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# Wild-Type Murine p53 Represses Transcription From the Murine *c-myc* Promoter in a Human Glial Cell Line

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**Abstract** Here we analyzed the effect of the suppressor proto-oncogene p53 on transcription from the P2 promoter of the murine *c-myc* gene. *c-myc* promoter constructs were coupled to the chloramphenical acetyl-transferase (CAT) gene and were transiently transfected into a human glial cell line along with plasmids overexpressing wild-type or mutant p53. It was found that significant repression of *c-myc* transcription took place following cotransfection with wild-type but not mutant p53. However wild-type p53 did not suppress transcription from the SV40 early promoter or from the MHC promoter. Promoter-CAT constructs containing only the ME1a2 or E2F elements, from the P2 promoter, were repressed by p53, indicating that p53 may exert its effect at these two sites within the P2 promoter. Finally, when the SV40 T antigen and wild-type p53 were expressed together in glial cells the repressive effect of p53 was abolished. 

**Key words:** P2 promoter, CAT, glial cell, plasmids, SV40

Controlled expression of the *c-myc* proto-oncogene has been broadly implicated in the control of cell proliferation (Cole, 1986). This controlled expression is commonly seen as induction of *c-myc* mRNA in response to growth stimuli. Evidence suggests that much of the transcriptional induction of *c-myc* in response to growth stimuli is due to increases in initiation at P2, the predominant *c-myc* promoter.

The P2 promoter sequences that are necessary for optimal transcription initiation in vitro and in vivo have only recently been identified (Asselin et al., 1989; Hall, 1990). In addition to the consensus TATA-box, three protein-binding sites have been mapped to this P2 proximal region (Asselin et al., 1989; Hall, 1990). These are termed ME1a2, E2F, and ME1a1 (Myc Exon 1, a1, and a2 sites) and are located at positions −85, −64, and −46, respectively, relative to P2 (Asselin et al., 1989; Hall, 1990). It has been demonstrated that these three elements are essential for optimal transcription from the P2 promoter (Asselin et al., 1989; Hall, 1990; Moberg et al., 1991, 1992). It has recently been demonstrated that the factor that binds the ME1a2 element is very similar or identical to the one that binds the ME1a1 element (Moberg et al., 1992). The factor that binds the E2F element undergoes cell-cycle dependent changes (Mudryj et al., 1990, 1991; Chellappan et al., 1991). It was found E2F is able to associate with the protein product of the retinoblastoma susceptibility gene (Rb) or the cyclin A factor (Mudryj et al., 1991; Chellappan et al., 1991; Bagchi et al., 1991). In keeping with this finding it has been shown that Rb functions to regulate transcription of *c-myc* (Pietenpol et al., 1990). These data indicate that elements within the *c-myc* promoter are the target of growth stimulatory signals.

Here we have analyzed the effect of another suppressor oncogene on *c-myc* transcription. This suppressor oncogene, termed p53, shares many characteristics with Rb (reviewed in Lane and Benchimol, 1990). In its wild-type form this protein suppresses oncogenic transformation however in its mutated form it acts as an activating oncogene (Finlay et al., 1989). Here we show that wild-type p53 represses transcription from the *c-myc* P2 promoter but that a mutated form does not. The implications for control of *c-myc* transcription and cell growth will be discussed.

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## MATERIALS AND METHODS

### Cell Culture and DNA Transfection and CAT Assays

The human glial cell line HTB14 was maintained in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum. All transfection experiments were initiated on 50% confluent monolayer cultures. Plasmids containing the bacterial chloramphenicol acetyltransferase gene (CAT), with or without *c-myc* promoter sequences, were transfected into the various cell lines by the calcium phosphate procedure (Ausubel et al., 1987) along with a plasmid that overexpresses the gene p53 gene. Plasmids containing the SV40 early promoter and enhancer or the H-2K$^b$ major histocompatibility locus promoter (Robbins et al., 1990) coupled to CAT were also transfected. Where indicated a plasmid expressing the SV40 large T antigen was also cotransfected into the glial cells. The cells were glycerol shocked 5–6 h after DNA addition. The cells were harvested approximately 24 h later and CAT activity assayed from the cytoplasmic extracts. Equal amounts of protein were assayed for CAT activity by thin layer chromotography. The autroradiographs from multiple experiements (3–5) were scanned with a densitometer and presented as an average (± standard deviation).

