Cell growth control by interferons (IFNs) involves up-regulation of the tumor suppressor interferon regulatory factor 1 (IRF1). To exert its anti-proliferative effects, this factor must ultimately control transcription of several key genes that regulate cell cycle progression. Here we show that the G1/S phase-related cyclin-dependent kinase 2 (CDK2) gene is a novel proliferation-related downstream target of IRF1. We find that IRF1, but not IRF2, IRF3, or IRF7, selectively represses CDK2 gene transcription in a dose- and time-dependent manner. We delineate the IRF1-responsive repressor element between nt −68 to −31 of the CDK2 promoter. For comparison, the tumor suppressor p53 represses CDK2 promoter activity independently of IRF1 through sequences upstream of nt −68, and the CDP/cut/Cux helix-turn-helix motif (HiNF-M), can function in the activation of histone H4 gene transcription at the G1/S phase transition through a phylogenetically conserved cell cycle regulatory element (9–16), which encompasses a canonical IRF consensus sequence (17). A characteristic N-terminal DNA binding domain spanning a winged helix-turn-helix motif (18–20) mediates the interaction of IRF factors with their cognate sites. The C-terminal region supports transcriptional enhancement or repression (21–23). The transcriptional activity of IRF factors is influenced by post-translational modifications, including phosphorylation and acetylations (24–27), as well as by protein/protein interactions with other IRF members and cofactors (1, 10, 28–31). The biological role of IRF factors in cell proliferation is reflected by the observation that forced expression of IRF factors perturbs normal cell growth and differentiation (32–36). Based on our studies that have revealed cell cycle regulatory roles for IRF proteins in control of histone gene expression at the G1/S transition (9, 10, 12), we have proposed that IRF factors may regulate cell proliferation through transcriptional mechanisms that operate parallel to and/or independent of E2F proteins (9, 10, 12, 35).

Cyclin-dependent kinases (CDKs) represent key factors that regulate major transitions during the cell cycle, and their enzymatic activities are controlled by activating cyclins and CDK inhibitors (CDIs) (37, 38). CDK2 is the critical enzyme controlling the G1/S phase transition and is required throughout S phase. Its serine/threonine kinase activity is activated by cyclins E and A and inhibited by the CDIs p21<sub>WAF1/Cip1</sub>, p27<sub>Kip1</sub>, and p50<sub>Kip2</sub>. The cyclin E and cyclin A promoters are controlled by E2F factors (39, 40), whereas the CDIs appear to be regulated independently of E2Fs. The CDK2 promoter is serum-inducible and is known to contain two SP1 binding sites that control basal transcription (41). However, after this initial characterization, the CDK2 promoter has remained unexplored. Because CDK2 activity is required at multiple cell cycle stages, the protein is stringently maintained at constitutive levels throughout the cell cycle. Consequently, the question arises whether control of cell growth may be influenced by anti-proliferative cell-signaling mechanisms that can suppress genetic evidence suggests IRF1, the prototypical member of the IRF class of transcription factors, functions as a tumor suppressor (4, 5) presumably by regulating cell growth-related target genes (6). There are few experimentally validated IRF1 target genes, and only a subset of these may contribute to the cell growth inhibitory potential of IRF1 (1, 6–10). To clarify the biological functions of IRF1 as a tumor suppressor, it is necessary to define additional cell growth regulatory genes that are IRF1-responsive.

Our laboratory has shown that IRF1, as well as the closely related protein IRF2 (also known as histone nuclear factor-M (HiNF-M)), can function in the activation of histone H4 gene transcription at the G1/S phase transition through a phylogenetically conserved cell cycle regulatory element (9–16), which encompasses a canonical IRF consensus sequence (17). A characteristic N-terminal DNA binding domain spanning a winged helix-turn-helix motif (18–20) mediates the interaction of IRF factors with their cognate sites. The C-terminal region supports transcriptional enhancement or repression (21–23). The transcriptional activity of IRF factors is influenced by post-translational modifications, including phosphorylation and acetylations (24–27), as well as by protein/protein interactions with other IRF members and cofactors (1, 10, 28–31). The biological role of IRF factors in cell proliferation is reflected by the observation that forced expression of IRF factors perturbs normal cell growth and differentiation (32–36). Based on our studies that have revealed cell cycle regulatory roles for IRF proteins in control of histone gene expression at the G1/S transition (9, 10, 12), we have proposed that IRF factors may regulate cell proliferation through transcriptional mechanisms that operate parallel to and/or independent of E2F proteins (9, 10, 12, 35).

