Mitogenic Regulation of p27<sup>Kip1</sup> Gene Is Mediated by AP-1 Transcription Factors<sup>*</sup>

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Ekta Khattar<sup>1</sup> and Vijay Kumar<sup>2</sup>

From the Virology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

The abundance of cyclin-dependent kinase inhibitor p27<sup>Kip1</sup> during the cell cycle determines whether cells will proliferate or become quiescent. Although the post-translational regulation of p27<sup>Kip1</sup> is well established, its transcriptional regulation is poorly understood. Here, we report that mitogenic stimulation of quiescent HEK293 and Huh7 cells showed a rapid decline in the levels of p27<sup>Kip1</sup> transcript by 2.4 ± 0.1-fold. Inhibition of the p27<sup>Kip1</sup> gene in response to mitogens involved transcriptional down-regulation and required newly synthesized protein(s). Mutation of the AP-1 element at position −469 in the human p27<sup>Kip1</sup> promoter abrogated the effect of mitogens. The recruitment of the AP-1 complex to the p27<sup>Kip1</sup> promoter was confirmed by in vitro DNA binding and chromatin immunoprecipitation studies. Reporter gene analysis combined with enforced expression of Jun/Fos proteins suggested the involvement of Jun/Fos heterodimer in the transrepression process. Both MAPK and phosphatidylinositol 3-kinase signaling pathways appeared to mediate p27<sup>Kip1</sup> transcription. Furthermore, hepatitis B virus X protein-mediated down-regulation of p27<sup>Kip1</sup> in a transgenic environment correlated with an increase in c-Fos levels, reiterating the physiological relevance of AP-1 in the transcriptional regulation of p27<sup>Kip1</sup>. Collectively, our studies present the first evidence demonstrating the role of the AP-1 complex in transcriptional down-regulation of the p27<sup>Kip1</sup> gene following mitogenic stimulation.

Cyclin-dependent kinase inhibitors (CKIs)<sup>3</sup> function as brakes for the cell division cycle by inhibiting cyclin/cyclin-dependent kinase complexes. CKIs belong to two different classes, INK4 and CIP/KIP proteins, depending on their sequence homology and mode of action. Although the INK4 proteins (p16<sup>INK4a</sup>, p15<sup>INK4b</sup>, p18<sup>INK4c</sup>, and p19<sup>INK4d</sup>) inhibit the kinase activities of CDK4 and CDK6 by interfering their association with D-type cyclins, the CIP/KIP proteins (p21<sup>CIPI</sup>, p27<sup>Kip1</sup>, and p57<sup>Kip2</sup>) inhibit the activities of cyclin D-, E-, A-, and B-associated cyclin-cyclin-dependent kinase complexes (1). The CIP/KIP proteins share an N-terminal domain that binds the cyclin/cyclin-dependent kinase subunits, but their C-terminal sequences are distinct, leaving them to be diversely regulated.

p27<sup>Kip1</sup> was identified as a CKI in G<sub>1</sub>-arrested cells (2). The p27<sup>Kip1</sup> levels are maximal in the G<sub>0</sub>-G<sub>1</sub> phase and progressively decline in the G<sub>1</sub> phase leading to cell cycle progression from G<sub>1</sub> to S phase (3). A number of post-translational mechanisms are known to control the stability of p27<sup>Kip1</sup> during different phases of the cell cycle. For example, phosphorylation at Tyr-88 by Src tyrosine kinase along with other signals can transform p27<sup>Kip1</sup> from an inhibitor of cyclin E-cyclin-dependent kinase complexes to its substrate (4). Likewise, cyclin E/CDK2 phosphorylates p27<sup>Kip1</sup> at Thr-187 (5). Phosphorylation at various residues targets p27<sup>Kip1</sup> for ubiquitination and proteasomal degradation by SCF<sup>Kip2</sup> ubiquitin ligase during the G<sub>1</sub>-S phase (6). However, phosphorylation at Ser-10 by Mirk/Dyrk kinase provides stability to p27<sup>Kip1</sup> and facilitates its CRM1-dependent nuclear export (7). In addition, some translational control mechanisms are also reported to regulate the levels of p27<sup>Kip1</sup> in cells (8). Mechanisms that regulate the transcription of the p27<sup>Kip1</sup> gene are poorly understood.

