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zrp2: a novel maize gene whose mRNA accumulates in the root cortex and mature stems

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Abstract

A near full-length cDNA clone (pZRP2) was isolated from a cDNA library constructed from maize root mRNAs. The predicted polypeptide has a calculated molecular mass of 66 975 Da, is largely hydrophilic, and contains 26 repeats of a motif the consensus sequence of which is RKATTSYG[S][D/E][D/E][D/E][D/E][P]. The function of the putative protein remains to be elucidated. The ZRP2 mRNA accumulates to the highest levels in young roots, and is also present in mature roots and stems of maize. Further analysis of young roots indicates that the lowest level of ZRP2 mRNA is near the root tip, with relatively high levels throughout the remainder of the root. *In situ* hybridization reveals that ZRP2 mRNA accumulates predominantely in the cortical parenchyma cells of the root. *In vitro* nuclear run-on transcription experiments indicate a dramatically higher level of *zrp2* gene transcription in 3-day old roots than in 5-day old leaves. A *zrp2* genomic clone, which includes the transcribed region and 4.7 kb of upstream sequence, was isolated and characterized.

Introduction

Root function is integrated with shoot function through transport and storage of water and nutrients needed for growth, as well as through translocation of signaling molecules affecting growth and development. One approach that has provided insight into the molecular basis of root development and function has been the identification of mutants in root development [1, 33, 38, 39]. The genetic approach is a powerful means to identify genes whose expression effects easily observed phenotypes, such as the production and morphology of root hairs. Another approach that has been employed to increase understanding of root development and function is the isolation of genes expressed in a root-specific or root-preferential manner [4, 7, 8, 9, 11, 13, 20, 23, 29, 34, 35, 46, 49, 50, 51]. This approach rests upon the assumption that expression of a gene in specific organs or tissues reveals an important role for that gene product in those organs or tissues.

Here we report on the isolation of a maize cDNA clone whose corresponding mRNA accumulates to high levels in both the roots and mature stems of the maize plant. The deduced ZRP2 polypeptide is predominantly composed of an unusual repeated motif and shares no extensive sequence similarity with any known protein. The ZRP2 mRNA was transcribed at a much higher rate in young roots than in young leaves. A genomic clone was isolated that includes the entire transcribed region of the *zrp2* gene, as well as 4.7 kb of the 5′-upstream region.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers U38790 (genomic) and U38791 (cDNA).

Materials and methods

Growth of plants

Maize (*Zea mays* cv. NKH31, a gift from Northrup King) plants were grown and organs or root segments were harvested as previously described [20, 23]. Seedlings were grown on germination paper for up to 9 days to obtain young roots and leaves for analysis. More mature maize organs were obtained from 3-week old plants grown in soil under greenhouse conditions, and from mature plants grown under field conditions.

Construction and screening of cDNA and genomic libraries

The ZRP2 cDNA was isolated from a maize root cDNA library constructed from poly(A)$^+$ isolated from 9-day old maize roots, screened with ^{32}P-labeled first strand cDNAs derived from 9-day old maize shoot and maize root poly(A)$^+$ RNA samples [23].

Genomic DNA was isolated from maize leaves as described by Saghai-Maroof *et al.* [37]. Southern analysis was performed as described by Held *et al.* [20]. For construction of a subgenomic library, genomic DNA was digested to completion with *Bam*HI and fractionated on a 1% agarose gel. DNA fragments (10–21 kb) were isolated by electro-elution, ligated into Lambda Dash II *Bam*HI-digested arms, and packaged into Gigapack II Packaging Extracts (Stratagene). The resulting subgenomic library, of about 100 000 plaques, was screened according to standard procedures [18]. The probe for both Southern analysis and screening of the subgenomic library was an *Eco*RI fragment of pZRP2 containing the entire ZRP2 cDNA, labeled with ^{32}P-dCTP using the multiprime DNA labeling System (Amersham) as specified by the manufacturer.

RNA isolation, gel blot analysis, and in situ *analysis*

Total RNA was isolated from whole-plant organs [15], or from 1 cm root segments as described by Chomezynki and Sacchi [6]. Poly(U) Sephadex columns were used to purify poly(A)$^+$ RNA from total RNA [30].

