The tumor suppressor protein p53 has been well documented as a transcriptional activator involved in the regulation of a number of critical genes involved in the cell cycle, response to DNA damage, and apoptosis. Activation by p53 requires the interaction of the protein with a consensus binding site consisting of two half-sites, each comprising two copies of the sequence PuPuPuC(A/T) arranged head-to-head and separated by 0–13 base pairs. In addition to activation, p53 has been shown to be a potent repressor of transcription. However, the basis for p53-mediated repression is not well understood and has been proposed to occur indirectly through interactions with other promoter-bound transcription factors. In the present study, we show that p53 can repress transcription directly by binding to a novel head-to-tail (HT) site within the MDR1 promoter. A mutation that disrupted p53 binding to the MDR1 HT site blocked p53-mediated repression of the MDR1 promoter in transfection assays. Replacement of the HT site with a head-to-head (HH) site converted the activity of p53 from repression to activation, indicating that simple recruitment of p53 to the promoter is not sufficient for repression and that the orientation of the binding element determines the fate of p53-regulated promoters.

The tumor suppressor protein p53 is mutated in over half of all human cancers, implicating the loss of this inducible phosphoprotein in the destabilization of the genome and the malignant transformation that follows (1). It is clear that a fundamental mechanism by which p53 regulates cell growth and death decisions is through its role as a transcriptional activator. However, p53 can also repress the transcription of a number of genes including those involved in regulatory cascades mediating cell proliferation and tumorigenesis (2). Indeed, analysis of transactivation-competent p53 mutant proteins that have lost repressor activity suggests that transcriptional repression by p53 plays a critical role in the execution of the apoptotic program (3).

p53 activates transcription by binding DNA in a sequence-specific manner through a highly conserved DNA-binding domain. The consensus p53 binding site consists of two half-sites, each comprising two copies of the sequence PuPuPuC(A/T) arranged head-to-head (HH) and separated by 0–13 nucleotides (4). Each half-site binds a dimer of p53, resulting in the formation of a functional p53 tetramer-DNA activator complex. The majority of p53 mutations found in human tumors occur within the DNA binding domain, emphasizing the importance of DNA-protein interactions in p53 function (5).

In contrast to the wealth of information with respect to p53 as an activator, the mechanism by which repression occurs is relatively unknown. This is due in large part to the lack of a consensus binding site within the vast majority of p53 promoters (1). Genes repressed by p53 fall into two general categories. In the first class of promoters, repression is mediated through upstream activators. In almost all of these cases, repression occurs in the absence of direct DNA binding and appears to be achieved through an interaction of p53 with a promoter-bound transcriptional activator. Activators reported to be inhibited through an interaction with p53 include Sp1 (hTERT (6) and IGF-1 (7) promoters), NF-Y (cdc2 (8), fibronectin (9), and Cox2 (10) promoters), C/EBPβ (albumin promoter (11)), and AP-1 (hMMP1 promoter (12)). In a variation of this mechanism, a few promoters have been shown to be repressed by a direct p53-DNA interaction (13–15). In these cases, p53 binds to a consensus site that overlaps the binding site of a more potent activator protein. Although promoter-bound p53 still activates transcription, it also displaces the more potent activator, resulting in a net decrease in transcriptional output, i.e. apparent “repression.” This mechanism has been shown to be responsible for the down-regulation of the Bel-2 promoter, whereby p53 displaces the Bm-3a activator (13). It has also been shown for the α-fetoprotein promoter, where p53 represses by binding to a consensus site and displacing HNF-3. Importantly, p53 activates through this site in cells that lack HNF-3 (14). Finally, p53 represses the HBV gene by binding to a consensus site adjacent to an enhancer element within the promoter region (15); mutation of the enhancer element abrogates repression and allows for transcriptional activation by promoter-bound p53, indicating that repression was again due to “activator occlusion.” In summary, it is important to note that in previous cases where p53 interacted directly with its consensus site to mediate repression, the repression was conditional, i.e. dependent on “de-activation” of adjacent factors, and was not dictated by the p53 binding site.

