Cell Cycle Regulation of the Human Polo-like Kinase (PLK) Promoter

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Plk (polo-like kinase) is a serine-threonine kinase that appears to function in mitotic control in mammalian cells. We demonstrated previously that PLK mRNA expression is low at the G1-S transition, increases during S phase, and is maximally expressed during G2-M. In the present study, we have cloned the human PLK gene and analyzed the structure and function of 2 kilobases of its 5'-flanking region. Using synchronized cultures of HeLa cells transfected with PLK promoter/luciferase constructs, we show that the promoter of PLK is activated at S phase and is maximal at G2-M phase. Using various PLK promoter/luciferase constructs, we show that three activating regions are located between 35 and 93 base pairs upstream of the transcription initiation site. We identified a repressor element (CDE/CHR) in the region of the transcription start site, and mutations within this element diminished cell cycle regulation of transcription.

The data accumulated during the last few years have clearly demonstrated that the cyclin-dependent kinases regulate many important functions during cell cycle progression, and new cyclin-dependent kinases continue to be identified. However, it has become evident that cell cycle progression also involves the activities of a variety of cyclin-independent kinases, some of which regulate cyclin-dependent kinase activity and some with activities entirely unrelated to cyclin-dependent kinase functions.

Recently, a family of cell cycle-regulated, cyclin-independent protein kinases, known as polo-related kinases, has been identified. Polo, the prototype enzyme, was originally identified in Drosophila as a cell cycle gene with an essential function during G2-M (1). Mutant alleles of the polo gene caused formation of monopolar and multipolar mitotic spindles and abnormal segregation of chromosomes. In Saccharomyces cerevisiae, mutant alleles of the polo homologue CDC5 cause impaired spindle formation, whereas mutations in the Shizosaccharomyces pombe polo homologue Plp1 caused failure both in the formation of the F-actin ring during cytokinesis and in deposition of septal material (2, 3). We and others have cloned a putative mammalian polo homologue, polo-like kinase (PLK) (4-8).

The kinetics of Plk kinase activation (9), its localization at the spindle poles and postmitotic midbody (10), and its association with CHO1/MKLP-1 (11) suggest that Plk, like polo, CDC5, and Plp1, is involved in chromosome segregation.

It has been shown previously that steady-state PLK message and protein levels are coordinately regulated, steadily rising from a low in G1 to a peak during G2-M (9, 11). Lake and Jelinek (8) suggested that posttranscriptional regulation is responsible for the cell cycle-associated fluctuations in PLK mRNA abundance based on the results of nuclear run-off experiments. More recently, Zwicker (12) showed that S-G2-specific transcription of cdc25C, cdc2, and cyclin A is regulated by two repressor elements known as CDE (cell cycle-dependent element) and CHR (cell cycle gene homology region). Mutation of the CDE and CHR elements in these genes allowed elevated transcription during G2 and consequent loss of cell cycle-regulated expression. Several cell cycle genes expressed at the G1-S transition are regulated by members of the E2F/DP family (13, 14). The E2F/DP transcription factors are repressed in G1, due to their association with RB family members. In late G1, transcription of E2F-regulated genes such as DHPR, E2F-1, and DNA polymerase is activated following dissociation of E2F/DP complexes from RB (15-17). Similarly, transcription of cdc2 is repressed during G1 by binding of E2F-4-p130 complexes at the CHR/CEDE region (18).

To investigate the mechanisms regulating fluctuations in steady-state PLK message, we have cloned the 5' region of the human PLK gene and analyzed its functions. Here we show that PLK expression is transcriptionally regulated in a cell cycle-dependent manner. This transcriptional regulation is achieved through interactions between repressor regions and at least three activating regions.

MATERIALS AND METHODS

Cell Culture and Transient DNA Transfections—NIH3T3 and HeLa cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin. For transient transfections, 3 x 10⁵ cells/dish (60-mm diameter) were plated 24 h prior to transfection. Cells were then transfected with 5 μg of various luciferase gene constructs using liposome-mediated N-[1-(2,3-dioleoyl)propyl-N,N,N-trimethylammoniummethyl sulfate reagent as described by the manufacturer (Boehringer Mannheim). After 6 h, the transfection media was replaced with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum for 48 h. Cells were then harvested using Reporter lysis buffer (Promega Corp.). For synchronization, cells were treated with mimosine (300 μM), thymidine (2 mM) or nocodazole (100 ng/ml) for 24 h to arrest them at G1, G1-S, or prometaphase, respectively.

