Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2444–2447, March 1992
Cell Biology

Cloning of a D-type cyclin from murine erythroleukemia cells

(CYL2 cDNA/cell cycle)

HIROAKI KIYOKAWA*, XAVIER BUSQUETS, C. THOMAS POWELL, LANG NGO, RICHARD A. RIFKIND, AND PAUL A. MARKS

DeWitt Wallace Research Laboratory, Memorial Sloan-Kettering Cancer Center and the Sloan-Kettering Division of the Graduate School of Medical Sciences, Cornell University, New York, NY 10021

Contributed by Paul A. Marks, December 13, 1991

ABSTRACT **We report the complete coding sequence of a cDNA, designated CYL2, derived from a murine erythroleukemia cell library. CYL2 is considered to encode a D-type cyclin because (*i*) there is cross hybridization with CYL1 (a murine homolog of human cyclin D_1) and the encoded protein has 64% amino acid sequence identity with CYL1 and (*ii*) murine erythroleukemia cell-derived CYL2 contains an amino acid sequence identical to that previously reported for the C-terminal portion of a partially sequenced CYL2. Transcripts of murine erythroleukemia cell CYL2 undergo alternative polyadenylylation like that of human cyclin D_1. A major 6.5-kilobase CYL2 transcript changes its expression during the cell cycle with a broad peak through G_1 and S phases and a decrease in G_2/M phases. The present findings suggest that CYL2 plays a role in the G_1 to S phase progression.**

A group of proteins that appear to be involved in regulating cell cycle progression, namely, the cyclins and the cyclin-associated $p34^{cdc2/CDC28}$, are highly conserved among eukaryotes from yeast to human (1–4). These protein complexes, with serine/threonine kinase activity, play a critical role at the major regulatory points in cell cycle progression, the G_1 to S and G_2 to M phase transitions in the cell cycle. The *cdc2/CDC28* gene, conserved among the eukaryotes tested to date, encodes the kinase catalytic subunit (5–8). Association of the cdc2/CDC28 protein with regulatory subunits known as cyclins activates the kinase. Cyclins were originally discovered in marine invertebrates as proteins that fluctuate in expression during cell division (9). B-type cyclins have been defined as mitotic cyclins that may trigger mitosis by activating the cdc2/CDC28 catalytic subunit (10–13). The function of A-type cyclins is less well understood, but it has been proposed (14–16) that A-type cyclins play a role at an earlier stage of the cell cycle, perhaps during S phase. In the budding yeast CLN-type cyclins (CLN1, CLN2, and CLN3) are required for G_1 to S phase progression (17–19). CLN1 and CLN2 mRNA levels fluctuate during the cell cycle, peaking in late G_1 phase. CLN2 protein was shown to associate with $p34^{CDC28}$ (20), suggesting that the kinase activity activated by CLN proteins regulates progression through the G_1 phase start point for commitment to initiate DNA replication (S phase) (21).

Human genes for cyclins C, D, and E have been cloned that rescue mutant yeast deficient in CLN functions (22–24). These are putative mammalian G_1 cyclins. *PRAD1* (25) was cloned as a gene rearranged in a parathyroid tumor and is identical to human cyclin D_1. Cyclin-like genes (CYL1, CYL2, and CYL3) have been isolated from murine macrophages induced with colony-stimulating factor 1 (26), and CYL1 is a murine counterpart to human cyclin D_1. These putative mammalian G_1 cyclins, especially D-type cyclin, could be important in regulating G_1 cell cycle progression, in governing S-phase commitment to differentiation, or in tumorigenesis (27, 28). We now report the isolation and nucleotide sequence of a D-type cyclin, designated CYL2,† from murine erythroleukemia cells (MELCs). The observed fluctuation in level of CYL2 mRNA during the cell cycle suggests a role in commitment to the G_1 to S phase transition.

MATERIALS AND METHODS

Cell Cultures. DS19/Sc9 MELCs, derived from 745A cells (29), were maintained in α-modified minimal essential medium supplemented with 10% (vol/vol) fetal calf serum.

