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Characterization and Distribution of a Maize cDNA Encoding a Peptide Similar to the Catalytic Region of Second Messenger Dependent Protein Kinases[1]

Brenda Biermann, Ellen M. Johnson, and Lewis J. Feldman*

Department of Plant Biology, University of California, Berkeley, California 94720

ABSTRACT

Maize (*Zea mays*) roots respond to a variety of environmental stimuli which are perceived by a specialized group of cells, the root cap. We are studying the transduction of extracellular signals by roots, particularly the role of protein kinases. Protein phosphorylation by kinases is an important step in many eukaryotic signal transduction pathways. As a first phase of this research we have isolated a cDNA encoding a maize protein similar to fungal and animal protein kinases known to be involved in the transduction of extracellular signals. The deduced sequence of this cDNA encodes a polypeptide containing amino acids corresponding to 33 out of 34 invariant or nearly invariant sequence features characteristic of protein kinase catalytic domains. The maize cDNA gene product is more closely related to the branch of serine/threonine protein kinase catalytic domains composed of the cyclic-nucleotide- and calcium-phospholipid-dependent subfamilies than to other protein kinases. Sequence identity is 35% or more between the deduced maize polypeptide and all members of this branch. The high structural similarity strongly suggests that catalytic activity of the encoded maize protein kinase may be regulated by second messengers, like that of all members of this branch whose regulation has been characterized. Northern hybridization with the maize cDNA clone shows a single 2400 base transcript at roughly similar levels in maize coleoptiles, root meristems, and the zone of root elongation, but the transcript is less abundant in mature leaves. *In situ* hybridization confirms the presence of the transcript in all regions of primary maize root tissue.

Roots are responsive to a wide variety of environmental stimuli, including light, gravity, and touch (2). For these and other stimuli the site of perception and transduction of the response appears to be the root cap, a structurally and physiologically distinct population of cells located at the tip of the root (2). It is hypothesized that these environmental stimuli affect root development via signal transduction pathways which operate within the cap and which, at least in part, may utilize calcium as a second messenger (13, 18).

In the eukaryotic signal transduction systems which have been characterized in detail at the biochemical level, activation of protein kinases and subsequent phosphorylation of substrate proteins generally are steps in the transduction pathway. In roots, light-, calcium-, and calmodulin-dependent phosphorylation of specific peptides have been reported, supporting the view that in plants, as in other eukaryotes, protein kinases transduce environmental stimuli (10, 13). Because these data suggest a role for calcium as a second messenger in plants (13) it is probable that specific calcium-dependent protein kinases ubiquitous in other eukaryotes (*e.g.* protein kinase C and/or calmodulin-dependent kinases) also operate in plants. The objectives of the work reported here are twofold: to obtain evidence for the existence within the root cap of second messenger-dependent kinases, and to characterize these kinases with regard to their location in the root and possible mode of action.

The research described here is based on the observation that amino acid sequences comprising the catalytic domains of protein kinases contain short regions that are highly conserved throughout the protein kinase family (4). For our work, DNA sequences encoding these conserved regions were used as probes to identify a maize root cDNA clone encoding a protein kinase. Probe sequences were designed to maximize the possibility of hybridization to serine/threonine protein kinases homologous to those which transduce extracellular signals (*e.g.* those in the cyclic nucleotide-, calcium-phospholipid-, and calcium-calmodulin-dependent subfamilies). Catalytic domains from protein kinases having similar modes of regulation or substrate specifities tend to have primary structures clustering together as branches in a phylogenetic tree of protein kinase domains (4). Therefore, identifying the phylogenetic relationships of putative protein kinase amino acid sequences can serve as a useful step in the functional characterization of new members of this family (4), such as the maize protein kinase described here.

To determine in which tissues the protein kinase might act, localization and abundance of the transcript in different regions of maize roots, in coleoptiles, and in mature leaves were examined by *in situ* hybridization or by Northern blotting analysis. Because the root cap is likely to be involved in the perception and transduction of a variety of environmental stimuli, we were interested in determining whether this kinase was localized specifically in the cap.

MATERIALS AND METHODS

Plant Tissues

Seed of *Zea mays* cv Merit (Asgrow Seed Co.) were germinated and root caps (500–750) collected from primary roots

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as described previously (3). Decapitated root tips (approximately 250/treatment) were prepared by excising the terminal 2 to 3 mm, after removal of the root cap. Tissue segments from the zone of elongation (approximately 200/treatment) were 3 to 4 mm long and located 5 to 10 mm from the root tip. Coleoptiles (approximately 100/treatment) were collected from seedlings 3 d after germination. Light treatments, where specified, were achieved using incandescent light and were applied for the entire 24 h period just prior to tissue collection.

cDNA Library Screening

Poly (A)[2] RNA prepared from primary root tips (2–3 mm, including the root cap) of 2-d-old maize seedlings was used to construct a cDNA library in lambda gt10 (cDNA synthesis and library construction by Clontech Co.). Recombinants (1×10^5) were plated at high density, and replica nylon filters successively screened with various oligonucleotide probes end-labeled with T4 polynucleotide kinase (9). Probe sequences, shown below, were degenerate; some also contained deoxyinosine residues at ambiguous codon positions (11).

