Isolation and Characterization of the cDNA for an A-like Cyclin in *Adiantum capillus-veneris* L.

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We have isolated and characterized the cDNA for a homolog of a cyclin from the fern *Adiantum capillus-veneris* L. Three fragments of cDNAs for cyclin homologs were amplified by the PCR from the cDNA of germinating spores with degenerate primers that encoded the highly conserved region in the so-called cyclin box. We isolated a full-length cDNA for cyclin from a λZAPII-cDNA library that has been derived from spores using an amplified fragment as a probe. The cDNA insert (CycAcI) in one positive clone was 2.0 kb in length, having an open reading frame of 1,599 bp that encoded 532 amino acids. The putative CycAcI protein had a cyclin box and a destruction motif, and it was homologous to A-type cyclins. Northern blot analysis was performed to study the expression of CycAcI during the first cell cycle in the haploid generation of the fern. CycAcI mRNA was not detectable in dormant spores, during the first G1 phase, and at the onset of the S phase in imbibed spores after breaking of dormancy. This mRNA became detectable after the termination of the S phase, and it accumulated during the second G1 phase. Although CycAcI was classified as an A-like cyclin, the failure to detect CycAcI mRNA at the onset of the S phase suggests that CycAcI might not play a role in the replication of DNA during the S phase.

**Key words:** *Adiantum capillus-veneris* — Cell cycle — Cyclin — Spore germination (fern).

The concept of a cell cycle was first proposed in plant cells by Howard and Pelc (1953), and the basic mechanisms associated with progression of the cell cycle have been studied extensively in yeast and animal cells over the past decade. Studies of cell division cycle (cdc) mutants in plant cells by Howard and Pelc (1953), and the basic mechanisms associated with progression of the cell cycle have been studied extensively in yeast and animal cells over the past decade. Studies of cell division cycle (cdc) mutants in yeast provided a general outline of these basic mechanisms (Hartwell et al. 1970). The cdc2 gene encodes the protein kinase p34cdc2 that is required for progression of both the S phase and the M phase and its activity is regulated by cyclins (Reed 1992). Homologs of these key components have been isolated from various species and their isolation demonstrates that the basic mechanisms that control progression of the cell cycle are universally conserved in all eukaryotes (Nurse 1994). Several homologs of key components in the cell cycle have been isolated from plants and the progress that has been made in studies of the cell cycle in plants has been repeatedly reviewed (e.g., Doerner 1994, Jacobs 1995).

Cell division in plants is regulated by genetic factors and by environmental factors, such as light, temperature and wound stress. Fern spores and protonemata are excellent systems for studies of the regulation of the cell cycle because the progression of the cell cycle can be synchronized and controlled by environmental light (Furuya 1983). In the protonemata of *Adiantum*, cell division is synchronously induced by brief irradiation with blue-UV light, whereas induction is prevented under red light (Wada and Furuya 1972). An unidentified blue/UV-light-absorbing pigment acts as the photoreceptor for the former reaction and phytochrome acts as the photoreceptor for the latter. Protonemata growing under continuous red light remain at the early G1 phase. After transfer to darkness or upon irradiation with far-red or blue light, they synchronously enter the S phase (Miyata et al. 1979). The rate of progression of the cell cycle is affected by different light conditions. The longer the cells are irradiated with blue light, the shorter is the duration of the G1 phase. By contrast, far-red light during the G1 phase extends the duration of the G2 phase (Miyata et al. 1979). There is a transition point for the prevention of progression of the cell cycle by red light in fern protonema (Wada et al. 1984). This point is called the "point of no return". Progression of the cell cycle in spores is also regulated by phytochrome and a blue/UV-photoreceptor, but the effects are essentially the opposite of those described above: (1) the progression from G1 to S is induced in spores by brief irradiation with red light rather than by the blue-UV light that is effective in protonema; and (2) this induction is photo-reversibly prevented by subsequent brief exposure to far-red light and is photo-irrever-

Abbreviations: MPF, maturation promoting factor; CTAB, cetyltrimethylammonium bromide; ORF, open reading frame.