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IRF1 interferes with this study is that the interferon-inducible transcription factor IRF1 interferes with CDK2 expression by decreasing the activity of SP1, which is critical for the basal activation of the CDK2 gene. We propose that IRF1 may be a key regulatory node in an IFN-responsive molecular network with positive and negative feedback loops to control cell proliferation.

EXPERIMENTAL PROCEDURES
Preparation of Reporter Gene Constructs and Expression Vectors—A panel of constructs containing deletions of the CDK2 promoter fused to the luciferase (LUC) reporter gene has been described previously (41). Constructs containing −2400CDK2/LUC (DSC37) contain the −24-kbp promoter region of the human CDK2 gene up to nucleotide +58 bp (i.e., downstream from the CDK2 mRNA capsite) inserted into the pGL2-basic plasmid (Promega). Additional constructs used in our studies are −683CDR2/LUC (DSC40), −440CDR2/LUC (DSC40A−1), −101CDR2/LUC (DSC40A−17), and −15CDR2/LUC (DSC40A−10) with the first numbers of each construct indicating the retained amount of the CDK2 promoter in base pairs (41).

To delineate the IRF1-responsive element, we prepared two additional CDK2 promoter deletion constructs designated −68CDR2/LUC and −31CDR2/LUC using PCR. To assess the contribution of SP1 to IRF1 responsiveness, we mutated the SP1 site in the −68 CDK2 promoter vector construct −68mtSP1 CDK2/LUC. The following forward primers were used to prepare the new constructs: (−68 forward/Neo1 primer, 5′-agt cta gcG AGG GCG GGC CCT C-3′; −31 forward/Neo1 primer, 5′-agt cta gcG AGG GCG GGC CCT C-3′; −68mtSP1 forward/Neo1 primer, 5′-tgc tag cCA GGC TGG AGC CTC TG-3′ (mutated nucleotides are underlined). A single reverse BglII primer was used with the above forward primers: 5′-ata gat ccG CTT TGG TCG GTT TTC a-3′.

Western Blot Analysis—Cell lysates were centrifuged at 14,000 × g at 4 °C for 30 min, and protein concentrations were determined using the Bio-Rad protein assay reagent (Pierce) according to the manufacturer’s instructions. Equal amounts of total cellular protein were mixed with loading buffer (26.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 2% β-mercaptoethanol, and bromphenol blue), boiled for 5 min, and subjected to 10% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp., Bedford, MA). The membranes were saturated with phosphate-buffered saline containing 0.05% Tween 20 (× PBS-T buffer) and 5% fat-free dry milk for 1 h at room temperature. Blots were then incubated overnight at 4 °C with primary antibodies using the indicated dilutions (α-IRF1 at 1:1,000, α-SP1 at 1:2,000, α-CDK2 at 1:5,000, and β-actin at 1:2,500) (Santa Cruz Biotechnology, Inc.). The membranes were washed with PBS-T, incubated for 1 h at room temperature with the same buffer containing horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.) diluted to 1:8,000–10,000. Blots were then washed three to five times in PBS-T buffer before visualization. The enhanced chemiluminescence (ECL) kit (Amersham Biosciences) was used for the detection of immunoreactive protein bands. Quantitation of the relative protein amounts, the level of CDK2 protein in untransfected cells was set as 1.0, whereas the levels of IRF1 in cells transfected with 1.6 μg of IRF1 expression vector was set as 1.0. Each experiment was repeated at least three times.

RESULTS
IRF1 Represses CDK2 Gene Expression by a Transcriptional Mechanism—The tumor suppressor IRF1 is a principal target of interferon signaling through JAK/STAT to inhibit cell growth. CDK2 activity is one of the major kinases that regulate cell cycle progression at the G1/S phase transition, and the protein is stringently maintained at constitutive levels throughout the cell cycle. We addressed whether IRF1 may

