Mdm2 Mutant Defective in Binding p300 Promotes Ubiquitination but Not Degradation of p53* 

EVIDENCE FOR THE ROLE OF p300 IN INTEGRATING UBQUITINATION AND PROTEOLYSIS

Turnover of the p53 tumor suppressor protein is mediated by Mdm2 through the ubiquitin proteolysis pathway. p300, a co-activator for p53, also participates in this process by complexing with Mdm2. We now report that the mutant Mdm2, defective in p33 binding, does not promote p53 ubiquitination and degradation in vivo but inhibit p53 transcriptional activation. By contrast, the mutant Mdm2, defective in p300 binding, still retains its activity to promote p53 ubiquitination and to inhibit p53 transcriptional activation but fails in promoting p53 degradation. We also show that both wild-type Mdm2 and the mutant Mdm2, defective in p300 binding, can promote the ubiquitination of cancer-derived p53 mutants, but only wild-type Mdm2 can cause their degradation. Furthermore, adenoviral oncprotein, 12S.E.1A, but not its deletion mutant that lacks p300 binding, was shown to decrease in vivo ubiquitination of mutant p53. Taken together, these results provide genetic evidence that p300 plays a pivotal role in the regulation of Mdm2-mediated p53 turnover by integrating the cellular ubiquitination and proteolytic processes.

The human tumor suppressor p53 plays a critical role in maintaining genomic stability and preventing tumorigenesis (1). Diverse mutations in the p53 gene constitute the most common type of genetic alterations in human cancers (2). Li-Fraumeni Syndrome (LFS)1 patients, suffering from a cancer-prone hereditary disorder, have been shown to harbor germ line mutations of the p53 gene (3). Studies on p53 knockout mice have provided supporting evidence for p53 functioning as a key tumor suppressor (4). In cellular response to DNA damage and other stresses, p53 protein accumulates and transcriptionally activates its target genes, which include p21WAF1/CIP1, GADD45, Mdm2, and other p53-inducible genes. The activation of these genes is believed to result in either cell cycle arrest or apoptosis (5, 6). Additionally, p53 has been demonstrated to have a role in the modulation of nucleotide excision repair (7–11).

It has been established that the turnover of p53 is regulated in part by the ubiquitin proteolysis pathway (12, 13). Ubiquitin is first sequentially transferred through the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme, and the ubiquitin-protein ligase (E3). The E3 enzyme then transfers the ubiquitin to one or more lysine residues in the substrate. Multiple ubiquitin molecules are attached to one another to form a polyubiquitin chain, which is deemed sufficient in targeting substrate proteins for destruction by the proteasome (reviewed in Ref. 14). It has been shown that Mdm2 can regulate the stability of p53 through the ubiquitin proteolysis pathway (15–17). More recent studies have demonstrated that Mdm2 has intrinsic E3 activity, which can ubiquitinate p53 and Mdm2 itself (18–21).

Although in vitro ubiquitination of p53 by Mdm2 only requires the addition of E1, ubiquitin-conjugating enzyme, and Ub (18, 21), participation of other cellular factor(s) can be envisaged in the complex processes of p53 ubiquitination and degradation in vivo. For example, adenoviral oncprotein E1A has been shown to cause p53 stabilization in multiple cell types through interaction with p300/CBP or with Rb family proteins (22–25). The p53 polyubiquitination activity in cell extracts has demonstrated a significant decrease upon the induction of E1A expression (26). It has been reported recently that most endogenous Mdm2 is bound to p300 and that specific interactions between p300/CBP, p53, and Mdm2 are intimately involved in Mdm2-mediated p53 degradation (27).