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sibly prevented by brief irradiation with blue light (M. Furuya, unpublished results). There is also a transition point in spores before the onset of the S phase, and at this point the red-far-red reversibility of commitment to progression of the cell cycle is lost (Iino et al. 1989).

Cyclin was first described as a protein whose levels oscillated with progression of the cell cycle in marine invertebrates (Evans et al. 1983). A cDNA for cyclin was first cloned from the clam, and the corresponding mRNA promoted the progression of the M phase, as does maturation-promoting factor (MPF) (Swenson et al. 1986). Cyclins in animals form a large gene family (Hunter and Pines 1994). Each cyclin binds to a specific type of cdk and controls the progression of the cell cycle at the G1/S or G2/M transition by regulating the kinase activity of the cdk (Sherr 1994). The cyclin-cdk complex acts to regulate translation via the retinoblastoma protein at the G1/S transition (Weinberg 1995), and it influences the initiation of DNA replication during the S phase (Girard et al. 1991, Cardoso et al. 1993), as well as the initiation of mitotic events via the activity of MPF at the G2/M transition (King et al. 1994).

cDNAs for cyclin homologs in plants were first cloned from soybean and carrot (Hata et al. 1991). Subsequently, cDNAs for a number of cyclins have been isolated from various plants, such as Arabidopsis (Hemerly et al. 1992, Day and Reddy 1994, Ferreira et al. 1994a), alfalfa (Hirt et al. 1992), maize (Renaudin et al. 1994), Antirrhinum (Fobert et al. 1994) and tobacco (Qin et al. 1993). Most of the encoded homologs are classified as mitotic cyclins, and Gl cyclins have been reported only in Arabidopsis (Son et al. 1995) and alfalfa (Dahl et al. 1996). The family of cyclin genes has been also analyzed in Arabidopsis (Ferreira et al. 1994a) and maize (Renaudin et al. 1994). Several cyclin homologs in plants were regulated at the transcriptional level, and they are expressed in actively dividing cells in alfalfa (Hirt et al. 1992), Arabidopsis (Ferreira et al. 1994b) and Antirrhinum (Fobert et al. 1994). The functional activity of cyclin mRNA of soybean (Hata et al. 1991), Arabidopsis (Hemerly et al. 1992) and maize (Renaudin et al. 1994) was demonstrated by their ability to induce the meiotic division of Xenopus oocytes (Swenson et al. 1986). Although several genes for A-type cyclin have been isolated from plants, little information is available about their expression and analysis of their functions has been limited.

Plant cyclins appear to be functionally homologous to animal cyclins, but it is not clear whether plant cyclins have a similar role during progression of the cell cycle in plants. Fern spores and protonemata are useful experimental materials, as described above, but they have not been exploited for a molecular approach to these issues. In this paper, we report the isolation of cDNA for cyclin from Adiantum spores and the results of an analysis of the expression of the corresponding transcript during the first cell cycle in spores.

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### Materials and Methods

**Plant material and aseptic culture—**Spores of Adiantum capillus-veneris L. were collected in a greenhouse at Tokyo Metropolitan University in June 1986, and they were stored dry at 4°C in darkness. Spores were sterilized by dipping in a 1% solution of sodium hypochlorite for 2 min. They were sonicated for one min and rinsed three times with sterilized water, then they were sown in plastic Petri dishes (30 mm i.d. x 10 mm) that contained 1.5 ml of liquid medium, namely, one-tenth strength Murashige and Skoog's mineral salts solution (Murashige and Skoog 1962), pH 5.7. The spores were incubated aseptically in darkness for 5 d at 25°C and then used for the experiments. Spores after imbibition were handled under a green safety light.

