Regulation of Transcription of the Human Presenilin-1 Gene by Ets Transcription Factors and the p53 Protooncogene*

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The expression of the human presenilin-1 cellular gene is suppressed by the p53 protooncogene. The rapid kinetic of the down-regulation has suggested that it may result from a primary mechanism. We show here that p53 also suppresses the transcription of a presenilin-1 promoter-chloramphenicol acetyltransferase reporter synthetic gene in transient infection assays in neuroblastoma (SK-N-SH) and hepatoma (HepG2) cell lines. Only a minimum promoter including sequences from −35 to +6 from the transcription initiation is sufficient to confer down-regulation. We have previously defined a crucial DNA element containing 90% of the expression of the gene within the same short area, and the identification of the transcription factors involved should also provide insights into the regulation of PS1 by p53. This region contains an Ets transcription factor binding motif, and a 2-base pair alteration within the core sequence (GGAA to TTAA) of the Ets consensus also reduced transcription by more than 90%. We now show that Ets1 and Ets2 indeed transactivate a PS1 promoter-chloramphenicol acetyltransferase reporter including the (−35 to +6) fragment. Furthermore, in vitro translated Ets2 binds specifically to the −10 Ets motif in electrophoretic mobility shift assays. Therefore, Ets1/2 factors bind specifically to the −10 Ets element and activate PS1 transcription. We also show that the coactivator p300 enhances the activation by Ets1 and Ets2 as well as the repression by p53. p300 is known to interact with p53 as well as with Ets1 and Ets2. We show that p33 does not bind directly to the PS1 promoter. Hence the repression of PS1 transcription by p53 is likely to be mediated through protein-protein interactions.

Presenilin genes (PS1 and PS2) encode highly homologous integral membrane proteins (1, 2). A majority of early onset or familial Alzheimer's disease (FAD)† cases results from mutations in PS1, PS2, or amyloid precursor protein (APP) with a majority of cases in PS1 (3, 4). The pathogenesis of FAD includes as an early invariant the development of amyloid plaques containing specifically Aβ42/43 polypeptide (3, 5). Aβ42 is produced by sequential proteolytic cleavage of β APP (6). PS1 appears to play a crucial role in the normal metabolism of APP as well as in the pathological increase of Aβ42 (7). The exact function of PS1 in the processing of APP is still unclear. PS1 appears to be tightly associated within a multiprotein complex with γ secretase, the second of two proteases that cleave APP and that has not yet been identified (8). Recent evidence has suggested that PS1 itself may contain γ secretase catalytic activity (9–11). Furthermore, evidence that an endoprotease activity crucial for normal biological function is contained in PS1 or requires PS1 has also been derived from examining the function of PS1 in Notch receptor cleavage and activation. The PS1 homologue in Caenorhabditis elegans, SEL-12, is required for Notch receptor signaling and cell fate determination (12). Similar to APP, Notch undergoes intramembrane proteolysis, freeing the Notch intracellular domain, which translocates to the nucleus and activates the transcription of specific genes by interacting with transcription factors (13). Mutations in SEL-12 can be rescued by human wild type PS1 or PS2 (14, 15). However, they cannot be rescued by AD mutant PS1. This demonstrates the homologous function of PS1 and SEL-12. In mice, knock out of the PS1 gene (PS1 KO) (16, 17) as well as single amino acid substitutions at aspartate residues in both PS1 and PS2 (18, 19) block the endoproteolytic activation of Notch. Because in mice PS1 KO can be rescued by FAD PS1 mutants (20), it appears that the point mutations in FAD lead to gain of malfunction of PS1. However, the global phenotype of PS1 KO indicates that PS1 function is required for mammalian embryogenesis including neural tube closure and central nervous system and skeletal development (21, 22). Down-regulation of PS1 also appears to induce apoptosis in cultured cells (23, 24). Since neuronal degeneration by apoptosis has been observed in postmortem brains from AD patients (25, 26), the down-regulation of the gene could contribute to the pathogenesis of FAD. Hence, the identification of the mechanisms controlling the expression of the PS1 gene should relate directly to further understanding of development and differentiation pathways and the pathogenesis of FAD.

