Cell, Vol. 117, 735–748, June 11, 2004, Copyright ©2004 by Cell Press

Ordered Cooperative Functions of PRMT1, p300, and CARM1 in Transcriptional Activation by p53

Woojin An, Jaehoon Kim, and Robert G. Roeder*
Laboratory of Biochemistry and Molecular Biology
The Rockefeller University
New York, New York 10021

Summary

Transcriptional coactivators that modify histones represent an increasingly important group of regulatory factors, although their ability to modify other factors as well precludes common assumptions that they necessarily act by histone modification. In an extension of previous studies showing a role for acetyltransferase p300/CBP in p53 function, we have used systems reconstituted with recombinant chromatin templates and (co)activators to demonstrate (1) the additional involvement of protein arginine methyltransferases PRMT1 and CARM1 in p53 function; (2) both independent and ordered cooperative functions of p300, PRMT1, and CARM1; and (3) mechanisms that involve direct interactions with p53 and, most importantly, obligatory modifications of corresponding histone substrates. ChIP analyses have confirmed the ordered accumulation of these (and other) coactivators and cognate histone modifications on the *GADD45* gene following ectopic p53 expression and/or UV irradiation. These studies thus define diverse cofactor functions, as well as underlying mechanisms involving distinct histone modifications, in p53-dependent gene activation.

Introduction

The gene encoding p53 is the most frequently mutated gene in human cancers, and p53 itself is a potent tumor suppressor that, in response to various cell stress signals, activates cell cycle checkpoints that arrest cell growth or apoptotic pathways that lead to cell death (Vogelstein et al., 2000). These functions are mediated largely through the action of p53 as a conventional DNA binding transcriptional activator of genes whose products regulate growth arrest or apoptosis. p53 contains an N-terminal activation domain, a central DNA binding domain, and a C-terminal regulatory domain (Ko and Prives, 1996). Stress-induced modifications of p53 variously implicated in protein stability and/or transcriptional activation include phosphorylation, acetylation, and ubiquitylation (Brooks and Gu, 2003).

Like many other transcriptional activators, p53 has been shown to act both through cofactors (e.g., TRAP/Mediator) that facilitate preinitiation complex formation or function (Gu et al., 1999) and through cofactors that modify chromatin structure. In the latter case, earlier demonstrations of physical and functional interactions between p53 and histone acetyltransferases (HATs) p300 and CBP have been confirmed and extended by recent studies showing p53-dependent recruitment of p300, as well as targeted acetylation, to p53 target genes (Barlev et al., 2001; Espinosa and Emerson, 2001). A distinct TRRAP- and Ada2-containing HAT complex(es), potentially containing GCN5, PCAF, or TIP60, has also been implicated in p53 function (Ard et al., 2002; Wang et al., 2001b). These results are consistent with many other studies that generally correlate histone acetylation with gene activation (Roth et al., 2001), although they do not prove, as generally assumed, that histones are obligate HAT targets.

Recent studies also have correlated histone lysine and arginine methylation events with gene activation (reviewed in Stallcup [2001]). Of special relevance to the present study are the protein arginine methyltransferases PRMT1 and CARM1, which, respectively, methylate H4-R3 and H3-R2, -R17, and -R26 (Strahl et al., 2001; Wang et al., 2001a). Stallcup and colleagues have demonstrated functional synergy between histone acetyltransferases and protein arginine methyltransferases, although the key protein targets responsible for the synergy were not defined (Koh et al., 2001; Lee et al., 2002). In relation to a possible mechanism involving histone modification, studies with isolated histones have shown that premethylation of H4 by PRMT1 stimulates acetylation by p300 (Wang et al., 2001a), whereas preacetylation of an H3 peptide by p300 stimulates methylation by CARM1 (Daujat et al., 2002). These results are reminiscent of similar cooperativity between histone acetylation and phosphorylation events in H3 (reviewed in Zhang and Reinberg [2001]).

To study the role of diverse histone-modifying cofactors in p53 function, we have used a cell-free system reconstituted with purified cofactors and recombinant chromatin templates (An et al., 2002). This system allows analyses of selectively mutated histones, direct versus indirect effects of cofactors, independent versus cooperative effects of different cofactors, and corresponding mechanisms on individual genes. In the case of p300-dependent activation by Gal4-VP16, earlier studies with this system demonstrated a repression mechanism that is intrinsic to the structure of the nucleosome core and selective requirements for H3 and H4 N-terminal tails, as well as corresponding acetylation events that are targeted to promoter proximal regions, for transcriptional activation (An et al., 2002). The present study shows independent and cooperative effects of p300, PRMT1, and CARM1 in mediating activation by p53 from a chromatin template with GADD45-derived p53 response elements, whereas chromatin immunoprecipitation (ChIP) assays have verified the accumulation of these (and other) cofactors and corresponding modifications on a natural GADD45 gene in response to UV damage.

*Correspondence: roeder@mail.rockefeller.edu

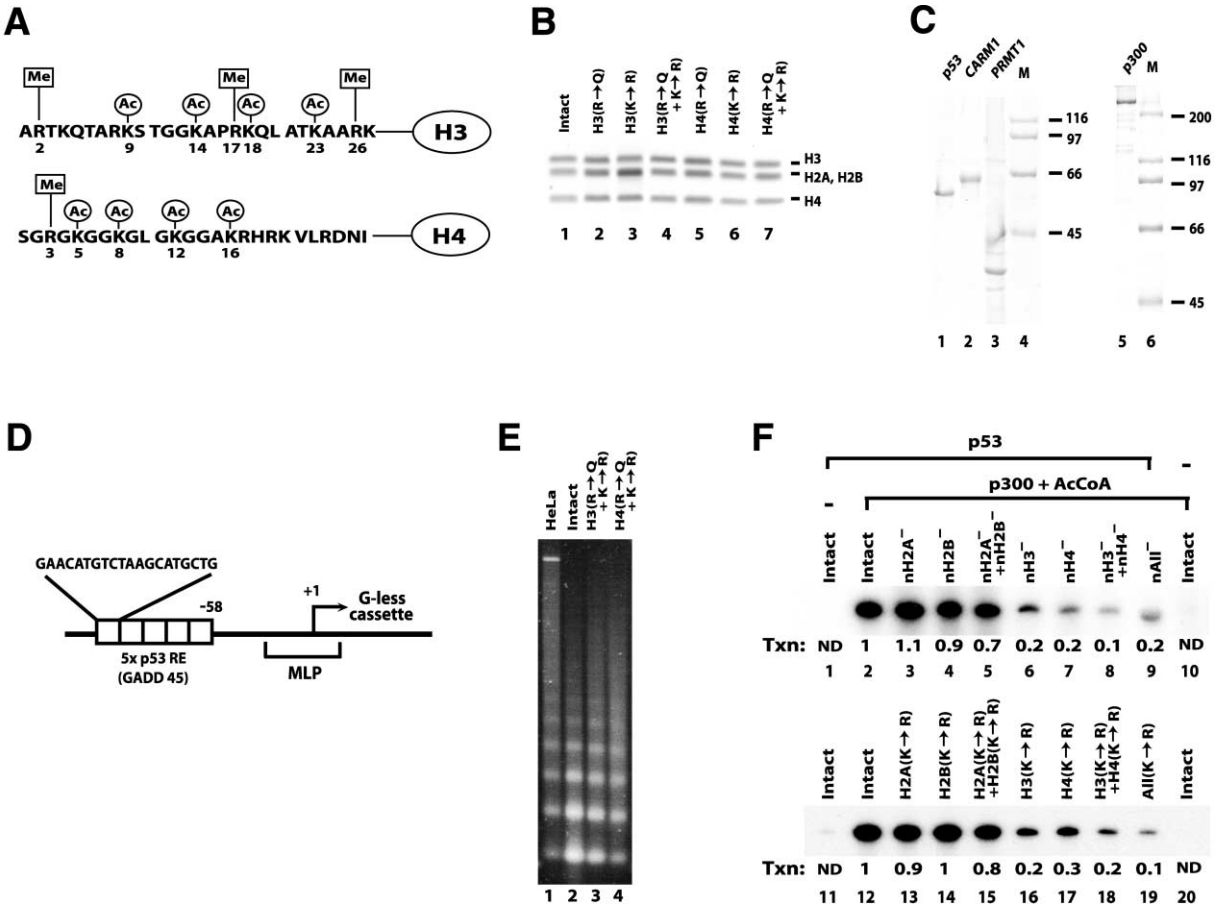


Figure 1. Recombinant Histones, Cofactors, and Histone Tail-Dependent Function of p300

(A) Schematic summary of histone tail sequences. "Me" indicates sites that are methylated by CARM1 and PRMT1 in H3 and H4, respectively. "Ac" indicates sites of acetylation. In any given histone mutant (B), all methylation or acetylation sites were collectively mutated.

(B) Analysis of reconstituted histone octamers by 15% SDS-PAGE and Coomassie blue staining. Singly or doubly mutated histones within the octamer are indicated at the top.

(C) Analysis of purified (co)activators by 10% SDS-PAGE and Coomassie blue staining.

(D) Schematic representation of the p53 binding sites adjacent to the AdML promoter in the pML array template.

(E) Micrococcal nuclease (MNase) analysis of assembled chromatin. Assembled chromatin was partially digested with MNase, and the recovered DNA was detected as described (An and Roeder, 2004).

(F) p53- and p300-dependent transcription from recombinant chromatin with intact and mutant histones. Chromatin templates contained singly or doubly mutated histones that lacked entirely the indicated tail(s) or contained the indicated K to R mutations. Mutant histones are as described in (A) and in An et al. (2002). Txn, relative transcription levels; ND, nondetectable.

