Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1575–1579, March 1987
Cell Biology

Cloning and sequence of the human nuclear protein cyclin: Homology with DNA-binding proteins

(gene expression/cell cycle/DNA synthesis/immunoscreening/hybrid-arrested *in vitro* translation)

J. M. ALMENDRAL, D. HUEBSCH, P. A. BLUNDELL, H. MACDONALD-BRAVO, AND R. BRAVO*

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany

Communicated by Severo Ochoa, November 10, 1986

ABSTRACT **A full-length cDNA clone for the human nuclear protein cyclin has been isolated by using polyclonal antibodies and sequenced. The sequence predicts a protein of 261 amino acids (M_r 29,261) with a high content of acidic (41, aspartic and glutamic acids) versus basic (24, lysine and arginine) amino acids. The identity of the cDNA clone was confirmed by *in vitro* hybrid-arrested translation of cyclin mRNA. Blot-hybridization analysis of mouse 3T3 and human MOLT-4 cell RNA revealed a mRNA species of approximately the same size as the cDNA insert. Expression of cyclin mRNA was undetectable or very low in quiescent cells, increasing after 8–10 hr of serum stimulation. Inhibition of DNA synthesis by hydroxyurea in serum-stimulated cells did not affect the increase in cyclin mRNA but inhibited 90% the expression of H3 mRNA. These results suggest that expression of cyclin and histone mRNAs are controlled by different mechanisms. A region of the cyclin sequence shows a significant homology with the putative DNA binding site of several proteins, specially with the transcriptional-regulator cAMP-binding protein of *Escherichia coli*, suggesting that cyclin could play a similar role in eukaryotic cells.**

The identification of the cellular proteins that are involved in the control of cell proliferation in normal cells is essential for understanding the mechanisms underlying growth regulation and cellular transformation. A nuclear protein, "cyclin" (M_r 36,000), whose synthesis correlates with the proliferative state of the cells, is potentially such a candidate (for reviews, see refs. 1 and 2). This protein is present in variable amounts in normal proliferating cells as well as transformed cells and tumors. It is highly conserved, as determined by one-dimensional peptide mapping, and it has been identified in several cell types of human, mouse, hamster, and avian origin. The level of cyclin fluctuates during the cell cycle, with a clear increase during the S phase (3, 4). Moreover, a coordinate synthesis of cyclin and DNA has been demonstrated in serum or growth factor-induced quiescent cells (5, 6). The proliferating-cell nuclear antigen (PCNA; refs. 7–10), a human protein that shares the same properties, has been shown to be identical to cyclin (9, 11). Immunofluorescence studies of the distribution of cyclin (PCNA) during the cell cycle have revealed dramatic changes in its nuclear localization during the S phase (7, 12, 13). Recent studies have demonstrated that these changes are not triggered by a mechanism involving direct phosphorylation of cyclin (4) and that they depend on DNA synthesis or events during the S phase (12).

To learn more about the structure and function of cyclin, we decided to isolate cDNA clones of the mRNA for cyclin. We report here the complete nucleotide sequence for human cyclin and its expression during the cell cycle.

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.

MATERIALS AND METHODS

Cells. Mouse 3T3 cells were routinely grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum and antibiotics (penicillin, 100 units/ml; streptomycin, 50 μg/ml). To obtain quiescent cultures, confluent cells were incubated for 48 hr in 1% fetal calf serum. MOLT-4 cells were grown in suspension in RPMI-1640 medium containing 10% fetal calf serum until they reached a density of 10^6 cells per ml.

Polyclonal Antisera. Antiserum against cyclin from MOLT-4 cells purified by two-dimensional gels was raised in rabbits (to be described elsewhere). The specificity of the antibody was tested on electrophoretic immunoblots containing a total cellular lysate of MOLT-4 cells resolved by two-dimensional gel electrophoresis. The antiserum used in these studies was proved to be monospecific for cyclin.

MOLT-4 cDNA Expression Library. Poly(A)$^+$ mRNA purified from MOLT-4 cells whose activity was tested by *in vitro* translation assay was copied into cDNA by priming with oligo(dT) essentially as described by Watson and Jackson (14). The cDNA was subsequently polished by consecutive treatments with phage T4 polymerase and Klenow enzyme and inserted into the *Bam*HI site of the expression vector pEX1 (15) by the adaptor cloning procedure (16). The recombinant cDNA was used to transform *E. coli* pop2136 cells, made competent by Hanahan's method (17). A library of 10^6 clones was obtained, with ≈95% showing inserts with an average length of 1 kilobase pair (kbp).