We have analyzed the control of transcription of the human PS1 gene, and we have previously identified a promoter fragment (−22/−178) that promotes efficient transcription in human neuroblastoma SK-N-SH cells as well as in hepatoma HepG2 cells (27). We have shown that a 16-base pair (−22/−6) fragment is required for more than 90% of the expression of the gene. An Ets consensus recognition sequence is present in this region and a mutation altering two nucleotides of the core consensus reduces transcription of the gene by more than 90%. We now present direct evidence indicating the role of Ets transcription factors in the regulation of PS1. We also show that the
transcription coactivator p300 acts as a coactivator of PS1 transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous PS1 gene (23). We now show that cotransfection of PS1 with a p53 expression vector decreases drastically PS1 transcription in our system as well. We have begun to examine the interactions of the p53 protein with the PS1 promoter and the transcription factors involved in its regulation.

**Experimental Procedures**

Plasmids and Expression Vectors—The construction of the human PS1 promoter-CAT reporter plasmids has been described previously (27). The vectors EVRF0-Ets1 and EVRF0-Ets2 express the human Ets1 and Ets2 transcription factors from the CMV promoter (28) and were a gift from Dr. B. J. Graves (University of Utah) and Dr. R. Maki (Burnham Institute). The empty vector EVPFO was used as a control for Ets1 and Ets2 in transient infection assays. The pC53-SN3 and pC53-SCX3 vectors expressing the wild type and mutant p53 were a gift from Dr. B. Vogelstein (John Hopkins University) (29). The control vector pCMV1 was obtained by releasing the p53 cDNA insert with BamHI and religation. The pCMV/p300 plasmid expressing the p300 protein from the CMV promoter was a gift from Dr. S. Grossman and Dr. D. Livingston (Dana Farber Cancer Institute) (30). The empty vector pCMV2 was obtained by releasing the p300 cDNA insert by double digestion with NotI and HindIII followed by treatment with the Klenow fragment of DNA polymerase I and religation. The Ets2 vector was constructed by releasing the Ets2 cDNA from EVRF0-Ets2 with BamHI and inserting it downstream from the T7 promoter in Bluescript KS. The pGEX-p53 wt and pGEX-p53 (m143) were constructed by inserting the p53 fragment of DNA polymerase I and religation. pEts2 was constructed by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2. p53 protein has been shown by others to down-regulate the expression of the endogenous transcription with Ets1 and Ets2.
Regulation of Presenilin-1 Gene

RESULTS

Repression of Transcription of the PS1 Promoter by p53 Protein—SK-N-SH cells were cotransfected with a PS1CAT reporter gene together with increasing amounts of p53 wild type expression vector, the p53 (m143) single amino acid mutant, or the empty vector pCMV1. Increasing the amount of wild type p53 vector from 0.25 to 2 μg resulted in greater suppression of PS1 expression. Promoter activity was reduced by more than 10-fold in the presence of 2 μg of p53 vector as compared with transfections with the control vector. Cotransfection with p53 (m143) did not affect significantly the expression of PS1 (Fig. 1). Virtually identical results were observed with HepG2 cells (data not shown). The production of p53 protein in transfected cells was verified by Western analysis of cellular proteins (Fig. 2A). Transfection of SK-N-SH cells with 2 μg of p53 expression vector increased the level of p53 protein in transfected cells (p53 lanes). As a result, the level of p53 in the transfected cell population appears significantly higher than the background of endogenous p53 (C lane). Furthermore, the transfection efficiency measured by transfecting the pSV-β-galactosidase vector and staining the cells for β-galactosidase activity was routinely 5 to 8%. Hence the increase in the level of p53 protein in cells transfected with p53 expression vector as compared with cells transfected with the control vector that appears in Fig. 2A is largely underestimated. p53 appears as multiple bands on our Western blots. The expression of p53 protein is tightly regulated, including at the posttranslational level, and covalent modifications by phosphorylation, acetylation, and glycosylation have been described that can potentially affect p53 activities, stability, and nuclear localization (33). Polyacrylamide gels such as that displayed in Fig. 2A typically enable resolution of different isoforms of p53 (34). Thus it is possible that the multiple bands observed represent alternative p53 isoforms differing by covalent modification. However we cannot rule out that some of the species represent degradation products. We also monitored the changes in specific DNA binding activity to a consensus p53 recognition motif (Fig. 2B). EMSAs including nuclear extracts from SK-N-SH cells transfected with the p53 expression vector (lanes p53) show a specific complex A that appears with a wild type probe (lane 2) but is absent with a mutant probe containing a four-base pair alteration within the p53 consensus motif (lane 7). The specific recognition of the p53 DNA binding motif by the complex A is also indicated by its competition in the presence of an excess of cold wild type p53 oligonucleotide (lane 3), whereas it remains virtually unaffected by the addition of the mutant competitor (lane 4). Furthermore, the amount of complex A is higher in extracts from p53-transfected cells (lane 2) as compared with cells transfected with the empty vector (lane 5). Hence the data suggest that this complex may represent or include p53, in which protein level and binding activity both increase markedly in p53-transfected cells.