Our previous studies have shown that interaction of p300/CBP with DNA repair protein hHR32A leads to the down-regulation of p53 (28). The current experiments investigated whether cellular p300 plays a role as a platform to bring together the necessary catalytic and regulatory factors needed for in vivo p53 ubiquitination and degradation (27). The data presented demonstrate that (i) the Mdm2 mutant defective in binding to p300/CBP can promote ubiquitination but not degradation of either wild-type or cancer-derived mutant p53, and (ii) wild-type E1A, but not its deletion mutant lacking p300/CBP binding, can decrease ubiquitination of p53 in vivo. These results provide evidence that p300 plays a pivotal role in Mdm2-mediated p53 degradation by integrating ubiquitination and proteolytic processes.

MATERIALS AND METHODS

Gene Constructs—Wild-type and mutant p53 expression vectors, p53Wt1, p53–143A (Val → Ala), p53–173H (Arg → His), and p53–248W (Arg → Trp) as well as pG13-luc were provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). Construct pG13-Luc is a luciferase reporter containing 13 copies of synthetic p53 consensus binding sites derived from the native p21WAF1/CIP1 promoter (29). Mdm2 constructs, pCMV-Mdm2, pCMV-Mdm2Δ, and pCOC-Mdm2ΔXM, were provided by Dr. Arnold Levine (Princeton University, Princeton, NJ), Dr. David Livingston (Harvard Medical School, Boston, MA), and

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* This work was supported by NIEHS, National Institutes of Health Grant ES03388. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1724 solely to indicate this fact.

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1 The abbreviations used are: LFS, Li-Fraumeni syndrome; E1, ubiquitin-activating enzyme; E3, ubiquitin-protein ligase; Ub, ubiquitin; HA, hemagglutinin; CBP, cAMP response element (CREB)-binding protein; CMV, cytomegalovirus; Mdm2, murine double minute; Wt, wild type.

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Dr. Moshe Oren (The Weizmann Institute of Science, Rehovot, Israel), respectively. The pCMV-Mdm2 encodes a full-length Mdm2 protein. The pCMV-Mdm2Δ4 encodes a mutant Mdm2 protein with a deletion from 217 to 246 amino acid positions. The pCOC-Mdm2ΔXHM contains an internal deletion and consequently encodes a truncated Mdm2 protein lacking 49 amino acid positions. The WI 125/8.A and mutant E1A constructs were obtained from Dr. Michael Mathews (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). The pBIND-p53–(1–71) and pBIND-p53–(19–71), encoding the corresponding p53 N-terminal domains, were constructed by in-frame fusion of the respective domains with yeast Gal4 DNA-binding domain. The specific p53 domains were generated by polymerase chain reaction, and individual fragments were inserted into the parent plasmid pB, pBIND (Promega). The pG5-Luc vector, containing five Gal4 binding sites upstream of a minimal TATA box followed by a firefly luciferase gene, was purchased from Promega. The expression vector for HA-Ub (31) was obtained from Dr. Dirk Bohmann (European Molecular Biology Laboratory, Heidelberg, Germany).