A complete genomic clone of wild-type murine p53 (ala at amino acid position 135) or mutant murine p53 (val at amino acid position 135) driven by the Hamster moloney sarcoma virus long terminal repeat was used in the cotransfection experiments (Eliyahu et al., 1985). For experiments involving the SV40 large T antigen, a construct containing the RSV-LTR driving the SV40 T antigen was cotransfected along with the *c-myc* promoter CAT constructs.

### Plasmid Constructions and Oligonucleotides

*c-myc* promoter-CAT constructions were engineered into the pGEM-1$^{R*}$ vector (Promega) as described (Moberg et al., 1991). The pGEM-1 vector containing promoterless CAT was used as a negative control. The double-stranded oligonucleotides representing the ME1a2 and E2F elements that were used in the gel-shift assays or in the construction of a modified *c-myc* promoter are

ME1a2

GATCGCCTCCTGAAGGGCAGGGCTTCGC
CGGAGGACTTCCCGTCCCGAAGCGCTAG,

E2F

AATTCGCTTGGCGGGAAAAC
GCGAACCGCCCTTTTGTTAA.

The underlined regions denote the sequences that are protected in DNAase I footprinting studies (Asselin et al., 1989; Hall, 1990). The ME1a2 oligonucleotide contained artificial flanking Mbo I ends (i.e., GATC). The E2F oligonucleotide has Eco R1 compatible artificial ends (i.e., AATT).

### Extracts and Electrophoretic Mobility-Shift Assays (EMSA)

Nuclear extracts from glial cells, NIH3T3 fibroblasts, or Hela cells were generated by the method of Dignam et al. (1983) with the additional protease inhibitors: pepstatin (2 μg/ml) and leupeptin (1 μg/ml). Electrophoretic-mobility shift assays (EMSA) were performed essentially as described by Carthew et al. (1985). Briefly, 0.5 ng of a $^{32}$P end-labeled promoter fragment or double-stranded oligonucleotide was incubated with nuclear extracts from the various cell lines in the presence of 2–3 μg of sheared salmon sperm DNA as a nonspecific competitor. The final buffer conditions for protein binding to the radiolabeled DNA were 16 mM Hepes, pH 7.9; 16% glycerol; 80 mM KCl; 0.16 mM EDTA; 0.4 mM DTT; and 0.4 mM PMSF. The reactions were then electrophoresed in a low-ionic strength (7 mM Tris, pH 7.9; 3.3 mM NaAcetate; and 1 mM EDTA) 4% polyacrylamide gel.

## RESULTS

### The ME1a2, E2F, and ME1a1 Elements Contribute Positively to Promoter Strength in Glial Cells In Vivo

To initiate studies on p53 mediated expression of *c-myc* in glial cells it was first necessary to establish the level of promoter activity following transfection with *c-myc* promoter:CAT constructs. As shown in Figure 1a the *c-myc* promoter contains three protein binding sites termed ME1a2, E2F, and ME1a1. It has been demonstrated that these sites are required for optimal initiation from P2 in NIH3T3 fibroblasts or Hela cells (Moberg et al., 1991, 1992). It has been shown that CAT activity is a measure of the level of correctly initiated P2 transcripts (Moberg et al., 1991, 1992). The *c-myc* promoter fragment used here does not contain the premature termination site at position +256 (Bentley and Groudine, 1988) so that only ef-

