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Isolation of Genes that Are Preferentially Expressed at the G_1/S Boundary during the Cell Cycle in Synchronized Cultures of *Catharanthus roseus* Cells[1]

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ABSTRACT

A cDNA library was screened for genes that may be involved in the progression of the cell cycle of cells of higher plants. The *Catharanthus roseus* L. (G) Don. cells were synchronized by the double phosphate starvation method, and a λgt11 cDNA library was prepared using poly(A)$^+$ RNA from cells in the S phase of the cell cycle. Two independent sequences, *cyc02* and *cyc07*, were identified by differential screening. The levels of *cyc02* and *cyc07* mRNAs increased dramatically, but transiently, at the G_1/S boundary of the cell cycle. High levels of *cyc02* mRNA, but not of *cyc07* mRNA, were also present in cells arrested at the G_1 phase by phosphate starvation. In an asynchronous batch culture, *cyc02* and *cyc07* mRNAs accumulated transiently at different stages of the growth cycle, *cyc02* mRNA early in the stationary phase, and *cyc07* mRNA in the midlogarithmic phase. When the proliferation of cells was arrested by nutrient starvation, *i.e.* by sucrose or nitrogen starvation, the relative amounts of the *cyc02* and *cyc07* mRNAs decreased. These results indicate that *cyc02* and *cyc07* contain nucleotide sequences from growth-related genes. The analysis of nucleotide sequence of *cyc02* shows that the predicted product of this gene is basic and is composed of 101 amino acids. No significant homology to other known proteins was detected.

One of the most fundamental problems in cellular biology is the elucidation of the molecular mechanisms that control the cell cycle. Many attempts to identify genes involved in the regulation of the cell cycle have been undertaken using mammalian or yeast cells. Some discrete, unique gene sequences that are involved in the cellular response to growth factors have been isolated and characterized (15). The availability of temperature-sensitive mutants in which proliferation is arrested at specific phases of the cell cycle at nonpermissive temperatures has allowed the isolation of genes that are essential for the progression of the cell cycle (10, 16). These studies of the molecular basis of the regulation of the cell cycle have been reviewed (5).

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Few reports dealing with gene expression during the cell cycle of higher plant cells have been published (12). The cells of the higher plant *Catharanthus roseus* L. (G) Don. (periwinkle) grown in suspension have been used in several investigations of cell proliferation (6, 8). Amino *et al.* (2) established a system of synchronous cell division in suspension-cultures of *C. roseus* cells. Using this system, Kodama *et al.* (13) have found changes in levels and rates of synthesis of various polypeptides during the cell cycle. Furthermore, variations in the population of mRNAs were also revealed by translation *in vitro* followed by two-dimensional gel electrophoresis. Such investigations revealed the altered expression of four species of mRNA during the cell cycle. Three mRNAs, a, b, and c, were found specifically in the S phase, and the level of another mRNA, d, increased preferentially during the progression from the G_2 phase to cytokinesis. These results indicate that some of genes are expressed specifically during certain phases of the cell cycle in *C. roseus* cells. In the present paper, the isolation is described of cDNAs corresponding to the mRNAs that vary in level during the cell cycle in synchronized cultures of *C. roseus* cells.

MATERIALS AND METHODS

Materials

All restriction enzymes, DNA-modifying enzymes, and nucleic acids were purchased from commercial sources. Nylon membranes (Biodyne A) were purchased from Pall Ultrafine Filtration Corporation (New York, USA). [α-^{32}P]dCTP and [^{3}H]thymidine were obtained from Amersham Japan (Tokyo, Japan).

Cells and Synchronized Growth

Cultures of cells of *Catharanthus roseus* L. (G) Don. were maintained at 27°C in the dark in Murashige-Skoog medium (19), which contained 3% (w/v) sucrose and 2.2×10^{-6} M 2,4-D. Cells were subcultured every 7 d, and their growth was synchronized in the early G_1 phase by the double phosphate starvation method (2). The modified procedure of synchronization of cell division was described previously (13).