Results

Assembly and Characterization of Recombinant Chromatin Templates and Cofactors

Chromatin was assembled from recombinant histone octamers (Figure 1B) and a plasmid DNA template (Figure 1D) using a recombinant ACF/NAP1 system (Ito et al., 1999) as described previously (An et al., 2002). In some cases, H3 and H4 were mutated in lysine residues known to be acetylated in vivo (H3 and H4), in an arginine residue (H4-R3) methylated by PRMT1, or in arginine residues (H3-R2, -R17, and -R26) methylated by CARM1 in order to assess the role of corresponding modifications in cofactor function (Figures 1A and 1B). Characterization of assembled chromatin by micrococcal nuclease digestion revealed a 200 bp ladder of kinetic intermediates (Figure 1E). The recombinant p53 (expressed in bacteria), p300 (expressed via baculovirus), CARM1 (expressed via baculovirus) and PRMT1 (expressed in bacteria) proteins utilized in conjunction with this template are shown in Figure 1C.

We initially determined requirements for core histone tails and corresponding acetylation events for p300-dependent activation by p53. As shown in Figure 1F, transcription from the template with intact histones was dependent upon p53 and p300/acetyl-CoA (lane 2 versus lanes 1 and 10). The transcription level was unaffected by independent or concomitant deletion of H2A and H2B tails (lanes 3–5) but dramatically reduced by independent or concomitant deletion of H3 and H4 tails or by removal of all tails (lanes 6–9). Parallel results were observed when the acetylatable lysines in corresponding histone tails were mutated to arginines (lanes 13–19). These results exactly mirror those reported for p300-

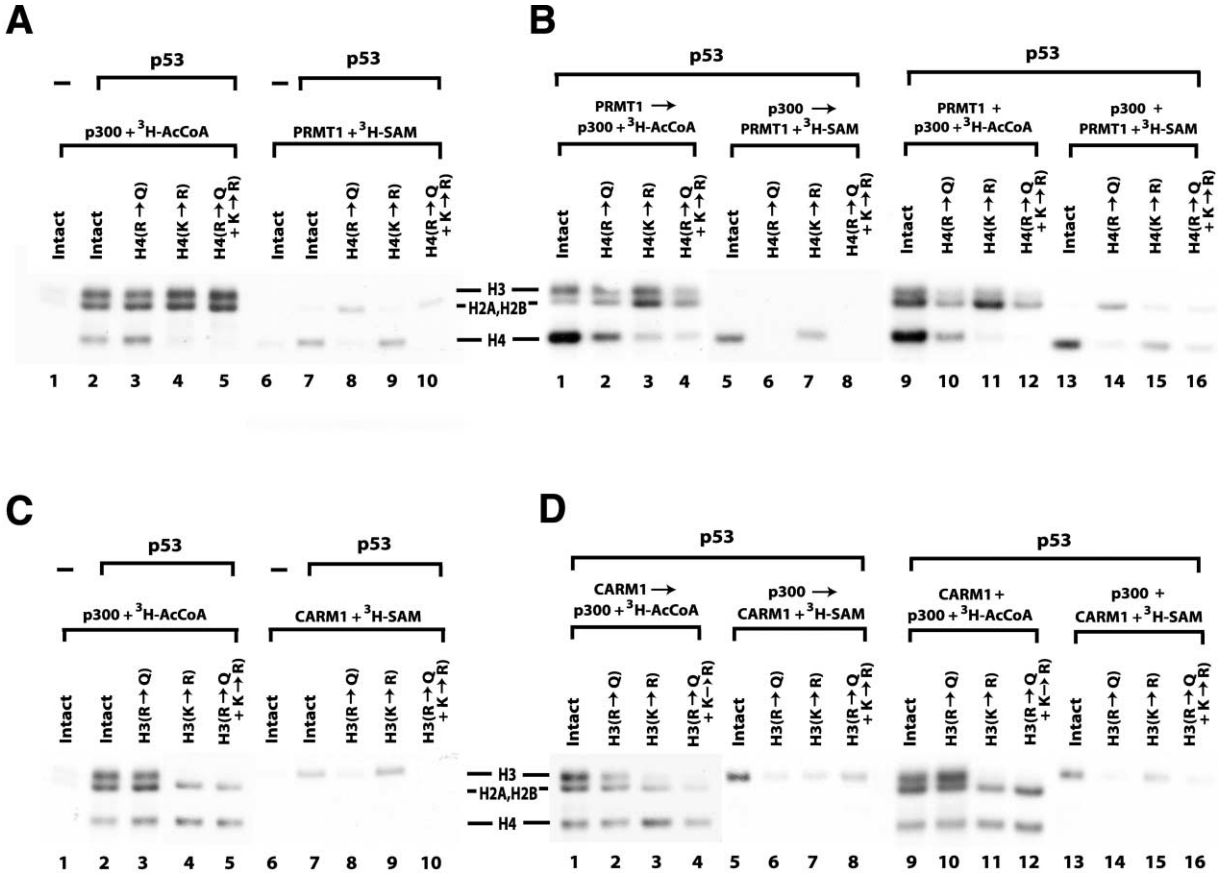


Figure 2. p53-Dependent Modifications of Nucleosomal Histones by Independent and Cooperative Functions of p300, CARM1, and PRMT1

Modification assays using [^{3}H]-SAM (S-adenosyl-L-[methyl-^{3}H]methionine) or [^{3}H]-acetyl-CoA were performed essentially as described (An and Roeder, 2004). All assays in (A) and (B) and in (C) and (D) were conducted simultaneously under identical conditions, such that the signals are directly comparable. (A) Independent modifications of nucleosomal H4 by p300 or by PRMT1. (B) Concomitant or sequential modifications of nucleosomal H4 by p300 and PRMT1. p300 and PRMT1 were incubated simultaneously (30 min) or sequentially (30 min for each) with chromatin templates in the presence of p53 and labeled/unlabeled acetyl-CoA and SAM, as indicated. (C) Independent modifications of nucleosomal H3 by p300 or by CARM1. (D) Concomitant or sequential modifications of nucleosomal H3 by p300 and CARM1. p300 and CARM1 were incubated with chromatin templates in the presence of p53 and labeled/unlabeled acetyl-CoA and SAM as described in Figure 2B.

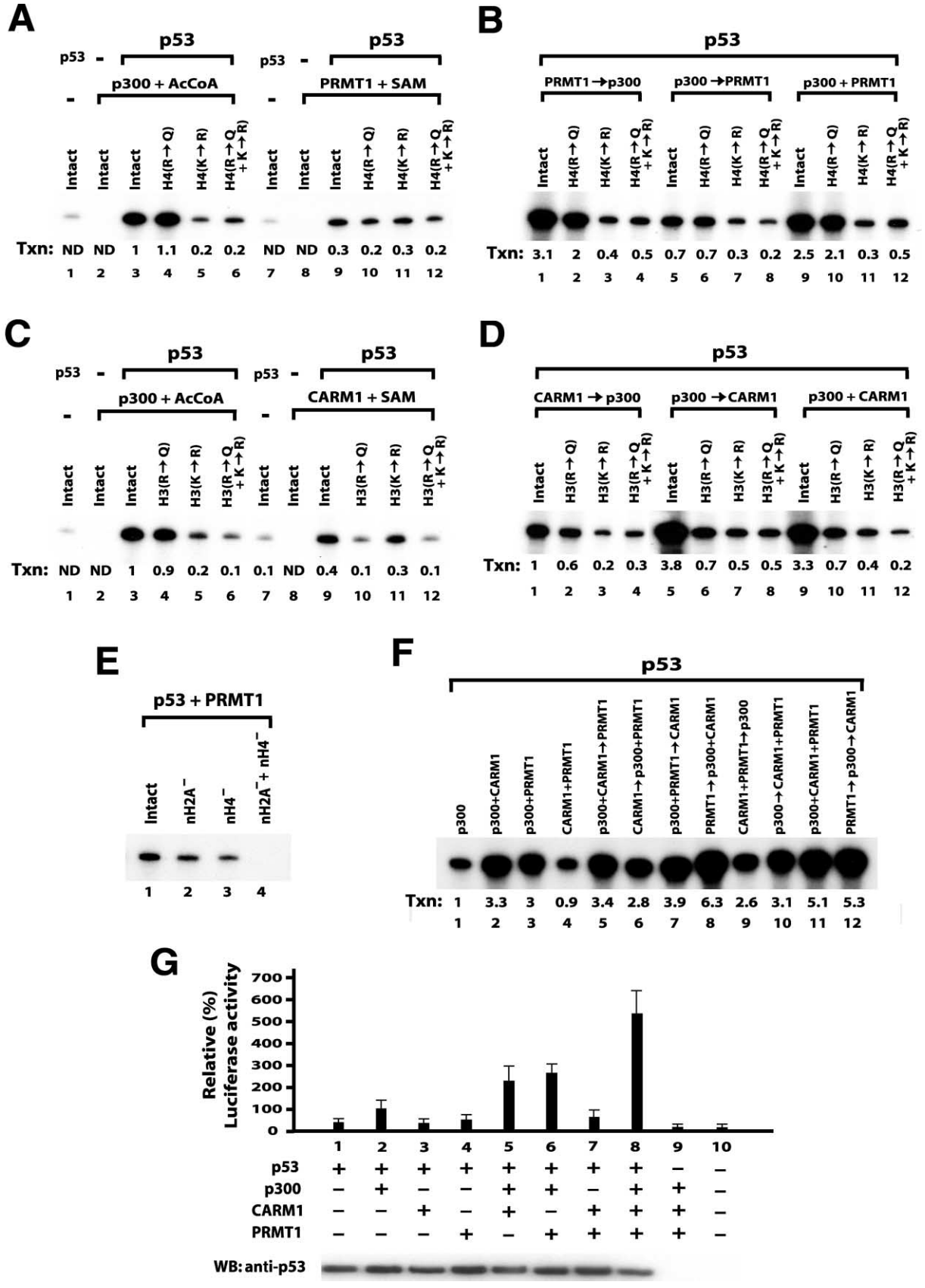
dependent transcription activation by Gal4-VP16 on a similar template (An et al., 2002). They argue for the generality of a selective requirement for H3 and H4 tails, and corresponding acetylation events, for p300-dependent transcription activation from these in vitro assembled chromatin templates.