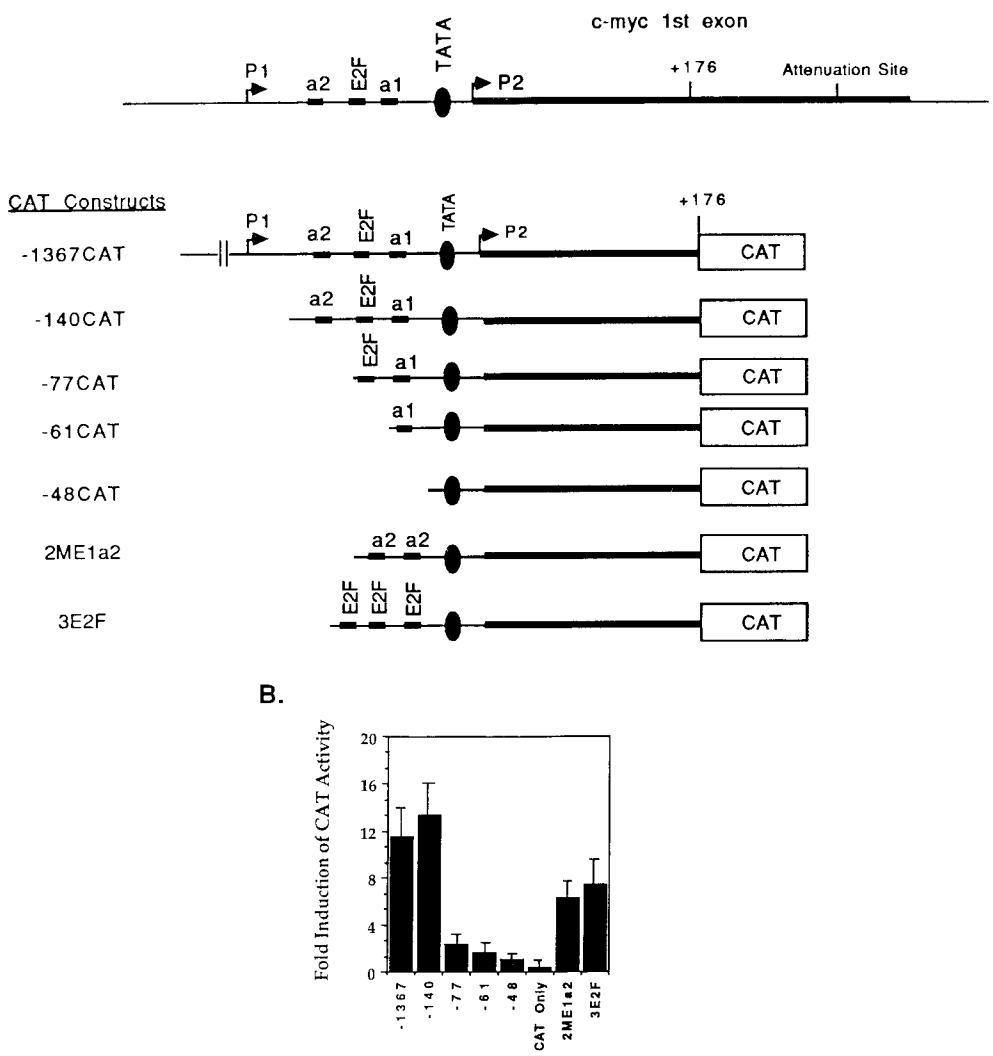


**Fig. 1.** In vivo analysis of *c-myc* promoter sequences in a human glial cell line. **A:** Outline of the *c-myc* promoter CAT constructs used in the transfections. The CAT constructs are identified by the position of their 5′ deletion (e.g., −140 CAT contains *c-myc* sequence to position −140 and −48CAT contains sequence to position −48). The CAT gene is not drawn to scale. Position +176 is the common 3′ boundary of the *c-myc* promoter constructs. **B:** CAT activity of the 5′ deletions. The CAT constructs outlined above were transiently transfected into glial cells along. "CAT Only" indicates the parent construct containing only the CAT gene with no *c-myc* sequence; 30 μg of DNA was transfected by the calcium phosphate method. Five hours after DNA addition the cells were glycerol shocked. Cytoplasmic extracts were generated after an additional twenty hours. Equal amounts of protein were assayed for CAT activity by thin layer chromatography (TLC) or for βGAL activity. The autoradiograms from multiple experiments were scanned and then averaged. "Fold Induction of CAT" is relative to the "TATA Only" construct which is arbitrarily set at 1. The results are presented ± the standard deviation.

fects on transcription initiation could be analyzed.

These deletions, as outlined in Figure 1A, were transiently transfected into the human glial cell line HTB14. As seen in Figure 1B the CAT activity diminishes markedly in the construct in which the ME1a2 site is deleted. Further deletion of the E2F and Me1a1 sites results in a drop in CAT activity to background levels. These results closely parrallel that found for transfection of these deletion constructs into NIH3T3 fibroblasts or Hela cells.

Artificial constructs were also generated that contained two ME1a2 or three E2F elements linked 5′ to the *c-myc* TATA box (Fig. 1B). These constructs produced significant CAT activity following transfection into glial cells, paralleling results seen for transfection into NIH3T3 fibroblasts and Hela cells (Moberg et al., 1991, 1992). Throughout these experiments the cells

were in the growth state such that the endogenous *c-myc* gene was expressed (data not shown).

