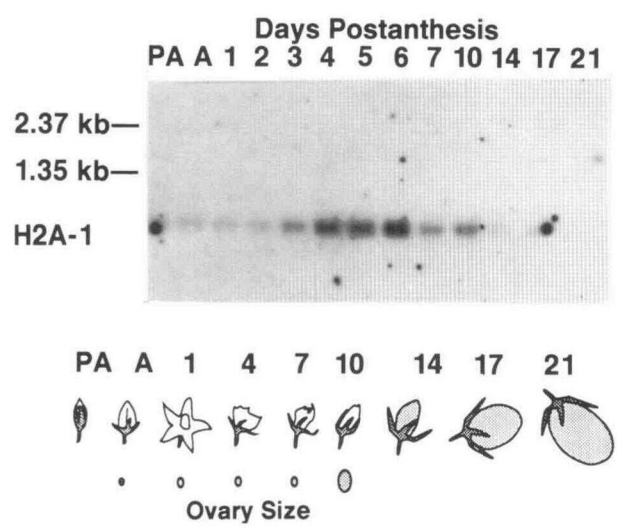

Figure 5. RNA Gel Blot Analysis of Histone H2A-1 in Developing Tomato Fruit.

Equal amounts of total RNA were loaded in each lane. PA, preanthesis; A, anthesis. The drawing at the bottom illustrates the developmental stages of the flower and of the fruit.

