p53 is known to repress transcription of a number of genes, but the mechanism of p53 recruitment to these target genes is unknown. The c-myb proto-oncogene product (c-Myb) positively regulates proliferation of immature hematopoietic cells, whereas p53 blocks cell cycle progression. Here, we demonstrate that p53 inhibits c-Myb-induced transcription and transformation by directly binding to c-Myb. The ability of c-Myb to maintain the undifferentiated state of M1 cells was also suppressed by p53. p53 did not affect the ability of c-Myb to bind to DNA but formed a ternary complex with the corepressor mSin3A and c-Myb. Thus, p53 antagonizes c-Myb by recruiting mSin3A to down-regulate specific Myb target genes.

The product of the TP53 tumor suppressor gene, p53, monitors the cellular response to various stresses, including genotoxic damage. In response to these stimuli, p53 becomes post-translationally stabilized and activated as a transcription factor (for review see Refs. 1–3). The cellular outcomes of this response are cell cycle arrest at the G1 or G2/M checkpoints and/or induction of apoptosis. This is related to its function as a sequence-specific transcription factor for the induction of a group of target genes, including p21^{trans} (Ref. 4). The consensus p53-binding element has been found in many promoters that are activated by p53. Transcriptional regulators, including the histone acetyltransferase p300/CBP, and arginine methyltransferases, PRMT1 and CARM1, have critical roles in p53-dependent transcriptional activation (5).

Conversely, p53 has been shown to repress a variety of cellular promoters. The genes found to be negatively regulated following p53 induction include Map4, DNA topoisomerase IIα, presenilin-1, bcl-2, and others (6–9). Certain human tumor-derived mutant forms of p53 are defective at trans-repression but not trans-activation (10). Deletion of the proline-rich region of p53 renders it defective at apoptosis induction and trans-repression, but not trans-activation (11, 12). Thus, transcriptional repression by p53 plays an important role in p53-dependent growth control. The interaction of p53 with the corepressor mSin3A is required for p53 to repress transcription (13). However, no consensus-binding element of p53 has been clearly identified in most of the p53-repressible promoters. Thus, the mechanism by which p53 is recruited to these target genes remains unknown; one possibility, addressed in this report, is that p53 interacts with other transcription factors bound to p53-repressed promoters.

The expression level of the c-myb proto-oncogene is high in immature hematopoietic cells, and its expression is turned off during differentiation (14). Similarly, the c-myb mRNA level is down-regulated during IL-6-transduced differentiation of M1 myeloblastic cells into macrophages, whereas constitutive expression of exogenous c-myb blocks this differentiation (15). A study of c-myb-deficient mice indicated that c-myb is essential for proliferation of immature hematopoietic cells (16), and analysis of tissue-specific c-myb KO mice revealed that c-myb is also required for T cell development at several stages (17).

The c-myb gene product (c-Myb) binds to a specific DNA sequence and regulates transcription (18–21). Various target genes of c-Myb, including c-myc, are involved in cell cycle control and blockage of apoptosis (22–25). In addition, c-Myb activates the chaperone gene promoters by interacting with heat shock transcription factor 3 without directly binding to the promoter itself (26). c-Myb has three functional domains that are responsible for DNA binding, transcriptional activation, and negative regulation (19). The DNA-binding domain (DBD) of c-Myb consists of three imperfect tandem repeats of 51–52 amino acids, each containing a variant helix-turn-helix motif (27). The transcriptional co-activator CBP binds to the c-Myb transcriptional activation domain (28). Deletion of the negative regulatory domain increases both trans-activation and transformation capability, implying that the negative regulatory domain normally represses c-Myb activity (19, 29–31). Recently, we reported that multiple corepressors, including Ski and mSin3A, bind directly to the DBD and the negative regulatory domain of c-Myb, and inhibit c-Myb-dependent trans-activation (32).

