A PSTTLRE-form of cdc2-like gene in the marine microalga *Dunaliella tertiolecta*

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**Abstract**

To understand the genetic control of algal cell division cycle that pertains to phytoplankton bloom dynamics in the sea, we cloned and analyzed a gene coding for a cyclin-dependent kinase (CDK) for the chlorophyte *Dunaliella tertiolecta*. The cDNA cloned, 1061 bp long, contained an open reading frame of 314 amino acids. FASTA and GAP analyses showed that this sequence was most homologous to cdc2 out of all known cdk$s$, with an identity of 54–68% and a similarity of 65–76% to cdc2 in higher plants, animals, and yeast. Several signature domains of cdc2 were identified from this sequence, although the PSTAIRE and GDSEID motifs were replaced with PSTTLRE and GDCELQ, respectively. Southern blot hybridization demonstrated that this gene occurred as a single copy in this species, and quantitative RT–PCR showed that the transcription of this gene was constitutive. The present results suggest that the universal cdc2 is conserved in the lower eukaryote with unique structural characteristics. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords**: Alga; cdc2; cdk; cDNA; Cell cycle; Phytoplankton

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1. **Introduction**

Cyclin-dependent kinases (CDK$s$) are the central components of the cell-cycle machinery in eukaryotes (Jacobs, 1992; Nigg, 1995). In association with cyclins, they trigger a well-coordinated sequence of cell-cycle events (e.g., G1/S, G2/M transitions) through a phosphorylation and dephosphorylation cascade. Nine members of this kinase family have been described for different organisms, six of which function in cell-cycle regulation through binding to their specific cyclins. CDK1 (p34cdc2) is the most universal and highly conserved member in this CDK family (Pines and Hunter, 1990; Nigg, 1995). It was first identified for yeast (Beach et al., 1982), as cdc2 for the fission yeast *Schizosaccharomyces pombe* and cdc28 for the budding yeast *Saccharomyces cerevisiae*. In yeast, the cdc2/cdc28 gene is required for both G1/S and G2/M transitions, whereas in higher organisms it is required for G2/M transition. The non-active form of p34cdc2 is synthesized constantly in the cell cycle and becomes undetectable in differentiated or growth-arrested plant tissues (e.g., Colasanti et al., 1991). The kinase activity, which oscillates in the cell cycle, is regulated by phosphorylation (by CDK-activating kinase or CAK) and dephosphorylation at characteristic threonine/tyrosine residues following binding to cyclins. The substrates of the p34cdc2 kinase, not well understood yet, include histone H1, nuclear lamin, microtubule-associated proteins (see Jacobs, 1992 for review), myosin II regulatory light chain (Komatsu et al., 1997), and other mitosis-related proteins (e.g., Resnick et al., 1997; Kimura et al., 1998). Cdc2 gene sequence has characteristic domains, such as EGVPSTAIREISLLKE (cyclin-binding domain, the so-called PSTAIRE/SLIKE), EGVTY (ATP-binding, HARDLNQ, WYRAPE, GDSEID, and sites for phosphorylation (Thr-161,167, Thr-14/Tyr-15) and dephosphorylation (Thr-14/Tyr-15).

In addition to yeast and other fungi (e.g., Osmani et al. 1994), cdc2 has been identified in as wide a range...
of organisms as slime mold (Michaelis and Weeks, 1992), invertebrates (e.g., Tang et al., 1993; Komatsu et al., 1997), and higher animals including humans (e.g., Lee and Nurse, 1987). Among photosynthetic organisms, known \textit{cdc2} genes are limited to higher plants (Colasanti et al., 1991; Ferreira et al., 1991; Feller and Jacobs, 1991; Hata, 1991; Hirayama et al., 1991; Hirt et al., 1991; Miao et al., 1993; Kvarnheden et al., 1995; Fobert et al., 1996; Lessard et al., 1999). Recent immunocytochemical evidence suggested the presence of p34\(^{\text{cd}2}\)-like proteins in several chlorophyte algae living in the aquatic environment (John et al., 1989; Lin et al., 1996). Histone H1 kinase activity associated with the p34\(^{\text{cd}2}\)-like proteins was detected in a photosynthetic dinoflagellate \textit{Gambierdiscus toxicus} (Van Dohlen et al., 1995). However, direct evidence from gene sequence is still lacking concerning these lower forms of photoautotrophs. It would be of great interest and significance to understand the regulation of the cell-division cycle for these organisms, because they (including chlorophyte, chromophyte, dinoflagellates, and others) constitute the foundation of the marine ecosystem. These organisms, collectively named microalgae despite their genetic diversity, sometimes cause the so-called harmful algal blooms that devastate the environment and the fishery industry.