	D	L	K	P	E	N	peptide
5′	GA(C/U)	(C/U)U(A/G)	AGG	CC(A/U)	GA(A/G)	AA	3′ code
3′	CT(A/G)	(A/G)A(C/T)	TTC	GG(A/T)	CT(C/T)	TT	5′ probe sequence

	G	T	P	D/E/G	Y	I/L	S/A	P	E	I/V	peptide
5′	GG(A/C/U)	AC(A/C/U)	CC(A/C/U)	G(A/C)(A/G/U)	UA(C/U)	A(C/U)(A/C/G/U)	G(A/C)C(A/C/U)	CC(A/C/U)	GA(A/G)	G(A/U)	3′ code
3′	CCI	TGI	GGI	C(C/T)(C/T)	ATG	IA(C/I)	A(C/G)I	GGI	CTT	(C/T)A	5′ probe sequence

	L	A	D	F	G	L	A	peptide
5′	(C/U)U(A/C/G/U)	GC(A/C/G/U)	GA(C/U)	UU(C/U)	GG(A/C/G/U)	(C/U)U(A/C/G/U)	GC	3′ code
3′	(A/G)A(C/I)	CG(C/I)	CTG	AAG	CC(C/I)	GA(C/I)	CG	5′ probe sequence

DNA Sequence Analysis

Restriction fragments of the cDNA (clone 90.7) were subcloned into Bluescript plasmid cloning vector (pBS-SK$^+$, Stratagene Co.), and plasmid 'miniprep' templates sequenced with Sequenase (United States Biochemicals) following manufacturer's protocols. Both strands were sequenced completely. FASTN and FASTA (12) were used for database searches.

RNA Gel Blot Analysis

Total RNA was prepared from plant tissues as described previously (3). A batch oligo dT chromatography procedure was used to purify poly (A) RNA (9). RNAs were separated on 1.5% agarose formaldehyde gels, then transferred onto nylon membranes in 10 × SSC buffer (9), but with no prior treatment of the gel. The cloned cDNA was labeled by the

[2] Abbreviation: poly (A), polyadenylated RNA.