RNA Preparation and Analysis. Mouse 3T3 cells grown in 500-cm^2 dishes were made quiescent as described above. After incubation for the indicated times with 10% fetal calf serum, cells were carefully rinsed with phosphate-buffered saline and lysed with guanidine hydrochloride. The lysate was then centrifuged over a cesium chloride cushion, extracted with phenol, and precipitated with ethanol (18). The RNA was separated on agarose gels containing 6% formaldehyde and blotted onto GeneScreen (New England Nuclear; ref. 19). In all cases, 15-μg samples were analyzed. RNA was quantitated by absorbance at 260 nm, and this subsequently was verified by staining the blots. [^{32}P]DNA probes were made by nick-translation labeling to a specific activity of 1–5 × 10^8 cpm/μg (20). Hybridizations were performed in 50% formamide/0.5% $NaDodSO_4$ containing 5× NaCl/Cit (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7) and 5× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin) at 42°C (40 hr), and filters were extensively washed in 0.1× NaCl/Cit containing 0.5% $NaDodSO_4$ at 56°C.

Hybrid-Arrested *in Vitro* Translation. Twenty-five nanograms of the DNA insert of the corresponding cyclin clone purified from low-melting agarose gels (21) was mixed with 0.5 μg of poly(A)$^+$ mRNA in a final volume of 3 μl. The mixture was boiled and hybridized as described by Paterson

*To whom reprint requests should be addressed.

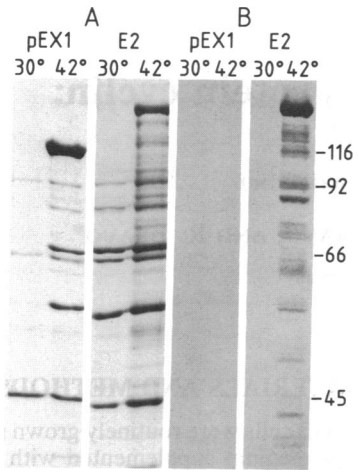


FIG. 1. Electrophoretic immunoblots of expressed hybrid protein. Logarithmic-phase cultures grown at 30°C from colonies carrying pEX1 or from positive colonies carrying pEX1 with insert E2 were induced for 2 hr at 42°C. The cells were lysed, and proteins were resolved in a 7.5% $NaDodSO_4$/polyacrylamide gel. (*A*) Coomassie-stained proteins. (*B*) Immunoblot using a rabbit polyclonal anti-cyclin. Molecular sizes are shown $\times 10^{-3}$.

et al. (22); after precipitation, the mRNA was translated in a reticulocyte cell-free system. A ×50 excess of double-stranded DNA with respect to cyclin mRNA was found to be optimal for its complete arrest without affecting the efficiency of translation of the other mRNAs.

RESULTS

Cloning of cDNA Molecules Coding for Cyclin Epitopes. To clone the mRNA for cyclin, we prepared a specific rabbit antiserum against human cyclin purified by two-dimensional gel electrophoresis (to be described elsewhere). The antiserum was monospecific, detecting only cyclin protein in immunoblots of two-dimensional gels. A cDNA library was constructed in the expression vector pEX1 with poly(A)$^+$ RNA from human lymphoma MOLT-4, as we have found that this cell line contains the highest levels of cyclin mRNA by analysis of *in vitro* translation products.

The cDNA library was screened by using the colony-blot procedure as described (23). A total of 15 positive colonies were detected out of 2.4×10^5 clones analyzed by antibody screening. The positive colonies were regrown and re-screened to obtain pure clones. Plasmid preparations were performed from all 15 positive independent colonies. All of them cross-hybridized, with the exception of 5 that proved to be false positives. The size of the inserts varied from 0.5 to

