Structure and cell cycle-regulated transcription of the human cyclin A gene

BERTHOLD HENGLEIN*†, XAVIER CHENIVESSE*, JIAN WANG*, DIRK EICK‡, AND CHRISTIAN BRÉCHOT*

*Institut National de la Santé et de la Recherche Médicale, Unité 370, Institut Necker, 156 rue de Vaugirard, 75015 Paris, France; and ‡Institut für Klinische Molekularbiologie und Tumorgenetik, GSF-Forschungszentrum, Marchioninistrasse 25, 8000 Munich 70, Germany

ABSTRACT   Cyclin A is a cell cycle regulatory protein that functions in mitotic and S-phase control in mammalian somatic cells. Its deregulated expression may have a role in cellular transformation. We have cloned and sequenced the human cyclin A gene and cDNAs representing its mRNAs and have characterized its promoter. Using synchronized cultures of NIH 3T3 cells stably transfected with cyclin A promoter/luciferase constructs, we show that the promoter is repressed during the G_1 phase of the cell cycle and is activated at S-phase entry. Cell cycle regulation of the cyclin A gene promoter is mediated by sequences extending from −79 to +100 relative to the predominant transcription start site. It does not require the presence of a functional retinoblastoma protein.

Cyclins control the transitions between the phases of the eukaryotic cell cycle as regulatory subunits of the cyclin-dependent kinases (cdk) (1). Phase-specific activation of the cdk is in part regulated by phase-specific expression of their cyclin component. In mammalian somatic cells, the G_1 cyclins of the D and E type regulate the passage of G_1 (2), cyclin A is required for S phase and passage through G_2 (3), and the kinase composed of a B-type cyclin and p34^{cdc2} is the key regulator of mitosis (4). Cyclin A was first identified in sea urchin embryos (5). It can bind and activate the kinases cdc2 and cdk2 (6, 7) and is involved in the control of mitosis and S phase as well. Thus, injection of cyclin A mRNA drives G_2-arrested *Xenopus* oocytes into meiotic metaphase (8), and degradation of the protein is necessary for the exit from mitosis (9). Cyclin A mutant *Drosophila* embryos undergo cell cycle arrest in G_2 (10), and somatic mammalian cells are blocked in G_2 upon ablation of cyclin A (11). In addition to its mitotic functions, cyclin A may have a role in the dependence of mitosis on S-phase completion (12). Cyclin A is required for S-phase passage in normal mammalian cells, while cyclin B is not (3, 12, 13). Cyclin A may indeed be directly involved in DNA replication: it colocalizes with the DNA replication complex (14, 15) and was found in a cellular activity that promotes large tumor-antigen-dependent simian virus 40 DNA replication in G_1-phase cell extracts (16); cyclin A/cdk2 may phosphorylate and activate the cellular DNA replication factor RPA (17, 18). During S phase, cyclin A forms a complex containing cdk2, the pRB-related protein p107, and the transcription factor E2F (19, 20). E2F regulates a number of growth-promoting genes (21) and is negatively controlled during G_1 by binding to pRB (22). In adenovirus 5-infected cells, the viral E1A oncoprotein dissociates the pRB/E2F complex (23, 24) and binds to cyclin A via p107 in S phase (25, 26). A possible role for cyclin A in cellular transformation was further suggested by our previous finding of a hepatitis B virus integration in the human cyclin A gene in a primary liver carcinoma (27). In this tumor, cyclin

A-specific mRNA is exclusively transcribed from a viral promoter (28), indicating that deregulated cyclin A expression might contribute to tumor formation. In primary epithelial cells (12), in diploid fibroblasts (29, 30), and in several other human and rodent cell types (31, 32), cyclin A transcripts and protein are low or absent in G_1 and appear abundantly at the G_1/S transition. We have cloned and characterized the human cyclin A gene and its promoter, and we show that cyclin A expression is cell cycle regulated through tight control of its promoter activity.§

MATERIALS AND METHODS

Cloning and DNA Sequencing. Using as a probe a 1.6-kb human cyclin A cDNA (27), we isolated genomic clones from a placenta DNA library constructed in the phage vector λEMBL3. cDNA clones were obtained by screening a λgt10 library of oligo(dT)-primed cDNA of poly(A⁺) RNA from human liver tissue. Phage inserts were subcloned into the plasmid vector pBluescript SK (Stratagene). DNA sequencing was performed on denatured double-stranded plasmid templates by the dideoxynucleotide chain-termination method with a modified T7 DNA polymerase (Sequenase; United States Biochemical) and primers derived from vector or insert sequences.

