Cyclin A1 is a recently cloned cyclin with high level expression in meiotic cells in the testis. However, it is also frequently expressed at high levels in acute myeloid leukemia. To elucidate the regulation of cyclin A1 gene expression, we cloned and analyzed the genomic structure of cyclin A1. It consists of 9 exons within 13 kilobase pairs. The TATA-less promoter initiates transcription from several start sites with the majority of transcripts beginning within a 4-base pair stretch. A construct containing a fragment from −190 to +145 showed the highest transcriptional activity. Transfection of cyclin A1 promoter constructs into S2 Drosophila cells demonstrated that Sp1 is essential for the activity of the promoter. Sp1, as well as Sp3, bound to four GC boxes between nucleotides −130 and −80 as observed by gel shift analysis. Mutations in two or more of the four GC boxes decreased promoter activity by >80%. The promoter was found to be cell cycle-regulated with highest activities found in late S and G2/M phase. Further analyses suggested that cell cycle regulation was accomplished by periodic repression of the GC boxes in G1 phase. Taken together, our data show that cyclin A1 promoter activity critically depends on four GC boxes, and members of the Sp1 family appear to be involved in directing expression of cyclin A1 in both a tissue- and cell cycle-specific manner.

A growing family of cyclin-dependent kinases (Cdk)s regulates a wide variety of cellular pathways (for review, see Ref. 1). Cdk 2 (Cdk 1) and Cdk 2 play a central role in the cell cycle of mammalian cells. Substrate specificity and activity of Cdks are controlled by their interaction with different cyclins that trigger the initiation of cell cycle events (2). Cdk 2 specifically interacts with cyclin E for G1/S progression and with cyclin A during S and G2/M phases. Cyclin B3 might be another partner for Cdk 2 and Cdk 2 during the G2/M phase (3). In accordance with their central role in mammalian cell cycle regulation, the levels of cyclins A and E (among other cyclins) oscillate in most if not all proliferating mammalian cells. Disruption of the murine cyclin A2 (the homolog of human cyclin A) leads to early embryonic death suggesting an essential role for this gene in embryonic cell cycle in mammals (4). The human cyclin A2 (also known as cyclin A) was initially cloned because its gene locus was the site of integration by the hepatitis B virus in a case of hepatocellular carcinoma (5). It has also been implicated to be important in the recurrence of hepatocellular carcinoma (6). Recently, we cloned a second human cyclin A-like partner for Cdk 2, termed cyclin A1, that exhibits a highly restricted pattern of expression (7). The high level tissue-specific expression of the human and murine cyclin A1 in testis suggests a specific role in meiosis (7, 8). Very low levels are detected in other tissues by reverse transcriptase-PCR; however, high levels of human cyclin A1 were also found in acute myeloid leukemia cell lines (7) and myeloid leukemia samples from patients (9). This intriguing observation might suggest a possible role for cyclin A1 in proliferation and differentiation of hematopoietic progenitors and/or in promotion of growth of leukemic cells.

Cyclin A1 shows homology to cyclin A2 and forms in vivo complexes with Rb as well as with E2F (10). Cyclin A1-Cdk 2 complexes phosphorylate these substrates in vitro (10). Our data showing that cyclin A1 is expressed in hematopoietic progenitors (9) and interacts with Rb family members and E2F, suggest that it may affect cell cycle progression in expressing cells.

The pattern of cyclin A1 expression indicates that the regulation of its expression is different from that of cyclin A2. Furthermore, overexpression of cyclin A1 in myeloid leukemia originates at the transcriptional level. To elucidate the transcriptional mechanisms that underlie the tissue-specific pattern of expression, we cloned and analyzed the genomic organization of the cyclin A1 gene and its promoter region. The highest transcriptional activity was assigned to a 335-bp fragment that required intact GC boxes located between −60 and −120 bp upstream of the main transcriptional start sites. These sites are also essential for cell cycle regulation of the promoter.