Increasing evidence now supports the role of transcriptional mechanisms that might control the levels of p27<sup>Kip1</sup>. The FOXO transcription factors have been shown to activate p27<sup>Kip1</sup> transcription leading to cell cycle arrest (9). Other activators of the p27<sup>Kip1</sup> promoter include Sp1, NF-Y, E2F1, and BRCA1 (10–12). Interestingly, c-Myc, Id3, Hes1, among others are known to inhibit the p27<sup>Kip1</sup> promoter (13–15). Considering the fact that growth factors initiate the cell cycle through destabilization of CKIs, in this study we investigate the role of serum and epidermal growth factor (EGF) on the regulation of the p27<sup>Kip1</sup> promoter in quiescent cells. We found that the AP-1 family of proteins modulated by the MAPK and PI3K signaling pathways are essential for the regulation and maintenance of basal transcriptional activity of the p27<sup>Kip1</sup> gene.

EXPERIMENTAL PROCEDURES

Expression Vectors and Reporter DNA Constructs—Expression constructs for RSV-Jun and c-Fos were kindly provided by Dr. A. Weisz (University of Napoli, Italy), and β-galactosidase plasmid (pCH110) was from GE Healthcare. Construction of the fos-dominant negative mutant (Fos-DN) has been described earlier (16). The full-length human p27<sup>Kip1</sup> luciferase
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reporter construct, p27PF, and its deletion (p27AfIII) construct
were kindly provided by Dr. T. Sakai (10). The luciferase reporter construct driven by a minimal promoter and two copies of 12-O-tetradecanoylphorbol-13-acetate-responsive element (2×TRE luc) was from Dr. M. Karin (17). The p27PF fragment (3.6 kb, XhoI fragment) was recloned in pCAT3 basic vector (Promega) to generate p27-1-CAT. p27-2-CAT was developed by cloning the KpnI-BglII fragment (~570-bp region) from p27AfIII, p27-III-CAT was created by generating a point mutation in the AP-1 element of p27-II-CAT using the QuickChange site-directed mutagenesis kit (Stratagene). The primers used to mutate the AP-1 site (5‘ to 3’) in the p27\(^{kip1}\) promoter are as follows: mutated nucleotides are underlined, forward (F) TTTCTTCTTGGTTCGCTCC and reverse (R) GGGAGGCCAACGGAAAGAAA.

Chemicals, Radiochemicals, and Antibodies—The chemicals and their working concentrations were as follows: PD98059 (20 \(\mu\)M), LY294002 (50 \(\mu\)M), 12-O-tetradecanoylphorbol-13-acetate (100 ng/ml), and EGF (10 ng/ml) were from Calbiochem; 5,6-dichloro-1\(^\beta\)-d-ribofuranosylbenzimidazole (DRB 10 \(\mu\)M), SYBR Green (0.25×), and cycloheximide (15 \(\mu\)g/ml) were from Sigma. \([\gamma^-P]ATP was supplied by PerkinElmer Life Sciences. All restriction enzymes were from New England Biolabs. Antibodies against c-Jun, c-Fos, total ERK, total Akt, p27\(^{kip1}\), pGSK-3\(^\beta\), pElk-1, GAPDH, and siRNAs against human c-Fos, human c-Jun, and control were as previously described (18). Sequences of the oligonucleotides (5‘ to 3’) used for EMSA were as follows: AP-1 consensus, F, CGCTTGATGAGTGCAGGCCGAA, and R, TCTCGGTGACTCATCAGCGG; p27AP-1, F, TTTCTTCTTGGTTCGCTCCTCC, and R, GGGAGGCCAACGGAAAGAAA; and p27AP-1 (mutated), F, TTTCTTCTTGGTTCGCTCCTCC, and R, GGGAGGCCAACGGAAAGAAA.

Chromatin Immunoprecipitation Assay (ChIP)—ChIP was performed as described earlier (21). The immune complexes were captured using protein A-Sepharose beads. After a series of washing steps, the beads were extracted in 500 \(\mu\)l of elution buffer (0.1 M NaHCO\(_3\), 1% SDS) and analyzed by PCR for AP-1 recruitment on the p27\(^{kip1}\) promoter. Primers used for PCR amplification (5‘ to 3’) were as follows: p27 F, CACACATGGATGTCTCTCCCT, and p27 R, GCCCTCTTCGACTCCTCCAAA spanning the region 525 to −24 relative to translation start site on the p27\(^{kip1}\) promoter.