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GCAAGCCTCACAAGTCAAACGACTCAAAGTGGGAAGCGATTCAGGTTGTC      50
  K  P  H  K  S  N  D  S  K  W  E  A  I  Q  V  V
CGAACCAAAGAAGGTTCTGTAGGTCTAGGTCATTTCAGGCTGCTCAAGAG     100
 R  T  K  E  G  S  V  G  L  G  H  F  R  L  L  K  R
GCTCGGTTGTGGTGATATTGGCAGTGTGTACTTATCAGAACTTAGTGGCA     150
  L  G  C  G  D  I  G  S  V  Y  L  S  E  L  S  G
CTAAATGCTATTTTGCCATGAAGATCATGGACAAGGCGTCATTAGCAAGC     200
T  K  C  Y  F  A  M  K  I  M  D  K  A  S  L  A  S
CGCAAAAAGCTGCTTAGAGCGCAGACTGAAAGGGAGATATTGCAATGCCT     250
 R  K  K  L  L  R  A  Q  T  E  R  E  I  L  Q  C  L
GGATCATCCTTTTCTTCCAACATTGTACACTCACTTTGAGACGGATAAGT     300
  D  H  P  F  L  P  T  L  Y  T  H  F  E  T  D  K
TCTCATGCTTGGTGATGGAGTTCTGCCCTGGAGGTGACCTACATACCCTA     350
F  S  C  L  V  M  E  F  C  P  G  G  D  L  H  T  L
AGGCAAAAGCAGCCTGGCAAGTATTTCCCTGAACAAGCTGCCAAGTTTTA     400
 R  Q  K  Q  P  G  K  Y  F  P  E  Q  A  A  K  F  Y
TGTAGCAGAGGTGCTTCTTGCTCTGGAATACCTGCACATGCTTGGTATCA     450
  V  A  E  V  L  L  A  L  E  Y  L  H  M  L  G  I
TATACCGTGACTTAAAGCCAGAGAATGTTCTTGTGAGGGAAGATGGACAT     500
I  Y  R  D  L  K  P  E  N  V  L  V  R  E  D  G  H
ATCATGCTGTCGGATTTCGATTTATCCCTCCGATGTGCTGTGAGCCCTAC     550
  I  M  L  S  D  F  D  L  S  L  R  C  A  V  S  P  T
TCTTCTCAGATCTTCTAACCCCAGTGGGGATAACCAAAAGGGCAACCCAG     600
  L  L  R  S  S  N  P  S  G  D  N  Q  K  G  N  P
CTTACTGTGTGCAGCCTGTGTGCATTGAGCCTGCCTGTATGCAGCCTTCT     650
A  Y  C  V  Q  P  V  C  I  E  P  A  C  M  Q  P  S
TGTGTCACGACAACCACATGCTTCTCTCCTCGCTTTTTCTCTTCCAAATC     700
 C  V  T  T  T  T  C  F  S  P  R  F  F  S  S  K  S
CAAGGAGAAAAAAGATAAAAAGGCAAAGGCAGACTGGGCAAATCAGGTCA     750
  K  E  K  K  D  K  K  A  K  A  D  W  A  N  Q  V
GACCACTTCCTGAGCTTGTTGCAGAGCCGACAGATGCAAAGTCTATGTCT     800
R  P  L  P  E  L  V  A  E  P  T  D  A  K  S  M  S
TTTGTTGGGCACCCATGAGTACTTGGCACCTGAAATTATAAAGGGCGAGGG     850
 F  V  G  T  H  E  Y  L  A  P  E  I  I  K  G  E  G
GCATGGAAGTGCTGTAGACTGGTGGACATTTGGCATATTCTTGTATGAGC     900
  H  G  S  A  V  D  W  W  T  F  G  I  F  L  Y  E
TTCTCTTCGGCAAGACACCATTCAAAGGTTCTGGGAATAGGGCGACATTG     950
L  L  F  G  K  T  P  F  K  G  S  G  N  R  A  T  L
TTCAACGTAGTTGGACAACCCTTAAGGTTCCCAGAATCACCTGTAGTGAG    1000
 F  N  V  V  G  Q  P  L  R  F  P  E  S  P  V  V  S
TTTTGCTGCAAGGGATCTTATTAGGGGATTGCTCATCAAGGAACCTCAGC    1050
  F  A  A  R  D  L  I  R  G  L  L  I  K  E  P  Q
ATCGATTAGCGTATAAGCGTGGAGCCACAGAAATAAAACAGCACCCGTTC    1100
H  R  L  A  Y  K  R  G  A  T  E  I  K  Q  H  P  F
TTTGAAGGTGTTAATTGGGCATTAATCAGGTGTGCCACTCCACCTGATAT    1150
 F  E  G  V  N  W  A  L  I  R  C  A  T  P  P  D  I
CCCCAAGCCAGTTGAGATCCCTCGTTCTGTAGCTTCATCAAGCCAGAAGG    1200
  P  K  P  V  E  I  P  R  S  V  A  S  S  S  Q  K
CTACATCAGCCGCTGAGAAAGGCTCAGATTATCTTGAATTGGAGTTTTTT    1250
A  T  S  A  A  E  K  G  S  D  Y  L  E  L  E  F  F
TAGTGGTCACGCATGATCTCCCATGTGCAAGTTGTAAAGCTCTACCTTTT    1300
 *
CCACTTAGACTTCTTTTTTTATGGGTTTTAATAACTTTATATGTAGATGC    1350
ATATTGTCGTACGTAGTGCTGTCCTCTTCAGTTGATTTCTCAGTTCATTC    1400
CTTTAGATGGGATCCTCAATCTGGTTGCAGATGCCCTTGTACAGATAATC    1450
TGCCCCTACCCAGTTTGTTTAAAACGCAAACAACGCCATTTTGGTTTTAG    1500
TTCCCTGGAGAAGGATAGAGGAGAAGGTGTGTGTCTTTGTAGTGTGAGAA    1550
GCATGGCTCTGTAGTGTTGTTGTTATAGTGTACTACTACTGTGCTAGCCA    1600
GCCGCATACACAGACTGTTTATTCTTTGGAGACGAATCAATTCTTTCATC    1650
TTGACTGGGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA    1700
AAAAAAAA                                              1708
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Figure 1. Nucleotide sequence and deduced amino acid sequence of cDNA clone 90.7. The deduced amino acid sequence is shown only for the long open reading frame. Sequences hybridizing to the oligonucleotide probes are underlined.

random hexamer primer method (Multiprime Kit, Amersham) for use as a hybridization probe. Standard protocols were followed for hybridizing and rinsing blots (9). Hybridization was at 50°C with formamide added to 50% (v/v); rinses were in 0.3 × SSC at 68°C.