MATERIALS AND METHODS

Cloning of the Genomic Fragment of the Human Cyclin A1—The cyclin A1 gene was cloned by screening a genomic Fix II lambda library made from placenta (Stratagene) using the cyclin A1 cDNA as a probe (7). Of the several phage clones obtained, one contained all the exons and included a 1.3-kb region upstream of the 5′ end of the cDNA. A
Cells were arrested in G2/M phase by nocodazole (0.1 μM) and aphidicolin treatment and release into fresh medium 6 h before harvest. Determination of the length of the G2/M phase was exchanged, and cells were synchronized essentially as described above. The positions of the cyclin A1 coding region with different primers (detailed primer information will be provided on request). Subsequently, PCR products were either subcloned using pGEM-T-Easy (Promega) or directly sequenced by cycle sequencing. Boundaries of the ~4.5 kb intron 2 were determined by direct sequencing of the cloned DNA. 

Generation of Luciferase Reporter Constructs—The initial luciferase constructs were generated by PCR amplification of the pRS316 plasmid containing the 2.2-kb cyclin A1 fragment. A BgIII site at the 5’ end and a BamHI site at the 3’ end were introduced and the Pfu-amplified fragment was cloned into the BgIII site of PGL3-Basic. The +145 fragment was generated to include the potential E2F site at +138. The ATG in the primer (the initiating codon for cyclin A1) was mutated to ATT to avoid the initiation of translation. All constructs were confirmed to have the correct sequence by DNA sequencing. The 5’ deletions were generated by exonuclease III treatment using KpnI/SalI-digested PGL3-Basic containing the −1299 to +145 fragment and the Erase-a-base kit (Promega). The end points of the deletions were determined by sequencing of the Pfu-amplified fragment by digoxigenin-labeled 190 to +145 or by PGL3-Basic with NcoI and HindIII and subsequent cloning of the 200-bp fragment into PGL3-Basic digested with NcoI and HindIII.

Cell Culture and Transfection—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum containing 100 units/ml penicillin and 100 μg/ml streptomycin. For transfection, 5 × 10⁵ cells were seeded into 60-mm plates 16 h before transfection. Transfection was carried out using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s protocol. Two μg of luciferase reporter plasmid was transfected together with 300 ng of a CMV-β-gal expression vector used for standardization. Cells were harvested and assayed for luciferase and β-galactosidase activity after 48 h. All experiments were carried out in duplicate and were independently performed at least three times. Data of luciferase assays are shown as mean ± S.E. of three independent experiments unless stated otherwise. The Drosophila cell line SL2 was obtained from ATCC and grown at room temperature in Schneider’s insect cell medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. Insect cells were transfected using Superfect (Qiagen). Briefly, 5 × 10⁵ cells were seeded into 60-mm plates and the mixture of the DNA and Superfect was added to the cells 16 h after addition. One μg of the luciferase reporter was transfected with or without 100 ng of the Sp1 expression vector pAC-Sp1, which was a kind gift from Dr. E. Stanbridge (University of California, Irvine). Luciferase activity was analyzed after 48 h. Luciferase values could not be standardized using β-galactosidase activity because the viral promoters in the available plasmids also depended strongly on Sp1 for adequate expression. All experiments were carried out in duplicate and independently performed at least three times.

Cell Cycle-dependent Promoter Activity—HeLa cells were transfected using LipofectAMINE as described above. After transfection, cells were cultured in 0.1% fetal calf serum containing medium. After 16 h, medium was changed, and cells were synchronized essentially as described (11). Cells were arrested in G0 by serum starvation (0.1% fetal calf serum), in early S phase by aphidicolin (2 μg/ml), and in S phase by aphidicolin treatment and release into fresh medium 6 h before harvest. Cells were arrested in G/M phase by nocodazole (0.1 μg/ml). Appropriate synchronization was confirmed by DNA quantitation using flow cytometry, and the experiments were performed at least three times. For the cell cycle release experiments, HeLa cells were arrested using aphidicolin as described above, and cells were harvested at the different time points after their release in fresh medium. The time course experiments were independently performed twice. To analyze cell cycle-regulated activity of different constructs, HeLa cells were arrested by serum starvation for 36 h followed by aphidicolin arrest and subsequently released for 18 h. At this time point, most cells were in late S or in G/M phase. The longer serum starvation of the cells led to a synchronous and a higher induction after release. The release experiments were independently performed at least three times. All luciferase values were normalized using β-galactosidase activity as described above.