Localization of the PS1 Promoter Sequences Required for the Repression of Transcription by p53—Next we compared the effects of p53 on the transcription of alternative PS1 promoter fragments in SK-N-SH cells (Fig. 3). Elimination of 5’ sequences from −687 to −22 or reduction of 3’ sequences from +178 to +6 did not eliminate repression by p53. Indeed, transcription from constructs including sequences from −22 to +178 or −35 to +6 was reduced by about 10-fold when cotransfected with p53, which is comparable with the repression observed with the (−687, +178) PS1 construct. Thus the p53 response element(s) appears to be located within the short −22 to +6 region around the initiation site, which also contains a major positive determinant of PS1 expression (27). Identical results were obtained in HepG2 cells.

p53 Does Not Bind Directly to DNA of the PS1 −10 Promoter Region—The −22 to +6 region does not contain any sequence similarity to the consensus motif reported for p53. However we have examined directly the binding of p53 to the PS1 promoter. We used a GST-p53 fusion protein synthesized in E. coli and tested its ability to bind to the −22/+6 PS1 fragment, simultaneously comparing its binding to a p53 consensus oligonucleotide probe in parallel EMSAs (Fig. 4). Incubation of GST-p53wt protein with a wild type p53 oligonucleotide probe produced a specific DNA-protein complex (lane 2), whereas the probe remained free in the presence of GST-p53(m143) (lane 3).
Fig. 3. Delineation of the minimum sequences required for the repression of the PS1 promoter by p53. SK-N-SH cells were cotransfected with a series of PSICAT reporter constructs containing various promoter fragments together with either a wild type p53 expression vector, a mutant p53 (m143), or the empty pCMV1. The 5′ and 3′ end points of the promoter fragments included in each construct are indicated on the y axis. Promoter activity is expressed in arbitrary units (x axis), assigning 100 to the activity of the maximum promoter construct cotransfected with the pCMV1 control vector. The corresponding numerical values for promoter activity are indicated in the table below the graph.

![Promoter Activity Graph](image)

The addition of DO-1 anti-p53 antibody to the GST-p53 protein resulted in a supershifted complex (lane 4) with no supershift observed in the present of buffer alone or an unrelated anti-Ets antibody (lanes 6 and 5, respectively). No complex was observed with wt or mutant GST-p53 with a p53 mutant oligonucleotide (lanes 8 and 9). No complex was observed using the −22/+6 PS1 probe (lanes 10–12). Therefore p53 does not appear to bind directly to PS1 promoter.

