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Isolation and characterization of cDNA clones encoding a functional p34^{cdc2} homologue from *Zea mays*

(cell division/plant development/*cdc2* gene/protein kinase)

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ABSTRACT **We describe the isolation of cDNA clones encoding a p34^{cdc2} homologue from a higher plant, *Zea mays* (maize). A full-length cDNA clone, *cdc2ZmA*, was isolated, sequenced, and shown to complement a *cdc28* mutation in *Saccharomyces cerevisiae*. Comparison of the deduced amino acid sequence of the maize p34^{cdc2} protein with other homologues showed that it was 64% identical to human p34^{cdc2} and 63% identical to *Schizosaccharomyces pombe* and *S. cerevisiae* p34^{cdc2} proteins. Studies of expression of the maize *cdc2* gene(s) by Northern blot analysis indicated a correlation between the abundance of *cdc2* mRNA and the proliferative state of the tissue. Southern blot analysis, as well as isolation of another cDNA clone, *cdc2ZmB*, which is 96% identical to *cdc2ZmA*, indicates that maize has multiple *cdc2* genes.**

The p34^{cdc2} protein kinase has been found in a wide variety of yeast and animal species and is believed to be a central component of the mechanism controlling cell division in eukaryotes (1, 2). The p34^{cdc2} protein kinase was first identified as the product of the *CDC28* gene of *Saccharomyces cerevisiae* (3), and later as the product of the *cdc2* gene of *Schizosaccharomyces pombe* (4). In both *S. cerevisiae* and *Sch. pombe* the product of this gene is required for progression through the G_1/S and G_2/M transitions of the cell cycle (5–8). The central role played by *cdc2* in the eukaryotic cell cycle became apparent when a *cdc2* homologue was identified as part of M-phase-promoting factor (MPF), the multiprotein complex required to stimulate *Xenopus* and starfish oocytes to undergo meiosis (9–11). The p34^{cdc2} protein kinase is activated at M phase, possibly by dephosphorylation of a tyrosine residue at its ATP binding site (12), and is thought to phosphorylate key proteins that lead to changes associated with M-phase-specific events, including chromosome condensation, spindle assembly and reorganization of the cytoskeleton, breakdown of the nuclear envelope, and changes in cell shape (2, 13). The catalytic activity of p34^{cdc2} is regulated by its interaction with a number of other proteins (1, 14), most notably the cyclins, which are required for activation of p34^{cdc2} and entry into M phase (14, 15), and the *suc1* gene product, which has a high affinity for p34^{cdc2} and appears to be required for exit from M phase (1). A remarkable aspect of the p34^{cdc2} kinase complex is the extent of conservation between species that are phylogenetically distant. The *cdc2* and *CDC28* genes of *Sch. pombe* and *S. cerevisiae* are functionally interchangeable (4); moreover, the human *cdc2* homologue can complement the *cdc2* defect in *Sch. pombe* (16, 17). The amino acid sequences of all *cdc2* homologues show regions of absolute conservation that are probably essential for interaction with other components of the cell cycle machinery.

Multicellular organisms require a highly regulated program of cell proliferation during the course of their development. Experiments with *Xenopus* and *Drosophila* have demonstrated that several proteins involved in mitotic control are of central importance to the development of these organisms (18, 19). We have initiated studies to determine how regulation of cell division is coupled to development in a higher plant. Immunological crossreactivity indicates that there is a 34-kDa protein in the green alga *Chlamydomonas*, as well as in *Arabidopsis* and oats, that is recognized by *cdc2*-specific antibodies; this suggests that a homologue of p34^{cdc2} exists in higher plants (20). Recently, antibodies to *Sch. pombe* cdc2 protein have been used to identify a polymerase chain reaction (PCR) product from pea cDNA that has strong homology to the internal conserved domain of both human and yeast cdc2 proteins (21). Here we describe the isolation and characterization of cDNA clones encoding cdc2 homologues from a higher plant, *Zea mays* (maize), and demonstrate that one of these clones encodes a functional p34^{cdc2} protein.*