RNA gel blot analyses were performed as described in Cotton *et al.* [10]. RNA size standards were from Gibco-BRL. The full-length ZRP2 cDNA in pBluescript (SK) was digested with *Hin*dIII and transcribed with T3 RNA polymerase (Promega) to produce a ^{32}P-labeled RNA probe used in RNA gel blot analyses. *In situ* hybridizations were performed as described in John *et al.* [23]. The antisense and sense ^{35}S-RNA probes used for the *in situ* analysis were synthesized from pZRP2.22 [22]. Sense-strand RNA probe was produced by linearizing pZRP2.22 with *Eco*RI and transcribing with T3 RNA polymerase. Antisense-strand RNA probe was generated by linearizing pZRP2.22 with *Hin*dIII and transcribing with T7 RNA polymerase.

DNA sequencing and analysis

The ZRP2 cDNA and genomic clones were sequenced at the Iowa State University Nucleic Acid Facility. Each strand of the DNA was sequenced at least twice. DNA sequence and predicted protein data were analyzed with the University of Wisconsin Genetics Computer Group (UWGCG) package [12]. Hydropathy analysis of the predicted ZRP2 polypeptide was performed using the DNA Strider program [32] as described [28].

Isolation of nuclei from shoots and roots

Maize seeds were germinated and grown under a 12 h light/dark cycle for 3 days (for roots) or 5 days (for leaves). Roots were excised with a razor blade and cut into pieces 5–10 mm long on an ice-cooled glass plate. The primary leaf was removed from the mesocotyl node with a gentle and gradual pull. All reagents and supplies were pre-cooled to 4 °C. Roots (4 g) were immersed in ethyl ether for 4 min, and leaves (2 g) were immersed in ethyl ether for 3 min. The ethyl ether was poured off and 5 ml of extraction buffer (2.5% (w/v) Ficoll 400, 4.0% (w/v) dextran T40, 250 mM sucrose, 25 mM Tris-HCl pH 7.8, 10 mM $MgCl_2$) was added to a mortar containing the roots or leaves [31]. The roots or leaves were thoroughly grounded with a pestle, and the homogenate was filtered through two layers of 149 μm and 60 μm nylon mesh into a 30 ml Corex tube. The mortar and filters were rinsed with a total of 10 ml extraction buffer. To the filtrate was added 500 μl of 20% (v/v) Triton-X-100. After mixing by inversion, tubes were centrifuged for 2 to 3 min at 2000 rpm in a HB-4 rotor. The supernatant was drained off, and the pellet was resuspended in 10 ml wash buffer (extraction buffer plus 0.1% (v/v) Triton-X-100) and centrifuged again. The supernatant was decanted and the pellet was resuspended in 300 μl extraction buffer, frozen in liquid N_2 and stored at -80 °C.

In vitro *run-on transcription assays*

The *in vitro* run-on transcription reaction included 48 μl of premix (325 μM ATP, CTP, GTP, and 50 mM NH_2SO_4), 12 μl (40 units/μl) RNASIN (Promega), 50 μl [^{32}P]UTP (500 μCi), and 290 μl of nuclei. The reaction was carried out for 30 min at 30 °C, with occasional mixing. The reaction mixture was centrifuged to pellet the nuclei. The supernatant was removed and the pellet resuspended in 30 μl sterile H_2O. Five units of DNase (Promega) were added and the mixture was allowed to incubate for 10 min at room temperature. The volume was brought up to 370 μl with elution buffer (20 mM Tris-HCl, 1 mM EDTA, 0.5% SDS, 5 μg/ml yeast RNA). This mixture was extracted with phenol/chloroform (1:1) two or three times and then passed over a Bio-gel P60 column to separate *in vitro* transcribed RNA from unincorporated nucleotides. The amount of radioactivity incorporated into RNA was determined with scintillation spectroscopy of 2 μl aliquots.