RACE and Primer Extension—The rapid amplification of 5’ cDNA ends (RACE) was performed using a 5’-RACE system (Life Technologies, Inc.). The procedure was performed as suggested in the manufacturer’s protocol using RNA of the myeloid leukemia cell lines ML1 and U937. RNA was reversely transcribed using the primer 5’-CCCTCTCA-GAACAGACATACA (positions +981 to +961 of the cDNA) and Superscript II reverse transcriptase (Life Technologies, Inc.). Gene-specific cDNA was PCR-amplified using the gene-specific primer 5’-CTGTCCACGAGAATACCGTTGGTCCGCGC and the universal primer 5’-RACE Abridged Anchor Primer 5’-GGCCACCCGTTGCAGC-TAGTACGCGGGGIGGGGGGIG. PCR amplifications from both RNA samples yielded a single band of ~450 bp. The entire PCR product was phenol/chloroform-extracted, precipitated using NH4 acetate, and finally cloned into pGEM-T-Easy and sequenced.

The rapid extension assay was carried out by reverse transcription of 10 μg of RNA (U937) using a 5’-TTCTTCCCCACGACACCAGAGGCA corresponding to +97 to +79 on the cDNA. Hybridization was carried out overnight at 58°C. Superscript II was used for reverse transcription at 42°C for 50 min. Extension products were resolved on a 8% sequencing gel with a sequencing reaction being run in parallel. As negative controls, we used tRNA and a sample without RNA.

Electrophoretic Mobility Shift Assays—Nuclear extracts from HeLa cells were prepared as described (12). For gel retardation experiments, 1 ng of 5’-TTCTTCCCCACGACACCAGAGGCA was incubated for 20 min at room temperature with 5 μg of HeLa nuclear extract (final reaction volume 10 μl) containing 400 ng of poly(dI-dC). For competition experiments, 100 ng of double-stranded oligonucleotide containing either a Sp1 consensus site (5’-ATTCCGATCGGCGCCCGACGACG) or Sp3 (D20, Santa Cruz Biotechnology) was preincubated with the nuclear extracts for 15 min at room temperature with the nuclear extracts before the addition of the labeled oligonucleotide. For supershift experiments, 2–3 μg of polyclonal antibody against Sp1 (Pep2, Santa Cruz Biotechnology) or Sp3 (D20, Santa Cruz Biotechnology) was preincubated with the nuclear extracts. Reactions were loaded on a 5% TBE, 4% nondenaturing polyacrylamide gel and run for 2–3 h at 10 V/cm. Gels were dried and autoradiographed.
were analyzed by PCR amplification and sequencing using sets of primers that span the entire coding region. The human cyclin A1 gene consists of 9 exons and 8 introns that extend over ~13 kb (Fig. 1). All the exon-intron boundaries adhere to the consensus sequence. The translation initiation codon is located in the first exon. A second in-frame ATG that resembles the starting codon of the murine cyclin A1 is found in exon 2, 50 nucleotides downstream of intron 1 of the human cyclin A1 gene.

Analysis of Transcription Start Sites—Transcription start sites were determined using primer extension and 5'-RACE (Fig. 2). Both methods demonstrated the existence of several transcription start sites. The PCR product from the RACE reaction consisted of a single band of ~450 bp. Sequencing of the inserts after cloning revealed that 80% of the RACE clones started at a particular base. The number of RACE clones (total 25) starting at a particular base is indicated by the number shown below the arrows. The site where 44% (11/25) RACE clones started was assigned +1.
products (20/25) started from a 4-base pair stretch, and thus the predominant start site was assigned +1 (Fig. 2A). This site is 130 bp upstream of the translation initiating ATG codon. Primer extension analysis identified the same start sites, but minor products were also seen further upstream (Fig. 2B). The major start site coincides with the RACE results of the 5' end of the initially described cDNA clone (7). We also looked for transcription start sites upstream of the second ATG in intron 1. However, neither RACE clones nor primer extension assays showed evidence for a second transcript in myeloid leukemia cells (data not shown).

