The Cell Cycle Inhibitor p21waf1 Binds to the myc and cdc25A Promoters upon DNA Damage and Induces Transcriptional Repression*

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Arnaud Vigneron, Julia Cherier, Benjamin Barre, Erick Gamelin, and Olivier Coqueret1
From INSERM U564, Cancer Center Paul Papin, 49033 Angers, France

In addition to its function as a cyclin-dependent kinase (cdk) inhibitor, p21waf1 fulfills additional roles involved in DNA replication and transcriptional regulation that could also contribute to cell cycle arrest. In this study, we have shown that p21waf1 functions as a transcriptional repressor of the myc and cdc25A genes. Ectopic expression of the cell cycle inhibitor down-modulates myc and cdc25A transcription but has no effect on cdk4 levels. Using chromatin immunoprecipitation, we found that p21waf1 is recruited to the promoters of these two genes together with the STAT3 and E2F1 transcription factors. Its presence on DNA is associated with an inhibition of the recruitment of the p300 histone acetylase and with a down-regulation of histone H4 acetylation. The same effect was also observed following DNA damage because topoisomerase inhibitors such as sn38 or doxorubicin also induce the association of p21waf1 with DNA. Following transcriptional repression of the myc and cdc25A genes, cells were arrested in the fraction with 4 N DNA content. By contrast, the expression of these two genes remains elevated in the absence of the cell cycle inhibitor, and p21waf1−/− cells re-replicate their DNA and become polyploid. In light of these results, we propose that p21waf1 simultaneously targets cdk and transcriptional regulators to prevent the expression of oncogenic pathways upon DNA damage.

Cell cycle progression relies on the activation of cyclin-dependent kinases (cdk)s that are controlled in part by cyclins and by two classes of cdk inhibitors that bind to and inactivate these kinases (1). The first class of inhibitors includes the INK4 proteins such as p16, which targets cdk4 and hampers its binding to D-type cyclins. The second class is composed of the Cip/Kip proteins, p21waf1, p27kip1, and p57kip2, which bind to and inhibit all cyclin-Cdk complexes. p21waf1 was originally identified as a transcriptional target of the p53 tumor suppressor gene, a cdk inhibitor and a protein induced upon senescence (2, 3). The essential role of p21waf1 relies upon its well known ability to inhibit cyclin-dependent kinases and DNA replication (4, 5), thereby inducing cell cycle arrest. Gene inactivation studies have also demonstrated essential roles of p21waf1 upon DNA damage, mediating G1 and G2 arrest as well as tetraploidy checkpoints (6, 7).

Besides its classical roles, p21waf1 is also involved in a number of other specific functions that may also contribute to growth arrest. Beyond its involvement with cyclin/cdks, p21waf1 functions as a transcriptional cofactor that regulates the activity of various DNA-binding proteins such as NF-κB, Myc, E2F, STAT3, and estrogen receptor (8–10). Interestingly, through a combined regulation of apoptosis and cell cycle progression, most of these transcription factors participate in cell transformation and induce carcinogenesis when constitutively activated. Given that p21waf1 binds to these transcription factors to regulate their activities, it is tempting to speculate that p21waf1 simultaneously targets growth-promoting genes and cdk activity to induce cell cycle arrest (8).

cDNA microarray analysis has demonstrated that the up-regulation of p21waf1 is correlated with the transcriptional repression of genes involved in cell cycle progression, DNA replication, and mitosis entry. For instance, p21waf1 can inhibit the expression of cdk1 as well as a set of genes involved in mitosis and DNA segregation such as the polo-like kinase I and the topoisomerase IIα (11, 12). In addition, it has been proposed that the cell cycle inhibitor modulates the activity of p300/CBP proteins (13–15). These proteins are essential coactivators that stimulate gene expression through their acetyl transferase activity or through their ability to interact with components of the transcriptional machinery (16, 17). For instance, it has been recently shown on the Wnt4 promoter that p21waf1 prevents the recruitment of p300, causing histone hypoacetylation and transcriptional repression of the Wnt4 gene (18). Upon estradiol signaling, p21waf1 has also been shown to form a ternary complex with estrogen receptor and CBP to regulate the expression of the progesterone receptor (10). Interestingly, a general correlation has been observed between CDE-CHR sequences and the p21waf1 inhibitory effects (19). Cell cycle-dependent element (CDE) and cell cycle gene homology region (CHR) are DNA sequences involved in cell cycle-dependent transcriptional regulation (20). These DNA sequences have been found in some promoters that are inhibited by p21waf1, such as PLKI, cyclin B1, or Topolla. In addition, mutating the CDE-CHR sequences prevents the transcriptional inhibition of PLKI and