We are trying to investigate the genetic uniqueness of these organisms regarding the cell-cycle control in the hope of gaining better understanding on what regulates the dynamics of phytoplankton populations. In this article, we report identification of a novel (PSTTLRE) form of the \textit{cdc2}-like gene for \textit{Dunaliella tertiolecta}, a marine chlorophyte alga.

2. Materials and methods

2.1. Algal culture and sample collection

A 2 L batch culture of \textit{Dunaliella tertiolecta} Butcher (Chlorophyceae) (clone CCMP 1302, Bigelow Laboratory) was grown with 1/2 medium in a 3 L glass flask. The temperature was controlled at 20 ± 1 °C and illumination was provided by cool white fluorescent bulbs with a photon flux of 100 \(\mu\text{E} \text{m}^{-2} \text{s}^{-1}\). A photocycle of 12 h light alternated with a 12 h dark period was used to phase the cell cycle such that mitosis and cell division occur mostly during the dark period. Growth rate was monitored daily by taking a 2 ml sample for determination of cell concentration with a Sedgwick-Rafter counting chamber.

When the culture reached the exponential growth phase (specific growth rate \(\mu \geq 0.6 \text{day}^{-1}\)), a sample of 250 ml was harvested for gene cloning purpose by centrifugation at 890 \(\times\) g at 4 °C. The cell pellet was resuspended at a final concentration of \(10^8\) cells ml\(^{-1}\) in 1.0–1.5 ml TRIzol Reagent (Gibco BRL, Gaithersburg, MD) and stored at −80 °C until RNA extraction within 2 days. To determine the pattern of transcription, two separate cultures were grown under the same conditions as described above. At the exponential growth phase (\(\mu = 0.63 \text{day}^{-1}\)), a 200 ml sample was collected from one of the cultures at each of the six time points, i.e., 3, 9, 15, 21, 3, 9 h since the onset of the light period. When the other culture entered stationary growth phase in a month (\(\mu = 0.21 \text{day}^{-1}\)), the same amount of sample was collected at 3. A final sample was taken at h 5 after another month, when there was essentially no apparent growth (\(\mu = 0.01 \text{day}^{-1}\)).

2.2. RNA extraction

Total RNA was extracted using TRIzol Reagent following the manufacturer’s instruction (Gibco BRL). After precipitation with ethanol and brief air-dry, RNA was dissolved in 50 µl of DEPC-treated distilled and deionized \(\text{H}_2\text{O}\). The concentration of RNA was measured spectrophotometrically at 260 nm wavelength.

2.3. cDNA synthesis and polymerase chain reaction

For gene cloning and sequencing purpose, about 5 µg of total RNA was used to synthesize complementary DNA (cDNA) using a Superscript Preamplification system (Gibco BRL) with an oligo (dT)\(_{15–18}\) primer. To ensure that no DNA contaminant was present and amplified, a negative control was constructed with the same amount of RNA but no reverse transcriptase was added (−RT control). Two microtubes of the cDNA or the −RT control was then used for PCR reaction performed with a Hybrid Omnis-E thermocycler (Hybaid Instruments, Holbrook, NY). Three degenerated primers were designed based on the three highly conserved regions, GEGTYG (forward: CDC2A), GDSEID (reverse: CDC2B), and EHPYFND (reverse: CDC2ER) (see Fig. 2). The sequences were CDC2A: 5′-GGG GAR GGI ACY TAY GG-3′; CDC2B: 5′-CTC AAT CTC GSA GTC TTC-3′; CDC2ER: 5′-TCR TYG AAG TAI GGG TGC TC-3′. Two internal precise-matched primers derived from the cloned sequence were used to confirm the fragments amplified: KLVALKK (forward: INT1F) and VMHRDLK (reverse: INT1R) (see Fig. 2). After obtaining the full-length cDNA sequence (see Section 3), a set of precise-matched primers were used to clone the full-length cDNA, DUNCDC23F (forward) and DUNCDC22R (reverse) (see Fig. 2). PCR reaction in a volume of 50 µl was hot-started (at 94 °C) with 2.5 U Taq polymerase (Gibco BRL) and continued for 30 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, and extending at 72 °C for 1 min. An additional extension was done at 72 °C for 10 min. At the end of the reaction, 8 µl from each tube was removed and resolved with 1% agarose gel electrophoresis. The
DNA bands of interest were excised and DNA recovered using Spin X columns (Costar, Cambridge, MA). After ethanol-precipitation, it was dissolved in 5 μl of ddH2O.