MATERIALS AND METHODS

Isolation of Maize *cdc2* cDNAs. For synthesis of cDNA, 2 μg of poly(A)$^+$ RNA from apical meristem tissue of the maize inbred strain B73 was incubated in PCR buffer (10 mM Tris·HCl, pH 8.3/50 mM KCl/1.5 mM $MgCl_2$/0.01% gelatin) containing 0.5 mM dNTPs, 40 units of RNasin (Boehringer Mannheim), 150 pmol of random hexamer primer mix and 60 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) in a final volume of 40 μl. The reaction mixture was incubated for 10 min at room temperature, shifted to 42°C for 30 min, and then heated at 95°C for 10 min to terminate the reaction. Half of the above reaction mix was brought to 50 μl with PCR buffer, 1 unit of *Taq* polymerase (Perkin–Elmer), and 8 μM each degenerate oligonucleotide. The degenerate oligonucleotides used were synthesized based upon two conserved regions of the cdc2 protein, GTYGVVYK and HRDLKPQN. The conditions for PCR were 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 30 cycles. A 360-base-pair (bp) maize *cdc2* probe generated by the PCR was used to screen a maize cDNA λZAP (Stratagene) library constructed with poly(A)$^+$ RNA of B73 seedlings (gift of Alice Barkan, University of California, Berkeley). Approximately 10^6 plaques were screened as described (22).

Complementation of a *S. cerevisiae cdc28* Mutation. To complement a *cdc28* mutation of *S. cerevisiae*, the open reading frame of the *cdc2ZmA* cDNA was cloned into the vector pMR438 so that expression was under the control of the yeast *GAL1* promoter. Several recombinant plasmids, with the *cdc2ZmA* open reading frame in both orientations

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Abbreviation: DAP, day(s) after pollination.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60526).

relative to the *GAL1* promoter, were isolated and used to transform a *S. cerevisiae* strain that carried the *ura3* and the temperature-sensitive *cdc28-1N* mutations (6, 7). Recombinant plasmid or pMR438 DNA was introduced into yeast cells by electroporation, and transformants were selected on minimal medium containing glucose and lacking uracil. Several transformants were then restreaked onto duplicate minimal medium plates containing 2% galactose and grown at 25°C or 37°C.

Histone H1 Kinase Assays. Recombinant $p13^{suc1}$ protein was purified from bacteria and coupled to CNBr-activated Sepharose CL-4B beads (Pharmacia), as described (23). Maize nuclear extract from mature leaf or apical meristems (Z. Zhao and V.S., unpublished work), or total yeast lysate from a logarithmic-phase culture of yeast cells (24), was incubated with 20 μl of a 50% (wt/vol) suspension of $p13^{suc1}$ beads for 2 hr at 4°C. The beads were washed and assayed for H1 kinase activity (25). Products of kinase assays were analyzed by SDS/10% PAGE followed by autoradiography.

RESULTS

Isolation of a *cdc2*-Homologous Sequence from Maize. The remarkable conservation of the $p34^{cdc2}$ kinase gene between highly diverse species allowed us to use the PCR to isolate homologous sequences from maize. The *cdc2* genes isolated from *Sch. pombe* (4), *S. cerevisiae* (3), and human (17) contain regions of identity at the amino acid level that are common to all *cdc2* genes isolated thus far. The amino acid sequence GTYGVVYK near the N terminus of the protein contains part of the motif (GXGXXGXV) that is characteristic of ATP-binding domains of other protein kinases (26). A region ≈100 amino acids further downstream, HRDLKPQN, is conserved in the *cdc2* gene family and contains several residues that are also conserved among many protein kinases. Degenerate oligonucleotide primers to the GTYGVVYK and HRDLKPQN regions were used in a PCR amplification with maize cDNA as template. The 360-bp PCR product was cloned into pUC118 and five recombinants were sequenced. Four of the clones contained the EGVPSTAIR motif characteristic of the *cdc2* gene family. The 360-bp insert from one of these clones was used as a probe in screening a cDNA library and for Southern and Northern blot analysis.