Isolation of Stable Transfectants—HeLa cells were transfected with PLK/luciferase constructs using N-[1-(2,3-dioleoyl)propyl-N,N,N-trimethylammoniummethyl sulfate reagent, and then a mixture of reporter plasmid (10 μg) and pRSV-neo (0.5 μg) was added. After 8 h, the medium was replaced with fresh medium. Following incubation for 24 h, the cells were incubated in selection medium containing 0.8 mg/ml

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Geneticin (G418) (Life Technologies, Inc.). Growing colonies (20–30 per 100 cells) were cloned, expanded, and tested for luciferase activity.

Synchronization and Cell Cycle Analysis—Untransfected and stably transfected HeLa cells were grown on 100-mm dishes to 80% confluence and synchronized at the G1-S boundary by a double thymidine block protocol (Fig. 1A). Cell cycle analysis, luciferase assays, and Northern blot analysis. For cell cycle analysis, cells were harvested, washed in ice-cold phosphate-buffered saline, and lysed in 135 mM NaCl, 5 mM KCl, 440 mM KH2PO4, 335 mM NaNH2PO4, 1 mM CaCl2, 50 mM MgCl2, 400 mM MgSO4, 240 mM Na3PO4, 0.02% w/v Nonidet P-40. Cells were then treated with RNase, and cellular DNA was stained with propidium iodide. Cell cycle determination was performed using a Becton-Dickinson fluorescence-activated cell analyzer, and data were interpreted using the SIFT model program provided by the manufacturer.

Northern Blot Analysis—Northern blot analysis was performed with 20 μg of total RNA prepared with RNeasy spin columns (Qiagen). After transfer to Hybond-N membrane (Oncor) and UV cross-linking, the blot was hybridized with human PLK cDNA probe generated by random primer labeling (Amersham Corp.). After hybridization, the blots were washed twice at 42 °C in washing buffer 1 (2 × SSC, 0.1% SDS) and twice in washing buffer 2 (0.2 × SSC, 0.1% SDS), and exposed to Kodak x-ray film.

Library Screening and Sequencing—A genomic library of the human fibroblast cell line was screened with a 5′-terminal probe (100 bp) derived from the human PLK cDNA sequence. Prehybridization and hybridization were performed as described previously (6). A PsiI fragment of 2 kb and an EcoRI overlapping fragment of 3 kb were subcloned into pBluescript, and the nucleotide sequence was determined by the dideoxy termination method (Sequenase; U. S. Biochemical Corp.) using synthetic primers as described previously (6).

Luciferase Assays—Cells were lysed in 200 μl of 25 mM Tris-phosphate buffer, pH 7.5, containing 1% Triton X-100. Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min). Soluble extracts were prepared by centrifugation (14,000 × g for 10 min).

Polymerase Chain Reaction (PCR)—PCR reactions contained 1 ng of total RNA prepared with RNeasy spin columns (Qiagen). After transfer to Hybond-N membrane (Oncor) and UV cross-linking, the blot was hybridized with human PLK cDNA probe generated by random primer labeling (Amersham Corp.). After hybridization, the blots were washed twice at 42 °C in washing buffer 1 (2 × SSC, 0.1% SDS) and twice in washing buffer 2 (0.2 × SSC, 0.1% SDS), and exposed to Kodak x-ray film.

PLK Promoter Fusion Construction and Mutant Derivatives—Inserts were cloned into the multiple cloning site of the pET-SacI site of pGAL2-GUS (Promega Corp.), and the nucleotide sequence was determined by the dideoxy termination method (Sequenase; U. S. Biochemical Corp.) using synthetic primers as described previously (6).

Luciferase Assays—Cells were lysed in 200 μl of 25 mM Tris-phosphate buffer, pH 7.5, containing 1% Triton X-100. Soluble extracts were prepared by centrifugation (14,000 × g for 15 s). Protein concentrations were determined with a protein assay kit (Bio-Rad), and concentrations were normalized. Luciferase assays were performed with luciferase assay reagent (Promega Corp.), and light intensity was measured for 10 s on a Berthold LB9510 luminometer. The luciferase assay results shown in the figures are representative of at least three independent experiments.

