Interferon regulatory factor-2 (IRF-2) is a transcription factor of the IRF family that represses interferon-mediated gene expression. In the present study, we show that human monocytic U937 cells express truncated forms of IRF-2 containing the DNA binding domain but lacking much of the C-terminal regulatory domain. U937 cells are shown to respond to phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) to induce expression of histone acetylases p300 and p300/CBP-associated factor (PCAF). In addition, TPA treatment led to the appearance of full-length IRF-2, along with a reduction of the truncated protein. Interestingly, full-length IRF-2 in TPA-treated U937 cells occurred as a complex with p300 as well as PCAF and was itself acetylated. Consistent with these results, recombinant IRF-2 was acetylated by p300 and to a lesser degree by PCAF in vitro. Another IRF member, IRF-1, an activator of interferon-mediated transcription, was also acetylated in vitro by these acetylases. Finally, we demonstrate that the addition of IRF-2 but not IRF-1 inhibits core histone acetylation by p300 in vitro. The addition of IRF-2 also inhibited acetylation of nucleosomal histones in TPA-treated U937 cells. Acetylated IRF-2 may affect local chromatin structure in vivo by inhibiting core histone acetylation and may serve as a mechanism by which IRF-2 negatively regulates interferon-inducible transcription.

Nucleosomal histones are post-translationally modified by various mechanisms including acetylation, deacylation, phosphorylation, and methylation (1, 2). High levels of histone acetylation have been linked to the transcriptionally active region of chromatin, while low histone acetylation is associated with the transcriptionally repressed regions (3). Consistent with a link between histone acetylation and transcriptional activation, several histone acetylases are recruited to transcriptionally active promoters by interacting with sequence-specific transcription factors (4, 5). Histone acetylases are classified into several groups including the conserved Gcn5-related N-acetyltransferase family and the p300/CBP, Myst, TAF, and nuclear receptor co-activator families (6). A number of transcription factors associate with p300/CBP, originally known as the global co-activators, and with PCAF and GCN5 belonging to the Gcn5-related N-acetyltransferase family. Recruitment of these histone acetylases is thought to alter chromatin structures, required as an integral part of transcriptional activation. As a result of interaction with histone acetylases, some transcription factors become themselves acetylated, which often results in enhanced transcriptional activity (7–11). Some non-DNA-binding regulatory factors are also acetylated by histone acetylases (6, 12).

In addition to transcription, the status of histone acetylation is thought to influence cell growth and differentiation (13, 14). In support of a link between histone modification and cell growth, histone acetylation is under control of growth and differentiation signals. For example, it has been shown that treatment of human tissue culture cells with epidermal growth factor increases phosphorylation and acetylation of histone H3 (15). In addition, p300/CBP, by virtue of the interaction with a number of cell cycle regulatory proteins, affects proliferation and differentiation of some cells (16). Activity of p300/CBP is also regulated during the cell cycle, peaking at G1/S transition (17).

Proteins of the interferon regulatory factor (IRF) family regulate type I interferon (IFN)-mediated transcription of many genes (18). Some members regulate IFN gene expression as well. All IRF proteins carry the conserved DNA binding domain (DBD) consisting of ~110 amino acids in the N-terminal region, through which they bind to the IFN-stimulated response element (ISRE) present in IFN-inducible genes. They have a less conserved C-terminal regulatory domain with variable lengths. IRF-1 and IRF-2 are founding members of the family, which have opposing activities. While IRF-1 activates transcription from promoters carrying the ISRE, IRF-2 represses transcription of these promoters. IRF-2, however, has a cryptic activation domain and can activate transcription from some promoters (19, 20). Moreover, IRF-1 can act as a tumor suppressor, while IRF-2 can act as an oncogenic factor (21). Some IRF members are shown to interact with histone acetylases. IRF-3, upon viral infection, interacts with p300/CBP, and this interaction is a necessary step for its functional activation (22–24). IRF-1 associates with p300/CBP and PCAF to form a part of multiprotein complexes that assemble on the IFN-β promoter (25). Recently, it has been shown that v-IRF of Kaposi’s sarcoma-associated herpesvirus that acts as a repressor of interferon inducible promoters also interacts with p300 (26–28). Previously, we have demonstrated that IRF-1 and IRF-2 both interact with PCAF in vivo and in vitro and that this interaction is a necessary step for functional activation (22–24). IRF-1 associates with p300/CBP and PCAF to form a part of multiprotein complexes that assemble on the IFN-β promoter (25). Recently, it has been shown that v-IRF of Kaposi’s sarcoma-associated herpesvirus that acts as a repressor of interferon inducible promoters also interacts with p300 (26–28). Previously, we have demonstrated that IRF-1 and IRF-2 both interact with PCAF in vivo and in vitro and that this interaction is a necessary step for functional activation.
interaction plays an important role in transcription from relevant promoters (29). We also observed that expression of histone acetylases p300/CBP was induced in response to phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) in human monocytic U937 cells (29). TPA has been shown to activate protein kinase C and stimulate U937 cells to differentiate into macrophage-like cells (30).