Isolation and Sequence Analysis of Maize *cdc2* cDNA Clones. Nine positive phage clones were isolated from the maize cDNA library; six had inserts of ≈1.4 kilobases (kb) and three had inserts slightly less than 1.4 kb. The 1.4-kb inserts corresponded in size to the specific band observed on Northern blots screened with the same 360-bp probe (see below). Inserts larger than 1.4 kb were not found, suggesting that this cDNA most likely represents the full-length or near-full-length transcript for the maize *cdc2* gene. Sequence analysis of the extreme 5′ and 3′ ends of each clone showed that there were two classes of closely related, but different, cDNA clones: eight of one class (*cdc2ZmA*) and one of the other class (*cdc2ZmB*). The eight clones from the *A* class varied at their 3′ ends only in the point of addition of the poly(A)tail, but they were identical where internal sequences overlapped. The single clone of the *B* class was truncated by ≈240 bp at its 5′ end as compared with the longest clone of the *A* class. One clone from class *A* and the only class *B* clone were sequenced and shown to be 96% identical at the nucleotide

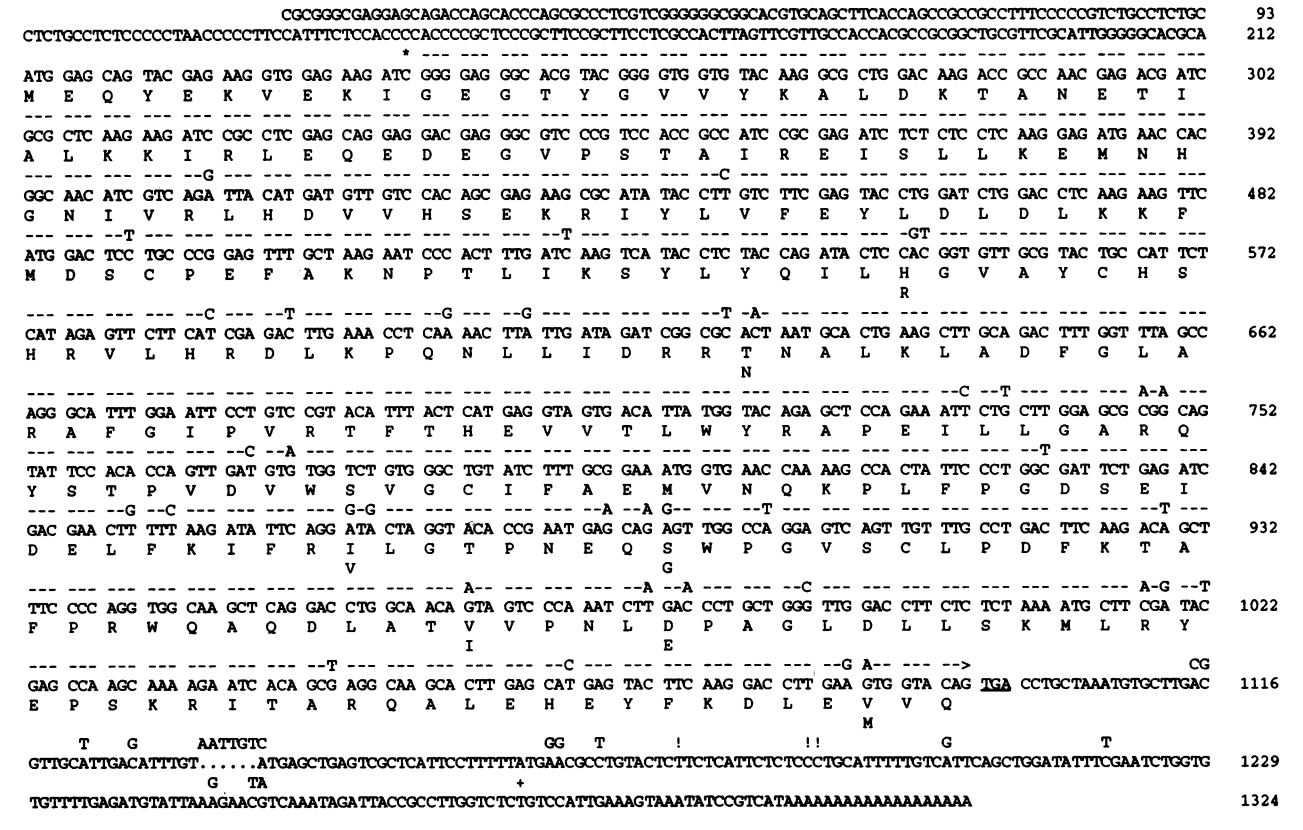

FIG. 1. Nucleotide and deduced amino acid sequences of the *cdc2ZmA* and *cdc2ZmB* clones. Nucleotide differences between *A* and *B* clones are shown above each line and amino acid differences are shown below. The beginning of the *cdc2ZmB* cDNA clone is indicated by an asterisk and the end of the clone by a plus sign; nucleotides absent in the *cdc2ZmB* sequence are shown by exclamation marks. The highly conserved cdc2 peptide domains upon which the degenerate oligonucleotides were based are amino acid residues 13–20 and 125–132. The 360-bp probe extends from the beginning of the first conserved region (nucleotide 248) and extends to the last codon of the last conserved region (nucleotide 608). The 927-bp probe, which contains the open reading frame of *cdc2ZmA*, begins at the ATG codon (nucleotide 213) and extends 43 bp beyond the TGA stop codon (nucleotide 1139).

level (Fig. 1). Within the open reading frame deduced from the cDNA sequence, clones *cdc2ZmA* and *cdc2ZmB* differ by only 7 amino acids; these substitutions are neutral or conservative except possibly for the His/Arg difference at position 113. The translated amino acid sequence suggests that the *cdc2ZmB* cDNA clone is missing only 10 amino acids at the N terminus. Maize therefore appears to have at least two closely related but distinct *cdc2* genes, with most of the differences in the C-terminal region of the protein; this region is usually the most variable between *cdc2* homologues from different species (27).