Primer Extension Analysis—Ten pmol of antisense oligonucleotide 5′-21 bp relative to the translation start site was end labeled with [32P]ATP. The labeled primer and HeLa poly(A) RNA or poly(U) were denatured for 10 min at 65 °C and then incubated at 42 °C overnight. Primer extension was carried out in a total volume of 20 μl containing 50 mM Tris, pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM MgCl2, 400 μM deoxynucleotide triphosphates, 2 units RNasin, and M-MuLV reverse transcriptase (Promega). After incubation for 30 min at 42 °C, the reaction was stopped with EDTA and purified with phenol-chloroform isoamyl alcohol. The resulting cDNA product was then separated by electrophoresis on a 6% polyacrylamide sequencing gel containing 8 M urea.

Transcriptional Regulation of the PLK Promoter

We first examined the expression of PLK mRNA by Northern blot analysis of synchronized cell populations. HeLa cells were synchronized at the G1-S boundary by double thymidine block and harvested at various times after release. Flow cytometric analysis was performed on propidium iodide-stained cells to determine DNA content as a control for synchrony.

HeLa cells entered S phase immediately after release from thymidine, were predominantly in G2 at 8 h, and had the highest mitotic index at 10–12 h (Fig. 1C). Northern blots were hybridized with PLK probes (Fig. 1A). PLK mRNA accumulates in S phase, peaks in M phase (10–12 h after release from the G1-S block), and decreases as cells enter G1 phase, 14–18 h after release. Levels of PLK mRNA varied 6–8-fold during the cell cycle (Fig. 1A). To confirm the drop in PLK message during G1, we examined PLK expression after release from a nocodazole-induced prometaphase block. HeLa cells synchronized with nocodazole (16 h) were released from nocodazole and harvested at indicated times. The level of PLK mRNA decreased after release of block and reached its nadir at 8 h (Fig. 1B). Flow cytometric analysis showed that 90% of cells arrested by no-
Further upstream at -70 is a GC stretch containing a putative SP1 consensus element. A c-myc binding site is located at -160.

The transcription initiation site was located using a primer extension assay. Using $^{32}$P-labeled oligonucleotide and 5 μg poly(A) RNA from HeLa cells, one major band was found 78 bp upstream of the ATG codon (Fig. 3). No primer-extended product was seen using tRNA as control. Previously, Brauninger et al. (21) reported that the human PLK gene contains three potential transcription initiation sites. The site we have identified corresponds to one of the three sites they identified, but we have found no indication of any other sites. This difference may be cell type-specific and was not further investigated.

Promoter Activity of the 5’ Upstream Region of PLK—To determine the upstream sequences required for promoter activity, we cloned the 5’ upstream sequences of the PLK gene into a Luciferase reporter vector, pGL2-Basic (Fig. 4A). The plasmid containing the 5’ upstream sequences was then transfected into HeLa cells using liposome reagent. To identify functional elements, a series of deletion mutants of the fragment were examined for transcriptional activity. Fragments deleted up to nucleotide position -93 exhibited full promoter activity (Fig. 4B). The -65 (pGL-65), -35 (pGL-35), and -19 (pGL-19) deletion mutants exhibited approximately 40, 4, and 0.5% activity with respect to -93 (pGL-93). These results indicate that the -93 to +63 fragment of the PLK promoter has full promoter activity and that there are at least two positive regulatory regions (-93 to -65 and -65 to -35) in the PLK promoter.

PLK Transcription Is Regulated during the Cell Cycle—To examine the cell cycle-regulated activity of the PLK promoter, PLK promoter-luciferase constructs (pGL-815, pGL-389, pGL-195, and pGL-93) that have full promoter activity were transfected into HeLa cells, and luciferase activity was assessed in cultures synchronized by a thymidine block and release. After release from the thymidine block, cells were harvested at indicated times (Fig. 5). Luciferase activity was measured and normalized for protein content. The cells were predominantly in G1-S at the start of the experiment. At this point, the level of luciferase activity was low. As the cells entered S phase, luciferase activity rose and peaked between 10 and 12 h after release, when the majority of cells were in G2-M. After 15 h, when the majority of cells entered G1, luciferase activity had substantially decreased. At 24 h after release, luciferase activity had fallen to slightly below the levels detected at the start of the experiment. The lowest level of luciferase activity was detected in G2, an increased level in S phase, and the maximal level in G1-M, corresponding to a pattern paralleling the level of PLK mRNA in cycling cells.