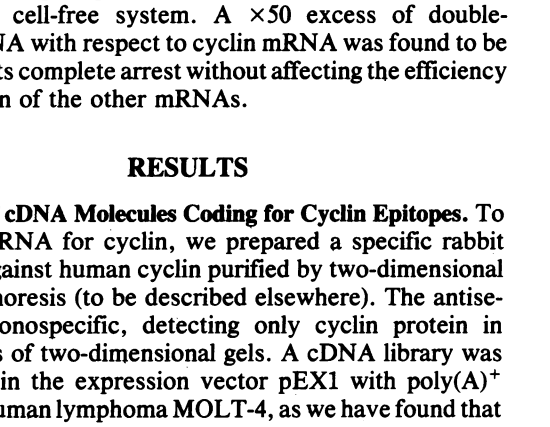
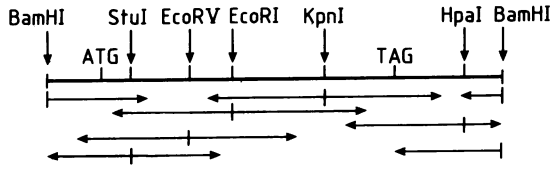


FIG. 2. Two-dimensional gel analysis of *in vitro* translation products from MOLT-4 poly(A)$^+$ RNA (*A*) and MOLT-4 poly(A)$^+$ RNA prehybridized with clone E2 (*B*) as described; 0.5 μg of poly(A)$^+$ RNA was used. Molecular sizes are shown $\times 10^{-3}$. Arrowhead, position of cyclin; a, actin; IEF, isoelectric focusing.




```
      AGGTCTCAGCCGGTCGTCGCGACGTTCGCCCGCTCGCTCTGAGGCTCCTGAAGCC   55
GAAACTAGCTAGACTTTCCTCCTTCCCGCCTGCCTGTAGCGGCGTTGTTGCCACTCCGCCACC  118

Met Phe Glu Ala Arg Leu Val Gln Gly Ser Ile Leu Lys Lys Val Leu   16
ATG TTC GAG GCG CGC CTG GTC CAG GGC TCC ATC CTC AAG AAG GTG TTG  166

Glu Ala Leu Lys Asp Leu Ile Asn Glu Ala Cys Trp Asp Ile Ser Ser   32
GAG GCA CTC AAG GAC CTC ATC AAC GAG GCC TGC TGG GAT ATT AGC TCC  214

Ser Gly Val Asn Leu Gln Ser Met Asp Ser Ser His Val Ser Leu Val   48
AGC GGT GTA AAC CTG CAG AGC ATG GAC TCG TCC CAC GTC TCT TTG GTG  262

Gln Leu Thr Leu Arg Ser Glu Gly Phe Asp Thr Tyr Arg Cys Asp Arg   64
CAG CTC ACC CTG CGG TCT GAG GGC TTC GAC ACC TAC CGC TGC GAC CGC  310

Asn Leu Ala Met Gly Val Asn Leu Thr Ser Met Ser Lys Ile Leu Lys   80
AAC CTG GCC ATG GGC GTG AAC CTC ACC AGT ATG TCC AAA ATA CTA AAA  358

Cys Ala Gly Asn Glu Asp Ile Ile Thr Leu Arg Ala Glu Asp Asn Ala   96
TGC GCC GGC AAT GAA GAT ATC ATT ACA CTA AGG GCC GAA GAT AAC GCG  406

Asp Thr Leu Ala Leu Val Phe Glu Ala Pro Asn Gln Glu Lys Val Ser  112
GAT ACC TTG GCG CTA GTA TTT GAA GCA CCA AAC CAG GAG AAA GTT TCA  454

Asp Tyr Glu Met Lys Leu Met Asp Leu Asp Val Glu Gln Leu Gly Ile  128
GAC TAT GAA ATG AAG TTG ATG GAT TTA GAT GTT GAA CAA CTT GGA ATT  502

Pro Glu Gln Glu Tyr Ser Cys Val Val Lys Met Pro Ser Gly Glu Phe  144
CCA GAA CAG GAG TAC AGC TGT GTA GTA AAG ATG CCT TCT GGT GAA TTT  550

Ala Arg Ile Cys Arg Asp Leu Ser His Ile Gly Asp Ala Val Val Ile  160
GCA CGT ATA TGC CGA GAT CTC AGC CAT ATT GGA GAT GCT GTT GTA ATT  598

Ser Cys Ala Lys Asp Gly Val Lys Phe Ser Ala Ser Gly Glu Leu Gly  176
TCC TGT GCA AAA GAC GGA GTG AAA TTT TCT GCA AGT GGA GAA CTT GGA  646

Asn Gly Asn Ile Lys Leu Ser Gln Thr Ser Asn Val Asp Lys Glu Glu  192
AAT GGA AAC ATT AAA TTG TCA CAG ACA AGT AAT GTC GAT AAA GAG GAG  694

Glu Ala Val Thr Ile Glu Met Asn Glu Pro Val Gln Leu Thr Phe Ala  208
GAA GCT GTT ACC ATA GAG ATG AAT GAA CCA GTT CAA CTA ACT TTT GCA  742

Leu Arg Tyr Leu Asn Phe Phe Thr Lys Ala Thr Pro Leu Ser Ser Thr  224
CTG AGG TAC CTG AAC TTC TTT ACA AAA GCC ACT CCA CTC TCT TCA ACG  790

Val Thr Leu Ser Met Ser Ala Asp Val Pro Leu Val Val Glu Tyr Lys  240
GTG ACA CTC AGT ATG TCT GCA GAT GTA CCC CTT GTT GTA GAG TAT AAA  838

Ile Ala Asp Met Gly His Leu Lys Tyr Tyr Leu Ala Pro Lys Ile Glu  256
ATT GCG GAT ATG GGA CAC TTA AAA TAC TAC TTG GCT CCC AAG ATC GAG  886

Asp Glu Glu Gly Ser *                                           261
GAT GAA GAA GGA TCT TAG GCATTCTTAAAATTCAAGAAAATAAAACTAAGCTCTTTG  943
AGAACTGCTTCTAAGATGCCAGCATATACTGAAGTCTTTTCTGTCACCAAATTTGTACCTCTA 1006
AGTACATATGTAGATATTGTTTTCTGTAAATAACCTATTTTTTTTCTCTATTCTCTCCAATTT 1069
GTTTAAAGAATAAAGTCCAAAGTCTGATCTGGTCTAGTTAACCTAGAAGTATTTTTGTCTCTT 1132
AGAAATACTTGTGATTTTTATAATACAAAAGGGTCTTGACTCTAAATGCAGTTTTAAGAAGTG 1195
TTTTTGAATTTAAATAAAGTTACTTGAATTTCAAACAAAAAAAAAAAAAAAAA            1248
```