We compared the amino acid sequence of the protein encoded by *cdc2ZmA* with the amino acid sequences of $p34^{cdc2}$ proteins from humans, *Sch. pombe*, and *S. cerevisiae* (Fig. 2). The maize *cdc2ZmA* transcript has a long untranslated leader sequence (at least 210 bases), but the beginning of the translated region appears to be in the same context as that of the *cdc2* genes from other organisms. In addition, the sequence surrounding the presumed ATG start codon concurs with the consensus sequence of AACAAUGGC for plant genes (28). At the amino acid level, the maize gene is 64% identical to human *cdc2* and 63% identical to the *cdc2/CDC28* homologues of *Sch. pombe/S. cerevisiae*. The 294-amino acid maize protein is also 86% identical to a 147-amino acid *cdc2*-homologous peptide from pea (21), and 83% identical to a 294-amino acid *cdc2* homologue from *Arabidopsis* (Callum Bell, University of Pennsylvania, personal communication). Several other notable features of this sequence lead us to conclude that *cdc2ZmA* is an authentic *cdc2* gene. There is absolute conservation of the ATP-binding-site motif (amino acids 12–18), the EGVPSTAIRISLKKE domain (amino acids 42–56), the domains typical of protein kinases (amino acids 124–129 and 145–147), and the four tryptophan residues found to be conserved in all $p34^{cdc2}$ proteins. In addition, the nine conserved residues found in all functional cdc2 homologues, but not in related proteins that lack cdc2 function (27), are present in *cdc2ZmA*. The *cdc2ZmA* sequence should help further define regions of the $p34^{cdc2}$ protein that are conserved and that are presumably of importance to cdc2 function.

Southern blots of genomic DNA from the maize inbred strain B73 probed with the 360-bp PCR fragment show four hybridizing bands under high stringency conditions, suggesting that there may be as many as four *cdc2* genes in the maize genome (Fig. 3). These data corroborate our finding of two different *cdc2* cDNAs. The same blot probed with a 927-bp fragment of the maize *cdc2* gene that spans the entire open reading frame (see *Materials and Methods*) showed many more hybridizing bands, but several of these were less intense and may correspond to genes for other protein kinases related to cdc2. The pattern of bands is also most likely complicated by the presence of introns within the genes.