2.4. RACE

Rapid amplification of cDNA end (RACE) was performed to extend the 3' and 5' end of the cDNA, essentially following Frohman et al. (1988) as modified slightly. The modification included that no radiolabeled nucleotide was used and amplification was run with the same temperature cycles as regular PCR, as described above. For 3'-end extension, the first-strand cDNA was synthesized as described earlier using oligo(dT)$_{17-20}$, which was primed by CDC2A (1 μM), (dT)$_{17-20}$, Adaptor (0.5 μM), and Adaptar (5'-GCG GCC GGC TCG AGA TCG AT-3', 1 μM) in the first PCR. A secondary PCR was performed using 0.5 μl of the primary PCR product as the template and INTIF and Adaptor as the primers. For 5'-end extension, the first-strand cDNA was synthesized with CDC2B as the primer. Subsequent 5'-tailing PCR reactions with this cDNA as a template were carried out using a 5'-RACE system (Gibco BRL), with CDC2B as gsp1 (gene specific primer 1) and INTIR as gsp2.

2.5. Cloning and sequence analysis

Three microliters of the DNA recovered from PCR or RACE was ligated to a pCR2.1 vector (Invitrogen, Carlsbad, CA) overnight at 14°C. Transformation of the ligate was carried out following instructions from Invitrogen. Cloning and sequencing was essentially according to Lin and Carpenter (1998).

Two to three clones from each cloning (PCR fragment, RACE, and full-length PCR product) were selected for DNA sequencing. Plasmid was isolated using the Plasmid MiniPrep kit (QIAGEN, Santa Clarita, CA) and sequenced using the dideoxynucleotide terminator method with an ABI Prism Dye-terminator terminator system (PE Biosystems, Foster City, CA). Both strands were sequenced with an overlapping scheme throughout the whole gene fragment. Sequences were analyzed using GCG programs such as FASTA, GAP, and MAP (Genetics Computer Group, Madison, WI), and a maximum parsimony tree was constructed using the PHYLLOWIN software package (Galtier et al. 1996).

2.6. Southern blot hybridization

Ten micrograms of DNA was digested with HindIII, PstI, EcoRI, and Accl for 4 h at 37°C. Agarose gel (0.8%) electrophoresis of the digestion product, transfer to nylon membrane, and Southern blot hybridization were performed essentially following Lin and Carpenter (1998). Briefly, the membrane was prehybridized at 68°C for 30 min with a buffer containing 5 × SSC buffer, 0.1% (w/v) Na-lauroyl sarcosine, 0.02% (w/v) SDS, 1% blocking agent (Boehringer Mannheim, Indianapolis, IN). Hybridization was then carried out overnight at 68°C with fresh buffer supplemented with the cDNA probe at a concentration of 125 ng ml$^{-1}$. The probe used was the Accl fragment of the cDNA amplified with CDC2A and CDC2B which was random-primed labeled with digoxigenin-tagged deoxynucleotide (Boehringer Mannheim). At the end of hybridization, the membrane was washed 2 × 5 min in 2× SSC buffer containing 0.1% SDS (w/v) at room temperature followed by washes 2 × 15 min in 0.1 × SSC buffer containing 0.1% SDS at 68°C under constant agitation. Finally, the membrane was subjected to immunological detection following the manufacturer’s instructions.

2.7. Quantitative RT-PCR

To determine the relative abundance of the gene transcript, RNA preparation was digested with RNase-free DNase I (Ambion, Austin, TX). Then 2–5 μg RNA equivalent to the same number of cells from each sample was primed by random hexamers for cDNA synthesis. One microliter of the cDNA product from each sample was used for PCR. In the PCR reaction, 18S rDNA was simultaneously amplified using a set of plant-specific primers (Ambion). Since 18S rRNA is predominantly abundant, it is necessary to prevent saturated amplification. A set of Competimer (Ambion) was added in the same amount as 18S primers (i.e., 1:1 ratio) to reduce amplification of 18S rDNA so that the increase rate of its amplified product would remain within the linear range. Competimer was essentially the same as regular 18S primers, except that the 3'-end was dephosphorylated so that no extension would occur even though they could prime to the DNA template.