Potential Transcription Factor Binding Sites in the 5' Upstream Region—Genomic sequences 1299 bp upstream of the transcription start site were cloned and sequenced. No TATA box was found in proximity to the putative transcription start site. The main transcriptional start site is likely to function as an initiator region (Inr) since the sequence “CCAGTT” is very similar to the consensus Inr sequence “TCA (G/T) T (T/C)” (14). No DPE element was found downstream of the main transcriptional start site (14). Several potential binding sites for transcription factors occur within the sequence (Fig. 3). An E2F site is located at +140 and another possible site at +68. A site that resembles the cycle-dependent element (CDE) of the cyclin A2 promoter was found at −28 (15). However, this element was located on the antisense strand. No cell cycle genes homology region (CHR) was found. Potential Myb sites are predicted at positions +2, −30, and −90. The nucleotide sequence contains two CpG islands of up to 90% GC content reaching from −1000 to −700 and from −550 to −50. Multiple GC boxes are found in this region, and six GC boxes grouped as three double sites are located between nucleotides −150 and −45.

Functional Analysis of the Basal Activity of the Cyclin A1 Promoter—Portions of the cyclin A1 promoter were Pfu PCR-amplified and cloned into the promoterless PGL3-Basic Luciferase vector. Promoter activity was analyzed after transient transfection into HeLa cells. The construct containing nucleotides from −1299 to −1145 from the 5' upstream region showed significant promoter activity when cloned in the sense direction (Fig. 4). The same fragment cloned in the opposite direction or a construct containing solely exon 1 and intron 1 did not show promoter activity (data not shown).

Deletions from the 5' end were made for the −1299 to +145 fragment using exonuclease III treatment (Fig. 4). Transient transfection and subsequent luciferase assays revealed the strongest activity occurred in the construct containing the fragment from −190 to +145 bp (Fig. 4). Both the −1299 and the −190 constructs exhibited promoter activity in a variety of cell lines including Cos-7, MCF-7, U937, KCL22, and Jurkat (data not shown). In all of these mammalian cell lines, luciferase activities generated by the −190 construct were higher than

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**Fig. 3.** 5' upstream region of the human cyclin A1 gene. Indicated are the first bases of the different fragments as well as potential transcription factor binding sites between −190 to −145. The transcriptional start site is marked with an arrow and the translational initiation codon is boldface.

**Fig. 4.** Transactivation activity of promoter fragments in HeLa cells. Activity of 5' deletion constructs was analyzed in luciferase assays. Values are expressed as fold activation (PGL3-Basic = 1), and means and S.E. of three independent experiments are shown.
those by the −1299 construct. Constructs with a 5’ end containing less than 190 bp upstream of the transcription start site showed a progressive loss of promoter activity. A construct containing bp −37 to +145 showed only 2-fold higher activity than the promoterless vector PGL3-Basic.