In the present study, we show that IRF-2 is truncated at the C-terminal domain in untreated U937 cells, and TPA treatment induces expression of full-length IRF-2. Coinciding with increased expression of p300/CBP and P300/CBP following TPA treatment, full-length IRF-2 became associated with these histone acetylases. As a result, IRF-2 was acetylated in vitro in TPA-treated U937 cells. Last, we demonstrate that acetylated IRF-2 inhibits p300-mediated acetylation of core histones. This inhibition may be a part of mechanisms by which IRF-2 represses transcription from some target genes and regulates cell growth.

EXPERIMENTAL PROCEDURES

Cell Culture—Human monocytic U937 cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and gentamicin (25 μg/ml). Cells were treated with 10 nM TPA or 3 μM protein kinase C inhibitor GF109203X (Calbiochem) (31) for the indicated periods of time. U937 cells stably expressing P300 were maintained in the above medium supplemented with 200 μg/ml Genticin (29). For in vitro labeling of [3H]acetate in U937 cells, 20 μCi of [3H]acetate (Amersham Pharmacia Biotech) were added to U937 cell culture incubated at 37 °C in the CO2 incubator 1 h before isolation of cell lysate.

Semiquantitative PCR—We used the methods of Colle et al. (32) with a small modification. cDNAs were synthesized using total RNA from TPA-treated U937 cells using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) in a reaction mixture containing 75 mM KCl, 3 mM MgCl2, 50 mM Tris-HCl (pH 8.5), 0.25 mM dNTPs, 0.8 units of RNaseA, and random hexamer primers (Promega). Each PCR mixture contained 10 μl of cDNA, 50 mM KCl, 3 mM MgCl2, 10 mM Tris-HCl (pH 9.0), 250 μM dNTPs, 1 unit of Taq DNA polymerase (Promega), and 1 μg of sense and antisense primers in a total volume of 50 μl. A total of 30 cycles were carried out for all samples. Southern blot hybridization was performed as in Ref. 29.

GST Fusion Proteins—A BamHI and SmaI fragment of IRF-2 cDNA corresponding to the DNA binding domain (amino acids 1–129) was subcloned into pGEX4T (Amersham Pharmacia Biotech). GST fusion proteins were isolated by using glutathione-Sepharose beads (Amersham Pharmacia Biotech) from bacteria extracts. In vitro translated, full-length or truncated IRF-2 were produced from pcDNA3.1 (+) (Invitrogen) harboring appropriate inserts.

Immunoblot Analysis—Rabbit antibody against the C-terminal domain of IRF-2 was described previously (29). Rabbit antibody against the DBD of IRF-2 was produced by using a purified bacterial protein produced in a pET-15b vector (Novagen) containing a PCR fragment corresponding to residues 1–129 of IRF-2. The antibody reacted with the DBD of IRF-2 but not with the DBD of IRF-1 or ICSBP. Mouse antibody to p300 and rabbit antibody to his tag were obtained from Upstate Biotechnology. Inc. Rabbit antibody to P300 was a gift from Dr. Y. Nakatani (Dana-Farber Cancer Institute). Monoclonal M2 anti-FLAG antibody was purchased from Eastman Kodak Co. For immunoblot analysis, the indicated amounts of nuclear extracts were resolved on SDS 10% polyacrylamide gel electrophoresis (PAGE) gels, gels were transferred to a Immobilon P polyvinylidene fluoride membrane (Millipore Corp.) and blocked with 1% skim milk in phosphate-buffered saline containing 0.1% Tween 20. The membranes were incubated with an appropriate dilution of primary antibodies and then with horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (Amersham Pharmacia Biotech). The membranes were then developed using the ECL detection kit according to the instructions provided by the manufacturer (Amersham Pharmacia Biotech).