We next examined luciferase activity in extracts made from cells transfected with various constructs (pGL-815, pGL-165, pGL-148, pGL-93, pGL-65, and pGL-19) and blocked at different points in the cell cycle. The transfected cells were treated with mimosine, thymidine, or nocodazole to arrest them in G1, G1-S or G2-M (prometaphase) phase, respectively, and flow cytometry was used to confirm the arrest points (data not shown). The arrested cells were harvested and assayed for luciferase activity (Fig. 6). The cells transfected with the four largest promoter fragments had 5–6-fold greater luciferase activity when arrested in G2-M (prometaphase) than cells arrested in G1 or at G1-S. The pGL-65 construct demonstrated about 50% of the promoter activity compared with pGL-93 when arrested at G1 and a 3–4-fold increase at G2-M (prometaphase). pGL2-Basic (data not shown) and pGL-19 had essentially no activity in transfected cells in any phase of the cell cycle. Thus, experiments using either double thymidine block and release, or simple overnight block with mimosine, thymi-
dine, or nocodazole, produced comparable results in the transiently transfected cells and indicated that the PLK promoter is cell cycle-regulated.

To confirm the results obtained from transient transfections, we established six cell lines that stably expressed four different promoter/luciferase constructs (pGL-815, pGL-241, pGL-195, and pGL-93) (Fig. 7A). These cell lines were synchronized at the G1-S boundary by double thymidine block and harvested at time intervals after release (Fig. 7B). Luciferase activity rose as the cells entered S phase and peaked between 10 and 12 h after release when the majority of cells were in G2-M. After 15 h, when the majority of cells entered G1, luciferase activity dropped. By 24 h after release, luciferase activity had decreased to approximately the same levels detected prior to release from the G1-S block.

Levels of PLK promoter activities varied 4–5-fold during the cell cycle. A control SV40 promoter enhancer construct (pGL-control) showed minimal differences between the mimosine- and nocodazole-blocked cell extracts (data not shown). Both the timing of PLK promoter activity and induction levels in the...
stable lines were essentially identical with the results obtained in transiently transfected cells. The results of the experiment in Fig. 7 confirm that the changes in luciferase activity during the time course are not simply due to elevation and subsequent decrease after transient transfections.

Identification of a Promoter Fragment Conferring Activation of the PLK Promoter—The studies described above indicate that the basic PLK promoter is present in the region downstream from −93. This region includes a GC stretch region containing a consensus SP1 site and a CCAAT box. To identify the sequences within this region responsible for conferring the cell cycle-regulated transcription of PLK, we generated a series of truncated or mutated plasmids spanning the PLK promoter (Fig. 8A). To assess the activity of the GC stretch (SP1) site and CCAAT box sequences, we introduced substitutional mutations in the sequences. GGCGGG was converted to GAATGG (pGL-93, M1), and CCAATCAG was converted to CAGCTCAG or CCAATTCA (pGL-93, M2 and pGL-93, M3, respectively). These constructs were subcloned into pGL-Basic and were transfected into HeLa cells. Transfected cells were then blocked with either mimosine or nocodazole.

We identified at least three positive regulatory elements in the PLK promoter. The first spans nucleotides −93 to −75 and has about 40% of the G₁ activity of the pGL-93 construct (Fig. 8B). Truncation of the SP-1 site in construct pGL-65 resulted in a decrease to about 20% of the G₁ activity of pGL-93, whereas the larger deletion in pGL-45 caused no additional effect. The second region spanning nucleotides −65 to −75 contains a portion of the 17-bp GC stretch sequence (GGCGGGG-GCGGGGGGG) containing a potential SP1 site (underlined). Mutation of the SP1 site (pGL-93, M1) did not reduce G₁ or G₀-M phase promoter activity. These results suggest that the GC stretch region is involved in transcriptional activation, but that the putative Sp1 site contained within it is not.

The third element spans nucleotides −45 to −33 and has about 30% of the G₁ activity of the pGL-93 construct (Fig. 8B).
Taking into account the lack of basal promoter activity with pGL-35 and pGL-19, or in mutants of pGL-65 (pGL-65, M2 or pGL-65, M3) which contain the putative CCAAT box, the results suggest that the CCAAT box binding factor NF-Y is likely important for \(PLK\) promoter activity.