Bioinformatic Analysis—The putative transcription factor binding sites on p27\(^{kip1}\) promoter was searched using TransFac Search software.

X15-myc Transgenic Mouse Model and in Vivo Regulation of p27\(^{kip1}\)—The development of X15-myc transgenic mouse model of hepatocellular carcinoma has been reported earlier (22). Phosphate-buffered saline (1 ml) containing 75 \(\mu\)M PD98059 was injected intravenously into transgenic and control mice 6 h before the sacrifice, following which livers were processed as described below. Injection of phosphate-buffered saline alone (mock injection) served as appropriate negative control. A portion of each liver was kept frozen for both the isolation of total RNA and the preparation of lysates for Western blotting. All experiments were independently repeated at least three times.

RESULTS

Mitogenic Stimulation Results in Transcriptional Repression of p27\(^{kip1}\) Gene—To understand the mechanism of p27\(^{kip1}\) gene regulation, serum-starved quiescent Huh7 cells were
incubated with serum or EGF, and the expression of the p27Kip1 gene was measured both at protein and mRNA levels. Fig. 1A shows that the p27Kip1 protein level was highest in quiescent cells, which gradually declined upon serum/EGF treatment. Analysis by real-time PCR showed that the p27Kip1 mRNA level was highest under the quiescent stage, which declined sharply within 1 h of serum/EGF treatment and was maintained at low levels throughout the period of observation (Fig. 1B). Thus, mitogenic stimulus negatively regulates the levels of p27Kip1 mRNA during the cell cycle.

To establish whether a mitogen-dependent decrease in the mRNA level was due to either destabilization or involved a transcriptional repression mechanism, the level of RNA was analyzed in the presence of DRB, a well-known inhibitor of mRNA synthesis. Treatment of quiescent cells with DRB or DRB along with serum starvation or after serum/EGF stimulation for the indicated times. The p27Kip1 mRNA levels suggesting mitogen-dependent p27Kip1 down-regulation was due to inhibition of mRNA synthesis rather than its destabilization (Fig. 1C). Further abrogation of serum/EGF-dependent effects in the presence of cycloheximide, a protein synthesis inhibitor, suggested the involvement of newly synthesized gene products in p27Kip1 transcriptional inhibition (Fig. 1D).

Transcriptional Repression of p27Kip1 Gene Is Mediated by AP-1 Elements—We used the chloramphenicol acetyltransferase reporter assay to understand the mechanistic details of transcriptional inhibition and/or involvement of repressor(s). Cells transfected with full-length p27Kip1 promoter reporter construct (p27-I-CAT) showed a time-dependent decline in the reporter activity following serum/EGF treatment and suggested that mitogenic effects were correctly reflected in our assays (Fig. 2A). As p27-II-CAT showed a similar reporter activity as compared with the full-length promoter (p > 0.1), it was inferred that the essential transcriptional regulatory elements were present within −571-bp region of the p27Kip1 promoter (Fig. 2B). Furthermore, similar levels of inhibition with p27-II-CAT in the presence of serum/EGF confirmed the presence of regulatory elements within −571-bp region of the p27Kip1 promoter (Fig. 2D).

As this transcriptional repression was observed immediately after mitogen treatment and required newly synthesized proteins, we speculated the involvement of immediate early proteins in this process. The TransFac search analysis of the human p27Kip1 gene promoter (−571 to +3 region) predicted binding sites for several transcription factors, including an
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AP-1 element (5′ CTTCCGTCAGC 3′) at position −469 (Fig. 2C). Interestingly, this element is located upstream of two major transcription start sites (−403 and −153) (23, 24). To test the role of AP-1 site in transrepression, this element was mutated to 5′ CTTCCGTGTCGG 3′ in p27-III-CAT. A significant decline in the reporter activity with p27-III-CAT confirmed the involvement of AP-1 element in the basal transcription of the p27<sup>Kip1</sup> gene (Fig. 2B). Interestingly, p27-III-CAT-transfected quiescent cells did not respond to mitogenic stimulus (Fig. 2E). Thus, the inhibitory effect of growth factors on the p27<sup>Kip1</sup> promoter is conferred by AP-1 elements.