In this study we demonstrate that p53 directly interacts with c-Myb bound to promoters of certain Myb target genes, and inhibits c-Myb-induced transcription of these genes by recruiting mSin3A. Thus, c-Myb-p53 interaction is critical in the selection of the target genes to be repressed.

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**S** The on-line version of this article (available at http://www.jbc.org) contains Figs. S1 and S2 and supplemental Files 1–3.

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1 The abbreviations used are: IL-6, interleukin-6; c-Myb, c-myb proto-oncogene product; DBD, DNA-binding domain; NRD, negative regulatory domain; v-Myb, viral myb gene product; GST, glutathione S-transferase; E3, ubiquitin-protein isopeptide ligase.
**EXPERIMENTAL PROCEDURES**

**In Vitro Binding Assay—GST pull-down assays were performed as described previously (33).** Binding buffer consisted of 20 mM Hepes (pH 7.5), 0.1% Nonidet P-40, 1 mM EDTA, 5 mM dithiothreitol, 150 mM NaCl. Sumoylated GST-p53 was prepared as follows. His-tagged Aos1-Uba2 fusion protein (34), His-GST-SUMO-1, and GST-SUMO-2 were co-expressed in *Escherichia coli* and purified. Aos1-Uba2 (0.2 μg), Ubc9 (3 μg), SUMO-1 or SUMO-2 (20 μg) and GST-p53 (10 μg) were incubated in the sumoylation buffer (25 mM Hepes, pH 7.5, 50 mM KCl, 1 mM dithiothreitol, 4 mM MgCl₂, 0.05% Triton X-100, 5 mM ATP, 5% glycerol) at 30 °C for 4 h.

**Co-immunoprecipitation Assay—To study the interaction between c-Myb and p53, c-Myb and the retroviruses encoding various forms of Myb were co-transfected with a mixture of the c-Myb expression plasmid, pact-c-Myb (3 μg), and the p53 expression plasmid, pact-p53 (0.5 μg).** Forty hours after transfection, cell lysates were prepared in lysis buffer A (50 mM Hepes, pH 7.4, 50 mM NaCl, 0.2 mM EDTA, 10 μg/mL NaF, 0.5% Nonidet P-40). After reducing the final NaCl concentration to 150 mM by adding lysis buffer A, which lacks NaCl, immunoprecipitation was performed with rabbit antibody against GST-p53 and the immunocomplexes were subjected to Western blotting with anti-Myb 1.1. For co-immunoprecipitation of endogenous c-Myb and p53, lysates were prepared from Molt-4 cells by mild sonication in LSDL buffer (50 mM Hepes, pH 7.4, 50 mM NaCl, 0.1% Tween 20, 20% glycerol), and immunoprecipitated with the rabbit antibody against GST-p53. The immunocomplexes were then analyzed by Western blotting with anti-p53 1.1.

For two-step co-immunoprecipitation, 293T cells were transfected with FLAG-c-Myb expression plasmid pact-FLAG-c-Myb (3 μg) and the p53 expression plasmid pact-p53 (5 μg). For first immunoprecipitation controls, the pact-c-Myb plasmid, which expresses untagged c-Myb was used. Forty hours after transfection, cell lysates were prepared using lysis buffer A. After reducing the final NaCl concentration to 150 mM, the lysates were incubated with anti-FLAG M2-agarose (Sigma), and the beads were washed with lysis buffer A containing 50 mM NaCl. The FLAG-linked complexes were eluted with lysis buffer A containing 150 mM NaCl and 3 × FLAG peptide (100 μg/mL). After decreasing the concentration of 3 × FLAG peptide to 25 μg/mL by adding lysis buffer A containing 150 mM NaCl, anti-p53 monoclonal antibodies (DO-1, Santa Cruz Biotechnology) or control IgG were used for second immunoprecipitation.