**Light sources—**Broad-range red light was provided by fluorescent tubes (FL 20SW-G; Hitachi Ltd., Tokyo, Japan) and their output was filtered through a 3-mm red filter (Acrylite; #102, Mitsubishi Rayon, Tokyo, Japan). The fluence rate was determined with a radiometer (PR200 Pyroelectric Radiometer; PR20 Detector; Molelectron, Sunnyvale, CA, U.S.A.). The green safety light was provided by a 10 W green fluorescent tube (National FL 10G; Matsuishita Electric Co., Ltd., Osaka, Japan), wrapped in four layers of lemon yellow-colored plastic film (#321C; Nakagawa Chemical Co., Tokyo, Japan), two layers of cerulean blue plastic film (#521C; Nakagawa Chemical Co.) and three layers of viridian plastic film (#431C; Nakagawa Chemical Co.).

**Cloning and sequencing of cDNA for a cyclin from Adiantum—**Spores (15 mg dry weight) were imbibed and irradiated with red light for 5 min to induce cell division, which occurred from 40 to 96 h after the irradiation with red light. Spores were collected 48 h after irradiation with red light. They were homogenized in an extraction buffer (200 mM Tris-HCl, pH 9.0; 100 mM NaCl; 10 mM EDTA; 0.5% SDS; 14 mM β-mercaptoethanol) and phenol. After three extractions with phenol, the RNA was precipitated with ethanol. Poly(A)+ RNA was purified from the resulting pellet with a QuickPrep Micro mRNA Purification Kit from Pharmacia (Uppsala, Sweden).

Single-stranded cDNA was synthesized from poly(A)+RNA with a random hexameric primer (Single-strand cDNA Synthesis Kit; Pharmacia). Two μl of the reaction mixture were then used in a 100 μl reaction mixture for amplification by PCR with AmpliTaq polymerase (Perkin Elmer, Branchburg, NJ, U.S.A.) according to the instructions from Perkin Elmer. The degenerate primers 5'-AT(ACT)(CT)T(AGGT)GT(ACGT)GA(CT)TG(TG)(AGT)- (AGT)-(AGT)(CGA)(AGT)GT-3', and 5'-GG(GAGG)(AGA)TA- (AGT)AT(TC)T(CA)T(CA)T(CA)T(T)-3', corresponding to the conserved motifs ILVDEWL(VI)DQVY and KYEIEYPYP in the cyclin box, were used. The conditions for PCR were forty cycles at 94°C for 1 min, at 55°C for 2 min, and at 72°C for 3 min, with a final extension for 7 min at 72°C. The amplified fragments were separated on a 4% agarose gel and the band of the expected size was cut from the gel. The extracted DNA was cloned directly into the pT7-Blue T vector (Novagene, Madison, WI, U.S.A.).

Double-stranded cDNA was synthesized from poly(A)+RNA with an oligo(dT) primer and attached to EcoRI/NotI adapters with a TimeSaver cDNA Synthesis Kit from Pharmacia. The cDNA was ligated with EcoRI-cleaved AZAP II vector from Stratagene (La Jolla, CA, U.S.A.). A Gigapack Gold Kit (Stratagene) was used for packaging. Approximately 5 × 10⁶ phages were screened with a 32P-labeled DNA fragment. Filters were allowed to hybridize overnight with the probe at 65°C in hybridization buffer that consisted of 6 × SSC (1 × SSC is 0.15 M
NaCl, 15 mM sodium citrate, pH 7.0), 5 × Denhardt's solution (1 × Denhardt's solution is 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 0.1% SDS, and 100 μg/ml salmon sperm DNA. They were washed with 5 × SSC plus 0.1% SDS at 65°C for 30 min and 2 × SSC plus 0.1% SDS at 65°C for 30 min. Inserts of plaque-purified phage clones were excised and ligated in vitro to pBluescript SK(−) in accordance with the instructions from Stratagene. cDNA inserts of phagemid colonies were examined by PCR.