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Maize   ....MEQYEKVEKIGEGTYGVVYKALDK..ATNET.IALKKIRLEQEDEGVP   45
Human   ....--D-T-I------------GRH-..T-GQV.V-M------S-E----   45
S.pom.  ....--N-Q----------------RH-..LSGRI.V-M------D-S----   45
S.cer.  MSGELAN-KRL--V-------------LRPGQGQRVV-M------S------   52

Maize   STAIREISLLKEMNHGNI....VRLHDVVHSE.KRIYLVFEYLDLDLKKFMD   92
Human   ------------LR-P--....-S-Q--LMQD.S-L--I--F-SM----YL-   92
S.pom.  ------------V-DE-NRSNC---L-IL-A-.SKL-----F--M----Y--   96
S.cer.  ------------LKDD--.....---Y-I---DAH-------F------RY-E  100

Maize   SCPEFAKN...PTLIKSYLYQILHGVAYCHSHRVLHRDLKPQNLLIDRRTNA  141
Human   -I-PGQYM..DSS-V--------Q-IVF---R---------------DKGT.  141
S.pom.  RIS-TGATSLD-R-VQKFT--LVN--NF---R-II------------KEG-.  147
S.cer.  GI-KDQPL..GADIV-KFMM-LCK-I-------I------------NKDG-.  149

Maize   LKLADFGLARAFGIPVRTFTHEVVTLWYRAPEILLGARQYSTPVDVWSVGCI  193
Human   I--------------I-VY----------S--V---SAR------I--I-T-  193
S.pom.  ----------S--V-L-NY---I---------V---S-H---G--I------  199
S.cer.  ---G---------V-L-AY---I---------V---GK----G--T--I---  201

Maize   FAEMVNQKPLFPGDSEIDELFKIFRILGTPNEQSWPGVSCLPDFKTAFPRWQ  245
Human   ---LATK----H------Q--R---A-----NEV--E-ES-Q-Y-NT--K-K  245
S.pom.  ----IRRS-----------I----QV------EV----TL-Q-Y-ST----K  251
S.cer.  ----C-R--I-S------QI-----V------AI--DIVY-----PS--Q-R  253

Maize   AQDLATVVPNLDPAGLDLLSKMLRYEPSKRITARQALEHEYFKDLEVVQ    294
Human   PGS--SH-K---EN---------I-D-A---SGKM--N-P--N--DNQIKKM  297
S.pom.  RM--HK----GEEDAIE---A--I-D-AH--S-KR--QQN-LR-FH       297
S.cer.  RK--SQ---S---R-I---D-L-A-D-IN--S--R-AI-P--QES         298
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FIG. 2. Comparison of amino acid sequences of $p34^{cdc2}$ proteins from maize, human, *Sch. pombe* (S. pom.), and *S. cerevisiae* (S. cer.). The maize sequence was deduced from the DNA sequence of *cdc2ZmA*. Dashes indicate identity with maize sequence, and dots indicate positions of spaces required to maximally align sequences.

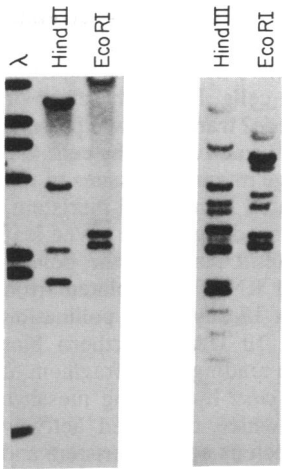

FIG. 3. Southern blot analysis of genomic DNA from maize inbred strain B73. Each lane contained ≈3 μg of DNA digested with the enzyme *Eco*RI or *Hin*dIII. Lanes were probed with a 360-bp PCR-generated fragment to the *cdc2* conserved region (*Left*) or with the 927-bp open reading frame fragment (*Right*). Labeled *Hin*dIII-cut λ phage DNA fragments were included as molecular size markers (far left lane).