### Cell-Type Dependent Binding to the E2F and ME1a2 Elements

Since these *c-myc* promoter:CAT constructs show similar patterns of transcriptional activity when transfected into glial, NIH3T3, and Hela cells, it could be predicted that extracts from these cells contain similar factors that recognize the *c-myc* promoter elements. To assay for protein binding to these elements electrophoretic mobility shift assays were performed (EMSA). The oligonucleotides used in the EMSA are outlined in Figure 2A. Because we have recently found that the factor that binds to the ME1a2 site is identical to the ME1a1 site, attention was focussed on protein binding to only the ME1a2 site. As seen in Figure 2B the major band appears in all lanes. The minor band is much reduced in the lane representing the Hela extracts. A novel band appears in the lane representing binding with the glial extracts (identified by an asterisk). This band is at exactly the same position as one identified previously in extracts of Hela cells in the presence of the protein dissociating agent desoxycholate (Moberg et al., 1992). Thus this protein appears in glial extracts devoid of a putative repressor protein (Moberg et al., 1992). All bands were eliminated by 100-fold molar excess unlabeled ME1a2 element indicating that binding is specific.

When binding to the E2F element was assayed multiple bands were seen (Fig. 2C). The band representing an association of E2F monomer with DNA (Mudryj et al., 1991; Moberg et al., 1992) is indicated. This band is sometimes present at low levels in the glial extracts. The uppermost band is a complex of E2F with other proteins (Mudryj et al., 1991; Moberg and Hall unpublished observations) and shows some variation between the three cell types. A novel faster migrating band also appears in the lane representing the glial extracts (identified by an asterisk). All bands were eliminated by 100-fold molar excess unlabelled E2F element indicating that binding is specific. Taken together these data indicate that there is some cell-type specificity in binding to both the ME1a2 site and the E2F site. It was also found that incubation of glial or 3T3 extracts with a monoclonal antibody specific for p53 did not inhibit binding or produce a supershift of these upper bands, indicating no direct interaction between E2F and endogenous p53 (data not shown). Throughout these experiments the cells were in the growth state such that the endogenous *c-myc* gene was expressed (data not shown).

### Wild-Type But Not Mutant p53 Represses Transcription From the P2 Promoter

In order to test the effect of p53 on transcription from the *c-myc* P2 promoter cotransfection experiments were performed. As shown in Figure 3A, the −140CAT construct was cotransfected into glial cells with increasing amounts of a wild-type p53 expressing plasmid. It is apparent that significant repression of transcription from the P2 promoter occurs, even at a level of 2 μg of p53 DNA. The same repressive effect is seen when a larger *c-myc* promoter:CAT construct is used (i.e., −1367CAT). To ensure that this is not a general repressive effect, the p53 plasmid was cotransfected into glial cells with an SV40 early promoter CAT construct (Fig. 3B) or an MHC promoter CAT construct (Fig. 3C). It is evident that p53 does not repress transcription from the SV40 early promoter or the MHC promoter; in fact it appears as if there is a slight enhancement of transcription from the SV40 promoter. These data indicate that p53 does not have a general repressive effect on transcription.

It would be predicted that p53 acts to repress transcription from the P2 promoter by either directly or indirectly acting at the level of specific *c-myc* promoter elements. To test this hypothesis the 2ME1a2CAT and 3E2FCAT constructs were cotransfected with the wild-type p53 expressing plasmid. As seen in Figure 4 transcription is significantly reduced in both of these constructs. These data indicate that p53 is able to exert its effect through both the ME1a2 and E2F elements.

It is known that mutations within the p53 gene can lead to a change in the function of the protein. For example a change from alanine to valine at amino-acid 135 results in the protein acting as an activator oncogene instead of a repressor oncogene. To determine if the repressive effect of wild-type p53 on *c-myc* transcription could be diminished through a mutation in amino-acid 135 (from alanine to valine) a mutant p53-expressing plasmid was used in cotransfection experiments. As seen in Figure 5, the mutant p53 does not repress the level of transcription from the *c-myc* promoter construct, indicating that the alanine-to-valine mu-