The cDNA of one plasmid was sequenced by the dideoxy sequencing method (Sanger et al. 1977) with dye-terminator according to the manufacturer's protocol with slight modifications, using a DNA sequencer (SQ-3000) from Hitachi Ltd. An analysis of sequence homologies was performed by MPsrch (IntelliGenetics Inc., Campbell, CA, U.S.A.) e-mail service, provided by the DNA Information and Stock Center of the National Institute of Agrobiological Resources (Tukuba, Japan). Swiss-Prot R31.0 was used as the data base. Mpsrch is based on Smith and Waterman's dynamic algorithm (Gotoh 1982). Analysis of the phylogenetic tree was performed with the DNASIS computer program (Hitachi Software Engineering, Tokyo, Japan), which is based on the CLUSTAL V multiple sequence alignment ( Higgins et al. 1992).

Analysis of the expression of cyclin mRNA in Adiantum—Materials were homogenized in an extraction buffer. After three extractions with phenol, the RNA was precipitated with ethanol. The precipitate was dissolved in TE buffer and the total RNA content was determined by measurement of the absorbance at 260 nm. Total RNA was fractionated on a 0.7% agarose gel that contained 0.66 M formaldehyde and blotted onto a nylon membrane (Hybond N; Amersham, U.K.) in 20 × SSPE (1 × SSPE is 0.15 M NaCl, 10 mM NaH2PO4, 1 mM EDTA, pH 8.0), layered onto a cushion of 5.7 M cesium chloride and centrifuged at 164,000 × g for 16 h at 16°C. The resulting pellet was dissolved in TE buffer and the total RNA content was determined by measurement of the absorbance at 260 nm. PCR amplification was performed using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980) with slight modifications and digested with various restriction enzymes. Digested DNA was fractionated on a 0.7% agarose gel and fragments were blotted onto a nylon membrane (Hybond N; Amersham). Preparation of the probe and hybridization conditions were the same as those for the Northern blot analysis described above.

Results

Isolation and sequencing of cDNA for a cyclin homolog in Adiantum spores—Three amplified fragments (CycAc-Frag1, CycAc-Frag2 and CycAc-Frag3) with homology to cyclin, in terms of their deduced amino acid sequences, were obtained by PCR with the degenerate primers that corresponded to the cyclin box. CycAc-Frag1, CycAc-Frag2 and CycAc-Frag3 were found in 14, 5 and 1 of 20 randomly sequenced clones. We chose the CycAc-Frag1 as the probe for screening of the cDNA library of germinating spores because this fragment was detected at significantly higher frequency than the other two. Eighty-one positive phages were obtained from approximately 500,000 plaques in the cDNA library. One cDNA insert of 2.0 kb was sequenced and the cDNA was designated CycAc1. CycAc1 was 1,981 bp long and included an open reading frame (ORF) of 1,596 bp that encoded 532 amino acids (Fig. 1). CycAc1 lacked putative polyadenylation signals, but another clone which was identical to the 3' region (1,696-1,981 bp) of CycAc1 had polyadenylation signals that began at position 2,029. The inserts in the rest of the 80 clones seemed to be identical to CycAc1 since all of them gave a band of the same mobility during agarose gel electrophoresis of PCR-amplified fragments obtained with primers that corresponded to the 3' region (1,316-1,338 and 1,901-1,923; data not shown).

The ORF of CycAc1 included a cyclin box (Nugent et al. 1991), namely, amino acids 298-450, and a destruction motif (RxxLxxIxN), which plays an important role in proteolysis with ubiquitination during the M phase (Glotzer et al. 1991). The sequence of this motif was RXALGVIXN (amino acids 96-104) and it was located near the amino terminus of the protein (Fig. 1). There were no PEST sequences (Rogers et al. 1986) in CycAc1. An analysis of homology using the MPsrch program showed that CycAc1 and its cyclin box were more closely related to A-type cyclins than to B-type cyclins (data not shown). The sequences of the cyclin boxes in plant mitotic cyclins were used to construct a phylogenetic tree (Fig. 2). This tree revealed that CycAc1 could be classified as an A-like cyclin (Renaudin et al. 1994).