FLAG Pull-down Assay and Immunoprecipitation—Extracts were lysed in buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mg/ml aprotinin and centrifuged at 15,000 rpm for 20 min. Supernatants were incubated with M2 anti-FLAG antibody (Sigma) or anti-IRF-2 polyclonal antibody conjugated to agarose beads for 2 h at 4 °C and washed three times in lysis buffer. Bound materials were eluted in SDS-sample buffer and resolved on SDS-10% PAGE and analyzed for immunoblot using antibodies to p300, PCAF, IRF-2, and histone deacetylase 1 (HDAC1). The gels were stained with Coomassie Brilliant Blue for loading (CBB; upper panel).

Analysis of Histone Acetylase Activity—Nuclear suspensions were prepared as described in Ref. 34. Core histones (500 ng; Roche Molecular Biochemicals) or nuclear preparations were incubated with 100 ng of baculovirus recombinant p300 or PCAF in the presence of [3H]acetate and the indicated amounts of baculovirus recombinant IRF-2, IRF-1, or ICSBP for 30 min at 30 °C in histone acetyltransferase reaction buffer as previously described (33). Reacted materials were mixed with SDS-sample buffer and electrophoresed on SDS-12.5% PAGE and subjected to PhosphorImager analysis using Fiji BAS 2000 or stained with Coomassie Brilliant Blue.

Affinity DNA Binding Assay and Electrophoretic Mobility Shift Assay (EMSA)—The DNA affinity binding assay was performed essentially as described (29). Briefly, the indicated amounts of baculovirus recombinant IRF-2 were incubated with recombinant p300 and PCAF in the presence of 100 μM acetyl-CoA for 30 min at 30 °C, and reaction mixtures were incubated with magnetic beads conjugated to biotinylated ISRE DNA from the ISG15 gene. Bound materials were immunoblotted with anti-IRF-2 antibody. Nuclear extracts were prepared by the method of Digman with modifications that included the addition of protease inhibitors to the buffers. Oligonucleotide probes were labeled with γ-32P]ATP (Amersham Pharmacia Biotech) by T4 polynucleotide kinase (Promega). EMSA and supershift analysis were performed as described (29).

RESULTS

TPA Treatment Increases Histone Acetylation in U937 Cells—Phorbol ester, TPA, mediates various biological effects through the activation of protein kinase C (30). Among other effects, TPA stimulates U937 human monocyctic cells to differentiate into macrophage-like cells (31). Consistent with the role of protein kinase C, TPA-induced differentiation is inhibited by protein kinase C inhibitor. Previously, we reported that TPA treatment induced expression of histone acetylases PCAF and p300 in U937 cells (29). We were interested in determining whether TPA induction of histone acetylation was affected by a protein kinase C inhibitor and whether TPA treatment changed the status of histone acetylation in U937 cells. Results in Fig. 1A show that while TPA treatment strongly induced expression of p300 and PCAF, the co-addition of a specific inhibitor, GF109203X, greatly inhibited expression of both histone acetylases. To address the functional conse-
Acetylation of IRF-2 by p300