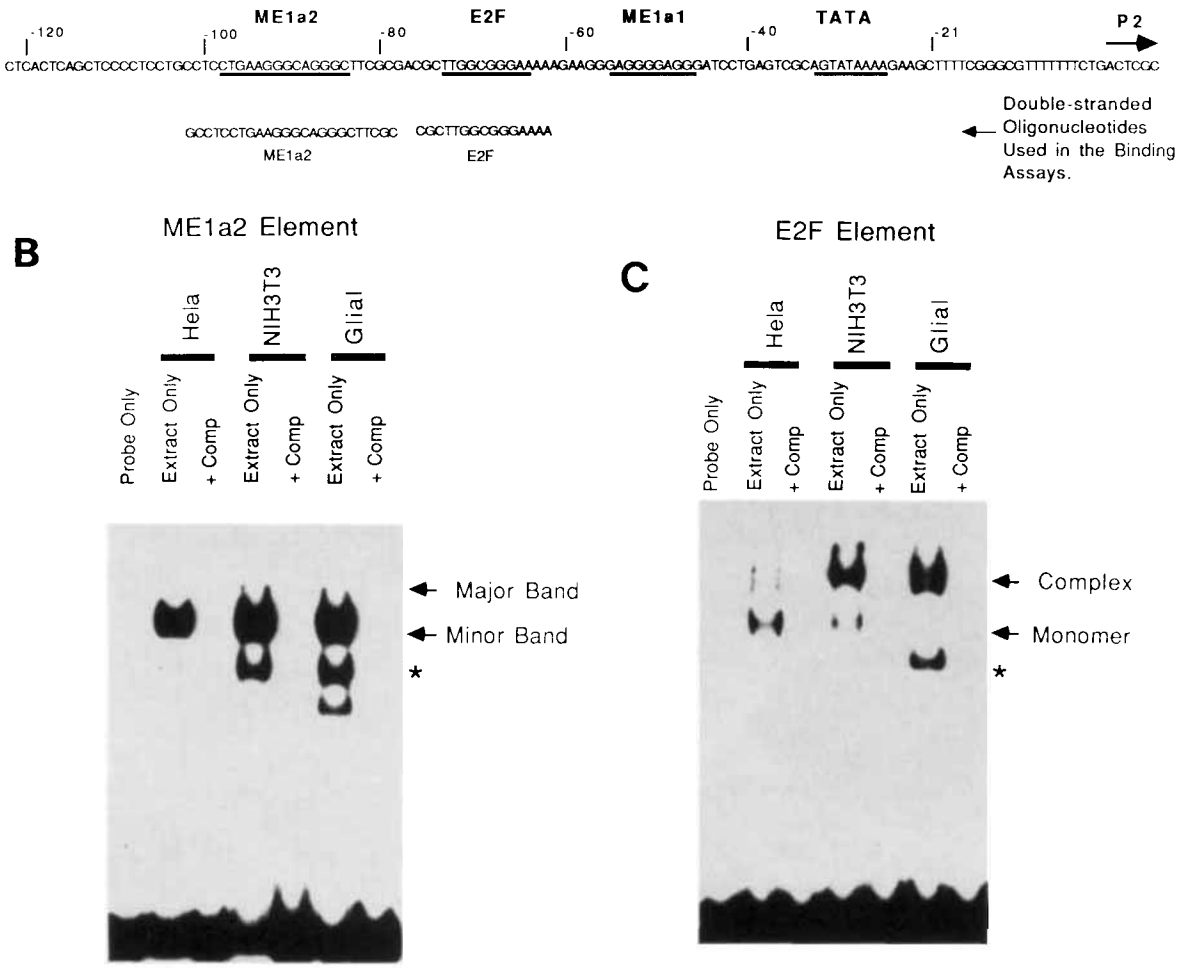


**Fig. 2.** Protein binding to the E2F element but not the ME1a2 element is cell-type specific. **A:** Diagram of *c-myc* promoter sequence and elements used in the electrophoretic mobility shift assay (EMSA). The designations ME1a2, E2F, ME1a1, and TATA indicate known protein binding sites. The double-stranded oligonucleotides used in the gel-shift assay are shown. Double-stranded oligonucleotides as in A above representing the ME1a2 (**B**) or E2F (**C**) element were $^{32}P$ end-labeled and used in the EMSA with nuclear extracts from Glial (10 μg), Hela (10 μg), or NIH3T3 fibroblasts (10 μg). "+ Comp" indicates that 100-fold molar excess of specific competitor double-stranded oligonucleotide was added to the binding reactions. The reactions were electrophoresed on a 4% low ionic strength polyacrylamide gel. The lane indicated as "Probe Only" contained no extract. For ME1a2 binding (B) the major and minor bands are indicated as is the position (asterisk) of a protein thought to be normally associated with a repressor in Hela cells. For E2F binding (**C**) the monomer and multiprotein complex with DNA are indicated while the asterisk marks the position of a novel band. Sheared salmon sperm DNA was used as a nonspecific competitor (2 μg) in all binding reactions. The intense band at the bottom of the gel is excess unbound probe.

tation adversely affects the ability of p53 to repress transcription from P2.