Role of Sp1 and GC Boxes for Transcriptional Activity of the Cyclin A1 Promoter—TATA-less promoters frequently depend on GC boxes to activate transcription (16, 17). One of the main factors binding to these sites are Sp1 family proteins (18–20). The cyclin A1 promoter contains at least six potential GC boxes between 190 and 37 bp upstream of the transcription start site. To analyze the importance of Sp1 for the activity of the cyclin A1 promoter, various promoter constructs were transfected into the Drosophila cell line S2 which lacks endogenous Sp1 and Sp3 (Fig. 5). When transfected alone, the activity of all cyclin A1 promoter fragments was not significantly different from the control (Fig. 6, dotted bars). The addition of an Sp1 expression plasmid strongly activated transcription by 15–25-fold from the cyclin A1 promoter (Fig. 5, solid bars). Interestingly, increased transcriptional activity was observed only for constructs containing sequences starting between −112 and −37 bp upstream of the transcription start site. The construct containing the nucleotide sequences between −37 and +145 did not show any increase in activity, suggesting that Sp1-binding sites between −112 and −37 are essential for Sp1-mediated transcriptional activity of the cyclin A1 promoter in Drosophila cells. This region contains four GC boxes that are grouped in two pairs (Fig. 3). To test whether Sp1 and other Sp1 family members could bind to these sites, gel-shift experiments were performed (Fig. 6). Several specific complexes in
HeLa cell nuclear extract bound to these sites (lanes 1 and 7). These complexes were competed away by an excess of cold oligonucleotides containing either the original site (lanes 2 and 8) or an Sp1 consensus site (lanes 3 and 9). A 100-fold excess of a nonspecific oligonucleotide did not alter complex binding (lanes 4 and 10). Supershift experiments with antibody against either Sp1 or Sp3 demonstrated the presence of Sp1 in one complex (lanes 5 and 11) and the presence of Sp3 (lanes 6 and 12) in two other complexes. The composition of the fastest migrating complex is unknown.
The relevance of these GC boxes for promoter activity was further studied by mutational analysis. Point mutations were made in each GC box. Each mutant was tested either alone with the remaining sites unaltered or in combination with the other mutant sites. Luciferase analyses demonstrated that a mutation in either GC box 1 or 2 reduced promoter activity by about 40 and 75%, respectively, whereas a single mutation of either GC box 3 or 4 did not have a major effect on promoter activity (Fig. 7). Mutation of GC box 1 and 2 together decreased promoter activity by 85%. The presence of at least one of the two upstream GC boxes (boxes 3 and 4) being intact was essential for cyclin A1 promoter activity as mutations in both reduced promoter activity by about 80%. Mutations of all four GC boxes reduced activity of the wild type reporter construct in G1 phase. How-

**Cell Cycle Regulation of Promoter Activity**—The concentration of cyclins varies during the cell cycle, and one mechanism of their regulation occurs at the transcriptional level (21). To analyze cell cycle regulation of promoter activity, transiently transfected cells were arrested in different phases of the cell cycle and were subsequently analyzed for luciferase activity. Cell cycle-regulated activity was found for the full-length promoter as well as for the construct containing the −190 to +145 fragment. The cyclin A1 promoter activity was relatively low during the G1/G0 phase. It increased after the cell cycle progressed beyond the G/S boundary (Fig. 8A). The highest levels of activity were observed in the S and G2/M phases. Recently, we showed that RNA levels of cyclin A1 accumulated during S phase with the highest levels present at the S and G2/M phases (10). When transiently transfected HeLa cells were released from an aphidicolin block, luciferase values started to increase after 6 h and reached a maximum after 12–16 h (Fig. 8B). The maximum promoter activity corresponded to the percentage of cells present in the S and G2/M phases (Fig. 8C).

To define the regions that are relevant for cell cycle regulation of the cyclin A1 promoter, we generated mutations in the presumed E2F sites and the suspected CDE. We also generated by PCR a 3′ deletion construct (−190 + 13) that deleted the two presumed E2F sites downstream of the transcriptional start site. Mutations in these two presumed E2F sites, the mutation in the inverted presumed CDE and the 3′ deletion, showed an indistinguishable pattern of cell cycle regulation when compared with the wild type (Fig. 9 and data not shown).

Hence, these E2F sites and the inverted CDE are unlikely to play a role in cell cycle regulation of the promoter. Analysis of 5′ deletions and the constructs containing the mutated GC boxes revealed that the four GC boxes are essential for cell cycle regulation (Fig. 9). Interestingly, the activity of the con-struct containing the mutated GC boxes showed 60% of the activity of the wild type reporter construct in G1 phase. How-ever, the activity of the construct failed to increase when cells entered S phase and showed only 4% of the wild type cyclin A1 promoter activity. Similar data were obtained for the 5′ deletion lacking the four GC boxes (Fig. 9).

**DISCUSSION**

Cyclin A2 (formerly cyclin A) is ubiquitously expressed in proliferating cells and is required for cell cycle progression (11, 22, 23). A second cyclin A-like protein, cyclin A1, shows a highly restricted pattern of expression suggestive not only of specific functional activities but also of distinct mechanisms of regulation (7, 8). The human cyclin A1 gene and its promoter were cloned from a genomic library to elucidate further the regions that regulate expression. Similar to other cell cycle regulatory genes (24–27), the cyclin A1 promoter does not possess a TATA box motif. The nucleotides surrounding the transcriptional start site are likely to function as an initiator. In addition, the upstream region contains a GC-rich region with multiple Sp1-binding sites that are essential for transcription from the cyclin A1 promoter. In contrast, predicted GC boxes in the cyclin A2 gene are located more than 120 bp upstream of the most 3′-transcriptional start site, and these have not been shown to be essential for gene expression.