Cell Culture, Transfection, Immunoprecipitation, and Immunoblotting for Ub-p53 Conjugates—The LFS 041 fibroblast strain MAH041 (p53-null, harboring a codon 184 frameshift mutation that results in premature termination of translation of the p53 protein) was kindly provided by Dr. Michael Tainsky (M.D. Anderson Cancer Center, Houston, TX). These fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and antibiotics at 37 °C in a humidified atmosphere of 5% CO₂. For the detection of Ub-p53 conjugates in vivo, exponentially growing LFS 041 fibroblasts were seeded at a density of 5 × 10⁶ cells/10-cm dish 18–20 h before transfection. The cells were transfected with expression vectors for p53, Mdm2 or mutant Mdm2, HA-UB, and 125/8.E1A or mutant E1A in different combinations as indicated in each figure legend. Transfections were performed using FuGENE 6 transfection reagent (Roche Molecular Biochemicals) according manufacturer instructions. The LFS 041 fibroblast strain MDAH041 (wild-type Mdm2), was obtained from Dr. Dirk Bohmann (European Molecular Biology Laboratory, Heidelberg, Germany). To evaluate the effects of E1A expression on Mdm2-mediated p53 degradation (27), we sought to understand the contributions of the specific protein interactions of Mdm2 and p300 in p53 ubiquitination in vivo. Thus, the initial experiments determined the ability of two structurally different Mdm2 mutants to promote p53 ubiquitination. As shown in Fig. 1, Mdm2ΔXM contains an N-terminal deletion, and the truncated Mdm2 protein starts at amino acid position 62. Because of its inability to bind p53, this truncated Mdm2 protein fails to (i) inhibit p53-dependent transcriptional activation, (ii) abolish p53-mediated apoptosis, and (iii) promote in vivo p53 degradation (15, 30). However, Mdm2Δ4 contains an internal deletion and the protein, while retaining its ability to bind p53, is defective in binding p300 in vivo. Consequently, Mdm2Δ4 is also defective in promoting p53 degradation in vivo (27). Thus, in vivo ubiquitination of wild-type and various mutant p53 proteins was determined upon co-expression with these Mdm2 molecules and by immunoprecipitation and Western blotting. As can be seen in Fig. 2A, an anti-HA-reactive ladder of bands, appearing as a smear of apparent molecular mass of ~66–220 kDa and representing HA-UB-p53 conjugates, was distinctly visible in lane 4 where p53 was co-transfected with Mdm2(Wt) and HA-UB. No such conjugates were apparent in samples of various control transfections without HA-UB (lanes 1, 2, and 3) or without Mdm2 (lane 7). The data suggest that the conjugates result from specific interaction with Mdm2. As expected, co-transfection of p53 with mutant Mdm2ΔXM did not produce any conjugates (lane 5), indicating that because of its inability to bind p53, Mdm2ΔXM is defective in mediating p53 ubiquitination. This observation is consistent with the early finding that Mdm2ΔXM does not promote p53 degradation in vivo (15, 30). Ubiquitinated p53 conjugates appeared with an even higher intensity when p53 was co-transfected with mutant Mdm2Δ4 (lane 6). Thus, this mutant, having an intact N terminus, can promote p53 ubiquitination. Overall results indicate that Mdm2 defective for p300 binding is not impaired in its ability to mediate p53 ubiquitination. This observation is novel and highly interesting, because as mentioned above, such mutant Mdm2 is defective in promoting p53 degradation (27).

**Results**

**Mutant Mdm2Δ4, Defective in p300 Binding, Retains Its Ability to Ubiquitinate p53 in Vivo—**p53 degradation depends on its ability to specifically interact with the N-terminal domain of Mdm2 (15, 17). Furthermore, the p300 protein, as part of a complex with Mdm2, is also shown to be involved in Mdm2-mediated p53 degradation (27). In view of recent studies on Mdm2 as a ubiquitin ligase (18, 19, 21, 33), we sought to determine the ability of two structurally different Mdm2 mutants to promote p53 ubiquitination. As shown in Fig. 1, Mdm2ΔXM does not promote p53 ubiquitination.
corresponding to allow ubiquitin conjugates to accumulate. Also, similar results were obtained using another highly specific and irreversible proteasome inhibitor, lactacystin (34) (data not shown).

Thus, it would seem that the inhibitory effect of proteasome inhibitors was manifested more prominently on mutant p53 than on p53(Wt) ubiquitin-protein conjugates, perhaps also because mutant p53 is more easily ubiquitinated.