Isolation of cDNA Clones. A cDNA library was prepared in λZAP (Stratagene) from an uninduced logarithmic-phase culture of DS19/Sc9 cells as described (30). The library contains oligo(dT)-primed cDNAs in a unique *Eco*RI site of the vector. Approximately a million recombinant plaques were screened with the entire coding region of CYL1 (murine cyclin D_1) cDNA (a generous gift from C. J. Sherr, St. Jude Children's Research Hospital, Memphis, TN) as a radiolabeled probe. Hybridization was performed in 5× SSPE (1× SSPE is 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) containing 5× Denhardt's solution, 10% (wt/vol) dextran sulfate, and denatured salmon sperm DNA (0.1 mg/ml) at 58°C, and filters were washed with 1× SSPE/0.1% SDS at 48°C. Isolated λ phages were converted to pBluescript plasmids (Stratagene) according to the manufacturer's instruction. Nucleotide sequences were obtained by the dideoxynucleotide chain-termination method (31) using double-strand DNA templates. Sequences in GenBank were searched (November 12, 1991) for homology with the FASTA program (32).

Northern Blot Analysis. Poly(A)$^+$ RNA was isolated from cells by using a FastTrack mRNA isolation kit (Invitrogen, San Diego). Total RNA was extracted by acid guanidinium thiocyanate/phenol/chloroform extraction (33). About 4 μg of poly(A)$^+$ RNA or 10 μg of total RNA was electrophoresed on 1.2% agarose/formaldehyde gels, transferred to nitrocellulose, and hybridized with randomly primed [α-^{32}P]dCTP radiolabeled probe in 0.1 M Pipes, pH 6.8/0.65 M NaCl/5 mM EDTA/0.1% SDS/5× Denhardt's solution/50% (vol/vol) formamide/denatured salmon sperm DNA (0.1 mg/ml)/10% dextran sulfate at 42°C. Filters were then washed with 1× standard saline citrate (SSC)/0.1% SDS at 65°C. After exposure to Kodak XAR films, filters were rinsed twice with distilled water at 95°C and hybridized with another probe.

Cell Cycle Synchronization. Approximately 4–6 × 10^8 DS19/Sc9 cells in logarithmic-phase growth were loaded onto a Beckman JE-6B elutriation rotor as described (34), and 13 fractions were elutriated by stepwise increases in the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MELC, murine erythroleukemia cell.