FIG. 3. (*Upper*) Restriction map of cyclin cDNA and strategy followed for sequencing. The initiator ATG and terminator TAG codons are indicated. (*Lower*) Nucleotide sequence of the insert from clone S14 and the amino acid sequence deduced for cyclin. The poly(A) addition signal and the consensus initiator sequence are underlined.

1.1 kilobases (kb). Clone E2 of 1.1 kb was selected for further studies.

Cultures of clone E2 grown at 30°C or at 42°C for 2 hr to induce the expression of the fusion protein were lysed, and proteins were analyzed by $NaDodSO_4$/polyacrylamide gels and detected by staining or immunoblotting. The Coomassie blue staining patterns from control cultures (pEX1) and from cells expressing the fusion protein (E2) grown at 30°C and at 42°C for 2 hr are shown in Fig. 1*A*. Control cultures only expressed β-galactosidase at 42°C as shown by the appearance of a M_r 116,000 protein. Cells containing the clone E2 expressed a protein of $M_r \approx 148{,}000$ at 42°C, suggesting that it was the hybrid molecule of β-galactosidase and cyclin. This was supported by the finding that only proteins from cells expressing the fusion protein (E2, 42°C) were positive in immunoblotting when tested with polyclonal anti-cyclin antibody (Fig. 1*B*). The major band detected corresponds to the M_r 140,000 protein present in Coomassie blue-stained gels. The bands of lower molecular weight possibly are degradation products from the fusion protein.

To establish further that the hybrid protein contained epitopes common to cyclin, polyclonal anti-cyclin antibody immunoabsorbed to fusion protein blotted onto nitrocellulose filters was eluted and used for the immunodetection of cyclin in a blot of total cellular lysate from MOLT-4 cells separated by two-dimensional gel electrophoresis. The results showed that the only protein recognized by the immunoaffinity-purified antibody was cyclin (not shown).