To estimate transcription levels, the *in vitro* transcribed RNA was allowed to hybridize with unlabeled DNA probes. The probes used were pZRP2.1 (a subclone including nucleotide 1264 to the 3′ end of the ZRP2 cDNA, Figure 1; [22]), and pGAB0.7 (a chlorophyll *a*/*b*-binding protein (*cab*) cDNA from maize; [15]). One μg of each plasmid was digested with *Pst*I (pGAB0.7) or *Kpn*I (pZRP2.1) to excise the cDNA insert, electrophoresed on a 1% agarose gel, and blotted onto a nylon (GeneScreen) membrane. The membrane was placed in prehybridization buffer (0.55 M NaCl, 40 mM Na_2PO_4 pH 6.8, 2% (w/v) SDS, 100 μg/ml denatured salmon sperm DNA, 100 μg/ml poly(A), 4 mM EDTA, 1% (w/v) BSA, 33% deionized formamide, 5× Denhardt's solution) for 4 h at 42–45 °C. Hybridizations were performed with equivalent amounts of leaf and root *in vitro* transcribed RNA (10 million cpm) in a hybridization buffer that was the same as the prehybridization buffer, but without SDS or BSA. Hybridizations were for 40 h at 42–45 °C. Blots were washed in 2× SSC, 0.1% SDS two times at room temperature for 5 min, and then in 0.1× SSC, 0.1% SDS two times at 65 °C for 30 min each, and exposed to X-ray film for 20–60 h.

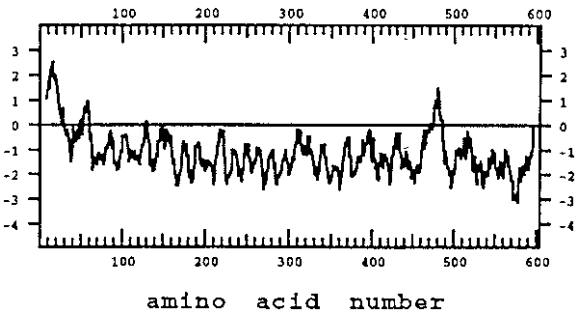

Figure 1. Hydropathy profile of the amino acid sequence predicted from the pZRP2 cDNA clone. A computer-generated hydropathy profile (window of 11 consecutive amino acids) of the predicted ZRP2 protein was plotted against the amino acid number using the DNA Strider program [32]. Hydrophobic regions have a positive sign, while hydrophilic regions have a negative sign.

Results

ZRP2 mRNA sequence and predicted ZRP2 amino acid sequence

ZRP2 was one of three root-preferential cDNA clones isolated by differential screening of a cDNA library constructed from poly(A)$^+$ RNA isolated from 9-day old maize roots [23]. The nucleotide sequence of the ZRP2 cDNA clone is available in the GenBank database. The largest open reading frame within the ZRP2 mRNA begins with an AUG initiation codon at nucleotide 63 and ends with a UAA stop codon at nucleotide 1865. The ZRP2 mRNA contains a putative polyadenylation signal sequence [24, 48] 43 nucleotides upstream of the poly(A) tail. The predicted ZRP2 protein consists of 600 amino acids with a calculated molecular mass of 66 975 Da. The ZRP2 protein contains a high percentage of serine (11%) and arginine (10%) residues. A BLAST search of the GenBank database revealed no significant amino acid sequence similarity between ZRP2 and previously reported sequences. A hydropathy plot for the predicted ZRP2 protein sequence indicates that much of the putative ZRP2 polypeptide is hydrophilic (Figure 1). Two short hydrophobic amino acid stretches are present, one at the amino terminus and the other between amino acids 470 and 480 (Figure 1).

The predicted ZRP2 protein can be divided into three domains based on characteristics of the primary sequence: a putative signal peptide at the amino terminus, a domain containing a highly repeated motif, and a basic domain at the carboxy-terminus. The amino terminal hydrophobic region has characterist-

ics of a signal peptide sequence specifying transport across the endoplasmic reticulum. The predicted cleavage site would be between amino acids Ala-24 and His-25 [45]. The amino terminal region (residues 1 to 5) of the ZRP2 polypeptide deviates from many [3], but not all [44], signal peptides in that it lacks a positively charged amino acid near the terminal methionine. The domain between Asp-55 and Ser-440 contains an amino acid motif repeated 26 times. The consensus sequence of this motif is: **RKATTSYG[S][D/E][D/E][D/E][D/E][P]**, with those amino acids not in brackets being most highly conserved. The consensus motif thus consists of: $[basic]_2$ [hydrophobic] $[polar]_4$ [hydrophobic] [polar] $[acidic]_4$ [proline]. Two putative glycosylation sites [26] are present at positions 276 and 375. The 143 amino acid domain at the carboxy terminus is highly basic; 37% of the amino acids in this domain are either histidine or arginine.