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M83749).

```
                                                    -270 GGCGAGCTGAGGAGAGCCGGGCAGTTCGGAGGG -238
AAGGACCGGTGCGAGTCAGGCGGCCCTTGAGGCTCCGCTCGCCCACCTTCCACTCTTCTCTCTCTCTCCCTCTCTCT -159
CTTTGCCATTTCTTTCCTCTCCCAAATCTCCCATTCAGCCAAAGGAAGGAGGTAAGGGAAGCACTCCCCGACTCTCTCT  -80
CTCTCTCCAGCTCAGTCCTCCAAAAAATAATAATAATAAAAAAAATTTACAGTCGGGACCGAGTGGTGGCCGGCTGGCT   -1

ATG GAG CTG CTG TGC TGC GAG GTG GAC CCG GTC CGC AGG GCC GTG CCG GAC CGC AAC CTG   60
Met Glu Leu Leu Cys Cys Glu Val Asp Pro Val Arg Arg Ala Val Pro Asp Arg Asn Leu   20

CTG GAA GAC CGC GTT CTG CAG AAC CTG TTG ACC ATC GAG GAG CGC TAC CTC CCG CAG TGT  120
Leu Glu Asp Arg Val Leu Gln Asn Leu Leu Thr Ile Glu Glu Arg Tyr Leu Pro Gln Cys   40

TCC TAT TTC AAG TGC GTG CAG AAG GAC ATC CAA CCG TAC ATG CGC AGG ATG GTG GCC ACC  180
Ser Tyr Phe Lys Cys Val Gln Lys Asp Ile Gln Pro Tyr Met Arg Arg Met Val Ala Thr   60

TGG ATG CTA GAG GTC TGT GAG GAA CAA AAG TGT GAA GAA GAG GTC TTT CCT CTG GCC ATG  240
Trp Met Leu Glu Val Cys Glu Glu Gln Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met   80

AAT TAC CTG GAC CGT TTC TTG GCT GGA GTC CCG ACT CCT AAG ACC CAT CTT CAG CTC CTG  300
Asn Tyr Leu Asp Arg Phe Leu Ala Gly Val Pro Thr Pro Lys Thr His Leu Gln Leu Leu  100

GGT GCA GTG TGC ATG TTC CTA GCT TCC AAG CTG AAA GAG ACC ATC CCG CTG ACT GCG GAA  360
Gly Ala Val Cys Met Phe Leu Ala Ser Lys Leu Lys Glu Thr Ile Pro Leu Thr Ala Glu  120

AAG CTG TGC ATT TAC ACC GAC AAC TCT GTG AAG CCC CAG GAG CTG CTG GAG TGG GAA CTG  420
Lys Leu Cys Ile Tyr Thr Asp Asn Ser Val Lys Pro Gln Glu Leu Leu Glu Trp Glu Leu  140

GTA GTG TTG GGT AAG CTG AAG TGG AAC CTG GCC GCA GTC ACC CCT CAC GAC TTC ATT GAG  480
Val Val Leu Gly Lys Leu Lys Trp Asn Leu Ala Ala Val Thr Pro His Asp Phe Ile Glu  160

CAC ATC CTT CGC AAG CTG CCC CAG CAA AAG GAG AAG CTG TCC CTG ATC CGC AAG CAT GCG  540
His Ile Leu Arg Lys Leu Pro Gln Gln Lys Glu Lys Leu Ser Leu Ile Arg Lys His Ala  180

CAG ACC TTC ATC GCT CTG TGC GCT ACC GAC TTC AAG TTT GCC ATG TAC CCG CCA TCG ATG  600
Gln Thr Phe Ile Ala Leu Cys Ala Thr Asp Phe Lys Phe Ala Met Tyr Pro Pro Ser Met  200

ATT GCA ACT GGA AGC GTG GGA GCA GCC ATC TGT GGG CTT CAG CAG GAT GAT GAA GTG AAC  660
Ile Ala Thr Gly Ser Val Gly Ala Ala Ile Cys Gly Leu Gln Gln Asp Asp Glu Val Asn  220

ACA CTC ACG TGT GAT GCC CTG ACT GAG CTG CTG GCC AAG ATC ACC CAC ACT GAT GTG GAT  720
Thr Leu Thr Cys Asp Ala Leu Thr Glu Leu Leu Ala Lys Ile Thr His Thr Asp Val Asp  240

TGT CTC AAA GCC TGC CAG GAG CAA ATC GAA GCT CTG CTG CTG AAC AGC CTG CAG CAG TTC  780
Cys Leu Lys Ala Cys Gln Glu Gln Ile Glu Ala Leu Leu Leu Asn Ser Leu Gln Gln Phe  260

CGT CAA GAG CAG CAT AAC GCC GGA TCC AAG TCT GTG GAA GAT CCG GAC CAA GCC ACC ACC  840
Arg Gln Glu Gln His Asn Ala Gly Ser Lys Ser Val Glu Asp Pro Asp Gln Ala Thr Thr  280

CCT ACA GAC GTG CGG GAT GTT GAC CTG TGA GGAAGCCATTCGGGCGGCAAGAGAGAGGCGTGTTCGTCA  909
Pro Thr Asp Val Arg Asp Val Asp Leu  *                                           289

TCTGCTAGCCCCTTCTCTCTCTAGTTATGTCTTGTTCTTTGTGTTTTTAGGATGAAACTTCAAAAAAAAAAAAAAA    985
```

FIG. 1. Sequence of murine CYL2 cDNA. The predicted protein sequence is shown. Amino acid numbers are given with the initiating methionine as residue 1, and the stop codon is indicated by an asterisk.

pump rate. One million cells from each fraction were stained with propidium iodide/10 mM Tris·HCl, pH 7.0/5 mM $MgCl_2$, treated with RNase A (100 μg/ml), and filtered, and fluorescence from propidium iodide–DNA complex was analyzed by flow cytometry. Percentages of cells in G_1, S, and G_2/M phases were calculated from the histogram of the relative DNA distribution.