Determination of Cell Number and Incorporation of [^{3}H]Thymidine

Cell number was estimated by counting protoplasts with a haemocytometer after enzymatic maceration (2). The rates of

DNA synthesis were measured by labeling cells with [^{3}H] thymidine (92.5 kBq/mL suspension, 185 kBq/mmol) for 60 min at 27°C and determining the amount of radioactive material that was soluble in hot 0.5 M perchloric acid, as described by Amino *et al.* (2).

Purification of RNA and Construction of cDNA Library

Total RNA was purified by the method of Schmidt *et al.* (23). Poly(A)$^+$ RNA was prepared from total RNA by chromatography on a column of oligo(dT)cellulose (Pharmacia, Uppsala, Sweden) (3). A λgt11 cDNA library was constructed from poly(A)$^+$ RNA isolated from the cells in the S phase. Double-stranded cDNA was prepared as described by Watson and Jackson (30). The cDNA was ligated to the vector λgt11 (9) and packaged *in vitro*. The packaged library was plated on *Escherichia coli* Y1090 and amplified by minimal passage through Y1090.

Differential Screening

The λgt11-cDNA library was plated at a low plaque density (about 3500 plaques per 140-cm^2 dish) and screened by differential hybridization. Duplicate nylon-membrane replicas were lifted from each of the plates. About 100,000 plaques on nylon membranes were screened by hybridization with ^{32}P-labeled single-stranded cDNA synthesized from the poly(A)$^+$ RNA isolated either from cells in the S phase or from cells during cytokinesis. Another 100,000 plaques were screened with ^{32}P-labeled probes prepared from the RNA isolated from the cells in the S phase and from phosphate-starved cells. Hybridization was performed according to the instructions from the manufacturer of the nylon membranes. Selected cDNA clones were isolated and cDNA was subcloned into the unique *Eco*RI site of the plasmid vector, pBluescript KS M13$^+$ (Stratagene, La Jolla, CA) to facilitate further analyses.

RNA Blot Analysis

Total RNA or poly(A)$^+$RNA was denatured with 6.3% (w/v) formaldehyde and 50% (w/v) formamide and then fractionated on a 1% (w/v) agarose gel that contained 2% (w/v) formaldehyde, by a modified version of the procedure of Lehrach *et al.* (18). RNAs were transferred to nylon membranes and hybridized with [^{32}P]cDNA fragments labeled by nick-translation. Prehybridization, hybridization, and post-hybridization steps were performed essentially in the same way as described in the instructions from the manufacturer of the nylon membranes. The fragments of cDNA that hybridized to corresponding RNAs were visualized by autoradiography. The optical density of each band was measured with a scanning densitometer, Digital densitol, DMU-33C (Advantec, Tokyo, Japan).

Nucleotide Sequencing

Deletion derivatives of clones were generated using a Double-Stranded Nested Deletion Kit (Pharmacia LKB biotechnology, Uppsala, Sweden). Nucleotide sequences were determined by the dideoxynucleotide-chain-termination method (22) with single-stranded DNA templates (7).

RESULTS

Construction and Screening of the cDNA Library Prepared from Poly(A)$^+$ RNA Isolated from Cells in the S Phase

The *Catharanthus roseus* cells were synchronized by the double phosphate starvation method (2). The phosphate-starved cells were arrested exclusively at the early G_1 phase, and readdition of phosphate induced the synchronized division of cells. Cells in the S phase were collected 15 h after the addition of phosphate and poly(A)$^+$ RNA was isolated. A λgt11 cDNA library was made from this poly(A)$^+$ RNA and was screened for genes that are preferentially expressed at the S phase by differential plaque hybridization. Plaques that gave positive hybridization signals with ^{32}P-labeled cDNA from cells in the S phase, but not with cDNA prepared from cells during cytokinesis or with cDNA prepared from phosphate-starved cells, were selected and then these selected plaques were rescreened. Of approximately 200,000 plaques screened in this manner, 64 plaques were selected as differentially hybridizing. The phage DNA of 64 clones was purified and each of the isolated cDNA inserts was labeled with [^{32}P]dCTP by nick-translation and hybridized to dot-blotted poly(A)$^+$ RNAs from the cells in each phase of the cell cycle and from phosphate-starved cells. At least 10 of 64 clones showed changes in the levels of their corresponding RNAs during the cell cycle (data not shown). These 10 cDNA inserts represented four independent gene sequences, as judged from the absence of cross-hybridization between them (data not shown). The four unique cDNA inserts were subcloned into pBluescript KS M13$^+$ and designated as pCYC01, 02, 06, and 07, respectively. The sizes of the cDNA inserts were 670, 690, 860 and 830 bp[3] for pCYC01, 02, 06, and 07, respectively. The genes, which are corresponded to pCYC01, 02, 06, and 07, are designated as *cyc01, 02, 06,* and *07*. However, the nucleotide sequences of *cyc01* and *cyc06* are very similar (about 90% homology) to those of the gene for rice 25S ribosomal RNA (data not shown). Because the cDNA inserts of pCYC01 and pCYC06 do not cross-hybridize to each other, these cDNAs are derived from different parts of the same rRNA. It is probable that some kinds of ribosomal RNA were included in a cDNA library by accident.