TPA Treatment—During the course of these studies, we observed that the molecular size of IRF-2 changes in U937 cells upon TPA treatment. This observation was made by the use of two anti-IRF-2 antibodies, one reacting with the DBD and another reacting with the C-terminal end of IRF-2. As shown in Fig. 1A, antibody to the DBD revealed two IRF-2 bands in untreated U937 cells, corresponding to ~41–46 kDa in size. These bands were smaller than a band expected of full-length IRF-2, which should run near 49 kDa. In TPA-treated U937 cells, this DBD antibody revealed a band of ~49 kDa along with weak smaller bands. The addition of the protein kinase C inhibitor inhibited the expression of the 49-kDa band (Fig. 1A). The smaller bands were not a product of fortuitous cross-reactivity, since they were not detected with sera absorbed with recombinant IRF-2 DBD (not shown). These results suggested that IRF-2 proteins in untreated U937 cells was truncated, and that TPA treatment restored expression of full-length IRF-2 protein. In light of the previous study documenting that IRF-2 undergoes C-terminal cleavage upon viral infection (35), we further investigated whether the smaller IRF-2 bands present in untreated U937 cells represent cleavage products of IRF-2. In Fig. 2A, reactivity of the two anti-IRF-2 antibodies was compared side by side. The antibody against the C-terminal region of IRF-2 did not reveal a discernible band in untreated cells, while it recognized a 49-kDa band in TPA-treated cells. This anti-C terminus IRF-2 antibody also reacted with a 49-kDa band in Namalwa B cell extracts run as a control. In contrast, the antibody against IRF-2 DBD (labeled N-terminal in Fig. 2A) revealed several bands ranging from 41 to 46 kDa in size in untreated U937 cells, but after TPA treatment the intensity of these bands was decreased, while the intensity of the 49-kDa band was markedly increased. Time course experiments using anti-IRF-2DBD antibody showed that the increase in full-length IRF-2 coincided with the decrease in truncated IRF-2 during 3 days of TPA treatment (Fig. 2B). Namalwa B cells expressed predominantly a full-length band of 49 kDa and a smaller 46-kDa band. These results indicate that untreated U937 cells express cleaved forms of IRF-2 and that TPA treatment causes expression of full-length IRF-2. It is unlikely that IRF-2 is degraded during extract preparations, because the sizes and levels of other proteins such as histone deacetylase 1 (HDAC1), ICSBP, and PU.1 (not shown) remained unchanged before and after TPA treatment, although this possibility cannot be formally excluded. As shown in Fig. 2C, TPA treatment increased IRF-2 mRNA expression beginning at around 16 h, and the levels remained high for the subsequent 48-h period. The increase in IRF-2 transcripts correlated with the expression of full-length IRF-2 protein (Fig. 2B), indicating that the TPA-induced change in the size of IRF-2 involves increased gene expression. In Fig. 2D, we examined whether TPA treatment of U937 cells changed factor binding activity for the ISRE, a target DNA element for the IRF family proteins. Extracts from untreated U937 cells produced several fast migrating bands (lane 2). On the other hand, extracts from TPA-treated cells did not reveal fast migrating bands. Instead, they produced three more slowly migrating bands (lane 4). The upper two bands contained IRF-2 and may represent a monomer and dimer of IRF-2, since these bands were supershifted by anti-C terminus IRF-2 antibody (Fig. 2D). The antibody did not react with bands from untreated cells. These results are consistent with the view that untreated U937 cells express truncated IRF-2 proteins that contain the DBD and that TPA treatment increases expression of full-length IRF-2, reducing the truncated species.

Full-length IRF-2 Is Complexed with p300 and PCAF in TPA-treated U937 Cells—We then investigated the interaction of IRF-2 with histone acetylase PCAF or p300 in vivo. Co-immunoprecipitation analysis performed with extracts of TPA-treated U937 cells using anti-C terminus IRF-2 antibody showed that full-length IRF-2 was co-precipitated with PCAF and p300 (Fig. 3A). However, neither IRF-2 nor the histone acetylases were precipitated from extracts of untreated cells. Control IgG did not precipitate these proteins (Fig. 3A). It should be noted here that it was not possible to test whether truncated forms of IRF-2 were also complexed with the histone acetylases, because the antibody reacting with the IRF-2 DBD did not work in immunoprecipitation experiments, although it did work for immunoblot experiments. It is possible that epitopes recognized by the antibody are sequestered in the native protein. However, based on the
low levels of histone acetylase expression in untreated U937 cells (Fig. 1) and data presented below, it seems unlikely that the truncated forms of IRF-2 are complexed with histone acetylases.

To further verify the formation of IRF-2-histone acetylase complex in U937 cells, we tested whether PCAF, known to interact with p300, also forms a complex with IRF-2. For this purpose, immunoprecipitation analysis was performed using U937 cells stably expressing FLAG-tagged PCAF (29). Anti-FLAG antibody precipitated full-length IRF-2 but not the truncated forms of IRF-2 (Fig. 3B, middle panel). These cells expressed significant levels of truncated IRF-2 as judged by immunoblot analysis (see Input, bottom panel). Interestingly, a mutant PCAF lacking the catalytic domain of PCAF, on the other hand, did not precipitate IRF-2. These results indicate that full-length IRF-2, but not the truncated counterpart, interacts with intact PCAF in vivo and forms a complex.

