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Mammalian p53 can function as a transcription factor in yeast

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ABSTRACT

p53 has previously been shown to contain a transactivation domain using GAL4 fusion proteins and to bind specifically to a 33 base pair DNA sequence in immunoprecipitation assays. We show here that mammalian p53 expressed in *S.cerevisiae* is able to activate transcription of a reporter gene placed under the control of a CYC1 hybrid promoter containing the 33 base pair p53-binding sequence. The activation is dependent on the orientation and number of copies of the binding site. Three p53 mutants commonly found in human tumours, 175H, 248W and 273H, are unable to activate transcription. A fourth human p53 mutant, 285K, is temperature-sensitive for transcriptional activation. Murine p53 activates transcription from the same sequence. The murine 135V mutant, which is temperature-sensitive for mammalian cell transformation, is also temperature-sensitive for transcriptional activation. There is a much better correlation between mutation and transcriptional competence than between mutation and the structure of p53 determined with conformation-sensitive antibodies. We have therefore developed a simple transcription assay for p53 mutation in which yeast are transfected with p53 PCR products and mutation is scored on X-gal plates.

INTRODUCTION

The p53 gene is the oncogene which is most frequently mutated in human tumours (1,2). Mutation converts p53 from a suppressor of transformation into a dominant transforming oncogene (3,4). There are two models for the biochemical action of p53: that p53 controls the onset of DNA replication (5,6,7,8); and that p53 is a transcription factor. There is mounting evidence that p53 is involved in transcriptional control. Wild type p53 stimulates transcription from the muscle-specific creatine kinase enhancer (9) and inhibits transcription from the interleukin 6, c-fos, β-actin, hsc70, c-jun and p53 promoters (10,11). p53::GAL4 fusion proteins containing the amino-terminal 73 amino acids of p53 can activate transcription in yeast and mammalian cells (12,13). The transactivation can be inhibited by mutations in the central part of the p53 molecule such as those which have been found in tumours and activate p53 for transformation (14,15). p53 also shows non-specific (16,17,18) and sequence-specific DNA binding activity which is abolished by the mutations which activate p53 for transformation (19,20). Wild type p53 protects GC rich sequences directly adjacent to the SV40 origin in DNaseI footprinting assays (19). This region contains three repeats of a 21 base pair sequence (21) and includes six repeats of the sequence bound by the Sp1 transcription factor [GGGCGG (22)]. Wild type p53 has also been shown in an immunoprecipitation assay to bind specifically to two restriction fragments containing repeats of the sequence TGCCT (20). One fragment is derived from a human extrachromosomal circular DNA related to a transposon-like repetitive element called THE-1 (23) and the other fragment is derived from the untranscribed ribosomal spacer region (24). The TGCCT consensus was shown to be important for p53 binding to the ribosomal spacer fragment by both methylation interference and mutational analysis (20). The SV40 origin is involved in both DNA replication and transcription, and the THE-1 and ribosomal spacer p53-binding sequences are thought to lie close to DNA replication origins (20).

The conflicting results obtained in mammalian cells suggested to us that a simpler system might be useful. Since the transcriptional machinery is highly conserved in yeast and mammals (25) we examined the possibility that p53 may be able to activate transcription in a sequence-dependent manner in yeast. We present clear evidence that wild type, but not mutant, p53 can function as a classical transcription factor when expressed in yeast.

METHODS

Strains and media

The protease deficient strain GA71 was used throughout (genotype Mata leu2 trp1 ura3-52 prb1-1122 prc1-407 pep4-3). The strain (previously called BJ2169) was obtained from S.Gasser and created by E.Jones. Routine manipulation of yeast was carried out as described (26). Plasmids were transfected into yeast by electroporation (26). For induction of p53 expression strains were

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grown overnight in 2% raffinose minimal medium and transferred to 2% galactose minimal medium five and a half hours before harvesting. Unless stated otherwise yeast were grown at 30°.

Plasmids

Plasmids were constructed using standard techniques (27) and checked for the presence of desired mutations by DNA sequencing using modified T7 DNA polymerase (Sequenase, United States Biochemical).

p53 was expressed in *S.cerevisiae* using P2, a vector containing the GAL1/10 promoter cloned into the ApaI site and the CYC1 terminator cloned into the SacII site of pRS314 (G.Micklem, personal communication). pRS314 is a yeast low copy number replicating plasmid containing the TRP1 gene, CEN6 and ARSH4 (28). p53 cDNAs containing various mutations were derived from bacterial expression plasmids constructed by Midgely et al (29). The human p53 cDNAs were transferred as XbaI/SmaI fragments and the mouse p53 cDNAs as XbaI/HindIII fragments into SpeI/NotI digested P2. The 5'-untranslated region in the p53 mRNA contains 55 nucleotides of the GAL1 mRNA followed by CCCCGGATCC ACTAGAAATA ATTTTGTTTA ACTTTAAGAA GGAGATATAC GC-ATG... in the plasmids expressing the human 175H, 248W and 273H cDNAs. In plasmids expressing the human wild type and 285K cDNAs the underlined A is replaced by CCCCTCGA. In the plasmids expressing murine p53 the underlined GC is replaced by AT.