### SV40 Large T Antigen Abolishes the Repressive Effect of Wild-Type p53

The retinoblastoma susceptibility gene product and p53 share the capacity to bind to a tumor virus specific protein, namely, SV40 large T antigen (reviewed in Lane and Benchimol, 1990). It is thought that this binding, in part, functions to confer oncogenic activity on T antigen (it is hypothesised by functionally eliminating p53 and Rb from their sites of action). Further, it would be predicted that T antigen would

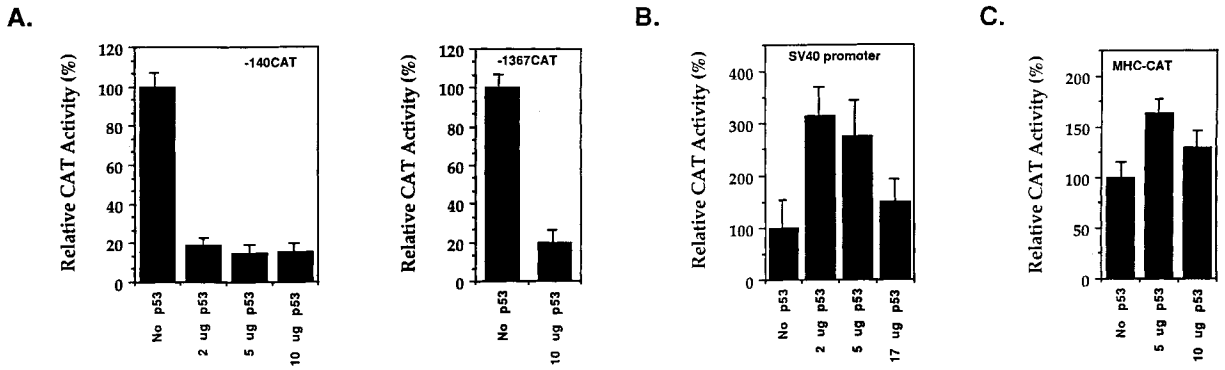


**Fig. 3.** Transcription from the *c-myc* P2 promoter but not the SV40 early or MHC promoters are repressed by wild-type p53. **A:** Effect of p53 on *c-myc*. *c-myc* promoter CAT constructs (15 μg) were cotransfected into glial cells with the indicated amounts of a plasmid expressing the wild-type form of p53. The *c-myc* promoter:CAT constructs used were as in Figure 1. CAT activity was assayed and CAT levels determined as in Figure 1. The levels were presented as relative to the condition without any p53 plasmid (arbitrarily set at 100%). **B:** Effect of p53 on SV40 early promoter. A plasmid containing the SV40 early promoter linked to CAT was cotransfected with the indicated amounts of the wild-type p53 expressing plasmid. CAT activity was assayed and presented as in (A) above. **C:** Effect of p53 on the MHC promoter. A plasmid containing the MHC, H-2K$^b$ class I promoter linked to CAT was cotransfected with the indicated amounts of the wild-type p53 expressing plasmid. CAT activity was assayed and presented as in (A) above.

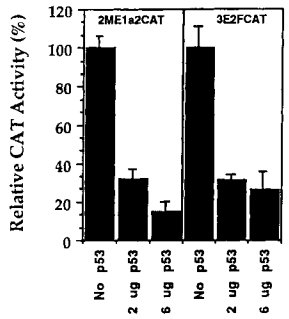


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**Fig. 4.** Wild-type p53 represses transcription from *c-myc* promoter constructs containing only the ME1a2 elements or only the E2F elements. The 2ME1a2 and 3E2F CAT constructs (15 μg) outlined in Figure 1 were cotransfected into glial cells along with the indicated amounts of a plasmid expressing wild-type p53. CAT activity was assayed and CAT levels determined as in Figure 1. The levels were presented as relative to the condition without any p53 plasmid (arbitrarily set at 100%).

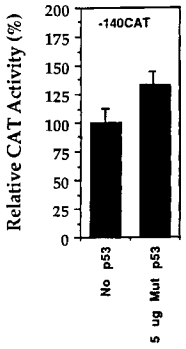


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**Fig. 5.** Mutant p53 does not repress transcription from the *c-myc* P2 promoter. The −140 *c-myc* promoter CAT construct (15 μg), as outlined in Figure 1, was cotransfected into glial cells along with the indicated amounts of a plasmid expressing a mutant p53 (ala to val change at amino acid 135). CAT activity was assayed and CAT levels determined as in Figure 1. The level was presented relative to the condition without any p53 plasmid (arbitrarily set at 100%).

be dominant to wild-type p53 when both are expressed in a given cell. Therefore, if T antigen and p53 were expressed in tissue culture cells along with a *c-myc* promoter CAT construct the repressive action of p53 would not be evident. The results of such an experiment are shown in Figure 6. It is clear that alone T antigen is capable of transactivating the *c-myc* promoter as was demonstrated recently (Moberg et al., 1992). However, addition of p53 does not appear to cause a significant reduction in the level of transcription from P2. These data indicate that T antigen is dominant to p53.