The *cdc2ZmA* Gene Complements a *cdc28* Mutation of *S. cerevisiae*. Definitive proof of the functionality of a *cdc2* homologue is defined by its ability to complement *cdc2* or *cdc28* mutations of *Sch. pombe* or *S. cerevisiae*, respectively (16, 19). We have put the maize *cdc2ZmA* gene under the control of the yeast *GAL1* promoter and shown that, under conditions of galactose induction, it rescues the temperature-sensitive *S. cerevisiae cdc28-1N* mutant and allows it to grow at 37°C (Fig. 4). The vector pMR438 alone or a construct with the *cdc2ZmA* gene in the reverse orientation relative to the *GAL1* promoter did not rescue the mutation. In addition, none of the recombinants could rescue the mutation in the presence of glucose, a repressor of the *GAL1* promoter (data not shown), confirming that transcription of the maize *cdc2* gene is required for this function.

Maize *cdc2* Transcripts Are Abundant in Actively Dividing Tissues. In the yeast *Sch. pombe* the level of *cdc2* mRNA does not vary with different stages of the cell cycle (29) and therefore does not appear to be regulated at the transcrip-

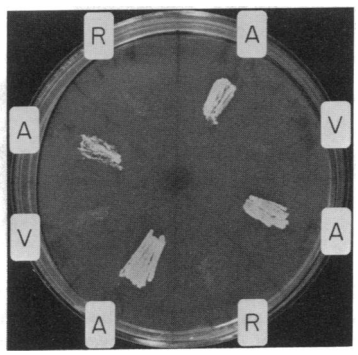

FIG. 4. *cdc2ZmA* complements a *cdc28* mutation. Several transformants of *S. cerevisiae cdc28-1N* were streaked onto a minimal medium plate containing galactose and lacking uracil and were grown at 37°C for 4 days. V, cells transformed with the vector pMR438 alone; A, strains that carry the maize *cdc2ZmA* cDNA clone transcribed under the control of the *GAL1* promoter; R, transformants with the maize gene in the reverse orientation relative to the *GAL1* promoter.

tional level. In contrast to a culture of actively dividing yeasts, a multicellular organism is composed of many types of cells in various states of replicative activity ranging from rapidly dividing cells to quiescent cells. It was therefore of interest to determine whether the *cdc2* transcript was present at levels that reflected the replicative activity of the cell. We examined the level of *cdc2* message in various tissues of the maize plant. Total RNA was isolated from apical meristem, immature leaf, and mature leaf of seedlings at the seven-leaf stage, and from mature leaf of adult plants at the pollen-shedding stage. In addition, total RNA was isolated from embryo and endosperm tissues at 18 days after pollination (DAP), and from endosperm at 10 DAP. Northern blot hybridization with the 927-bp open reading frame fragment of *cdc2ZmA* showed that a 1.4-kb *cdc2*-hybridizing message was abundant in those tissues which contained actively dividing, undifferentiated cells, such as apical meristem and immature leaf, but almost absent from tissues composed of terminally differentiated tissue, such as mature leaf from either seedlings or adult plants (Fig. 5*A Lower*). The size of the hybridizing mRNA is consistent with the size of the cDNA clone for *cdc2ZmA*. There was, however, a low level of *cdc2*-hybridizing 1.4-kb mRNA in mature leaf, suggesting that transcription of the *cdc2* gene does not cease completely in cells of terminally differentiated tissues (Fig. 5*B*). We might expect that even if some $p34^{cdc2}$ synthesis occurs in these nondividing cells, there should be no *cdc2* kinase activity. Therefore, we examined the levels of $p34^{cdc2}$-specific histone H1 kinase activity in mature leaf and apical meristems by specific precipitation of protein extracts from these tissues with $p13^{suc1}$-Sepharose beads (23). We found extensive histone H1 kinase activity in the apical meristem but not in the mature leaf (Fig. 6), as expected from the level of mitotic activity in the two tissues. The productive interaction of $p13^{suc1}$ from *Sch. pombe* with histone H1 kinase activity in maize is further evidence of the functional conservation of the mechanism of cell cycle control in higher plants.