**Luciferase Reporter Assay—Using the LipofectAMINE Plus reagent (Invitrogen), 293T or HepG2 cells were co-transfected with a mixture of the 6MBS-Luc reporter (0.2 μg), the c-Myb expression plasmid pCDNA-c-Myb (0.1 μg), the p53 expression plasmid pCDNA-p53 (0.1, 0.3, or 1.0 μg), and internal control plasmid pRL-TK (Invitrogen) (0.05 μg).** Cells were also transfected with the c-myc-Luc reporter (0.5 μg) together with the c-Myb expression plasmid (1 μg), the p53 expression plasmid (0.1, 0.3, or 1.0 μg), and internal control plasmid pRL-TK (0.05 μg). DNA plasmid was added to 2.0 μg with diluted transfection mixture. The luciferase assay was performed using the dual-luciferase assay system (Promega). Luciferase reporter assays were performed using various forms of *in vitro* translated c-Myb and GST-p53 fusion proteins. The results indicate that the region containing repeats 2 and 3 in the DBD of c-Myb bind to GST-p53 (Fig. 1A and supplemental Fig. S1). The three hydrophobic amino acids on the surface of repeat 2 of c-Myb DBD, which are mutated into non-hydrophobic amino acids in the v-Myb encoded by avian myeloblastosis virus, are involved in interactions with various proteins, including C/EBPβ and co-repressors such as Ski (38, 39). However, the mutant R23 fragment of c-Myb (R23–3M), in which these amino acids are changed to the same non-hydrophobic amino acids found in v-Myb, efficiently bound to GST-p53 (Fig. 1A), as did the bacterially expressed R123 fragment of c-Myb (Fig. 1A). Thus, p53 directly binds to the DBD of c-Myb.

**RESULTS**

**Direct Interaction of c-Myb with p53—We previously demonstrated that p53 binds to heat shock transcription factor 3 and inhibits c-Myb-induced heat shock transcription factor 3 activation (33).** During analysis of the interactions between p53, c-Myb, and heat shock transcription factor 3, we have found that p53 also directly binds to c-Myb. To further examine the interaction between c-Myb and p53, we performed GST pull-down assays using various forms of *in vitro* translated c-Myb and GST-p53 fusion proteins. The results indicate that the region containing repeats 2 and 3 in the DBD of c-Myb bind to GST-p53 (Fig. 1A and supplemental Fig. S1). The three hydrophobic amino acids on the surface of repeat 2 of c-Myb DBD, which are mutated into non-hydrophobic amino acids in the v-Myb encoded by avian myeloblastosis virus, are involved in interactions with various proteins, including C/EBPβ and co-repressors such as Ski (38, 39). However, the mutant R23 fragment of c-Myb (R23–3M), in which these amino acids are changed to the same non-hydrophobic amino acids found in v-Myb, efficiently bound to GST-p53 (Fig. 1A), as did the bacterially expressed R123 fragment of c-Myb (Fig. 1A). Thus, p53 directly binds to the DBD of c-Myb.

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**p53 Inhibits c-Myb-dependent trans-activation—To determine whether p53 can affect c-Myb-induced trans-activation, we performed luciferase reporter assays using a reporter construct containing the Myb-binding sites (6MBS-Luc). Transfection of 293T cells or HepG2 cells with 6MBS-Luc, c-Myb expression vector and increasing amounts of p53 expression vector indicated that p53 suppressed c-Myb-induced trans-activation in a dose-dependent manner in both cell types (Fig. 2A and B). Adriamycin treatment, which induces p53 protein expression, also inhibited c-Myb-induced luciferase expression from the 6MBS-Luc reporter in a dose-dependent manner (Fig. 2C).