## DISCUSSION

Here we have examined the role of wild-type and mutant p53 in regulating *c-myc* transcription. It was found that overexpression of the wild-type form of p53 in the human glial cell line HTB14 resulted in a repression of transcription from the *c-myc* P2 promoter yet had no repressive effect on the MHC promoter or the SV40 early promoter. The effect of wild-type p53 could be localized to the more proximal region of the P2 promoter since a −140 deletion was repressed to the same level as a −1367 deletion.

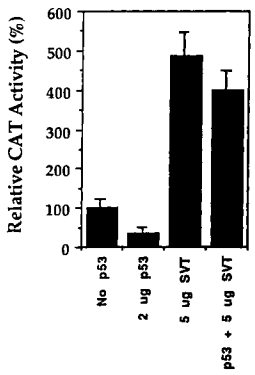


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Fig. 6. Expression of SV40 T antigen eliminates the repressive effect of wild-type p53 on *c-myc* transcription. A −140 *c-myc* promoter CAT construct (15 μg) was cotransfected with wild-type p53 expressing plasmid and an SV40 T antigen expressing where indicated. CAT activity was assayed and CAT levels determined as in Figure 1. The levels were presented as relative to the condition without any p53 plasmid (arbitrarily set at 100%).

The action of p53 could be further localized to two positions within the P2 promoter; p53 inhibited transcription from *c-myc* promoter constructs containing only ME1a2 elements or only E2F elements. Both of these sites are required for optimal transcription initiation from P2 (Asselin et al., 1989; Hall, 1990; Moberg et al., 1991, 1992). That wild-type p53 inhibits transcription from the E2F construct is consistent with previous findings that a number of regulatory factors act through the E2F site. It has been recently demonstrated that the adenovirus E1a gene product (Heibert et al., 1989; Lipp et al., 1989; Thalmeier et al., 1989) and the SV40 T antigen (Moberg et al., 1992) transactive *c-myc* transcription through the E2F site. Further it has also been demonstrated that the retinoblastoma susceptibility gene product (Chellappan et al., 1991; Bagchi et al., 1991) and cylcin A (Mudryj et al., 1991) interact with E2F. Although E2F appears to be an important site for regulation of *c-myc* it does not appear as if p53 acts directly through E2F (our preliminary experiments using a p53 monoclonal antibody in the EMSA show no direct interaction of p53 with E2F, data not shown).

While the ME1a2 site is required for optimal transcription initiation from P2 (Moberg et al., 1991, 1992), little is known of its role as a target for regulatory proteins. The fact that wild-type p53 suppresses transcription from a promoter construct containing only ME1a2 elements indicates that it plays such a role.

In the studies presented here transcription from the SV40 early promoter was not inhibited by wild-type p53 in vivo. This is surprising in light of recent evidence that wild-type p53 binds to the region encompassing the transcription factor SP1 binding sites in the early promoter (Bargonetti et al., 1991). Since SV40 large T antigen can inhibit binding of p53 to the early promoter region, it is possible that cellular factors exist that also prevent p53 from interacting with the SV40 DNA, allowing transcription to proceed.

A recent study has also revealed that p53 can block replication of SV40 DNA in an in vitro assay (Wang et al., 1989); p53 appeared to effect the ability of T antigen to function optimally in replication from the SV40 origin, likely due to an interaction of SV40 T antigen with p53 protein. Here we found that expression of SV40 T antigen along with wild-type p53 abolishes the repressive effect of p53 on *c-myc* transcription. One possible mechanism is that T antigen interacts with p53, thereby eliminating its repressive function (Lane and Benchimol, 1990). These data would indicate that SV40 T antigen is dominant to p53 in trans-activating *c-myc* transcription. Yet these results point to an apparent paradox in that replication in vitro is inhibited by p53 while transcription in vivo is not. However it is possible that the roles of T antigen in SV40 replication and in transcriptional transactivation are distinct or that cellular factors in vivo play a role in overcoming p53 repression by T antigen.

While wild-type p53 repressed transcription from the P2 promoter, a mutant form of p53 produced no such effect. This mutant form of p53 results from a point mutation at amino-acid position 135 (an ala to val change) and changes the function of p53 from a suppressor of oncogenic transformation to an activator of transformation (Finlay et al., 1989). These data indicate that the repressive action of p53 on *c-myc* transcription mirrors the effects on cell growth. Thus, it may be that an alteration in p53 structure, by a point mutation for example, results in derepression of *c-myc* expression. As a result normal cell growth controls may be lost.