Independent p53-Dependent Modifications of Nucleosomal Histones by p300, PRMT1, or CARM1

p53-dependent histone modifications by cofactors were monitored following independent incubation of templates with various cofactors and corresponding radiolabled substrates. As shown in Figures 2A and 2C, all core histones were acetylated by p300 in a p53-dependent manner (lanes 1 and 2), consistent with previous in vitro results of Espinosa and Emerson (2001), and modifications in H3 and H4 were blocked by K to R but not R to Q mutations (lanes 3–5). H4 and H3 were selectively methylated by PRMT1 and CARM1, respectively, in a p53-dependent manner (lanes 6 and 7), and corresponding R to Q but not K to R mutations blocked these modifications (lanes 8–10). Notably, however, H2A was methylated by PRMT1 when the normal PRMT1 methylation site (R3) in H4 was mutated (Figure 2A, lane 8), suggesting that H2A might serve as a back-up substrate in a PRMT1-mediated signaling pathway (Cheung et al., 2000). These results document p53-dependent modifications of nucleosomal histones by PRMT1 and CARM1 as well as p300 and further confirm that these modifications occur at specific lysine and arginine residues in H3 and H4.

Cooperativity between p300 and PRMT1 or CARM1 in Modifying Nucleosomal Histones

Consistent with earlier results with isolated H4 (Wang et al., 2001a), nucleosomal H4 premethylation by PRMT1 selectively stimulated H4 acetylation by p300 (Figure 2B, lane 1, versus Figure 2A, lane 2). This stimulatory effect of PRMT1 was lost upon mutation of the major PRMT1 methylation site in H4 (Figure 2B, lane 2 versus lane 1), indicating an effect of the modification, per se. Similarly, nucleosomal histone acetylation by p300 mod-


A
p53
p53 - p53 -
p300 + AcCoA
- PRMT1 + SAM
Intact Intact Intact H4(R→Q) H4(K→R) H4(R→Q + K→R) Intact Intact Intact H4(R→Q) H4(K→R) H4(R→Q + K→R)
Txn: ND ND 1 1.1 0.2 0.2 ND ND 0.3 0.2 0.3 0.2
1 2 3 4 5 6 7 8 9 10 11 12
B
p53
PRMT1→p300 p300→PRMT1 p300 + PRMT1
Intact H4(R→Q) H4(K→R) H4(R→Q + K→R) Intact H4(R→Q) H4(K→R) H4(R→Q + K→R) Intact H4(R→Q) H4(K→R) H4(R→Q + K→R)
Txn: 3.1 2 0.4 0.5 0.7 0.7 0.3 0.2 2.5 2.1 0.3 0.5
1 2 3 4 5 6 7 8 9 10 11 12
C
p53
p53 - p53 -
p300 + AcCoA
- CARM1 + SAM
Intact Intact Intact H3(R→Q) H3(K→R) H3(R→Q + K→R) Intact Intact Intact H3(R→Q) H3(K→R) H3(R→Q + K→R)
Txn: ND ND 1 0.9 0.2 0.1 0.1 ND 0.4 0.1 0.3 0.1
1 2 3 4 5 6 7 8 9 10 11 12
D
p53
CARM1→p300 p300→CARM1 p300 + CARM1
Intact H3(R→Q) H3(K→R) H3(R→Q + K→R) Intact H3(R→Q) H3(K→R) H3(R→Q + K→R) Intact H3(R→Q) H3(K→R) H3(R→Q + K→R)
Txn: 1 0.6 0.2 0.3 3.8 0.7 0.5 0.5 3.3 0.7 0.4 0.2
1 2 3 4 5 6 7 8 9 10 11 12
E
p53 + PRMT1
Intact nH2A− nH4− nH2A−+ nH4−
1 2 3 4
F
p53
p300 p300+CARM1 p300+PRMT1 CARM1+PRMT1 p300+CARM1→PRMT1 CARM1→p300+PRMT1 p300+PRMT1→CARM1 PRMT1→p300+CARM1 CARM1+PRMT1→p300 p300→CARM1+PRMT1 p300+CARM1+PRMT1 PRMT1→p300→CARM1
Txn: 1 3.3 3 0.9 3.4 2.8 3.9 6.3 2.6 3.1 5.1 5.3
1 2 3 4 5 6 7 8 9 10 11 12
G
Relative (%) Luciferase activity
700 600 500 400 300 200 100 0
1 2 3 4 5 6 7 8 9 10
p53 + + + + + + + + − −
p300 − + − − + + − + + −
CARM1 − − + − + − + + + −
PRMT1 − − − + − + + + + −
WB: anti-p53

erately stimulated H4 methylation by PRMT1 (Figure 2B, lane 5, versus Figure 2A, lane 7). This p300 stimulatory effect was lost when the acetylation sites in H4 were mutated (Figure 2B, lane 5 versus lane 7), indicating that the stimulatory effect is dependent upon H4 acetylation events. Similar effects of comparable or greater magnitude were observed following simultaneous incubation of p300 and PRMT1 (Figure 2B, lanes 9–16, versus Figure 2A) with nucleosomal histone templates.

In parallel experiments with p300 and CARM1 and consistent with results of studies with an H3 peptide (Daujat et al., 2002), nucleosomal H3 preacetylation by p300 significantly stimulated H3 methylation by CARM1 (Figure 2D, lane 5, versus Figure 2C, lane 7). By contrast, H3 premethylation by CARM1 had no effect on subsequent H3 acetylation by p300 (Figure 2D, lane 1, versus Figure 2C, lane 2). The effect of p300 on H3 methylation by CARM1 is dependent upon H4 acetylation events, since the effect was lost when all major acetylation sites were mutated (Figure 2D, lane 5 versus lane 7). Somewhat milder effects of histone preacetylation on methylation by CARM1 were observed when CARM1 and p300 were simultaneously incubated with nucleosomal templates (Figure 2D, lane 13, versus Figure 2C, lane 7), suggesting that the optimal effect of p300 on CARM1-mediated modification may depend on the prior action of p300.

Although these studies are generally consistent with prior studies of modifications of isolated histones (or derived peptides) by p300, CARM1, and PRMT1 (Daujat et al., 2002; Wang et al., 2001a), it is important to note that the cooperative effects observed here are manifest in the more physiological context of nucleosomal substrates. Further, all the modifications are dependent upon the presence of p53.

Independent Effects of p300, PRMT1, and CARM1 on p53-Dependent Transcription

In contrast to transfection assays that do not allow an effect of a given coactivator to be ascribed to an independent action, owing to the presence of other endogenous cofactors, the in vitro assay with isolated cofactors and mutated histone substrates does allow this determination. In the recombinant chromatin transcription assay, a high level of p53- and coactivator-dependent activity was observed with p300 (Figures 3A and 3C, lanes 1-3), with PRMT1 (Figure 3A, lanes 7–9), and with CARM1 (Figure 3C, lanes 7–9). Moreover, the p300-dependent activity was dramatically reduced by H3 and H4 K to R mutations, consistent with the results in Figure 1F, but not by R to Q mutations (Figures 3A and 3C, lanes 4–6). CARM1-dependent activity was significantly reduced by H3 R to Q mutations but not by H3 K to R mutations (Figure 3C, lanes 10–12). These results argue that the corresponding histone acetylation and methylation events are essential, respectively, for p300 and CARM1 function (i.e., that histone tails are obligatory substrates). The PRMT1-dependent activity was unaffected by H4 K to R mutations and, somewhat surprisingly, only moderately affected by the H4 R to Q mutation (Figure 3A, lanes 10–12). That this latter result reflects the proposed back-up function of H2A, which is modified only when H4 is mutated (Figure 2A, lanes 7 and 8), is indicated by the complete loss of transcription upon joint H2A and H4 tail deletions but not upon individual tail deletions (Figure 3E).

These results are consistent with the abilities (Figure 2) of p300, CARM1, and PRMT1 to independently effect histone modifications in a p53-dependent manner. Moreover, the mutational analyses rule out potential contributions of endogenous histone acetyltransferases or endogenous histone arginine methyltransferases, in the nuclear extract-based transcription assay, on functions of purified PRMT1 and CARM1 or purified p300, respectively. Also of note is that while the tested coactivators elicit p53-dependent activity far above the background level, their absolute levels of activity differ (p300 $>$ CARM1 $>$ PRMT1).

Cooperative Effects between p300 and CARM1 or PRMT1 on p53-Dependent Transcription

Given demonstrated cooperativity between p300 and CARM1 in mediating nuclear receptor function (Koh et al., 2001; Lee et al., 2002), as well as cooperativity between p53-dependent acetylation and methylation events (Figure 2), we next assessed p53-dependent transcription from recombinant chromatin templates following simultaneous incubation with p300 and PRMT1 or p300 and CARM1. The p53-dependent activity observed with p300 and PRMT1 was significantly greater than the sum of the independent p300 and PRMT1 activities (Figure 3B, lane 9, versus Figure 3A, lanes 3 and 9). H4 K to R mutations reduced this activity to a level comparable to that observed with PRMT1 alone, while

Figure 3. p53-Dependent Transcription from Chromatin Templates in Response to Independent and Cooperative Functions of p300, PRMT1, and CARM1

Transcription was carried out following prior histone modifications by independent (20 min), simultaneous (20 min), or sequential (20 min each) incubation of chromatin templates and coactivators, as indicated, in the presence of p53 and acetyl-CoA and/or SAM as appropriate (An and Roeder, 2004). Data were quantitated by phosphorimager, and averaged results from three independent experiments are shown under a single representative autoradiographic analysis. Txn, relative transcription; ND, nondetectable. All transcriptions in (A) and (B) and in (C) and (D) were conducted simultaneously under identical assay conditions, such that the signals are directly comparable. All values in (A) and (B) are normalized to lane 3 in (A), and all values in (C) and (D) are normalized to lane 3 in (C). (A) Independent functions of p300 and PRMT1 in mediating p53-dependent transcription. (B) Cooperative and sequential function of PRMT1 and p300 in mediating p53-dependent transcription. (C) Independent functions of p300 and CARM1 in mediating p53-dependent transcription. (D) Cooperative and sequential functions of CARM1 and p300 in mediating p53-dependent transcription. (E) Functional complementarity between H4 and H2A tails in mediating PRMT1 function. (F) Combined effects of PRMT1, p300, and CARM1 on p53-dependent transcription. Prior to transcription, chromatin templates were incubated simultaneously (30 min) or sequentially (20 min each) with coactivators and p53 as indicated. (G) The effects of ectopic PRMT1, p300, and CARM1 on intracellular p53-dependent transcription. p53-deficient H1299 cells were transfected with luciferase reporter with the p53 response element from the *GADD45* gene and expression vectors for p53 and coactivators, as indicated.

the H4 R to Q mutations had only a modest effect on the activity because of the apparent back-up effect of H2A (above) (Figure 3B, lanes 10–12). Addition of PRMT1 prior to p300 resulted in an even greater activity than that observed with simultaneous addition of both coactivators (Figure 3B, lane 1), while addition of p300 prior to PRMT1 resulted in a dramatically lower level of activity (Figure 3B, lane 5). These results are consistent with the results of the histone modification analyses (Figure 2) and suggest that, for optimal activity, PRMT1 modification of nucleosomal histones must precede modifications by p300.