The transcription reporter plasmids contain the URA3 gene, the 2μ replication origin and the *E.coli* lacZ gene coding sequence fused to the *S.cerevisiae* CYC1 gene transcription control elements (30). pLGΔ312 contains the complete CYC1 upstream region ('intact CYC1 promoter'); pLGΔ178 contains a truncated version lacking the upstream activating sequences ('minimal promoter'). There is a XhoI site at the 5'-end of the promoter in pLGΔ178 into which DNA fragments can be cloned to test them for enhancer activity. A double-stranded oligonucleotide adaptor with the sequence 5'-TCGA-CCTTGCCTGGAC-TTGCCTGGCCTTGCCTTT-TCGA-3' (20) was cloned into the XhoI site in pLGΔ178 and a set of plasmids containing different multimers and orientations of the adaptor was obtained. For the experiments presented in table 1, a single copy of the adaptor with the indicated 3'-end adjacent to the CYC1 promoter was used ('orientation-1'). For table 2, orientation-1 was used in the constructs marked '1, 2, 3 copies p53 oligo'; the adaptor was reversed (ie, with the indicated 5'-end adjacent to the CYC1 promoter, 'orientation-2') in the construct '1 copy p53 oligo*'; the palindrome construct contains two copies of the adaptor with the indicated 5'-end in the middle of the palindrome. For table 3 and figures 1 and 5, a single copy of the adaptor in orientation-2 was used. For figures 3 and 4, three copies of the adaptor in orientation-1 were used.

Antibodies

PAb240 (31), PAb246 (32), PAb1620 (33,34,35) and PAb421 (36) are mouse monoclonal antibodies against p53. CM1 is a rabbit polyclonal antibody against p53 (37). BG2 (D.Lane), PAb204 (38) and 7.10 (S.Lindquist) are monoclonal antibodies against β-galactosidase, SV40 T antigen and hsp70, respectively.

Immunoprecipitation and immunoblotting were performed as described (39). Cells were lysed by vortexing for 1 minute with acid-washed glass beads in 150 mM NaCl, 50 mM Tris pH8.0, 5 mM EDTA, 1 mM DTT and 0.1 mg/ml PMSF. NP40 was then added to a final concentration of 1% and the extract was left on ice for 15 minutes. After clarification by centrifugation at 100,000×g for 15 minutes the extract was preabsorbed with Protein G-Sepharose beads (Pharmacia) for 30 minutes. The extract was divided into equal aliquots and monoclonal antibody-coated Protein G beads were added. After incubation at 4° for one hour beads were collected by centrifugation, washed three times in lysis buffer and resuspended in SDS gel sample buffer. For immunoblotting without prior immunoprecipitation cells were resuspended in 150 mM NaCl, 50 mM Tris pH 8.0, 1% NP40, 0.5% Na deoxycholate and 0.1% SDS, lysed with glass beads, and an equal volume of 2×SDS gel sample buffer was added.

β-galactosidase assays

β-galactosidase assays were performed as described (40) using SDS/chloroform lysis. 10^7 cells were incubated with o-nitrophenyl-β-D-galactopyranoside (ONPG) for 5 minutes. Samples were spun at 10,000×g for 5 minutes to remove debris before measurement of OD. Results are expressed as (1000×A_{420}) per minute after deduction of the absorbance of a control sample lacking ONPG. For analysis of temperature-sensitive mutants cells were not preincubated on ice. Each value is the mean of three assays ± standard deviation.