Identification of Cell Cycle-regulated Repressor Elements in the \(PLK\) Promoter—Zwicker et al. (12, 22) reported recently that transcription of \(S_G2\)-specific genes such as \(cdc25C\), \(cdc2\), and \(cyclin A\) are mediated by a cell cycle-regulated repressor element (CDE) and a cell cycle gene homology region (CHR). An alignment of the \(PLK\) promoter sequence with the CDE and CHR of these \(S_G2\)-specific genes shows that the \(PLK\) promoter sequence is perfectly matched with the CHR consensus sequence (Fig. 9). Three base pairs at the center of a putative \(PLK\) CDE region (GCG) are identical with the consensus sequence, but the perimeter is different. However, the spacing between these two putative elements in \(PLK\) and their locations within the promoter region are very similar to that reported for the other genes. These results suggest that the putative CDE and CHR region of \(PLK\) function as a \(G_1\)-specific repressor as they do in other \(S_G2\)-specific genes. To confirm the repressor function of these elements, we performed mutagenesis of CDE and CHR in the \(PLK\) promoter-luciferase construct pGL-93 (Fig. 10A). Wild-type and two mutant constructs were transiently transfected into HeLa cells, and luciferase activity was determined in cells arrested with mimosine (G1) or nocodazole (prometaphase) for 16 h (Fig. 10B). The wild-type construct showed low activity in extracts from cells in G1, but this activity was elevated approximately 5-fold in extracts from prometaphase cells. The CHR mutant constructs had elevated activity in the G1 extracts but demonstrated only 1.5-fold increases in the prometaphase extracts. In contrast, the CDE mutant constructs caused insignificant elevation in G1 extracts. The CHR mutations decreased the ratio of the promoter activity measured in prometaphase versus G1 cells by approximately 3-fold, whereas the CDE mutations decreased the ratio by less than 2-fold (Fig. 10C). These results indicate that the CHR element in \(PLK\) acts as a \(G_1\)-specific repressor, whereas the CDE-like element in \(PLK\) makes little contribution to cell cycle-specific transcription.

**DISCUSSION**

Several groups have reported that steady-state \(PLK\) message levels vary dramatically during cell cycle progression, from very low or undetectable amounts in \(G_0-G_1\), steadily increasing amounts detected during S phase onward, to a peak at mitosis (5, 8, 11). Recently, Brauninger et al. (21) have analyzed the
structure of both the human and murine PLK promoter regions and have shown that the 5' flanking sequences contain two regions of homology. The largest region extends about 400 bp upstream of the translation initiation site and includes both a CCAAT box and an SP1 site. Deletion of the 3' 112 bp, including the CCAAT box and SP1 site from a human promoter/CAT construct, abolished promoter activity. The second region included 90 bp located 1.8 kb 5' of the core homology region. Deletion of the 5'-most 500 bp, including the conserved 90 bp, reduced the promoter activity of the full 2.3-kb clone by 50%. Their studies thus identified two regions that significantly contributed to basal human PLK promoter activity and showed that these elements are conserved in both the human and mouse genes.

Our work, to the extent that it overlaps with the results of Brauninger et al. (21), is in good agreement with their findings. However, in addition to addressing the question of basal PLK promoter activity, we have examined cell cycle-specific regulation of promoter function and have identified the responsible sequence elements within the core promoter region. The results of our experiments demonstrate that cytoplasmic PLK mRNA levels, like those of several other cell cycle-regulated genes, correlate with the cell cycle. The hypothesis that these fluctuations in mRNA levels are based, at least in part, on promoter function is supported by results from both transient transfections with various promoter/luciferase constructs and by experiments using the stably transfected cell lines. Finally, both the presence in the PLK promoter of CDE and CHR sequences similar to those found in cdc2, cyclin A, and cdc25C promoters (12, 22, 23), and the fact that mutations of the CDE and CHR sequences of PLK caused elevated G1 luciferase activity, also support a role for promoter function in the control of mRNA levels.

Our results and conclusions conflict with the nuclear run-off results of Lake and Jelinek (8), who detected no indication of cell cycle-specific transcriptional regulation of PLK after serum stimulation of starved NIH 3T3 cells. However, in their experimental system, they only tested cells starved for 48 h and at 6 and 24 h following serum stimulation. The first two points comprise cells in G0 and G1 when little PLK mRNA is detected, whereas the 24-h point was expected to be mainly G2-M but could possibly have contained substantial numbers of G1 cells. Thus, the limited nature of the time course they examined may account for the differences between their data and the results presented here.