Hybrid-Arrested *in Vitro* Translation of Cyclin mRNA. The immunological analyses established that clone E2 codified for a protein with one or more common epitopes to cyclin. To investigate if clone E2 contained the cyclin sequence, hybrid-arrested *in vitro* translation (HART) experiments were done. For this, poly(A)$^+$ mRNA from MOLT-4 cells was prehybridized with clone E2, under conditions in which only highly homologous sequences (>90%) would hybridize, before *in vitro* translation analyses. The radioactive products were resolved by two-dimensional gel electrophoresis (24, 25). Cyclin was one of the most abundant products of *in vitro* translation from MOLT-4 poly(A)$^+$ mRNA (Fig. 2*A*, arrowhead). Prehybridization with clone E2 completely inhibited the translation of cyclin mRNA (Fig. 2*B*), demonstrating that clone E2 contained a sequence homologous to that codifying for cyclin. As can be seen from the figure, the inhibition was specific because no other changes in the *in vitro* translation products were detected.

Predicted Sequence of Cyclin. We used the insert of clone E2 to isolate longer cDNA clones from the pEX1 cDNA library. The cyclin sequence was found to be represented once in 1000 clones, and the longest insert of the isolated clones was from recombinant S14 (1.3 kb), which was used for sequencing. The S14 insert, which efficiently arrested *in vitro* translation of cyclin mRNA, was cloned into pUC19 and sequenced by the method of Maxam and Gilbert (26) modified as suggested by Chuvpilo and Kravchenko (27). The strategy used for the sequencing is outlined in Fig. 3. Fig. 3 *Lower* shows that the cDNA clone extends 118 nucleotides to the 5′ side of the starting codon. The coding region contains 786 nucleotides, and the 3′ untranslated region contains 327 nucleotides. The position of the terminator codon TAG was confirmed by sequencing the insert of clone E2. The poly(A) tail is 17 nucleotides long. The 5′ nucleotide residues directly preceding the ATG codon match the consensus initiator sequence -CC$\overset{A}{G}$CC- discussed by Kozak (28). A potential mRNA processing signal, AATAAA (29), is present 18 nucleotides upstream from the poly(A) sequence. The amino acid sequence predicted from the open reading frame starting at nucleotide 119 is identical to that obtained from the analysis of the N terminus of cyclin from human and rabbit origin (our unpublished observations; ref. 30).

The open reading frame contains 262 codons and would encode a protein of M_r 29,261. There is a large excess of acidic amino acids (41, glutamic and aspartic acids) over basic amino acids (24, lysine and arginine) giving the protein its acidic properties.

Possible DNA-Binding Domain. A search of the NBRF protein database for protein sequences homologous to cyclin using the VWGCG programs (J. Deveraux) revealed that cyclin has a domain (amino acids 66–80) that is homologous to the α-helix-turn-α-helix putative DNA-binding domain of several proteins (ref. 31; Fig. 4). Interestingly, the secondary structure of cyclin predicted as described by Argos *et al.* (32, 33) would also contain two short α-helices in the region of amino acids 61–80. The degree of homology including conservative residues between cyclin (amino acids 61–80) and the cAMP-binding protein (amino acids 169–188) is as high as 55%. The highest degree of homology (77%) is in the region of helix 3, which is the helix that fits into the major groove of DNA and determines the specificity of the binding (34). Moreover, cyclin also has the highly conserved glycine residue between both helices.

Induction of Cyclin mRNA After Stimulation with Serum. Blot-hybridization analysis revealed that the size of human cyclin mRNA is ≈1.3 kb, which is similar to the size of clone S14, suggesting that this clone possibly contains the complete 3′ and 5′ noncoding sequence of cyclin mRNA. Clone S14 hybridized with mouse cyclin mRNA showed a similar size to the human cyclin mRNA (not shown). Previous studies have shown that cyclin synthesis increases several fold after serum stimulation of quiescent cells (1). To study if the induction of cyclin synthesis is due to changes in the levels of cyclin mRNA, quiescent cells were stimulated with 10% fetal calf