Gap repair assay

The recipient strain for the gap repair experiments was initially transfected with a pLGΔ178-derived lacZ reporter plasmid containing 3 copies of the p53 DNA-binding site adaptor oligonucleotide (in orientation-1, see above). 1 ng of the human wild type and 273H mutant p53 cDNA plasmids proSp53 (41) and pR4.2 (42) were amplified for 30 cycles (92° for 30", 60° for 30", 72° for 150") with 2 units AmpliTaq DNA polymerase in buffer containing 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton-X100, 200 μM deoxynucleotides, 10% dimethyl sulfoxide, 100 μM tetramethylammonium chloride and 5 μg/ml primers, and the products were extracted with chloroform and precipitated with ethanol. The polymerase chain reaction (PCR) primers used were TGGATTGGCAGCCAGAC-TGCCTTCC (11 nucleotides before the initiator methionine codon) and GTGGGAGGCTGTCAGTGGGGAACAA (14 nucleotides after the stop codon). The yeast expression plasmid containing the human p53 175H mutant (described above) was digested with StyI and the vector fragment was gel purified away from the p53 stuffer fragment (codons 160 to 347, see figure 7). The gapped plasmid and PCR products overlap by 477 base pairs at the 5'-end and 280 base pairs at the 3'-end. ~100 ng of gapped plasmid was mixed with ~500 ng of PCR product, transfected by electroporation into the strain containing the lac Z reporter plasmid and 1/100th of the transfection mix was spread on plates containing 1 M sorbitol, 2% glucose, 0.67% yeast nitrogen base (Difco), 1% casaminoacids (Difco) and 2.5% agar (Difco). To detect β-galactosidase activity individual colonies were streaked onto plates containing 40 mg/l X-gal (Sigma), 0.1 M phosphate buffer pH 7.0, 2% galactose, 0.67% yeast nitrogen base (Difco), 1% casaminoacids (Difco) and 2.5% agar (Difco).

RESULTS

Human p53 expression in yeast

The ability of p53 to activate transcription was tested by cotransfecting yeast with a p53 expression plasmid and lacZ reporter plasmids (table 1). The positive control contains the intact CYC1 promoter fused to the lacZ gene coding sequence.

Table 1. β-galactosidase assays. Units of β-galactosidase were calculated as described in the methods section and represent the mean of three independent assays ± standard deviation. The intact CYC1 promoter is mildly repressed by glucose (76).

Expression plasmid	lacZ reporter plasmid	Glucose	Galactose
No insert	Intact CYC1 promoter	180 ± 14	546 ± 23
Wild type human p53	Intact CYC1 promoter	205 ± 27	523 ± 20
No insert	Minimal promoter	1 ± 1	1 ± 1
Wild type human p53	Minimal promoter	1 ± 1	2 ± 2
No insert	p53::CYC1 promoter	1 ± 1	3 ± 1
Wild type human p53	p53::CYC1 promoter	1 ± 1	139 ± 1

Table 2. β-galactosidase assays using different multimers and orientations of the p53-binding oligonucleotide. *The orientation of the oligonucleotide was reversed in this construct.

lacZ reporter plasmid	Glucose	Galactose
Minimal promoter	2 ± 1	1 ± 1
Intact CYC1 promoter	228 ± 9	340 ± 19
1 copy p53 oligo	3 ± 1	98 ± 8
2 copies p53 oligo	2 ± 2	338 ± 42
3 copies p53 oligo	2 ± 1	639 ± 63
1 copy p53 oligo *	2 ± 1	441 ± 54
Palindrome p53 oligo	3 ± 1	653 ± 28

Table 3. β-galactosidase assays using different p53 mutants. Cells were grown in medium containing galactose.

Human p53 gene	Minimal promoter	p53::CYC1 promoter
wild type	3 ± 1	334 ± 13
175H	2 ± 2	3 ± 1
248W	3 ± 1	2 ± 1
273H	3 ± 1	3 ± 1
285K	3 ± 1	24 ± 4

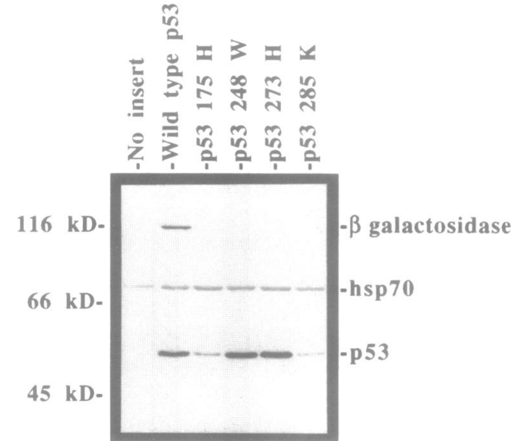


Figure 1. Immunoblot of yeast probed with antibodies against p53 (PAb240 and PAb421), hsp70 (7.10) and β-galactosidase (BG2). Strains containing different p53 expression plasmids and a lacZ reporter plasmid were grown in galactose to induce p53 expression. A faint band of β-galactosidase was visible with the 285K mutant after prolonged development of the blot. hsp70 acts as a loading control.