Jun/Fos Heterodimers Inhibit p27<sup>Kip1</sup> Expression—The AP-1 complexes involved in interaction with their cognate elements on the p27<sup>Kip1</sup> promoter were further characterized by EMSA. The nuclear extracts from asynchronously growing HEK293 and Huh7 cells showed two major protein-bound DNA complexes with both consensus and p27<sup>Kip1</sup> promoter-derived AP-1 elements suggesting the involvement of AP-1 proteins in p27<sup>Kip1</sup> gene expression (Fig. 3A). The specificity of this interaction was further confirmed in a competition experiment where 50- and 100-fold molar excess of unlabeled wild type probes competitively displaced the interaction but not the mutated p27AP-1 probe (Fig. 3A, compare lanes 5 and 6 with 3 and 4). The composition of AP-1 proteins in the complexes was determined by supershift assay using c-Jun and c-Fos antibodies. As reported by others (20), two major bands of slow mobility corresponding to Jun/Fos heterodimer and Jun/Jun homodimers were observed (Fig. 3B). As evident from the in vitro DNA binding assay, there was accumulation of Jun/Fos heterodimers following mitogenic stimulation, which correlated with increased levels of Jun and Fos proteins after mitogenic treatment (Fig. 3, C and D).

Taking a cue from the in vitro DNA binding results, the interaction of the AP-1 complexes with its cognate element in the p27<sup>Kip1</sup> promoter was investigated in a chromatin environment. The ChIP assay using anti-c-Jun and anti-c-Fos antibodies suggested that both c-Jun and c-Fos were recruited on the p27<sup>Kip1</sup> promoter in response to mitogen stimulation (Fig. 3E). Furthermore, we observed that RNA polymerase II was constitutively bound to the p27<sup>Kip1</sup> promoter in a transcriptionally repressed state.

**Jun-dependent p27<sup>Kip1</sup> Promoter Activation Can Be Titrated by Fos**—The functional significance of c-Jun and c-Fos binding to the p27<sup>Kip1</sup> promoter was evaluated through enforced expression of c-Jun and c-Fos along with p27-II CAT or 2X-TRE luc reporter plasmids. We observed that overexpression of c-Jun in asynchronously growing HEK293 cells led to a 2–3-fold increase in the reporter activity, although c-Fos alone had no effect (Fig. 4A). Nonetheless, c-Fos significantly inhibited Jun-mediated transactivation possibly through formation of Jun/Fos heterodimers. c-Fos alone did not repress the p27<sup>Kip1</sup> promoter perhaps due to the presence of saturating levels of endogenous c-Fos in serum-fed cycling cells (Fig. 4A). However, overexpression of c-Fos in quiescent cells resulted in a significant repression of the p27<sup>Kip1</sup> promoter (Fig. 4B). The inhibitory effect of AP-1 proteins was also evident from the stimulation of the p27<sup>Kip1</sup> transcription in Fos-DN-expressing cells (Fig. 4C). Interestingly, we also observed the transcriptional down-regulation of p27<sup>Kip1</sup> in nontransformed AML-12 hepatocytes in the presence of serum suggesting a common mitogen response mechanism operational in cells. Furthermore, as expected, this down-regulation was also abrogated by Fos-DN overexpression (Fig. 4D). The functionality of the recombinants c-Jun, c-Fos, and Fos-DN was validated by performing activity assay using 2X-TRE luc reporter construct (Fig. 4E).

Furthermore, siRNA-mediated interference with c-Jun and c-Fos expression abrogated the effect of mitogens on p27<sup>Kip1</sup> at mRNA (Fig. 5A) and protein levels (Fig. 5B). As expected, the control siRNA did not rescue the levels of p27<sup>Kip1</sup> in these experiments. Thus, our data clearly indicated that Jun/Fos heterodimers are required for transcriptional down-regulation of p27<sup>Kip1</sup> following cell cycle entry.

**Mitogenic Signaling Pathways Mediate p27<sup>Kip1</sup> Gene Expression**—Mitogens activate several common signaling events that drive the cells toward proliferation. It has been reported that Raf-MEK-ERK and PI3K-AKT pathways act cooper-
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As we established a strong correlation between AP-1 expression and p27\(^{Kip1}\) transcription, we analyzed the binding activity of Jun and Fos to AP-1 elements of the p27\(^{Kip1}\) promoter in the presence of MAPK and PI3K inhibitors. As shown in Fig. 6D, Jun and Fos binding declined sharply in the presence of PI3K inhibitors. Effectiveness of these pathways was also observed (Fig. 6B). We observed that inhibition of either MAPK or PI3K pathways abrogated the effects of serum/EGF on p27\(^{Kip1}\) expression (Fig. 6A, compare lane 2 with 3 and 4 and lane 6 with 7 and 8). Furthermore, real time PCR analysis of p27\(^{Kip1}\) mRNA in quiescent cells treated with serum/EGF in the presence of LY294002 or PD98059 confirmed the rescue of p27\(^{Kip1}\) expression (Fig. 6C). Interestingly, there was no combinatorial effect of PI3K/MEK inhibitors on the p27\(^{Kip1}\) mRNA level suggesting the independent role of these pathways in the regulation of p27\(^{Kip1}\) promoter (data not shown).