In Situ RNA Analysis

Hybridizations were performed *in situ* using ^{35}S-UTP-labeled transcripts as probes. The plasmid, p90.7, which contained cDNA 90.7 cloned into the *Eco*RI site of pBluescript SK$^+$ (Stratagene Co.), was linearized with *Xba*I or *Hin*dIII, and used as a template for *in vitro* transcription of antisense or sense transcripts, respectively. Limited alkaline hydrolysis was used to reduce transcript size to approximately 100 to 150 bases. Roots from 2-d-old, dark-grown maize seedlings (cv Merit) were fixed and embedded by standard techniques (6), then sectioned to 10 μm. Pretreatment, hybridization, and autoradiography were carried out as described by Raikhel *et al.* (15), except that hybridization and washes were performed at 42°C, and slides were developed after 1 week of exposure. A Zeiss Axiophot microscope (Carl Zeiss Co.) was used to observe cell morphology. Silver grains were detected using differential interference contrast optics. Silver grain density was determined by manually counting grains in computer images representing equivalent areas from different regions of the root (NIH Image version 1.23 for the Macintosh computer). These values were used to calculate the 'fold increase' in grain density in a given region, relative to grain density in background areas.

RESULTS

Partially degenerate inosine-containing oligonucleotides corresponding to the three peptide sequences, peptide sequence DLKPEN, GTP(D/E/G)Y(I/L)(S/A)PE(I/V) and LADFGLA, were used to successively probe a cDNA library from maize root tips. These probes encode conserved regions of protein serine/threonine kinase catalytic regions. Out of 10^5 λ clones screened, several clones hybridized to each individual probe, and one clone hybridized to the first two probes. This cDNA clone (90.7) was further characterized by DNA sequence analysis (Fig. 1). It contains an open reading frame from nucleotides 3 to 1253. The lack of the 5′ untranslated region, and size of the mRNA (see below) indicates that the cDNA is incomplete at the 5′ end. A 3′ untranslated region 407 bases in length precedes the 3′ poly (A) sequence. No canonical poly (A) signal sequence was detected.

The deduced polypeptide of 90.7 contains all but one of the conserved features characteristic of protein kinases (Fig. 2). These include amino acids corresponding to the consensus Gly^{50}XxxGly^{52}XxxXxxGly^{55}, which forms part of the nucleotide binding site; Lys^{72}, which appears to be necessary for ATP binding and phosphotransfer, based on mutagenesis studies; Asp^{166}, Asn^{171}, and Asp^{184}, which are also implicated in ATP binding; and Ala^{206}Pro^{207}Glu^{208}, a key protein kinase catalytic domain indicator (as reviewed by Hanks *et al.* [4]). (Amino acid positions are referred to using the residue numbering for bovine cAMP-dependent protein kinase, Fig. 2). The 90.7 deduced polypeptide also contains the other highly conserved amino acids corresponding to Val^{57}, Ala^{70}, Glu^{91}, Phe^{185}, Asp^{220}, Gly^{225}, and Arg^{280}. The only invariant amino acid not conserved in the maize homolog is Gly^{186}, which is replaced by an aspartate residue (Fig. 2). The peptide encoded by cDNA clone 90.7 contains all of the 18 nearly invariant residues described by Hanks *et al.* (4) as characteristic of protein kinase catalytic domains (data not shown). Alignment of all 65 known protein kinase catalytic domain sequences showed alternating regions of high and low amino acid conservation, with 11 major conserved subdomains evident (4). The regions of highest peptide sequence homology between