Given a number of reports that some transcription factors are acetylated in vivo (7, 8, 10–12, 36), we sought to test the possibility that IRF-2 is acetylated in vivo. U937 cells were pulse-labeled with [14C]acetate for 60 min, and extracts were immunoprecipitated with anti-C terminus IRF-2 antibody. As shown in Fig. 4A, both p300 and PCAF were labeled with [14C]-acetate, although acetylation was more extensive for p300 than PCAF, indicating autoacetylation. In addition, IRF-2 was acetylated by both p300 and PCAF. Interestingly, p300 was more potent in acetylating IRF-2 than PCAF. IRF-1 was also acetylated by p300, although to a much lesser degree than IRF-2. On the other hand, PCAF did not acetylate IRF-1 to an appreciable degree. In contrast, a detectable level of acetylation was not seen with ICSBP by either p300 or PCAF. Coomassie Blue staining of reaction mixtures shown in Fig. 4B confirmed that each reaction contained equivalent amounts of proteins.

Since a number of transcription factors are shown to gain increased DNA binding activity upon acetylation (6, 8, 10, 37), it was of interest to test whether acetylation of IRF-2 led to higher ISRE binding activity. To this end, we carried out DNA affinity binding assay in which increasing amounts of recombinant IRF-2 and p300 or PCAF were incubated with the ISRE conjugated to magnetic beads in the presence or absence of acetyl-CoA, and bound IRF-2 was measured by immunoblot analysis. As shown in Fig. 4C, binding of IRF-2 to the immobilized ISRE DNA was not significantly affected by acetylation, since the addition of acetyl-CoA did not change the amount of bound IRF-2. We favored the above DNA affinity binding assay over EMSA, since in the latter assays binding of IRF-2-p300 complex or IRF-2-PCAF to the ISRE was not detected, although binding of IRF-2 itself to the ISRE was seen. Nevertheless, IRF-2 DNA binding activity tested in EMSA was not affected by the histone acetylases in the presence of acetyl-CoA either (not shown).

We previously noted that IRF-2 interacted with PCAF through the DNA binding domain (29). Thus, it was of interest to determine whether IRF-2 DBD is acetylated in vitro by p300 and PCAF. In Fig. 5A, a GST-IRF-2 DBD fusion protein was incubated with recombinant p300 or PCAF in the presence of [14C]acetate, indicating that it is acetylated. IRF-2 DBD peptide but not the control GST peptide was strongly acetylated by p300 and weakly by PCAF. Acetylation of IRF-2 DBD was also demonstrated by the reactivity with antiacetyl lysine antibody (Fig. 5C). IRF-2 DBD peptides were co-incubated with p300 or PCAF and reacted with the antibody in the presence of acetyl-CoA. IRF-2 DBD reacted with the acetyl lysine antibody when incubated with p300 or PCAF only in the presence of acetyl-CoA and not in the absence. IRF-2 DBD showed stronger reactivity when incubated with p300 than with PCAF, consistent with more efficient acetylation of IRF-2 by p300 than by PCAF. As shown in EMSA analysis in Fig. 5D, binding of IRF-2 DBD to DNA was not significantly affected by acetylation, similar to the results with full-length IRF-2. We noted that the addition of p300 and PCAF increased the apparent binding of IRF-2 DBD to DNA in the presence and absence of acetyl-CoA. The basis of this increase is not clear at present.

**IRF-2 DBD Binds to p300**—Previous studies have shown that some IRF family proteins interact with p300/CBP, including IRF-1, IRF-2, and v-IRF (23–27, 38). Given the observation that p300 can acetylate IRF-2, it was anticipated that p300 directly interacted with IRF-2 DBD. In experiments shown in Fig. 6, we tested binding of IRF-2 DBD to full-length IRF-2, IRF-2 DBD, or IRF-2 without DBD (ΔDBD) to FLAG-tagged p300 immobilized to M2 antibody beads. Full-length IRF-2 and IRF-2 DBD bound to p300 but not to control M2 beads. In contrast, ΔDBD and in vitro translated luciferase did not bind to p300-conjugated beads. These results show that...
IRF-2 interacts with p300 through the DBD. Our results are consistent with the recent report that v-IRF interacts with p300 through the N-terminal region corresponding to the DBD (28). These results are analogous with our previous observation that interaction of IRF-1 and IRF-2 with PCAF is dependent on the DBD (29).