FIG. 1. IRF1 inhibits CDK2 gene expression. A, Western blot analysis performed 24 h after transfection show that endogenous CDK2 expression is inhibited upon increasing IRF1 expression. β-Actin was used as a control for protein loading. The reduction in CDK2 protein levels was consistently observed in multiple experiments, and results of four representative experiments (#1 to #4) are shown. B, Quantitative analysis of multiple Western blots shows that IRF1 down-regulates CDK2 protein levels up to 5-fold. The relative protein levels of IRF1 and CDK2 were normalized using β-actin. To facilitate a comparison of relative protein amounts, the level of CDK2 protein in untransfected cells was set as 1.0, whereas the level of IRF1 in cells transfected with 1.6 μg of IRF1 expression vector was set as 1.0. Each experiment was repeated at least three times.
transduce the anti-proliferative signals of IFNs by suppressing the basal expression of the CDK2 gene. We expressed IRF1 above its normal levels in fibroblasts and observed a dose-dependent inhibition of endogenous CDK2 gene expression at the protein level (Fig. 1A). Semi-quantitative analysis of our data reveals that the maximal effect of IRF1 on endogenous CDK2 protein levels ranges from 2- to 5-fold in four independent transient transfection experiments (Fig. 1B). This range represents a conservative estimate of the IRF1-dependent reduction of CDK2 levels, because untransfected cells, which continue to express the CDK2 protein unabatedly, generate background signals.

To address whether IRF1 influences the levels of CDK2 by a transcriptional mechanism, we co-transfected an IRF1 expression plasmid and a luciferase reporter gene construct under control of the CDK2 promoter. Transfections were performed with different amounts of expression vector, and cells were harvested at various times after transfection (Figs. 2 and 3, as well as data not shown). Our results show that IRF1 reduces CDK2 promoter activity in a manner that is proportional to time after transfection (Fig. 2) and the amount of expression construct (Fig. 3). We conclude that IRF1 inhibits the CDK2 promoter activity in a time- and dose-dependent manner.

We note that, although our data indicate that IRF1 regulates CDK2 transcription, we can not exclude the possibility that CDK2 protein expression may be additionally regulated at post-transcriptional levels in response to IRF1. In addition, it is not possible to directly compare the quantitative effects of IRF1 on endogenous CDK2 protein levels (Fig. 1) with those on transfected reporter genes (Fig. 2) due to the intrinsic differences in the experimental approaches. The main finding of Figs. 1 and 2 is that both CDK2 protein levels and CDK2 promoter activity decline in tandem. This finding indicates that down-regulation of CDK2 protein levels is mediated at least in part by a transcriptional mechanism.

IRF1 is an activator of many genes (1) and we have previously shown that IRF1 activates the cell cycle-regulated histone H4 gene (9, 10). However, the inhibition of the CDK2 promoter by IRF1 suggests that it may function as a repressor. To demonstrate directly that IRF1 can exert bifunctional effects on transcription, it is necessary to establish that IRF1 can mediate both activation and repression under identical biological conditions. Therefore, we co-transfected an IRF1 expression plasmid together with the CDK2 promoter and a histone H4-related promoter each fused to distinct reporter genes (i.e. CDK2/LUC and 4xIRF/H4-Site II/CAT constructs) into the same NIH3T3 cells. Because both reporter gene constructs are present in the same cells, modulations in LUC and CAT activities can be directly compared as a function of elevations in IRF1. Our data show that IRF1 inhibits the CDK2 promoter while simultaneously activating the H4-related promoter in the same population of cells (Fig. 2). These results establish that IRF1 is capable of selectively increasing or decreasing transcription depending on promoter context.

We note that, apart from 4xIRF/H4-Site II/CAT, IRF1 also activates a 4xIRF/H4-Site II/LUC reporter gene (data not shown) (9, 10), indicating that IRF1-dependent transcriptional
modulations are observed irrespective of the CAT or LUC reporter gene constructs. Although we could reduce intra-experimental variation by normalizing the LUC and CAT data with a standard Renilla luciferase construct, which we routinely include in our transfection assays, this calculation would affect both reporter genes in the same manner and thus not be informative. The effects of forced expression of IRF1 are clearly evident in the primary data and the LUC and CAT reporters are, respectively, increased and decreased as the amount of IRF1 expression vector is increased. Hence, we are confident that the IRF1-dependent repression of CDK2/LUC and activation of 4XIRF1/H4-Site II/CAT are reproducible properties that are attributable to the promoters present in the respective constructs. More importantly, our observations rule out general squelching of transcription factors as a mechanism for IRF1-mediated repression and provide the basis for the studies described below which are aimed at defining the transcriptional events that are responsible for the inhibitory effect of IRF1 on the CDK2 promoter.