In the second class of promoters, repression is believed to occur through the minimal promoter region, leading to the
proposal that p53 inhibits transcription of this class of genes by interfering with the basal machinery. This hypothesis was based initially on the observation that p53 can repress transfected reporter constructs in which the promoter in question has been reduced to the minimal sequence required for transcription. In addition, in vitro studies have shown that p53 interacts directly with the TATA-binding factor, TBP (16, 17), as well as with the TBP-associated factors dTAF40 and dTAF460 (18) and other basal factors (19). However, there is little evidence as yet to suggest that this is a general mechanism of repression in vivo. The p53-repressed MDR1 (P-glycoprotein) promoter has been included in this category by some investigators.

The MDR1 gene encodes P-glycoprotein, a transmembrane glycoprophophitin in which overexpression mediates resistance to chemotherapeutic agents (20) and other apoptotic inducers (21). In 1992, Gottesman and co-workers (22) showed that wild-type p53 represses transcription of MDR1 promoter constructs. Soon thereafter, Ling and co-workers (23) confirmed this observation and extended it to the hamster homologue of MDR1, the pgp1 promoter. Notably, p53 has also been shown to repress expression of the endogenous MDR1 gene. By stably introducing a trans-dominant negative p53 into wild-type p53-expressing cells, Shuetz and co-workers (24) showed that down-regulation of wild-type p53 led to up-regulation of the endogenous MDR1 RNA and protein, consistent with the role of p53 in MDR1 repression. More recently, it has been shown that induction of wild-type p53 activity in human cancer cells using ribozymes that repair mutant p53 proteins results in reduced activity of the MDR1 promoter (25).

Although the first evidence that wild-type p53 repressed transcription of the MDR1 promoter was reported almost a decade ago, the mechanism by which this occurs has remained elusive. Investigators delineated the region responsible for repression to the minimal promoter; unable to identify a consensus p53 binding site within this region, they suggested that p53 was repressing transcription indirectly by inhibiting the basal machinery (22, 23). However, this hypothesis has never been tested. In the present study, we have identified a novel mechanism of p53 repression that involves the specific binding of p53 to an atypical site within the MDR1 promoter, which in turn dictates the repressive function of the bound protein.

MATERIALS AND METHODS

Cell Culture and Transfections—The human colon carcinoma SW620 (ATCC no. CCL227) and human osteosarcoma Saos-2 (ATCC no. HTB85) cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mm l-glutamine at 37 °C in 5% CO2. 1.5 × 105 cells/well were plated in six-well plates and allowed to grow overnight at 37 °C in RPMI with 10% fetal calf serum prior to transfection. Saos-2 cells were transfected using calcium phosphate transfection protocols as described previously (26). SW620 transfections were performed using LipofectAMINE as described by the manufacturer (Life Technologies, Inc.) at a 10:1 LipofectAMINE:DNA ratio. For all transfections, a total DNA concentration of 2 μg of DNA/well was used (salmon sperm DNA was used as a carrier when needed). The reporter constructs pMDR1-HT (previously referred to as pMDR1–1202), pMDR1-mutC1, and pMDR1-mutGC have been described (27, 28), pMDR1-HTmut and pMDR1-HH were engineered through site-directed mutagenesis of pMDR1-HT using the QuickChange site-directed mutagenesis kit (Stratagene) and the following oligonucleotides: pMDR1-HTmut, 5'-GATTGGACAGCAGGCTGGGGCATGTTCCAGCCGC-3'; pMDR1-mutGC, 5'-GGTGCTGGAACAGGCCGCCCCGCAGGCACAGC-3'; pMDR1-HTmut, 5'-GGCTGCCAGAACCGCTGCCACCACAGC-3'; pMDR1-HTmut, 5'-GCTGAGGGAAACCGCTGCCACCACAGC-3'.