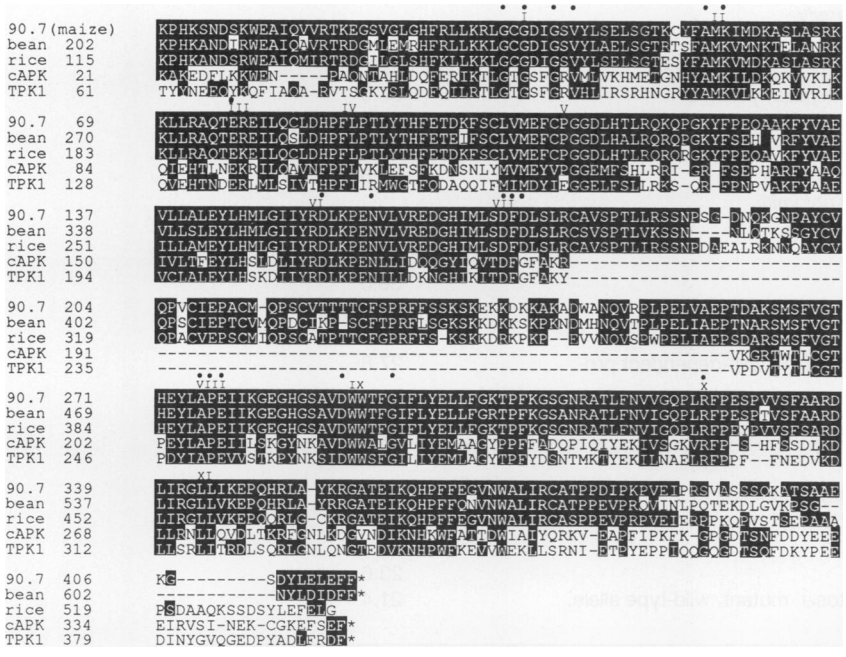

Figure 2. Comparison of the deduced amino acid sequence of maize cDNA clone 90.7, protein kinase homologs cloned from bean and rice (8), α-form of the catalytic subunit of bovine cAMP-dependent protein kinase (cAPK, ref. 19), and type 1 yeast cAMP-dependent protein kinase catalytic subunit (TPK1, ref. 20). Amino acids identical or chemically similar to 90.7 are in shaded boxes. Chemically similar groupings used are: nonpolar chain R groups (C, M, L, I, V); aromatic or ring-containing R groups (F, Y, W, and H); small R groups with near neutral polarity (A, G, S, T, and P); acidic and uncharged polar R groups (D, E, N, and Q), and basic polar R groups (K, R, and H). Dashes indicate gaps introduced to optimize the alignment. Roman numerals indicate the eleven major conserved subdomains of protein kinases denoted by Hanks *et al.* (4). Dots are placed above residues that are referred to in the text.

the deduced 90.7 gene product and animal and yeast protein serine/threonine kinases include all of the conserved subdomains (Fig. 2).

The catalytic domain encoded by maize cDNA 90.7 is most similar to that of the cyclic nucleotide- and calcium-phospholipid-dependent subfamilies of serine-threonine kinases (Table I), having 35% or more sequence identity to all members of that branch of protein kinases. The similarity is slightly greater to cyclic nucleotide-regulated than to calcium-phospholipid-regulated protein kinases (Table I). The region comprised of subdomains VI to IX, in which different protein kinases have greatest homology (4), was also the region in which the 90.7 deduced polypeptide and catalytic domains of second messenger-regulated protein kinases had the greatest frequency of conserved residues (Fig. 2). There was less similarity between the maize kinase peptide sequence described here and catalytic domains of protein serine-threonine kinases in other subfamilies, including members of the calcium-calmodulin-dependent protein kinase subfamily, relatives of yeast protein kinases involved in cell division cycle, and relatives of yeast SNF1 protein kinase (Table I). Peptide sequence identities for these kinases range from approximately 23 to 28% (Table I). Similarity to protein tyrosine kinases was considerably less (data not shown).

The deduced maize peptide sequence contains a region of 81 amino acids inserted into the catalytic domain in a location similar to that of a nonhomologous 79 amino acid insert in the protein kinase encoded by the yeast CDC7 gene (Fig. 2).

The peptide encoded by cDNA clone 90.7 is 77% and 81% identical, respectively, to peptides encoded by cDNA clones from bean cell suspension cultures and from rice leaves, described recently by Lawton *et al.* (8) (Fig. 2). Regions of high peptide sequence identity include the complete catalytic domain, most of the inserted region, and part of the short region C-terminal to the catalytic region.

Northern analysis showed roughly similar levels of the predominant hybridizing transcript of 2400 bases in maize root tips, root cells in the region of elongation, and in coleoptiles (Fig. 3A). Mature leaves contained proportionately less of this transcript (Fig. 3A). Expression in coleoptiles and in terminal root sections including tissue from the root cap and meristematic zone was not regulated by light (Fig. 3B). Examination of the transcript accumulation within specific regions of the root was accomplished through *in situ* hybridization analysis, which permits increased resolution compared to that provided by Northern blotting. The results of this analysis showed that the transcript was present in the root cap and accumulated to levels which, although relatively low, were nevertheless consistently above background. A similar level of accumulation of the transcript was found in most other dark-grown root tissues, including meristematic tissue, immature vascular and cortical cells and mature tissues within the zone of elongation (Figs. 4, 5). Moreover, illumination of the root did not alter transcript accumulation, either in absolute amount or in location (Fig. 6). Thus, the localization of the 90.7 transcript is not confined to the root cap. Rather this transcript is more generally expressed in a variety of cell types within the root.

Table I. *Sequence Identity between the Polypeptide Encoded by Maize cDNA Clone 90.7 and the Catalytic Domains of Serine/Threonine Protein Kinases*

To conserve space, sequence references and alignments are not shown, because both can be found in a review by Hanks *et al.* (4).