Cyclin A RNA Stability Determination. HL60 cells were treated with the transcription inhibitor actinomycin D (5 µg/ml). Northern blots of total RNA prepared at different time points after addition of the drug were hybridized with the 1.6-kb cyclin A cDNA (27) and subsequently with a 1.4-kb *Cla* I/*Eco*RI fragment from the third exon of the human *MYC* gene.

RNA 5′-End Mapping. The cyclin A RNA cap sites were localized by nuclease S1 protection analysis as described (33). We used two double-stranded probes (see Fig. 3), a 613-bp *Sac* I/*Xho* I fragment (probe 1), and an 827-bp *Sac* I/*Nhe* I fragment (probe 2), which were 5′-³²P-labeled at the *Xho* I and *Nhe* I sites, respectively. RNA was prepared from the cell lines HL60 (promyelocytic leukemia), Raji (Burkitt lymphoma), and EW1 (Ewing sarcoma) and from human diploid fibroblasts. Cellular RNA (40 µg) and tRNA as a control were hybridized with either probe and digested with nuclease S1 (Boehringer Mannheim). Protected fragments were separated on a 6% polyacrylamide sequencing gel and visualized by autoradiography.

In Vitro **Promoter Assay.** The luciferase reporter plasmid pL was constructed by insertion of a polylinker sequence into the unique *Hind*III site of pSVOALΔ 5′ (34). Genomic restriction fragments from the cyclin A upstream region were cloned into pL; 2 × 10⁵ cells per 35-mm dish were transfected

5490
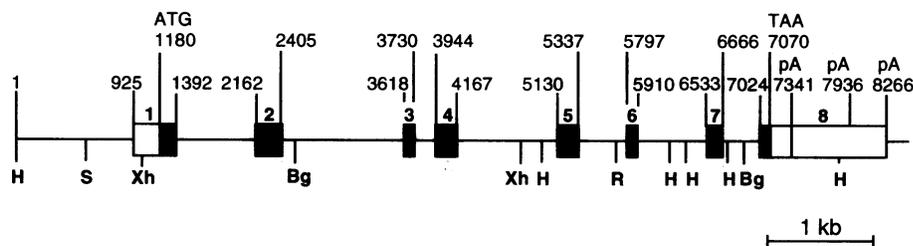
FIG. 1. Structure of the human cyclin A gene. Restriction map of the gene. Boxes designate exons; solid boxes represent coding sequences. H, *Hind*III; S, *Sac* I; Xh, *Xho* I; Bg, *Bgl* II; R, *Eco*RI; pA, major polyadenylylation sites; ATG, translation start site; TAA translation stop site. Base numbering corresponds to numbering of the complete sequence. The 5' end of exon 1 corresponds to the 3'-most transcription start site (cf. Fig. 4).

by the calcium phosphate method (35) for 8 h with 1.5 μg of reporter plasmid and 1 μg of the β-galactosidase expression plasmid pCH110 (Pharmacia). Thirty-six hours posttransfection, cells were lysed in 400 μl of buffer containing 0.6% Nonidet P-40, 0.15 M NaCl, 10 mM Tris·HCl (pH 7.9), 1 mM EDTA. The luciferase activity of 100 μl of cleared lysate was determined by using a Berthold LB 9501 luminometer, by injection of 50 μl of lysis buffer containing 0.45 mM D-luciferin (Sigma) and 1.2 mM ATP. Luciferase activity was normalized for transfection efficiency as determined by β-galactosidase activity (36). The luciferase activity in extracts of cells transfected with the promoterless pL plasmid did not exceed the machine background level. For each reporter plasmid, at least four independent transfections were performed.

Cell Lines. Transient transfections were performed into human cell lines HeLa, SAOS-2, Huh7, and HepG2; the simian cell line Vero; the mouse cell line NIH 3T3; and the hamster cell line CCL39 (all from the American Type Culture Collection). Cells were maintained in minimal essential medium or Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) in a humidified atmosphere with 5% CO_2/95% air.

Stable Transfectants and Synchronization. Ten 10-cm plates, each containing 10^6 NIH 3T3 cells, were transfected with the cyclin A promoter/luciferase construct PALUC (the full promoter fragment A; see Fig. 4B) and with PGLUC (the minimal promoter fragment G; see Fig. 4B), respectively, together with a plasmid conferring puromycin resistance, derived from pBABEPuro (37). Three days posttransfection, puromycin (1 μg/ml) was added to the medium. Puromycin-resistant colonies were pooled and expanded.

These cells, termed PALUC/3T3 and PGLUC/3T3, respectively, were analyzed for luciferase content per cell, which stayed constant during a 2-week observation period. Both populations were then synchronized by serum deprivation (0.5% FCS) for 60 h and refed with 10% FCS. At 2-h intervals, we recorded cell cycle stage and cell number by flow cytometry (see below) and luciferase activity as described above.