In similar experiments with p300 and CARM1, the activity observed following simultaneous addition was significantly higher than the sum of the independent p300 and CARM1 activities (Figure 3D, lane 9, versus Figure 3C, lanes 3 and 9). H3 R to Q mutations reduced this activity to a level comparable to that observed with p300 alone (Figure 3D, lane 10, versus Figure 3C, lane 3), while H3 K to R mutations reduced the activity to a level comparable to that observed with CARM1 alone (Figure 3D, lane 11, versus Figure 3C, lane 9). Significantly, addition of p300 prior to CARM1 resulted in an even greater activity than that observed upon simultaneous addition (Figure 3D, lane 5 versus lane 9), while the level of transcription was dramatically lower (equivalent to the p300-only level) when incubation with CARM1 preceded incubation with p300 (Figure 3D, lane 1 versus lane 9). These results are consistent with the histone modification analyses (above) and suggest that, for optimal activation, p300 action must precede CARM1 action.

We next investigated the joint effects of p300, CARM1, and PRMT1 on p53-dependent transcription when added according to the various protocols indicated at the top of Figure 3F. Levels of activity approximately 2-fold above those observed with p300 plus CARM1 (lane 2) or p300 plus PRMT1 (lane 3) were observed when all three coactivators were added simultaneously (lane 11), in the sequence PRMT1→p300→CARM1 (lane 12), or in combinations and orders (lanes 7 and 8) consistent with the results of Figures 3B and 3D. Other combinations and orders of addition (lanes 5, 6, 9, and 10) only gave levels of activity equivalent to those observed with pairwise combinations of p300 and PRMT1 or CARM1. Also of note, PRMT1 and CARM1 together showed no significant synergy in the absence of p300 (lane 4). Hence, the presence of all these cofactors results in higher levels of p53-dependent activity than are observed with any pairwise combination of cofactors.

Cooperative Effects of p300, PRMT1, and CARM1 In Vivo

To gain support for the relevance of the in vitro findings to intracellular gene activation by p53, p53-deficient H1299 cells were transfected with a luciferase reporter gene bearing p53 response elements from the *GADD45* gene and vectors variously expressing p53, p300, CARM1, and PRMT1. As shown in Figure 3G, p53 expression produced a moderate increase in activity that was enhanced about 3-fold by ectopic expression of p300 alone (lane 2) but not by ectopic expression of CARM1 and PRMT1 alone or together (lanes 3, 4, and 7). In contrast, expression of CARM1 or PRMT1 with p300 increased activity several-fold over that observed with p300 alone (lanes 5 and 6 versus lane 2), while the joint expression of p300, CARM1, and PRMT1 increased activity a further 2-fold (lane 8). No significant activity was observed with all three coactivators in the absence of p53 (lane 9). These results showing functional synergy between coactivators on a p53-responsive promoter in vivo are consistent with and strongly support the in vitro data. Collectively, our results indicate an ordered accumulation of distinct histone modifications in response to PRMT1, p300, and CARM1 and a functional association with p53-dependent transcription.

CARM1 and PRMT1, Like p300, Bind Directly to p53

To test the possibility that the independent functions of the coactivators might reflect direct interactions with p53, we examined interactions of purified coactivators with GST-p53 fusion proteins (Figure 4A). PRMT1 and p300 both showed direct interactions with N-terminal fragments (residues 1–43 and 1–83) of p53, although it appears that PRMT1 may interact preferentially with residues 1–43 and p300 with residues 44–83 (Figures 4B and 4C). PRMT1 and p300 showed similar interactions when assayed together (Figure 4D). CARM1 showed interaction exclusively with the C terminus of p53 (residues 300–393) but with a strong dependency on residues 370–393 (Figure 4E). When assayed together with p300, CARM1 showed a similar interaction with the p53 C-terminal fragment, although p300 now showed binding both to C-terminal and N-terminal fragments of p53 (Figure 4F). This latter result suggests an interaction between CARM1 and p300, as recently reported by Xu et al. (2001). Most importantly, all these cofactors show direct p53 interactions as originally shown for p300/CBP (Gu et al., 1997; Scolnick et al., 1997).

To document coactivator interactions with p53 in vivo, anti-Flag-p53 immunoprecipitates from H1299 cells coexpressing Flag-p53 proteins and individual coactivators were analyzed by Western blot. As shown in Figure 5, p300 (lanes 1 and 2), CARM1 (lanes 5 and 6), and PRMT1 (lanes 9 and 10) were all coimmunoprecipitated in a Flag-p53-dependent manner. Consistent with the in vitro binding data, a p53 C-terminal deletion mutant (lacking residues 351–393) showed markedly lower CARM1 binding (lane 8) but a wild-type level of PRMT1 binding (lane 12), whereas a p53 N-terminal deletion mutant (lacking residues 1–80) showed a wild-type level of CARM1 binding (lane 7) but no PRMT1 binding (lane 11). Somewhat surprisingly, the same N- and C-terminal deletion mutants both showed wild-type levels of p300 binding (lanes 3 and 4). This may indicate the presence in p53 of p300 interaction sites not scored in previous studies or p300 interactions through intermediate p53 binding proteins. As indicated in the control analyses (lanes 13–48), these results cannot be attributed to differential levels of expression of cofactors or to differential levels of expression or immunoprecipitation of intact and mutant forms of p53. These results thus document in vivo interactions of p53 with the various coactivators (see also below), whereas the results of Figure 4 argue that most of these interactions may be direct.

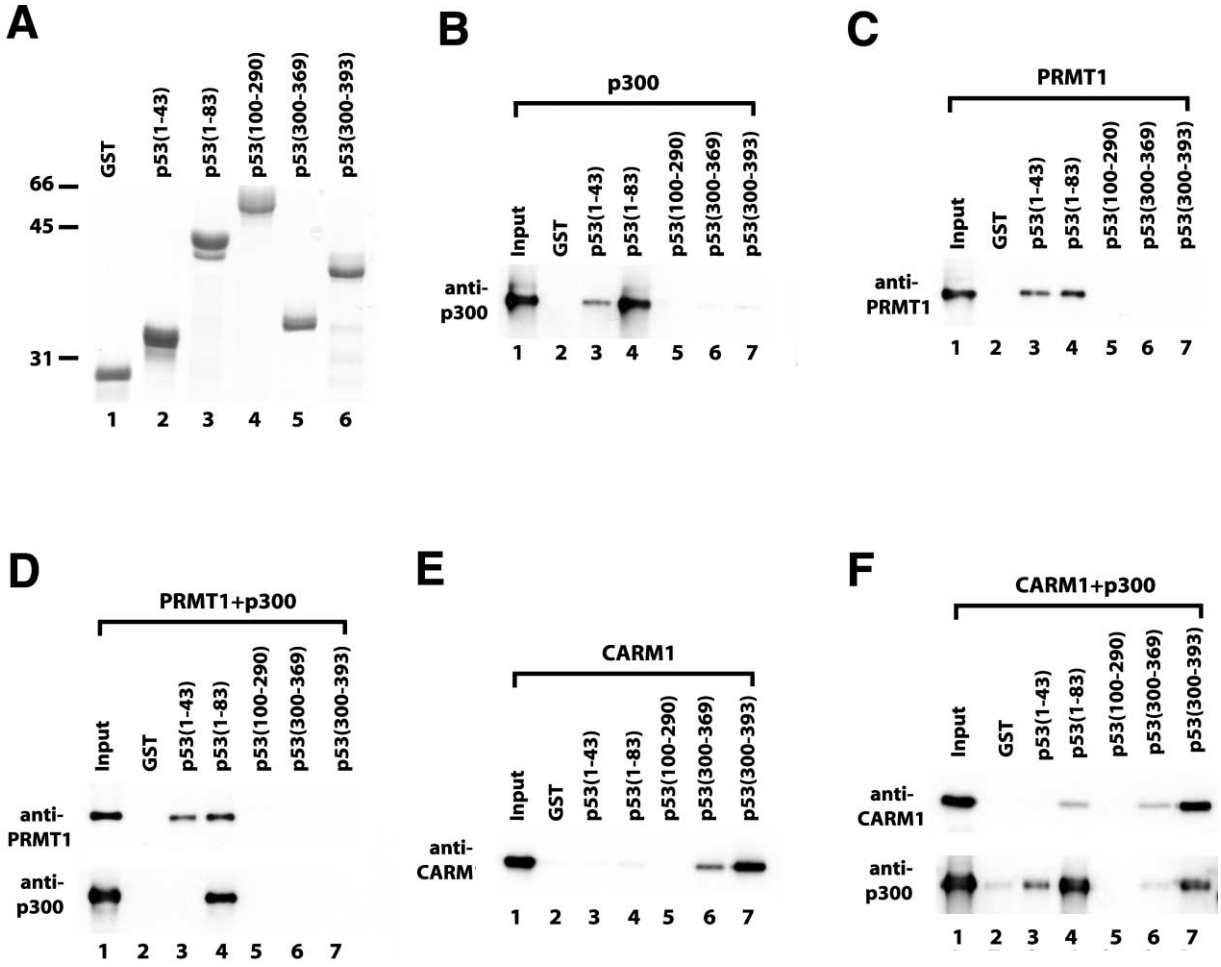


Figure 4. Direct Interactions of PRMT1, p300, and CARM1 with p53 In Vitro

Recombinant p300, PRMT1, and CARM1 were tested for binding to GST or GST-p53 fusion proteins. Bound proteins were scored by immunoblot. In each case, 5% of the input protein(s) is shown. (A) Purified GST-p53 fusion proteins. (B) Independent p300 interactions with p53 derivatives. (C) Independent PRMT1 interactions with p53 derivatives. (D) Joint interactions of PRMT1 and p300 with p53 derivatives. (E) Independent CARM1 interactions with p53 derivatives. (F) Joint interactions of CARM1 and p300 with p53 derivatives.