Subfamily and Member Peptide	Percent Identity to 90.7
Cyclic nucleotide-dependent subfamily	
cAMP-dependent protein kinase, α-subunit, bovine	38.2
cAMP-dependent protein kinase, β-subunit, bovine	39.0
TPK1, cAMP-dependent protein kinase, yeast	37.0
TPK2, cAMP-dependent protein kinase, yeast	35.4
TPK3, cAMP-dependent protein kinase, yeast	37.0
cGMP-dependent protein kinase, bovine	36.6
Calcium-phospholipid-dependent subfamily	
PKC, protein kinase C, α-form, bovine	36.2
PKC, β-form, bovine	37.0
PKC, γ-form, bovine	36.6
Drosophila gene product related to PKC	35.8
Calcium-calmodulin-dependent subfamily	
CaM II-a, multifunctional calcium-calmodulin-dependent protein kinase, α-subunit, rat	27.8
SNF1 subfamily	
kin1, putative yeast protein kinase	26.9
CDC28-cdc2+ subfamily	
CDC28, 'cell-division-cycle' gene product, yeast	25.4
CDC2HS, functional homolog of cdc2, human	26.1
No close relatives	
CDC7	23.6
wee 1^+, 'reduced cell size at mitosis' mutant, wild-type allele, yeast	21.4

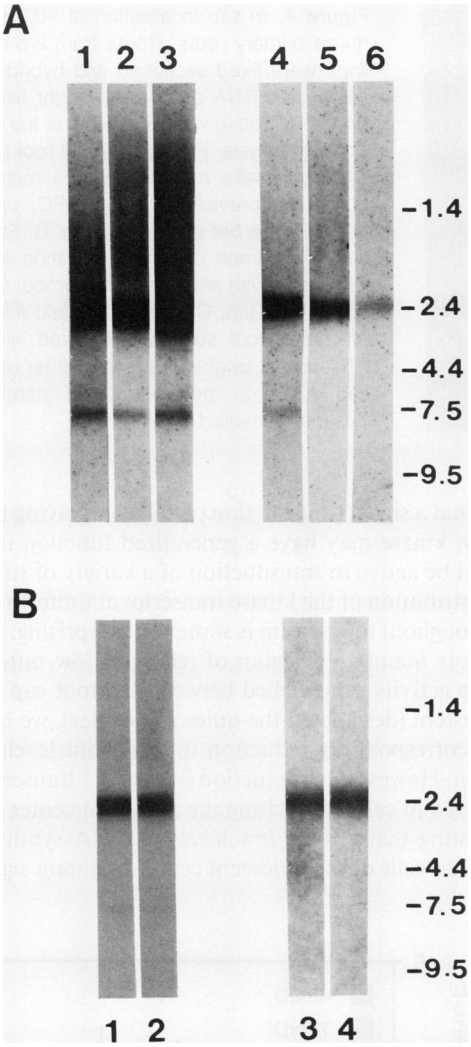

Figure 3. RNA gel blot analysis of transcripts hybridizing to cDNA clone 90.7. A, Two (lanes 1–3) or 0.5 (lanes 4–6) μg poly (A) RNA isolated from maize coleoptiles (lanes 1 and 4), decapitated root tips (lanes 2 and 5), root elongation zone (lane 3), or mature leaves (lane 6) were electrophoresed. B, Samples electrophoresed were 2 μg poly (A) RNA isolated from maize coleoptiles (lanes 1 and 2), or 0.5 μg poly (A) RNA from root tips (lanes 3 and 4). Tissues were from plants grown in darkness (lanes 1 and 3) or given a 24 h light treatment (lanes 2 and 4).

DISCUSSION

In animal and fungal systems protein phosphorylation/dephosphorylation is known to be an integral part of the basic mechanism regulating cellular responses to environmental stimuli (5). There is ample evidence that phosphorylaton of a wide variety of plant proteins by kinases occurs *in vivo*, and that this posttranslational modification modifies the activity of some plant enzymes (16). However, progress in understanding the regulatory role of plant protein phosphorylation has been limited by lack of defined phosphorylated substrates of known protein kinases (16). In plants the most detailed *in vivo* studies on the effects of environmental stimuli on protein phosphorylation have been conducted on light- and gravity-stimulated maize roots. For both of these stimuli the root cap is believed to be the site of perception, and also is hypothesized involved with the transduction of the stimulus leading to a particular developmental response. Recent studies have revealed that a few specific proteins are rapidly phosphorylated in response to light and calcium application, and that calmodulin is probably involved in this response (10, 14). Here we present further evidence for the existence of a second messenger-dependent protein kinase in maize roots, and also describe the primary structure and the spatial distribution of gene expression within a tissue known to transduce a variety of environmental stimuli.

A maize cDNA clone similar to protein serine/threonine kinases was isolated from a root tip cDNA library by probing with degenerate deoxyinosine-containing oligonucleotides corresponding to conserved regions of the catalytic domain. (Probe sequences were designed to maximize the possibility of hybridization to protein kinases homologous to those which function in transduction of extracellular signals in animals and fungi). The deduced polypeptide sequence contains all but one of the highly conserved amino acids characteristic of the catalytic region of protein kinases, some of which are thought necessary for ATP binding, phosphate transfer and target peptide binding (4). Regions of frequent amino acid similarities between the deduced maize polypeptide and mammal and yeast protein kinases correspond to catalytic region subdomains which are conserved in all known protein kinases (Fig. 2).