Genomic Southern blot analysis—A Southern blot experiment was performed with a genomic DNA from a diploid leafy sporophyte of Adiantum. One major fragment of 3.8 kb was obtained when DNA was digested with BamHI, for which there was one restriction site in CycAc1 cDNA. Fragments of 2.3 kb and 2.5 kb were obtained with Saci, for which there was one restriction site, and fragments of 2.5 kb and 1.7 kb were obtained with EcoRI for which there were two restriction sites (Fig. 3). The pattern of bands was very similar under lower-stringency conditions, although the background was higher (data not shown). It appeared, therefore, that there was only one copy of the CycAc1 gene in the Adiantum genome.

Expression of CycAc1 mRNA during the cell cycle in germinating spores—We performed Northern blot analysis to characterize the expression of CycAc1 mRNA during the first cell cycle in germinating spores. Total RNA was extracted from imbibed spores between 0 and 50 h after the onset of red-light irradiation at intervals of 10 h. Under continuous red-light irradiation, the S phase of the first cell cycle began at about 20 h and lasted for 8 h, and mitosis occurred synchronously in all spores from 30 to 38 h after the
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Fig. 1 Nucleotide and deduced amino acid sequence of CycAcl cDNA. The underlined sequence corresponds to the cyclin box and dotted underlining shows the destruction box.

The transcript was not detectable at 0 or at 10 h during the first Gl phase of the haploid generation, nor was it detectable at 20 h during the G1/S transition phase. It was detectable at 30 h at the G2/M phase and accumulated from 40 to 50 h during the second Gl phase. Patterns of expression obtained with the ORF region of the cDNA and the 3' region (1,326-1,923 bp) as probes were...
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Fig. 2 Phylogenetic tree for mitotic cyclins from plants. The tree was constructed from the sequences of cyclin boxes using the CLUSTAL V program (Higgins et al. 1992) in DNAIS (Hitachi Software Engineering). *Antirrhinum* Cyclin 1 and Cyclin 2 are from *Antirrhinum* (Pobert et al. 1994); *Cyc1At*, *Cyc2aAt*, *Cyc2bAt*, *Cyc3aAt* and *Cyc3bAt* are from *Arabidopsis* (Hemerly et al. 1992, Ferreira et al. 1994a); *CyclaZm*, *CyclbZm*, *CyclIZm* and *CycllIZm* are from maize (Renaudin et al. 1994); *CycMs2* is from alfalfa (Hirt et al. 1992); and *S13-6* and *C13-1* are from soybean and carrot, respectively (Hata et al. 1991). The *Adiantum* cyclin characterized in this report is identified in boldface. Cyclins in groups I and III are B-like and those in group II are A-like (Renaudin et al. 1994).

Fig. 3 DNA gel blot analysis of *CycAcl*. Genomic DNA was isolated by the CTAB method. Ten μg of digested DNA were fractionated in each lane and bands were transferred to a nylon membrane and probed with 32P-labeled *CycAcl* cDNA. Probe was allowed to hybridize at 65°C. The membrane was washed with 0.1× SSC plus 0.1% SDS at 65°C. Restriction enzymes used was as follows: B, *Bam*HI; S, *Sac*I; E, *Eco*RI.

Fig. 4 Expression of *CycAcl* mRNA during the first cell cycle. Ten μg of total RNA were subjected to electrophoresis in each lane of an agarose gel, transferred to a nylon membrane and probed with 32P-labeled *CycAcl* cDNA. The arrow indicates mRNA of 2.2 kb, as calculated from the mobility of RNA size markers. The lower panel shows ethidium bromide-stained residual ribosomal RNA that was blotted onto the nylon membrane.