RESULTS

Cloning of Murine CYL2 cDNA. A cDNA library constructed from uninduced MELCs was screened with the complete coding region of murine CYL1 cDNA (26) as a probe. Under mildly stringent conditions, ≈40 out of a million plaques hybridized with the probe. Nineteen clones have been characterized, and none was identical to CYL1. Two 1.3-kilobase (kb) cDNA clones and two 4.0-kb clones were found to have identical open reading frames of 870 base pairs (Fig. 1). The two 1.3-kb clones have identical poly(A) tails except that one has an additional 104 nucleotides at the 5′ end (residues −270 to −167). An in-frame stop codon was found (residues −210 to −208) upstream of the assigned initiator codon. One of the 4.0-kb clones contains a sequence identical to the 1.3-kb clones and an additional 2.7-kb untranslated sequence at its 5′ side (sequence not shown). The other 4.0-kb clone has the sequence of the 1.3-kb clone from the 5′ end. Instead of the poly(A) tail found on the shorter clone, it has a long additional sequence after residue 970 and ends with another poly(A) stretch (sequence not shown). This heterogeneity in 3′ untranslated sequences among these clones suggests alternative polyadenylylation of the transcript, which has been reported for human cyclin D_1 (22).

Northern blot analysis with MELC poly(A)$^+$ RNA showed a major 6.5-kb band and two smaller bands at 2.9 kb and 1.3 kb (Fig. 2). By using the 1.3-kb cDNA probe, Northern blot analysis of total RNA from human promyelocytic leukemia HL-60 cells showed a 6.5-kb mRNA and a 4.5-kb band. The smaller RNA could reflect 28S rRNA. We failed to detect the

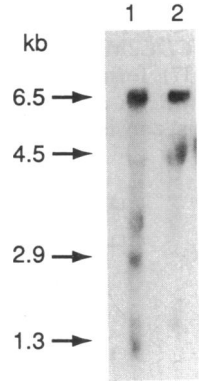

FIG. 2. CYL2 gene expression in MELCs and HL-60 cells. Approximately 4 μg of poly(A)$^+$ RNA prepared from DS19-Sc9 MELCs was loaded on lane 1, and 10 μg of total RNA isolated from human promyelocytic leukemia HL-60 cells was loaded on lane 2. The 1.3-kb clone of murine CYL2 was used as a probe for Northern blot analysis.

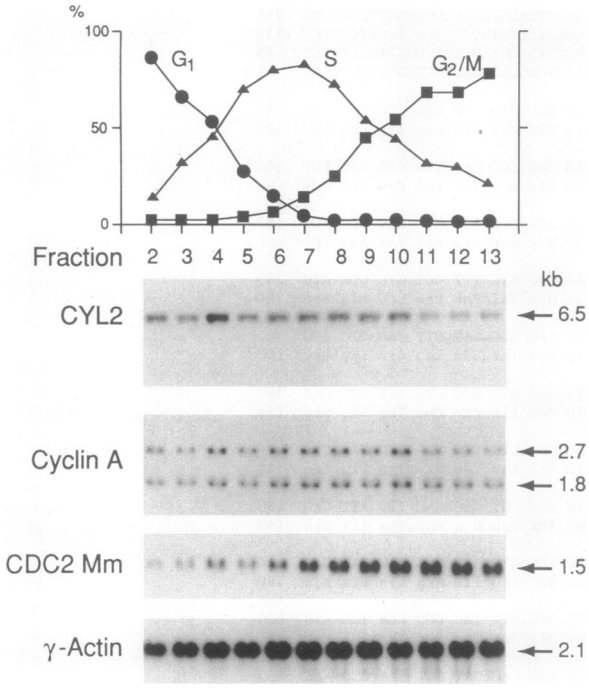

FIG. 3. Cell-cycle-dependent expression of CYL2, cyclin A, and CDC2Mm. DS19-Sc9 MELCs were fractionated by centrifugal elutriation and the nuclear DNA content of each fraction was analyzed by flow cytometry. (*Upper*) Percentages of cells in G_1 (●), S (▲), and G_2/M (■) phases. (*Lower*) Total RNA (10 μg) isolated from each fraction was separated by electrophoresis and analyzed on a Northern blot. The calculated sizes of the transcripts are shown on the right.

6.5-kb RNA in HeLa cells under the identical condition (data not shown).