p53-Dependent Coactivator Recruitment and Cognate Histone Modifications on the *GADD45* Gene

The results of the in vitro/in vivo interactions and transcription assays (above) suggested that p53 may facilitate recruitment of these cofactors to a p53-responsive gene such as *GADD45* in vivo. To test this possibility, p53-deficient H1299 cells were transiently transfected with p53 and subjected to chromatin immunoprecipitation (ChIP) analyses (Figure 6). The analysis in Figure 6B shows low levels of H3 and H4 acetylation on *GAPDH* (control) and on the *GADD45* distal region that are largely unaffected following p53 expression, whereas low levels of H3 lysine and H3/H4 arginine methylation are induced by p53 in the distal *GADD45* region. In contrast, greatly increased levels of H3 and H4 acetylation; H3-R17, H4-R3, and H3-K4 methylation; and p53, p300, CARM1, and PRMT1 proteins were observed on the *GADD45* p53-responsive element following ectopic expression of p53 (Figure 6B). Thus, these analyses show a targeted recruitment of p300, CARM1, and PRMT1, as well as targeted accumulation of corresponding histone acetylation and methylation marks, on the natural *GADD45* gene in response to p53 overexpression. These results, suggesting corresponding roles in transcription of the *GADD45* gene in vivo, are consistent with the in vitro data. The in vivo ChIP analysis also shows targeted, p53-dependent accumulation of the H3-K4 methylation mark (Figure 6B), which appears to be a general mark for active genes (Sims et al., 2003).

A similar analysis (Figure 6C) with a p53 mutant lacking N-terminal residues 1–80 showed levels of p53 (lane 6), CARM1 (lane 8), H3-R17 methylation (lane 3), and H3 acetylation (lane 1) at the GADD45 PRE comparable to those observed with wild-type p53 but markedly reduced levels of PRMT1 (lane 9), p300 (lane 7), H4-R3 methylation (lane 4), H3-K4 methylation (lane 5), and H4 acetylation (lane 2). A similar analysis with a p53 mutant lacking C-terminal residues 351–393 showed a reduced but nonetheless significant level of p53 binding to the PRE relative to wild-type p53 (lane 15). However, when normalized to the level of GADD45-associated mutant p53,

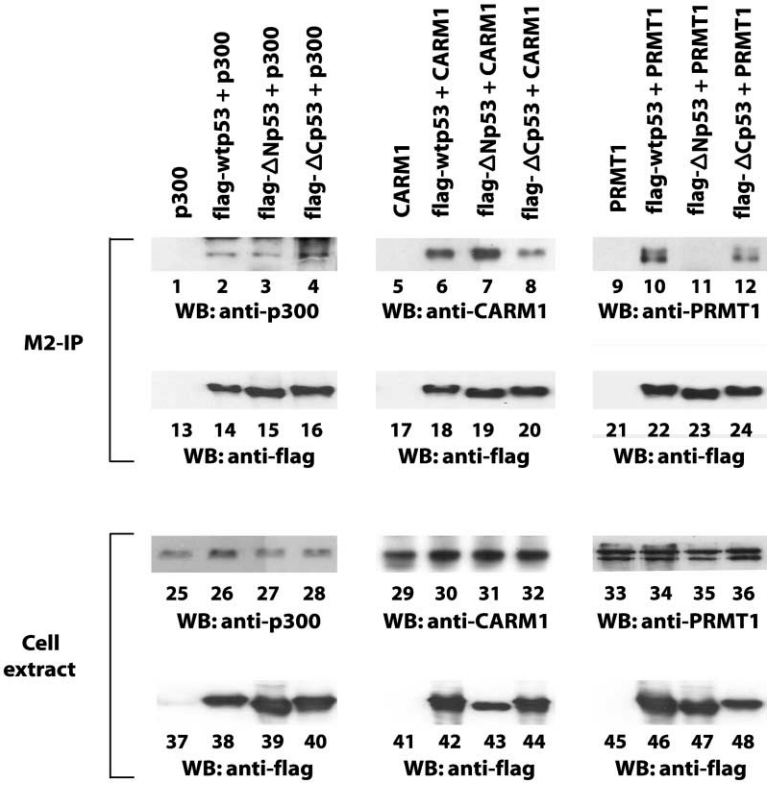


Figure 5. Interactions of PRMT1, p300, or CARM1 with p53 In Vivo

Flag-tagged wild-type or mutant p53 was transiently coexpressed with p300, PRMT1, or CARM1 in H1299 cells. Cell extracts were incubated with M2-agarose, and immunoprecipitated proteins (M2-IP) were analyzed by Western blot using p300, PRMT1, CARM1, or Flag antibodies as indicated. The cell extract represents 1/10 volume of the input used for immunoprecipitation.

the levels of PRMT1 (lane 18), p300 (lane 16), H4-R3 methylation (lane 13), H3-K4 methylation (lane 14), H3 acetylation (lane 10), and H4 acetylation (lane 11) associated with the GADD45 PRE were nearly normal (or elevated), while those of CARM1 and H3-R17 methylation were markedly reduced. These results strongly support earlier indications (Figures 4 and 5) that the p53 C terminus plays the dominant role in PRE recruitment and function of CARM1, whereas the N terminus plays the major role in PRE recruitment and function of PRMT1, p300, and the unidentified cofactor effecting H3-K4 methylation. The results also show that H4 acetylation but not H3 acetylation is correlated with p300 recruitment to the GADD45 PRE and, further, that the cofactor responsible for enhanced H3 acetylation may be recruited through a distinct (internal) p53 domain.

Temporal Accumulation of Coactivators and Histone Modifications on the p53-Induced *GADD45* Gene upon DNA Damage

To further validate the conclusions from the in vitro and in vivo studies described above, the temporal accumulation of various coactivators and histone modifications on the *GADD45* gene were monitored following UV irradiation of U2OS cells. UV irradiation resulted in large increases (from low basal levels) in p53 protein and *GADD45* mRNA levels by two hours, with further increases over the next several hours (Figure 7). ChIP assays over the same time period showed an elevated (and maximal) accumulation of p53, as well as R3-methylated and acetylated H4, by two hours. p300 also showed maximal accumulation in the PRE by this time and presumably mediates the H4 acetylation. Although PRMT1 accumulation could not be detected in these cells by ChIP analysis, possibly due to transient interactions of PRMT1 and/or lower p53 levels in U2OS-irradiated cells relative to p53-transfected H1299 cells (Figure 7), other studies indicate that H4-R3 methylation is mediated mainly by PRMT1 (Strahl et al., 2001). These results are consistent with the H4-lysine acetylation/H4-R3 methylation-dependent synergy of PRMT1 and p300 observed in vitro.

Also consistent with the in vitro transcription results indicating CARM1 function subsequent to PRMT1 and p300 function, enhanced CARM1 accumulation on the PRE was first evident, and maximal, at 4 hr and declined thereafter. Somewhat surprisingly, the major histone modification (H3-R17 methylation) ascribed to CARM1 was barely detectable at 4 hr but increased continuously over the next few hours. Whether this apparent discontinuity between the presence of maximal levels of CARM1 and its modifications truly reflects delayed modifications (due perhaps to synergy with another modification/enzyme) or a delayed epitope unmasking remains unclear.

Also of significance is the greatly enhanced accumulation, by four hours of irradiation, of GCN5, acetylated H3, and K4-methylated H3. Along with previous indications that GCN5 preferentially acetylates nucleosomal H3 in vitro (Roth et al., 2001), and consistent with the data in Figure 6C, these observations suggest that the enhanced accumulation of acetylated H3 is due at least