In preliminary experiments, 0.25, 0.5, 1, and 2 μl of cDNA products were used for PCR to test for the linear relationship between the volume of cDNA used and the level of the target PCR product. We observed a linear increase in PCR product for up to 1 μl and a plateau was reached when 2 μl was used (results not shown), and 1 μl was thus chosen for quantitative PCR. The PCR was run with conditions described earlier for a total of 30 cycles. At cycles 10 and 20, 5 μl was removed from each tube. At the end, 5 μl of the three sets of PCR products were electrophoresed on 1% agarose gel prepared with a small-well comb. The gel was stained with ethidium bromide and documented with a Polaroid camera system. The samples collected at cycle 20 yielded a specific faint band on the gel for both 18S and cdc2, respectively, whereas those from cycle 10 did not produce any bands for cdc2 and barely visible bands for 18S. This result confirmed our preliminary observation.
that the amplification was still exponential within 30 cycles, which produced clear bright DNA bands of both cdc2 and 18S.

For quantification, 8 μl of the 30 cycle product was resolved by gel electrophoresis. The bands of the specific PCR products were scanned using a FotoTouch ScanMan and the relative intensity of the bands was measured using SigmaGel software (Jandel Scientific, San Rafael, CA).

3. Results and discussion

The degenerated primers yielded specific fragments of the cdc2-like gene in Dunaliella tertiolecta (Fig. 1). Absence of amplified products in the −RT negative control (Fig. 1, lane 1) indicates that these amplified products were truly of cDNA rather than genomic DNA that might be present in the RNA preparation. The molecular size of the amplicon was about 620 bp from primers CDC2A/CDC2B (Fig. 1, lane 2), 850 bp from CDC2A/CDC2ER (Fig. 1, lane 4) and 310 bp from INT1F/INTIR (Fig. 1, lane 3). All these sizes were as expected for the cdc2 gene.

Rapid amplification of cDNA ends (RACE) produced a dominant band mixed with several light bands in the secondary PCR in each of the 3′-end and 5′-end extensions (not shown). A single full-length cDNA of 1061 bp was obtained from the dominant bands from RACE (Fig. 2). Based on the gene sequence obtained, primers DUNCDC23F and DUNCDC22R were designed and the full-length cDNA was also generated directly from one PCR (Fig. 1, lane 5). Sequencing of all the PCR and RACE products cloned showed that they all came from the same gene with identical sequence.

3.1. High homology to cdc2

Comparison analyses revealed similar amino-acid composition between the cDNA cloned and cdc2 in other organisms, with leucine being dominant (results not shown). This gene contained an open reading frame (ORF) coding for 314 amino acids, ending with the stop codon of TGA (Fig. 2). The C-terminus has several additional amino-acid residues compared to the cdc2 genes in other organisms, which results in a predicted molecular mass (35.5 kDa) higher than 34 kDa. Nevertheless, the amino-acid sequence of this ORF had a highest homology (68% identity and 76% similarity) with cdc2B in Arabadopsis thaliana and shared 59% identity and 71% similarity with cdc2-1 in rice (Oryza sativa) (Fig. 3). In comparison to cdc2/cdc28 in fission and budding yeast, S. cerevisiae and S. pombe, the algal gene shares an identity of 54% and a similarity of 65% and 69%, respectively. An identity of 48–57% and a similarity of 55–70% were found in comparison with other higher plants and animals. The homology of this algal gene to cdc2 in higher plants agrees with the close phylogenetic relatedness between chlorophyte algae and higher plants.

Highly conserved domains, characteristic of cdc2 and related genes, such as GEGTYG, HARDLKPQNL, and WYRAPE, were identified in D. tertiolecta (Fig. 3). The phosphorylation residues, Thr-14 and Tyr-15, were also present. There was a threonine at position 169, which appeared to be a homolog of Thr-161 in fission yeast, S. pombe. The algal gene shares an identity of 54% and a similarity of 65% and 69%, respectively. An identity of 48–57% and a similarity of 55–70% were found in comparison with other higher plants and animals. The homology of this algal gene to cdc2 in higher plants agrees with the close phylogenetic relatedness between chlorophyte algae and higher plants.