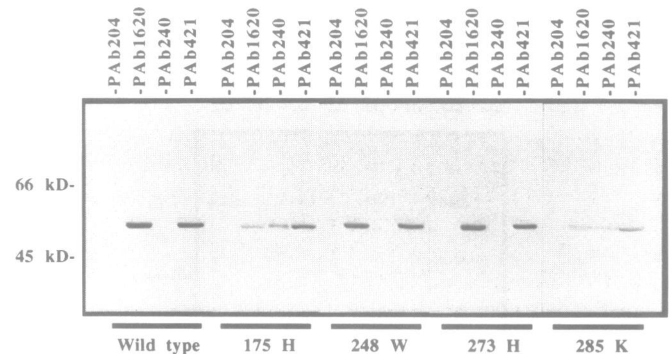


Figure 2. Immunoblot of immunoprecipitates. Cell extracts from strains containing different human p53 expression plasmids were immunoprecipitated with the monoclonal antibodies indicated and the blot was probed with a rabbit antibody against p53 (CM1).

Truncation of this promoter at the XhoI site removes upstream activating sequences which normally control the activity of the CYC1 promoter ('minimal promoter'). The test promoter ('p53::CYC1 promoter') contains a 33 base pair p53 DNA-binding sequence (20) cloned into the XhoI site of the minimal promoter. The p53 expression plasmid contains a wild type human p53 cDNA under the control of the GAL1 promoter: p53 expression is repressed by glucose and stimulated by galactose. Table 1 shows that p53 stimulates lacZ expression, and that the effect requires both the presence of p53 protein (compare glucose with galactose) and the presence of the p53 DNA-binding site (compare the minimal promoter with the p53::CYC1 promoter). The effect of orientation and copy number of the 33 base pair sequence on wild type human p53-dependent transcription were analysed (table 2). Transcription from a single copy is four-fold more effective in one orientation than the other, and is strongly dependent on the number of copies of the binding site oligonucleotide.

The ability of human p53 mutants to stimulate transcription was tested with the reporter plasmid containing a single copy of the binding site oligo in the more effective orientation. Four mutants were examined (175H, 248W, 273H and 285K). Amino acids 175, 248 and 273 are hotspots for mutation in human tumours (1). Amino acid 248 is commonly mutated in the Li-Fraumeni hereditary cancer syndrome (43). The 175H mutant cooperates efficiently with ras in transformation assays and binds to hsc70, whereas the 273H mutant cooperates inefficiently with ras and fails to bind to hsc70 (44). The 285K mutant was chosen because it binds well to the mutant p53-specific antibody PAb240 and fails to bind to the wild type p53-specific antibody PAb1620 in immunoprecipitation assays using mammalian cell extracts (45). Three of the mutants (175H, 248W and 273H) are completely inactive in transcription assays in yeast whereas the fourth mutant is weakly active (285K, table 3). The inactivity of the mutants does not result from a general inhibition of transcription or failure to enter the nucleus (data not shown). All of the mutants express detectable amounts of p53 protein (figure 1), although the level of the 175H and 285K mutants is lower than that of wild type p53, presumably due to instability of the mutant protein. Since the 285K mutant is partially active despite its low level of expression the inactivity of the 175H mutant can not be due solely to its low, but comparable, level of expression. The 285K mutant is analysed in detail below.

The epitopes displayed by human p53 expressed in yeast were examined by immunoprecipitation with conformation-sensitive antibodies to see if there was a correlation between epitope display, mutation and transcriptional activity (figure 2). In