We next investigated the effect of p53 on c-Myb-dependent trans-activation of the promoter of a known c-Myb target gene, c-myc. Using a c-myc promoter-containing luciferase reporter (c-myc-Luc) it was found that p53 did suppress c-Myb-dependent transcription from this promoter (Fig. 2D). To investigate whether p53 also inhibits the activity of oncogenically activated forms of Myb, we used v-Myb, 3M, and CT3 in the luciferase reporter assays. v-Myb is encoded by avian myeloblastosis vi-
rus, whereas CT3 is a C-terminally truncated form of c-Myb; both of these forms of Myb have a strong transforming capacity. In the 3M mutant, the three hydrophobic amino acids in repeat 2 of the DBD have been replaced with non-hydrophobic amino acids, analogous to the avian myeloblastosis virus-encoded v-Myb. p53 inhibited c-myc promoter activity induced by all three forms of Myb (Fig. 2E), suggesting that oncogenic activation of c-Myb is not due to the abrogation of negative regulation of c-Myb activity by p53.

**Fig. 1.** p53 directly binds to c-Myb. 
A, p53 binds to the DBD of c-Myb. Upper panel, the domain structure of c-Myb and various forms of c-Myb used are shown. The results of binding assays are summarized on the right. Lower left panel, GST fusion protein containing full-length p53 was analyzed by SDS-PAGE followed by Coomassie Blue staining. Lower middle panel, binding of two forms of in vitro translated c-Myb to GST-p53 resin was analyzed by SDS-PAGE followed by autoradiography. Lower right panel, GST pull-down assays were performed using GST-p53 and the bacterially expressed recombinant c-Myb containing three repeats of the c-Myb DBD (R123). B, c-Myb binds to the C-terminal region of p53. Upper panel, the domain structure of p53 and the three forms of p53 used are shown. The results of binding assays are summarized on the right. Lower left panel, GST-c-Myb fusion proteins were analyzed by SDS-PAGE followed by Coomassie Blue staining. The arrow indicates the full-length GST-c-Myb. Lower middle and right panels, binding of three forms of in vitro translated p53 to GST-c-Myb was examined. C, co-immunoprecipitation of overexpressed c-Myb and p53. Lysates from 293T cells transfected with the plasmid encoding c-Myb and p53 were precipitated with the antibody shown above, and the immunocomplexes were analyzed by Western blotting with anti-c-Myb antibody. The left-most lanes contain samples of the whole cell lysates. D, co-immunoprecipitation of endogenous proteins. Lysates from Molt-4 cells were precipitated with the antibody shown above, and the immunocomplexes were analyzed by Western blotting with anti-c-Myb antibody.

**p53 Inhibits c-Myb Capacity to Control Cellular Proliferation**—We next asked whether p53 plays a biological role as a negative regulator of c-Myb. To address this, we first examined the effect of p53 on the transforming capacity of Myb. Transforming recombinant retroviruses that express full-length wild-type c-Myb or the oncogenically activated C-truncated form CT3 were generated (36), and the capacity of these viruses to transform fetal liver cells prepared from wild-type, p53<sup>+/−</sup>, or p53<sup>−/−</sup> mice was compared. Loss of p53 enhanced transform-
mation by these viruses in a dose-dependent manner (Fig. 3A), demonstrating that p53 does suppress the transforming capacity of Myb.

One element of the ability of Myb to transform hematopoietic cells is its capacity to suppress differentiation. We therefore next investigated the effect of overexpressing p53 on the capacity of c-Myb to maintain the undifferentiated state of M1 myeloblastic leukemia cells, which lack functional endogenous p53. Upon IL-6 stimulation, M1 cells undergo terminal differentiation into macrophage-like cells. However, constitutive expression of c-Myb in M1 cells blocks this IL-6-induced differentiation (15). We generated M1 cell clones that constitutively express c-Myb alone, or both c-Myb and the temperature-sensitive mutant of p53 (p53ts), as well as control clones contain-
p53 inhibits the ability of c-Myb to enhance cellular proliferation. A, p53 suppresses the Myb-induced transformation of fetal liver cells. Fetal liver cells were prepared from the wild-type, p53+/−, and p53−/− mice, and infected with retroviruses that express full-length c-Myb, oncogenically active CT3, or no protein. The average number of colonies formed in three experiments are indicated (± S.D.). p values are also shown. N.S., no significant difference. Note that full-length c-Myb can transform fetal liver cells depending on the cell density (36) and that under the condition used here full-length c-Myb also has a transforming capacity. B and C, p53 blocks the ability of c-Myb to maintain the undifferentiated state of M1 cells. M1 cell clones infected with either the retrovirus vector that expresses c-Myb and a neomycin-resistant marker, or p53ts and an enhanced green fluorescence protein marker were isolated, and the expression of c-Myb or p53 in typical clones was analyzed by Western blotting (B). M1 cell clones that constitutively express c-Myb with or without p53ts were treated with IL-6 or the control solvent and CD14 expression was examined by flow cytometry (C). Note that the CD14 expression level after IL-6 treatment was lower than previously published data due to cultivation at 32 °C.