Ets1 and Ets2 Transactivate the PS1 Promoter—To further analyze the mechanism of action of p53, we have begun to identify the transactivators acting over the −22 to +6 region. We directly tested the function of known Ets transcription factors since an Ets motif present at −10 plays a crucial role in the control of the gene. The analysis of the PS1 promoter was originally carried out in SK-N-SH and HepG2 cells where at least one of the protein-DNA complexes formed with the PS1 promoter appears to include a protein sharing an antigenic determinant with Ets2 (Fig. 10D of Ref. 27). Therefore we have examined whether Ets2 and the closely related factor Ets1 affect the expression of PS1 in transient infection assays. Co-transfection of a (−687, +178) PS1CAT promoter fusion gene with Ets1 or Ets2 resulted in 3–10-fold activation of PS1 transcription as compared with the empty EVRF0 vector (Fig. 5). Similar data were obtained in both SK-N-SH and HepG2 cell lines. Thus it appears that both Ets1 and Ets2 can act as transactivators of PS1. The deletion of promoter sequences outside of the interval −35 to +6 did not affect the activation by Ets1 or Ets2. Indeed Ets1 or Ets2 activated by 3–4-fold the expression of the (−35, +6) PS1CAT construct. Hence it is likely that the −10 Ets motif functions as a target site for Ets transcription factors. The considerable reduction in promoter activity resulting from deletion of 3′ sequences does not appear to result from deletion of Ets sites since point mutations eliminating individual or all Ets motifs present downstream did not affect PS1 expression.

Transfection of p53 together with Ets1 or Ets2 expression vectors reduced transcription in similar proportion whether promoter activity was stimulated by Ets1 or Ets2, or not (data not shown). Indeed the induction effect of Ets1/2 is conserved in both SK-N-SH and HepG2 cells where at least one of the protein-DNA complexes formed with the PS1 promoter appears to include a protein sharing an antigenic determinant with Ets2 (Fig. 10D of Ref. 27). Therefore we have examined whether Ets2 and the closely related factor Ets1 affect the expression of PS1 in transient infection assays. Co-transfection of a (−687, +178) PS1CAT promoter fusion gene with Ets1 or Ets2 resulted in 3–10-fold activation of PS1 transcription as compared with the empty EVRF0 vector (Fig. 5). Similar data were obtained in both SK-N-SH and HepG2 cell lines. Thus it appears that both Ets1 and Ets2 can act as transactivators of PS1. The deletion of promoter sequences outside of the interval −35 to +6 did not affect the activation by Ets1 or Ets2. Indeed Ets1 or Ets2 activated by 3–4-fold the expression of the (−35, +6) PS1CAT construct. Hence it is likely that the −10 Ets motif functions as a target site for Ets transcription factors. The considerable reduction in promoter activity resulting from deletion of 3′ sequences does not appear to result from deletion of Ets sites since point mutations eliminating individual or all Ets motifs present downstream did not affect PS1 expression.

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Ets2 Binds Specifically to the −10 Ets Element—As a corollary to these functional assays we have verified that Ets transcription factors indeed bind to the −10 region of PS1. The specific binding of in vitro translated Ets2 protein to an oligonucleotide including sequences from −22 to +6 was tested in EMSAs. The specific protein-DNA complex formed (bottom arrow) (Fig. 6, lane 2) could be eliminated by competition with an excess of unlabeled oligonucleotide containing a heterologous Elk1 binding site (27) (lanes 5 and 6), whereas the same oligonucleotide with a two-base pair mutation within the Ets consensus was no longer an effective competitor (lanes 3 and 4).

Fig. 4. GST-p53 does not bind to the −22/+6 region of the PS1 promoter. Wild type GST-p53 (+) or the mutant GST-p53 (m143) (m) were incubated with wild type (lanes 1–6) or mutant (lanes 7–9) oligonucleotide probes or with a PS1 (−22/+6) probe (lanes 10–12). Wild type GST-p53 protein was added in lanes 2, 4, 5, 6, 8, and 11. GST-p53 (m143) was added in lanes 3, 9, and 12. No protein was added in lane 1, 7, and 10. An arrow indicated the position of the GST-p53-DNA complex. Anti-p53 antibody DO-1 (D) was added in lane 4. Anti-Ets1 antibody (E) or buffer alone (C) was added in lanes 5 and 6. The position of the supershifted complex is marked (*).