**FIG. 2. In vivo ubiquitination of p53 by Mdm2.** LFS 041 cells were transfected with the indicated expression vectors for p53 (1 µg), Mdm2(Wt), or mutant Mdm2 (4 µg) along with HA-Ub (4 µg). The total amount of DNA for each transfection was kept at 10 µg by the addition of empty vector. After 24 h of transfection, cells were treated for 10 h with the proteasome inhibitor MG132 (10 µM). Whole-cell extracts were made in radioimmune precipitation buffer, and immunoprecipitates were prepared by using the anti-p53 monoclonal antibody DO6 and protein A/protein G agarose beads. Immunoprecipitates (i.p.) were separated by 8% SDS-polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride membrane, and probed with an anti-HA antibody. A, p53(Wt) and wild-type or mutant Mdm2 were co-expressed with or without HA-Ub as indicated. KD, kilodalton. B, mutant p53 and the indicated wild-type or mutant Mdm2 were co-expressed with HA-Ub. C, wild-type and mutant p53 and Mdm2(Wt) were co-expressed, immunoprecipitated, and analyzed simultaneously. Lanes 4* and 8* show the longer time exposure of HA-Ub-p53 conjugates seen in the corresponding lanes 4 and 8.

To confirm that Mdm2Δ4 specifically mediates ubiquitination of p53, various cancer-derived p53 mutants were tested for ubiquitination upon transfection by Mdm2 and Mdm2Δ4 (Fig. 2B). The p53 mutants 143A (Val→Ala), 173H (Arg→His), and 248W (Arg→Trp) were seen to ubiquitinate to approximately equivalent levels when co-expressed with Mdm2(Wt). More importantly, mutant Mdm2Δ4, with an intact N terminus, also ubiquitinated the various p53 mutants tested to an extent comparable with Mdm(Wt). These results reproducibly demonstrate that Mdm2Δ4 can ubiquitinate both wild-type and mutant p53 proteins. It should be noted that these mutant forms of p53 are devoid of sequence-specific DNA binding activity and were unable to activate the expression of a gene under the control of p53-responsive DNA binding site (29). Thus, these results suggest that the p53 transcriptional activation process is not associated with p53 ubiquitination.

For direct comparison, the ubiquitination of wild-type and cancer-derived mutant p53 by Mdm(Wt) in concomitant transfections and identical exposure conditions is shown in Fig. 2C. Surprisingly, ubiquitin conjugates of p53 mutants were found to accumulate in vivo much more efficiently than those of p53(Wt) (lanes 5, 6, and 7 versus lane 4). Nevertheless, ubiquitin conjugates of p53(Wt) (as shown above (Fig. 1A)), could be visualized with longer time exposures (lane 4* and 8*). It should be noted that in all these experiments, the proteasome inhibitor MG132 had to be used to treat cells after transfection to allow ubiquitin conjugates to accumulate. Also, similar results were obtained using another highly specific and irreversible proteasome inhibitor, lactacystin (34) (data not shown).

Thus, it would seem that the inhibitory effect of proteasome inhibitors was manifested more prominently on mutant p53 than on p53(Wt) ubiquitin-protein conjugates, perhaps also because mutant p53 is more easily ubiquitinated.

**Mutant Mdm2Δ4, Defective in p300 Binding, Abrogates Transactivation by p53**—Mdm2 is known to bind p53 and inhibit its transcriptional activation (30). Here we investigated the effect of mutant Mdm2Δ4 overexpression on the ability of p53 for transactivation. LFS 041 cells were co-transfected with p53(Wt) and the pG13-luc reporter in the presence of Mdm2(Wt), Mdm2ΔXM, or Mdm2Δ4. Luciferase activity of the cell extracts from various transfections is shown in Fig. 3. As expected, p53(Wt) dramatically elevated luciferase activity relative to the control pCMV vector (Fig. 3A). Co-transfection of Mdm2(Wt) blocked the ability of p53 to transactivate the reporter construct. By contrast, mutant Mdm2ΔXM failed to inhibit p53 activity, confirming that the inhibition requires the binding of Mdm2 to p53 (30). On the contrary, mutant Mdm2Δ4, in keeping with its ability to bind p53, was found to block transactivation by p53 to an extent comparable with that of Mdm2(Wt) (27). Similar experiments were then carried out to verify the effects of Mdm2 and its mutants on transcriptional activity of the p53 N-terminal activation domain. As shown in Fig. 3B, co-transfection of Mdm2(Wt) or mutant Mdm2Δ4 inhibited luciferase activity transcribed from reporter pG5-luc to a comparable extent. However, mutant Mdm2ΔXM failed to exhibit any inhibitory effects but instead increased luciferase activity ~1.5-fold. Deletion of the Mdm2-binding site (from 1 to 19 amino acid positions) caused a dramatic decrease in the transactivation activity of Gal4-p53 to a level that was slightly above that achieved with Mdm2(Wt). Both Mdm2(Wt) and mutant Mdm2Δ4 were unable to exhibit a significant inhibitory effect on the transactivation activity of the truncated Gal4-p53 (19–71). These results show that the amino acids, spanning positions 1–19 in the p53 activation domain, contribute a very important component to the transactivation by p53 and provide the needed binding site for Mdm2 to cause inhibition of p53 activity. Taken together, it can be concluded that the binding of Mdm2 to p53, but not to p300, is responsible for the inhibition of p53-dependent transcriptional activation. Therefore, the binding of Mdm2 to p53 is required for both the ubiquitination and inactivation of p53.