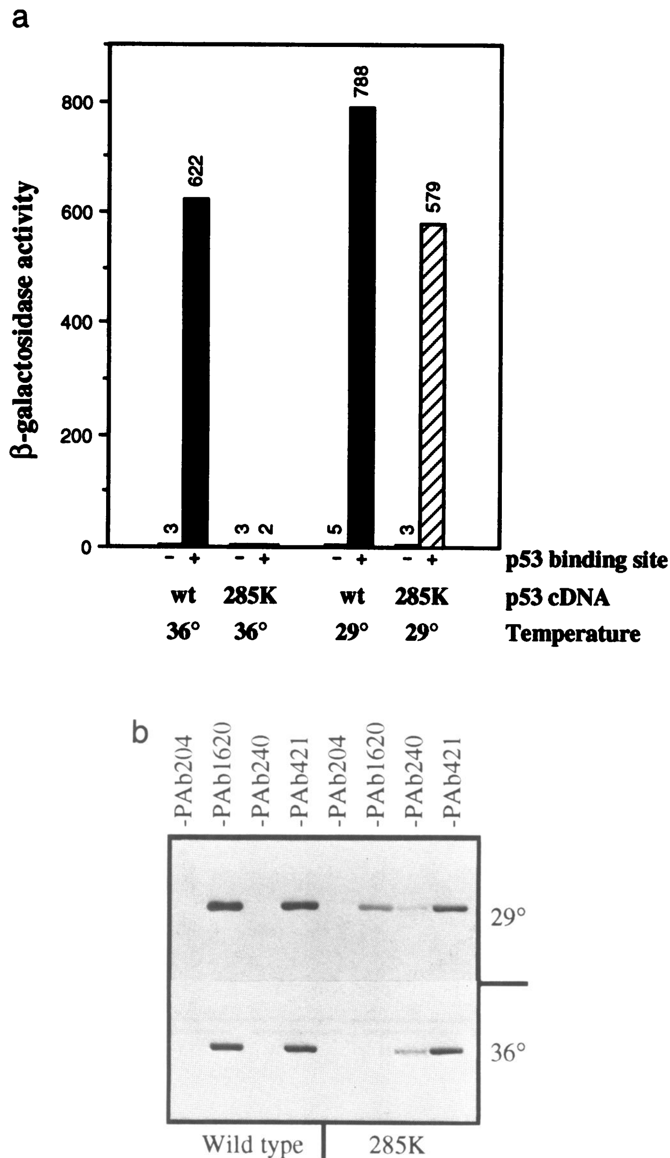


Figure 3. a. β-galactosidase assays using human wild type and 285K mutant p53. '+/− p53 binding site' refers to the presence or absence of a p53-binding oligonucleotide cloned upstream of the minimal CYC1 promoter. b. Immunoblot of immunoprecipitates. Cell extracts from strains expressing human wild type or 285K mutant p53 were immunoprecipitated with the monoclonal antibodies indicated and the blot was probed with a rabbit antibody against p53 (CM1).

mammalian cells PAb240 and PAb1620 preferentially recognise mutant and wild type p53 respectively (31,45). PAb421, which recognises all p53 molecules, and PAb204, an anti-T antigen antibody, were used as positive and negative controls, respectively. Only the 175H and 285K mutants express significant levels of the PAb240 epitope; the other mutants and wild type p53 express almost exclusively the PAb1620 ('pseudo-wild type') epitope. Since two of the mutants are indistinguishable from wild type when probed with antibodies but are completely inactive in transcription assays it is clear that immunoreactivity can not be used to predict transcriptional activity.

The epitope structure of several p53 mutants has previously been shown to be temperature-dependent (46,47,48,49,50,51). The dual phenotype of the 285K mutant described above, ie weak activity in transcription assays and the expression of both wild type and mutant epitopes, prompted us to test it for temperature-sensitivity. Figure 3a shows that the mutant is transcriptionally highly active at 29° and completely inactive at 36°. It is also temperature-sensitive for expression of the PAb1620 epitope (figure 3b). The level of protein is low at 30° (figure 1) and falls further at 36° (data not shown). A detailed analysis of transcriptional activity and protein expression was therefore performed. The mutant is transcriptionally active at 29° and inactive at 33° (figure 4a). In this temperature range the level of protein does not change significantly (figure 4b). The human 285K mutant is thus intrinsically temperature-sensitive for transcriptional activity.

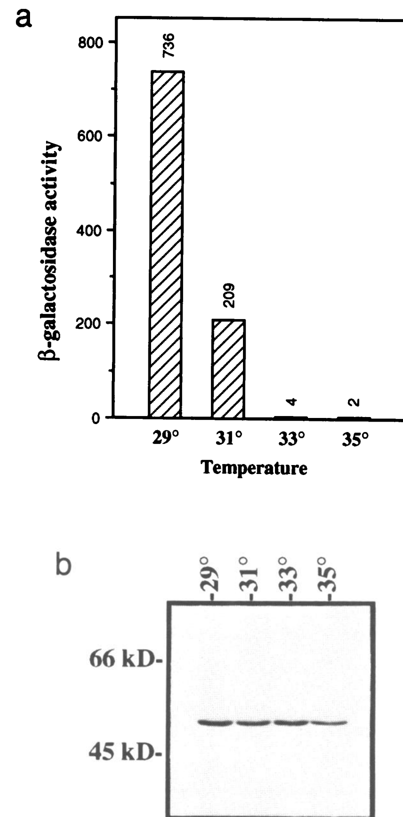
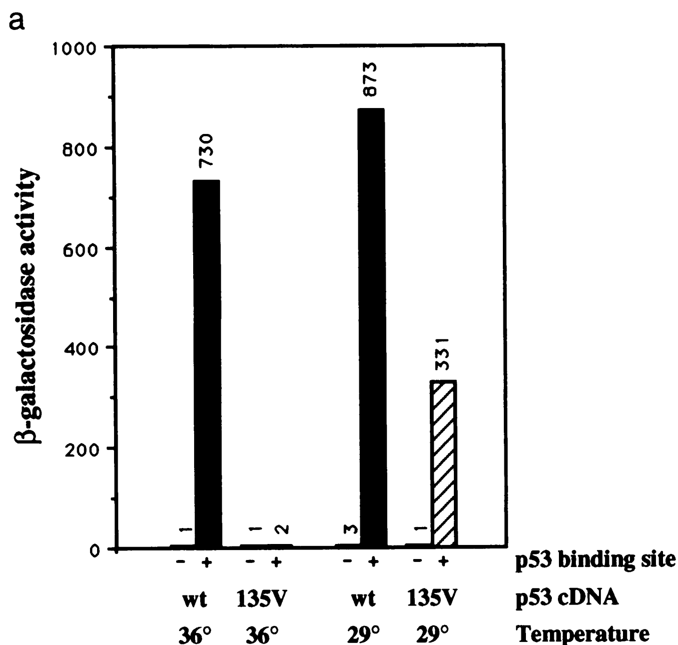