Isolation and analysis of the zrp2 *genomic clone*

Genomic Southern blot analysis using *Hin*dIII, *Eco*RI and *Bam*HI indicated that *zrp2* is a low- to single-copy gene (Figure 2A). *Bam*HI endonuclease digestion produced a single band of ca. 20 kb. A maize subgenomic library, enriched for *Bam*HI-digested fragments ranging from ca. 10 to 20 kb in size, was constructed and screened with a ^{32}P-labeled ZRP2 cDNA probe. A single hybridizing clone (designated Z2B) was isolated. Partial sequencing of this genomic clone revealed a perfect match to the 5′-untranslated and the 3′-untranslated region of the ZRP2 cDNA sequence.

Restriction mapping of the genomic clone revealed an insert of about 14 kb that contains two internal *Sal*I restriction sites, which divide the *zrp2* genomic clone into fragments of 4.5 kb, 8.0 kb, and 1.3 kb (Figure 2B). The 8.0 kb fragment hybridized to the 5′ end of the ZRP2 cDNA and the 1.3 kb fragment hybridized to the 3′ end of the ZRP2 cDNA (data not shown). DNA sequencing of the 3′ end of the 1.3 kb *zrp2* fragment verified that it was identical to the ZRP2 cDNA. Partial sequencing of the *zrp2* genomic clone revealed that the 4.5 kb *Sal*I fragment was upstream of the 8.0 kb fragment, and that the 5′ end of the ZRP2 cDNA clone was very near the 5′ end of the 8.0 kb *Sal*I fragment [19]. The ZRP2 transcribed region appears to span ca. 8 kb of genomic sequence, of which about 6 kb represent intron sequences. The 5′-most intron (a; Figure 2B) was determined to be 90 nucleotides in length, while the 3′-most intron (c) was 760 nucleotides in length (data not shown). The length of the other

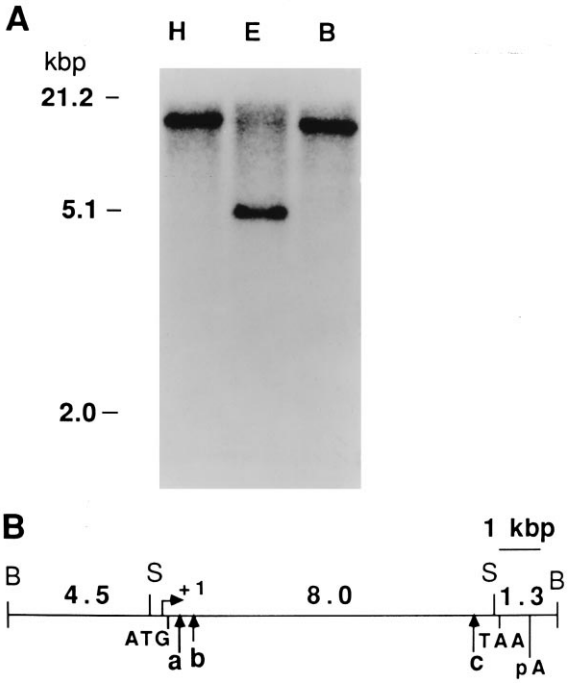

Figure 2. Characterization of the *zrp2* gene. A. Genomic Southern blot analysis of the *zrp2* gene. Maize total DNA from leaves (ca. 10 μg) was digested with *Hin*dIII (H), *Eco*RI (E), or *Bam*HI (B), fractionated by agarose gel electrophoresis, transferred onto a nylon membrane, and hybridized with the ^{32}P-labeled cDNA insert of pZRP2. *Eco*RI/*Hin*dIII-digested λ DNA fragments were used as size standards. B. Partial restriction enzyme map of the *zrp2* genomic clone and structure of the *zrp2* gene. The *Bam*HI (B), and *Sal*I (S) restriction sites are displayed. The positions of the putative transcription start site (+1), the translation start and stop codons (ATG and TAA, respectively), and the polyadenylation site (pA) are indicated. The arrows (a, b, and c) indicate the positions of known introns.

intron (b) was greater than 1.3 kb. The position and size of other introns that might be present within the *zrp2* gene is currently unknown.