In a similar way, we obtained the cells PALUC/C33A by using the human cervical carcinoma cell line C33A (38). For synchronization, we treated PALUC/C33A cells with 2.5 mM thymidine for 16 h and, after an 8-h incubation in drug-free medium, with 60 ng of nocodazole per ml for 6 h. Mitotic cells were shaken off the plate, washed repeatedly, and reseeded in normal medium. After 3 h, nonadherent cells were discarded, and adherent cells were analyzed at 2-h intervals for number, cell cycle status, and luciferase content.

Flow Cytometry. Cells were fixed in 90% methanol (−20°C), pelleted, and resuspended in 45 mM $MgCl_2$/30 mM sodium citrate/20 mM 4-morpholinopropanesulfonate, pH 7.0/0.1% Triton X-100/20 mM 2-mercaptoethanol/3 μg of 4',6-diamidino-2-phenylindole per ml. The stained nuclei were counted and their DNA content was assessed with a

Coulter Epics instrument; the flow cytometric data were analyzed by the computer program MULTICYCLE (Phoenix Flow Systems, San Diego).

RESULTS AND DISCUSSION

Structure of the Human Cyclin A Gene. The cyclin A gene locus was cloned as a set of overlapping phage inserts, which were subcloned and sequenced. A restriction map of the gene is shown in Fig. 1. Numbering of bases refers to the complete sequence. On Northern blots of oligo(dT)-selected RNA, the 1.6-kb cyclin A cDNA probe (27) detects a predominant 2.7-kb, a minor 1.8-kb, and a weak 2.5-kb species (Fig. 2A). Using this probe, we isolated 22 cDNA clones from a liver tissue cDNA library. Fifteen clones contained a poly(A) tract and were partly or entirely sequenced. Nine of 15 cDNAs were polyadenylylated at nucleotide position 8266 (Fig. 1), 18 bp downstream of a consensus polyadenylylation signal (AATAAA). Five of 15 cDNA clones represented mRNAs polyadenylylated at position 7341, 17 bp downstream of a polyadenylylation signal. One cDNA represented an RNA polyadenylylated at nucleotide 7936, a polyadenylylation signal being present 23 bp upstream. Given the position of the cyclin A cap sites in the region 877–925 (see below), the size and relative cloning frequency of the three types of cDNA are in good correspondence with the size and relative abundance of cyclin A mRNAs observed on Northern blots. Comparison of cDNA and genomic sequence shows that the cyclin A gene is organized in eight exons displaying canonical intron/exon and exon/intron borders. The mRNAs differ only in the eighth exon by the length of their 3' noncoding region.
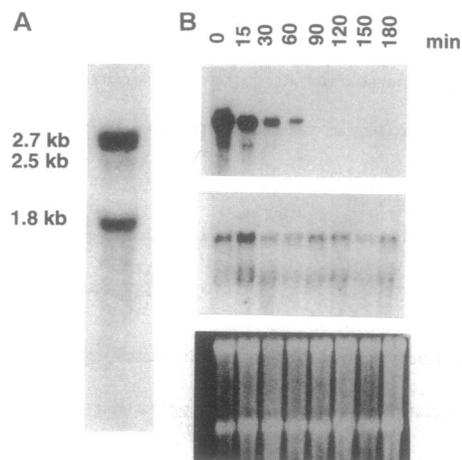
FIG. 2. Stability of cyclin A transcripts. (A) Northern blot of oligo(dT) selected Raji RNA, hybridized with the 1.6-kb cyclin A cDNA. (B) Northern blot of HL60 RNA prepared at the indicated time points of actinomycin D treatment and hybridized with a c-myc probe (*Top*) and a cyclin A probe (*Middle*). (*Bottom*) RNA loading (ethidium bromide stain).
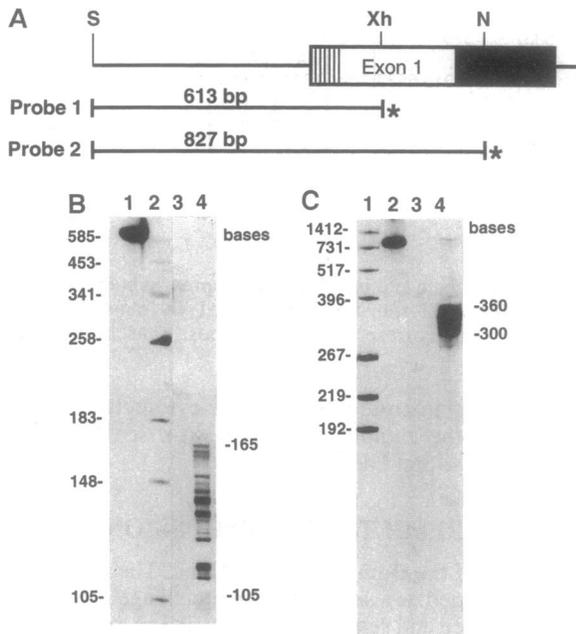
FIG. 3. Mapping of cyclin A transcription start sites. (*A*) Map of the first exon showing the two probes used for nuclease S1 protection analysis. S, *Sac* I; Xh, *Xho* I; N, *Nhe* I. (*B*) Nuclease S1 protection of probe 1. Lanes: 1, probe 1 undigested; 2, molecular size marker (bp); 3, tRNA hybridized with probe 1 and digested with nuclease S1; 4, RNA from cell line Raji hybridized with probe 1 and digested with nuclease S1. Size range of protected fragments is indicated. (*C*) Protection of probe 2. Lanes: 1, molecular size marker (bp); 2, probe 2 undigested; 3, tRNA hybridized with probe 2 and digested with nuclease S1; 4, Raji RNA hybridized with probe 2 and digested with nuclease S1. Size range of protected fragments is indicated.