We systematically compared transcriptional inhibition versus enhancement by IRF1 during a time course up to 48 h after transfection to assess the temporal aspects of IRF1-dependent repression and to determine the earliest stage at which we observe either transcriptional effect. Our data indicate that repression of the CDK2 promoter occurs as early as 8 h after transfection but is more pronounced at 10 h and later time points (Fig. 2A and data not shown). The IRF1-dependent reduction in CDK2 promoter activity is maximal at 48 h. In contrast, IRF1-dependent activation of the H4 promoter is already apparent at 6 h after transfection and remains high at later time points (Fig. 2B and data not shown). The data indicate that the repressive effects of IRF1 on the CDK2 promoter are slightly delayed relative to the activating effects on the histone H4 gene promoter. Furthermore, the time-dependent concomitant increases in repression and activation properties of the IRF1 protein indicates that IRF1 levels remain within a range that supports physiological control of transcription.

**FIG. 4. Deletion analysis of the IRF1-responsive element in the CDK2 promoter.** A shows a schematic diagram of the CDK2/LUC reporter gene constructs used in this study. Inverted triangles indicate SP1 binding sites, and the asterisks indicate p53 consensus elements. B and C show reporter gene expression results obtained with NIH3T3 cells that were co-transfected with the various CDK2/LUC deletion constructs in the presence or absence of the IRF1 expression vector. B shows relative luciferase values in the absence (light gray bars) or presence (dark gray bars) of IRF1 to permit evaluation of effects of the deletions on basal expression. C shows the fold repression of each CDK2 promoter construct by IRF1 (expressed as ratio of the LUC values obtained in the presence and absence of IRF1). The luciferase assays reveal that deletion of the segment between nt -68 and -31 results in loss of the IRF1 response. Construct pGL2 represents the promoterless luciferase reporter construct.

**Delineation of an IRF1-dependent Repressor Element between nt -68 and -31.—** To define the region of the CDK2 promoter that supports IRF1-specific transcriptional inhibition, we tested a series of deletion mutants of the CDK2 promoter (Fig. 4A) for responsiveness to IRF1. The transfection results indicate that each of the promoter deletion mutants exhibits lower levels of basal transcription in control cells (Fig. 4B), consistent with the loss of putative elements that support CDK2 gene transcription. Deletion mutants up to nt -2400, -683, -440, -101, and -68 are inhibited by IRF1 (Figs. 4B), which is reflected by the ratios of LUC values in cells with elevated IRF1 levels and control cells (Fig. 4C). However, deletion mutants containing sequences up to nt -31 and -15 (Fig. 4, B and C) or that have a deletion between nt -141 to +61 (data not shown) are not responsive to IRF1. Thus, these
data indicate that the IRF1-responsive repressor element is located between nt −68 and −31. Inspection of this segment of the promoter indicates absence of a canonical IRF binding site suggesting that IRF1 controls CDK2 transcription through a novel mechanism involving other factors that interact with the CDK2 promoter.

**The CDK2 Promoter Is Repressed by IRF1, p53, and CDP/cut through Distinct Mechanisms**—Because the CDK2 promoter does not contain IRF1 binding sites, we postulated that IRF1 either functions through another repressor protein or interferes with the positive activity of an activating factor. However, there is only limited insight into the factors and elements that regulate CDK2 promoter activity (41). To define a molecular framework for the repressive actions of IRF1, we assessed the contributions of a panel of different gene regulatory factors to control of CDK2 promoter activity. Dose-response curves with increasing amounts of expression vectors were established for each of these factors, and adequate doses that result in specific measurable transcriptional effects were used in our experiments (Fig. 5 and data not shown).

One of the proteins we tested was p53, which, like IRF1, represents a cell growth inhibitory protein. Both factors are known to interact with the p21<sup>waf1/cip1</sup> gene in response to anti-proliferative signals; in each case, induction of the p21 protein inhibits CDK2 kinase activity (7, 48). Therefore, we tested whether, apart from IRF1, p53 can also regulate the CDK2 promoter as a component of a two-pronged growth-suppressive mechanism. It has been reported that the CDK2 promoter contains two putative p53 binding sites (41), but the functional role of p53 on the CDK2 promoter was not previously investigated. Our transfection data reveal that the tumor suppressor p53 represses the −683/CDK2 promoter, which spans two putative p53 binding sites, but not the −68/CDK2 promoter, which lacks these p53 consensus elements (Fig. 5A).

Therefore, p53-dependent repression requires elements distinct from those utilized by IRF1.