RESULTS

p53 Represses MDR1 Transcription through a Novel DNA Element—Unsatisfied with the untested hypothesis that p53 repressed MDR1 transcription through the basal machinery, we re-addressed the possibility that p53-mediated repression could be “direct,” i.e. involve binding to a promoter element. We began with the hypothesis that conversion of p53 from an “activator” to a “repressor” might occur through interaction with a variant DNA binding site, perhaps an element in which all four quarter sites were present but arranged in a different orientation. With this in mind, we scanned the MDR1 promoter (~2000 to +100) using a sequence analyzer program that allows for independent permutations of quarter site orientations (HH, TT, HT, TH) with the 0–13 base pair linker region found in the consensus site and a maximum of a single mismatch. We identified four near perfect p53 quarter-sites (spanning −72 to −40) with a novel head-to-tail (HT) orientation instead of the consensus head-to-head (HH) alignment found in activated promoters (Fig. 1A). To test the importance of this putative binding site in p53-mediated repression, either a wild-type MDR1 promoter/luciferase construct (pMDR1-HT, −1202 to +118) or a construct containing a mutation within the HT site (pMDR1-HTmut) was transfected into either a p53 mutant cell line (SW620, Fig. 1B) or a p53 null cell line (Saos-2, Fig. 1C) in the presence (pCMV-wtp55) or absence (pCMV.5) of exogenous wild-type p53. As expected from earlier studies (22, 23), expression from the wild-type promoter was repressed −4–5-fold in both cell lines. Most importantly, the mutation of the HT site significantly reduced the p53 effect, indicating an involvement of this site in p53-mediated MDR1 repression. This repression required the DNA binding domain of p53 because a protein mutated in this domain was incapable of repressing MDR1 transcription and, in fact, activated transcription through an upstream Ets transcription factor binding site as previously shown (30) (Fig. 1D).

As discussed above, p53 had been found to mediate transcriptional repression of other promoters through its interactions with either NF-Y or Sp1. We (27, 28) and others (31, 32) have previously shown that binding sites for both Sp1 (the GC element) and NF-Y (the inverted CCAAT box) are present within the MDR1 promoter region required for p53 repression (Fig. 1E). Because these sites are in close proximity to the HT
p53 Repression through Direct Binding to a Novel Site

FIG. 1. A novel p53 site oriented head-to-tail mediates repression of the MDR1 promoter. A, comparison of the consensus p53 HH binding site and the MDR1 HT site. a, sequence of the conventional p53 HH site with arrows indicating the orientation of the quarter-sites; \( R = \) purine, \( Y = \) pyrimidine, \( W = A or T \), sequence of the MDR1 HT site (bold type) with arrows showing quarter-site orientation. c, sequence of the mutated MDR1 HT site (MDR1-HTmut). b, transfection analysis of the wild-type and mutant MDR1 HT promoters in SW620 cells. For each transfection, 0.5 \( \mu g \) of either the pMDR1-HT or the pMDR1-HTmut luciferase reporter plasmids along with 0.5 \( \mu g \) of the empty vector, pCMV5 (dark grey bars), or the wild-type p53 expression plasmid, pCMV-p53 (white bars), were introduced into SW620 cells. Luciferase activity was normalized to protein content and to the expression of an internal Renilla luciferase reporter construct, pRL-TK (20 \( \mu g \) (Promega), the expression of which was unaffected by p53. The activity level of the reporter construct in the absence of exogenous p53 was arbitrarily set at 1. Experiments were performed a minimum of two times in triplicate. C, transfection analysis of the wild-type and mutant MDR1 HT promoters in Saos-2 cells. Transfections were performed as described in B, except 1.5 \( \mu g \) of reporter plasmid was used. D, a mutant p53 incapable of DNA binding cannot repress the MDR1 promoter. SW620 cells were transfected as described in B with pMDR1-HT alone (dark grey bar) or with 0.5 \( \mu g \) of wild-type p53 (white bar) or a mutant p53 incapable of DNA binding (pCMV.mt248 Arg→Trp) (black bar). E, structure of the proximal MDR1 promoter used for these studies. The inverted CCAAT box (striped box), the GC element (solid box), and the p53-HT half-sites (stippled boxes) are shown. F, neither the Sp1 nor the NF-Y binding site mediates repression by p53. Saos-2 cells were transfected with 1.5 \( \mu g \) of pMDR1-HT, pMDR1-mutC1 (an MDR1 luciferase reporter plasmid containing a mutation within the inverted CCAAT box (27)), or pMDR1-mutGC (an MDR1 luciferase reporter plasmid containing a mutation within the GC element (28)) with or without wild-type p53 (Fig. 1F). Mutation of either the CCAAT or GC elements had little impact on repression of the MDR1 promoter by p53, indicating that repression was not due to a functional inhibition of Sp1- or NF-Y-mediated activation.