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1 To whom correspondence should be addressed: Centre Régional de Lutte Contre le Cancer Paul Papin, INSERM U564, 2 Rue Moll, 49033 Angers, France. Tel.: 33-2-41-35-27-00 (ext. 2564); Fax: 33-2-41-48-31-90; E-mail: olivier.coqueret@univ-angers.fr.

2 The abbreviations used are: cdk, cyclin-dependent kinase; IPTG, isopropyl-1-thio-β-D-galactopyranoside; IL, interleukin; ChIP, chromatin immunoprecipitation; CBP, cAMP-response element-binding protein-binding protein.
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cdk1 by p21<sup>waf1</sup>. Therefore, one can speculate that the effects of p21<sup>waf1</sup> are probably related to the inhibition of the transcription factors that bind to the CDE-CHR sequences. Determining the composition of these complexes and the associated role of CBP/p300 acetylases will be an important step in understanding the transcriptional functions of p21<sup>waf1</sup>. Following mitogenic stimulation of quiescent cells, the Cdc25A phosphatase is activated by DNA-binding proteins involved in cell cycle progression such as Myc, STAT3, or E2F1 (21–23). It has been proposed that Myc binds to the cdc25A promoter to up-regulate its expression, thereby activating the cyclin E-CDK2 complexes. To ascertain the physiological relevance of p21<sup>waf1</sup> transcriptional functions, we characterized the effects of the cell cycle inhibitor on the Myc-cdc25A pathway. Using chromatin immunoprecipitation experiments, we have observed that p21<sup>waf1</sup> is recruited to the myc and cdc25 promoters and that this binding is correlated with the inhibition of p300 recruitment and with the down-regulation of histone H4 acetylation. As a consequence, the ectopic expression of p21<sup>waf1</sup> with an isopropyl-1-thio-β-D-galactopyranoside (IPTG)-inducible vector, induced a down-regulation of the Myc and cdc25A mRNAs. Importantly, this effect was also shown when cells were treated with DNA-damaging drugs, indicating that p21<sup>waf1</sup> not only binds to cyclin-cdk complexes but also to the promoter of cell cycle genes upon DNA damage. We therefore propose that kinase inhibition and transcriptional repression are both necessary for p21<sup>waf1</sup> to prevent cell cycle progression in response to genomic insults.

**MATERIALS AND METHODS**

**Antibodies, Cell Lines, and Cell Stimulation**—Antibodies against STAT3 (C20), E2F1 (C20), p21<sup>WAF1</sup> (C19), c-Myc (N262), and p53 (FL393) were obtained from Santa Cruz Biotechnology. Anti-α-tubulin (T9026) was obtained from Sigma.

**Cell Extracts and Immunoblotting**—All experiments were carried out in a 10-μl volume containing 1× LightCycler Fast-start DNA master SYBR Green 1 (Roche Applied Science) and 4 mM MgCl<sub>2</sub>. Fluorescent products were monitored by real-time PCR using a LightCycler. The PCR reactions were carried out in a 10-μl volume containing 1× LightCycler Fast-start DNA master SYBR Green 1 (Roche Applied Science), 5 pmol for each forward and reverse primer, 2 mM MgCl<sub>2</sub>, and 5 μl of the cDNA diluted 10-fold. After an initial denaturation step at 95 °C for 10 min, each cycle consisted of a denaturation step at 95 °C for 15 s, an annealing step at 55 °C for 11 s, and an elongation step at 72 °C for 22 s. A total of 40 cycles were performed. The fluorescent signal was acquired at the end of each elongation step. A fusion curve was performed at the end of the PCR cycle to determine the specificity of the primers. Data analysis was performed as indicated by Roche Applied Science.
The relative quantification of gene expression was performed using the “Fit Point Method” in the LightCycler software 3.3. Flow Cytometry Analysis—For DNA content analysis, 2 × 10^5 cells were washed twice with phosphate-buffered saline and fixed in 70% ethanol. Cells were treated with 100 units/ml Ribonuclease A for 20 min at 37 °C, resuspended in phosphate-buffered saline containing 50 μg/ml propidium iodide, and immediately analyzed by flow cytometry (BD Biosciences).