About 2.6 kb of the 4.7 kb upstream region of the *zrp2* gene was sequenced [19, 43] . A putative TATA box is present 22 bp upstream of the mapped transcription start site [19]. The CATC box, which is located near position −90 in some promoters of genes from various grain species [27], is not present in the *zrp2* promoter. Comparison of the *zrp2* genomic sequence to sequences in the GenBank database revealed 72% identity over a stretch of 159 bp (from −365 to −208 of *zrp2*) with the first intron from an α-tubulin gene from maize [36]. This region contains 77% A+T, and includes a 36 bp stretch of continuous A+T. Beginning at position −113 is a tandem repeat of the sequence ACGT.

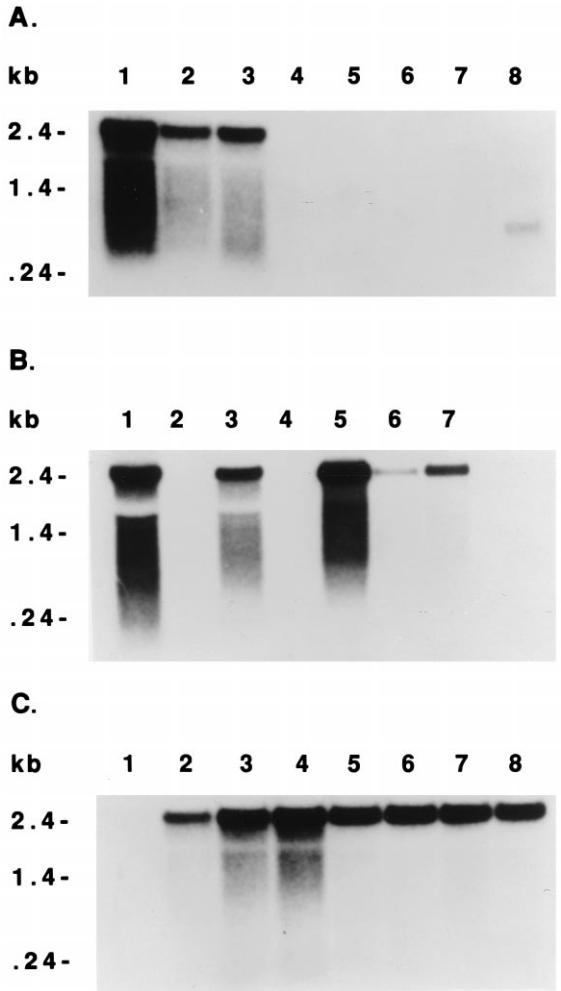

Figure 3. Analyses of ZRP2 mRNA accumulation in the root and other maize organs. RNA was fractionated by electrophoresis in a 3% formaldehyde/1% agarose gel. After electrophoresis the RNA samples were transferred to a nylon membrane and hybridized with ^{32}P-labeled antisense RNA probe. A. Total RNA (10 μg) was isolated from roots of 3-week old greenhouse grown plants (lane 1), and from other organs that were harvested from field-grown plants at pollination: root (lane 2), stem (lane 3), leaf (lane 4), ear at pollination (lane 5), ear 10 days after pollination (lane 6), silk (lane 7), tassel (lane 8). B. Total RNA (10 μg, lanes 1 and 2; 5 μg, lanes 3 and 4) isolated from 3-day old roots (lane 1), 5-day old leaves (lane 2), 3-week old roots (lane 3), and leaves (lane 4), or poly(A)$^+$ RNA samples (0.5 μg) from 9-day old roots (lane 5), light-grown 9-day old shoots (lane 6), and etiolated 9-day old shoots (lane 7). C. Total RNA (5 μg) isolated from the root tip (lane 1), 1–2 cm (lane 2), 2–3 cm (lane 3), 3–4 cm (lane 4), 4–5 cm (lane 5), 5–6 cm (lane 6), 6–7 cm (lane 7), and 7–9 cm (lane 8) of 4-day old seedlings.