Kinetics of the Appearance of *cyc02* and *cyc07* mRNAs in Synchronous Cultures

To determine the time course of appearance and decay of *cyc02* and *cyc07* mRNAs during the cell cycle, total RNA was isolated at various times from *C. roseus* cells in a synchronized culture, and the RNA was analyzed by Northern blot hybridization (Fig. 1). Clones *cyc02* and *cyc07* corresponded to 0.9-kb RNA and 1.2-kb RNA, respectively. The *cyc02* mRNA was present at high levels in phosphate-starved cells which were arrested in the G_1 phase; its level fell sharply within 2 to 4 h after addition of phosphate; and after 4 to 8 h it was barely detectable. The *cyc02* mRNA reappeared rapidly within 8 to 10 h after the addition of phosphate. By contrast, the *cyc07* mRNA was not detected in phosphate-starved cells,

[3] Abbreviations: bp, base pair; kb, kilobase pair.

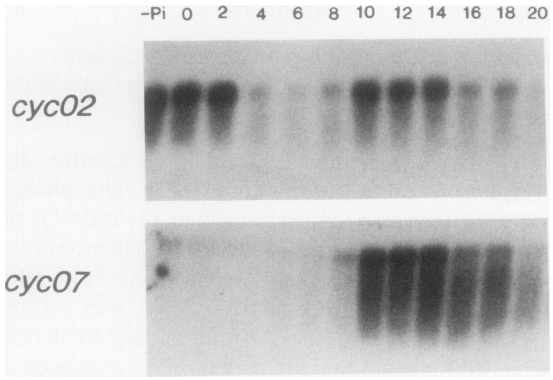

Figure 1. Induction of *cyc02* (upper) and *cyc07* (lower) gene expression in synchronized cultures. Total RNA was extracted from phosphate-starved cells and from cells at indicated times (h) after the addition of phosphate. Samples (4.5 μg) were subjected to electrophoresis in the various lanes of a 1% agarose/formaldehyde gel and subsequently transferred to nylon membranes. The cDNA inserts of pCYC02 and pCYC07 were labeled with ^{32}P, by nick-translation, and used as probes.

but it appeared within 8 to 10 h after addition of phosphate. The level of *cyc07* mRNA reached maximum values at 10 to 14 h. The levels of both *cyc02* and *cyc07* mRNAs declined after 14 to 16 h. The changes in the relative amounts of *cyc02* and *cyc07* mRNAs as a fraction of the total RNA and in terms of cell number are presented in Figure 2, together with changes in the amount of total RNA, the incorporation of [^{3}H]thymidine into the DNA fraction, and cell numbers. DNA synthesis began to increase after 8 h, reached a maximum level 12 to 16 h, and decreased 20 h after the addition of phosphate. The division of cells was observed 18 to 23 h after the addition of phosphate. These results indicate that a dramatic increase in levels of *cyc02* and *cyc07* mRNA occurs at the G_1/S boundary of the cell cycle in these synchronized cultures.

Growth Stages and Levels of RNA

The growth cycle of suspension-cultured *C. roseus* cells consisted of a lag phase of about 1 d, a logarithmic phase of 4 d, and a stationary phase which is reached about 6 d after subculture (Fig. 3). The level of *cyc02* mRNA decreased in the lag phase, increased gradually during the mid- and late-logarithmic phase, reached a maximal value after 5 to 6 d of subculture, and then it decreased again in the subsequent culture period (7–8 d, Fig. 4). By contrast, the level of *cyc07* mRNA showed a sharp peak at the midlogarithmic phase (3–4 d, Fig. 4).