p53 down-regulates multiple c-Myb target genes—To inves-

FIG. 3. p53 inhibits the ability of c-Myb to enhance cellular proliferation. A, p53 suppresses the Myb-induced transformation of fetal liver cells. Fetal liver cells were prepared from the wild-type, p53+/−, and p53−/− mice, and infected with retroviruses that express full-length c-Myb, oncogenically active CT3, or no protein. The average number of colonies formed in three experiments are indicated (± S.D.). p values are also shown. N.S., no significant difference. Note that full-length c-Myb can transform fetal liver cells depending on the cell density (36) and that under the condition used here full-length c-Myb also has a transforming capacity. B and C, p53 blocks the ability of c-Myb to maintain the undifferentiated state of M1 cells. M1 cell clones infected with either the retrovirus vector that expresses c-Myb and a neomycin-resistant marker, or p53ts and an enhanced green fluorescence protein marker were isolated, and the expression of c-Myb or p53 in typical clones was analyzed by Western blotting (B). M1 cell clones that constitutively express c-Myb with or without p53ts were treated with IL-6 or the control solvent and CD14 expression was examined by flow cytometry (C). Note that the CD14 expression level after IL-6 treatment was lower than previously published data due to cultivation at 32 °C.

FIG. 4. p53 down-regulates a significant number of the c-Myb target genes. RNAs were prepared from the control M1 cells and M1 cells that constitutively express c-Myb, both of which had been treated with IL-6 at 32 °C. The cDNA probes prepared from these RNAs were then used for DNA array analysis. The expression levels of 122 genes were more than 10-fold higher in the c-Myb-expressing cells than in the control cells. RNAs were also prepared from the IL-6-treated M1 cells, which constitutively express c-Myb with or without p53ts, and DNA array analysis was performed. The expression levels of 642 genes were more than 2-fold lower in the p53-expressing cells relative to the control cells. Comparison of these 642 genes with the 122 genes up-regulated in c-Myb-expressing cells identified 28 genes in common, indicating that these 28 genes are up- and down-regulated by c-Myb and p53, respectively. These 28 genes are indicated on the right. Dio2, iodothyronine deiodinase type II; Nphp1, nephronophthisis 1 (juvenile); Polo, DNA polymerase ε; Atp6ip1, H+ transporting ATPase lysosomal interacting protein 1; Flt3, FMS-like tyrosine kinase 3, Skp2, 5-phosphate kinase-associated protein 2 (p45); MEF2C, myocyte enhancer factor 2. The degrees of activation and repression of c-myc by c-Myb and p53, respectively, are also shown.
Negative Regulation of c-Myb Activity by p53