**RESULTS**

**p21waf1** Suppresses myc Gene Transcription—In addition to inhibiting cyclin/cdk, p21waf1 participates in several protein-protein interactions to prevent cell cycle progression and DNA replication. In particular, p21waf1 binds to transcription factors and coactivators to regulate their functions (8, 9). An attractive possibility could be that p21waf1 inhibits cell proliferation through transcriptional inhibition of cell cycle genes. To test this hypothesis, the effect of the cell cycle inhibitor was investigated on the expression of myc, a well known inducer of G1–G0 transition. The HT1080 fibrosarcoma cell line used in the present study (p21–9) (11) carries p21waf1 in an IPTG-inducible vector (Fig. 1A, lanes 1–4). Up-regulation of p21waf1 was detected as early as 12 h after IPTG induction and remained constant for the next 48 h. A 24-h stimulation was used for the following experiments. To determine whether p21waf1 regulates myc expression, cells were either maintained in serum (Fig. 1C) or serum starved and stimulated with two different mitogens, IL-6 or 10% serum (Fig. 1, B and D, respectively).

Results presented in Fig. 1B indicate that p21waf1 prevents myc induction in response to IL-6 stimulation. Treatment of cells with IPTG suppressed Myc mRNA as shown by quantitative real-time PCR and protein expression by Western blotting (Fig. 1B, lanes 3–4 and 7–8). In addition, p21waf1 prevented the IL-6-mediated induction of the Myc HBM-Luc promoter (24), confirming its inhibitory functions at the transcriptional level (Fig. 1B, lanes 11 and 12). By contrast, no effect was observed in the absence of cytokine stimulation. Interestingly, the same effects were also observed in growing cells, where p21waf1 was found to

**FIGURE 1.** p21waf1 down-regulates myc expression. A, growing HT1080 cells were left untreated or treated with IPTG (50 μM) for the indicated times. Overexpression of p21waf1 was verified by Western blot analysis. B, HT1080 cells were serum starved, left untreated or treated with IPTG (24 h, 50 μM), and then stimulated with IL-6 (20 ng/ml, last 12 h) as indicated. Total RNA was prepared and Myc mRNA levels were analyzed by real-time PCR (lanes 1–4). The expression of myc was then analyzed by Western Blot, and tubulin expression was monitored as a control (lanes 5–8). In parallel, cells were transfected with the HBM-Luc reporter gene (5 μg), starved, and treated or not with IPTG for 24 h, in the presence or absence of IL-6 for the last 12 h. Cytoplasmic extracts were then prepared and processed to measure luciferase activity (lanes 9–12); the mean of five transfections ± S.D. is shown. C, asynchronously growing HT1080 cells were either left untreated or treated with IPTG (24 h, 50 μM), and the expression of myc was analyzed as described in panel A by real-time PCR (lanes 1–2) or Western Blot (lanes 3–4). D, HT1080 cells were serum starved for 2 days, left untreated or treated with IPTG (24 h, 50 μM), and then stimulated or not with 10% serum for the last 12 h. The expression of myc was analyzed by real-time PCR (lanes 1–4), Western blot (lanes 5–8), or reporter gene experiments (lanes 9–12); the mean of five transfections ± S.D. is shown.