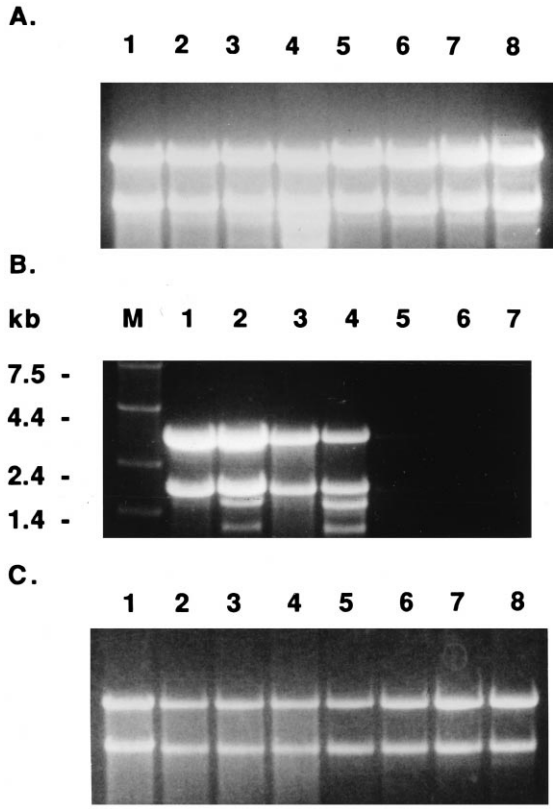

Figure 4. Loading controls for RNA blot analyses. Lane designations are the same as for Figure 3 except that M in panel B corresponds to the RNA molecular weight markers.

ZRP2 mRNA accumulation

ZRP2 mRNA accumulation was analyzed in the organs of 3-week old greenhouse-grown and mature, field-grown, maize plants (Figure 3A). A prominent band of ca. 2.4 kb (the expected size of the ZRP2 mRNA) was evident. Most of the total RNA samples containing ZRP2 mRNA also had a distribution of smaller ZRP2 RNA fragments (Figure 3A, lanes 1–3). These smaller fragments were also evident in poly(A)$^+$ RNA samples (Figure 3B, lane 5). Such fragments could be produced *in vitro* during RNA isolation, or could be *in vivo* degradation products of the ZRP2 RNA as have been described for other plant mRNAs [21, 40]. When the same RNA samples were used to analyze other mRNAs (ZRP3 and ZRP4) extensive amounts of smaller fragments were not evident [20, 23]. ZRP2 mRNA was most abundant in total RNA isolated from the root systems of 3-week old greenhouse grown plants (Figure 3A, lane 1). The ZRP2 mRNA was also present in total RNA from prop roots and stems of mature field-grown plants (Figure 3A, lanes 2 and 3). Other organs of mature maize plants lacked detectable levels of ZRP2 mRNA (Figure 3A, lanes 4–8). The signific-

ance of the faint band (ca. 1 kb) in tassel RNA (Figure 3A, lane 8) is unclear. This band was not detected when ^{32}P-labeled ZRP2 DNA was used as the hybridization probe [19].

Comparison of ZRP2 mRNA accumulation in roots and leaves at different developmental stages confirmed that ZRP2 mRNA accumulates preferentially in the roots, but is not root-specific even in younger maize plants (Figure 3B). ZRP2 mRNA was quite abundant in roots of 3-day old and 3-week old light-grown plants, but was not detectable in total RNA from leaves of these plants (Figure 3B, lanes 1–4). However, poly(A)$^+$ RNA isolated from shoots of 9-day old light-grown maize (consisting almost entirely of leaves) did have low levels of ZRP2 mRNA (Figure 3B, lane 6). The abundance of ZRP2 mRNA in shoots appears to be environmentally influenced. ZRP2 mRNA abundance was several-fold greater in shoots of dark-grown 9-day old maize than in shoots of 9-day old light-grown maize (Figure 3B, lanes 6 and 7).

The longitudinal distribution of ZRP2 mRNA accumulation in roots of 4-day old maize seedlings was investigated (Figure 3C). Roots were dissected into 1 cm segments, and total RNA isolated from each of these segments was subjected to RNA gel blot analysis. Lane 1 (Figure 3C) corresponds to the first centimeter of the root, which includes the root cap and apical meristem. The other lanes (2–8) correspond to successive 1 cm segments. ZRP2 mRNA accumulation was lowest at the root tip, increased in the second centimeter, was highest at the third and fourth centimeters, and remained at a high level throughout the rest of the 4-day old root. Loading controls for the RNA blot analyses presented in Figure 3 are provided in Figure 4.