Cyclin A expression is cell cycle regulated on the RNA and protein levels. At prometaphase, the protein is degraded by the ubiquitin pathway (39). In HeLa cells, cyclin A mRNA is completely degraded 4 h after release from a nocodazole block (6). Together with our result that cyclin A RNA is stable during at least 3 h in asynchronously growing HL60 cells treated with actinomycin D (Fig. 2*B*), these data suggest that cyclin A RNA is specifically degraded at mitosis. We observed no difference in stability between the 2.7- and the 1.8-kb cyclin A mRNAs, and the ratio of abundance of the

two species is constant throughout S and G_2 in normal cells (12). The presence of three ATTTA mRNA degradation signals (40) on the 2.7-kb species may lead to its preferential decay in mitosis, as observed for the longer one of the maternal cyclin A transcripts in the early embryonic cleavage divisions of *Drosophila* (41).

Localization and Functional Characterization of the Cyclin A Promoter. Primer extension and RNase protection experiments showed heterogenous transcription initiation but gave no clear signals for the 5'-most start sites. We could map the cyclin A transcription start sites by nuclease S1 protection experiments using two different probes. We localized 10 major and some minor transcription start points to the genomic region 877–925 with a precision of ±2 bp (Fig. 3; data not shown). The start sites were found to be identical in all cell types analyzed. In HL60 cells, however, >90% of transcription initiation occurs at the 3'-most start site (nucleotide 925; data not shown). We therefore designated this position as +1. Fig. 4*A* shows the sequence of the cyclin A upstream region. The absence of a TATA box is consistent with the observed heterogeneity of transcription initiation, since the TATA box is believed to direct RNA polymerase II to a defined start position (42). There is an inverted CAAT box or NF-1/CTF (43) binding site (at position −55). Four Sp1 (44) sites (centered around positions −193, −170, −138, +118), an ATF (45) site (at −80), and two overlapping sites (at +108 and +112) for the murine G_1/S-specific transcription factor Yi (46) are present (consensus: AGNGNNNGGG). A near-consensus AP-1 site (47) is found at position −279. Two nested TGCCT motifs (at +53) could be targets for the binding of p53 (48). The octamer TTTGGCTC (at +31) has one mismatch with an E2F binding site in the *NMYC* gene, while the octamer TTTGGCCG (at +165) differs in 1 base from an E2F site in the *MYB* promoter (21). At position −300 one observes a 10-mer repeated from position −310.

To delineate regulatory sequences in the cyclin A promoter, we cloned different restriction fragments from the cyclin A upstream region in front of the firefly luciferase cDNA as a reporter gene. These constructs were transiently transfected into the cell lines HeLa, Huh7, HepG2, and SAOS-2 (human); Vero and COS (simian); and NIH 3T3 and CCL39 (rodent). The respective luciferase activities in NIH 3T3 cells (normalized to transfection efficiency) are shown in Fig. 4*B*. The values were similar in all other cell lines tested (data not shown). Since fragment B, a 3.6-kb *Bam*HI/*Sma* I fragment (−3400 to +245), gave the same results as the 7.5-kb