**Mutant Mdm2Δ4, Defective in p300 Binding, Fails to Promote Degradation of p53 in Vivo**—Failure to promote in vivo p53 degradation by the mutants Mdm2ΔXM and Mdm2Δ4 was confirmed by co-transfection experiments in LFS 041 cells and..
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Materials and Methods.
Mdm2. Relative luciferase activity was measured as described under "Materials and Methods." The data points indicate the mean ± S.E. of expression vector for indicted wild-type or mutant Mdm2 expressed in triplicate. The data points indicate the mean ± S.E. of expression vector for wild-type or mutant Mdm2 as indicted. Transfection, relative luciferase activity, and data presentation was as described for A.

Analyzing the protein steady-state levels by Western blotting. As expected from previous studies (15, 27), the p53 steady-state level was reduced markedly in cells co-transfected with Mdm2(Wt). No such decrease was, however, seen in cells co-transfected with Mdm2(Wt) but not mutants Mdm2XM or Mdm2Δ4 shift mutants. This was expected from previous studies (15, 27), the p53 steady-state level is increased in cells co-transfected with Mdm2(Wt). Thus, expression vectors for wild-type or mutant Mdm2 were co-transfected into LFS 041 cells in the absence or presence of expression vectors for wild-type or mutant Mdm2 as indicted. Transfection, relative luciferase activity, and data presentation was as described for A.

FIG. 3. Wild-type and mutant Mdm2 differentially inhibit the transcriptional activation by p53. A, p53 reporter pG13-luc and p53 expression vectors were co-transfected into LFS 041 cells in the absence (−) or presence (+) of expression vector for indicted wild-type or mutant Mdm2. Relative luciferase activity was measured as described under "Materials and Methods." The figure shows representative data of at least three independent experiments, with each transfection performed in triplicate. The data points indicate the mean ± S.E. B, Gal4 reporter pG5-luc and expression vectors Gal4-p53(1–71) or Gal4-p53(19–71) were co-transfected into LFS 041 cells in the absence or presence of expression vectors for wild-type or mutant Mdm2 as indicted. Transfection, relative luciferase activity, and data presentation was as described for A.

FIG. 4. Mdm2, defective in CBP/p300-binding, fails to diminish p53 steady-state level. 1.0 μg of expression vector for wild-type (A) or various tumor-derived mutant p53 (B) proteins were transfected into LFS 041 cells together with 4.0 μg of empty vector (control) or expression vectors for wild-type or the mutants Mdm2ΔXM or Mdm2Δ4. After 24 h of transfection, the cells were lysed in SDS sample buffer, and the proteins were quantitated and processed by SDS-polyacrylamide gel electrophoresis and Western blotting for p53, Mdm2, and actin as a loading control.