Figure 4. a. β-galactosidase assays using human 285K mutant p53. b. Immunoblot of yeast expressing human 285K mutant p53 probed with antibodies against p53 (PAb240 and PAb421).

Murine p53 expression in yeast

The murine p53 protein has not previously been tested for binding to the 33 base pair DNA sequence identified by Kern et al (20). We show here that murine p53 is clearly able to activate transcription from that sequence in yeast (figure 5a). The murine 135V mutant is temperature-sensitive for transformation of mammalian cells (49) and its transactivation domain is temperature-sensitive in GAL4 fusion proteins (15). Figure 5a shows that this mutant is also temperature-sensitive for transcriptional activation from the p53 binding site oligonucleotide. The difference in transcriptional activity is not accounted for by changes in the level of p53 expression at the two temperatures (figure 5b). As in mammalian cells the epitope structure of the mutant changes following temperature shift: the



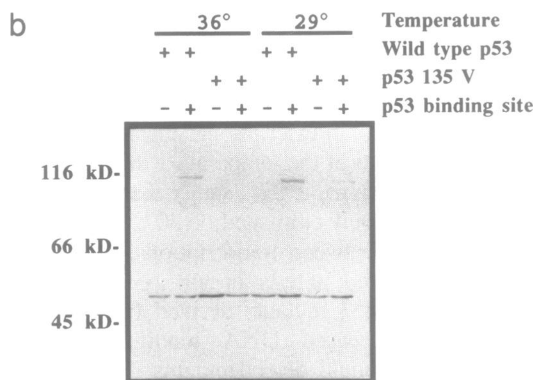


Figure 5. a. β-galactosidase assays using mouse wild type and 135V mutant p53. '+/− p53 binding site' refers to the presence or absence of a p53-binding oligonucleotide cloned upstream of the minimal CYC1 promoter. b. Immunoblot of yeast probed with antibodies against p53 (PAb240 and PAb421) and β-galactosidase (BG2). Strains containing mouse wild type or 135V mutant p53 expression plasmids and a lacZ reporter plasmid were grown in galactose to induce p53 expression. '+/− p53 binding site' refers to the presence or absence of a p53-binding oligonucleotide cloned upstream of the minimal CYC1 promoter.

PAb240 epitope is relatively more abundant at 36° and the PAb1620 and PAb246 (murine wild type-specific) epitopes are relatively more abundant at 29° (figure 6).

A transcriptional screening assay for p53 mutation

The correlation between mutation and transcriptional inactivation suggests that it should be possible to detect p53 mutation using a simple yeast colony colour assay. Yeast will repair double stranded breaks in transfected plasmids by homologous recombination ['gap repair' (26)]. If yeast are transfected with both a gapped plasmid and a suitable linear template they use the transfected template to repair the gap. It is thus possible to cotransfect yeast with a fragment of p53 cDNA and a gapped p53 expression plasmid (figure 7), select for the auxotrophic

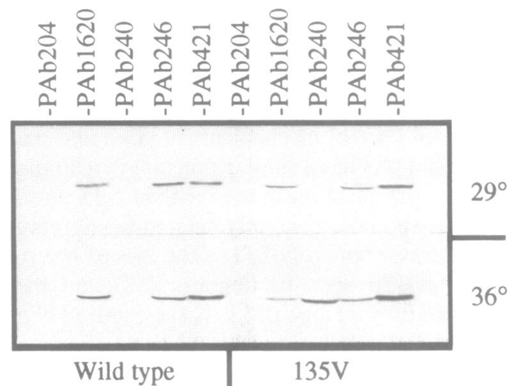


Figure 6. Immunoblot of immunoprecipitates. Cell extracts from strains expressing mouse wild type or 135V mutant p53 were immunoprecipitated with the monoclonal antibodies indicated and the blot was probed with a rabbit antibody against p53 (CM1).