cyclin A upstream fragment linked to luciferase cDNA		relative luciferase activity
A	−7300 to +245	100%
B	−3400 to +245	100%
C	−924 to +245	92%
D	−516 to +245	94%
E	−215 to +245	247%
F	−79 to +245	16%
G	−79 to +100	6%
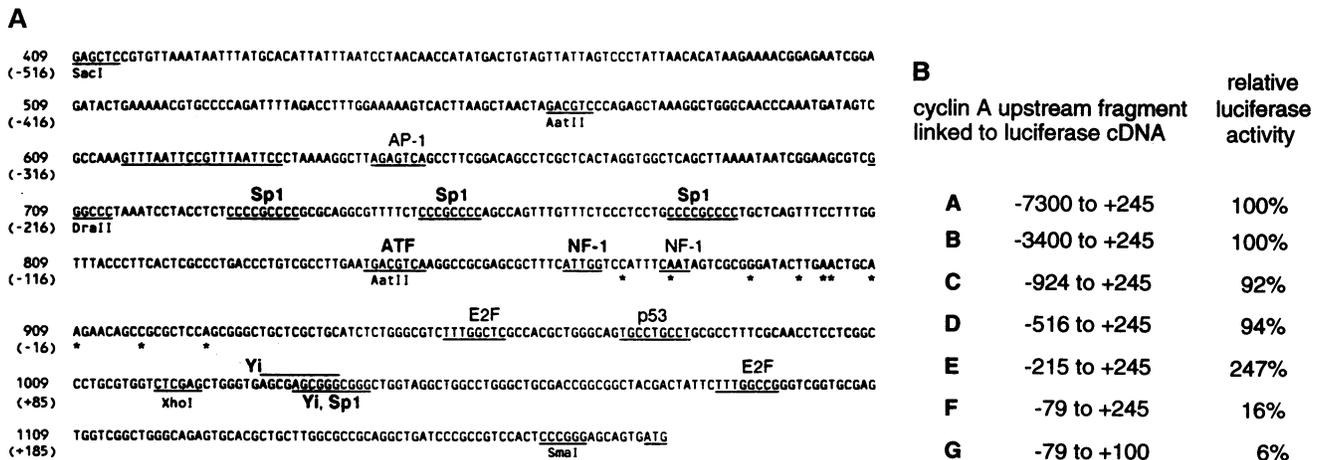
FIG. 4. Characterization of cyclin A promoter region. (*A*) Sequence of cyclin A promoter region. Asterisks indicate major transcription start points (±2 bp). Numbers in parentheses refer to the 3'-most start site (position 925) as +1. Consensus (boldface) or near-consensus transcription factor binding sites are indicated and underlined. A 10-bp repeat and the translation start site are underlined. (*B*) Promoter activity of different cyclin A upstream fragments in NIH 3T3 cells. Relative luciferase activities of fragments A–G are given as percentage of activity of fragment A; values are rounded means of at least four independent transfections normalized to β-galactosidase expression. SDs did not exceed 18%.

fragment A, we considered fragment A to be the full promoter of the gene. The smallest promoter fragment with activity in all cell lines tested was fragment G, an *Aat* II/*Xho* I fragment (−79 to +100). Deletion of the transcription start sites from fragment F (*Xho* I/*Sma* I fragment, +95 to +245; data not shown) completely abolished its activity.

Fig. 4*B* shows the presence of a negative regulatory sequence (−516 to −215, fragment D vs. E) and of positive regulatory elements located between −215 and −79 (E vs. F) and between +100 and +245 (F vs. G), respectively. The sequence −215 to −79 harbors a group of three Sp1 sites, known to act synergistically (49). Prominent features of the positive regulatory sequence +100 to +245 are an E2F site and two Yi sites. This setting is found in several other TATA-less promoters of "late response" genes. Sp1 site clusters are present in the genes for thymidine kinase (TK) (50), proliferating cell nuclear antigen (PCNA) (51), and cyclin D1 (52). E2F, whose activity is repressed during G_1 by the negative growth regulators pRB (22) and p107 (53), was shown to mediate G_1/S-specific transcriptional activation of the *TK, DHFR*, and *BMYB* genes (54–56) either by derepression or by direct activation. Two sites for the transcription factor Yi (46) are present on a 15-mer (+106 to +120) conserved (with one C to G transversion) in the 70-bp fragment from the human TK promoter, which confers cell cycle regulation to a heterologous promoter (50). The binding of an E2F-like complex to a Yi site on the murine TK promoter was shown to induce TK transcription at the G_1/S border (54). Consistent with a role of E2F in cyclin A transcription control are the observation that MYC overexpression up-regulates E2F activity and cyclin A mRNA (57) and our recent finding that type β transforming growth factor (TGF-β) down-regulates cyclin A mRNA (32), since TGF-β inhibits the phosphorylation of pRB in cells growth arrested by TGF-β (58). The only promoter sequence of a mammalian cyclin gene available to date is that of human cyclin D1 (52). Its TATA-less promoter shares with the cyclin A promoter the presence of partially clustered Sp1 sites and an E2F site. However, the gene is expressed well before cyclin A (29), and cell cycle regulation of its promoter has not been demonstrated.