Cyclin	61	Arg	*Cys*	Asp	*Arg*	*Asn*	*Leu*	Ala	*Met*	Gly	Val	Asn	*Leu*	*Thr*	*Ser*	*Met*	Ser	Lys	Ile	Leu	Lys			
CAP	169	Arg	*Gln*	*Glu*	*Ile*	*Gly*	*Gln*	*Ile*	*Val*	Gly	*Cys*	*Ser*	*Arg*	*Glu*	*Thr*	*Val*	*Gly*	*Arg*	Ile	Leu	Lys			
ftz	287	Arg	*Ile*	Asp	*Ile*	*Ala*	*Asn*	Ala	*Leu*	*Ser*	*Leu*	*Ser*	*Glu*	*Arg*	*Gln*	*Ile*	*Lys*	*Ile*	*Trp*	*Phe*	*Gln*			
λcro	16	*Gln*	*Thr*	*Lys*	*Thr*	*Ala*	*Lys*	*Asp*	*Leu*	Gly	Val	*Tyr*	*Gln*	*Ser*	*Ala*	*Ile*	*Asn*	Lys	*Ala*	*Ile*	*His*			
Lac Rep	6	*Leu*	*Tyr*	Asp	*Val*	*Ala*	*Glu*	*Tyr*	*Ala*	Gly	Val	*Ser*	*Tyr*	*Gln*	*Thr*	*Val*	Ser	*Arg*	*Val*	*Val*	*Asn*			
P22cI	26	*Gln*	*Arg*	*Lys*	*Val*	*Ala*	*Asp*	Ala	*Leu*	Gly	*Ile*	Asn	*Glu*	*Ser*	*Gln*	*Ile*	Ser	*Arg*	*Trp*	*Lys*	*Gly*			
									Helix 2										Helix 3					

FIG. 4. Putative DNA-binding domain within the cyclin sequence. The number to the left of each sequence indicates the position of the first amino acid within the complete sequence. The sequences have been taken from ref. 31. Sets of identical residues are indicated in bold letters and sets of conservative residues are in italics and underlined. Conservative substitutions are defined according to ref. 39. The positions of the two α-helices of the DNA-binding domain [numbered 2 and 3 according to the convention for the phage λ cro protein (λcro)] are shown at the bottom. CAP, cAMP-binding protein; ftz, fushi tarazu protein; Lac Rep, Lac repressor protein; P22cI, phage P22 cI protein.

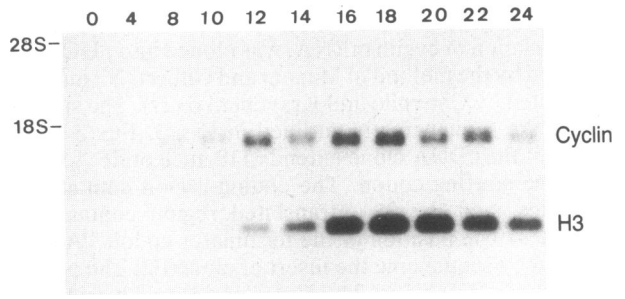


FIG. 5. Kinetics of induction of cyclin mRNA in serum-stimulated quiescent 3T3 cells. Quiescent cells were stimulated with 10% fetal calf serum (FCS) at the hours indicated above the lanes. RNA was extracted and analyzed in blots as described. The probes used were insert S14 and recombinant histone H3.

serum, and every 2 hr the level of cyclin mRNA was determined by blot-hybridization analysis (Fig. 5). An increase in cyclin mRNA expression was detected 8–10 hr after serum stimulation, reaching a maximum induction of ≈10-fold at 18–20 hr. The increase in cyclin expression preceded the synthesis of histone H3 mRNA. The abundance of cyclin mRNA was ≈5–10 times lower than that of H3 mRNA.

Induction of Cyclin mRNA Expression Is Independent of DNA Synthesis. The difference in the kinetics of induction between cyclin and H3 mRNAs suggested that the expression of these genes could be regulated by different mechanisms. Because it is well established that histone synthesis is dependent on DNA replication, we decided to investigate if cyclin mRNA expression was controlled by the synthesis of DNA. For this, quiescent cells were stimulated with 10% fetal calf serum in the presence of 5 mM hydroxyurea in order to inhibit DNA replication and block the cells in the G_1/S boundary (35). The levels of cyclin mRNA were analyzed every 2 hr after serum addition. Cyclin mRNA expression was detected after 10 hr, reaching a maximum after 18–20 hr similar to that of control cells without hydroxyurea (Fig. 6, lanes 1–3). In contrast, H3 mRNA expression was inhibited >90% in hydroxyurea-treated cells compared to control cells (Fig. 6, lanes 1–3). Under these conditions, DNA synthesis, as determined by [^{3}H]thymidine incorporation was 90–95% inhibited (not shown). Cells released from the hydroxyurea block rapidly increased DNA (not shown) and H3 mRNA synthesis, reaching a maximum level of expression between 4 and 6 hr (Fig. 6, lanes 4 and 5). However, the expression of cyclin mRNA remained constant for the first 4–6 hr after hydroxyurea removal (Fig. 6, lanes 4 and 5). Both cyclin and H3 mRNAs rapidly decreased at the end of the S phase (not shown).