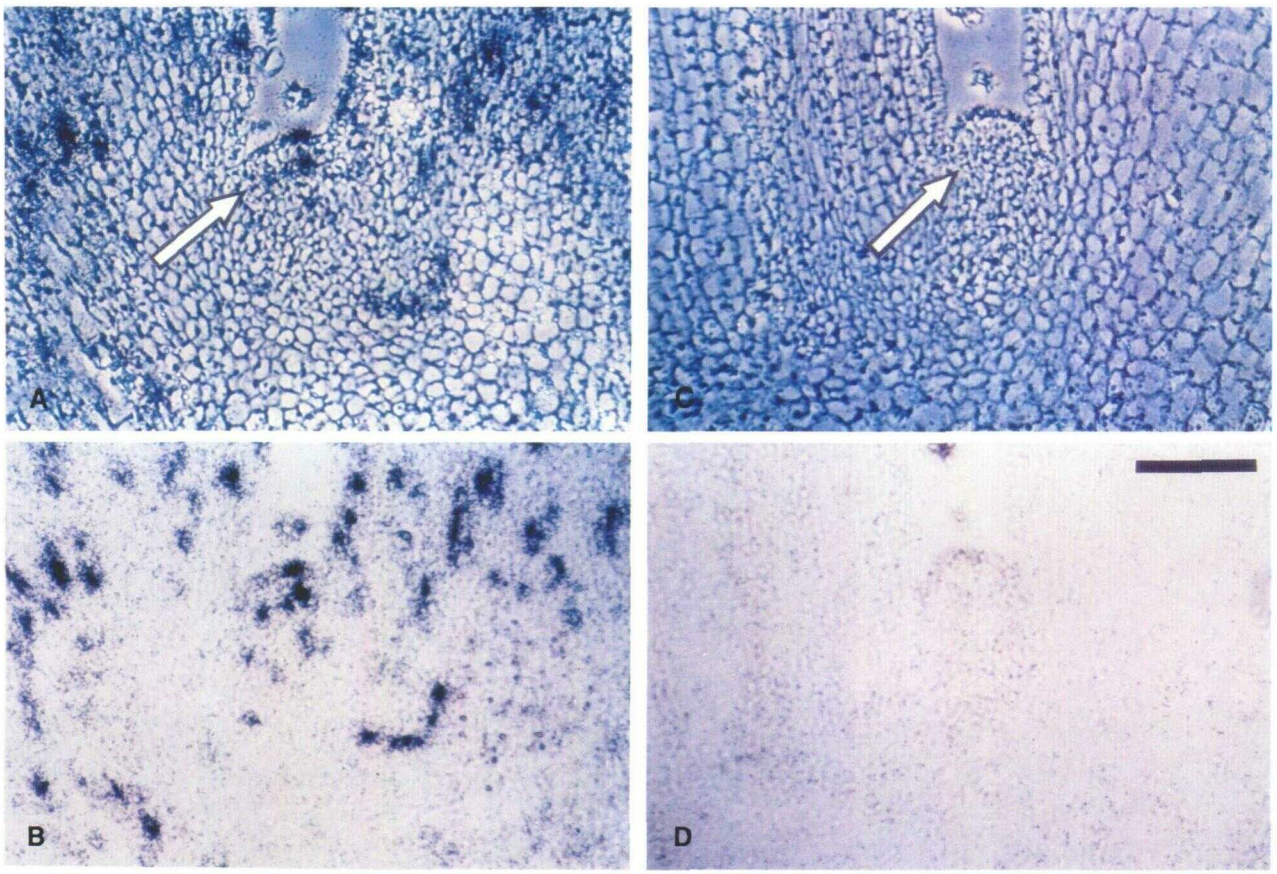

Figure 6. Localization of H2A mRNA in the Tomato Axillary Bud.

(A) In situ mRNA hybridization of an antisense H2A-1 probe to fixed sections of tomato shoot apex showing buds in the first leaf axils, viewed by phase contrast.
(B) The same section as in **(A)** viewed by bright-field optics.
(C) Another section of tomato shoot apex hybridized to a sense H2A-1 probe as control, viewed by phase contrast.
(D) The same section as in **(C)** viewed by bright-field optics.
The arrows point to the axillary bud. The bar in **(D)** = 100 μm.

where evolutionary pressure is relaxed, provide a phylogenetic picture consistent with common wisdom. Tomato, pea, and wheat H2As have a remarkable feature: an SPKK motif in their carboxy terminus. This motif is reiterated in both termini of histone H1 and in the amino terminus of the sea urchin histone H2B but not in animal or yeast H2A. Recently, Churchill and Suzuki (1989) demonstrated that the SPKK motif interacts with the minor groove of A/T-rich sites. The presence of this motif in the carboxy-terminal region seems to be unique to plant H2A and may reflect a different role of plant H2A histones in nucleosome assembly and phasing and, possibly, gene regulation.

DNA gel blot analysis revealed that the tomato genome contained as few as two and no more than five histone H2A loci. We can neither exclude the possibility that other H2A loci having little homology to H2A-1 exist nor that multiple copies of the gene exist at a given locus. The isolated tomato H2A clone represents 95% of the H2A's mRNA cloned in the shoot tip cDNA library and it is, therefore, a highly expressed locus. As reported for H3 and H4 (Tabata et al., 1983; Chaboute et al., 1988; Chaubet et al., 1988; Wu et al., 1989), tomato H2A is transcribed as a polyadenylated mRNA. The mRNAs of plant histones H3 and H4 differ from those of animal histones by having nonpolyadenylated 3′ ends with a hairpin structure. This difference may reflect different regulatory strategies.

RNA gel analysis and in situ hybridization studies of H2A expression led to several conclusions. The main one is that mRNA expression is not constitutive but is controlled according to cell position. Cells in mature tissues showed