Cell Cycle Regulation of the Cyclin A Promoter. To study cyclin A transcription initiation as a function of cell cycle progression, we used synchronized NIH 3T3 cells stably transfected with luciferase constructs PALUC and PGLUC, carrying the full promoter fragment A and the minimal promoter fragment G, respectively. During exponential growth, the luciferase activity level was constant at 10 relative light units (RLU) per cell in PALUC/3T3 (0.5 RLU per cell in PGLUC/3T3). This value decreased to 0.2 RLU per cell in PGLUC/3T3) after 60 h of serum starvation, when 97% of the cells were in the G_1 phase of the cell cycle. Serum was then added to the cultures; at 2-h intervals, cells were counted and analyzed for DNA content by flow cytometry, and the relative luciferase activity per cell was determined. Fig. 5 shows that PALUC/3T3 cells enter S phase with remarkable synchrony and that activation of the full cyclin A promoter coincides with the beginning of DNA synthesis in 3T3 cells. The cell cycle passage of PGLUC/3T3 cells after synchronization was indistinguishable from PALUC/3T3. Fig. 5 shows that luciferase expression in PGLUC/3T3 follows the kinetics of that in PALUC/3T3, except that G_1/S induction is less abrupt. We conclude that the essential targets for G_1 repression of the cyclin A promoter are located on fragment G.

In view of the putative binding sites for pRB/E2F in the cyclin A upstream sequence, we asked whether G_1 repression of the cyclin A promoter may require the presence of a functional RB protein. C33A cervical carcinoma cells express a mutant RB protein unable to bind to E1A and constitutively

hypophosphorylated (38), thus being either unable to bind E2F or unable to release E2F upon phosphorylation. In a way similar to PALUC/3T3, we generated PALUC/C33A—i.e., stably PALUC-transfected C33A cells. PALUC/C33A were synchronized by a thymidine block followed by a nocodazole
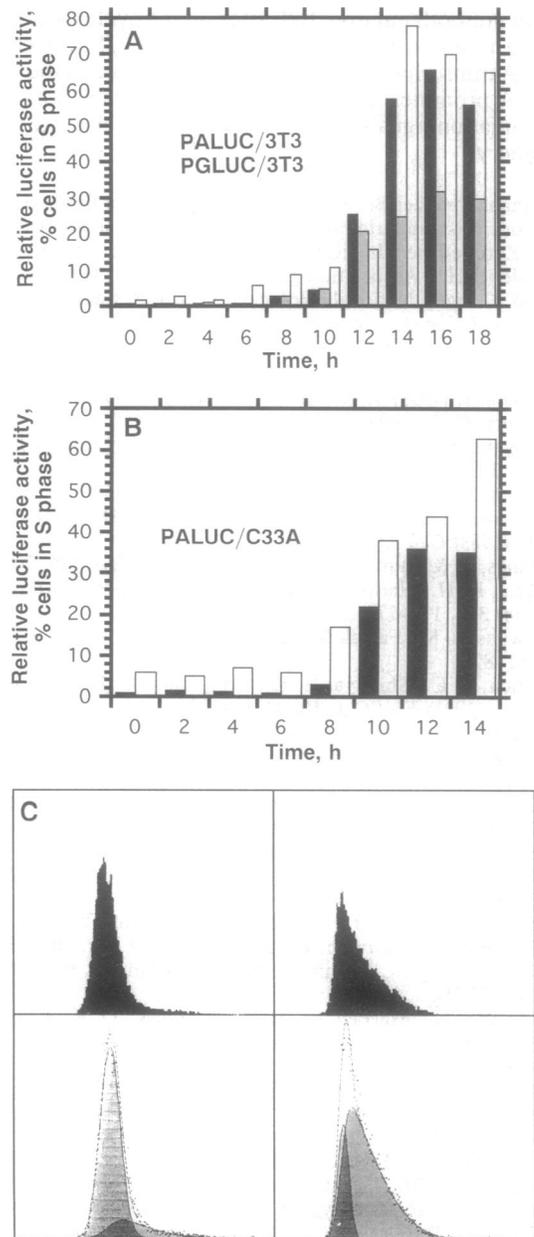
FIG. 5. S-phase-specific induction of cyclin A promoter. (*A*) Luciferase activity in PALUC/3T3 (solid bars) and PGLUC/3T3 (shaded bars) at 2-h intervals after release from serum starvation, relative to luciferase activity at *t* = 0 h. Open bars, percentage of S-phase cells in PALUC/3T3; values for PGLUC/3T3 were very similar (data not shown). At *t* = 0 h, 97% of the cells were in G_1 phase. Bars represent mean values from three (two for PGLUC/3T3) independent experiments. (*B*) Relative luciferase activity (solid bars) and percentage of S-phase cells (open bars) in PALUC/C33A released from a thymidine/nocodazole block. At *t* = 0 h, 92% of the cells were in G_1 phase. Bars represent mean values from three independent experiments. (*C*) (*Upper*) Raw data from flow cytometry of PALUC/3T3 cells at time points *t* = 12 h and *t* = 14 h. (*Lower*) Computer analysis of flow cytometric data at the same time points, demonstrating good synchrony of S-phase entry. Shaded area designates cells in G_1; hatched area designates cells in S phase. At *t* = 12 h (*Left*), 83% of the cells are in G_1 and 16% are in S phase; at *t* = 14 h (*Right*), 21% are in G_1 and 78% are in S phase.