Similar to IRF1 and p53, the CCAAT displacement protein CDP/cut-like homeodomain protein (CUTL1/cut/cux) is also known to repress transcription of the p21 gene (49). In addition, CDP functions together with IRF proteins in the cell cycle regulation of histone H4 genes (9, 10, 50), as well as in regulation of the myeloid-specific gp91<sup>phox</sup> gene (45, 51). To assess whether CDP/cut contributes to transcriptional control of the CDK2 promoter, we performed co-transfection experiments in which we analyzed the effects of increasing CDP levels on CDK2 gene transcription (Fig. 5, B and C). The results show that CDP represses the CDK2 promoter in a dose-dependent manner (Fig. 5B) and that repression requires sequences located between −31 and −15 (Fig. 5C). Based on the sequence of the CDK2 promoter (41), the −31/−15 segment contains a CAAT element, and this motif is known to mediate binding of CDP/cut to its target genes (52, 53). The CDP-responsive −31/−15 segment is downstream of the region that mediates the IRF1 effects and thus is not the primary target of the IRF1 response.

We also tested the NPAT protein, which represents a key substrate of the CDK2/cyclin E kinase (46). Similar to CDP/cut and IRF1, NPAT is involved in control of histone gene transcription (46), and recent data from our laboratory have demonstrated that NPAT functions as a transcriptional cofactor of HNF-P, the H4 subtype-specific regulatory protein that mediates up-regulation of H4 gene transcription at the onset of S phase (54). We postulated that NPAT could potentially participate in feedback regulation of CDK2 gene transcription. However, our transfection results indicate that the CDK2/CLN-E-responsive NPAT protein does not influence CDK2 promoter activity (Fig. 5D). Taken together, the results presented in Figs. 4 and 5 establish that IRF1, p53, and CDP/cut, but not NPAT, are capable of transducing inhibitory signals to the CDK2 promoter. This repression occurs in each case through distinct elements, because p53 and CDP/cut repress the CDK2 promoter through sequences that are, respectively, upstream and downstream of the −68/−31 element, which mediates IRF1 repression.

**IRF1 Interferes with SP1-dependent Activation of the CDK2 Promoter through an SP1-responsive Element**—Previous studies identified three SP1 consensus elements (GC boxes) in the CDK2 promoter centered at approximately nt −160, −90, and −60 (41). Mutation of either the −90 or −60 element within the context of the −101/CDK2 promoter was shown to reduce basal transcription. Both the −90 and −60 elements were experimentally validated by DNase I protection analysis to represent high affinity SP1 binding sites in vitro (41). We extended these previous studies by addressing directly whether SP1 levels are rate-limiting for basal transcription of the CDK2 promoter.

Forced expression of SP1 results in a dose-dependent activation of the full-length −2400/CDK2 promoter (data not shown). SP1 activation is also observed with the −68/CDK2 promoter, which contains only one of the three SP1 consensus elements (Fig. 6). However, SP1 activation is not observed when the SP1 promoter element at nt −60 is mutated within the context of the −68/CDK2 promoter (Fig. 6). Thus the SP1 binding site at nt −60 is required and sufficient for the SP1 response of the proximal region (i.e. downstream from nt −68) of the CDK2 promoter.

Because the −68 to −31 segment lacks a canonical IRF binding site, but contains a functional SP1 binding site, we addressed the hypothesis that IRF1 may counteract SP1 activation of the CDK2 promoter. We co-transfected the IRF1 expression vector and the LUC reporter gene driven by either the
IRF1 inhibits the SP1-dependent enhancement of CDK2 promoter activity. NIH3T3 cells were cotransfected with the reporter gene constructs −68 CDK2/LUC (left bars), −68 (mt SP1) CDK2/LUC (middle bars), and pGL2/LUC (right bars) as indicated, as well as SP1 or IRF1 expression vectors (0.4 µg/well per construct; presence or absence is indicated by plus/minus signs) to analyze the responsiveness of the CDK2 promoter to SP1, IRF1, or the combination of both. SP1 activates the −68 CDK2 promoter, and co-transfection of IRF1 inhibits the SP1-dependent enhancement of CDK2 promoter activity.

Fig. 6. IRF1 interferes with the SP1-dependent activation of CDK2 gene transcription. NIH3T3 cells were cotransfected with the reporter gene constructs −68 CDK2/LUC (left bars), −68 (mt SP1) CDK2/LUC (middle bars), and pGL2/LUC (right bars) as indicated, as well as SP1 or IRF1 expression vectors (0.4 µg/well per construct; presence or absence is indicated by plus/minus signs) to analyze the responsiveness of the CDK2 promoter to SP1, IRF1, or the combination of both. SP1 activates the −68 CDK2 promoter, and co-transfection of IRF1 inhibits the SP1-dependent enhancement of CDK2 promoter activity.