Fig. 1. RT-PCR products resolved by electrophoresis on 1% agarose gel stained with ethidium bromide. Lane M is 1 kb DNA molecular weight marker (Roche Applied Science, marker X), from the top are (in kb) 8.14, 7.13, 6.11, 5.09, 4.07, 3.05, 2.04, 1.64, 1.32, 0.51, 0.40, 0.34, 0.30, 0.22. Lanes 1–4: 8 μl (16% of total) of the PCR product was loaded to each lane. 1: negative control (−RT); 2: CDC2A/CDC2B as primers; 3: INT1F/INTIR as primers; 4: CDC2A/CDC2ER as primers; 5: DUNCDC23F/DUNCDC22R as primers. Arrows and numbers at the right indicate the size of the four PCR products in Lanes 2–5.
Fig. 2. The nucleotide and deduced amino-acid sequences of the algal p34<sub>cdc2</sub> homolog. Highly conserved domains such as the ATP-binding domain and cyclin-binding domain are shown in bold type. Sequences used for primer design are underlined or overlined. The small asterisks denote the phosphorylation sites and the large asterisk denotes the stop codon. The gene sequence has been deposited in GenBank under the accession number AF038570.

described previously, but is close to PSSALRE found in <i>Xenopus cdc2</i>-2 and CDK5 (Fig. 4) and PSTAIRE in <i>Dictyostelium discoideum</i> cdc2 (Michaelis and Weeks, 1992). Finally, it was also noted that another motif characteristic of cdc2, GDSEID, was present as sequences analyzed, D. tertiolecta was clustered with plant/yeast cdc2/cdc28. The clustering pattern is in accordance to sequence similarity analyzed with GCG programs, except that rice cdc2-1 was far separated from <i>D. tertiolecta</i> despite its high similarity. The disparity may arise from the insertions occurring in the alga, yeast and <i>A. thaliana</i>, but absent in rice and other organisms (Fig. 3). Based on a partial cDNA sequence obtained using PCR-based cloning, a chromophyte alga,
Fig. 3. Alignment of the algal amino-acid sequence with \textit{cdc2} in other organisms. Abbreviated species names and databank accession numbers: \textit{Dun} = \textit{D. tertiolecta} (this study, AF038570); \textit{ARATH} = \textit{Arabidopsis thaliana}, \textit{cdc2B} (P25859); \textit{Maiz} = \textit{Zea mays}, \textit{cdc2} (P23111); \textit{Rice} = \textit{Oryza sativa}, \textit{cdc2}-1 (P29618); \textit{Human}, \textit{cdc2} (P06493); \textit{SCHPO} = \textit{Schizosaccharomyces pombe}, \textit{cdc2} (P04551); \textit{SCERE} = \textit{Saccharomyces cerevisiae}, \textit{cdc28} (P00546); \textit{XENL} = \textit{Xenopus laevis}, \textit{cdc2}-2 (P24033). Dark shade indicates identical residues; gray shade indicates similar residues; unshaded are dissimilar residues.
Fig. 4. Phylogenetic tree derived from Maximum Parsimony analysis using PHYLOWIN. Bootstrap values over 50% are shown inside the nodes; results were based on 500 replicates. Dunaliella tertiolecta is shown in bold type. Abbreviated species names and databank accession numbers: ARATH, cdc2B gene of Arabidopsis thaliana (P25839); CAEEL, Caenorhabditis elegans, cdc2 (Q02399); CANAL, Candida albicans, cdc28 (P40663); ENTHI, Entamoeba histolytica, cdc2 (Q04770); Human, cdc2 (P06493), cdc2 (P24941), cdc3 (Q05326), cdk5 (Q00535); ISO, Isochrysis galbana (unpubl.); LEIME, Leishmania mexicana, cdc2 (Q06309); Maize, Zea mays, cdc2 (P23111); MEDSA, Medicago sativa, cdc2-2 (Q05066); Mouse, cdk5 (P46815); TRVO, cdc2-1 gene of Trypanosoma congoense (P34664); PLAFK, cdk2 of Plasmodium falciparum (Q07865); Rat, cdk2 (P39951), cdk5 (Q05114); Rice, Oryza sativa, cdc2-1 (P59615); SCERE, Saccharomyces cerevisiae, cdc2 (P05486); SCHPO, Schizosaccharomyces pombe, cdc2 (P05451); TRYBB, T. brucei brucei, cdk1 gene (P54664); VIGUN, Vigna unguiculata, cdc2 (P52389).