Cell-Cycle-Dependent Expression of CYL2. To evaluate the expression of murine CYL2 during the cell cycle, MELCs were fractionated by centrifugal elutriation. Fig. 3 shows the cell cycle distribution (by DNA content) of cells in each fraction. Expression of the major 6.5-kb CYL2 transcript fluctuated during the cell cycle. There is a broad elevation of expression during G_1 and S phases and a decrease in G_2/M phase. Northern blot analysis with human cyclin A cDNA (35) as a probe showed two transcripts, 2.7 kb and 1.8 kb, in MELCs. The expression of both transcripts varied through the cell cycle with a peak in S phase. Cyclin A mRNA was most abundant in S, decreased in G_2/M, and further decreased in G_1 to 40% of the peak value in S. This observation was reproduced in three experiments.

The expression of *CDC2*Mm, which encodes the kinase catalytic subunit (36, 37), forms a striking contrast with that of CYL2. CDC2Mm mRNA accumulates predominantly in G_2/M phase, which is consistent with the observation in HeLa cells (38).

DISCUSSION

We report the complete coding sequence of a D-type cyclin cDNA, CYL2, isolated from MELCs. The nucleotide sequence is 67% homologous to murine CYL1, now recognized as the murine counterpart of human cyclin D_1. The C terminus part of the predicted amino acid sequence from Met-54 was found to be identical to the partial sequence that was reported as CYL2 by Matsushime *et al.* (26). The entire predicted sequence of 289 amino acids of CYL2 is 64% homologous to the 292-residue CYL1 sequence (26) (Fig. 4). As shown for CYL1 (26), the sequence of CYL2 is not homologous to that found in the N-terminal regions of A- and B-type cyclins and shown to facilitate ubiquitin-dependent degradation (39). CYL2 contains no apparent PEST (Pro-Glu-Ser-Thr) sequences, which were identified in rapidly degraded proteins (40) and have been found in the C-terminal regions of yeast CLN proteins (17) and human cyclins C, D_1, and E (23).

The highest homology of CYL2 with other cyclins is found at amino acids 54–139, which corresponds to the N-terminal half of the evolutionarily conserved cyclin box identified in a comparison of yeast CLN cyclins with A- and B-type cyclins (17). Table 1 shows the percent identity of amino acids 54–139 of CYL2 with the corresponding regions in several other cyclins. Clearly the highest homology in this region of CYL2 is with the other murine CYL proteins, CYL1 and CYL3, and with cyclin D_1, the human counterpart of CYL1, indicating that CYL2 is a member of D-type cyclins. There is much less homology with the other cyclins (Table 1), although the homology is greater with human cyclins A, B, and E than with yeast G_1 cyclins, CLN1, CLN2, CLN3, and puc1$^+$ (41).

During the screening process we did not isolate any clones identical to CYL1. Further, we did not detect CYL1 mRNA or protein in MELCs by Northern blot and immunoblot analyses using a N-terminus-specific cyclin D_1 antiserum (data not shown). Matsushime *et al.* (26) report that CYL1 mRNA is expressed in proliferating macrophages, but not in interleukin (IL) 2-dependent T-lymphoid, IL-7-dependent early pre-B-lymphoid, or IL-3-dependent myeloid cell lines. CYL2 mRNA was detected, however, in all four of these cell lines. CYL2 cDNA detects a 6.5-kb mRNA in human promyelocytic leukemia HL-60 cells (Fig. 2) but not in HeLa cells. These results suggest tissue-specific expression of both CYL1 and CYL2. Interestingly, CYL2 mRNA was found in all the hematopoietic cell lines tested, including erythroid, myeloid, and lymphoid cells, suggesting that CYL2 may be the G_1 cyclin active in hematopoietic cells.