Fig. 5 Expression of *CycAcl* mRNA at various stages of the life cycle of *Adiantum*. S, Dry spores; PR(2d), protonemata and rhizoids 50 h after the beginning of red-light irradiation (0.7 W m⁻²); PR(6d), elongated protonemata and rhizoids 6 d after the beginning of red-light irradiation (0.3 W m⁻²); P, prothallia collected after culture of spores for one month in continuous white light (5 W m⁻²); YS, shoot-like sporophytes. Ten μg of total RNA were subjected to electrophoresis in each lane of an agarose gel, transferred to a nylon membrane and probed with 32P-labeled *CycAcl* cDNA. See legend to Figure 4 for other details.

In control experiments, spores were imbibed in the same manner and kept in darkness instead of under red-light irradiation. Samples were collected at the same time points. In the control samples, no signals were obtained in the Northern analysis (data not shown). During imbibition of spores for 5 d in darkness, no *CycAcl* mRNA was detectable (data not shown).
If germinated spores are kept under continuous red light at 0.3 W m\(^{-2}\), protonemata and rhizoids elongate without mitosis and they remain in the second G1 phase (Wada and Furuya 1970). CycAcl mRNA was remained detectable during the second G1 phase under red light for 6 d after the onset of red-light irradiation, but the level of the CycAcl mRNA decreased (Fig. 4).

It is noteworthy that CycAcl mRNA was detectable throughout both haploid and diploid generations in Adiantum, with the exception of the G1-S phases of the first cell cycle in the haploid generation (Fig. 5).

Discussion

Identification of CycAcl as an A-like cyclin—The cyclins that have previously been found in plants have been classified structurally as A-like, B-like and D-like cyclins, and each class consists of a few molecular species (Renaudin et al. 1994, Ferreira et al. 1994a, Soni et al. 1995). The sequences of plant mitotic cyclin cannot easily be classified as A-like, B-like and D-like cyclins, and each class consists of a few molecular species. The sequences of plant mitotic cyclin have previously been found in plants have been classified structurally as A-like, B-like and D-like cyclins that have previously been found in plants have been classified structurally as A-like, B-like and D-like cyclins. CycAcl mRNA appeared to be an A-like cyclin from our phylogenetic analysis (Fig. 2). In a comparison of the deduced amino acid sequence within the cyclin box of CycAcl with those of other plant cyclins, CycAcl exhibited higher homology to A-type cyclins than to A-type or B-type cyclins. CycAcl appeared to be an A-like cyclin from our phylogenetic analysis (Fig. 2). In a comparison of the deduced amino acid sequence within the cyclin box of CycAcl with those of other plant cyclins, CycAcl exhibited higher homology to A-type cyclins than to B-type cyclins since the distinct amino acid residues conserved in A-type cyclins were found in the cyclin box of CycAcl (Fig. 6). The LVEVXEEYKL motif (Fig. 6, amino acids 9 to 18) found in the cyclin box of cyclin A was completely conserved in CycAcl, whereas the GRPLXLHFLR-RXSK motif (Fig. 6, amino acids 98-110), found in cyclin B, was not well conserved. Sixty-one amino acids in CycAcl were identical to those in the 70 amino acids that are conserved in the cyclin box of cyclin A, and 40 amino acids in CycAcl were identical to those in the 67 amino acids that are conserved in the cyclin box of cyclin B (Fig. 6, asterisks). A phylogenetic tree revealed that CycAcl belongs to the same group as C13-1, an A-like cyclin in carrot (Hata et al. 1991). Thus, CycAcl seems also to be an A-like cyclin.