In situ hybridization of root cross-sections taken ca. 4 cm behind the root tip revealed that ZRP2 mRNA accumulated in the cortex parenchyma cells of the maize root (Figure 5). Little or no ZRP2 mRNA accumulated in epidermal and vascular tissues. The parenchyma cells of the pith also lacked detectable ZRP2 mRNA.

Analysis of ZRP2 transcription

In vitro nuclear run-on assays were performed to investigate *zrp2* transcription in roots and leaves of maize (Figure 6). Nuclei isolated from roots of 3-day old maize actively transcribed the *zrp2* gene. In contrast, *zrp2* transcription could not be detected in the nuclei isolated from leaves of 5-day old maize. As a control, transcription of the *cab* genes was analyzed in both the roots and leaves. Primary leaves were harvested within 4–6 h of the onset of light to ensure maximal *cab* transcription [42]. As reported in previous studies [42], a high level of transcription for *cab* was observed in nuclei isolated from the leaf, but not in nuclei isolated from the root.

Discussion

The ZRP2 mRNA accumulates to high levels in both the root and stem of mature maize plants. In the root, accumulation is maximal in the region 3 to 4 cm from the root tip. However, excluding the root apex, ZRP2 mRNA accumulates to relatively high levels throughout the length of the 4-day old maize root. ZRP2 mRNA accumulates to the highest levels in roots of seedlings and young plants, but is also present at relatively high levels in prop roots and stems of mature plants. *In situ* hybridization indicates that the cortex is the predominant site of accumulation in young roots.

The putative signal peptide in ZRP2 indicates that the ZRP2 protein may be transported into, or across, the endoplasmic reticulum, and may be further targeted to other locations within the cell. As the major site of accumulation of the ZRP2 mRNA is the root cortex, it is likely that the ZRP2 protein is localized and functions primarily in this region. The accumulation of ZRP2 mRNA in the stem of mature plants indicates that the ZRP2 protein functions in stems as well. It seems likely that the ZRP2 mRNA will be localized in the parenchyma cells composing the ground tissue of the stem, analogous to its location in the cortical parenchyma cells of the root.

At present we do not know the function of the ZRP2 polypeptide. The majority of the protein is composed of variations of a motif which has not been previously described, and which bears no sequence similarity to the protein products of any previously isolated genes. Indeed, the presence of such a high number of repeats is unusual in proteins. The group 3 *lea* proteins, which accumulate in embryos just prior to desiccation, and in some instances accumulate in adult plants under desiccation and other stresses, also have high numbers of repeated sequences [14, 16]. Mammalian ribosome receptor proteins contain a repeated motif that is thought to play a role in the binding of the ER and the ribosome [47]. Ice nucleation proteins have a series of complex repeated regions that may function to orient water crystals as well as interact with the membrane [25]. Extracellular extensins, which are