block; 92% of the cells were then in G_1, and their luciferase activity had dropped from 8 RLU per cell (in cycling cells) to 0.4 RLU per cell. PALUC/C33A cells entered S phase with sufficient synchrony to show that the cyclin A promoter is repressed during G_1 and induced at the G_1/S transition (Fig. 5). Taken together, these results indicate that neither the RB protein nor both putative E2F sites on the cyclin A promoter may be required for G_1 repression and G_1/S induction of cyclin A transcription. Further studies of the regulation of the cyclin A promoter may help to understand how growth and differentiation signals are transmitted to the cell cycle control machinery.

1. Pines, J. (1993) *Trends Biochem. Sci.* **18**, 195–197.
2. Sherr, C. J. (1993) *Cell* **73**, 1059–1065.
3. Pagano, M., Pepperkok, R., Verde, F., Ansorge, W. & Draetta, G. (1992) *EMBO J.* **11**, 961–971.
4. Nurse, P. (1990) *Nature (London)* **344**, 503–508.
5. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T. (1983) *Cell* **33**, 389–396.
6. Pines, J. & Hunter, T. (1990) *Nature (London)* **346**, 760–762.
7. Tsai, L.-H., Harlow, E. & Meyerson, M. (1991) *Nature (London)* **353**, 174–177.
8. Swenson, K., Farrel, K. M. & Ruderman, J. V. (1986) *Cell* **47**, 861–870.
9. Luca, F. C., Shibuya, E. K., Dohrmann, C. E. & Ruderman, J. V. (1991) *EMBO J.* **10**, 4311–4320.
10. Lehner, C. F. & O'Farrell, P. H. (1990) *Cell* **61**, 535–547.
11. Walker, D. H. & Maller, J. L. (1991) *Nature (London)* **354**, 314–317.
12. Zindy, F., Lamas, E., Chenivesse, X., Sobczak, J., Fesquet, D., Wang, J., Henglein, B. & Bréchot, C. (1991) *Biophys. Biochem. Res. Commun.* **182**, 1144–1154.
13. Girard, F., Strausfeld, U., Fernandez, A. & Lamb, N. J. C. (1991) *Cell* **67**, 1169–1179.
14. Sobczak-Thépot, J., Harper, F., Florentin, Y., Zindy, F., Bréchot, C. & Puvion, E. (1993) *Exp. Cell Res.* **206**, 43–48.
15. Cardoso, M. C., Leonhardt, H. & Nadalginard, B. (1993) *Cell* **74**, 979–992.
16. D'Urso, G., Marraccino, R. L., Marshak, D. R. & Roberts, J. M. (1990) *Science* **250**, 786–791.
17. Dutta, A. & Stillman, B. (1992) *EMBO J.* **11**, 2189–2199.
18. Elledge, S. J., Richman, R., Hall, F. L., Williams, R. T., Lodgson, N. & Harper, J. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2907–2911.
19. Shirodkar, S., Ewen, M., DeCaprio, J. A., Morgan, J., Livingston, D. M. & Chittenden, T. (1992) *Cell* **68**, 157–166.
20. Devoto, S. H., Mudryj, M., Pines, J., Hunter, T. & Nevins, J. R. (1992) *Cell* **68**, 167–176.
21. Mudryj, M., Hiebert, S. W. & Nevins, J. R. (1990) *EMBO J.* **9**, 2179–2184.
22. Chellappan, S. P., Hiebert, S., Mudryj, M., Horowitz, J. M. & Nevins, J. R. (1991) *Cell* **65**, 1053–1061.
23. Bandara, R. L. & La Thangue, N. B. (1991) *Nature (London)* **351**, 494–497.
24. Bagchi, S., Raychaudhuri, P. & Nevins, J. R. (1990) *Cell* **62**, 659–669.