In conclusion it appears that wild-type p53 plays a role in regulating *c-myc* transcription. Since p53 is a target of the $p34^{cdc2}$ kinase (Milner et al., 1990) it is possible that the activity of p53 is regulated by the cell cycle. This cell cycle regulation may then affect expression of *c-myc*

and thereby have an effect on the growth state of the cell.

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## REFERENCES

Asselin C, Nepveu A, Marcu KB (1989): Molecular requirements for transcriptional initiation of the murine *c-myc* gene. Oncogene 4:549–558.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1987): Current Protocols in Molecular Biology. John Wiley and Sons.

Bagchi S, Weinmann R, Raychaudhuri P (1991): The retinoblastoma protein copurifies with E2F-I and E1A-regulated inhibitor of the transcription factor E2F. Cell 65: 1063–1072.

Bargonetti J, Friedman PN, Kern SE, Vogelstein B, Prives C (1991): Wild-type but not mutant p53 immunopurified proteins bind to sequences adjacent to the SV40 origin of replication. Cell 65:1083–1091.

Bentley DL, Groudine M (1988): Sequence requirements for premature termination of transcription in the human *c-myc* gene. Cell 53:245–256.

Carthew RW, Chodosh LA, Sharp PA (1985): An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. Cell 43: 439–448.

Chellappan SP, Hiebert S, Mudryj M, Horowitz JM, Nevins JR (1991): The E2F transcription factor is a cellular target for the RB protein. Cell 65:1053–1061.

Cole, MD (1986): The myc oncogene: it's role in transformation and differentiation. Annu Rev Genet 20:361–384.

Dignam JD, Lebovitz RM, Roeder RG (1983): Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nuc Acids Res 11:1475–1489.

Eliyahu D, Michalovitz D, Oren M (1985): Overproduction of p53 antigen makes established cells highly tumorigenic. Nature 316:158–160.

Finlay CA, Hinds PW, Levine AJ (1989): The p53 proto-oncogene can act as a suppressor of transformation. Cell 57:1083–1093.

Hall DJ (1990): Regulation of *c-myc* transcription in vitro: dependence on the guanine-rich promoter element ME1a1. Oncogene 5:47–54.

Heibert SW, Lipp M, Nevins JR (1989): E1A-dependent trans-activation of the human MYC promoter is mediated by the E2F factor. Proc Natl Acad Sci USA 86:3594–3598.

Lane DP, Benchimol S (1990): p53: Oncogene or anti-oncogene? Genes Dev 4:1–8.

Lipp M, Schilling R, Bernhardt G (1989): Trans-activation of human MYC: The second promoter is target for the stimulation by adenovirus E1a proteins. Oncogene 4:535–541.

Milner J, Cook A, Mason J (1990): p53 is associated with $p34^{cdc2}$ in transformed cells. EMBO J 9:2885–2889.

Moberg KH, Tyndall WA, Pyrc J, Hall DJ (1991): Analysis of the *c-myc* P2 promoter. J Cell Phys 148:75–84.

Moberg KH, Logan TJ, Tyndall WA, Hall DJ (1992): Three distinct elements within the murine *c-myc* promoter are required for transcription. Oncogene. In press.

Mudryj M, Hiebert S, Nevins JR (1990): A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. EMBO J 9:2179–2184.

Mudryj M, Devoto SH, Hiebert SW, Hunter T, Pines J, Nevins JR (1991): Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. Cell 65:1243–1253.

Pietenpol JA, Stein RW, Moran E, Yaciuk P, Schlegel R, Lyons RM, Pittelkow MR, Munger K, Howley PM, Moses HL (1990): TGF-b1 inhibition of *c-myc* transcription and growth in keratinocytes is abrogated by viral transforming protein with pRB binding domains. Cell 61:777–785.

Robbins PD, Horowitz JM, Mulligan RC (1990): Negative regulation of human c-fos expression by the retinoblastoma gene product. Nature 346:668–671.

Thalmeier K, Synovzik H, Mertz R, Winnacker E-L, Lipp M (1989): Nuclear factor E2F mediates basic transcription and transactivation by E1a of the human MYC promoter. Genes Dev 3:527–536.

Wang EH, Friedman PN, Prives C (1989): The murine p53 protein blocks replication of SV40 DNA in vitro by inhibiting the initiation functions of SV40 large T antigen. Cell 57:379–392.