CYL2 mRNA oscillates during the cell cycle in MELCs, increasing broadly in G_1 and S and decreasing in G_2/M phases. This pattern is consistent with a role for CYL2 in cell cycle progression in the G_1 and S phases. The oscillation of CYL2 during the cell cycle differs from the pattern reported for human cyclin D_1 (25). PRAD1 mRNA (identical to human cyclin D_1) displays in HeLa cells a broad elevation after S phase, a pattern resembling that of B-type cyclin. CYL1 is

```
CYL2  1 ME--LLCCEVDPVRRAVPDRNLLEDRVLQNLLTIEERYLPQCSYFKCVQKDIQPYMRRMVATWMLEVCEEQKCEE
        ::  ::::::* *::: :: ::: ::::   :  ::  *:  :::::::::*: : ::* ::::::::::::::::
CYL1  1 MEHQLLCCEVETIRRAYPDTNLLNDRVLRAMLKTEETCAPSVSYFKCVQKEIVPSMRKIVATWMLEVCEEQKCEE

     74 EVFPLAMNYLDRFLAGVPTPKTHLQLLGAVCMFLASKLKETIPLTAEKLCIYTDNSVKPQELLEWELVVLGKLKW
        ::::::::::::::*  :: :**:::::: :::*::: :::::::::::::::::::**: :::  ::*** ::::
     76 EVFPLAMNYLDRFLSLEPLKKSRLQLLGATCMFVASKMKETIPLTAEKLCIYTDNSIRPEELLQMELLLVNKLKW

    149 NLAAVTPHDFIEHILRKLPQQKEKLSLIRKHAQTFIALCATDFKFAMYPPSMIATGSVGAAICGLQQDDEVNTLT
        :::: ::::::::: : : :   :    :::::::::*:::::: ::   ::::*: ::: :: ::*     : :*
    151 NLAAMTPHDFIEHFLSKMPEADENKQTIRKHAQTFVALCATDVKFISNPPSMVAAGSVVAAMQGLNLGSPNNFLS

    224 CDALTELLAKITHTDVDCLKACQEQIEALLLNSL---QQFRQEQHNAGSKSVED-PDQATTPTDVRDVDL 289
        :   :  :*** * : :::*:::::::::::  ::   ::            ::*    : :::::::::*
    226 CYRTTHFLSRVIKCDPDCLRACQEQIEALLESSLRQAQQNVDPKATEEEGEVEEEAGLACTPTDVRDVDI 295
```

FIG. 4. Sequence comparison between murine CYL2 and CYL1 (26). Dots indicate identical amino acids and asterisks show conservative substitutions.

Table 1. Homology between murine CYL2, amino acids 54–139, and the most homologous region of other cyclins

	CYL1	humD$_1$	CYL3	humA	humB	humC	humE	CLN1	CLN2	CLN3	puc1
Residue positions	72–157	72–157	17–102	226–311	217–302	75–161	145–231	130–220	133–223	122–210	128–213
% identity	80	80	73	37	29	21	37	13	15	14	23

CYL1, murine CYL1 (26); humD$_1$, human cyclin D$_1$ (22, 25); CYL3, murine CYL3, partial sequence of 236 amino acids (26); humA, human cyclin A (35); humB, human cyclin B (12); humC, human cyclin C (23); humE, human cyclin E (23, 24); CLN1 and CLN2, *Saccharomyces cerevisiae* CLN1 and CLN2 (17); CLN3, *Saccharomyces cerevisiae* CLN3 (WHI1) (19); puc1, *Schizosaccharomyces pombe* puc1$^+$ (41).

induced late in G$_1$ in murine macrophages exposed to colony-stimulating factor 1 (CSF-1), and its level remains unchanged throughout subsequent cell cycles in the presence of CSF-1 (26).

Thus the present studies identifying CYL2 are consistent with it playing a role in regulation of G$_1$ to S transition. It remains to be determined whether CYL2 protein can activate p34^{cdc2} kinase or a closely related catalytic subunit by its association, in a fashion analogous to A-, B-, and CLN-type cyclins.

We thank Dr. Charles J. Sherr for CYL1 cDNA, Drs. Jonathon Pines and Tony Hunter for human cyclin A cDNA, Dr. Christian Bréchot for cyclin A cDNA, Drs. Paul Nurse and Christopher J. Norbury for CDC2Mm cDNA, and Dr. Fred Cross for helpful discussion. This work was supported, in part, by grants from the National Cancer Institute (CA-0874823), the Japanese Foundation for the Promotion of Cancer Research, Japan Heart Foundation, and the Roberta Rudin Leukemia Research Fund.