Cells were arrested by starvation for sucrose or nitrogen in the medium (14), and changes in the levels of *cyc02* and *cyc07* mRNAs were examined by Northern blot analysis (Fig. 5). Our previous study demonstrated that cells were arrested at the G_1 phase as a result of nitrogen starvation, while sucrose starvation caused the arrest of cells in the G_1 and G_2 phases (14). In these growth-arrested cells, the levels of both *cyc02* and *cyc07* mRNAs decreased considerably. These results indicate that the expression of genes that encode *cyc02* and *cyc07* mRNA is correlated with the proliferation of cells.

Sequence of Full Length *cyc02* cDNA

The *cyc02* cDNA was sequenced since the size of *cyc02* mRNA (0.9 kb) is most similar to the size of the cloned cDNA (690 bp) among the isolated four clones. Figure 6 shows both the DNA and the predicted protein sequences of *cyc02*. The consensus sequence for plant translation start sites (11), CAATGGC, was observed in the putative translation start site of *cyc02* cDNA. The coding region contains 303 nucleotides, equivalent to 101 codons, and encodes a prótein with a molecular mass of 11067 D. Fifteen hydrophobic amino acids are contained in the amino-terminal region. Repeated amino acid sequences are found in residues 42 to 64 and in 79 to 101. Of the 23 amino acids found in residues 42 to 64, 18 amino acids were identical with the amino acid sequence found in residues 79 to 101. This repeated sequence was

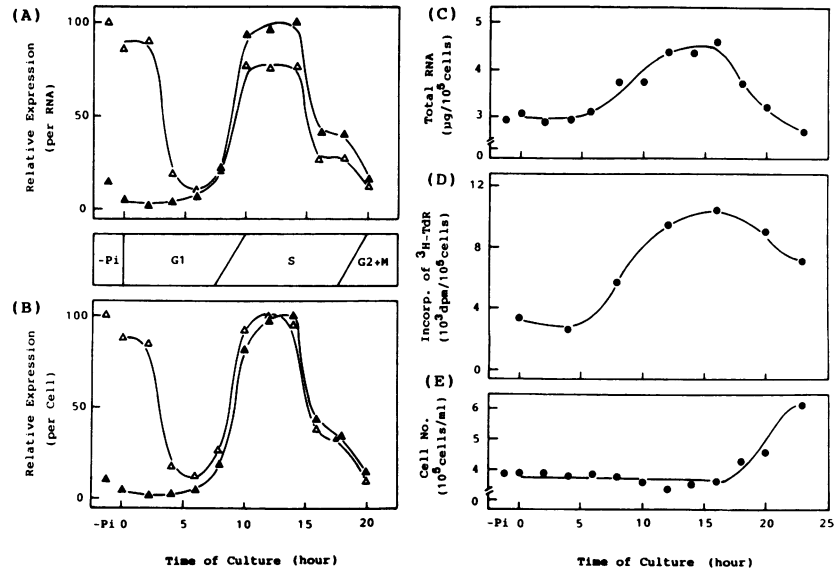

Figure 2. Levels of expression on the basis of the amount of RNA (A) and per cell (B) of isolated gene sequences, *cyc02* and *cyc07*; levels of total cellular RNA (C); incorporation of [^{3}H]thymidine into the DNA fraction (D); and cell numbers (E) in synchronous cultures. Cells were synchronized and total RNA was extracted and hybridized as shown in Figure 1. DNA synthesis was measured by pulse-labeling the cultures with [^{3}H]thymidine for 60 min and determining the amount of radioactive material that was soluble in hot 0.5 M perchloric acid. Autoradiograms were scanned densitometrically. The data are expressed as a percentage of maximal expression. (△), Expression of *cyc02*; (▲), expression of *cyc07*.