**FIG. 4.** Acetylation of IRF-2 by p300 and PCAF in vitro. A, 200 ng of recombinant IRF-2 (lanes 1, 4, and 7), ICSBP (lanes 2, 5, and 8), or IRF-1 (lanes 3, 6, and 9) was incubated with [14C]acetyl-CoA in the presence of 100 ng of BSA (lanes 1–3), p300 (lanes 4–6), or PCAF (lanes 7–9) for 30 min at 30 °C. Reaction mixtures were separated on SDS 10%–PAGE and autoradiographed. B, gels in A were stained with Coomassie Brilliant Blue. C, affinity DNA binding assay for acetylated IRF-2. Indicated amounts of recombinant IRF-2 (rIRF-2) were mixed with 100 ng of p300 or PCAF for 30 min in the absence (lanes 1–4) or presence (lanes 5–8) of acetyl-CoA and incubated with magnetic beads conjugated to biotinylated ISRE. Bound IRF-2 was detected by immunoblot assay.

**FIG. 5.** Acetylation of IRF-2 DBD by p300 and PCAF in vitro. A, 200 ng of GST-IRF-2-DBD peptides (lanes 1, 3, and 5) or GST peptides (lanes 2, 4, and 6) were incubated with 100 ng of recombinant p300 (lanes 1 and 2) or PCAF (lanes 3 and 4) in the presence of [14C]acetyl-CoA for 30 min at 30 °C and analyzed as in Fig. 4A. B, gels in A were stained with Coomassie Brilliant Blue. C, 200 ng of GST-IRF-2-DBD peptides (lanes 1, 3, and 5) or GST peptides (lanes 2, 4, and 6) were incubated with 100 ng of recombinant p300, PCAF, and BSA in the presence or absence of 100 μM acetyl-CoA for 30 min at 30 °C. Reaction mixtures were immunoblotted using antiacetyl lysine antibody. D, DNA binding activity of IRF-2 DBD. 200 ng of GST-IRF-2-DBD peptides or GST peptide were incubated with 100 ng of p300, PCAF, or BSA in the presence or absence of acetyl-CoA and [35S]labeled ISRE probe.

**DISCUSSION**

We show here that TPA treatment of U937 cells induces expression of p300 and PCAF, leading to increased acetylation...
of histones in U937 cells. It has been shown that TPA activates protein kinase C and stimulates differentiation of U937 cells toward macrophage-like cells (31). Supporting the importance of protein kinase C activation in histone acetylation, the inhibitor GF109203X greatly inhibited histone acetylase induction and blocked increased histone acetylation in TPA-treated U937 cells. These results imply that TPA (and perhaps other differentiation signals) influence the overall levels of core histone acetylation in the cells by regulating histone acetylase expression. Consistent with the idea that histone acetylation is subject to growth and differentiation signals, Cheung et al. (15) showed that epidermal growth factor triggers phosphorylation and acetylation of histone H3 in tissue culture cells. Several lines of evidence support a link between increased histone acetylation and differentiation. First, various histone deacetylase inhibitors are shown to cause differentiation in many types of cancer cells (13). Second, quinidine-induced differentiation of breast cancer cells is reported to coincide with histone H4 hyperacetylation (14).

Along with changes in histone acetylase expression, truncated forms of IRF-2 predominantly expressed in untreated U937 cells were largely replaced by full-length IRF-2 after TPA treatment. Since there was no evidence for alternative splicing of IRF-2 mRNA, it is likely that in untreated U937 cells IRF-2 is cleaved by an endogenous protease(s). Although the protease(s) that specifically cleaves IRF-2 has not been identified, a previous report demonstrated that full-length IRF-2 was cleaved in some cells upon viral infection (35). Interestingly, like the truncated IRF-2 seen in U937 cells, the IRF-2 cleaved after viral infection is shown to lack the C-terminal domain, while retaining the intact DBD. The truncated IRF-2 in virus-infected cells acts as a negative regulator of intact IRF-2. The emergence of full-length IRF-2 after TPA treatment is likely to be accounted for by newly synthesized IRF-2, since there was a significant increase in the levels of IRF-2 mRNA expression after TPA treatment. In addition, it is possible that increased stability of IRF-2 may have also contributed to the appearance of full-length IRF-2.