1. Murray, A. W. & Kirschner, M. W. (1989) *Science* **246,** 614–621.
2. Hunt, T. (1989) *Curr. Opinion Cell Biol.* **1,** 268–274.
3. Pines, J. & Hunter, T. (1990) *New Biol.* **2,** 389–401.
4. Draetta, G. (1990) *Trends Biochem. Sci.* **15,** 378–383.
5. Nurse, P. & Bissett, Y. (1981) *Nature (London)* **292,** 558–560.
6. Piggott, J. R., Rai, R. & Carter, B. L. A. (1982) *Nature (London)* **298,** 391–393.
7. Simanis, V. & Nurse, P. (1986) *Cell* **45,** 261–268.
8. Reed, S. I., Hadwiger, J. A. & Lörincz, A. T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4055–4059.
9. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T. (1983) *Cell* **33,** 389–396.
10. Nurse, P. (1990) *Nature (London)* **344,** 503–507.
11. Draetta, G. & Beach, D. (1988) *Cell* **54,** 17–26.
12. Pines, J. & Hunter, T. (1989) *Cell* **58,** 833–846.
13. Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M. & Kirschner, M. W. (1990) *Cell* **63,** 1013–1024.
14. Pines, J. & Hunter, T. (1990) *Nature (London)* **346,** 760–763.
15. Minshull, J., Golsteyn, R., Hill, C. S. & Hunt, T. (1990) *EMBO J.* **9,** 2865–2875.
16. D'Urso, G., Marraccino, R. L., Marshak, D. R. & Roberts, J. M. (1990) *Science* **250,** 786–791.
17. Hadwiger, J. A., Wittenberg, C., Richardson, H. E., Lopes, M. B. & Reed, S. I. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6255–6259.
18. Cross, F. R. (1988) *Mol. Cell. Biol.* **8,** 4675–4684.
19. Nash, R., Tokiwa, G., Anand, S., Erickson, K. & Futcher, A. B. (1988) *EMBO J.* **7,** 4335–4346.
20. Wittenberg, C., Sugimoto, K. & Reed, S. I. (1990) *Cell* **62,** 225–237.
21. Richardson, H. E., Wittenberg, C., Cross, F. & Reed, S. I. (1989) *Cell* **59,** 1127–1133.
22. Xiong, Y., Connolly, T., Futcher, B. & Beach, D. (1991) *Cell* **65,** 691–699.
23. Lew, D. J., Dulić, V. & Reed, S. I. (1991) *Cell* **66,** 1197–1206.
24. Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M. & Roberts, J. M. (1991) *Cell* **66,** 1217–1228.
25. Motokura, T., Bloom, T., Kim, H. G., Jüppner, H., Ruderman, J. V., Kronenberg, H. M. & Arnold, A. (1991) *Nature (London)* **350,** 512–515.
26. Matsushime, H., Roussel, M. F., Ashmun, R. A. & Sherr, C. J. (1991) *Cell* **65,** 701–713.
27. Pardee, A. B. (1989) *Science* **246,** 603–608.
28. Hunter, T. & Pines, J. (1991) *Cell* **66,** 1071–1074.
29. Ohta, Y., Tanaka, M., Terada, M., Miller, O. J., Bank, A., Marks, P. A. & Rifkind, R. A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1232–1236.
30. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
31. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
32. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
33. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
34. Gambari, R., Marks, P. A. & Rifkind, R. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4511–4515.
35. Wang, J., Chenivesse, X., Henglein, B. & Bréchot, C. (1990) *Nature (London)* **343,** 555–557.
36. Lee, M. G. & Nurse, P. (1987) *Nature (London)* **327,** 31–35.
37. Lee, M. G., Norbury, C. J., Spurr, N. K. & Nurse, P. (1988) *Nature (London)* **333,** 676–679.
38. McGowan, C. H., Russell, P. & Reed, S. I. (1990) *Mol. Cell. Biol.* **10,** 3847–3851.
39. Glotzer, M., Murray, A. W. & Kirschner, M. W. (1991) *Nature (London)* **349,** 132–138.
40. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234,** 364–368.
41. Forsburg, S. L. & Nurse, P. (1991) *Nature (London)* **351,** 245–248.