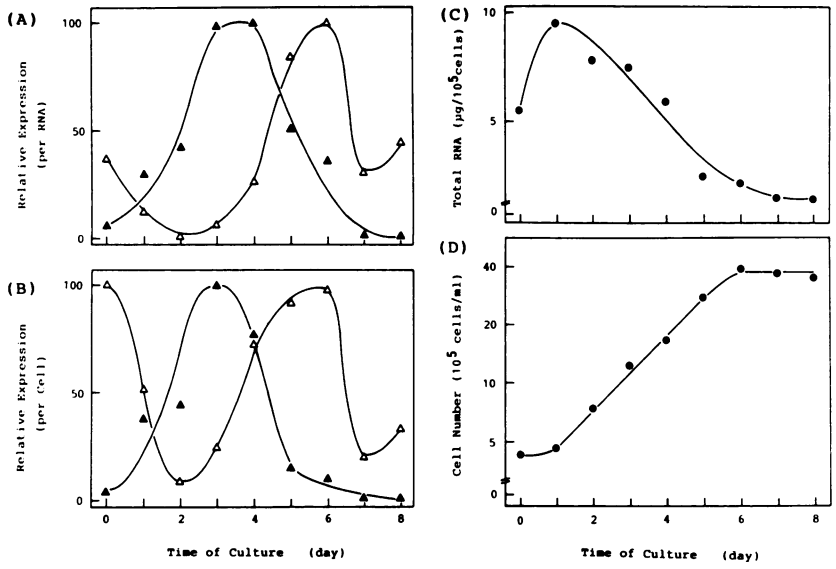

Figure 3. Levels of expression on the basis of the total amount of RNA (A) and per cell (B) of *cyc02* and *cyc07*; levels of total cellular RNA (C); and cell numbers (D) during a growth cycle. Cells were grown asynchronously and total RNA was extracted and hybridized as shown in Figure 4. Densitometry was performed as described in the legend to Figure 2. (△), Expression of *cyc02*; (▲), expression of *cyc07*.

enriched with Cys and basic amino acids such as His and Arg. The predicted amino acid sequence of *cyc02* suggests that the entire *cyc02* protein is basic. The putative polyadenylation signal ATTAAA was found 30 bp upstream of polyadenylated nucleotide sequence. Five copies of sequence ATTT are found in 3′-untranslated region. The results of sequence analysis of *cyc07* cDNA will be published elsewhere.

DISCUSSION

Several gene sequences have been isolated from a cDNA library which showed cell-cycle-dependent expression during the growth of synchronized cultures of cells of a higher plant, *Catharanthus roseus*. The *cyc02* mRNA is found preferentially in phosphate-starved cells and in cells in the early S phase. A unique gene sequence (*gas1*) has been reported in mammalian cells (24). This *gas1* gene is expressed at high levels in serum-starved NIH 3T3 cells. After the addition of serum, levels of *gas1* mRNA decrease to a minimum at 3 h, and then they increase again within 6 to 10 h (S phase). This time course of disappearance and appearance of *gas1* mRNA is surprisingly similar to that of *cyc02* mRNA. Furthermore, *cyc02* mRNA disappeared when cells began to divide during the lag phase of the growth cycle. These features of *cyc02* and the *gas1* gene suggest that, in the G_1 phase, the expression of several growth-related genes is transiently turned off at the time of the reinitiation of cell division. However, at present,

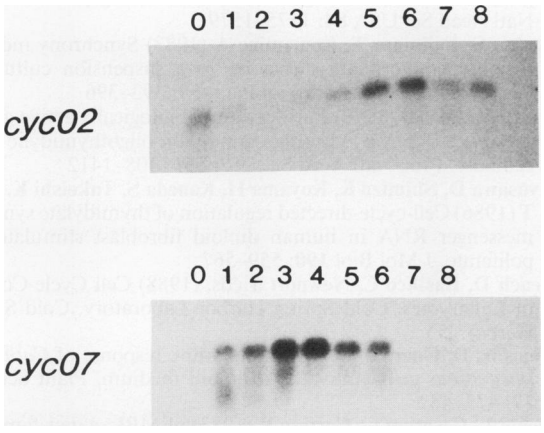

Figure 4. Regulation of the expression of *cyc02* (upper) and *cyc07* (lower) during the growth cycle. Total RNA was extracted at the indicated times after subculture, and 6-μg samples were subjected to electrophoresis on a 1% agarose/formaldehyde gel. The bands were transferred to nylon membranes and then hybridized as described in the legend to Figure 1.