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In Vivo Transcriptional Down-regulation of p27\(^{Kip1}\) by Viral HBx—Next, we investigated the role of AP-1 proteins in the regulation of the p27\(^{Kip1}\) gene in a tumor environment using the HBx-myc mouse model of hepatocellular carcinoma (16, 22). HBx, which is a multifunctional protein involved in transcription, cellular transformation, apoptosis, growth stimulation, among others, also behaves like a growth factor for cells and is also known to destabilize p27\(^{Kip1}\) protein by increasing its proteasomal degradation (18). However, its involvement in the transcriptional regulation of p27\(^{Kip1}\) is not known. We analyzed the p27\(^{Kip1}\) transcript levels in the liver of control and HBx-myc transgenic mice. Interestingly, we observed lower levels of p27\(^{Kip1}\) transcripts in the transgenic samples (Fig. 7A), which were reflected at the pro-
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Cell division cycle is driven by sequential activation of cyclin/cyclin-dependent kinase complexes, and the cyclin/cyclin-dependent kinase activity is controlled by CKI. Thus, regulation of CKI levels may be an important step that controls cell division or cell death. $p27^{kip1}$ is well known to function as a negative regulator of the cell cycle by inhibiting cyclin-dependent kinase rapidly down-regulated in the presence of growth factors such as serum or recombinant EGF. It seems that a similar regulatory mechanism is also operative in nontransformed cells as mitogenic stimulation of AML12 cells, an immortalized mouse hepatocytic cell line, showed a similar decline curve for $p27^{kip1}$ mRNA as observed in case of human hepatoma Huh7 cells. Interestingly, enforced expression of growth-promoting factors like viral oncoprotein HBx and cellular c-Myc in a transgenic environment also resulted in a marked decline in the levels of $p27^{kip1}$ mRNA and protein. Thus, down-regulation of $p27^{kip1}$ levels by growth factors seems to be a built-in mechanism that allows the cell cycle to proceed.

The deletion analysis of the $p27^{kip1}$ promoter suggested the presence of mitogen-responsive element(s) within the −571-bp region, whereas the TransFac analysis identified binding sites for important transcription factors like AP-1, SP1, and NF-Y. Because SP1 and NF-Y are reported to bind constitutively to the $p27^{kip1}$ promoter and stimulate its basal transcription (10), it was unlikely that the two factors would regulate the promoter under mitogenic stimulation. On the other hand, it is well established that AP-1 binding to DNA is rapidly induced by growth factors, cytokines, and oncoproteins resulting in proliferation, differentiation, and/or transformation of cells (33). Besides AP-1 proteins are considered important regulators of early G1 phase in the cell cycle. Although the TransFac search predicted two AP-1-binding sites (at positions −2242 and −2224 and −2202 and −2184).
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FIGURE 7. \textit{In vivo} regulation of the p27\textsuperscript{Kip1} gene in the liver of control and X15-\textit{myc} transgenic mice. A, real time PCR analysis of p27\textsuperscript{Kip1} mRNA expression. B, Western blot (WB) of p27\textsuperscript{Kip1}, c-Jun, c-Fos, HBx, and GAPDH. C, real time PCR analysis of p27\textsuperscript{Kip1} mRNA levels in transgenic mice 6 h post treatment with phosphate-buffered saline (PBS) or PD98059. D, WB of p27\textsuperscript{Kip1}, c-Jun, c-Fos, pERK, total ERK, and GAPDH after treatment as in C. *, statistically significant difference at $p < 0.05$.