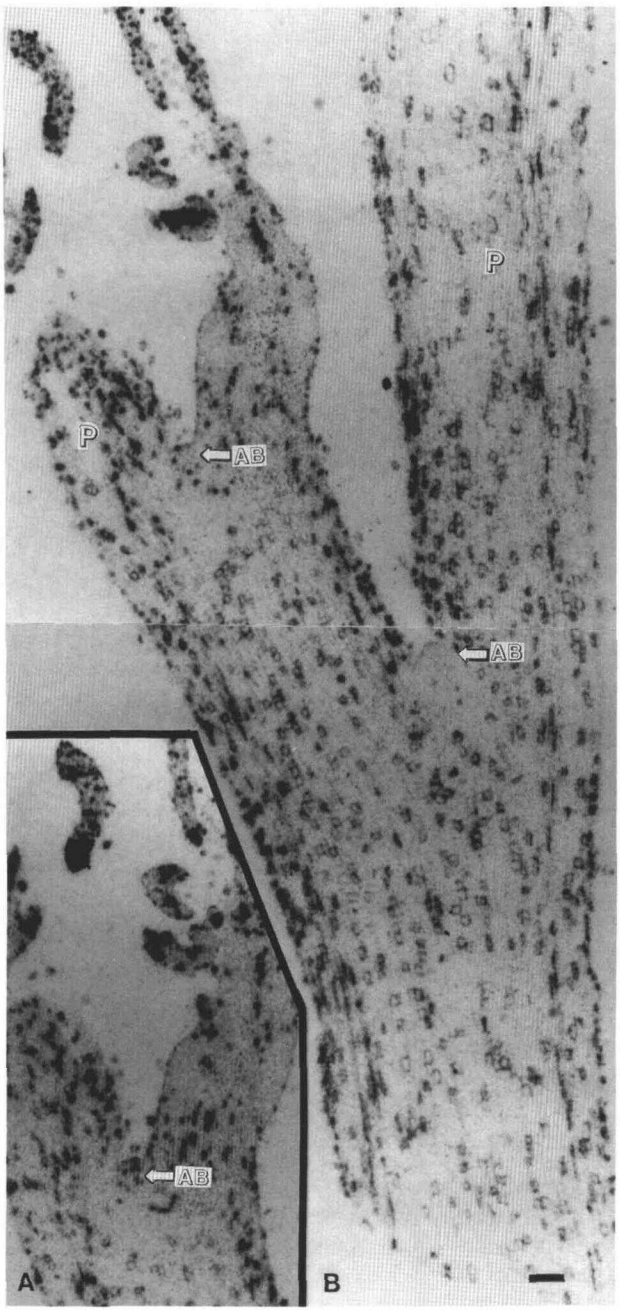

Figure 7. Localization of H2A mRNA in the Tomato Shoot Apex.

(A) In situ mRNA hybridization of an H2A-1 probe to fixed sections of the tomato shoot apex. The bud is in the first leaf axil. AB, axillary bud.

(B) Serial section from the same apex as in **(A)** showing a larger area. A dormant axillary meristem is seen in the second leaf axil. AB, axillary buds; P, petiole.

The doughnut-shaped signals over differentiated cells are due to the presence of a vacuole within the cell confining the cytoplasm to the cell periphery. Bar = 100 μm.

little expression, whereas cells in meristematic tissues showed higher levels of expression. In addition, only some of the cells within expressing tissues contained high levels of H2A mRNA. In vegetative meristems, cells expressing H2A were surrounded by neighboring cells that did not show any detectable expression. We can rule out that the variegated pattern of H2A expression is due to artifacts in several ways. First, both the tomato and the pea H2A probe gave the same in situ pattern. Second, we performed this analysis on different organs with similar results. Third, to date we have analyzed more than 15 different genes that are expressed preferentially or exclusively in meristems without finding this pattern. Because all cells contained the histone H2A gene, production of mRNA must take place at different times in different cells where, in response to unknown cues, accumulation of H2A mRNA is initiated and terminated. This result indicates that the expression of H2A-1 is tightly regulated, both temporally and spatially, and that mRNA levels increase and decrease rapidly. We estimated from RNA gel blot analysis and screening of the cDNA library that the level of H2A mRNA in apical plumule preparation is about 0.5% of total mRNA. Because the fraction of expressing cells is estimated at less than 10%, it follows that the level of H2A mRNA in positive cells is at least 5%. Rapid induction and depletion of histone mRNA are achieved in metazoans by transcriptional induction of reiterated genes, followed by mRNA degradation. An unusual 3′ structure of animal histone mRNAs allows specific recognition by an RNase species. Because plant histone mRNAs lack this structure, they may employ a different regulatory mechanism.

We have examined the expression of H2A in root, vegetative shoot, and flower meristems. In vegetative shoot meristems, positive cells are not numerous and lack any obvious organization. The pattern of cell division within apical vegetative meristems of tomato was described by Malayer and Guard (1964) who, by examining 50 apices at the beginning of the fifth plastochron (the time between the formation of fourth and fifth true leaf), found that mitotic figures are limited to a crescent-shaped region with a downward-facing concave side located immediately below the meristem dome. The numbers of mitotic figures that they observed (24 figures out of 50 apices) is within an order of magnitude of the number of H2A-expressing cells that we observed. Dormant axillary meristems, inhibited by apical dominance, showed neither mitotic figures nor H2A positive cells. Interestingly, there are a high number of nondividing cells in the shoot that are still expressing H2A mRNA. These cells have already initiated their differentiation and have a vacuole, as indicated by the doughnut shape of the hybridizing cytoplasm and by microscopic observation. We suspect that expression in these nondividing cells may be associated with DNA endoduplication. Cells in several mature plant tissues undergo DNA endoduplication (D'Amato, 1964; Evans and Van't Hof, 1975; Cavallini and Cionini, 1986). Recently, cells with ploidy