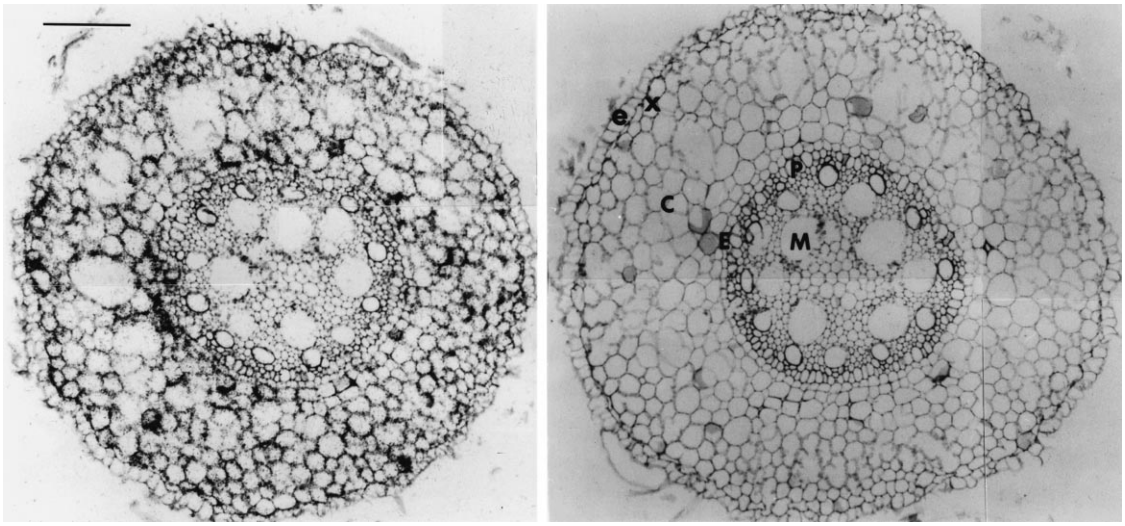

Figure 5. Localization of ZRP2 mRNA in transverse sections of the maize root using *in situ* hybridization. Root cross-sections were made ca. 4 cm behind the root tip and hybridized either to antisense-strand ZRP2 RNA probe (left panel) or sense-strand ZRP2 RNA probe (right panel). Labels: e, epidermis; x, exodermis; c, cortex; E, endodermis; P, pericycle; M, metaxylem. The bar equals 150 μm.

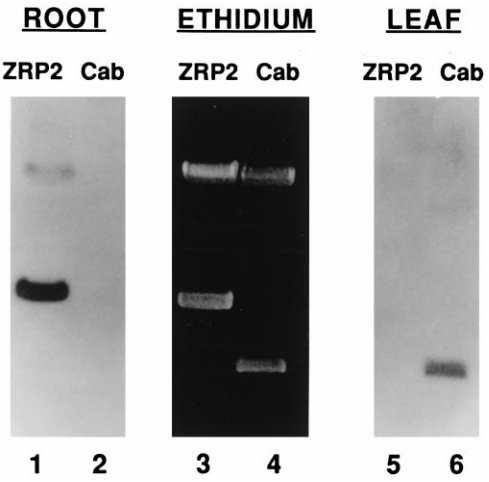

Figure 6. Transcription of the *zrp2* gene in nuclei of maize roots and leaves. ^{32}P-labeled *in vitro* transcribed RNAs from 3-day old root nuclei (lanes 1 and 2) and 5-day old leaf nuclei (lanes 5 and 6) were hybridized to subclones of *cab* (lanes 2 and 6) and ZRP2 (lanes 1 and 5). An ethidium bromide-stained gel of the ZRP2 (lane 3) and *cab* (lane 4) subclones prior to transfer to the nylon membranes is shown in the middle panel. Both the cDNA insert and plasmid vector bands are visible.

thought to have a strutural role in the plant cell wall, have a highly repeated hydroxyproline-rich motif [2, 5]. Extensins are thought to interact with metals, ions, or other proteins. The repeated motifs of these previously described proteins are completely distinct both from each other and from that of ZRP2. However, they all have in common that they are implicated as having structural rather than enzymatic roles. Non-covalent interactions with ions or other molecules is a common theme among these proteins. Plant storage proteins (e.g., zeins) also contain repeated amino acid sequences [41]. Conceivably, ZRP2 could function in root cortical cells as a previously undescribed type of storage protein.

Preliminary analysis of the 5′-upstream region has revealed some sequence features that may play a role in regulating transcription of the *zrp2* gene. Beginning at position -113 is a tandem repeat of ACGT. The ACGT sequence is the core of *cis*-acting DNA sequence elements that have been identified in many plant genes regulated by diverse environmental and physiological cues [17]. The ACGT core has been suggested to be necessary for maximal transcriptional activation and interacts with DNA-binding factors classified as bZIP proteins. A second sequence feature is an A+T-rich region between -365 and -208 of the *zrp2* gene. This region has 72% identity to the first intron of an α-tubulin gene that is expressed preferentially in the roots of maize [36]. It may be that this region contains an enhancer-like element that activates transcription in the root. DNA gel-shift assays have revealed root proteins capable of binding the *zrp2* DNA in this region [43].

The *in vitro* run-on transcription results, combined with the high level of ZRP2 mRNA detected in the root, indicate that the *zrp2* promoter may be suitable for directing a high level of transcription in the

roots of transgenic maize plants. Preliminary transient expression studies have revealed that the 4.7 kb *zrp2* upstream region does function as a promoter in maize roots [19]. Experiments involving maize plants stably transformed with *zrp2*-promoter/reporter gene constructs are on-going.

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