Tigate whether p53 induction leads to suppression of c-Myb target genes, including c-myc, we determined by DNA array analysis how many and which c-Myb-induced genes are regulated by p53. RNAs were prepared from the M1 cells constitutively expressing c-Myb and from control M1 cells, which were treated with IL-6 for 2 days and subjected to DNA array analysis with the Affymetrix Mouse Genome Array 430 2.0, which represents 39,000 transcripts. Because IL-6 treatment leads to down-regulation of c-myc mRNA, the expression level of c-Myb target genes should be lower in the control M1 cells, relative to the c-Myb-expressing cells. The results indicate that 533 genes are up-regulated more than 5-fold in the c-Myb-expressing cells compared with the control M1 cells (data not shown), and among these, 122 genes were up-regulated more than 10-fold (Fig. 4, supplemental File 1). These genes may include the direct and indirect Myb target genes and the genes that are regulated as a function of the differentiation process. We then prepared RNAs from the IL-6-treated M1 cells, which constitutively expressed either c-Myb or both c-Myb and p53, and subjected them to micro-array analysis. The results indicate that 628 genes are down-regulated more than 5-fold by constitutive expression of c-Myb compared with the control M1 cells (2.0–3.7-fold) is relatively low compared with the level of up-regulation by c-Myb expression (10.6–147-fold). This may be due to the relatively low level of p53 expression in the M1 cells (high levels of p53 induced a rapid apoptosis; data not shown).

Comparison of the 3,157 genes, which were down-regulated 40% or more by p53 expression, with the 533 genes, which were up-regulated more than 5-fold by constitutive expression of c-Myb, identified 285 genes (53%) in common (data not shown). These include c-myc, which was up-regulated 34.0-fold by constitutive expression of c-Myb and down-regulated 1.8-fold by p53 expression. Thus, a considerable number of c-Myb target genes are negatively regulated by p53.

p53 Recruits mSin3A to c-Myb—To investigate the mechanism by which p53 inhibits c-Myb-induced trans-activation, we first examined the effect of p53 on the DNA binding capacity of c-Myb (Fig. 5A). In gel mobility-shift assays, the recombinant R123 protein, which contained only the DBD of c-Myb, bound to the Myb binding site-I probe and generated a retarded band. Addition of GST-p53 to this reaction gave rise to an additional band, whereas GST-p53 alone did not generate a retarded band. An anti-Myb antibody super-shifted both of these bands, whereas anti-p53 antibody only slightly diminished the band generated by addition of GST-p53, possibly due to the low affinity of the p53 antibody to p53 in the complex. Addition of
control IgG did not affect either of these bands. These results indicate that p53 interacts with c-Myb bound to the Myb binding site-I DNA, generating a p53-Myb-DNA complex and that p53 does not inhibit the DNA binding of c-Myb.

It has been previously reported that p53 represses transcription by recruiting the corepressor mSin3A (13). In addition, we recently found that mSin3A also directly binds to c-Myb and inhibits c-Myb-induced trans-activation (32). Therefore, we speculated that p53 may suppress c-Myb-dependent trans-activation by recruiting mSin3A to c-Myb. To investigate this possibility, we first examined the effect of p53 and mSin3A on c-Myb-dependent trans-activation. Co-expression of both p53 and mSin3A resulted in an additive effect on suppression of the c-Myb-dependent transcription from the Myb site containing promoter (Fig. 5B), suggesting that p53 and mSin3A act together to suppress c-Myb-dependent transcription.

To examine whether c-Myb, p53, and mSin3A form a complex, a two-step co-immunoprecipitation was performed (Fig. 5C). 293T cells were co-transfected with expression plasmids for FLAG-tagged or untagged c-Myb, and p53. Cell lysates were prepared and first immunoprecipitated with an anti-FLAG antibody. This immunocomplex was eluted with a FLAG peptide prepared and first immunoprecipitated with an anti-FLAG antibody. This immunocomplex was eluted with a FLAG peptide and found to contain p53, endogenous mSin3A, and FLAG-c-Myb. The FLAG-peptide eluates were then immunoprecipitated with anti-p53 or control IgG. The anti-p53 immunocomplex contained endogenous mSin3A, but the IgG complex did not. Thus, c-Myb, p53, and mSin3A can form a complex.