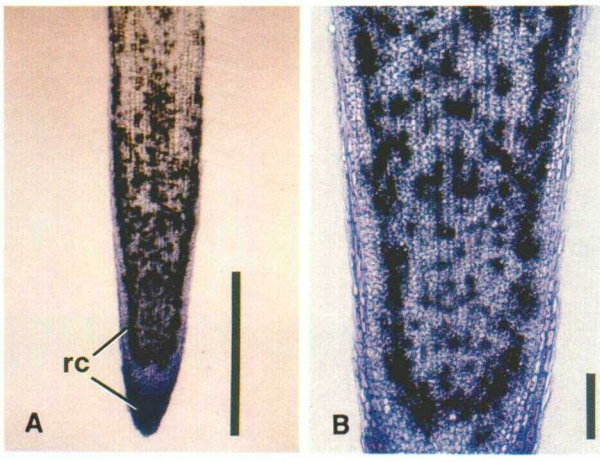

Figure 8. Localization of H2A mRNA in Pea Roots.

In situ mRNA hybridization of antisense H2A RNA to a median longitudinal section of a pea root.
(A) The apical 8-mm section of the root. rc, root cap. The vertical bar = 1.5 mm.
(B) A closeup of the meristem region. The vertical bar = 200 μm.

levels as high as 32C were described in several tomato tissues including the stem (Dr. M.G. Noteboos and Dr. M.-L. Tan, Zaadunie B.V., The Netherlands, personal communication).

To distinguish clearly between dividing and endoduplicating cells, we investigated the histological expression pattern of H2A in pea root apex. The position of dividing and endoduplicating cells in this organ is well characterized. Rost et al. (1988) studied the organization of cell files in root apices of pea and showed that most cell divisions occur in the apical 4 mm. We isolated the pea histone H2A cDNA using the tomato clone as a probe. We showed by RNA gel blot hybridization that the highest level of H2A mRNA is found in the apical 1.5 mm; however, moderately high amounts are still found in the 1.5- to 5.5-mm region. The histological expression pattern confirms the notion, suggested by RNA gel blot analysis, that H2A expression is found in some cells that are cycling but not dividing anymore. This type of cell is abundant in the 4- to 5.5-mm region. The pattern described for mitotic figures is similar to the pattern of H2A-expressing cells but limited to the first 4 mm of the root apex. In addition, expression in roots continues beyond dividing cells to the region of cell enlargement and differentiation. Expressing cells are also found in the root pericycle up to 1 cm away from the apex. Tomato flower meristems show clusters of positive cells in regions of sympodial growth. All of these different expression patterns are consistent with the present understanding of the organization of these different meristems (Francis and Lyndon, 1979; Nougarede et al., 1987; Bernier, 1988; Rost et al., 1988; Sussex, 1989). The expression of H2A-1 after fertilization provides an additional example of how the expression of this gene is modulated during a phase of intense cell division followed by enlargement. We observed that expression increased rapidly day 3 postanthesis, peaking at days 4 to 6, decreasing to a lower plateau until day 10, and becoming nondetectable by day 21. The onset of ovary enlargement between day 7 and day 10 is accompanied by a reduction in the number of cell divisions.

The observed expression pattern suggests that the H2A-1 gene is cell cycle regulated and probably expressed during late G1 and S phases of mitosis and endomitosis. This pattern of expression has been documented clearly for many histone genes, and it is expected to hold true for a DNA replication-dependent histone (Stein et al., 1984). We are now trying to document this hypothesis by localizing DNA synthesis and H2A mRNA in serial sections of pea root apices. In future studies, we plan to investigate the mechanism of H2A regulation during the cell cycle.

METHODS

Nucleic Acid Manipulations

Unless specified, procedures described in Sambrook et al. (1987) were followed. Tomato plants (*Lycopersicon esculentum* var UC82B) were grown at 25°C, with 8-hr nights, 500 $\mu E/m^2$. Shoot tips averaging 5 mm in size, roots, and mature leaves were collected from plants 3 to 4 weeks old and immediately frozen in liquid N_2. RNA was extracted using the guanidinium thiocyanate method of Colbert (1983), as modified by Facciotti et al. (1985). Poly(A^+) RNA was purified using oligo(dT)-cellulose chromatography. The cDNA library was constructed from shoot tip mRNA using a vector primer method (Alexander, 1987) and transformed into *Escherichia coli* DH5α. Colony lifts (Taub and Thompson, 1982) were probed with first-strand cDNA from shoot tips, roots, and mature leaves (Gubler and Hoffman, 1983). Clones highly expressed in the shoot tip and root were chosen for further characterization. This library was transferred into λgt10 by digestion with EcoRI and ligation to λ arms; in the screen with H2A cDNA (see results), we used the phage library.