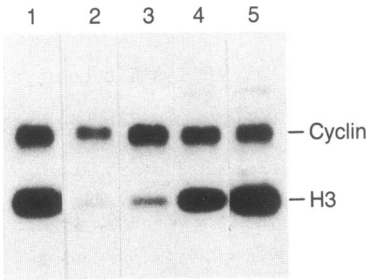


FIG. 6. Effect of DNA synthesis inhibitors in cyclin mRNA expression. Quiescent 3T3 cells were stimulated with 10% fetal calf serum for 18 hr (lane 1); quiescent cells were stimulated with 10% fetal calf serum in the presence of 5 mM hydroxyurea for 10 hr (lane 2) and 18 hr (lane 3). Cells stimulated as for lane 3 were washed and incubated in fresh medium without hydroxyurea for 2 hr (lane 4) and 6 hr (lane 5). RNA was extracted, and cyclin expression and H3 expression were analyzed by blot-hybridization analysis. Probes were as in Fig. 5.

DISCUSSION

We have used a cyclin antiserum to isolate pEX1 human cDNA recombinants capable of expressing cyclin epitopes. The authenticity of the cDNA was confirmed by the observation that *in vitro* translation of cyclin mRNA was specifically arrested by prehybridizing with the purified inserts.

The predicted amino acid sequence of human cyclin, determined by sequencing both strands of insert S14 and confirmed by the sequence of the independent clone E2, shows that this protein is composed of 261 residues with a corresponding M_r of 29,261. There is a significant excess of acidic amino acids accounting for the acidic pI (≈4.8) of cyclin observed during isoelectric focusing (9). The predicted molecular weight differs from the apparent M_r of 36,000 determined by $NaDodSO_4$/polyacrylamide gels. This discrepancy has been observed for several proteins (36).

We have found that the predicted N-terminal sequence (first 18 amino acids) is identical to that of the N terminus of human and rabbit cyclin as determined by protein sequencing (our unpublished results; ref. 30), demonstrating that the initiator methionine assigned in these studies is correct. This, together with the observation that the *in vitro* translation product of cyclin mRNA is identical in size and pI to the *in vivo* synthesized protein as determined by comigration in two-dimensional gel electrophoresis (not shown), indicates that cyclin is not processed posttranslationally.

The pattern of expression of cyclin mRNA as determined by blot-hybridization analysis agrees with our previous observations of cyclin protein synthesis levels (1), the expression of cyclin mRNA being very low in nonproliferating cells and increasing after 8–10 hr of serum stimulation. This indicates that the expression of cyclin is largely regulated at the transcriptional level.

The studies with DNA synthesis inhibitors showed that the control of cyclin and histone mRNA expression (37) is different and that it is possibly similar to that of thymidine kinase, thymidylate synthase, and dihydrofolate reductase, which have been demonstrated to increase in essentially the same manner in cells stimulated in the presence of DNA synthesis inhibitors (38).

The finding that cyclin has a homologous sequence to the putative α-helix-turn-α-helix DNA-binding domain only common to transcription-regulating proteins is interesting considering that cyclin is a nuclear protein whose synthesis correlates with cell proliferation. Immunofluorescence studies together with autoradiographic analysis have shown that the nuclear localization of cyclin changes throughout the S phase and that its nuclear distribution coincides with the sites of DNA replication (12, 13). Interestingly, in contrast to its induction, the changes in nuclear distribution of cyclin depend on DNA synthesis (12). Altogether this would suggest that cyclin could have an important role in the events leading to or occurring during the S phase.

We thank Dr. R. Baserga for providing us with the cDNA clone of H3; Dr. R. Frank for determining the N-terminal sequence of human cyclin; Dr. K. Stanley for introducing us to the use of the pEX expression vector system; Dr. S. Fuller for computer assistance, and I. Benner for helping in the preparation of the manuscript. J.M.A. was supported by a European Molecular Biology Organization fellowship.