The unique pattern of expression of CycAcl mRNA—The pattern of expression of cyclin mRNA during the cell cycle is another indicator of the specific type of cyclin, because plant genes for cyclins are regulated transcriptionally during the cell cycle (Hirt et al. 1992, Fobert et al. 1994). The pattern of expression of CycAcl mRNA was not typical of A-type or B-type cyclins. In alfalfa cultured cells, mRNA for a B-type cyclin gradually appears during the G2 phase and disappears during the next G1 phase; the mRNA for another cyclin homolog appears at the onset of the S phase and vanishes during the M phase before that for a B-type cyclin disappears. The pattern of expression of the latter cyclin is typical of an A-type cyclin (Hirt et al. 1992). CycAcl mRNA appeared around the onset of the M phase and accumulated during the second G1 phase (Fig. 4). In our Northern analysis, a very weak signal was detected 25 h after the start of red-light irradiation (data not shown). Expression of CycAcl mRNA started around the end of the S phase.

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![Sequence alignment](https://example.com/sequence_alignment.png)

- The sequence of CycAcl from Adiantum is compared with those of other plant mitotic cyclins. C13-1 from carrot (Hata et al. 1991) and Cycl21 from Zea mays (Renaudin et al. 1994) are A-type cyclins, Cycl1At from Arabidopsis and S13-6 from soybean (Hata et al. 1991) are B-type cyclins (Renaudin et al. 1994). Conserved (Con) amino acids in A-type and B-type cyclin are shown above and below the sequences, respectively. Asterisks show residues in CycAcl that are identical to those conserved in A-type or B-type sequences.
Phase and the mRNA accumulated thereafter, even during the second G1 phase.

Mitotic cyclins such as A-type cyclins are generally degraded at the end of the M phase. CycA1 mRNA was expressed after the first mitosis (Fig. 4). We do not yet know whether CycA1 is degraded after mitosis, but it does have a destruction motif.

Possible role of CycA1 in the first cell cycle—Cyclin A has been shown to be involved in the progression of the S phase in animal cells. Microinjection of antibodies against cyclin A prevents both the onset of the S phase and entry into the M phase in mammalian cells (Girard et al. 1991, Pagano et al. 1992). Both cyclin A and cdk2 are localized at nuclear foci of replication (Cardoso et al. 1993). Although CycA1 was structurally classified as an A-like cyclin, the corresponding mRNA was not detected at the onset of the S phase in the first cell cycle. We suspect that CycA1 might not be involved in the progression of the S phase and might not be involved in the entry into the S phase of the first cell cycle.

It is possible that CycA1 protein is inherited maternally from mother cells in amounts sufficient to drive the first cell cycle. Thus, transcription of the gene would not be required in this cycle. However, since CycA1 has a destruction motif, it might be difficult to store CycA1 protein without degradation at the end of meiosis.

It is an interesting coincidence that the G1/S progression of the first cell cycle in spores is induced by phytochrome and does not require CycA1 mRNA, whereas the G1/S progression of the next cell cycle occurs in the presence of abundant CycA1 mRNA and is induced by a blue/UV photoreceptor. The developmental regulation of the expression of mRNA for cyclin A was reported in the early development of Xenopus (Howe et al. 1995). The cloning and analysis of expression of genes for other cyclins are needed if we are to determine whether diverse cyclins are required at different developmental stages in the fern.

The signal transduction pathway from phytochrome to the regulatory switch for entry into the S phase in the first cell cycle of Adiantum spores has not been characterized. In fact, there are no publications, to our knowledge, on the roles of any cyclins in the phytochrome-dependent progression of the cell cycle. The A-type cyclin is a candidate for relevant analysis among the members of the cyclin family, because A-type cyclins are involved in the onset of the S phase in animal cells (Girard et al. 1991). G1 cyclins, in particular D-like cyclins that were cloned from Arabidopsis (Soni et al. 1995) and alfalfa (Dahl et al. 1996), are other candidates for future study.

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References
Hunter, T. and Pines, J. (1994) Cyclins and cancer II: cyclin D and CDK in-
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