Isochrysis galbana, was clustered with group B. Its PSTAIRE motif occurred as PVTTLRE (Fig. 4). The comparison suggests that among microalgae, the cdc2 sequence may be as variable as their general genetic identities.

Ability to complement yeast cdc2 mutants has been commonly used to define a cdc2 gene (Lee and Nurse, 1987; Ferreira et al., 1991; Osman et al., 1994; Hirt et al., 1991; Michaelis and Weeks, 1992). Whether the algal gene will complement yeast cdc2 remains to be examined. Nevertheless, the analysis described above suggests that this gene is likely a cdc2 variant.

3.2. Single-copy gene

Southern blotting of the genomic DNA using an algal cDNA clone as a probe showed a single band for digestion with HindIII, PstI, and AccI, respectively (Fig. 5); all the restriction enzymes, except AccI, do not have internal restriction sites in the cDNA cloned. The restriction digestion with EcoRI in this case appeared to generate two close bands, but in a repeated blot, only the band with lower molecular size was apparent (not shown), suggesting that the two bands were the result of incomplete digestion. Cdc2 genes also occur in single copy in some higher plants (e.g., Ferreira et al., 1991) and lower organisms (e.g., Michaelis and Weeks, 1992), although multiple copies have been found in some other
species (e.g., Tang et al., 1995). It is noteworthy that \textit{Acc}I did not produce a band of 525 bp from genomic DNA as it did from cDNA (restriction results not shown, but \textit{Acc}I restriction site can be located on the cDNA sequence shown in Fig. 2); a larger fragment was generated instead. This suggests that an intron is probably present and disrupts the restriction site. Further research with genomic clones is warranted to identify the intron.

3.3. Expression pattern

Using quantitative RT-PCR technique, we demonstrated that the transcription of this \textit{cdc}2-like gene varied only by a factor of 2 in different phases of growth rate or cell-division cycle (Fig. 6). In the exponential culture in which the cell cycle was phased (Fig. 6A), the transcript level per cell was lower at h 3 (3 h from the onset of the light period), before mitosis began (Fig. 6B, C). The mRNA level was similar to levels detected for the stationary culture in which most of cells were in the G1/G0 phase (Fig. 6B, C). At h 9 and 15 when mitosis and cell division were underway (Fig. 6A), the \textit{cdc}2 transcript increased by nearly 2-fold (Fig. 6B, C). In contrast, 18S rDNA that was amplified simultaneously using primers 18S1, 18S2, and Competimers remained somewhat constant in the diel cycle (proxy of cell-division cycle) (Fig. 6B, C). In the stationary growth phase, the 18S transcript decreased significantly (Fig. 6B, C). The ratio of the \textit{cdc}2 transcript to 18S transcript displayed a pattern similar to \textit{cdc}2 for the exponential culture (Fig. 6C), but increased markedly for the h 15 sample of the stationary culture due to the dramatic decrease in the 18S rRNA.

Variability of \textit{cdc}2 transcription with growth phase seems to vary with species. In the fission yeast \textit{S. pombe}, the \textit{cdc}2 transcript level does not change between proliferating and stationary stages (Durkacz et al., 1986). Similarly, \textit{cdc}2A transcription in \textit{Arabidopsis} also appeared to be invariant in different organs (Ferreira et al., 1991). In contrast, \textit{cdc}2 mRNA is more abundant in actively growing tissues of humans (Dalton, 1992), some higher plants (Colasanti et al., 1991), and the exponential stage of the slime mold \textit{D. discoideum} (Michaelis and Weeks, 1992) and the ciliate \textit{Paramecium tetraurelia} (Tang et al., 1995). Protein abundance also varies dramatically with growth phase. Dramatic decrease in the CDC2 abundance has been observed widely when cells transform from proliferating to resting (or differentiated) states (e.g., John et al., 1989). The same pattern was found for the protein homolog in \textit{D. tertiolecta} detected with an anti-p34\textit{cdc}2, which decreased dramatically from its high level in the exponential growth phase to nearly negligible level in the late stationary stage (Lin et al., 1996). Taken together, it is suggested that growth phase-specific cellular content of \textit{cdc}2 mRNA is regulated differentially in different organisms. Although it is generally held that CDC2 protein abundance is constant, abundance of mRNA may vary in the cell-division cycle. \textit{Cdc}2 transcript level was
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