p53 Binds Directly to the MDR1 HT Element—Although all four quarter sites are present in the HT site, the functional interaction of p53 with such a site had never been tested. To determine whether p53 could indeed bind to this promoter element, electrophoretic mobility shift assays were performed using purified baculovirus-expressed p53. As shown in Fig. 2A, p53 formed a complex with an oligonucleotide that included the sequence of the conventional p53 HH oligonucleotide (lane 1) and co-migrated with the complex formed on a consensus p53 HH oligonucleotide lacking the intervening 13 base pairs (MDR1-HH0) (lane 2) or the same sequence containing an MDR1 luciferase reporter plasmid (lane 3). The luciferase activity level of reporter constructs with or without wild-type p53 (Fig. 2C). Co-transfection assays were performed in Saos-2 cells using these pMDR1 promoter constructs with or without wild-type p53 (Fig. 2F). p53 binds specifically to the MDR1 HT site. A, gel shift analysis using a \( ^{32}P \)-radiolabeled double-stranded MDR1-HT (0.2 \( \mu g \)) or MDR1-HH (0.2 \( \mu g \)) oligonucleotide with 50 \( ng \) of purified baculovirus-expressed p53. Lanes 1 and 7, mouse IgG; lanes 2 and 8, PAb1801; lanes 3 and 9, PAb240; lanes 4 and 10, PAb245; lanes 5 and 11, PAb1820; lanes 6 and 12, DO-1. B, supershift analysis using 50 \( ng \) of baculovirus-expressed p53 protein and the MDR1-HT oligonucleotide (lane 1) or the same sequence lacking the intervening 13 base pairs (MDR1-HT0, lanes 2–4). Lanes 1 and 2, no antibody; lane 3, 200 \( ng \) of mouse IgG; lane 4, 200 \( ng \) of p53-specific PAb421. C, gel shift analysis of Bac-p53 (50 \( ng \)) bound to radiolabeled MDR1-HT (lane 1), or competed with 1, 2.5, 5, or 10 \( ng \) of unlabeled MDR1-HT0, lanes 2–5 or MDR1-HTmut (lanes 6–9). D, gel shift analysis of Bac-p53 (50 \( ng \)) bound to radiolabeled MDR1-HT (lane 1) and competed with 2.5, 5, or 10 \( ng \) of an unlabeled oligonucleotide containing the 5′ half of the HT site (MDR1-HT-U) (lanes 2–4) or the 3′ half of the HT site (MDR1-HT-D) (lanes 5–7).
We have identified a novel/alternative p53 DNA binding site within the MDR1 promoter in which the relative orientation of the four consensus quarter-sites defines p53 as a transcriptional repressor (Fig. 3B). Identical or near identical HT sites are also present within the p53-repressed promoters of the cyclin A (34), cyclin B1 (35), and ARF (36) genes (Fig. 3C). Moreover, we have identified sites with complete identity to the MDR1 HT site in several other promoters that have not yet been analyzed with respect to p53 regulation (see Supplementary Material). Therefore, the MDR1 promoter may be the prototype for a new class of p53-repressible genes. Although the mechanism by which repression at an HT site occurs remains to be determined, it should be noted that simple recruitment of p53 to the MDR1 promoter is not sufficient for repression, because replacement of the HT site with a consensus HH site resulted in transcriptional activation. Moreover, in contrast to observations in other promoters (13–15), the HT site cannot support transcriptional activation by p53 even when linked to a heterologous promoter (data not shown). Thus, there appears to be a fundamental difference between the conformation of the p53-HT complex and that of the p53-HT complex.