1. Bravo, R. (1986) *Exp. Cell Res.* **163,** 287–293.
2. Celis, J. E., Bravo, R., Mose Larsen, P. & Fey, S. J. (1984) *Leuk. Res.* **8,** 143–157.
3. Bravo, R. & Celis, J. E. (1980) *J. Cell Biol.* **84,** 795–802.
4. Bravo, R. & Celis, J. E. (1985) *FEBS Lett.* **182,** 435–440.

5. Bravo, R. (1984) *FEBS Lett.* **169,** 185–188.
6. Bravo, R. & Macdonald-Bravo, H. (1984) *EMBO J.* **3,** 3177–3181.
7. Miyashi, K., Fritzler, M. J. & Tan, E. M. (1978) *J. Immunol.* **121,** 2228–2234.
8. Takasaki, J., Deng, J. S. & Tan, E. M. (1981) *J. Exp. Med.* **154,** 1899–1909.
9. Takasaki, J., Fischwild, D. & Tan, E. M. (1984) *J. Exp. Med.* **159,** 981–992.
10. Tan, E. M. (1982) *Adv. Immunol.* **33,** 167–240.
11. Mathews, M. B., Bernstein, R. M., Franza, B. R. & Garrels, J. I. (1984) *Nature (London)* **309,** 374–376.
12. Bravo, R. & Macdonald-Bravo, H. (1985) *EMBO J.* **4,** 655–661.
13. Celis, J. E. & Celis, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3262–3266.
14. Watson, C. J. & Jackson, J. F. (1985) in *DNA Cloning: Practical Approach*, ed. Glover, D. M. (IRL, Arlington, VA), pp. 79–88.
15. Stanley, K. K. & Luzio, J. P. (1984) *EMBO J.* **3,** 1429–1434.
16. Haymerle, H., Herz, J., Bressan, G., Frank, R. & Stanley, K. K. (1986) *Nucleic Acids Res.* **14,** 8615–8624.
17. Hanahan, D. (1983) *J. Mol. Biol.* **166,** 557–579.
18. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
19. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
20. Roop, D. R., Tsai, M. J. & O'Malley, B. W. (1980) *Cell* **19,** 63–68.
21. Langridge, J., Langridge, P. & Bergquist, P. L. (1980) *Anal. Biochem.* **103,** 264–271.
22. Paterson, B. M., Roberts, B. E. & Kuff, E. L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4370–4374.
23. Stanley, K. K. (1983) *Nucleic Acids Res.* **11,** 4077–4092.
24. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
25. Bravo, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4848–4850.
26. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 489–560.
27. Chuvpilo, S. A. & Kravchenko, V. V. (1984) *FEBS Lett.* **179,** 34–36.
28. Kozak, M. (1984) *Nucleic Acids Res.* **12,** 857–872.
29. Birnstiel, M. L., Busslinger, M. & Strub, K. (1985) *Cell* **41,** 349–359.
30. Ogata, K., Ogata, Y., Nakamura, R. M. & Tan, E. M. (1985) *J. Immunol.* **135,** 2623–2677.
31. Laughon, A. & Scott, M. P. (1984) *Nature (London)* **310,** 25–31.
32. Argos, P., Hanei, M., Wilson, J. M. & Kelly, W. N. (1983) *J. Biol. Chem.* **258,** 6450–6457.
33. Argos, P. (1985) *EMBO J.* **4,** 1351–1355.
34. McKay, D. B., Weber, I. T. & Seitz, T. A. (1982) *J. Biol. Chem.* **257,** 9518–9524.
35. Adams, R. L. P. & Lindsay, J. G. (1967) *J. Biol. Chem.* **242,** 1314–1317.
36. Lambin, P. (1978) *Anal. Biochem.* **85,** 114–125.
37. Baumbach, L. L., Marashi, F., Plumb, M., Stein, G. & Stein, J. (1984) *Biochemistry* **23,** 1618.
38. Johnson, L. F. (1984) in *Recombinant DNA and Cell Proliferation*, ed. Stein, G. S. & Stein, J. L. (Academic, New York), pp. 25–39.
39. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. (1978) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Washington, DC), Vol. 5, Suppl. 3, pp. 345–352.