The catalytic domains from protein kinases having similar modes of regulation or substrate specifities tend to have similar structures; thus, they are clustered together in a phylogenetic tree deduced from amino acid alignment data (4). Therefore, the protein kinase subfamily affiliation of the protein kinase catalytic domain deduced from the maize cDNA sequence was determined in order to predict its functional properties. Generally, sequences found within a branch cluster or subfamily of protein kinases share 35% or more identical amino acids with other sequences in the cluster, whereas catalytic domain sequences which do not map within the same branch or subfamily have identities about 20 to 25% (4). The encoded catalytic domain of the maize cDNA described here is most similar to the branch of serine/threonine protein kinases containing the cyclic nucleotide- and calcium-phospholipid-dependent protein kinases, and the degree of homology is equal to that predicted if 90.7 encoded a new member of this branch (Table I). All members of this group transduce extracellular signals via regulation of their activity by second messengers (cAMP, cGMP, calcium, and diacylglycerol), indicating that the 90.7 homolog may mediate transduction of extracellular signals in maize roots. It is unclear whether the maize gene encodes a regulatory region as well as the catalytic domain. The size of the mRNA to which cDNA 90.7 hybridizes indicates that the complete clone could encode as many as 200 amino acids N-terminal to the catalytic region. The corresponding regions of homologous rice and bean clones (sequenced by Lawton *et al.* [8]) are not similar to each other nor to any known sequences (including protein kinases). Furthermore, the size of the mRNA to which 90.7 hybridizes limits the size of the unsequenced portion to

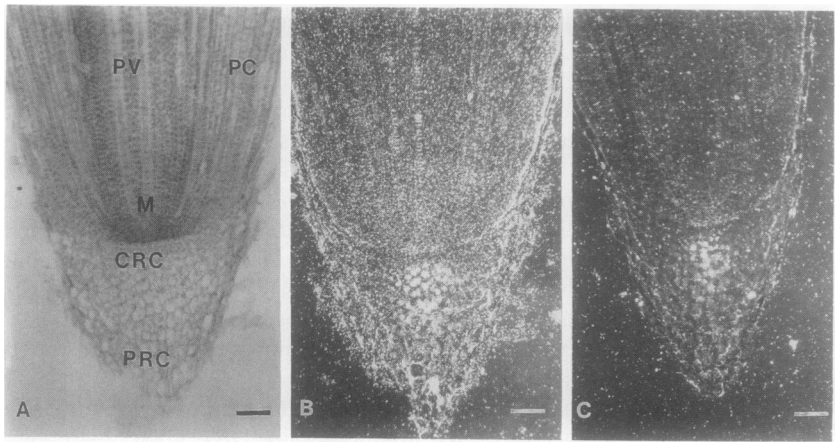

Figure 4. *In situ* localization of 90.7 mRNA in maize primary roots. Roots from 2-d-old seedlings were fixed sectioned and hybridized with ^{25}S-labeled RNA probes. A, Bright field micrograph illustrating various regions of the root chosen for analysis. PRC, peripheral root cap cells; CRC, columellar root cap cells; M, meristematic zone; PV, prevascular tissue; PC, precortical tissue. Scale bar equals 100 μm. B, False dark field micrograph of identical section as in (A), hybridized with antisense RNA probe. Scale bar equals 100 μm. C, False dark field micrograph of control root section, hybridized with sense RNA probe. Bright area in the center of the root cap is due to the presence of starch grains. Scale bar equals 100 μm.

a size too small to contain the conserved regulatory regions known for second messenger-regulated kinases.

The protein kinase catalytic domain from maize is highly similar to protein kinase catalytic domains from bean and rice recently described by Lawton *et al.* (8). indicating strong selective pressure for conservation of its structural features. The catalytic region is not appreciably more similar to rice (which, like maize, is a monocot) than to bean (a dicot), thus suggesting that the function of the protein kinase may be similar in both monocots and dicots.

Because the kinase transcript is not preferentially localized to the root cap it would seem unlikely that it functions exclusively in the transduction of those stimuli perceived and processed by the cap (*e.g.* light, gravity, touch). Rather, the distribution of the transcript in both root and shoot tissues suggests that a signal transduction pathway involving this type of protein kinase may have a generalized function in plants and could be active in transduction of a variety of stimuli.