The addition of DO-1 anti-p53 antibody to the GST-p53 protein resulted in a supershifted complex (lane 4) with no supershift observed in the present of buffer alone or an unrelated anti-Ets1 antibody (lanes 6 and 5, respectively). No complex was observed with wt or mutant GST-p53 with a p53 mutant oligonucleotide (lanes 8 and 9). No complex was observed using the −22/+6 PS1 probe (lanes 10–12). Therefore p53 does not appear to bind directly to PS1 promoter.

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* M. Pastorcic, unpublished information.
The identity of the complex was confirmed by the supershift produced (top arrow) in the presence of the antibody sc-351 raised against Ets2 (lane 7) or by its inhibition in the presence of the antibody sc-112 raised against a protein region conserved between Ets1 and Ets2 (lane 8). No complex was formed with an oligonucleotide probe containing a two-base pair substitution from GGAA to TTAA within the Ets consensus (data not shown). Together with the data from the transfection assays above, this suggests the −10 Ets element is crucial for the transactivation of the PS1 gene by Ets transcription factors.

p300 Is a Coactivator of Transactivation of the PS1 Promoter by Ets1 and Ets2—p300 is a transcription coactivator that was recently shown to cooperate with Ets1 and Ets2 to activate several promoters (35, 36). p300 acts by mediating interactions between basal transcription factors and DNA binding transcription factors as well as numerous other regulatory proteins (37). In particular, p300 is known to bind and synergize with p53 (38–40). Hence we sought to explore a mechanism by which p53 would inhibit the transcription of PS1 by altering the interactions of Ets1 and Ets2 with p300 or by altering the interaction of p50 with other factors within the initiation complex. First we asked whether p300 functions as a coactivator for the PS1 promoter. Cotransfection of p300 together with Ets2 resulted in a drastic increase in the activation of transcription of the PS1 promoter-reporter construct as compared with cotransfection of Ets2 with the pCMV2 control vector (Fig. 7). However, p300 affected only marginally the expression of PS1 when the EVRF0 control vector was substituted for Ets2 (lane 8). This suggests that the −10 Ets element is crucial for the transactivation of the PS1 promoter by Ets transcription factors.

p300 Synergizes with p53 to Repress Transcription of PS1—The PS1CAT construct was cotransfected in HepG2 cells together with p300 or the pCMV2 empty vector in the presence of increasing amounts of p53. Transcription of PS1 decreased by 2–10-fold when 0.3 to 2.4 μg of p53 were transfected in the presence of p500, whereas it was not significantly affected by any amount of p53 in the presence of the pCMV2 empty vector (Fig. 8). Cotransfections with the p53(m143) mutant did not alter PS1 expression as compared with the control pCMV1 vector. Thus, under these conditions, the repression by p53 appears to be dependant on the presence of p300. The lack of inhibition of PS1 by p53 in the presence of pCMV2 vector contrasts with data in Fig. 1 showing suppression of PS1 by p53. This may be the result of interactions of the CMV promoter with the endogenous p300 which may be “scavenged” in transcription complexes bound to the relatively high concentration of CMV promoter transfected in the experiments. This would be consistent with the lower level of expression observed for the same PS1 construct in the presence of the pCMV2 vector.