In the present work, we have demonstrated that IRF-2 in TPA-treated U937 cells was acetylated and occurred as a complex with p300 and PCAF (See Fig. 8 for a diagram). Since PCAF is shown to directly interact with p300 (39), IRF-2 may be part of a large PCAF-p300 complex. Recently, PCAF and GCN5 have been shown to occur as a large stable complex containing many other factors (40, 41). However, neither IRF-2 nor p300 is shown to be a component of a stable PCAF complex. It is likely that the IRF-2-containing complex we detected by co-immunoprecipitation is a subset of heterogeneous PCAF complexes that may be less stable than other complexes analyzed in the previous studies. Although we do not have conclusive data, it seems likely that only full-length IRF-2 is acetylated by p300 and PCAF in vivo, based on the results that FLAG-tagged PCAF was not co-precipitated with truncated IRF-2 in untreated U937 cells. Furthermore, the paucity of histone acetylases expressed in untreated cells would also make it less likely to cause acetylation of truncated IRF-2, although in vitro acetylation experiments showed that the IRF-2 DBD can be acetylated by p300. Interestingly, although both IRF-1 and IRF-2 were acetylated in vitro, the level of acetylation was significantly higher for IRF-2 than IRF-1. Sehgal et al. (42) recently reported that IRF-2 inhibits the recruitment of CBP to IFN-β gene. Although our results are seemingly
Acetylation of IRF-2 by p300

Acetylation of IRF-2 by p300 may be reasonable to anticipate that the mode with which IRF-2 interacts with a target DNA element is modified by acetylation in the native, chromatinized promoters. Acetylation of IRF-2 may have a significant functional consequence in transcription. We showed that the addition of recombinant IRF-2 but not IRF-1 inhibited core histone acetylation by p300 in vitro as well as nucleosomal acetylation of U937 cells after TPA treatment. We noted that inhibition was more prominent for histone H-2A and H-2B than histones H3 and H4, suggesting that IRF-2 inhibited histone acetylase activity of p300 more strongly than PCAF, since p300 is capable of acetylating all four histones, while PCAF/GCN5 acetylates predominantly H3 and H4 (46). A simple interpretation of the observed inhibition would be that IRF-2, by acting as a substrate for histone acetylases, competitively inhibits histone acetylation. Consistent with this interpretation, appreciable inhibition of histone acetylation was not observed by IRF-1 and ICSBP, a weaker substrate for the acetylases. Together, it is attractive to assume that IRF-2 inhibition of histone acetylation is relevant to transcriptional repression of IFN-responsive genes by IRF-2 (47, 48). It is of note that although for many factors acetylation leads to enhanced transcriptional activity, it can have a negative regulatory effect, as reported for T-cell factor in Drosophila (49). Interestingly, another IRF member, v-IRF, derived from Kaposi’s sarcoma-associated herpesvirus, also interacts with p300 and inhibits histone acetylation, resulting in repressed transcription of interferon-responsive genes (28, 50). It would be of interest to determine whether v-IRF inhibits core histone acetylation by acting as a competitor. Among several transcriptional activators involved in IFN-β enhancer element, IRF-2 acts on CBP recruitment negatively. In contrast, IRF-2 binds to p300 directly and inhibits its histone acetyltransferase activity after acetylation by 300 or PCAF. Nevertheless, for IRF-2, we did not observe increased DNA binding activity and enhanced transcriptional activity upon acetylation. For IRF-2, we noted that inhibition was observed inhibition would be that IRF-2, by acting as a substrate for histone acetylases, competitively inhibits histone acetylation. Consistent with this interpretation, appreciable inhibition of histone acetylation was not observed by IRF-1 and ICSBP, a weaker substrate for the acetylases. Together, it is attractive to assume that IRF-2 inhibition of histone acetylation is relevant to transcriptional repression of IFN-responsive genes by IRF-2 (47, 48). It is of note that although for many factors acetylation leads to enhanced transcriptional activity, it can have a negative regulatory effect, as reported for T-cell factor in Drosophila (49). 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