using the “Fit Point Method” in the LightCycler software 3.3. The relative quantification of gene expression was performed using the comparative C_{T} method, with normalization of the target gene to the endogenous housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The induction factor was determined for the three reverse transcriptions, and an average induction factor was then calculated.
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One proposed mechanism whereby p21<sup>waf1</sup> can specifically regulate transcription is through physical association with transcription factors that could provide a bridge with the initiation complex. We and others have previously shown that p21<sup>waf1</sup> interacts with E2F1 or STAT3 to prevent their transcriptional activity (25, 26). To assess whether p21<sup>waf1</sup> binds to the myc proximal promoter in association with these two transcription factors, ChIP experiments were performed following IL-6 stimulation or in growing cells. Upon cytokine stimulation, results demonstrated binding of endogenous STAT3 to the proximal Myc promoter, whereas no binding was detected in the control region (Fig. 2A, lanes 1–4 and data not shown). In parallel, the ChIPs assay also showed that p21<sup>waf1</sup> binds specifically to this region of the myc gene upon IPTG induction (Fig. 2A, lanes 5–8). As expected, STAT3 DNA binding was associated with the recruitment of p300 and with histone H4 acetylation on the myc proximal promoter (Fig. 2B, lanes 2 and 6). ChIP assays showed a significant down-modulation of p300 binding as well as a reduction of H4 acetylation upon p21<sup>waf1</sup> induction (Fig. 2B, compare lanes 2, 4 and 6, 8).

The same effects were also observed in growing cells, where p21<sup>waf1</sup> was also found to inhibit myc expression (Fig. 1C). Under these conditions, p21<sup>waf1</sup> bound to the myc promoter upon IPTG induction (Fig. 2C, lanes 7 and 8). Confirming the above results, down-modulation of p300 binding as well as inhibition of histone H4 acetylation in p21<sup>waf1</sup>-expressing cells was shown. As a consequence, the association of the elongating form of the RNA polymerase with DNA was also inhibited (Fig. 2D, lanes 1–8). In growing cells, only E2F-1 was recruited to the myc promoter because neither STAT3 nor ets1/2 was detected on DNA (Fig. 2C, lanes 1–6).

We also observed that the DNA binding activities of E2F-1 and STAT3 were unaffected by increased p21<sup>waf1</sup> expression (Fig. 2A, lane 4, and 2C, lane 2). To confirm that p21<sup>waf1</sup> down-

Inhibit the expression of the steady-state level of Myc mRNA and its corresponding protein (Fig. 1C). The same experiments were also performed using serum-starved cells that were restimulated with 10% serum (Fig. 1D). In contrast, p21<sup>waf1</sup> had no effect on myc mRNA (Fig. 1D, lanes 1–4), protein (lanes 5–8), or promoter (lanes 9–12) under these conditions, further confirming the specificity of its transcriptional functions.
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regulates transcription through histone deacetylation, cells were pretreated with trichostatin A, a histone deacetylase inhibitor. Under these conditions, the ability of \( p21^{\text{waf1}} \) to down-modulate endogenous Myc mRNA expression was inhibited. This effect was observed in growing cells or upon cytokine stimulation (Fig. 2E, lanes 3–4 and 7–8).

Transcriptional Regulation of myc Target Genes by \( p21^{\text{waf1}} \).—Having shown that \( p21^{\text{waf1}} \) down-regulates Myc expression, we investigated whether the cell cycle inhibitor also prevents the expression of Myc target genes involved in cell cycle progression. To this end, we focused on the expression of \( \text{cdk4} \) and \( \text{cdc25A} \), two genes involved in S-phase progression and previously shown to be directly regulated by Myc (21, 22, 27). Results presented in Fig. 3A indicate that the up-regulation of \( p21^{\text{waf1}} \) effectively prevents the expression of \( \text{cdc25A} \) mRNA. As a control, Myc steady-state levels were also inhibited under these conditions (Fig. 3A, lanes 1–4). Surprisingly, \( p21^{\text{waf1}} \) did not affect the expression of \( \text{cdk4} \) (Fig. 3A, lanes 5 and 6). It has been shown previously that Myc and E2F1 regulate the expression of \( \text{cdc25A} \) gene (21–23). Using ChIP experiments, we observed that the two transcription factors were present on the \( \text{cdc25A} \) promoter in growing cells (Fig. 3B and 3C, lane 1). As expected, p300 binding and histone H4 acetylation were also detected (Fig. 3C, lanes 3 and 5). Upon IPTG addition, \( p21^{\text{waf1}} \) was recruited to the \( \text{cdc25A} \) promoter, probably in association with E2F1, still bound on the DNA (Fig. 3B, lanes 3 and 4). Down-modulation of p300 binding and inhibition of histone H4 acetylation were also observed in the presence of the cell cycle inhibitor (Fig. 3C, lanes 4 and 6).