In the case of the developing kernel, the level of 1.4-kb *cdc2*-hybridizing mRNA varied considerably between the embryo and endosperm tissues (Fig. 5*A*). In the 18-DAP kernel, *cdc2*-hybridizing 1.4-kb mRNA was highly abundant

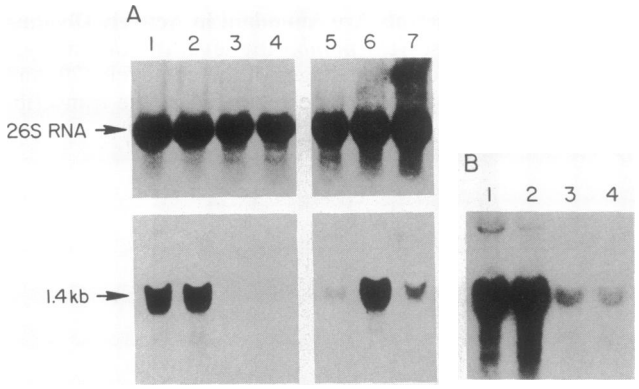

FIG. 5. (*A*) Northern blot analysis of RNA from various tissues of maize plants (inbred strain B73) probed with the 927-bp open reading frame fragment of *cdc2ZmA* (*Lower*). Each lane represents 10 μg of total RNA electrophoresed in a 0.8% agarose/formaldehyde gel. Lanes 1–3, apical meristem, immature leaf, and mature differentiated leaf, respectively, of young plants at the seven-leaf stage; lane 4, mature differentiated leaf of an adult plant at the pollen-shedding stage; lane 5, endosperm at 10 DAP; lanes 6 and 7, embryo and endosperm, respectively, at 18 DAP. The same blot was stripped and hybridized with a maize 26S rDNA probe to show that approximately equal amounts of RNA were loaded in each lane (*Upper*). (*B*) Longer exposure of the 1.4-kb *cdc2*-hybridizing signal in lanes 1–4.

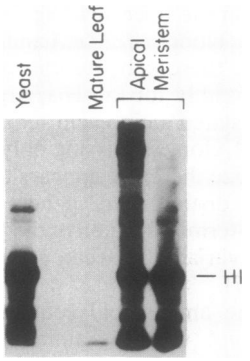

FIG. 6. Autoradiogram of SDS/polyacrylamide gel showing histone H1 kinase activity in different cell extracts. Nuclear extract from maize tissue (300 μg of total protein) or total cell lysate from *S. cerevisiae* (300 μg of total protein) was incubated with $p13^{suc1}$ beads and assayed as described in *Materials and Methods*. Two independant isolations of apical meristem protein are shown. The position of histone H1 migration (32 kDa) is indicated. Note that endogenous substrates associated with $p34^{cdc2}$ are also phosphorylated in both yeast extracts and apical meristem extracts.

in the embryo but at a much lower level in the endosperm. The level of *cdc2*-hybridizing 1.4-kb mRNA at 10 DAP was not significantly different from that in endosperm at 18 DAP. (We have not examined embryos at this stage because of the difficulty of obtaining enough tissue for Northern analysis.) This difference in transcript levels may reflect the nature of the cell division activity in this tissue (ref. 30; see *Discussion*).