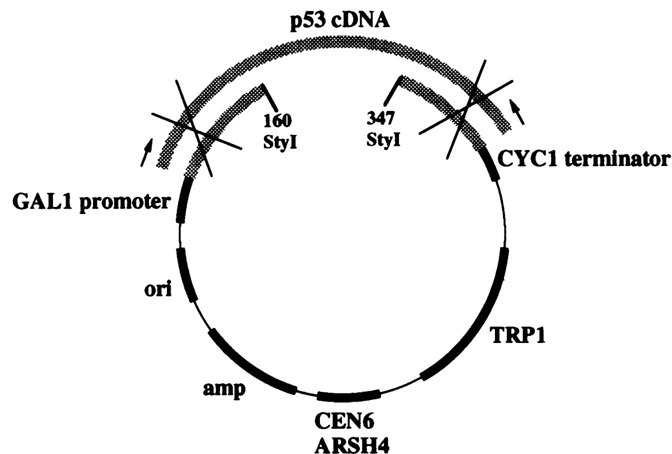


Figure 7. Diagram depicting the DNA molecules transfected into yeast for the gap repair assay. The p53 expression plasmid containing the 175H mutation was cut with StyI and the indicated fragment was gel purified. p53 cDNAs were amplified by PCR using primers lying just outside the open reading frame. Crosses indicate the homologous recombination events required to repair the plasmid. Self-ligated plasmid molecules can not produce functional p53 molecules because the region encoding amino acids 160 to 347 has been deleted.

marker on the plasmid and test colonies for β-galactosidase activity on X-gal plates. To test this strategy, wild type and 273H mutant p53 cDNA plasmids (proSp53 and pR4.2) were amplified by the polymerase chain reaction using primers which span the open reading frame and cotransfected with a gapped expression plasmid into yeast containing a lacZ reporter plasmid. Non-homologous repair and integration events are expected to give rise to a small number of colonies in transfections with plasmid alone. Addition of the p53 PCR product produced a 75-fold increase in the number of colonies relative to that seen with gapped plasmid alone. 35 randomly picked colonies from the primary transfection plates were transferred to plates containing galactose and X-gal to induce p53 expression and assay β-galactosidase activity. All colonies from the plate transfected with plasmid alone were white. One out of 35 colonies transfected with the 273H mutant was blue, and 30 out of 35 colonies transfected with the wild type PCR product were blue.

DISCUSSION

The main conclusion from this study is that mammalian p53 can function as a transcription factor in yeast. The strong conservation of transcriptional control mechanisms in yeast and mammalian cells suggests that p53 has a similar function in mammalian cells (25). However, the situation in mammalian cells appears more complex because p53 has generally been found to repress rather than stimulate transcription (10,11). The loss of heterozygosity of chromosome 17p seen in tumours (52) and the growth inhibition caused by wild type p53 in tissue culture (49,53,54,55) are easily explained if p53 stimulates the transcription of growth inhibitory genes, for example GAS, GADD or TA1 arrest genes (56,57,58). However, no simple growth suppression model can accommodate the observation that p53 expression is induced 10 to 20-fold by serum stimulation (59). Furthermore, the transcriptional inactivity of the p53 mutants in yeast does not readily explain the positive transforming action of these mutants in p53-null cell lines (60). It is possible that mutant and wild type p53 show different sequence specificity and hence bind to different promoters. Alternatively, mutant p53 may act indirectly to transform mammalian cells. For example, it may produce inactive heterodimers with other transcription factors.

The involvement of p53 in transcription does not rule out a role in replication. Many experimental systems exist in which effects on replication and transcription overlap and many proteins are known which are apparently involved in both processes (61,62,63,64,65,66,67,68). Attractive models to explain p53's dual role as both a recessive and a dominant oncogene thus include mutation altering the specificity of DNA binding, mutation converting a transcriptional activator into a repressor and mutation converting a transcription factor into a replication factor.