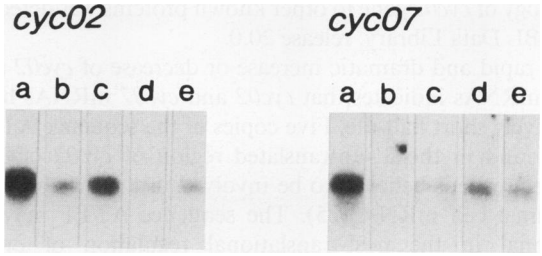

Figure 5. The presence of *cyc02* and *cyc07* mRNA in growth-arrested cells. Cells were arrested by nutrient starvation as described previously (14). In each lane, 100 ng of poly(A)$^+$ RNA was separated on a 1% agarose/formaldehyde gel, transferred to a nylon membrane, and then hybridized as described in the legend to Figure 1. Lane a, cells in the logarithmic phase. Lane b, sucrose-deprived cells. Cells in the logarithmic phase were transferred to fresh medium without sucrose and cultured for 2 d. Lane c, sucrose-limited cells. Cells were subcultured in sucrose-limited medium that contained about 0.5% (w/v) sucrose. Standard Murashige-Skoog medium contains 3% (w/v) sucrose. After 5 d in culture, proliferation of cells ceased completely and poly(A)$^+$ RNA was extracted. Lane d, nitrogen-deprived cells. Cells in the logarithmic phase were transferred to fresh medium without nitrogen and cultured for 2 d. Lane e, nitrogen-limited cells. Cells were transferred to nitrogen-limited medium which contained 1.2 mM nitrogen. Standard Murashige-Skoog medium contains 60 mM nitrogen. In 5-d-old cultures, growth of almost all cells was arrested and poly(A)$^+$ RNA was extracted.

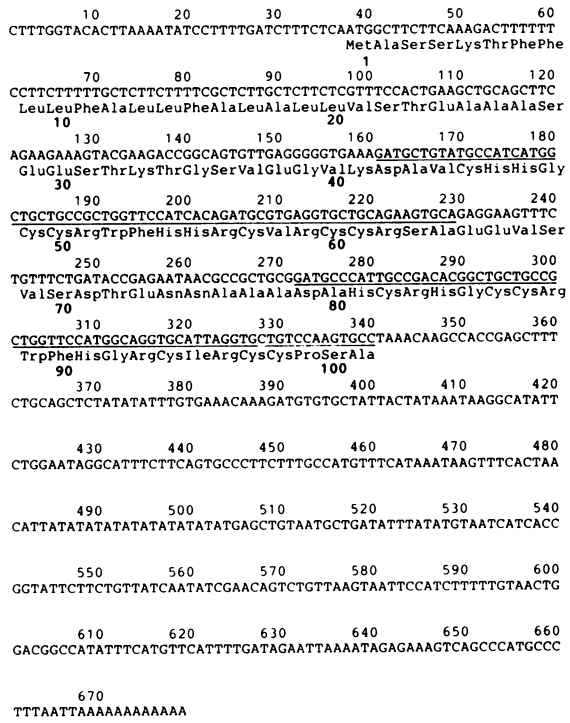

Figure 6. Nucleotide sequence of *cyc02* cDNA. The nucleotide sequence of the 5′ to 3′ strand is shown. The amino acid sequence is shown in the three letter notation under the nucleotide sequence. Repeated sequences in the open reading frame are underlined.

it is unknown whether or not any similarity in nucleotide sequence is found between these two genes, because the sequence of *gas1* has not been published. No significant homology of *cyc02* gene to other known proteins was detected in EMBL Data Library, release 20.0.

The rapid and dramatic increase or decrease of *cyc02* and *cyc07* mRNAs indicates that *cyc02* and *cyc07* mRNAs have a relatively short half-life. Five copies of the sequence ATTT were found in the 3′-untranslated region of *cyc02* cDNA. This sequence is believed to be involved in the instability of an animal cell mRNA (25). The sequence ATTT may be functional in the post-translational regulation of *cyc02* mRNA.