DISCUSSION

The central role of the $p34^{cdc2}$ kinase in the regulation of the cell cycle in yeast and in animals is well established. We have demonstrated that a functional homologue of the *cdc2* gene is present in higher plants by isolating a cDNA clone from maize that encodes a protein, closely related to both yeast and human cdc2 proteins, that can complement a temperature-sensitive *cdc28* mutation in *S. cerevisiae*. Therefore it is likely that the cell cycle in higher plants is regulated by mechanisms similar to those in animal and yeast cells, as suggested by earlier studies (20, 21). Evidence derived from the studies in animal systems suggests that *cdc2* activity can be controlled at several levels (23, 25). The development of a complex organism from a unicellular zygote requires precisely coordinated cell divisions that must be controlled by the regulation of mitosis (18). Studies of *cdc2* expression in mammalian tissue culture and during chicken embryonic development suggest that, while the basic mechanism that controls cell division may be universal, the regulation of this mechanism during growth could vary among organisms (19, 31). In *Sch. pombe*, for example, the level of *cdc2* mRNA does not vary appreciably, even in cells arrested in the G_1 phase of the cell cycle (29), whereas serum stimulation of quiescent mouse cells in culture causes significant induction of *cdc2* mRNA (31). In multicellular organisms the $p34^{cdc2}$ kinase appears to be regulated at the transcriptional level as well. This was shown in a study by Krek and Nigg (19), who found that in the development of a chicken embryo, there is a correlation between the abundance of *cdc2* mRNA and the proliferative state of a tissue. We have found a similar pattern of regulation in maize tissues. There was a large amount of *cdc2*-hybridizing mRNA in apical meristem tissue and young leaf tissue as compared with mature leaf, a tissue composed of terminally differentiated cells. It is interesting that mature leaf did show a low but detectable level of *cdc2*-hybridizing mRNA (Fig. 5*B*). In contrast, no detectable *cdc2* mRNA was

found in any of the differentiated adult tissues examined in chicken (19). This difference might relate to differences in the terminally differentiated state between plants and animals. In several plant systems, "terminally differentiated" cells can be induced to divide in culture and in some cases to regenerate new plants (although this has not been accomplished with maize). However, mature maize leaf did not have any detectable $p34^{cdc2}$-specific histone H1 kinase activity, as expected from its nondividing state (Fig. 6). This result suggests either that the protein is present but not active or that there is no cdc2 protein despite the low-level transcription of the gene. It will be possible to distinguish between regulation of protein kinase activity vs. regulation of protein abundance once specific antibodies to the maize cdc2 protein have been generated. Correlation of *cdc2*-hybridizing RNA levels with the proliferative state was also found in endosperm and embryo tissues. The endosperm tissue at 10 and 18 DAP had substantially less *cdc2*-hybridizing mRNA than embryos at 18 DAP. This difference may reflect some interesting properties about the mechanism of cell proliferation in this tissue. At 18 DAP, cell division activity in the endosperm is very low and is confined mostly to the outer layers of the endosperm (30). Surprisingly, the level of *cdc2*-hybridizing mRNA in the endosperm at 10 DAP was not significantly different from that at 18 DAP, even though the endosperm is growing rapidly at this stage. In the developing maize endosperm, a large number of cells are polyploid, particularly toward the center of the endosperm, because they undergo chromosome replication and cell enlargement without cell division (32, 33). It is possible that polyploidization is accomplished by switching off *cdc2* transcription. Transcription of other cell cycle genes may also be turned off in these cells. In this context, we note that polytenization occurs in many cells of the *Drosophila* embryo after mitosis 16, and no string (*cdc25*) or cyclin mRNA was detectable in those cells (18); *cdc2* mRNA was not examined.

Our results also raise the possibility that another level of *cdc2* regulation may exist within a species. We have isolated two closely related but different *cdc2* cDNAs from maize. That both genes are transcribed and highly conserved suggests that both of them are important in the development of the organism. It is possible that different *cdc2* genes are used in different developmental programs; for example, adult vs. juvenile, or meiosis vs. mitosis. Our preliminary results (i.e., the isolation of eight clones of *cdc2ZmA* and only one of *cdc2ZmB*) suggest that the *cdc2ZmA* gene may be expressed at a greater level than the *cdc2ZmB* gene, at least in seedling tissue.

Finally, while cell division in higher plants resembles that in animals in many respects (e.g., in both cases there is nuclear envelope breakdown during mitosis), there are also many significant differences between the two systems (34). It is now clear that the $p34^{cdc2}$ kinase plays a central role in the initiation of mitosis in animal cells (2). The isolation of clones encoding plant homologues of $p34^{cdc2}$ is a step toward elucidating the mechanisms of mitosis and cytokinesis in higher plants.

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