Three potential binding sites for Myb proteins are present within 100 bp of the transcription start sites of the cyclin A1 gene, and one of them is located at +2. No consensus Myb sites have been found for either the murine or human cyclin A2 promoter (24, 28). The Myb-binding sites may play an important role in expression of cyclin A1 during spermatogenesis as well as hematopoiesis.

One major finding of this study demonstrates the importance of the GC boxes and the Sp1 family transcription factors in the regulation of cyclin A1 expression. Six GC boxes were found in the first 200 bp upstream of the transcription start site. The 5′ deletions revealed that omitting the four GC boxes between −112 and −37 almost completely abrogated promoter activity. Analysis of these fragments in insect cells demonstrated that Sp1 can reconstitute cyclin A1 promoter activity in all fragments that involve the GC boxes 1–4. These experiments show that Sp1 (or at least a member of the Sp1 family) is required for cyclin A1 promoter activity through interaction with elements located between −112 and −37. A more detailed analysis of the GC boxes 1–4 showed that the two closest to the transcriptional start sites are most critical. Of the GC boxes 3 and 4, only one of these was necessary for a basal level of transcriptional activity of the promoter. Gel-shift experiments demonstrated that Sp1 and Sp3 can bind to GC boxes 1 + 2 and 3 + 4.

The finding that these GC boxes are essential for expression of the cyclin A1 gene raises some interesting questions. Is Sp1, as the main activating factor of the Sp1 family, involved only in
the basic transcriptional activation of the cyclin A1 gene, or do Sp1 family proteins also play a role in the tissue-specific expression of this gene? Sp1 has been shown to serve distinct roles in transcriptional activation as follows: it can directly interact with the basal transcription complex (29), and it can determine the transcription start site in TATA-less promoters (16). However, Sp1 can also function as a more general transcriptional activator. Whereas these functions are not necessarily exclusive, recent studies demonstrated that Sp1 and its other family members can play an important role in directing tissue-specific expression (30–32). Levels of the ubiquitously expressed Sp1 vary up to 10-fold in different tissues (33). This could provide a basis for directing tissue-specific expression, especially if the affinity of the cis-acting Sp1-binding sites differ. For example, induction of Sp1 was found to be associated with differentiation of embryonal carcinoma cells, and Sp1 was causally linked to expression of the fibroinectin gene, providing evidence for a role of Sp1 in differentiation (34). In adult tissue, high levels of Sp1 have been reported in hematopoietic progenitors and in the later stages of spermatogenesis (33). This pattern does not entirely coincide with the much more restricted expression pattern of cyclin A1, but Sp1 is obviously expressed at high levels in tissues where cyclin A1 expression is found (8, 9). In addition, Sp1 was found to bind in vivo to two myeloid-specific promoters only in myeloid cells and was thus implicated in directing myeloid-specific promoter activity (31, 35).

Another mechanism of tissue-directed expression depends on the ratio of Sp1 family members to each other resulting in either activation or repression of transcription (19, 36). We showed that Sp3 can bind to the GC boxes in the cyclin A1 promoter. The Sp3 protein is known to function either as transcriptional activator or repressor depending on the context of the binding site in the promoter (37, 38). When Sp3 binds to a single site, it can activate transcription, but binding to multiple sites can lead to strong transcriptional repression (36).

Gene expression from the cyclin A1 promoter is cell cycle-regulated with the lowest promoter activity found in G2/G1 and highest activity during the S and G2/M phases. Fragments containing nucleotides −1299 to +145, −190 to +145, or −190 to +13 performed similarly in these experiments (data not shown). The levels of cyclin A1 mRNA and protein paralleled the promoter activity (10). Both increased during S phase and peaked at G2/M phase (10).