Maximal expression of the *cyc07* gene was observed at the G_1/S boundary. In mammalian cells, several genes have been shown to be expressed specifically at the G_1/S boundary. These genes include the genes for thymidine kinase (28), thymidylate synthase (4), histones (21), proliferating-cell nuclear antigen (1), and the human cdc2$^+$ homolog (17). Expression of most of these genes at the G_1/S boundary is transient and these genes play important roles in the cell cycle and in DNA replication. The *cyc07* mRNA also shows transient expression at the G_1/S boundary. The function of a protein encoded by *cyc07* mRNA may also be involved in regulating the progression of higher plant cells from the G_1 phase to the S phase.

We (13) previously reported the identification of four mRNAs, a, b, c, and d, the levels of which increase transiently during the cell cycle. In addition, in our previous paper, two mRNAs, l and s, were identified that were detected commonly and preferentially in actively cycling cells (14). However, neither of the two clones, *cyc02* and *cyc07*, correspond to any of the six previously reported mRNAs, namely, a, b, c, d, l, and s, as judged from the size of the corresponding mRNAs and the analysis of translation products of *cyc02* and *cyc07* (our unpublished data). It is, thus, necessary to isolate the cDNA clones that correspond to these 6 mRNAs in order to elucidate the more detailed pattern of gene expression during the cell cycle of higher plant cells.

In conclusion, two gene sequences, *cyc02* and *cyc07*, have been isolated by exploitation of their characteristic levels of expression during the cell cycle of synchronized cultures of cells of a higher plant. Northern blot analysis, using RNA isolated from cells during the growth cycle and from growth-arrested cells, indicated that the clones *cyc02* and *cyc07* contain sequences of growth-related genes. In higher plants, there are a few reports of the expression of specific genes during the cell cycle. It is well established that the histone genes are expressed in association with the synthesis of DNA, and the *cis*-acting elements involved the cell-cycle-dependent regulation of expression of histone genes have been investigated in wheat (20). Recently, the gene for plant homolog of proliferating-cell nuclear antigen was isolated (26). As auxin-regulated genes, several cDNAs were isolated in tobacco suspension cultures (29). These cDNAs are expressed when auxin was applied in the suspension. Cell division was induced by applied auxin. Another auxin-regulated gene, which is expressed during the transition from the G_0 to the S phase, was isolated from tobacco mesophyll protoplast cDNA library (27). The analysis of these genes mentioned above and the clones described in this paper should provide new insight into the regulation of the progression of the cell cycle and the regulation of gene expression during the cell cycle in the cells of higher plants.

LITERATURE CITED

1. **Almendral JM, Huebsch D, Blundell PA, MacDonald-Bravo H, Bravo R** (1987) Cloning and sequence of the human nuclear protein cyclin: homology with DNA-binding proteins. Proc Natl Acad Sci USA **84:** 1575–1579
2. **Amino S, Fujimura T, Komamine A** (1983) Synchrony induced by double phosphate starvation in a suspension culture of *Catharanthus roseus*. Physiol Plant **59:** 393–396
3. **Aviv H, Leder P** (1972) Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA **69:** 1408–1412
4. **Ayusawa D, Shimizu K, Koyama H, Kaneda S, Takeishi K, Seno T** (1986) Cell-cycle-directed regulation of thymidylate synthase messenger RNA in human diploid fibroblast stimulated to poliferate. J Mol Biol **190:** 559–567
5. **Beach D, Basilico C, Newport J** eds, (1988) Cell Cycle Control in Eukaryotes. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. **Courtois D, Guern J** (1980) Temperature response of *Catharanthus roseus* cells cultivated in liquid medium. Plant Sci Lett **17:** 473–482
7. **Dente L, Cesareni G, Cortese R** (1983) pEMBL: a new family of single-stranded plasmids. Nucleic Acids Res **11:** 1645–1655
8. **Ettlinger C, Lehle L** (1988) Auxin induces rapid changes in phosphatidylinositol metabolites. Nature **331:** 176–178
9. **Huynh TV, Young RA, Davis RW** (1985) Constructing and screening cDNA libraries in λgt10 and λgt11. *In* DM Glover, ed, DNA Cloning—A Practical Approach, Vol 1. IRL Press, Oxford, pp 49–78