25. Ewen, M. E., Faha, B., Harlow, E. & Livingston, D. M. (1992) *Science* **255**, 85–87.
26. Giordano, A., Whyte, P., Harlow, E., Franza, B. R., Beach, D. & Draetta, G. (1989) *Cell* **58**, 981–990.
27. Wang, J., Chenivesse, X., Henglein, B. & Bréchot, C. (1990) *Nature (London)* **343**, 555–557.
28. Wang, J., Zindy, F., Chenivesse, X., Lamas, E., Henglein, B. & Bréchot, C. (1992) *Oncogene* **7**, 1653–1656.
29. Baldin, V., Lukas, J., Marcote, M. J., Pagano, M. & Draetta, G. (1993) *Genes Dev.* **7**, 812–821.
30. Lucibello, F. L., Sewing, A., Brüsselbach, S., Bürger, C. & Müller, R. (1993) *J. Cell Sci.* **105**, 123–133.
31. Sewing, A., Bürger, C., Brüsselbach, S., Schalk, C., Lucibello, F. L. & Müller, R. (1993) *J. Cell Sci.* **104**, 545–554.
32. Barlat, I., Fesquet, D., Strausfeld, U., Bréchot, C., Henglein, B., Vié, A. & Blanchard, J. M. (1993) *Cell Growth Differ.* **4**, 105–113.
33. Eick, D. (1990) *Nucleic Acids Res.* **18**, 1199–1205.
34. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7**, 725–737.
35. Graham, F. L. & van der Eb (1973) *Virology* **52**, 456–467.
36. Rosenthal, N. (1987) *Methods Enzymol.* **152**, 704–720.
37. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**, 3587–3596.
38. Scheffner, M., Münger, K., Byrne, J. C. & Howley, P. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5523–5527.
39. Glotzer, M., Murray, A. W. & Kirschner, M. W. (1991) *Nature (London)* **349**, 132–137.
40. Shaw, G. & Kamen, R. (1986) *Cell* **46**, 659–667.
41. Lehner, C. F. & O'Farrell, P. H. (1989) *Cell* **56**, 957–968.
42. Jones, N. C., Rigby, P. W. J. & Ziff, E. B. (1988) *Genes Dev.* **2**, 267–281.
43. Santoro, C., Mermod, N., Andrews, P. C. & Tjian, R. (1988) *Nature (London)* **334**, 413–419.
44. Kadonaga, J. T. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5889–5893.
45. Lee, K. A. W., Hay, T. J., SivaRaman, L., Thimmappaya, B., Hurst, H. C., Jones, N. C. & Green, M. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8355–8359.
46. Dou, Q.-P., Fridovich-Keil, J. L. & Pardee, A. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1156–1161.
47. Angel, P., Imagawa, M., Chin, R., Stein, B., Imbra, R. J., Ramsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49**, 721–739.
48. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C. & Vogelstein, B. (1991) *Science* **252**, 1708–1711.
49. Anderson, G. M. & Freytag, S. O. (1991) *Mol. Cell. Biol.* **11**, 1935–1943.
50. Kim, Y. K. & Lee, A. S. (1991) *Mol. Cell. Biol.* **11**, 1185–1194.
51. Morris, G. F. & Mathews, M. B. (1990) *J. Biol. Chem.* **265**, 16116–16125.
52. Motokura, T. & Arnold, A. (1993) *Genes Chromosomes Cancer* **7**, 89–95.
53. Zhu, L., van den Heuvel, Z., Helin, K., Fattaey, A., Ewen, M., Livingston, D., Dyson, N. & Harlow, E. (1993) *Genes Dev.* **7**, 1111–1125.
54. Dou, Q.-P., Markell, P. J. & Pardee, A. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3256–3260.
55. Means, A. L., Slansky, J. E., McMahon, S. L., Knuth, M. W. & Farnham, P. J. (1992) *Mol. Cell. Biol.* **12**, 1054–1063.
56. Lam, E. W.-F. & Watson, R. J. (1993) *EMBO J.* **12**, 2705–2713.
57. Jansen-Dürr, P., Meichle, A., Steiner, P., Pagano, M., Finke, K., Botz, J., Wessbecher, J., Draetta, G. & Eilers, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2547–2552.
58. Laiho, M., DeCaprio, J. A., Ludlow, J. W., Livingston, D. M. & Massagué, J. (1990) *Cell* **62**, 175–185.