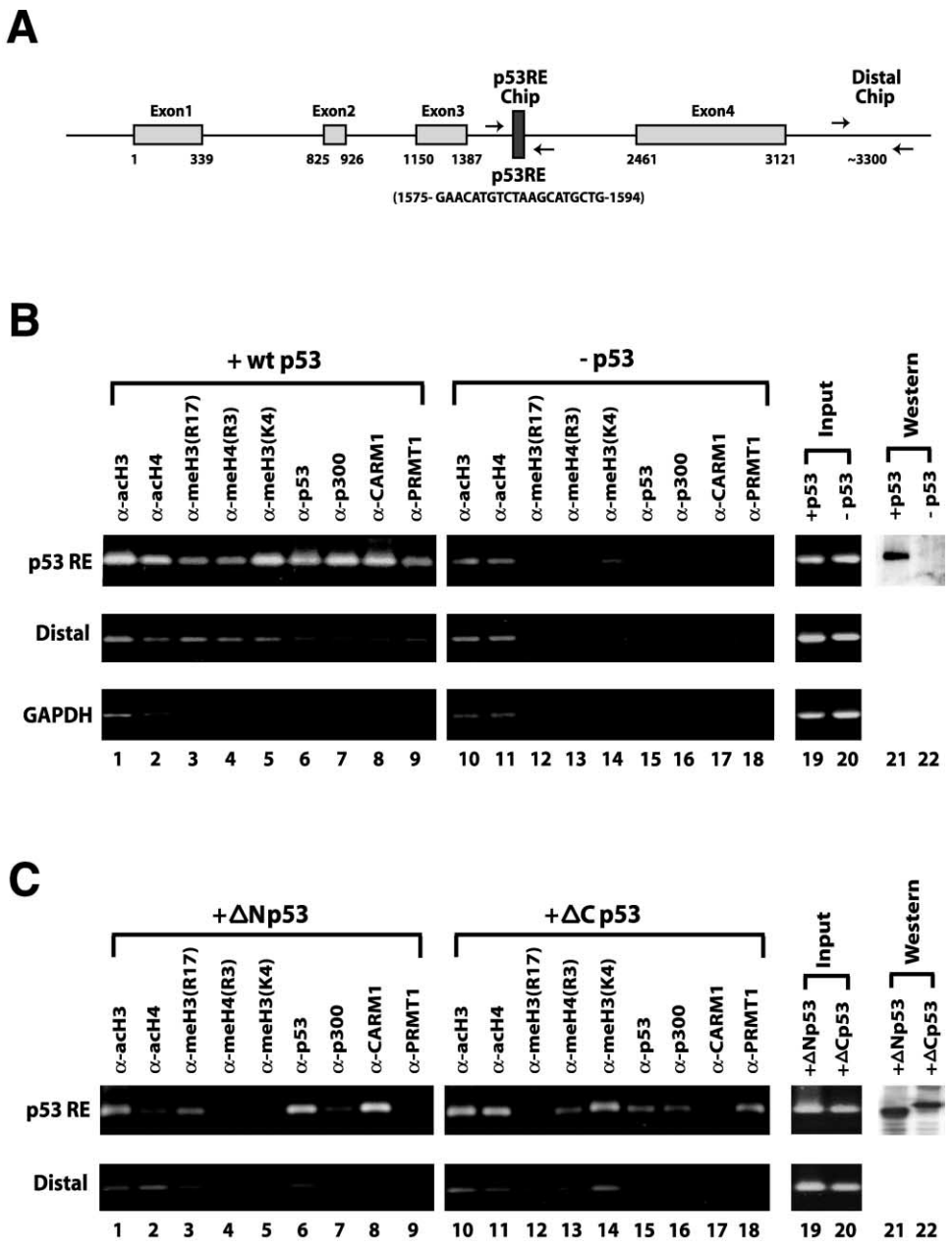


Figure 6. p53-Induced Accumulation of Coactivators and Histone Modifications on the *GADD45* Gene

(A) Schematic of *GADD45* and the p53 response element. Arrows indicate positions of PCR primers in and around the p53 response element (RE).

(B) p53-induced accumulation. p53-negative H1299 cells transfected with a control (−p53) or a p53-expression (+p53) vector were subjected to ChIP analysis with antibodies to the indicated proteins and modified histone residues (lanes 1–18). The selected PCR primers (A) scored sequences at the p53 response element and 2 Kb distal. PCR analysis with identical primers on input chromatin confirmed that equal amounts were used for all reactions (lanes 19 and 20). A Western blot (lanes 21 and 22) confirmed ectopic p53 expression.

(C) Mutant p53-induced accumulation. ChIP was performed as described in Figure 6B, except that mutant p53 lacking the N terminus (residues 1–80) or the C terminus (residues 350–393) was expressed as indicated.

in part to enhanced recruitment of GCN5 rather than p300. Although we have not yet correlated the appearance of K4-methylated H3 with a corresponding histone methyltransferase, candidates include Set1, Set9/Set7, and MLL (Sims et al., 2003). Moreover, the coincidental appearance and decrease of K4-methylated H3 and acetylated H3 raise the possibility of a functional synergy. The apparent decline in K4-methylated H3 could be due to epitope masking and/or histone replacement, as no histone demethylases are yet known.

These studies thus confirm the presence on a natural UV-induced p53-dependent gene of those cofactors im-

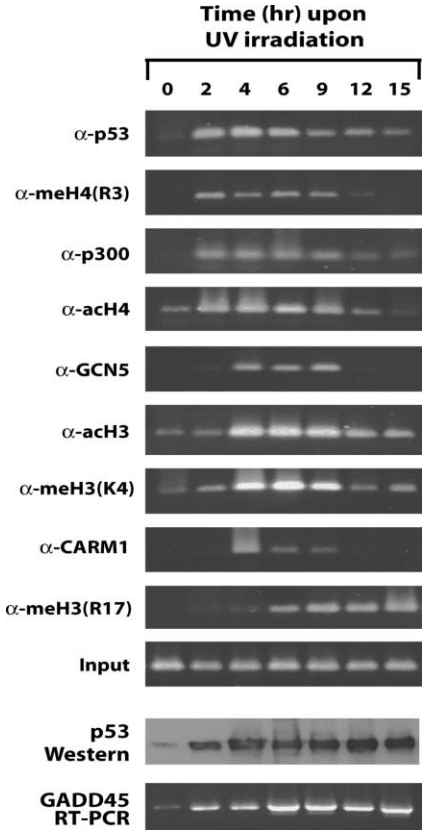


Figure 7. UV-Induced Accumulation of Coactivators and Histone Modifications on the *GADD45* Gene

U2OS cells irradiated with UV were subjected to ChIP analysis after the indicated times with primers surrounding the p53 response element of *GADD45* as in Figure 6. The two lower panels show p53 protein and *GADD45* RNA accumulation.

plicated in the in vitro function of p53 on chromatin templates. They further suggest the stepwise function of these and other cofactors during UV-induced DNA damage.

Discussion

Although modulations of the abundance and activity of p53 play a key role in target gene activation, various coactivators that are typically utilized by activators offer additional targets for the integration of signaling pathways and could be important in target gene discrimination. Beyond earlier studies implicating p53-interacting histone acetyltransferases (p300/CBP and PCAF/GCN5 or TIP60) in p53 function (Introduction), we demonstrate (1) the additional involvement of protein arginine methyltransferases PRMT1 and CARM1 in p53 function, (2) cooperative and ordered functions of PRMT1 and CARM1 with p300, and (3) for all these coactivators, mechanisms that include direct interactions with p53 and obligatory modifications of cognate histone substrates. The results of the more detailed biochemical analyses with in vitro reconstituted chromatin templates containing p53 response elements from the *GADD45* gene are substantiated by ChIP analysis indicating ordered accumulation of cofactors and corresponding histone modifications on the *GADD45* gene during UV-induced DNA damage.

Independent Functions of p300, PRMT1, and CARM1

While the cooperative functions of these (and other) cofactors on p53-activated genes may represent the more physiological situation (below), the recombinant chromatin/purified cofactor-based cell free system has allowed us to assess independent functions and mechanisms for the various cofactors. Thus, in addition to confirming a role for p300 in p53 function through *GADD45* p53-response elements, we have shown independent functions for PRMT1 and CARM1. Heretofore, CARM1 and PRMT1 coactivator functions were shown mainly for nuclear hormone receptors (Koh et al., 2001; Lee et al., 2002), and it was not established whether CARM1 or PRMT1 could act alone or only in conjunction with other ectopic or endogenous cofactors or, indeed, whether histones or other proteins were the essential substrates for the activities observed (Stallcup, 2001). The latter point is critical, since CARM1 and PRMT1, like p300, have alternate transcription-related substrates (Gu and Roeder, 1997; Kwak et al., 2003; Mowen et al., 2001; Xu et al., 2001). Importantly, by using recombinant chromatin templates with mutant histones, we have been able to verify that the histone modifications (and corresponding enzymatic functions) are essential for p53-dependent functions of PRMT1 and CARM1, as well as p300. This represents a significant extension of previous reports of p53-dependent p300 function (Espinosa and Emerson, 2001) and nuclear receptor-dependent PRMT1 and CARM1 function (Koh et al., 2001; Lee et al., 2002) that did not establish histones as essential substrates. Moreover, the independent functions of p300, PRMT1, and CARM1 also indicate that the distinct modifications introduced by a given coactivator must have an effect beyond any ability to stimulate histone modifications by the other two coactivators.

In confirmation of our previous results, recombinant chromatin lacking all tails was completely repressed (An et al., 2002). This again demonstrates a repressive mechanism that is intrinsic to the core nucleosome and independent of any repression that might be superimposed by tail-dependent higher order nucleosome interactions (reviewed in An et al. [2002]). Further, p300-dependent transcriptional activation by p53 was strongly dependent upon both the H3 and H4 tails and associated acetylation events, indicating nonredundant functions, but completely independent of the H2A and H2B tails. These results parallel those observed for Gal4-VP16- and p300-dependent transcription (An et al., 2002) and argue for their generality for p300-dependent function, despite contrasting results from another study (Georges et al., 2002). However, emphasizing the lack of a general requirement for the H4 tail for all histone modifying coactivators, as well as distinct mechanisms for PRMT1 and p300, deletion of the H4 tail had a minimal effect on PRMT1-dependent transcription (Figure 3E) owing to an apparent back-up function by the H2A tail. This interesting result points to the need for caution in the interpretation of results that show no apparent func-

tional defect following mutations of primary histone/transcription factor modification sites.

Ordered Cooperative Functions of p300, PRMT1, and CARM1

Pairwise combination of p300 and PRMT1 or p300 and CARM1 resulted in a functional synergy in effecting p53-dependent transcription from recombinant chromatin templates. That the cooperative functions, like the independent functions, depend upon corresponding histone modifications was established by analysis of chromatin templates with intact versus mutated histones. Relevance to intracellular p53 function was established through transfection assays showing a similar cooperativity between p300 and CARM1 and between p300 and PRMT1 but with no demonstrable cooperativity between PRMT1 and CARM1 in the absence of ectopic p300. Moreover, in both the in vitro and in vivo assays, the highest levels of p53-dependent activity were observed in the presence of p300, PRMT1, and CARM1.