The conformation of wild-type p53 is flexible, and the protein can exist in multiple states under different physiological conditions. Moreover, it has been proposed that, to allow for the alignment of the tetrameric p53 DNA binding domains with the pentanucleotide inverted repeats in the HH site, the quaternary structure of p53 must undergo an energetically unfavorable conformational switch (37). It is therefore possible that binding of p53 to the HT site is more energetically favorable, perhaps providing novel surfaces for interaction with other transcription factors. Interestingly, a conformational “switch” dictated by a variant consensus p53 binding site has been identified in the p21 promoter where p53 protein bound to the upstream site interacts with the p53-specific Ab421 antibody, whereas p53 bound to the downstream site does not. It should be noted, however, that both binding sites support activation by p53 (29). In light of recent studies showing that p53 interacts with co-regulators to effect transcriptional activation and repression, one can speculate that the HH-p53 complex favors interactions with co-activators such as p300/CBP and PCAF (38, 39) whereas the HT-p53 complex is incapable of interacting with co-activators and may instead interact preferentially with co-repressors such as mSin3a and histone deacetylases (40). Studies testing this hypothesis are underway.

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REFERENCES

1. May, P., and May, E. (1999) Oncogene 18, 7621–7636
2. el-Deiry, W. S. (1998) Semin. Cancer Biol. 8, 345–357
3. Venot, C., Maratrat, M., Dureuil, C., Conseiller, E., Bracco, L., and Debussche, L. (1998) EMBO J. 17, 4663–4679
4. Levine, A. J. (1997) Cell 88, 323–331
5. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994) Science 265, 346–355
6. Kanaya, T., Kyo, S., Hamada, K., Takakura, M., Kitagawa, Y., Harada, H., and Inoue, M. (2000) Clin. Cancer Res. 6, 1239–1247
7. Ohlsson, C., Kley, N., Werner, H., and LeRoith, D. (1998) Endocrinology 139, 1101–1107
8. Yun, J., Chae, H. D., Choy, H. E., Chung, J., Yoo, H. S., Han, M. H., and Shin, D. Y. (1999) J. Biol. Chem. 274, 29677–29682
9. Iotsova, V., and Stehelin, D. (1996) Cell. Growth Differ. 7, 629–634
10. Subbaramaiah, K., Altorki, N., Chung, W. J., Mestre, J. R., Sampat, A., and Dannenberg, A. J. (1999) J. Biol. Chem. 274, 10911–10915
11. Kubicka, S., Kuhnel, F., Zender, L., Rudolph, K. L., Plumpe, J., Manns, M., and Trautwein, C. (1999) J. Biol. Chem. 274, 32137–32144
12. Sun, Y., Wenger, L., Rutter, J. L., Brinckerhoff, C. E., and Cheung, H. S. (1999) J. Biol. Chem. 274, 11535–11540
13. Budhram-Mahadeo, V., Morris, P. J., Smith, M. D., Midgley, C. A., Boxer, L. M., and Latchman, D. S. (1999) J. Biol. Chem. 274, 15237–15244
14. Lee, K. C., Crowe, A. J., and Barton, M. C. (1999) Mol. Cell. Biol. 19, 1279–1288