DNA gel blot analysis was performed using 10 μg of tomato genomic DNA isolated according to Dellaporta et al. (1983), digested with various enzymes, electrophoresed on agarose gel, and blotted onto Zeta-Probe nylon membranes according to procedures recommended by the manufacturer (GIBCO-BRL, Grand Island, NY). RNA gel blots were made according to the protocol described by Fourney et al. (R.M. Fourney, J. Miyakoshi, R.S. Day, and M.C. Paterson, 1988. Northern blotting: Efficient RNA staining and transfer. Focus, Vol. 10, 5–7) with total RNA isolated as described above. The restriction fragments to be used as probes were restriction digested and separated from other fragments by gel electrophoresis. They were purified from agarose by electroelution (Sambrook et al., 1987) and nick translation using a GIBCO-BRL nick-translation kit. Hybridizations were carried out in a solution containing 50% formamide, 1 M NaCl, 1% SDS, and

100 μg/mL sheared salmon sperm DNA, at 22°C (DNA gel blot 1, not shown; also plaque hybridization) to 37°C (DNA gel blot 2, Figure 3) for low-stringency conditions, or at 42°C for high-stringency conditions. Low-stringency washes were in 2 × SSC, 0.1% SDS at 37°C, and high-stringency washes were at 68°C in 0.2 × SSC, 0.1% SDS. Sequencing was done by the use of the dideoxy chain termination reaction employing a Sequenase sequencing kit (US Biochemical Corporation, Cleveland, OH). All listed (tomato) or deposited (pea) DNA sequences were verified on the opposite strand.

Pea seedlings (*Pisum sativum* var Alaska) were germinated axenically in moist vermiculite in the dark at 25°C for 4 to 5 days. Root tips were collected from 4-cm-long seedling roots. RNA was extracted as above from whole root tips (1 to 1.5 cm) or from roots that were dissected into sections 0 to 1.5 mm, 1.5 to 5.5 mm, and 5.5 to 11 mm (as measured from the root tip) on a root slicer designed to hold root tips stationary and properly aligned for bulk dissection. RNA was also extracted from greenhouse-grown mature leaves and 10-cm whole shoots (18°C daytime, during January in Davis, CA). DNA and RNA manipulations and probe construction were performed as above. Pea RNA gel blots were loaded with 2.5 μg of poly(A^+) RNA per lane from the respective tissues. Nick-translated probes were synthesized from agarose gel-isolated inserts of XbaI-EcoRI-digested plasmids carrying the pea H2A-1 cDNA.

In Situ RNA Hybridization

The cDNA sequence in pCGN7108 was excised from the vector backbone using XbaI and ligated into XbaI-cut pBluescript SK+ (Stratagene, San Diego, CA) to create pCGN7113. In this vector, use of T7 or T3 RNA polymerases resulted, respectively, in transcription of sense or antisense H2A RNA. In situ hybridizations were performed on sections of tomato shoot tip and roots according to Pokalsky et al. (1989). The plants were grown as specified above. Antisense and sense ^{35}S-labeled RNA transcripts were made from pCGN7113 using the Stratagene transcription kit. Hybridizations were at 37°C. Hybridized sections were washed sequentially for 20 min each in the following buffers: at 37°C, 2 × SSC, 50% formamide, then 37°C, 1 × SSC, 50% formamide, and finally at 30°C, 1 × SSC and no formamide.

Pea roots were fixed as above but were manipulated for in situ hybridizations as in Raikhel et al. (1989). Pea cDNA was synthesized from poly(A^+) RNA isolated from whole 1.5-cm root tips, and it was cloned into LambdaGEM-4 (Promega). The pGEM1-cDNA segments of LambdaGEM-4 were excised from purified λ by SpeI digestion, ligated, and transformed into DH5α. For in situ hybridizations, the plasmids (pGEM1 plus the cDNA insert) were linearized with EcoRI or XbaI digestion yielding the sense or antisense template for in vitro RNA transcription from T7 or SP6 promoters, respectively.

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