Because AP-1 proteins bind to the p27\textsuperscript{Kip1} promoter, the involvement of selective members of AP-1 family was further investigated following their enforced expression. We observed that c-Jun was able to up-regulate p27\textsuperscript{Kip1} expression in serum-fed cycling cells. Although c-Fos had no effect in asynchronous cells, it was able to repress in quiescent cells. Such a differential effect may relate to higher basal AP-1 activity in asynchronous cells that may keep the promoter in a repressed state, although it may be very low in quiescent cells. Thus, in quiescent cells where p27\textsuperscript{Kip1} promoter is active, overexpression of c-Fos could down-regulate the transcription. Similar to this observation, G"uller \textit{et al}. (37) recently reported that Fos overexpression can cause an increase in p27\textsuperscript{Kip1} protein levels but does not affect p27\textsuperscript{Kip1} transcription in immortalized human hepatocytes. However, they found these changes in asynchronously growing cells as also observed by us. This type of Jun/Fos cooperation where Jun behaves as an activator while Fos titrates this activity has been reported earlier in the case of phosphoenolpyruvate carboxykinase gene regulation (38). The regulatory role of AP-1 was further evident from the fact that mitogen-dependent p27\textsuperscript{Kip1} down-regulation could be abrogated by overexpressing Fos-DN or RNA interference against c-Fos and c-Jun by specific siRNAs. Interestingly, Fos-DN was able to significantly up-regulate p27\textsuperscript{Kip1} transcription in response to mitogens suggesting that in the absence of the AP-1 complex other positive regulatory factors might be driving p27\textsuperscript{Kip1} transcription. However, presently it is not clear whether Fos interacts with other transcription factors/repressors to inhibit p27\textsuperscript{Kip1} transcription. It is possible that Fos may bind to such protein(s) in a manner that would prevent subsequent DNA binding or alter the levels or activities of transcription factors available for binding to the p27\textsuperscript{Kip1} promoter. Besides, Fos may also function as an adaptor to modify the function of pre-existing factors.

AP-1 is a well known downstream target of the RAF-MEK-ERK pathway. Therefore, our studies on the inhibition of this pathway on AP-1 binding and regulation of the p27\textsuperscript{Kip1} promoter showed a dramatic rescue of p27\textsuperscript{Kip1} expression with a concomitant reduction in Jun/Fos binding to AP-1 elements. A similar increase in the abundance of p27\textsuperscript{Kip1} mRNA and improved stability of the protein has been reported following pharmacological inhibition of MAPKs (39). PI3K-dependent Akt signaling has also been implicated as a regulator of p27\textsuperscript{Kip1} levels (9). Furthermore, platelet-derived growth factor-dependent DNA synthesis in response to Akt kinase is known to involve the expression of p27\textsuperscript{Kip1} and c-Fos (40). In agreement with this, we observed that PI3K inhibition also abrogated the mitogen-dependent inhibition of p27\textsuperscript{Kip1} transcription. Akt kinase is known to inactivate FOXO transcription factors by nuclear exclusion. Furthermore, overexpression of FOXOs is known to stimulate the p27\textsuperscript{Kip1} promoter to induce growth arrest of cells (9). However, the Akt activation/FOXO inactivation model is unlikely to be applicable here because there are no FOXO-binding sites in the minimal promoter used by us that were responsive to mitogenic stimuli. Besides, this model cannot reconcile for the requirement of \textit{de novo} synthesis of pro-
The viral oncoprotein HBx has been reported to promote cell cycle progression by destabilizing the p27\textsuperscript{kip1} protein (18). Furthermore, it is known to up-regulate c-Fos levels by stabilizing c-Myc (16). To study the involvement of AP-1 and p27\textsuperscript{kip1} in tumorigenesis in the X15-myc transgenic mice, we analyzed the levels of two proteins in the liver of transgenic versus control animals. We observed a significant decline in levels of p27\textsuperscript{kip1} protein and mRNA and a marked increase in the levels of Jun and Fos proteins in the transgenic liver thus reiterating the involvement of Jun/Fos in regulating p27\textsuperscript{kip1} expression. Interestingly, MAPK inhibition resulted in a dramatic increase in p27\textsuperscript{kip1} expression with a concomitant decline in Jun/Fos levels substantiating our earlier findings that AP-1 proteins negatively regulate p27\textsuperscript{kip1} gene expression during cell cycle.

Thus, this study has been able to unveil a novel role of AP-1 in cell cycle progression via regulating the levels of p27\textsuperscript{kip1}. It will be interesting to investigate the role played by other transcription factors during this molecular concert. As AP-1 transcription factors are well known targets in tumor development, it may be a promising target for cancer therapy (41).

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