Stabilization of p53 Protein and Inhibition of p53 Ubiquitination by Adenoviral 12S.E. 1A Protein—E1A can stabilize p53 via binding to p300 (22–25). This binding is known to disrupt the transcriptional co-activation mediated by CBP/p300 (35). In an effort to explore the role of p300 in the regulation of p53 stability, we next examined whether E1A could stabilize p53 protein in the presence of Mdm2. As shown in Fig. 5A, Mdm2 expression drastically decreased the steady-state level of p53. Co-expression of 12S.E. 1A overcame the Mdm2-affected decrease of p53 steady-state levels in a dose-dependent manner and restored it to the level observed in control without any Mdm2. Interestingly, co-expression of E1AΔ(2–36) lacking p300-binding also resulted in the restoration of the p53 expression level as reported earlier (36) (Fig. 5B). Because E1AΔ(2–36) is known to possess the ability to bind several members of the Rb family (37), this result would seem to suggest the involvement of other cellular factor(s), e.g. Rb, in this process.

To examine the effect of E1A on the in vivo ubiquitination of p53 by Mdm2, we took advantage of our previous observation that the proteolytic degradation of ubiquitin conjugates of p53 mutants is more susceptible to inhibition by proteasome inhibitors. Because detection of cellular ubiquitin conjugates of p53(Wt) is poor even in the presence of proteasomal inhibitors (Fig. 2C, lane 4), the logical use of mutant p53 makes such an examination practical. Thus, expression vectors for p53(1–143A), Mdm2, and HA-Ub were co-transfected into LFS 041 cells along with increasing amounts of 12S.E.1A or E1AΔ(2–36) expression vectors (Fig. 6). Transfected cells were either treated with MG132 or mock-treated with Me2SO to allow the detection of p53 ubiquitin conjugates. The conjugates were immunoprecipitated with an anti-p53 antibody and then immunodetected in Western blots with an anti-HA antibody. No p53-ubiquitin conjugates appeared in lanes where mutant p53 or Mdm2 were transfected alone (lanes 1, 2, 8, and 9) or in lanes of mock-
The regulation of p53 activity is in large part mediated through the changes in protein stability, which is of major significance in the response of cells to DNA damage and other cellular stresses. The tight control of metabolic stability of p53 has been hypothesized to disrupt p53-dependent transactivation by binding to p300 through a three-way interaction between p53, Mdm2, and p300. The binding of p53 to p300 is therefore important to such a role. It can be envisaged that the p300 protein temporally stabilizes the multiple protein complex of ubiquitination/proteolysis processes needed for p53 ubiquitination/degradation (27). In view of such a role, it can be envisaged that the p300 protein temporarily stabilizes the multiple protein complex of ubiquitination/degradation. Disruption of such multiple protein complexes through specific protein modification, e.g. phosphorylation or viral oncoprotein binding, would be expected to affect ubiquitination and/or degradation that in turn leads to the stabilization of p53. An examination of the effects of adeno-viral 12S.E.1A on Mdm2-mediated p53 ubiquitination and degradation fully supports this scenario. Because the 12S.E.1A protein is known to disrupt p53-dependent transactivation by binding to the co-activator CBP/p300, a similar binding would disrupt the complexes of ubiquitination/degradation presumably assembled on p300. In the present study, both 12S.E.1A and E1AΔ-(2–36) were shown to stabilize the p53 protein in the presence of Mdm2. However, consistent with its effects on p53 polyubiquitination activity in vitro, only the 12S.E.1A protein inhibited the Mdm2-mediated ubiquitination of p53 in vivo. It has been shown that the N-terminal transactivation domain of p53 is necessary and sufficient to drive Mdm2-mediated degradation (15). However, ubiquitination of p53 by Mdm2 does not require the direct binding of Mdm2 to p300. Biochemically then, the actual ubiquitination process must be occurring on p300 through a three-way interaction between p53, Mdm2, and p300. The binding of p53 to p300 is therefore important to such a ubiquitination process. Nevertheless, it is not fully clear as to how the E1A protein might stabilize p53. It is noted that in addition to interaction with p300 and Rb, E1A can also interact with Sug1 and the 19S proteasome and thereby reduce the ability of human papillomaviral E6 protein to target p53 for ubiquitin-mediated proteasomal degradation (41). Under con-
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The cancer-derived p53 mutants tested in the present study are unable to bind sequence-specific DNA to activate the expression of a gene adjacent to the p53 DNA binding site (29). These mutants are efficiently ubiquitinated and degraded by co-expression of Mdm2. The results suggest that the inability to transactivate the Mdm2 gene is the main reason for protein accumulation of these mutants in cancer cells. We can not surmise why ubiquitin conjugates of these p53 mutants, compared with those of p53(WT), were easier to accumulate when transfected cells were treated with MG132. In a recent study, p53 mutants 143A and 248W were shown to be ubiquitinated by Mdm2 to an approximately equal level to p53(WT) without treatment by a proteasome inhibitor (33). One possibility is that the proteolysis of ubiquitin conjugates of these p53 mutants is more susceptible to the inhibition by proteasome inhibitors. However, the alternative that these mutants are inherently more prone to ubiquitination than the wild type cannot be ruled out.