These results are generally consistent with the demonstrated functions of these coactivators in mediating nuclear receptor-dependent transcription, in conjunction with p160 coactivators (e.g., GRIP1), in transfection assays. Thus, Stallcup (2001) and colleagues have shown cooperativity between p300 and CARM1 (but not PRMT1) and between PRMT1 and CARM1 in these assays (Koh et al., 2001; Lee et al., 2002). Some differences, such as their failure to see cooperativity between p300 and PRMT1 or their observation of synergy between PRMT1 and CARM1 in the absence of ectopic p300 may be attributed either to intrinsic differences between p53 and nuclear receptors with respect to cofactor utilization or to differences in assay conditions. Overall, however, the cooperative functions of p300, PRMT1, and CARM1 are consistent with the distinct substrate specificities of these cofactors. Apart from our specific demonstration of new coactivator functions and synergies in mediating p53 function, these results are reminiscent of earlier demonstrations of cooperativity between other histone modifications such as phosphorylation and acetylation (reviewed in Zhang and Reinberg [2001])

Somewhat surprisingly, in pairwise assays, the addition of one cofactor prior to the other either increased or decreased overall p53-dependent activity relative to that observed when p300 and CARM1 or p300 and PRMT1 were simultaneously added to reaction mixtures. The results of these studies (Figure 3) indicate a preferred order of function for optimal activity: PRMT1 first, p300 second, and CARM1 third. These conclusions from the biochemical analyses are supported by the results of ChIP assays monitoring accumulation of cofactors and histone modifications on the p53-dependent *GADD45* gene during UV-induced DNA damage. In particular, an early accumulation of R3-methylated H4, p300, and acetylated H4 was followed by the accumulation of CARM1 and R17-methylated H3. In further support of this proposed pathway, estrogen induction of an estrogen receptor-activated gene was accompanied by an early accumulation of CBP and K18-acetylated H3, followed by accumulation of CARM1 and R17-methylated H3 (Daujat et al., 2002). In vitro histone modification studies (below) also support the prediction from the functional assays and the ChIP assays.

Although our functional studies have focused on a restricted set of coactivators, the ChIP assays on the UV-induced *GADD45* gene also have shown induced accumulation of GCN5 and accompanying acetylated H3, as well as K4-methylated H3. These results are consistent with other studies implicating components (TRRAP, Ada3, GCN5) of GCN5-, PCAF-, or TIP60-containing HAT complexes in p53 function on diverse (*p21* and *MDM2*) genes (Ard et al., 2002; Barlev et al., 2001). The positive function of a histone lysine methyltransferase (e.g., Set1, Set7/9, or MLL)-containing complex on the *GADD45* gene is also suggested by the UV-induced accumulation of K4-methylated H3 (Sims et al., 2003). Thus, these analyses point to the possible interplay of a diverse group of cofactors in mediating activation of *GADD45* and other p53-dependent genes. Of special importance will be determination of the complete repertoire of cofactors employed in p53 function and possible gene-, cell type-, and signal-specific variations in their utilization.

Mechanisms Involved in p53-Dependent Coactivator Function

Although p300, CARM1, and PRMT1 could function either through histone modifications or through modifications of other factors, as first established for p53, our studies with mutant histones have established unequivocally that the observed coactivator functions are dependent, minimally, upon histone modifications and, hence, cognate coactivator enzymatic activities. The further demonstration of direct interactions of unmodified (bacterially expressed) p53 with CARM1 and PRMT1 as well as p300 suggests a mechanism for direct recruitment to promoter bound p53 through these interactions. This mechanism is further supported by our demonstration of p53-dependent modifications of nucleosomal histones on a recombinant chromatin template with p53 binding sites. The utilization of recombinant, bacterially expressed p53 and histones in these assays indicates that prior p53 or histone modifications are unnecessary either for p53 binding to chromatin or for cofactor recruitment and function. This possibility was not excluded in previous studies of p53-dependent p300 function on chromatin templates with native histones and baculovirus-expressed p53 (Espinosa and Emerson, 2001) or in transfection studies showing ectopic p53-dependent recruitment of p300, PRMT1, and CARM1 (Figure 6) or of TRRAP (Ard et al., 2002).

Interestingly, studies with purified proteins have shown that PRMT1 and p300 bind to N-terminal domains in p53, whereas CARM1 binds to the C terminus, and chromatin immunoprecipitation assays have confirmed the role of these domains in p53-dependent recruitment of the corresponding factors to the endogenous GADD45 gene. While the role of the C terminus in p53 function is controversial (Prives and Manley, 2001), it appears to be required for p53 function in vitro (Espinosa and Emerson, 2001; W.A. and R.G.R., unpublished data). The apparent recruitment of p300, CARM1, and PRMT1 to promoters through direct interactions with p53 contrasts with their recruitment to nuclear receptor-acti-

vated promoters through interactions with receptor-interacting p160 coactivators (Lee et al., 2002; Ma et al., 2001). The role of p300-mediated acetylation in p53 binding and coactivator recruitment is also controversial, with acetylation being implicated in promoter recruitment of both p53 (Luo et al., 2004) and coactivators (Barlev et al., 2001) in vivo but without apparent function in vitro (Espinosa and Emerson, 2001). In agreement with the latter results, joint mutations in the major PCAF and p300 acetylation sites in p53 were also without effect in our in vitro transcription assays (W.A. and R.G.R., unpublished data).

Given that the corresponding histone acetylation and methylation events are critical for p53-dependent functions of p300, PRMT1, and CARM1, a major question concerns their subsequent roles. The simplest notion, based on the histone code hypothesis (Strahl and Allis, 2000), is that they serve as recognition sites for other proteins. One possibility is that a given modification makes the histone a better substrate for recognition and modification by other cofactors. Relative to the present data, studies with isolated H4 have shown that premethylation by PRMT1 stimulates acetylation by p300, whereas preacetylation by p300 represses methylation by PRMT1 (Wang et al., 2001a). Similarly, preacetylation of an H3 peptide was reported to stabilize CARM1 binding and to enhance methylation (Daujat et al., 2002). These results are consistent with the cooperative and ordered coactivator functions predicted by the in vitro analysis of both p53-dependent histone modifications and p53-dependent transcription, and cooperativity between histone acetylation and methylation events may explain at least part of the effect on transcription. Ultimately, however, some or all of the modifications must have downstream effects on transcription, per se.

A second and highly likely role for modified histones is in the recruitment of other transcriptional factors or cofactors. While there is yet no information regarding factors that recognize acetylated or methylated histones in p53-dependent transcription, precedent from other studies argues strongly for this probability. Thus, H3-K9 methylation potentiates chromatin condensation and silencing through its ability to bind HP1 (reviewed in Sims et al. [2003]). In relation to gene activation events, K4- or K20- methylated H3 provides part of a binding site for the ATP-dependent chromatin remodeling complex BRAHMA and a mark for long-term maintenance of a transcriptionally active state (Beisel et al., 2002). Similarly, activation of the IFN-β promoter has been reported to involve stepwise acetylation of H4 and H3 residues that are involved, respectively, in recruitment of the SWI/SNF and TFIID complexes—potentially through interactions of acetylated lysines with bromodomain containing components of the complexes (Agalioti et al., 2002).

Histone methylation might also facilitate transcriptional activation by antagonizing the association of (co)-repressors, as suggested by recent reports that H3-K4 methylation can indirectly enhance H3 acetylation by blocking association of the histone deacetylase-containing NURD complex with the H3 tail (Nishioka et al., 2002; Zegerman et al., 2002). Consistent with this possibility for p53-dependent transcription of the *GADD45* gene, H3-K4 methylation is coincident with GCN5 recruitment and H3 acetylation after UV irradiation (Figure 7). Thus, an H3-K4 methylation-mediated dissociation of an HDAC complex might conceivably result in enhanced H3 acetylation and consequent promotion of CARM1-mediated H3-R17 methylation. However, the ability of an N-terminal p53 mutant to enhance both H3 acetylation and H3-R17 methylation in the absence of H3-K4 methylation (Figure 6C) argues against this possibility for the GADD45 gene.

Since our analyses have been restricted to the GADD45 gene, future studies must determine the extent to which the cofactors and mechanisms described here pertain to other p53-responsive genes. It is likely that cofactor usage and mechanisms will vary for different stress responses and promoters, as recently reported by Espinosa et al. (2003).

Experimental Procedures

Plasmids and Expression Vectors

Bacterial vectors for core histone expression and histone purification were as described (Luger et al., 1999). Mutations (Figure 1A) were introduced by site-directed mutagenesis according to the manufacturer's instructions (Stratagene). Flag-tagged p53 and his-tagged PRMT1 were expressed in bacteria from pET28a (Novagen) vectors and Flag-tagged CARM1 and p300 were expressed in Sf9 cells from baculovirus vectors. Recombinant proteins were purified by standard procedures with Ni-NTA- or M2-agarose. For in vitro transcription assays, five copies of the p53 response element from the *GADD45* gene were inserted upstream of the adenovirus major late (AdML) promoter in the pML array plasmid (An et al., 2002), as summarized in Figure 1D. For transfection assays, a *GADD45*-luciferase reporter was constructed by inserting five copies of the *GADD45* p53-responsive element into the pGL3 Vector (Promega), and cDNAs encoding CARM1, PRMT1, p300, or p53 were subcloned into a CMV-driven expression vector, pIRESneo (Clontech). To generate p53 mutants, cDNA fragments encoding amino acids 81–393 (for ΔNp53) and 1–350 (for ΔCp53) were amplified and subcloned into pIRESneo.

Nucleosomal Histone Modifications and Chromatin Transcription Assays

Chromatin assembly and histone modification assays with coactivators were as described (An and Roeder, 2004; Ito et al., 1999). Transcription assays using activator (20 ng) and coactivators (15 ng for CARM1, 20 ng for PRMT1, and 10 ng for p300) were as recently described (An and Roeder, 2004).

GST Pull-Down Assay

Production and purification of glutathione S-transferase (GST)-p53 fusion proteins were as described (Gu and Roeder, 1997). Binding reactions contained 4 μg of GST-p53 proteins and 1 μg of purified coactivator(s) in a total volume of 0.2 ml. Reaction mixtures also included 250 mM NaCl, 0.5% NP-40, 25 mM HEPES, 10% glycerol, and a protease inhibitor cocktail (Roche Diagnostics). After an overnight incubation at 4°C, bound proteins were analyzed by immunoblot.

Cell Culture and Transfection

For transient transfection experiments, H1299 cells were grown to 50% confluency in RPMI medium supplemented with 10% fetal bovine serum. Transfection assays utilized, as indicated, 100 ng of *GADD45* p53 response element-driven luciferase reporter plasmid, 20 ng of p53 expression vector, and 200 ng of CARM1, PRMT1, or p300 expression vector. Cells were harvested at 36 hr and analyzed for luciferase activity.