SP1 form stable complexes, we performed immunoprecipitation experiments, but precipitates obtained with IRF1 antibodies did not contain detectable levels of SP1 (data not shown). One caveat of this result is that the IRF1-dependent decrease in SP1 levels may preclude detection of SP1 in these precipitates. In addition, a direct interaction between IRF1 and SP1 may not be necessary to influence SP1 levels, because IRF1 may target proteolytic factors that promote SP1 degradation. Taken together, our data suggest that one mechanism by which IRF1 mediates the anti-proliferative effect of interferon γ is by interfering with the SP1-dependent activation of CDK2 gene transcription by decreasing SP1 protein levels.

DISCUSSION

In this study, we have obtained several lines of evidence that support the concept that the tumor-suppressive effects of IRF1 may be exerted at least in part by reducing the endogenous protein levels of CDK2, a key regulator of the G1/S phase transition. These data complement previous studies by other laboratories (7), which have indicated that p21, a physiological inhibitor of CDK2 kinase, is activated by IRF1. Thus, one major mechanism that may be operative in the biological function of IRF1 is the potential to mediate a two-pronged effect that can simultaneously decrease the levels of CDK2 protein and increase the levels of its cognate CDK inhibitor p21.

Transcriptional Repression of the CDK2 Promoter by IRF1, p53, and CDP/ctn Occurs through Three Distinct Mechanisms—Our data show that IRF1 and p53 suppress the CDK2 promoter through distinct mechanisms, whereas both proteins function as positive regulators of the CDK inhibitor protein p21 through their cognate sites (7, 55). Hence, both tumor suppressor proteins operate through analogous dual growth suppres-
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Pleiotropic Effects of the IRF1-dependent Reduction in SP1 Levels on Other Components of Cyclin-dependent Kinase Complexes—The basal transcription of both the CDK2 and p21 genes is regulated through SP1 binding sites, yet only the promoter of the p21 gene appears to contain IRF binding sites. Thus, IRF1-dependent degradation of SP1 may convert the p21 promoter from a constitutively active SP1-responsive promoter to an IRF1-inducible promoter that responds to feedback mechanisms of the IFN and JAK/STAT signaling pathway. The absence of IRF1 binding sites at the CDK2 promoter ensures that this gene is no longer activated by SP1 without becoming IRF1-responsive. We note that the genes for the CDK2 regulator subunits, cycin E and cycin A, as well as the CDK2 inhibitor p27Kip1, are also controlled by SP1 binding sites (64–69). Hence, transcription of these genes may be affected by reductions in SP1 levels. Redundancy of SP1 with other transcriptional mechanisms that regulate the promoters of these genes will dictate the extent to which loss of SP1 interactions translates into reduced promoter activity. The expected reduction of the levels of cycins E and A, together with increased levels of the CDK inhibitor p21 and decreased levels of CDK2, would provide a highly effective mechanism to control CDK2 kinase activity.

Interconnected Autoregulatory Feedback Loops Involving IRF1, SP1, and CDK2 Levels—Recent data indicate that SP1 is required for prolactin activation of the IRF1 gene in T cells (70). Hence IRF1 may attenuate its own protein levels by down-regulating SP1 following the induction of IRF1 in response to extracellular signals. CDK/cyclin A complexes increase SP1 activity (71, 72). Thus, the IRF1-dependent degradation of SP1 levels may also initiate a second autoregulatory feedback loop. We have shown that decreased SP1 protein results in reduced transcription of the CDK2 gene and is expected to reduce expression of the cyclin A gene. The resulting decrease in CDK2/cyclin A protein levels is thus expected to diminish further the transcriptional activity of SP1 and its activation of the CDK2 promoter.

In summary, our findings provide insight into the cell growth inhibitory effects of interferons as well as the normal role of IRF1 as a tumor suppressor. We propose that the up-regulation of IRF1 by interferon γ causes repression of the CDK2 promoter by diminishing the levels of the activating protein SP1. The reduction in CDK2 kinase activity may affect cell growth by inhibiting CDK2-mediated events late during G1 and/or at the G1/S phase transition.

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IRF1 Suppresses the Human CDK2 Promoter
The Tumor Suppressor Interferon Regulatory Factor 1 Interferes with SP1 Activation to Repress the Human \textit{CDK2} Promoter

Rong-Lin Xie, Sunita Gupta, Angela Miele, Dov Shiffman, Janet L. Stein, Gary S. Stein and Andre J. van Wijnen

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