15. Ori, A., Zauberman, A., Doitsh, G., Paran, N., Oren, M., and Shaul, Y. (1998) EMBO J. 17, 544–553
16. Sato, E., Ushera, A., Zambetti, G. P., Momand, J., Horikoshi, N., Weinmann, R., Levine, A. J., and Shenk, T. (1999) Proc. Natl. Acad. Sci. U. S. A. 99, 12028–12032
17. Truant, R., Xian, H., Ingles, C. J., and Greenblatt, J. (1993) J. Biol. Chem. 268, 2248–2257
18. Farmer, G., Colgan, J., Nakatani, Y., Manley, J. L., and Prives, C. (1996) Mol. Cell. Biol. 16, 4295–4304
19. That, C. J., Chen, J. J., Klemm, R., and Tjian, R. (1995) Science 267, 100–104
20. van Veen, H. W., and Konings, W. N. (1998) Adv. Exp. Med. Biol. 456, 145–158
21. Johnstone, R. W., Ruelli, A. A., and Smyth, M. J. (2000) Trends Biochem. Sci. 25, 1–8
22. Chin, K. V., Ueda, K., Pastan, I., and Gottesman, M. M. (1992) Science 255, 459–462
23. Zastawny, R. L., Salvino, R., Chen, J., Benchimol, S., and Ling, V. (1993) Oncogene 8, 1529–1535
24. Thottassery, J. V., Zambetti, G. P., Arimori, K., Schuetz, E. G., and Schuetz, J. D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11037–11042
25. Watanabe, T., and Sullenger, B. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8430–8434
26. Morrow, C. S., Nakagawa, M. E., Goldsmith, M. J., Madden, M. J., and Cohen, K. H. (1994) J. Biol. Chem. 269, 10739–10746
27. Jin, S., and Scotto, K. W. (1998) Mol. Cell. Biol. 18, 4377–4384
28. Hu, Z., Jin, S., and Scotto, K. W. (2000) J. Biol. Chem. 275, 2979–2985
29. Resnick-Silverman, L., St. Clair, S., Maurer, M., Zhao, K., and Manfredi, J. J. (1998) Genes Dev. 12, 2102–2107
30. Sampath, J., Gandhi, A., Shapiro, L. H., Scotto, K. W., Zambetti, G. P., and Schuetz, J. D. (2000) Proc. Am. Assoc. Cancer Res. 41, 841
31. Cornwell, M. M., and Smith, D. E. (1993) J. Biol. Chem. 268, 19505–19511
32. Sundseth, R., MacDonald, G., Ting, J., and King, A. C. (1997) Mol. Pharmacol. 51, 963–971
33. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E., and Shay, J. W. (1992) Mol. Cell. Biol. 12, 2866–2871
34. Desdouets, C., Ory, C., Matesic, G., Soussi, T., Brechot, C., and Scozak-Thepot, J. (1996) FEBS Lett. 385, 34–38
35. Innocente, S. A., Abrahamsen, J. L., Cogswell, J. P., and Lee, J. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2147–2152
36. Robertson, K. D., and Jones, P. A. (1998) Mol. Cell. Biol. 18, 6457–6473
37. Waterman, J. L., Shenk, J. L., and Halazonetis, T. D. (1995) EMBO J. 14, 512–519
38. Gu, W., and Roeder, R. G. (1997) Cell 46, 595–606
39. Sakaguchi, K., Herrera, J. E., Saito, S., Miki, T., Bustin, M., Vassilev, A., Anderson, C. W., and Appella, E. (1998) Genes Dev. 12, 2831–2841
40. Murphy, M., Ahn, J., Walker, K. K., Hoffman, W. H., Evans, R. M., Levine, A. J., and George, D. L. (1999) Genes Dev. 13, 2490–2501
Transcriptional Repression by p53 through Direct Binding to a Novel DNA Element
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