As stated above, there was no significant effect of \( p21^{\text{waf1}} \) on \( \text{cdk4} \) expression. In addition, we were unable to detect any association of E2F1 with the \( \text{cdk4} \) promoter (data not shown). To determine whether \( p21^{\text{waf1}} \) was recruited to the \( \text{cdk4} \) promoter, we then used real-time PCR to compare by a quantitative assessment the ChIP signals obtained on the myc, \( \text{cdc25A} \), and \( \text{cdk4} \) promoters (Fig. 3D). As expected, \( p21^{\text{waf1}} \) was not recruited to DNA in the absence of IPTG, but real-time
PCR analysis indicated that the cell cycle inhibitor could be found associated with the myc and cdc25A promoters upon induction. By contrast, p21<sup>waf1</sup> was not detected on the cdk4 promoter (Fig. 3D). As a control, no amplification was detected in the presence of a control IgG antibody or when immunoprecipitations were performed in the presence of the p21<sup>waf1</sup> immunogenic peptide. In addition, we observed that despite Myc down-regulation, residual levels of the transcription factor were still found associated with the cdk4 promoter upon IPTG addition (Fig. 3E, lanes 3 and 4). Accordingly, p300 binding and histone H4 acetylation were not affected by the up-regulation of the cell cycle inhibitor (Fig. 3E). This indicates that the transcriptional effects of p21<sup>waf1</sup> are specific to the Myc-cdc25A pathway and do not affect the expression of cdk4.

**p21<sup>waf1</sup> Binds to the myc and cdc25A Promoters upon DNA Damage**—We then studied whether p21<sup>waf1</sup> also binds to the myc and cdc25A promoters upon DNA damage. To this end, cells were exposed to moderate doses of two topoisomerase I/II inhibitors, sn38 (the active metabolite of irinotecan) or doxorubicin, two genotoxic drugs that induce the expression of p21<sup>waf1</sup> (Fig. 4A, lanes 1–3). Using real-time PCR analysis, we observed that the steady-state levels of the Myc and cdc25A mRNAs in growing cells were inhibited upon drug treatment (Fig. 4B, lanes 1–4 and 5–8). As expected, the same effect was also observed when IPTG was used as a control to up-regulate p21<sup>waf1</sup>. Western blot analysis also confirmed that these treatments induce the down-regulation of the Myc and cdc25A proteins (Fig. 4B, lanes 9–12). Note that the two drugs induce the expression of the cell cycle inhibitor to the same extent as IPTG.

Importantly, ChIP experiments indicated that both drugs induced a significant association of p21<sup>waf1</sup> with the myc and cdc25A promoters (Fig. 4C and 4D, lanes 4–6). Confirming the above results, we also observed that the recruitment of p21<sup>waf1</sup> to DNA was associated with a down-modulation of p300 and RNA polymerase binding to the myc gene (Fig. 4C, lanes 7–9 and 10–12). On the cdc25A promoter, the loading of the cell cycle inhibitor was also correlated with an inhibition of Myc and p300 binding. In addition, p21<sup>waf1</sup> prevented the elongating form of the polymerase from reaching the 3' part of the gene (Fig. 4D, lanes 7–9, 10–12, and 13–15).

To confirm this result, we then used the human colorectal cancer cell line HCT116 and its p21<sup>−/−</sup> derivative cell line in which both p21<sup>waf1</sup> alleles have been deleted by homologous recombination (6). Whereas sn38 and doxorubicin reduced cdc25A mRNAs in parental cells, real-time PCR showed that this down-regulation was not observed in HCT116 p21<sup>−/−</sup> cells (Fig. 5A, lanes 1–6). Interestingly, Myc was only partially repressed in the absence of p21<sup>waf1</sup> (Fig. 5A, lanes 7–12). It has been recently demonstrated that p53 binds to the Myc promoter to repress its expression (28).
**p21<sup>waf1</sup> Binds to the myc and cdc25A Promoters**

**FIGURE 5. Regulation of myc and cdc25A expression in cells lacking p21<sup>waf1</sup>.**

A, HCT116 wild-type cells or their p21<sup>−/−</sup> derivative were treated with SN38 (5 ng/ml) or doxorubicin (30 nm) for 36 h, and the expression of the cdc25A (lanes 1–6) and myc (lanes 7–12) mRNAs was analyzed by real-time PCR. B, soluble chromatin was prepared from asynchronously growing HT1080 cells treated or not with SN38 (5 ng/ml) or doxorubicin (30 nm) for 36 h. ChIP experiments were performed to analyze the recruitment of p21<sup>waf1</sup> and p53 to the proximal myc promoter in parental (lanes 1–6) or p21<sup>−/−</sup> cells (lanes 7–12).