Several lines of evidence converge in supporting the integration of ubiquitination and proteolysis through a complex assembled on the N-terminal end of p300. First, we have already established that hHR23A interacts with the CH1 region (aa 325–528) of p300/CBP to down-regulate p53 (28). Moreover, overexpression of wild-type hHR23A inhibits the p53 transcriptional activity and results in a decreased steady-state protein level of cellular p53 (28). Second, it is known that hHR23A/B interact with the 26S proteasome through its N-terminal ubiquitin-like domain and that these proteins co-purify with proteasomes in human cells. Specifically, hHR23A/B is shown to interact with S5a, a subunit of the human 26S proteasome (42). Third, the ubiquitin-like domain of RAD23 is obviously responsible both for UV-damage response (43) and to interact with the 26S proteasome in yeast (44, 45). Last, our preliminary studies indicate that the p300-(1–595) segment is very unstable and co-expression of hHR23A causes a prominent decrease in the steady-state levels of p300-(1–595) and p300-(325–528) segments. Therefore, a model is proposed to suggest that the hHR23A protein, through interaction with CH1 domain, enables the docking of proteasome onto p300 (Fig. 7).

The finding that p300 integrates ubiquitination and proteolysis underscores the complexity of the regulation of the metabolic stability of p53. However, it provides a rationale as to why p300 would be required for the stabilization of p53 in the cellular response to DNA damage (28, 46, 47). Interestingly, the p19ARF tumor suppressor is another participant in the regulation of Mdm2-mediated p53 degradation. It is noted that the p19ARF protein physically and functionally interacts with Mdm2 and p53 (48). The p19ARF stabilizes p53 and restores its transcriptional activity while promoting Mdm2 degradation (49). It will be worthwhile to explore whether p19ARF plays any part in p300/Mdm2-mediated p53 degradation. More importantly, it will be quite revealing to investigate how such an integrated process responds to DNA damage and cell cycle progression. Because p300 serves as a co-activator for a large group of transcription factors, more in-depth studies on the role of p300 in protein degradation are clearly warranted for understanding its diverse biological function.

Acknowledgments—We thank Drs. Vogelstein, Levine, Livingston, Oren, Mathews, Bohmann, and Tainsky for providing valuable reagents used in our various experiments. We are also thankful to Drs. Andrea...

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2 Q. Zhu, J. Yao, G. Wani, M. A. Wani, and A. A. Wani, unpublished observation.
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Doseff and Maqood Wani for careful reading of the manuscript and helpful suggestions.

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J. Biol. Chem. 2001, 276:29695-29701.
doi: 10.1074/jbc.M102634200 originally published online May 4, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M102634200

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