Further analyses of the mechanism of cell cycle regulation revealed that the four GC boxes are critical for cell cycle-regulated transcription from the cyclin A1 promoter. No increase in promoter activity in S phase was found when the four sites were mutated. Interestingly, the effect of the GC box mutations on promoter activity in G1 phase was not prominent with only a 40% reduction compared with the wild type construct. These findings are consistent with repression of Sp1-mediated activity in the G1 phase of the cell cycle. Selective repression of Sp1-mediated activity by Sp3 has been demonstrated to be relevant in cell cycle-regulated promoters containing several Sp1 sites. The dihydrofolate reductase promoter contains four Sp1 sites and is specifically repressed by Sp3 (36). Besides repression by Sp3, other mechanisms probably contribute to repression of the cyclin A1 promoter in G1. Studies have shown that repression of glutamine-rich activators such as Sp1 and NF-Y is the predominant mechanism of cell cycle regulation for several promoters (39, 40). However, none of the known repressor elements (CDE, CHR, E2F) appears to be relevant for the cyclin A1 promoter. Similar to the cyclin A2 gene, two potential E2F sites are downstream of the transcriptional start site of cyclin A1. These E2F sites are not required for repression of cyclin A2 transcription in the G1 phase (15, 28). The introduction of mutations in these sites in the cyclin A1 promoter did not alter cell cycle regulation. Further evidence that these E2F sites are not relevant for cell cycle regulation was shown using a 3' deletion (−190 to +13) that showed cell cycle regulation similar to the constructs containing both E2F sites (data not shown). Likewise, an 8-bp sequence that resembles the CDE of the human cyclin A2 gene was found in an antisense direction at position −19 to −24 (TCGCGG) of the cyclin A1 promoter. No significant differences in cell cycle regulation were found when these nucleotides were mutated (Fig. 9). This is consistent with the finding that these elements need to be in a 5′→3′ orientation to be functional (15, 41, 42). Taken together, our data regarding the mechanisms of cell cycle regulation of the cyclin A1 promoter suggest that cell cycle regulation is accomplished by periodic repression of the Sp1 site mediated activity. Repression is likely to be accomplished by Sp3 and an as yet unidentified repressor mechanism that does not depend on E2F, CDE, or CHR elements.

Besides the Sp1 family members, other mechanisms are likely to be important for expression of cyclin A1 in vivo. So far, the promoter of cyclin A1 showed activity in all the cell lines that we have analyzed including MCF-7, PC3, Cos-7, U937, KCL22, Jurkat, and others. Most of these cells expressed relatively low levels of cyclin A1 mRNA. Three possibilities could explain the discordance between cyclin A1 expression in vivo and promoter activity in vitro. First, regulatory sequences further upstream of the cloned fragment could repress expression in vivo in selected types of tissue. However, negatively regulating sequences are often located within a few hundred bases of the transcription start site as is the case for cyclin A2 (15, 41, 43) including those for many myeloid-specific genes (31, 44). In addition, preliminary data from a transgenic murine model suggest that the cyclin A1 promoter fragment −1299 to +145 is sufficient to direct tissue-specific expression. Second, some genes (e.g. c-myb) that show limited or low levels of expression in vivo are frequently expressed at higher levels in cell lines (45–47). The broad range of expression of such a transcription factor in cell lines could provide an explanation for the prominent promoter activity in cell lines, despite the limited expression pattern in the tissues from which the cell lines were derived. Third, methylation within the CpG island upstream of the start site or variations in the chromatin structure might contribute to the tissue-specific expression of cyclin A1 in vivo.

Taken together, we have cloned and analyzed the genomic organization of the human cyclin A1 gene and its promoter region. The analyses of the promoter region revealed that the cyclin A1 promoter critically depends on four GC boxes upstream of the transcriptional start site. The promoter is cell cycle-regulated with maximum activity in the S and G2/M phases. Our data suggest that cell cycle regulation depends on periodic repression of promoter activity in the G1 phase. The binding of both Sp1 and Sp3 implicates Sp1 family members in the regulation of tissue-specific and periodic expression of the cyclin A1 gene.

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