Using ChIP experiments, we effectively found in wild-type or p21<sup>−/−</sup> cells that p53 was recruited to the Myc promoter upon drug treatment (Fig. 5B, lanes 1–3 and 7–9). Although additional studies are required to confirm these findings, these results suggest that the residual repression of Myc in p21<sup>−/−</sup> cells was probably due to p53 binding.

**p21<sup>waf1</sup> Prevents Aneuploidy upon Genotoxic Treatment—**

The effect of doxorubicin was also studied on HCT116 wild-type or p21<sup>−/−</sup> cells. To this end, cells were synchronized in G<sub>1</sub>/S with hydroxyurea, released, and further treated with 30 nm doxorubicin for 15–48 h. This treatment is well known to induce the expression of p21<sup>waf1</sup> in HCT116 cells, which remained growth arrested with a 4 N DNA content (Fig. 6A and 6B, top panel) (6, 7). As previously shown (29), most of the p21<sup>waf1</sup><sup>−/−</sup> cells started to float and died by apoptosis under these conditions (data not shown). However, 30 h after synchronization, a substantial fraction of these cells remained attached to the plastic dish and became polyploid (30–40%). By 30 h, 44% of these surviving cells were detected with a DNA content between 4 and 8 N (Fig. 6B, bottom panel), suggesting that these cells re-replicate their DNA. Accordingly, a significant expression of the Myc and cdc25A mRNAs was detected in this attached p21<sup>−/−</sup> subpopulation. By contrast, most of the HCT116 control cells were still arrested within 48 h in the fraction with 4 N DNA content (Fig. 4B, top panel). Additionally, we were not able to detect the expression of myc and cdc25A in these cells even after 48 h. Although further experiments are needed to fully demonstrate this point, these results suggest that the presence of p21<sup>waf1</sup> prevents polyploidization through the down-regulation of cell cycle genes.

**DISCUSSION**

Cell cycle progression relies on the activation of cyclins and cyclin-dependent kinases (cdk) that successively act in G<sub>1</sub> to initiate S-phase and in G<sub>2</sub> to initiate mitosis. To prevent abnormal proliferation, cyclin-cdk complexes are precisely regulated by cell cycle inhibitors that block their catalytic activity. Among
These inhibitors, p21^waf1^ is induced by p53-dependent and -independent mechanisms to bind all cyclin-cdk complexes and prevent cell cycle progression. This protein is therefore well known to inhibit E2F function through cdk2 inhibition and Rb dephosphorylation, but it can also prevent DNA replication through proliferating cell nuclear antigen binding and DNA replication inhibition (2, 4). Besides these classical functions, several studies have shown that p21^waf1^ also interacts with various DNA-binding proteins involved in cell cycle progression. This has led to the hypothesis that p21^waf1^ might simultaneously target cdks and transcriptional regulators to inhibit the expression of growth-promoting genes (8, 9).

In this study, we have shown that p21^waf1^ functions as a transcriptional regulator that physically associates with the promoter of the myc and cdc25A genes. By ChIP assays, we found that p21^waf1^ binds the same region of these promoters as STAT3 or E2F1, consistent with a model whereby these two transcription factors provide a bridging mechanism to targeted promoters (25, 26). Determining whether these two transcription factors are the only targets of p21^waf1^ will be an important issue to resolve. Several cyclin-cdk complexes are involved in transcriptional regulation, and one can speculate that the cell cycle inhibitor might also be recruited to DNA through cyclin-cdk bridges. For instance, the transcriptional functions of p21^waf1^ have been initially correlated to the inhibition of p300-associated cdk2 activity (30). In addition, cdk7 and cdk9 phosphorylate the carboxyl-terminal domain of the large subunit of RNA polymerase, a critical mechanism in the regulation of pre-mRNA elongation (31–33). Importantly, we have recently shown STAT3 regulates the elongation of transcription through its interaction with cdk9 (34). Therefore, these observations suggest that p21^waf1^ might also interact with proximal promoters through cyclin-cdk bridges. Serial ChIP analysis as well as RNA interference should help to evaluate the relative contributions of STAT3, E2F1, and cyclin-cdks in the transcriptional functions of the cell cycle inhibitor.