We then examined the p53-dependent recruitment of mSin3A to the c-myc gene promoter by chromatin immunoprecipitation (ChIP) assays. We recently demonstrated that c-Myb binds to the c-myc promoter region using ChIP assays (32). We used M1 cell clones that constitutively express c-Myb and p53ts. Anti-p53 and anti-mSin3A antibodies precipitated the mouse c-myc promoter 343-bp DNA fragment (−782 to −11249), which contains two Myb-binding sites (40), from cells cultivated at 37 °C (Fig. 5D, left three lanes). Other regions of c-myc DNA, which contained no Myb-binding sites (−705 to −945 and −5 to −235) were not precipitated (data not shown). These results indicate that some amount of p53, which does not have a functional protein conformation, and mSin3A proteins bind to the c-myc promoter region via c-Myb. Activation of p53 by cultivation of cells at 32 °C increased the amounts of c-myc promoter fragment precipitated by anti-p53 and anti-mSin3A antibodies (Fig. 5D, middle three lanes), suggesting that the number of mSin3A molecules that bound to the c-myc promoter increased in p53-overexpressing M1 cells. Thus, p53 recruits mSin3A to the c-myc promoter. Because the c-myc promoter does not contain the consensus p53-binding sequence, p53 is likely to bind to the c-myc promoter via c-Myb.

Sumoylation of p53 Suppresses the Interaction with c-Myb—The C-terminal 78-amino acid region of p53, which contains the tetramerization domain directly interacts with c-Myb (Fig. 1B). Interestingly, lysine 386 in this region is known to be sumoylated (41, 42). To examine whether the sumoylation of p53 at K386 affects its interaction with c-Myb, we used sumoylated GST-p53 for in vitro binding assays. Bacterially expressed GST-p53 was bound to glutathione beads and incubated with SUMO-1 or SUMO-2 and E1 and E2 enzymes. SDS-PAGE analysis indicated that about 90% of GST-p53 was sumoylated (Fig. 6A). These sumoylated GST-p53 proteins were used for pull-down assays with in vitro-translated c-Myb and mSin3A. Binding efficiency of c-Myb and mSin3A to the sumoylated GST-p53 was about half that of control GST-p53 (Fig. 6B). Thus, sumoylation of p53 suppresses the ability of p53 to inhibit c-Myb-dependent trans-activation.

**DISCUSSION**

The mechanism by which p53-dependent repression of transcription is translated into cell cycle arrest or induction of apoptosis by p53 is not well understood. The present study indicates that p53 inhibits a group of c-Myb target genes by
recruiting mSin3A (Fig. 7). c-Myb induces the expression of various genes that are involved in G1/S progression and suppression of apoptosis, and the induction of these target genes mediates the capacity of c-Myb to induce cell cycle progression and to protect cells against apoptosis. We have identified 28 genes that are up-regulated more than 10-fold by c-Myb expression and down-regulated more than 2-fold by p53. These include Fli3 (fms-like tyrosine kinase 3), which is required for proliferation of immature hematopoietic cells. These results suggest that p53 suppresses multiple c-Myb target genes. We previously showed that p53 induces c-Myb degradation by inducing the ubiquitin E3 ligase Siah-1, which directly binds to c-Myb (33). Therefore, p53 negatively regulates c-Myb activity by two mechanisms; an inhibition of c-Myb-dependent trans-activation by direct binding and by Siah-1-mediated degradation of c-Myb. The former may act immediately after p53 induction, whereas the latter may function in the later stage after Siah-1 induction. In leukemia, colon cancers, and breast cancers where Myb is expressed, p53 is frequently mutated. In addition, it was reported that loss of p53 allows the immortalization of hematopoietic cells by the myc gene, which is a direct target of Myb (43). Together with these reports, our results may support the idea that loss of p53 function in these cases contributes to transformation by up-regulating Myb activity. Because p53 is known to interact with various transcription factors, p53 may repress transcription of additional cell-cycle or apoptosis-related genes by mechanisms similar to those identified here.

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