Immunoprecipitation

Approximately 2×10^7 H1299 cells were transfected with Flag-p53 (2 μg), CARM1 (5 μg), PRMT1 (5 μg), and/or p300 (7 μg) expression plasmids. 48 hr posttransfection, cells were lysed in 2 ml of BC150 containing 1% Nonidet P-40. Expressed Flag-p53 was captured by

incubation of cell extracts with M2-agarose beads (Sigma) and eluted with SDS sample buffer. The levels of PRMT1, CARM1, or p300 coimmunoprecipitated with Flag-p53 were analyzed by Western blot. The anti-Flag antibody was from Sigma, and other antibodies were as described for ChIP assays.

Chromatin Immunoprecipitation Assay

ChIP assays were performed essentially as described (Barlev et al., 2001). Primers used for PCR were from the GADD45 p53 response element region (5′ primer, 5′-GGATCTGTGGTAGGTGAGGGTC AGG-3′; 3′ primer, 5′-GGAATTAGTCACGGGAGGCAGTGCAG-3′) or from a region 2 kb downstream (5′ primer, 5′-GGAGTTGGAGTTG TCAGGAAAAAGGG-3′; 3′ primer, 5′-GGTTGTGGTCTTTCAGGCCT CCACACC-3′). An identical analysis on the human *GAPDH* promoter was included as a control. For ChIP studies with UV-treated cells, U2OS cells were irradiated with 50 J/m^2 UV. Antibodies against diacetylated H3-K9, K14, tetraacetylated H4-K5, 8, 12, 16, dimethylated H4-R3, and CARM1 were from Upstate Biotechnology. Antibodies for PRMT1, dimethylated H3-R17, and trimethylated H3-K4 were from Abcam. Antibodies for p300, GCN5, and p53 were from Santa Cruz Biotech. To monitor expression of the *GADD45* gene, harvested cells were subjected to RT-PCR analysis (Qiagen). p53 protein levels were monitored by immunoblot with antibodies against p53. To discriminate amplification of the mRNA from pre-mRNA, the 5′ primer was located within exon 3, and the 3′ primer was in exon 4. In all cases, aliquots of PCR products at shorter (28) or longer (38) cycles were analyzed to ensure that amplification was maintained in the linear range.

Acknowledgments

We thank Dr. J.T. Kadonaga for ACF baculovirus vectors; Drs. K. Luger and T.J. Richmond for histone expression vectors; Dr. M.R. Stallcup for CARM1 cDNA; Dr. Y. Zhang for PRMT1 cDNA; and Drs. V.P. Palhan and Y. Dou for helpful discussions and critical readings of the manuscript. W.A. is supported by National Research Fellowship Award. This work was supported by the Rockefeller University and a grant from the NIH to R.G.R.

Received: November 24, 2003
Revised: April 19, 2004
Accepted: April 20, 2004
Published: June 10, 2004

References

Agalioti, T., Chen, G., and Thanos, D. (2002). Deciphering the transcriptional histone acetylation code for a human gene. Cell *111*, 381–392.

An, W., and Roeder, R.G. (2004). Reconstitution and transcriptional analysis of chromatin in vitro. Methods Enzymol. *377*, 460–474.

An, W., Palhan, V.B., Karymov, M.A., Leuba, S.H., and Roeder, R.G. (2002). Selective requirements for histone H3 and H4 N termini in p300-dependent transcriptional activation from chromatin. Mol. Cell *9*, 811–821.

Ard, P.G., Chatterjee, C., Kunjibettu, S., Adside, L.R., Gralinski, L.E., and McMahon, S.B. (2002). Transcriptional regulation of the mdm2 oncogene by p53 requires TRRAP acetyltransferase complexes. Mol. Cell. Biol. *22*, 5650–5661.

Barlev, N.A., Liu, L., Chehab, N.H., Mansfield, K., Harris, K.G., Halazonetis, T.D., and Berger, S.L. (2001). Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Mol. Cell *8*, 1243–1254.

Beisel, C., Imhof, A., Greene, J., Kremmer, E., and Sauer, F. (2002). Histone methylation by the Drosophila epigenetic transcriptional regulator Ash1. Nature *419*, 857–862.

Brooks, C.L., and Gu, W. (2003). Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. Curr. Opin. Cell Biol. *15*, 164–171.

Cheung, P., Tanner, K.G., Cheung, W.L., Sassone-Corsi, P., Denu, J.M., and Allis, C.D. (2000). Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol. Cell *5*, 905–915.

Daujat, S., Bauer, U.M., Shah, V., Turner, B., Berger, S., and Kouzarides, T. (2002). Crosstalk between CARM1 methylation and CBP acetylation on histone H3. Curr. Biol. *12*, 2090–2097.

Espinosa, J.M., and Emerson, B.M. (2001). Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. Mol. Cell *8*, 57–69.

Espinosa, J.M., Verdun, R.E., and Emerson, B.M. (2003). p53 functions through stress- and promoter-specific recruitment of transcription initiation components before and after DNA damage. Mol. Cell *12*, 1015–1027.

Georges, S.A., Kraus, W.L., Luger, K., Nyborg, J.K., and Laybourn, P.J. (2002). p300-mediated tax transactivation from recombinant chromatin: histone tail deletion mimics coactivator function. Mol. Cell. Biol. *22*, 127–137.

Gu, W., and Roeder, R.G. (1997). Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell *90*, 595–606.

Gu, W., Shi, X.L., and Roeder, R.G. (1997). Synergistic activation of transcription by CBP and p53. Nature *387*, 819–823.

Gu, W., Malik, S., Ito, M., Yuan, C.X., Fondell, J.D., Zhang, X., Martinez, E., Qin, J., and Roeder, R.G. (1999). A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol. Cell *3*, 97–108.

Ito, T., Levenstein, M.E., Fyodorov, D.V., Kutach, A.K., Kobayashi, R., and Kadonaga, J.T. (1999). ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev. *13*, 1529–1539.

Ko, L.J., and Prives, C. (1996). p53: puzzle and paradigm. Genes Dev. *10*, 1054–1072.

Koh, S.S., Chen, D., Lee, Y.H., and Stallcup, M.R. (2001). Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. J. Biol. Chem. *276*, 1089–1098.

Kwak, Y.T., Guo, J., Prajapati, S., Park, K.J., Surabhi, R.M., Miller, B., Gehrig, P., and Gaynor, R.B. (2003). Methylation of SPT5 regulates its interaction with RNA polymerase II and transcriptional elongation properties. Mol. Cell *11*, 1055–1066.

Lee, Y.H., Koh, S.S., Zhang, X., Cheng, X., and Stallcup, M.R. (2002). Synergy among nuclear receptor coactivators: selective requirement for protein methyltransferase and acetyltransferase activities. Mol. Cell. Biol. *22*, 3621–3632.

Luger, K., Rechsteiner, T.J., and Richmond, T.J. (1999). Preparation of nucleosome core particle from recombinant histones. Methods Enzymol. *304*, 3–19.

Luo, J., Li, M., Tang, Y., Laszkowska, M., Roeder, R.G., and Gu, W. (2004). Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. Proc. Natl. Acad. Sci. USA *101*, 2259–2264.

Ma, H., Baumann, C.T., Li, H., Strahl, B.D., Rice, R., Jelinek, M.A., Aswad, D.W., Allis, C.D., Hager, G.L., and Stallcup, M.R. (2001). Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. Curr. Biol. *11*, 1981–1985.

Mowen, K.A., Tang, J., Zhu, W., Schurter, B.T., Shuai, K., Herschman, H.R., and David, M. (2001). Arginine methylation of STAT1 modulates IFNalpha/beta-induced transcription. Cell *104*, 731–741.

Nishioka, K., Chuikov, S., Sarma, K., Erdjument-Bromage, H., Allis, C.D., Tempst, P., and Reinberg, D. (2002). Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev. *16*, 479–489.

Prives, C., and Manley, J.L. (2001). Why is p53 acetylated? Cell *107*, 815–818.

Roth, S.Y., Denu, J.M., and Allis, C.D. (2001). Histone acetyltransferases. Annu. Rev. Biochem. *70*, 81–120.

Scolnick, D.M., Chehab, N.H., Stavridi, E.S., Lien, M.C., Caruso, L., Moran, E., Berger, S.L., and Halazonetis, T.D. (1997). CREB-binding protein and p300/CBP-associated factor are transcriptional coacti-

vators of the p53 tumor suppressor protein. Cancer Res. *57*, 3693–3696.

Sims, R.J., III, Nishioka, K., and Reinberg, D. (2003). Histone lysine methylation: a signature for chromatin function. Trends Genet. *11*, 629–639.

Stallcup, M.R. (2001). Role of protein methylation in chromatin remodeling and transcriptional regulation. Oncogene *20*, 3014–3020.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Strahl, B.D., Briggs, S.D., Brame, C.J., Caldwell, J.A., Koh, S.S., Ma, H., Cook, R.G., Shabanowitz, J., Hunt, D.F., Stallcup, M.R., and Allis, C.D. (2001). Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. Curr. Biol. *11*, 996–1000.

Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. Nature *408*, 307–310.

Wang, H., Huang, Z.Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B.D., Briggs, S.D., Allis, C.D., Wong, J., Tempst, P., and Zhang, Y. (2001a). Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. Science *293*, 853–857.

Wang, T., Kobayashi, T., Takimoto, R., Denes, A.E., Snyder, E.L., el-Deiry, W.S., and Brachmann, R.K. (2001b). hADA3 is required for p53 activity. EMBO J. *20*, 6404–6413.

Xu, W., Chen, H., Du, K., Asahara, H., Tini, M., Emerson, B.M., Montminy, M., and Evans, R.M. (2001). A transcriptional switch mediated by cofactor methylation. Science *294*, 2507–2511.

Zegerman, P., Canas, B., Pappin, D., and Kouzarides, T. (2002). Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. J. Biol. Chem. *277*, 11621–11624.

Zhang, Y., and Reinberg, D. (2001). Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev. *15*, 2343–2360.