Although overexpressed p21^waf1^ prevents both Myc and cdc25A mRNA expression, cdk4 levels were unaffected by the cell cycle inhibitor. Accordingly, p21^waf1^ binding does not occur at the cdk4 promoter, indicating that the transcriptional functions of this protein are promoter specific. Although this remains to be fully demonstrated, our results also indicate that p21^waf1^ might prevent the recruitment of the p300 histone acetylase to inhibit histone H4 acetylation. We speculate that this finally prevents the loading of the initiation complex, converting the myc and cdc25A promoters from a transcriptionally active state to an inactive one. Future experiments will determine whether the presence of the cell cycle inhibitor is associated with a decreased accessibility of these promoters through chromatin remodeling.

Through activation and/or repression of its target genes, up-regulation of the Myc oncogene leads to hyperproliferation, tumorigenesis, and genomic instability (35, 36). It is well known that tumor suppressor genes, such as p53, p16^INK4a^, or p19^ARF^, prevent the effects of Myc on cell cycle progression through Rb dephosphorylation and E2F inactivation. However, recent results have also shown that p19^ARF^ binds to the activation domain of Myc to prevent its transcriptional functions (37). As a consequence, the tumor suppressor blocked the ability of Myc to transactivate the telomerase reverse transcriptase promoter. Interestingly, p19^ARF^ does not inhibit the ability of Myc to induce apoptosis or repress transcription, suggesting that its inhibitory functions are directed against growth-promoting genes. In addition, p53 can repress myc expression (28). Chromatin immunoprecipitation experiments have shown that p53 binds to the myc promoter to prevent its expression. This effect is associated with histone H4 deacetylation and recruitment of the mSin3a corepressor. Although this remains to be demonstrated for p16^INK4a^, it therefore appears that p53, p19^ARF^, and p21^waf1^ have transcriptional functions that also control the effect of Myc on tumorigenesis.

Recent results have suggested that the balance between Myc and p21^waf1^ plays an important role in cell cycle regulation. For instance, the cell cycle inhibitor binds to the activation domain of Myc to prevent the formation of the Myc-Max heterodimer and repress its transcriptional activity (38). Conversely, Myc can be recruited to the p21^waf1^ promoter by Myz-1 to inhibit its expression and prevent cell cycle arrest (39, 40). Through recruitment of the Dnmt3a corepressor, the Myc-Myz complex leads to DNA methylation and subsequent silencing of the p21^waf1^ promoter (41). Therefore, it appears that p21^waf1^ can inhibit cell cycle arrest through Myc down-regulation but also that Myc prevents p21^waf1^ expression to induce proliferation. In line with this observation, we have recently shown that the

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**FIGURE 7. Proposed model for the p21-mediated cell cycle arrest upon DNA damage.** Upon DNA damage, p21^waf1^ is induced by p53-dependent mechanisms and binds to cyclin-cdk complexes. As a consequence, Rb is dephosphorylated, and this protein binds to E2F to prevent cell cycle progression. In addition, p21^waf1^ is recruited to the promoters of myc and cdc25A to inhibit their activation. The same effect has also been reported on mitotic genes to prevent inappropriate chromosome segregation upon DNA damage (11, 12).
expression of p21<sup>WAF1</sup> is lost in glioblastoma cells, whereas p21 levels remain unaltered (42).

Recent observations have demonstrated that the up-regulation of p21<sup>WAF1</sup> leads to the down-regulation of multiple genes, most of which are involved in chromatin assembly and mitosis. As illustrated Fig. 7, we now propose that in p53 and p19<sup>ARF</sup>, p21<sup>WAF1</sup> functions as a transcriptional inhibitor of the Myc-cdc25A pathway. Therefore, in addition to its ability to inhibit cyclin-dependent kinases and DNA replication, the transcriptional functions of p21<sup>WAF1</sup> might also play an important role in the control of DNA damage and tumorigenesis.

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