DISCUSSION

We have shown that Ets1 and Ets2 bind to the −10 motif of the PS1 promoter and activate its transcription. Ets factors recognize specific DNA motifs including a central GGA. However the flanking sequences determine a more narrow specific recognition by selective members of the Ets family. The sequence surrounding the −10 GGA element in PS1 is most similar to the sequences preferred by Ets1, ER81, and GABPal (41). We have first tested the function of Ets1 and Ets2. We also show that p300 acts as a coactivator of Ets1 and Ets2 for the PS1 gene. Ets1 and Ets2 have recently been shown to interact with p300/CBP (35, 36). The regions of p300/CBP that are required to bind to Ets factors have been mapped by deletion,
and they correlate well with the areas required for transcription activation. Ets1 interacts with an N-terminal fragment extending from amino acids 357 to 452 (35) or amino acids 328 to 596 (36). It also interacts with a C-terminal fragment from amino acids 1459 to 1892 (35) or 1678 to 2370 (36), both including a cysteine- and histidine-rich region. Ets1, Ets2, or the transactivation domain of Ets2 recognize both fragments, whereas the DNA binding domain of Ets2 only binds to the C-terminal fragment. The binding of p300/CREB mutants has been tested in pull-down experiments using proteins expressed in E. coli. However, the wild type p300/CREB indeed binds to Ets1 and Ets2 in vivo, since the proteins can be coimmunoprecipitated in crude nuclear extracts. Hence the association of p300/CREB with Ets1 and Ets2 occurs in the absence of DNA. Therefore one would expect the coactivation of Ets1 and Ets2 by p300 to be relatively promoter-independent. However, the ability of p300 to act as a coactivator of Ets factors shows some degree of promoter specificity. For example, p300 did not act as a coactivator of PEA3 in the context of the stromelysin promoter, whereas it did coactivate with PEA3 in the context of a synthetic promoter including two palindromic Ets sites upstream from a minimal promoter containing 56 base pairs of upstream sequence from the c-fos gene (36). Furthermore, PEA3 did transactivate both promoters. The dependence of p300/CREB coactivation on the promoter context can be simply interpreted as a requirement for specific transcription factors that typically partner with Ets monomers to activate synergistically promoters where their binding site is present near the Ets motif. Therefore it is now important to identify the sequence element(s) outside the Ets binding site that is required for p300 coactivation of Ets1/2. In particular, an AP1 motif is present in immediate proximity (Δ25) of the Ets motif. This structure is apparently similar to the oncogene-responsive domain of the polyoma enhancer where Ets factors cooperate with c-Fos and c-Jun (42). A detailed analysis of PS1 sequences required for p300 coactivation of Ets1/2 involving localized mutagenesis is in progress.

Either the N-terminal amino acids 1 to 1097 or the C-terminal amino acids 1678 to 2442 can function independently as Ets2 coactivators (36). Hence the ability of Ets1/2 to interact independently with the N- and C-terminal domain of p300 may suggest some degree of redundancy. However, interactions with the C- and N-terminal domains of p300/CREB may not be equivalent. Indeed, the Ets2 activation domain binds to ei-

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**FIG. 6.** *In vitro* translated Ets2 binds specifically to the −10 region of the PSI promoter. Ets2 transcription factor was synthesized by *in vitro* transcription and translation, and its specific binding to an oligonucleotide probe bearing *PS1* sequences from −22 to +6 was tested by EMSAs. DNA binding reactions included 2-μl aliquots of *in vitro* translation reaction (lanes 2–9) or 2 μl of control reaction where the empty KS replaced pEts2 (lane 10). No extract was added in lane 1. Lanes 5 and 6 contained 10 and 20 ng of a heterologous oligonucleotide competitor containing an Elk1 binding site. Lanes 3 and 4 contained 10 and 20 ng, respectively, of the same competitor with a two-base pair mutation within the Elk1 binding motif (m). Antibodies raised against Ets2 (αEts2) and a region conserved in Ets1 and Ets2 (αEts1/2) were added in lanes 7 and 8. The same aliquot of antibody buffer was added in the control lane 9 (C). Arrows mark the position of the specific complex.

**FIG. 7.** p300 acts as a coactivator of Ets1 and Ets2. A PS1CAT reporter construct (6 μg) containing sequences from −118 to +178 was cotransfected in HepG2 cells with expression vectors for Ets1, Ets2, or EVRF0 (V) (2 μg) together with a p300 expression vector or the empty pCMV2 vector (CMV) (A, 3 μg; B, 9 μg). Promoter activity was expressed in arbitrary units, assigning 100 to the activity in transfections with EVRF0 and 3 μg pCMV2.
ther N- or C-terminal domains of p300, whereas the Ets domain recognizes only the C-terminal region (36). This may indicate that alternative interactions might enable various cooperating factors known to act in concert with Ets to contact p300 as well, depending on promoter context. Indeed different sets of transcription factors recognize the N-terminal or the C-terminal regions of p300/CBP (37).