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The results of our studies support the hypothesis that the increase in PLK mRNA levels seen at G2-M is largely if not entirely due to cell cycle-specific transcriptional regulation. In our experiments using transient transfections and stable cell lines containing the full promoter region, we observed approximately a 5-fold increase of luciferase activity at G2-M phase. We also observed that the PLK promoter sequences between construct, abolished promoter activity. The second region included 90 bp located 1.8 kb 5' of the core homology region. Deletion of the 5'-most 500 bp, including the conserved 90 bp, reduced the promoter activity of the full 2.3-kb clone by 50%. Their studies thus identified two regions that significantly contributed to basal human PLK promoter activity and showed that these elements are conserved in both the human and mouse genes.

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The results of our studies support the hypothesis that the increase in PLK mRNA levels seen at G2-M is largely if not entirely due to cell cycle-specific transcriptional regulation. In our experiments using transient transfections and stable cell lines containing the full promoter region, we observed approximately a 5-fold increase of luciferase activity at G2-M phase. We also observed that the PLK promoter sequences between...
nucleotides −93 and +65 were sufficient to cause similar 5–6-fold induction during G₂-M, which is comparable to the increases in steady-state levels of the endogenous human PLK mRNA.

Using deletion analysis within this region, we have identified elements responsible for transcriptional regulation of the PLK gene. We identified a critical region required for minimal expression of the PLK gene between nucleotide position −35 and −95. This region contains at least three positive regulatory elements, the first with no known consensus site, the second containing a GC-rich element that includes an SP1 site, and the third containing the CAATT box.

The first positive element spans nucleotides −93 to −75 and has about 40% of the activity of the largest PLK construct. As mentioned above, we did not identify any consensus sequences for known transcription factors in this region. However, Tommasi and Pfeifer (18), using genomic footprinting, identified 11 protein binding sites in the cdc2 promoter. Six of these sites correspond to known regulatory elements, including an inverted SP1, inverted CCAAT box, and est-2 and E2F-4 sites. The identities of the other five factors are unknown. We found that the PLK promoter region between −93 to −75 contains a sequence similar to one of the binding sites for unknown factors identified in the cdc2 promoter.

The second positive element contains a putative SP1 site (24). Potential binding sites for SP1 were previously identified in the promoters of cyclin A, cdc2, and cdc25C genes (12, 22). However, we did not detect cell cycle regulated binding to the GC-rich SP1 containing PLK oligonucleotide in electrophoretic mobility shift assays, nor did mutation of the SP1 site block cell cycle regulation of the promoter/luciferase construct (data not shown). Nevertheless, deletion of the entire GC stretch region reduced promoter activity by 20%.

The third positive element found between −45 and −33 contains a CCAAT box (NF-Y binding site) (25–28). This region of the basal promoter has approximately 40% of the activity of the largest PLK construct. (Fig. 4). Mutation of the NF-Y binding site did not abolish cell cycle-regulated transcription (Fig. 5). Also, we did not observe cell cycle-regulated binding to the NF-Y oligonucleotide in electrophoretic mobility shift assay, although we confirmed that NF-Y does bind to the site by supershift analysis (data not shown).

Transcription of the cdc2 gene is controlled by multiple regulatory regions, including upstream activating sequences and one repressing (CDE/CHR) element. The isolated regulatory region of the cdc25C promoter contains SP1 and NF-Y binding sites and CDE and CHR repressor elements (12, 22, 23). The CDE/CHR element of the cdc25C gene inhibits the transcription activity of the SV40 promoter (23), demonstrating that repressor elements that interfere with the basal transcription machinery will affect all promoters.

Plk kinase activity is subject to very tight controls during cell cycle progression. In parallel with increased steady-state message and protein levels, kinase activity rises gradually from S phase to an abrupt peak at M phase that is a consequence of activating phosphorylation on one or more serine residues. At the end of mitosis, Plk protein levels drop with only slightly slower kinetics than cyclin B protein (11).2 The present manuscript demonstrates that cell cycle-specific transcriptional regulation is at least partly responsible for the tight regulation of Plk kinase activity. One area of future interest will be to determine whether specific programs exist to degrade Plk protein at the end of mitosis or if postmitotic loss of the midbody is sufficient to account for the decrease in protein levels.

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