The 33 base pair oligonucleotide which was used as a p53 binding site in this study was previously shown to bind to p53 in immunoprecipitation assays (20). The sequence is present in the ribosomal non-transcribed spacer region 3.5 kb upstream of the 45S rRNA start site. It has not been shown to function as a classical enhancer in this context. Since it lies near a putative origin of DNA replication it has been postulated that p53 binding to it may reflect a role for p53 in the control of DNA replication (20). Proof that p53 is a transcription factor requires identification of an endogenous promoter whose activity is dependent on p53 binding. p53's ability to stimulate transcription in a sequence-specific manner in yeast should greatly facilitate this task because it is possible to screen libraries of potential p53-binding fragments using 'enhancer trap' technology in yeast (69).

The *in vitro* SV40 DNA replication system is inhibited by wild type p53 (7,8,70). It will clearly be of interest to see whether p53 can influence the transcriptional activity of T antigen and vice versa. The yeast transcription system should allow rapid progress in characterising this interaction. It should also facilitate studies on the interaction between wild type and mutant p53 and that between p53 and proteins such as adenovirus E1B (71) and papilloma virus E6 (72).

Immunoprecipitation with conformation-sensitive antibodies is widely used as an assay for p53 mutation. The results presented here support the idea that mutation can lead to changes in the structure of the p53 protein, but argue strongly that induction of these changes is not the primary target of tumorigenic p53 mutations: all of the mutants were abnormal in transcription assays, whereas two of the mutants were indistinguishable from wild type in immunoprecipitation assays. The structure of p53 determined with conformation-sensitive antibodies is similar in yeast and mammalian cells. There are clear instances in mammalian cells where the epitopes displayed by the same p53 mutant can vary in different cell lines or at different temperatures (31,45,46,47,48,49,50,51,73,74). The most plausible explanation for the variation is that p53 can fold in different ways. It is possible that all mutants which display both the PAb1620 epitope and the PAb240 epitope in immunoprecipitation assays will prove to be temperature-sensitive. If that were the case the 273H mutant (which displays both epitopes in some mammalian cell lines) and the 175H mutant (figure 2), would have to be extremely heat sensitive since they are completely inactive in transcription assays performed at 30° (table 3). Since there is good evidence that certain mutants can adopt both the PAb240-positive and the PAb1620-positive conformations at a single temperature the conclusion that a cell line is heterozygous for p53 mutation because it contains both forms of the protein is unwarranted (75). The existing panel of conformation-specific anti-p53 antibodies needs to be used with great care: many p53 mutants bind only to PAb1620, the 'wild type-specific' antibody, in immunoprecipitation assays; and wild type p53 binds well to PAb240, the 'mutant-specific' antibody, following denaturation of the antigen, as occurs during immunoblotting or fixation for cell staining.

In contrast with the results using antibodies, all of the mutants tested here score unequivocally as mutant in transcription assays. The only note of caution is that some mutants may be temperature-sensitive for transcriptional inactivation. It makes sense to test p53 mutants at the temperature at which they were presumably selected *in vivo*, ie 37°, rather than the temperature at which yeast are routinely cultivated, ie 30°. That there should be a tight correlation between transcriptional inactivation and mutation is not unexpected: in tests for non-specific DNA binding fifteen out of fifteen p53 mutants derived from cell lines or tumours showed decreased DNA binding activity (18). Transcription assays should detect mutants defective in either DNA-binding or transactivation (or both) and hence should be even more sensitive than DNA-binding assays. The overall conclusion is that transcriptional activity is a more sensitive indicator of mutation of the p53 gene than immunoreactivity and thus more probably reflects a biologically relevant phenotype.

Many studies have shown overexpression of p53 protein in human tumours (37, and references therein). Although the p53 gene has been sequenced from a large number of tumours there is still a discrepancy between the ease with which overexpression can be detected by cell staining and that with which mutations can be detected by DNA sequencing. We describe a simple colony colour assay which can be used to detect p53 mutations. The assay inevitably gives a small number of false positives and false negatives, presumably because of mutations introduced by Taq polymerase. The false negative in this study presumably contains an intragenic suppressor (ie, a second mutation in p53 which reverts the phenotype of the 273H mutation). Such suppressors are potentially useful tools to study p53 conformation. By transfecting yeast directly with p53 PCR products the difficulties associated with direct sequencing and the additional steps required for subcloning in bacteria are avoided. However, the assay does require cDNA and hence tumour RNA because *S.cerevisiae* does not splice mammalian introns. Nevertheless, the gap repair assay should greatly facilitate the detection of p53 mutations in tumours because it lends itself readily to the analysis of large numbers

of samples. The assay using the 273H mutant was performed at 30°; given the known temperature-sensitivity of some mutants it would be clearly be appropriate to induce p53 expression on X-gal plates at 37°.

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