We have shown that p53 represses the transcription of the PS1 promoter and that the short promoter area from -118 to +178 was cotransfected with an expression vector for p300 or the empty control vector pCMV2 (9 μg) together with increasing amounts (0 to 2.4 μg) (x axis) of an expression vector for the wild type p53 protein, p53m(143), or pCMV1. The amount of DNA was kept constant by the addition of pCMV1. Promoter activity was reported, assigning 100 to the value obtained by cotransfecting PS1CAT with the control vectors (y axis).

**Fig. 8.** p300 acts as a corepressor of p53. A PS1CAT reporter construct (6 μg) containing promoter sequences from -118 to +178 was cotransfected with an expression vector for p300 or the empty control vector pCMV2 (9 μg) together with increasing amounts (0 to 2.4 μg) (x axis) of an expression vector for the wild type p53 protein, p53m(143), or pCMV1. The amount of DNA was kept constant by the addition of pCMV1. Promoter activity was reported, assigning 100 to the value obtained by cotransfecting PS1CAT with the control vectors (y axis).

**Fig. 9.** Nuclear factors bind specifically to the PS1 promoter and recognize the p53 binding motif. Nuclear extracts from SK-N-SH cells transfected with the p53 expression vector (p53) or the empty vector (C) were included in EMSAs with a PS1 -22/+7 probe, including a wild type GGAA Ets motif (wt) or a mutant TTAA motif (m). Lanes 2–6 and 8, 10, 12, and 14 included 2 μl of nuclear extract (5 mg/ml). Lanes 7, 9, 11, and 13 included 1 μl of nuclear extract. No protein was added in lane 1. An excess of cold wild type (lanes 3 and 4) or mutant (lanes 5 and 6) p53 oligonucleotide was added as a competitor; 10 ng were added in lanes 3 and 5, and 30 ng were added in lanes 4 and 6. An arrow indicates the position of the complex A, which is selectively competed by the p53 wild type oligonucleotide.

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| Probe | wt | m |
|-------|----|---|
| Extract | 0 | p53 | C | p53 | C | p53 |
| Competitor | p53+ | p53m | 10 | 30 | 10 | 30 |

It is also interesting to note that unlike p53, the p53-like protein p73 interacts with N-terminal region of p300 (43). However, p300/CBP interacts with the N-terminal transactivation domains of both p53 (38–40) and p73; this is consistent with the lower (29%) degree of conservation of the transactivation regions between the p53 homologues (44, 45). Interestingly, p73 has been mapped to a region found frequently deleted in cancers, including a large proportion of neuroblastomas (45). It will be interesting to compare how transactivation of PS1 by Ets factors is affected by p53 and its homologues.
The relevance in vivo of the inhibition of PS1 by p53 is supported by the strong anti-apoptotic function of PS1. Furthermore, PS1 was identified as one of the genes down-regulated during p53-induced apoptosis (46). p53-dependent apoptosis is a response to a variety of stress signals including DNA damage, hypoxia, and the induction of oncogenes. Hence the repression of PS1 is likely to be a key factor in developmental regulation, the elimination of damaged cells, and cancer prevention. Both the down-regulation as well as the activation of PS1 are likely to be critically regulated during development, and it appears that Ets transcription factors may play a pivotal role in both aspects. Ets factors are usually implicated in the regulation of genes involved in development and the control of cell growth (47). Interestingly, a first example of their role in the differentiation of a neuronal system has just been reported (48). The ER81 gene was shown to define the assembly of neuronal circuits in the development of the vertebrate central nervous system. The cell-specific expression of ER81 was identified as one of the genes down-regulated during p53-induced apoptosis (46). p53-dependent apoptosis induced during p53-induced apoptosis (46).

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