10. **Ittmann M, Greco A, Basilico C** (1987) Isolation of the human gene that complements a temperature-sensitive cell cycle mutation in BHK cells. Mol Cell Biol **7:** 3386–3393
11. **Joshi CP** (1987) An inspection of the domain between putative TATA box and translation start site in 79 plant genes. Nucleic Acids Res **15:** 6643–6653
12. **Kodama H, Komamine A** (1988) Regulation and gene expression in the cell cycle of higher plants. *In* BJ Miflin, ed, Oxford Surveys of Plant Molecular and Cell Biology, Vol 5. Oxford University Press, Oxford, pp 185–193
13. **Kodama H, Kawakami N, Watanabe A, Komamine A** (1989) Phase-specific polypeptides and poly(A)$^+$ RNAs during the cell cycle in synchronous cultures of *Catharanthus roseus* cells. Plant Physiol **89:** 910–917
14. **Kodama H, Ando S, Komamine A** (1990) Detection of mRNAs cerrelated with proliferation of cells in suspension cultures of *Catharanthus roseus*. Physiol Plant **79:** 319–326
15. **Lau LF, Nathans D** (1985) Identification of a set of genes expressed during the G_0/G_1 transition of cultured mouse cells. EMBO J **4:** 3145–3151
16. **Lee MG, Nurse P** (1987) Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. Nature **327:** 31–35
17. **Lee MG, Norbury CJ, Spurr NK, Nurse P** (1988) Regulated expression and phosphorylation of a possible mammalian cell-cycle control protein. Nature **333:** 676–679
18. **Lehrach H, Diamond D, Wozney JM, Boedtker H** (1977) RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16:** 4743–4751
19. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–497
20. **Nakayama T, Ohtsubo N, Mikami K, Kawata T, Tabata T, Kanazawa H, Iwabuchi M** (1989) *Cis*-acting sequences that modulate transcription of wheat histone H3 gene and 3′ processing of H3 premature mRNA. Plant Cell Physiol **30:** 825–832
21. **Rickles R, Marashi F, Sierra F, Clark S, Wells J, Stein J, Stein G** (1982) Analysis of histone gene expression during the cell cycle in HeLa cells by using cloned human histone genes. Proc Natl Acad Sci USA **79:** 749–753
22. **Sanger F, Nicklen S, Couson AR** (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467
23. **Schmidt GW, Bartlett SG, Grossman AR, Cashmore AR, Chua N** (1981) Biosynthetic pathway of two polypeptide subunits of the light-harvesting chlorophyll a/b protein complex. J Cell Biol **91:** 468–478
24. **Schneider C, King RM, Philipson L** (1988) Genes specifically expressed at growth arrest of mammalian cells. Cell **54:** 787–793
25. **Shaw G, Kamen R** (1986) A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediate selective mRNA degradation. Cell **46:** 659–667
26. **Suzuka I, Daidoji H, Matsuoka M, Kadowaki K, Takasaki Y, Nakane PK, Moriuchi T** (1989) Gene for proliferating-cell nuclear antigen (DNA polymerase δ-auxiliary protein) is present in both mammalian and higher plant genomes. Proc Natl Acad Sci USA **86:** 3189–3193
27. **Takahashi Y, Kuroda H, Tanaka T, Machida Y, Takebe I, Nagata T** (1989) Isolation of an auxin-regulated gene cDNA expressed during the transition from G_0 to S phase in tobacco mesophyll protoplasts. Proc Natl Acad Sci USA **86:** 9279–9283
28. **Thompson CB, Challoner PB, Neiman PE, Groudine M** (1985) Levels of *c-myc* oncogene mRNA are invariant throughout the cell cycle. Nature **314:** 363–366
29. **Van der Zaal EJ, Memelink J, Mennes AM, Quint A, Libbenga KR** (1987) Auxin-induced mRNA species in tobacco cell cultures. Plant Mol Biol **10:** 145–157
30. **Watson CJ, Jackson JF** (1985) An alternative procedure for the synthesis of double-stranded cDNA for cloning in phage and plasmid vectors. *In* D Glover, ed, Practical Approach, Vol I. IRL Press, Oxford, pp 79–88