The distribution of the kinase transcript at uniform but low levels throughout the root tip is somewhat surprising. Because maize roots maintain a region of relatively low mitotic and metabolic activity sandwiched between the root cap and the root meristem (designated the quiescent center), we expected to see a corresponding reduction in transcript levels within this region. However, no reduction in the 90.7 transcript level was detected in cells comprising the quiescent center (Figs. 4, 5), suggesting that despite low levels of DNA synthesis and mitosis, that cells of the quiescent center maintain significant

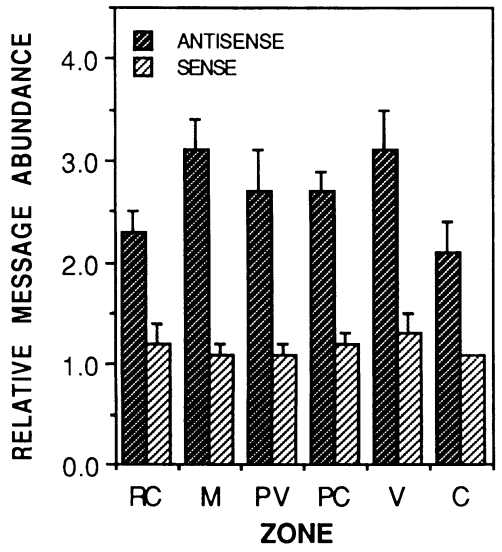

Figure 5. Graph depicting the relative message abundance (or 'fold increase' in silver grain density above background) in various regions of the root (RC, root cap; M, meristem; PV, prevascular tissue; PC, precortical tissue; V, vascular tissue; C, cortical tissue). Silver grain density was determined for all regions in each of five replicates for both antisense- and sense-probed sections. Values shown represent the mean ± SE.

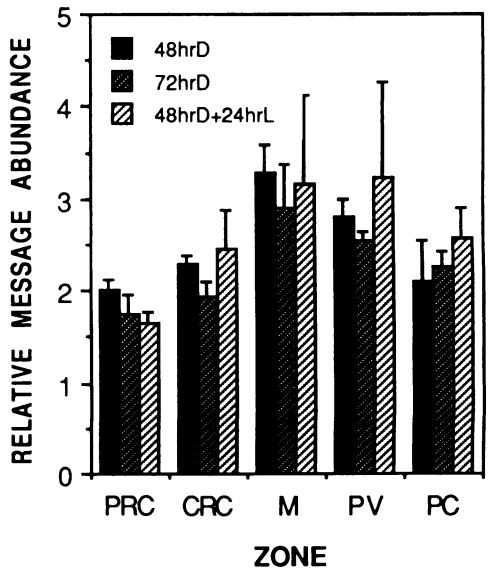

Figure 6. Graph depicting the relative message abundance in five regions of interest in root tips (PRC, peripheral root cap cells; CRC, columellar root cap cells; M, meristematic zone; PV, prevascular tissue; PC, precortical tissue) exposed to one of the following light conditions: 48 h darkness (48D), 72 h of darkness (72D), or 48 h of dark followed by 24 h of continuous white light (48D + 24W). For each light treatment, all zones were quantified in each of three replicates. Values shown represent the mean ± SE.

levels of transcriptional activity, at least for the 90.7 transcript. (Similar results, however, have been obtained for transcripts for alcohol dehydrogenase, calmodulin, phytochrome, and α-amylase [our unpublished results]). Thus, it should be emphasized that the notion of 'quiescence' is based mainly on observations which reflect cell division activity and that the characterization of certain cells in the meristem as quiescent should not imply an overall lack of transcriptional activity. Light did not regulate the level of the mRNA encoding this protein kinase. This finding contrasts with data showing that light can regulate the levels of many mRNAs within the root cap, including alcohol dehydrogenase, aldolase, and the α and β subunits of mitochondrial ATPase (3). Hence it is probable that, if the 90.7 kinase protein is associated with light transduction, the mechanism by which the light affects root development is not at the level of the regulation of 90.7 transcript abundance.

In plants, other components of a hypothetical second messenger-regulated transduction system have recently been identified. Tobacco plants contain enhancer elements identical to those involved in cAMP-regulated transcription in mammals (7). Plants also contain proteins which bind to these enhancer elements, and which show peptide homology to the protein that binds to the mammalian cAMP-responsive enhancer elements (7). These findings suggest the possible operation of a similar signal transduction system in higher plants (7). There is strong evidence that activation of cyclic nucleotide- and calcium-phospholipid-dependent protein kinases is part of the pathways leading to transcriptional induction of mammalian genes containing second messenger-responsive enhancer elements (1, 17). Our work showing the existence of a plant protein similar to the catalytic region of second-messenger-regulated protein kinases is further evidence for the operation of such a pathway in plants. We plan to characterize substrates and second messenger regulators of the maize protein kinase, thus defining particular steps of the pathway.

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